Predictive Biomarker Profiling of > 6000 Breast Cancer Patients Shows Heterogeneity in TNBC, With Treatment Implications

Sherri Z. Millis,1 Zoran Gatalica,1 Josiah Winkler,1 Semir Vranic,2 Jeffery Kimbrough,1 Sandeep Reddy,1 Joyce A. O’Shaughnessy3

Abstract

We evaluated 6341 consecutive breast cancer samples across multiple platforms to identify biomarkers of potential drug response. Subgroups of triple-negative breast cancers were identified, with different gene mutations, protein expression levels, and patterns in co-incidence, which might inform individualized treatment options.

Background: Triple-negative breast cancer (TNBC) is an aggressive disease without established targeted treatment options for patients with metastatic disease. This study was undertaken to evaluate potentially actionable biomarkers in a large cohort of TNBC and compare them with non-TNBCs. Materials and Methods: We evaluated 6341 (2111 TNBC and 4230 non-TNBC) breast cancer samples at a central laboratory for biomarkers of potential drug response across multiple platforms, including gene sequencing, protein expression, and gene copy number. Results: TNBC expresses androgen receptor (AR) in a significantly (P < .05) lower percentage of cases (17%) than hormone receptor (HR)-positive and human epidermal growth factor receptor 2 (HER2)-positive breast carcinomas (59% and 79%, respectively), and gene comutations were differentially associated with AR-positive versus AR-negative cases. Higher AR expression levels in TNBC predicted for lower Ki-67 levels. Seventy percent of TNBC harbored a phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA), v-akt murine thymoma viral oncogene homolog 1 (AKT1), or phosphatase and tensin homolog (PTEN) aberration. TNBC patients had a significantly lower PIK3CA mutation rate (13%) than all other subtypes (P < .05) and a higher tumor protein p53 (TP53) mutation rate (64%) than the estrogen receptor (ER)-positive cases (approximately 30%; P < .05). Topoisomerase 2 (TOP2A) amplification was observed in 1.3% of TNBC and in 1.6% of HER2-negative, HR-positive cancers; in contrast, HER2-positive, HR-negative or HR-positive cancers exhibited TOP2A amplification in 19% and 40% of cases, respectively (P < .05).

Conclusion: Multi-platform molecular profiling identifies subgroups of TNBC with different biomarker profiles, suggesting numerous potentially targetable alterations in TNBC. TNBC is further characterized by different gene mutations and proliferative activity relative to AR expression, highlighting a need for comprehensive pathologic examination with potential to develop different, individualized treatment options.

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Introduction

Breast cancer is the leading cause of cancer-related mortality in women worldwide.1 It is a heterogeneous disease at the molecular—genetic and clinical levels, resulting in variable responses to existing treatment modalities.2 Recent gene expression profiling studies have subclassified breast cancer into at least 4 molecular subtypes including at least luminal A and B, human epidermal growth factor receptor (HER2)-positive, and triple-negative breast tumors.

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Triple-negative breast cancer is typically an aggressive subtype of breast cancer. Approximately 30% of TNBC patients experience a rapid relapse (within 1 to 3 years) after standard adjuvant chemotherapy with a median survival of 12 to 18 months with metastatic disease. Treatment options are limited because of an absence of the more common molecular targets in breast cancer (ER, PR, HER2). The largest comprehensive analysis of TNBC to date consisted only of disease from primary tumors. Few data are available on metastatic and locally recurrent TNBCs. Therefore, an unmet medical need exists to identify TNBC biomarkers that might predict therapy response.

In the present study we reviewed a database of 6341 breast carcinomas profiled at a single institution (Caris Life Sciences) in an effort to further differentiate TNBC from other breast cancer subtypes and to identify potentially targetable molecular characteristics within TNBC.

**Materials and Methods**

**Tissue Samples**

A cohort of 6341 consecutive breast cancer samples, comprising 6206 unique patients, were profiled at Caris Life Sciences from 2009 through February 2014. Of those, 5823 unique breast cancer patients with known ER, PR, and HER2 status were evaluated for similarities and differences in gene mutations (Sanger or next-generation sequencing [NGS]), protein expression (immunohistochemistry [IHC]), and/or gene amplification using DNA in situ hybridization (ISH; fluorescent [FISH], and chromogenic [CISH]).

**Multiplatform Testing**

Testing was performed on formalin fixed paraffin embedded tissue samples using the Caris Molecular Intelligence (CMI) tumor profiling service (http://www.carismolecularintelligence.com). CMI uses multiple standard platforms and methodologies, including protein expression (IHC), gene mutation analysis (NGS and Sanger sequencing), and gene copy number alterations (FISH and CISH; list available at: http://www.carismolecularintelligence.com/next-generation-sequencing-profile).

Per clinical recommendations for breast cancer, ER and PR were considered positive when ≥ 1% of tumor cells exhibited nuclear positivity (College of American Pathologists [CAP]/American Society of Clinical Oncology [ASCO] 2010). HER2/neu was positive when > 10% of cells exhibited strong complete membranous staining (score 3+); alternatively, FISH or CISH was used for evaluation of the HER2/neu (HER2/chromosome 17 centromere [CEP17] probe); HER2/CEP17 ratio > 2.0 was considered amplified (CAP/ASCO guideline 2013). Thresholds used for other IHC and in situ hybridization assays are listed in Supplemental Table 1 in the online version.

**Predictive Biomarker Profiling and Heterogeneity in TNBC**

The cohort was grouped according to ER and PR IHC status (performed centrally), and HER2/neu IHC or ISH status (Figure 1). Metaplastic breast cancer cases were excluded; ER and PR were considered positive when ≥ 1% of tumor cells exhibited nuclear positivity. The cohort was divided into ER-negative (ER−; HER2−; PR−), ER-positive (ER+; HER2−; PR−), and ER-positive (ER+; HER2−; PR+) groups. The ER− (HER2−; PR−) group was further divided into hormone receptor-positive (HR+) and hormone receptor-negative (HR−) subtypes, defined by PR status (Table 1). The ER+ (HER2−; PR−) group was further divided into HR+ and HR− subtypes, defined by PR status (Table 1).
positivity (CAP/ASCO 2010). HER2/neu was positive when > 10% of cells exhibited strong complete membranous staining (3+) or when FISH or CISH evaluation of the HER2/neu (HER-2/CEP17 probe) HER2/CEP17 ratio was > 2.0 (CAP/ASCO guideline 2013).

Depending on tissue availability, physician preference, and technology standards over the course of sample receipt at the laboratory, the testing breakdown varied by case (TNBC detailed in Figure 2). For example, gene sequence analysis was performed using Sanger sequencing on a limited number of relevant genes before the availability of NGS technology, which then led to hot spot analysis of larger numbers of genes simultaneously.

All methods used in this study were clinically validated to at least Clinical Laboratory Improvement Amendments, CAP, and International Organization for Standardization (ISO) 15189 standards. The therapeutic drug associations were determined using recommendations from published clinical evidence, which includes peer-reviewed literature and/or the National Comprehensive Cancer Network guidelines, not restricted to cancer type. No preclinical or experimental drug associations were made; however, biomarker associations with drugs in advanced-stage clinical trials were made.

Statistical Methods

The 2-tailed Fisher exact test and $\chi^2$ test were used to test when proportions of positive results were different between TNBC and non-TNBC (significance level $\leq .05$). JMP version 10.0 (SAS Institute Inc, Cary, NC) and R version 2.15 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analysis.

Results

The 5823-patient study included 2111 (36%) TNBC patients. The high percentage of TNBC patients in this breast cancer cohort is likely due to the aggressive nature of TNBC, which led to a higher percentage of cases submitted for molecular profiling than in the general breast cancer population. Of the TNBC cohort 1302 (62%) consisted of tissue from metastatic cancers. Of the overall patient cohort 560 cases (14%) were HER2-positive, and within that cohort 229 (4%) were positive for ER and PR (Figure 1).

Multiplatform Profiling and Relevance to Therapeutic Targets

There were 2300 breast cancer cases in this cohort, including 760 TNBC cases, that were profiled by either NGS (n = 450) or Sanger sequencing (n = 310). Mutations were detected in 39 of 45 genes tested (87%; Figure 3). Because of the extremely low incidence of many of the mutations, no clear mutation pattern difference was identified between the subtypes, nor was a significant difference in the mutational load between metastatic cases and primary breast cancers identified across all subtypes, with the exception of TNBC. The TNBC metastatic tissues had significantly more cases with gene mutations, cases with comutations, and overall more total gene mutations than did the primary/recurrent TNBCs (215 of 276 vs. 147 [P = .0005]; 305 vs. 147 [P = .001], respectively). Notable genes that were mutated more frequently in metastatic cases included TP53, PIK3CA, and PTEN (Figure 4).

The most common mutations in TNBC included TP53 (278 of 437; 64%) and PIK3CA (93 of 702; 13%). TP53 mutations were less frequently identified in hormone receptor (HR)-positive cases (P < .05), especially in the absence of HER2 positivity. In contrast, PIK3CA mutations were 2 to 3 times higher in the non-TNBC population (P < .05) than in the TNBC group. Compared with luminal breast cancers (HR-positive), TNBC had a statistically
significant greater frequency of \textit{PIK3CA} mutations in exon 20. TNBC had a statistically significant lower frequency of mutations in exon 9 compared with luminal and HER2-positive/HR-negative cases. TNBC also had a higher frequency of mutations outside of hotspot exons 9 and 20, compared with other subtypes (Figure 5).

Phosphatase and tensin homolog protein loss (0 and <50%) was observed in 66% (1368 of 2069) of TNBC and in 53% (1513 of 2884) of non-TNBC tumors ($P < .0001$), despite the infrequent rate of PTEN mutations (4% [78 of 1950]) in both tumor types. Mutations in another Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3) kinase pathway gene, \textit{v-akt murine thymoma viral oncogene homolog 1} (\textit{AKT1}), were not seen in any HER2-positive cancers, but were seen at low frequency in HER2-negative cancers (3.8% [14 of 369] in ER-positive/PR-positive; 5.2% [10 of 192] in ER-positive/PR-negative, and 2.9% [5 of 172] in TNBC). Within the TNBC cohort, PI3 kinase pathway involvement measured using either \textit{AKT1}, \textit{PIK3CA}, or \textit{PTEN} aberration was identified in 70% (1478 of 2111) of cases, regardless of AR expression levels. This trend in PI3 kinase pathway involvement was seen in HER2-negative and HR-positive/HER2-positive or -negative cancers as well.

\textit{HER2} mutations were found in 9 of 448 TNBCs tested (see Supplemental Table 2 in the online version), similar to rates in the other subtypes. Twenty-four \textit{HER2} mutations were identified across all subtypes, 5 of which were unique to these breast cancers and had not been observed in 10,000 cases across all solid tumors tested at this laboratory. Two of these unique \textit{HER2} mutations were found in the TNBC cohort. Of the \textit{HER2} mutations in the TNBC cohort 67% (6 of 9) were observed in primary tumors whereas only 20% (3 of 15) of non-TNBC \textit{HER2} mutations were found in primary sites ($P = .036$).

In general, mutations in other genes were not frequently observed. \textit{cKIT} mutations were very rare in TNBC (1 of 547; 0.2%) despite the common expression of \textit{cKIT} protein (329 of 1239; 26%). Adenomatous polyposis coli was found in 3% (14 of 448) of cases, ataxia telangiectasia mutated in 2% (7 of 442), F-box and WD repeat domain containing 7 in 1% (3 of 447), Harvey rat sarcoma viral oncogene homolog in 1% (4 of 368), KRAS in 1% (10 of 739), retinoblastoma 1 in 2% (9 of 445), serine/threonine kinase 11 in 1% (6 of 418), and Braf in 0.3% (2 of 743) of cases. Ten of the 13 mutations identified in the RAS/RAF pathway were activating. AR positivity (≥ 10% cells with nuclear AR) was significantly lower (340 of 1951; 17%) in TNBC (Figure 6) than in HER2-positive and/or HR-positive tumors (46%-79%, $P < .05$). Additionally, a significant difference in the percentage of AR positivity was seen in the primary/recurrent versus metastatic TNBC samples (14% [104 of 735] vs. 19% [236 of 1215], respectively, $P = .01$).

Androgen receptor status and concurrent PI3 kinase alterations were also evaluated across subtypes (Table 1). Fifty-eight percent (1997 of 340) of TNBCs positive for AR also harbored either \textit{PIK3CA} or \textit{PTEN} mutation/protein loss. Similar rates of \textit{PIK3CA} and \textit{PTEN} mutation/protein loss were observed in AR-positive non-TNBC cancers (58% vs. 59%). Across all subtypes, AR-positive cases had a 36% (398/1004) \textit{PIK3CA} mutation rate, and only 9% (47/524) of the AR-negative cases had a \textit{PIK3CA} mutation, similar to a recent report. The types of \textit{PIK3CA} mutations differed between AR-positive and AR-negative cancers (see Supplemental Table 3 in the online version). AR-negative TNBC cases had a
greater percentage of mutations in exon 20 (68% vs. 62%) than AR-positive cases, and AR-positive cases had more mutations in exon 9 (24% vs. 19%). As noted, AKT1 mutations were found only in HER2-negative subtypes; interestingly, these few instances of AKT1 mutations were nearly always mutually exclusive of PIK3CA/PTEN mutation/loss. Overall, 91% (1921/2111) of TNBC cases had changes in AR status and/or mutations in PIK3CA/PTEN/AKT1, or loss of PTEN.

Triple-negative breast cancer with higher degrees of AR positivity assessed according to Allred score\(^16\) exhibited significantly lower proliferation rates (Ki-67) compared with AR-negative TNBC (\(P < .05\); Figure 7).

Amplification of the Topoisomerase 2 (TOP2A) gene was observed in only 1% (10 of 778) of TNBC and 2% (18 of 1127) of HER2-negative, HR-positive cancers, despite the common observation of Topo2z protein expression (67% [865 of 1289] and 41% [774 of 1869], respectively). HER2-positive, HR-negative or HR-positive cancers harbored EGFR and cMYC amplification in only 8% (54 of 650) and 13% (82 of 604) of cases, respectively. Additionally, in TNBC, EGFR amplification was associated with a significant decrease in expression of TS (\(\geq 1+\)) and \(\geq 10\%\); 26% of EGFR nonamplified cases vs. 10% of EGFR amplified cases; \(P = .0001\)) and RRM1 (\(\geq 2+\)) and \(\geq 50\%\); 36% of EGFR nonamplified cases vs. 20% of EGFR amplified cases; \(P = .0003\), respectively). Of the EGFR amplified TNBC 95% overexpressed TOP2A and 90% were AR-negative. EGFR amplification was almost always associated with HER2 amplification in non-TNBC breast cancers, although the converse was not seen.

Transducin-like enhancer of split 3 (TLE3) overexpression was observed in 33% (587 of 1772) of TNBC. Significantly greater TLE3 overexpression was observed in HER2-positive and HR-
positive cancers (156 of 330; 47%; 58% [1425 of 2457]-70% [147 of 210]; \( P = .0001 \)). TS overexpression (\( \geq 1 + \) and \( \geq 10\% \)) was seen in 26% (489 of 1899) of TNBC cases and in 17% (717/4283) of HER2-positive cases, whereas only 10% (301/3011) of HR-positive cancers exhibited significant TS expression (\( P < .05 \)). Pgp overexpression (\( \geq 1 + \) and \( \geq 10\% \)) was similarly distributed across molecular subtypes and was generally uncommon (12% of TNBC, 11% of HER2-positive, and 5% of HR-positive cases). The percentage of cases that displayed topoisoerase 1 expression (\( \geq 2 + \) and \( \geq 30\% \)), RRM1 expression (\( \geq 2 + \) and \( \geq 50\% \)), MGMT expression (\( \geq 1 + \) and \( \geq 35\% \)), and SPARC overexpression (\( \geq 2 + \) and \( \geq 30\% \)) were fairly evenly distributed across the molecular subtypes, including TNBC. Other changes in protein expression and or gene amplification status associated with potential therapeutic options are shown (Figure 6).

**Discussion**

Protein expression and gene sequencing profiling have contributed significantly to the characterization of the genomic and molecular landscape of breast cancer, especially in characterization of primary tumors. However, with few exceptions such as breast cancer 1 and 2 mutations, a substantial proportion of breast cancer patients remain without targetable gene mutations, particularly those with aggressive subtypes such as TNBC. In this study, which is the largest known cohort published to date (compared with Lehmann with 587 TNBC \(^{18} \) and Teng with 653 TNBC \(^{19} \)), we
Figure 7 (A) Androgen Receptor (AR)-Positive and (B) AR<sub>+</sub> (<10%) Correlation With Ki-67 in Triple-Negative Breast Cancer

characterized breast cancers using multiple platforms to measure gene mutations, gene amplification, and protein expression to identify potentially actionable targets previously not considered or not considered in combination. Additionally, we evaluated differences in mutation rates between primary and metastatic breast cancers. Analysis of this large cohort, of which 62% presented with metastatic tissue, identified numerous predictive biomarkers for targeted agents, cytotoxic therapies, and suggests potential treatment combinations.

A limited number of actionable somatic mutations characterize breast cancers including TNBC. In the current study, NGS of TNBC identified mutations in 29 of 45 genes tested, and in only 22% of cases tested, of which 15% were potentially clinically actionable mutations (defined as a mutation for which there is a Food and Drug Administration-approved therapy). Specifically, PIK3CA, PTEN, and APC mutations were the most common in TNBC using NGS. TP53 mutations, although not considered clinically actionable, were also commonly seen and have been associated with poor response to anthracyclines and radiotherapy. TP53, PIK3CA, and PTEN were found at greater frequencies in metastatic TNBC compared with primary disease. All other mutations occurred at very low levels (≤ 1% of cases), similar to what has been reported in previous studies on somatic mutations in breast cancer. This affirms the limited number of actionable targets in TNBC when only gene mutations are evaluated. For example, we identified EGFR mutations in only 2 of 466 TNBC cancers (1 primary and 1 metastatic), in contrast to EGFR gene amplification, which was observed in 22% of TNBC breast cancers. Germline mutations, including BRCA1/2, were not evaluated. Somatic BRCA1/2 mutations evaluated at this laboratory were identified at an incidence similar to what was previously reported and were not a part of this analysis.

Epidermal growth factor receptor amplification was also seen at significant rates in other subtypes. Of HER2-positive/HR-negative breast cancers 23% (30% in primary and 20% in metastatic tissues) harbored EGFR amplifications, as did 10% of poor-prognosis ER-positive breast cancers. These results indicate that single (anti-EGFR) and dual therapeutic targeting (anti-HER2/EGFR) might be worthy of exploration in TNBC and HER2-positive patients, respectively.

HER2 mutations have been described in a small percentage of various human tumors including lung, colorectal, head and neck, ovarian, and gastric carcinomas. These mutations can also occur in HER2-amplified and non-amplified breast carcinomas as confirmed in a small proportion of the cases in our study. More HER2 mutations were found in primary TNBCs compared with other breast cancers, suggesting perturbation of the HER2 pathway in some TNBC. Inhibition of HER2 mutation neratinib in patients with HER2 non-amplified breast cancer is currently being investigated.

The proportion of AR positivity in TNBCs (17%) was significantly lower than in non-TNBCs (46%-79% range). ER-negative HER2-positive breast cancers had a significantly lower incidence of AR-positive cases (46%) compared with ER-positive cases (79%). Expression of AR in breast cancers might be associated with response to AR inhibitors such as bicalutamide or enzalutamide. An ongoing study with enzalutamide is currently being explored in a phase II trial in patients with TNBC, and data presented at the San Antonio Breast Cancer Symposium (SABCS) 2014 showed effectiveness (P5-19-09, Traina, et al. Stage 1 results from MDV3100-11: A 2-stage study of enzalutamide [ENZA], an AR inhibitor, in advanced AR+ TNBC). Apocrine breast tumors (AR-positive) tend to harbor PIK3CA and/or PTEN alterations. PI3 kinase pathway inhibitors might be of benefit in combination with inhibitors of AR in these patients. As has been previously reported, our analysis also showed PIK3CA mutant cancers more commonly expressed AR than do PIK3CA wild type cancers. A retrospective study showed a significant association between AR positivity and the presence of PIK3CA mutations in ER/PR-negative, AR-positive tumors.

Furthermore, we found a 2-fold increased frequency of PIK3CA/PTEN alterations in metastatic compared with primary TNBCs. This finding corroborates previous reports. Apart from its potential predictive role, we found higher levels of AR expression to be significantly associated with decreased proliferation in TNBC as has been recently reported. This observation highlights the heterogeneity of TNBC and suggests a potentially more favorable clinical course in a subset of more indolent AR-positive TNBCs.
Predictive Biomarker Profiling and Heterogeneity in TNBC

The AR is emerging as an important new target in ER-positive and TNBC, yet it is not known which subtypes of patients might benefit and should therefore be selected for clinical trial enrollment of antiandrogen therapy. A relationship between the degree of AR positivity and tumor biology (eg, Ki-67), has been elucidated for ER-positive disease, yet few data exist from central reference laboratories that describe this relationship. This is the only study to date with a large number of TNBC with Ki-67 and AR status evaluated in a single laboratory.

Among cytotoxic agent response biomarkers, our study revealed high topo1 expression in TNBC, which could potentially refine the use of therapy with topoisomerase 1 inhibitors in breast cancers. Some topoisomerase 1 inhibitors (eg, etirinotecan, irinotecan) have been previously evaluated in metastatic breast cancer and have evidence of activity, including in TNBC.24 Topo1 has not been shown to select for TNBC or non-TNBC patients that benefit from topoisomerase 1 inhibitors, but this hypothesis is being pursued in the ongoing phase III studies of etirinotecan in TNBC (NCT01492101).

We also noted that TOP2A amplification is more common in ER-positive breast cancers. Whether this marker predicts for benefit from anthracycline therapy will be evaluated in the ongoing National Surgical Adjuvant Breast and Bowel Project B49 study. The finding that 91% of TNBC have either increased AR protein expression, loss of PTEN, or mutations in PIK3CA, PTEN, or ATM1 indicates potential for new therapies or combination therapies for most of the patients. When other changes in protein expression in this patient cohort are added to the evaluation, 98% of the analyzed patient population has potentially targetable biomarkers, which is comparable with the non-TNBC population.

Early evidence supports predictive utility for many of the biomarkers reported in this study, and studies are ongoing to evaluate these proteins as predictors of response and resistance. The protein status is not used as part of standard of care algorithms, but rather as guidance when standard agents have failed the patient.

**Conclusion**

In summary, evaluating genomic alterations and protein expression identifies potentially actionable targets in up to 98% of TNBC cases. Targetable mutations/amplicons such as PIK3CA and EGFR were identified, and expression of AR, Ki-67, PTEN, and topoisomerase 1. Although treatment and outcomes were not available for this large retrospective analysis, future studies using biomarker status as part of trials will bring the field forward and answer some of the outstanding questions relative to the potential therapeutic options identified in this study.

**Clinical Practice Points**

- Triple-negative breast cancer is a heterogeneous disease associated with poor prognosis and having in common a lack of expression of ER, PR, and HER2 proteins. Some molecular characterization has been performed, specifically in primary TNBC.
- Differences in molecular characteristics have been found between primary versus metastatic disease.
- Novel patterns in co-incidence or exclusivity of incidence of genomic and/or proteomic biomarker alterations have been found.
- Triple-negative breast cancer molecular subtypes will dictate different responses to conventional therapeutic strategies, different therapies, and novel therapies based on identified molecular targets.

**Disclosure**

Sherri Z. Millis, Zoran Gatalica, Josiah Winkler, Jeffery Kimbrough, and Sandeep Reddy disclose employment at Caris Life Sciences. The remaining authors have stated that they have no conflicts of interest.

**Supplemental Data**

Supplemental tables accompanying this article can be found in the online version at http://dx.doi.org/10.1016/j.clbc.2015.04.008.

**References**


### Predictive Biomarker Profiling and Heterogeneity in TNBC

**Supplemental Table 1 | Immunohistochemistry Thresholds**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Localization</th>
<th>IHC Thresholds (Staining Intensity and Percent Tumor)</th>
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( ) indicates primary tissue and number of times identified.

aSeen in 19% of HER2 mutations across tumor types (in 10,000 cases tested at Caris).
bSeen in 8% of HER2 mutations across tumor types (in 10,000 cases tested at Caris).
cNot seen elsewhere across tumor types (in 10,000 cases tested at Caris).
dSeen in 4% of HER2 mutations (1 = bladder, 1 = kidney, 1 = cancer of unknown primary; in 10,000 cases tested at Caris).
eSeen in 4% of HER2 mutations (presumed benign1 = CRC,1 = ovarian,1 = endometrial; in 10,000 cases tested at Caris).
### Supplemental Table 3: Specific PIK3CA Mutations Identified

<table>
<thead>
<tr>
<th>PIK3CA Mutation, Protein Change</th>
<th>AR⁻ (48 Cases; 2 With 2), n</th>
<th>AR⁻, %</th>
<th>AR⁺ (37 Cases, 2 With 2), n</th>
<th>AR⁺, %</th>
<th>Exon</th>
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<tbody>
<tr>
<td>P104_V105delinsL</td>
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<td></td>
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<tr>
<td>G106V</td>
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<td>2.7</td>
<td></td>
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</tbody>
</table>

Comparison between AR⁻ triple-negative breast cancer (TNBC) cases and AR⁺ TNBC cases.