Durable Complete Response With Immune Checkpoint Inhibitor in Breast Cancer With High Tumor Mutational Burden and APOBEC Signature

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ABSTRACT

Increasing data support the importance of preexisting host immune response and neoantigen burden for determining response to immune checkpoint inhibitors (ICIs). In lung cancer and melanoma, tumor mutational burden (TMB) has emerged as an independent biomarker for ICI response. However, the significance of TMB in breast cancer, particularly in the context of PD-L1 negativity, remains unclear. This report describes a patient with HER2-negative breast cancer with high TMB and an apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) trinucleotide signature; her disease was refractory to multiple lines of treatments but achieved durable complete response using ICIs and capecitabine. Additional analysis of the tumor revealed a low amount of stromal tumor-infiltrating lymphocytes (sTILs) and PD-L1 negativity, reflecting a poor preexisting host immune response. In collaboration with Foundation Medicine, comprehensive genomic profiling from 14,867 patients with breast cancer with the FoundationOne test was evaluated. Using the cutoff of ≥ 10 mutations/megabase (mut/Mb) for high TMB, PD-L1 positivity and TMB-high populations were not significantly overlapping (odds ratio, 1.02; $P = .87$). Up to 79% of TMB-high tumors with > 20 mut/Mb were PD-L1-negative. Our study highlights that despite having low TILs and PD-L1 negativity, some patients may still experience response to ICIs.

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Case Presentation

A white woman aged 66 years initially presented with a large fungating right breast mass with bleeding requiring blood transfusion. CT scan of the chest, abdomen, and pelvis showed a 14-cm mass in her right breast along with bulky right axillary lymphadenopathy. A punch biopsy of the tumor revealed grade 3 infiltrating ductal carcinoma, estrogen receptor (ER)-positive by immunohistochemistry (IHC) at 76% to 100%, progesterone receptor (PR) focally positive at 10%, HER2 nonamplification by fluorescence in situ hybridization, and a Ki-67 level of 70%. Further staging using a PET/CT scan, bone scan, and brain MRI did not show definite evidence of distant metastatic disease.

The patient received neoadjuvant chemotherapy with doxorubicin and cyclophosphamide every 2 weeks for 4 cycles followed by weekly paclitaxel for 12 weeks. Follow-up CT of the chest, abdomen, and pelvis after neoadjuvant chemotherapy showed a partial response with no evidence of distant metastases. The patient subsequently underwent right modified radical mastectomy with axillary lymph node dissection. Pathology results showed multifocal residual disease measuring up to 16.5 cm, with > 10 additional satellite tumors and skin involvement. Among resected axillary lymph nodes, 7 of 9 sampled lymph nodes were positive for metastatic disease, with the largest deposit measuring 4.5 cm with extracapsular extension. After surgery, the patient received adjuvant radiation with 5,040 cGy to the right chest wall, 6,040 cGy to the right chest wall scar, and 4,500 cGy to the right regional lymphatics. After completion of radiation, she began adjuvant endocrine therapy using letrozole.

Approximately 10 months later, the patient presented again with skin nodules on the right chest wall while on letrozole. Subsequently, a punch skin biopsy was performed that showed recurrent infiltrating ductal carcinoma. At that time, a PET/CT scan showed hypermetabolic activity in the right chest wall mass with no

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definite evidence of distant metastases. The patient then underwent resection of the right chest wall lesion. Pathology results showed recurrent invasive adenocarcinoma consistent with breast primary, and ER-positive by IHC at 76% to 100%, PR-negative <1%, and no HER2 overexpression (IHC 0). Further quantification of stromal tumor-infiltrating lymphocytes (sTILs) based on the International TILs Working Group¹ showed <2% sTILs (Figure 1). The PD-L1 IHC 22C3 pharmDx IHC assay (Dako; Carpinteria, CA) showed PD-L1 expression <1% in both tumor and immune cells (Figure 2). The chest wall lesion was further evaluated for potentially actionable genomic alterations using next-generation sequencing-based comprehensive genomic profiling (CGP) with the FoundationOne test. CGP showed high tumor mutational burden (TMB) (40 mutations/megabase [mut/Mb]) and likely pathogenic alterations in *PIK3CA* (E453Q, E545K), *ESR1* (E380Q), *MEN1* (S313*), *CTCF* (E145*), *GATA3* (M294K), *MAP3K1* (S782*), *MLL3* (S2025*), *NUP93* (Q15*), *SPEN* (E376*), *SPTA1* (Q2195*), and *TP53* (R248Q) and *ZNF703* amplification, but no evidence of mismatch repair deficiency (dMMR). At that time, her systemic treatment was switched to fulvestrant and palbociclib.

Approximately 3 months later, the patient's follow-up PET/CT scan unfortunately showed progressive disease with contralateral left axillary lymphadenopathy, which was confirmed by fine-needle aspiration biopsy showing metastatic disease consistent with breast primary, ER >90%, PR >50%, and no HER2 overexpression (IHC 0). Given that this was the only site of recurrence or progressive disease, the patient underwent excision of these lymph nodes. Pathology results showed that 1 of 7 left axillary lymph nodes was positive for metastatic

moderately differentiated adenocarcinoma consistent with breast primary, measuring 3.0 cm with extranodal extension. She was continued on fulvestrant and palbociclib after surgery. The patient had another follow-up PET/CT scan 3 months later that unfortunately showed progressive disease with 2 new soft tissue lesions in the right fourth and fifth intercostal space and a soft tissue nodule in the right fourth costochondral junction. Her treatment was then switched to weekly dosing of capecitabine at 1,500 mg orally twice daily, 7 days on and 7 days off. Because of high TMB seen on the FoundationOne testing, off-label use of nivolumab was requested through the patient access program with Bristol-Myers Squibb and was added 43 days later after the patient was started on capecitabine. At the time of the last follow-up, the patient remained on both capecitabine and nivolumab. She had achieved complete response with no evidence of disease lasting >26 months (Figure 3). The patient's informed consent was obtained for this case report.

In collaboration with Foundation Medicine, we further evaluated hybrid capture-based CGP of up to 395 cancer-related genes using the FoundationOne assay in 14,867 histologically defined breast carcinomas sequenced at Foundation Medicine. Molecular subtype was available for a subset of patients. ER status was available in 2,394 patients, of whom 1,371 (57%) had ER-positive (ER+) disease. HER2 status was available in 14,867 patients, of whom 1,522 (10%) had HER2-positive (HER2+) disease. Among 2,394 patients with available ER and HER2 status, 917 (38%) had ER-negative/HER2-negative breast cancer. There were 5,241 samples from locoregional lesions and 6,710 samples from metastatic lesions. PD-L1 expression status using the VENTANA

Figure 1. Chest wall mass consistent with adenocarcinoma. Hematoxylin-eosin stain showing <2% stromal tumor-infiltrating lymphocytes (original magnification, $\times 100$).

Figure 2. Biopsy of the chest wall lesion showing negative PD-L1 expression with 22C3 pharmDx IHC assay (original magnification, $\times 40$).

Figure 3. PET/CT from (A) August 2017 showing hypermetabolic lesion in the left axilla (white arrow), and (B) August 2019 showing complete resolution of hypermetabolic lesions in the left axilla (white arrow).

PD-L1 assay with the SP142 antibody (E. Hoffmann-La Roche Ltd) was available in 1,425 samples. We defined PD-L1 positivity as $\geq 1\%$ tumor staining. We found that PD-L1 expression data were available in 99 triple-negative breast cancer (TNBC) samples, 134 HER2+ breast cancer samples, and 129 ER+ breast cancer samples. High TMB was defined as ≥ 10 mut/Mb.

Consistent with previous reports,² the incidence of PD-L1 positivity was highest in TNBC at 14%, followed by 6.0% in HER2+ disease and 5.1% in invasive lobular carcinoma, and lowest in ER+ disease at 2.3%. Interestingly, the percentage of PD-L1-positive cases was significantly lower in metastatic disease compared with local disease (6.3% vs 11.1%; $P=.005$). The frequency of high TMB was highest in invasive lobular carcinoma at 13.6%, followed by HER2+ disease at 9.9%, TNBC at 7.0%, and ER+ disease at 6.9%. High TMB was associated with older age, with 3.7% in patients aged ≤ 45 years, 9.3% in patients aged 46 to 60 years, and 12.8% in patients aged ≥ 61 years ($P<.001$). Using the cutoff of ≥ 10 mut/Mb, PD-L1 positivity and TMB-high populations were not significantly overlapping (odds ratio, 1.02; $P=.87$). High TMB was also associated with metastatic disease, with 11.1% of metastatic lesions harboring high TMB compared with 5.3% with localized disease ($P<.001$). Among patients with very high TMB, defined as >20 mut/Mb, there was a significant association between high TMB and PD-L1 positivity (odds ratio, 2.6; $P=.023$). Nevertheless, even with a high cutoff of >20 mut/Mb, 79% of the TMB-high samples were PD-L1-negative.

There are several mechanisms underlying genomic instability in breast cancer, including homologous recombination deficiency, dMMR, and apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like (APOBEC) cytidine deaminase.³ Emerging data have shown that dysregulation of APOBEC enzyme can result in tumors with high TMB.⁴ Further evaluation of this particular patient's tumor genomic landscape showed no evidence of dMMR or homologous recombination repair

deficiency. Using the trinucleotide mutational signatures described by Zehir et al,⁵ we found that this patient's tumor harbored a dominant APOBEC trinucleotide signature, with a fit of 91%. In the FoundationOne cohort, most of the TMB-high (≥ 10 mut/Mb) breast samples with at least 10 assessable mutations were APOBEC mutational signature-positive (62.3%; 909 of 1,460). Across molecular subtypes, the fraction of TMB-high cases with an APOBEC signature was relatively similar, including 56.3% (27 of 48) in TNBC, 69.2% (54 of 78) in ER+ disease, and 62.4% (111 of 178) in HER2+ disease. Comparing between ductal and lobular carcinoma, we noted that TMB-high lobular carcinomas exhibited a higher proportion of APOBEC positivity (85.8%; 121 of 141) relative to TMB-high ductal carcinomas (56.0%; 200 of 357; $P<.0001$). In addition, TMB-high samples from metastatic sites harbored a significantly higher fraction of APOBEC positivity relative to TMB-high samples from primary tumors (67.3% vs 52.3%; $P<.0001$). Approval for this particular part of the study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (protocol number 20152817).

Discussion

Immunotherapy using immune checkpoint inhibitors (ICIs) has truly revolutionized cancer therapy in the past decade. ICIs exert their activity mainly through enhancing preexisting host immune response. Multiple clinical trials have shown long durable responses using ICIs, particularly in immunogenic or "hot" tumors such as melanoma or lung cancer. In contrast, results of single-agent ICIs in less-immunogenic tumors, including breast cancer, have been less promising, particularly in immunologically "cold" tumors such as hormone receptor-positive, HER2-negative, or luminal breast cancer.

One way to evaluate preexisting host immune response is by measuring TILs. A higher percentage of TILs has been shown to be associated with improved outcomes in patients treated with ICIs in multiple tumor types.⁶

In breast cancer, several studies have also shown the prognostic value of TILs beyond other conventional pathologic characteristics and staging.⁷⁻⁹ An initial study showed that each 10% increase in TILs was associated with a 15% to 17% reduction in risk of relapse in patients with TNBC.⁷ More recently, 2 additional pooled individual analyses from multiple large clinical trials confirmed the prognostic significance of TILs in patients with early-stage breast cancer, particularly in TNBC.^{8,9} Nevertheless, similar to our case report, a number of studies have shown a lower preexisting host immune response measured by TILs in cold luminal breast cancer.^{8,10}

Several clinical trials have evaluated the efficacy of ICIs in breast cancer.¹¹ For example, TNBC is a subtype for which ICIs seem to have promising activity. The objective response rates (ORRs) of single-agent ICIs were reported to be as high as 23.1% in patients with TNBC.¹² In contrast, the previous phase IB JAVELIN trial showed that the ORR of the single-agent anti-PD-L1 avelumab was only 2.8% in patients with unselected luminal breast cancer.¹³

Currently, there are several potential biomarkers to identify patients who are more likely to benefit from ICIs. In addition to baseline TILs, PD-L1 expression and microsatellite instability status or dMMR have been shown to correlate with an improved outcome using ICIs.⁶ Impairment of MMR can result in genomic instability and hypermutated tumors with highly immunogenic frameshift peptide neoantigens, which can trigger immune response and increase T-cell activation.¹⁴ In 2017, the FDA approved pembrolizumab for the treatment of patients with solid tumors with dMMR regardless of their tumor type, based on the phase II trial by Le et al,¹⁵ which reported an ORR of 40% in patients with dMMR colorectal cancer and 71% in those with dMMR non-colorectal cancer. In a subsequent report, ORRs were observed in 53% of patients with dMMR across 12 tumor types, with 21% being complete responses.¹⁶ However, the incidence of dMMR in breast cancer has been reported to be fairly low at 1.53% compared with other cancers, such as 31.37% in endometrial cancer and 19.72% in colon cancer.¹⁷

In addition to dMMR status, recent studies have shown that TMB itself is also predictive of improved outcome in patients receiving ICIs. Initial studies in melanoma have shown that high TMB was associated with improved outcomes in patients receiving anti-CTLA-4 antibodies.^{18,19} Similar results were also observed in non-small cell lung cancer,²⁰ melanoma,²¹ and urothelial cancer²² treated using anti-PD-1/PD-L1 therapies. More recently, Samstein et al²³ examined the predictive value of TMB across multiple tumor types, with 1,662 advanced cancers treated using ICIs. Overall, higher TMB was associated with improved survival in patients receiving ICIs in most tumor types. However, TMB cutoffs

seemed to be markedly varied between different cancer types.²⁴

Our unique case report highlights the benefit of next-generation sequencing beyond standard histopathologic features. Although this patient's tumor is considered to be immunologically cold by conventional clinicopathologic factors, including luminal breast cancer histology, low sTILs, and PD-L1 negativity, the patient did derive prolonged durable benefit from ICIs. Further evaluation in the larger breast cancer cohort showed that TMB-high PD-L1-positive populations were not significantly overlapping. Unexpectedly, up to 79% of TMB-high tumors were PD-L1-negative. Similar findings were reported previously by Barrett et al²⁵ in a smaller group of patients with TNBC, wherein PD-L1 expression by IHC did not seem to correlate with TMB, dMMR status, and amount of predicted neoepitopes. Nonetheless, profound response to ICIs in patients with TNBC with PD-L1-negative tumors harboring dMMR has been reported.²⁶ Similar observations were also previously reported by Rizvi et al²⁰ in non-small cell lung cancer. Overall, patients with both TMB-high and PD-L1-positive tumors had the highest durable clinical benefit rate of 91%. Interestingly, among patients with low PD-L1 expression, 75% of those with TMB-high tumors had durable clinical benefit compared with 11% with TMB-low tumors. Based on these intriguing data, additional studies will be needed to evaluate the efficacy of ICIs in this group of patients with breast cancer with high TMB but PD-L1-negative tumors.

Notably, given that our patient also received capecitabine, it is unclear whether her disease would have responded to single-agent capecitabine or single-agent ICI. Nevertheless, because her disease progressed rather rapidly with multiple lines of previous therapies, it is unlikely that she would have benefited from single-agent capecitabine alone. Because several recent studies showed the benefit of combining chemotherapy with immunotherapy, it is conceivable that our patient derived the most benefit from the combination of capecitabine and nivolumab. For treatment of TNBC, the FDA recently approved combination nab-paclitaxel and atezolizumab for patients with unresectable locally advanced or metastatic TNBC, based on the report from the phase III IMpassion130 trial.²⁷ Nevertheless, this particular combination seemed to only benefit patients with PD-L1-positive tumors. In luminal breast cancer, similar results using the combination of ICI and chemotherapy were also reported in the I-SPY 2 trial (ClinicalTrials.gov identifier: NCT01042379).²⁸ In this trial, the addition of pembrolizumab to the standard neoadjuvant chemotherapy resulted in a higher complete pathologic response rate of 34% compared with 13% using standard neoadjuvant chemotherapy alone.

Conclusions

This case report highlights that tumors with high TMB may still respond to ICIs in combination with chemotherapy, despite patients having tumors that may have been considered immunologically cold based on standard clinicopathologic assessment. Although the tumor in this case was PD-L1-negative and had no evidence of dMMR, the patient's disease exhibited a durable complete response to the combination of nivolumab and capecitabine for a prolonged period. Further evaluation with CGP in a large breast cancer cohort showed that PD-L1 positivity and TMB-high populations were only moderately overlapping. A considerable number of breast tumors with high TMB were PD-L1-negative. Future studies will be needed to further evaluate the efficacy of ICIs in patients with breast cancers with high TMB and/or an APOBEC

trinucleotide signature that are considered immunologically cold based on other conventional pathologic assessments.

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