**CANCER GENOMICS**

### APOBEC Mutational Signatures in Hormone Receptor–Positive Human Epidermal Growth Factor Receptor 2–Negative Breast Cancers Are Associated With Poor Outcomes on CDK4/6 Inhibitors and Endocrine Therapy

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**abstract**

**PURPOSE** APOBEC mutagenesis underlies somatic evolution and accounts for tumor heterogeneity in several cancers, including breast cancer (BC). In this study, we evaluated the characteristics of a real-world cohort for time-to-treatment discontinuation (TTD) and overall survival on CDK4/6 inhibitors (CDK4/6i) plus endocrine therapy (ET) and immune checkpoint inhibitors.

**METHODS** Comprehensive genomic profiling results from 29,833 BC samples were analyzed for tumor mutational burden and APOBEC signatures. For clinical outcomes, a deidentified nationwide (United States–based) BC Clinico-Genomic Database (CGDB) was evaluated with log-rank and Cox models. Patients with hormone receptor–positive (HR+) human epidermal growth factor receptor 2–negative (HER2–) BC who received first-line ET and CDK4/6i were included. Eligible patients from Mayo Clinic and Duke University were HR+ HER2– BC with sequencing data between September 2013 and July 2020.

**RESULTS** Of 29,833 samples sequenced, 7.9% were APOBEC+ with a high rate in invasive lobular carcinoma (16.7%) and in metastatic tumors (9.7%) relative to locally biopsied BC (4.3%; \( P < .001 \)). In CGDB, 857 patients with HR+ HER2– BC received ET plus CDK4/6i in the first line. APOBEC+ patients had significantly shorter TTD on ET plus CDK4/6i than APOBEC– patients, 7.8 (95% CI, 4.3 to 14.6) versus 12.4 months (95% CI, 11.2 to 14.1; hazard ratio, 1.6; 95% CI, 1.03 to 2.39; \( P = .0036 \)). Clinical benefit to immune checkpoint inhibitors was observed in HR+ HER2–, APOBEC+, tumor mutational burden–high patients, with four of nine CGDB patients (TTD 0.3-11.3 months) and four of six patients in Duke/Mayo cohorts (TTD 0.9-40.5 months) with a TTD of ≥ 3 months.

**CONCLUSION** APOBEC+ HR+ HER2– patients had shorter TTD on first-line ET plus CDK4/6i relative to APOBEC– patients. Further research is needed to optimize the treatment of APOBEC+ HR+ HER2– BC and to investigate the efficacy of immunotherapeutic strategies in this population.

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**INTRODUCTION**

APOBEC or apolipoprotein B mRNA-editing enzyme catalytic polypeptides are a family of cytidine deaminases, which protect against viral infection by degrading viral genomes via cytosine deamination.1,2 Besides mutating viral genomes, APOBEC enzymes also have significant mutagenic effects on human genomes leading to primarily cytosine-to-thymine (C-to-T) mutations. More recently, dysregulation of APOBEC has been shown to serve as a major driver of tumor mutations in human cancer leading to intratumoral heterogeneity, clonal evolution, and tumor adaptation in response to therapy in multiple tumor types.3 Several studies demonstrated that APOBEC mutagenesis exists in many cancer types, including breast cancer (BC), and is associated with poor prognosis and resistance to cancer therapy.2 In BC, APOBEC mutagenesis seems to be the predominant mechanism for endogenous mutations. We previously reported that up to 62.3% of breast tumors with high tumor mutational burden (TMB) harbored APOBEC signature.4 However, data related to APOBEC mutagenesis and outcomes, particularly in patients with BC treated with endocrine therapy (ET) with CDK4/6 inhibitors, are currently limited.
In hormone receptor–positive (HR+) human epidermal growth factor receptor 2–negative (HER2−) BC, a previous preclinical study demonstrated that APOBEC3B regulates growth by promoting estrogen receptor transcriptional activity. Furthermore, overexpression of APOBEC3B has been shown to promote tamoxifen resistance in HR+ BC. Evaluation of circulating tumor DNA in peripheral blood of patients with advanced BC revealed that subclonal mutations were enriched in patients with APOBEC mutational signatures. These results suggest that dysregulation of APOBEC promotes genetic diversity resulting in a larger pool of subclones for tumor evolution and endocrine resistance in HR+ HER2− BC. Nevertheless, the prognostic value of APOBEC signatures in the setting of first-line ET and CDK4/6 inhibition (CDK4/6i) is unknown.

Given that dysregulation of APOBEC is a dominant mechanism of tumor mutagenesis, APOBEC signatures have generally been evaluated in the context of TMB. High TMB (TMB-H), which is associated with generation of neoantigens, has emerged as a biomarker for response to immune checkpoint inhibitors (ICIs) across multiple solid tumors. In June 2020, pembrolizumab obtained tumor agnostic approval from the Food and Drug Administration for the treatment of patients with advanced solid tumors with TMB-H defined as ≥ 10 mutations/megabase (muts/Mb). This approval was based on the results of the KEYNOTE-158 trial, which demonstrated an objective response rate (ORR) of 34.3% (95% CI, 28.3 to 40.85). However, only 2.1% of patients on this trial had BC, and their subtypes were not reported. Currently, there are limited data on efficacy of ICI in TMB-H HR+ HER2− metastatic BC, and no additional biomarkers for selection of response exist in this population.

In this study, we evaluated clinical characteristics and outcomes of patients with HR+ HER2− BC with and without APOBEC signatures treated with first-line ET with CDK4/6i in real-world data (RWD) cohort from a deidentified Clinico-Genomic Database (CGDB). The CGDB cohort and a Mayo Clinic and Duke University cohort were evaluated to assess outcomes of TMB-H patients treated with ICIs with or without chemotherapy.

METHODS

Patient Cohorts

This study consists of three distinct cohorts: a comprehensive genomic profiling (CGP) data set, a CGDB, and a combined Mayo Clinic and Duke University ICI-treated cohort. Detailed information from patients in each cohort is described in the flow diagrams in Figures 1A and 1B.

CGP BC Cohort

CGP using hybrid capture-based next-generation sequencing (NGS) was performed on tissue samples from all comers with BC (N = 29,833) submitted to Foundation Medicine from 2014 to 2021 during routine clinical care. Approval for this study, including a waiver of informed consent and Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (protocol no. 20152817).

Flatiron Health-Foundation Medicine CGDB

The Flatiron Health (FH)-Foundation Medicine (FMI) CGDB is a real-world deidentified database originating from approximately 280 cancer clinics (800 sites of care) across the United States. Retrospective longitudinal clinical data were derived from electronic health record data, comprising patient-level structured and unstructured data, curated via technology-enabled abstraction, and were linked to deidentified CGP data in the FH-FMI CGDB by deidentified deterministic matching as previously
Linking of patient data between FH and FMI databases was performed via tokenized protected health information by a third party in an Institution Review Board–approved, Health Insurance Portability and Accountability Act–compliant manner. The resulting data set contains both clinical RWD and comprehensive genomic profiles, immunohistochemistry, and annotations. Patients’ clinical characteristics and treatment history data were obtained via technology-enabled abstraction of clinician notes and radiology/pathology reports. For outcomes (time-to-treatment discontinuation [TTD] and overall survival [OS]), patients with HR+/HER2–BC who received first-line ET with CDK4/6i from January 2011 to December 2020 were included. Only patients with CGP results ≤60 days after the last FH network visit, diagnosis ≤90 days before first FH visit, and available tissue-based CGP reports were included. TTD was defined as the difference between the first and last drug episodes within a given line of treatment (LOT). LOT was derived based on FH algorithms. OS was defined as the time from LOT start to the date of death or data cutoff. The log-rank test and the Cox model were used to evaluate the difference in outcomes. To reduce the impact of confounding variables, inverse probability of treatment weighting was used and adjusted for age at diagnosis, stage at diagnosis, tumor type, metastatic sites, TMB group (≥10 vs <10), treatment group, and PIK3CA mutation status. Institutional Review Board approval of the study protocol was obtained before study conduct and included a waiver of informed consent since this study was a noninterventional study using deidentified, routinely collected data.

**Duke/Mayo Cohort**

TMB-H patients defined as TMB ≥10 muts/Mb, who had HR+ HER2–metastatic BC from Mayo Clinic and Duke University, with tumor sequencing at FMI between September 2013 and July 2020, were included. Clinical data were manually extracted from electronic health records. Similar to the CGDB cohort, TTD was measured as the difference between the first and last dates of treatments received. This study was approved by the IRB from Mayo Clinic (IRB no. 18-008231) and Duke University (IRB no. 00106741).

**NGS**

CGP using hybrid capture-based NGS was performed on BC tissue specimens submitted to FMI during routine clinical care. All classes of alterations (short variants, copy number alterations, and rearrangements) were examined for at least 324 genes. Only known and likely pathogenic alterations were included. Approval for the CGP portion of this study, including a waiver of informed consent and Health Insurance Portability and Accountability Act waiver of authorization, was obtained from the Western Institutional Review Board (protocol #20152817). TMB was calculated on at least 0.8 Mb (megabases) of sequence.

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**FIG 1.** Flow diagrams of patients from (A) the CGDB cohort and (B) Mayo Clinic and Duke University Cohorts. CGDB, Clinico-Genomic Database. *May overlap: There were overlapping cases in the HR+ HER2- received first-line standard of care group (n = 907) and the group that received ICIs in metastatic setting (n = 390).
Mutational signatures, including APOBEC, were calculated as previously described with a minimum of 10 assessable alterations to make a positive signature call. Predicted ancestry was calculated using a SNP-based approach. Programmed death-ligand 1 (PD-L1) IHC status was available for a subset of samples (VENTANA SP142: PD-L1-positive threshold IC ≥ 1%, DAKO 22C3 CPS ≥ 10). Microsatellite instability status was calculated as previously described.

RESULTS

Prevalence of APOBEC Signatures in a CGP Cohort

Of the 29,833 BC samples in the FMI CGP cohort, 7.9% harbored an APOBEC mutational signature (APOBEC+). APOBEC+ tumors were enriched in invasive lobular carcinoma (ILC; 16.7%) compared with invasive ductal carcinoma (4.9%; \( P < .001 \)) as well as in metastatic biopsies (9.7%) relative to breast biopsies (4.3%; \( P < .001 \)). Of patients with metastatic ILC, 22.4% harbored an APOBEC signature. Among tumors with TMB-H, 64.4% harbored an APOBEC mutational signature.

Pathological and Clinical Characteristics of APOBEC+ Versus APOBEC– HR+/HER2– BC in the CGDB Cohort

In the CGDB cohort, 857 patients with HR+ HER2– BC met the eligibility criteria and were included in the analysis (Fig 1A). Similar to the CGP cohort, APOBEC mutational signatures were observed in 69 patients (8.1%; Table 1) with a strong overlap with the TMB-H patients in the cohort (Appendix Fig A1). ILC represented a significant fraction of the APOBEC+ HR+ HER2– tumors, 30 of 69 (43.4%), which is significantly higher than the ILC prevalence in the APOBEC– cohort (17.5%; \( P < .001 \)). Median age at diagnosis was similar between APOBEC+ and APOBEC– groups (median 59 and 56 years, respectively; \( P < .102 \)). Grade, stage, predicted ancestry, Eastern Cooperative Oncology Group performance status, and distribution of metastatic sites did not differ between APOBEC+ and APOBEC– groups (all \( P > .05 \)).

As expected, APOBEC+ tumors had a higher median TMB of 11.3 (8.8-18.8) muts/Mb compared with 2.5 (1.3-3.8) muts/Mb in APOBEC– tumors. There was no difference in PD-L1 positivity, microsatellite instability, and BRCA 1 and BRCA 2 mutational status between the two groups. However, PIK3CA mutations were present at a greater frequency in APOBEC+ versus APOBEC– groups (66.7% vs 43.3%, respectively; \( P < .001 \)). PIK3CA mutation E545K, caused by a C>T mutation in a canonical TCA APOBEC trinucleotide context, was notably more common at 23.2% versus 11.7% in APOBEC+ relative to APOBEC– samples (\( P \)-adjusted false discovery rate = .022).

Inferior Outcomes in Patients With APOBEC+ HR+/HER2– BC Treated With ET and CDK4/6i in CGDB Cohort

In the CGDB cohort, 38 of 69 patients (55%) in the APOBEC+ group received first-line fulvestrant in combination with CDK4/6i compared to 278 of 788 patients (35%) in APOBEC– cohort (\( P = .005 \)). Nevertheless, after adjusting for age at diagnosis, stage at diagnosis, tumor type, metastatic sites, TMB group (≥ 10 < 10), treatment group, and PIK3CA mutation status, APOBEC+ patients had significantly inferior TTD with ET and CDK4/6i compared with the APOBEC– group with TTD of 7.8 (95% CI, 4.1 to 14.6) versus 12.1 (95% CI, 11.1 to 13.8) months, respectively (hazard ratio, 1.6; 95% CI, 1.03 to 2.39; log-rank \( P = .036 \)), as shown in Figure 2A, APOBEC+ patients also had a trend toward shorter OS compared with APOBEC– patients with OS of 32.4 (95% CI, 15.9 to 44.4) versus 39.8 (95% CI, 36.5 to 45.8) months, respectively (hazard ratio, 1.96; 95% CI, 1.2 to 3.3; \( P = .06 \)), as shown in Figure 2B.

Responses With ICIs in TMB-H Microsatellite Stable HR+/HER2– BC in CGDB

In the CGDB cohort, 18 patients with HR+ HER2– BC had TMB-H and received ICIs (Fig 3A). The median TTD was 2.8 (range, 0.3-17.8) months, with 5 of 18 patients (28%) having TTD ≥ 6 months. Notably, 4 of 5 (80%) patients with TTD ≥ 6 months had TMB higher than the median (21 muts/Mb). APOBEC mutational signatures were observed in 10 patients, all of whom had TMB-H. Nine of 10 of these patients had evaluable TTD. As of December 2020, four of nine patients were still on ICI treatment. Five patients received ICI monotherapy, and four patients received ICIs in combination with chemotherapy or ET. At the cutoff point, four of nine patients were able to continue ICIs without disease progression longer than 3 months. Two patients received ICI monotherapy, and two patients received combination therapy. TTD ranged from 0.3 to 11.3 months. The patient with the longest TTD of 11.3 months received fulvestrant in combination with pembrolizumab.

Pathological, Clinical Characteristics, and Outcomes of TMB-H HR+ HER2– BC in Mayo Clinic/Duke University Cohorts

There were 63 patients in Mayo Clinic/Duke University cohorts with TMB-H who had tumor sequenced at FMI. Of those, 38 patients had HR+ HER2– BC, with APOBEC+ in 28 patients (74%) and APOBEC– in 10 patients (26%). Among TMB-H HR+ HER2– patients who received ICIs, the median TTD was 5.8 (range, 0.9-40.6) months, with two patients ongoing as of August 1, 2021 (Fig 3B). Half of the patients (4 of 8, 50%) had TTD ≥ 6 months. Seventy-five percent of patients with TTD ≥ 6 months had TMB higher than the median (32.5 muts/Mb).

In the Mayo Clinic and Duke University cohort, six patients with TMB-H APOBEC+ HR+/HER2– BC received ICIs. All patients were microsatellite-stable (MSS). The median TTD was 6.34 (range, 0.96-44.07) months. At the data cutoff, two patients remained on ICI therapy. Clinical benefit longer than 3 months was observed in four of six patients, with all four patients receiving ICI in combination with
| Characteristics                        | APOBEC+ (n = 69) | APOBEC– (n = 788) | P Adjusted (FDR) |
|---------------------------------------|-----------------|-------------------|-----------------|
| Median age at diagnosis (interquartile range) | 59.0 (53.0-65.0) | 56.0 (47.0-65.0) | .102            |
| Female                                | 69 (100.0)      | 776 (98.5)        | .491            |
| Ancestry, No. (%)                     |                 |                   | .645            |
| African                               | < 5             | 77 (10.2)         |                 |
| Admixed American                      | < 5             | 45 (6.0)          |                 |
| East Asian                            | < 5             | 13 (1.7)          |                 |
| European                              | 59 (85.5)       | 611 (81.2)        |                 |
| South Asian                           | < 5             | 6 (0.8)           |                 |
| Stage at Dx, No. (%)                  |                 |                   | .491            |
| 0-III                                 | 52 (75.4)       | 505 (64.1)        |                 |
| IV                                    | 11 (15.9)       | 245 (31.1)        |                 |
| Not documented                        | 6 (8.7)         | 38 (4.8)          |                 |
| Tumor grade, No. (%)                  |                 |                   | .104            |
| 1                                     | 2 (2.9)         | 49 (6.2)          |                 |
| 2                                     | 34 (49.3)       | 259 (32.9)        |                 |
| 3                                     | 11 (15.9)       | 163 (20.7)        |                 |
| Not documented                        | 22 (31.9)       | 317 (40.2)        |                 |
| Histologic type, No. (%)              |                 |                   |                 |
| Invasive ductal                       | 7 (10.1)        | 251 (31.9)        |                 |
| Invasive lobular                      | 30 (43.5)       | 138 (17.5)        |                 |
| Other/unknown                         | 32 (46.4)       | 399 (50.6)        |                 |
| Metastases sites, No. (%)             |                 |                   | .734            |
| Bone only                             | 17 (24.6)       | 169 (21.5)        |                 |
| CNS                                   | 11 (15.9)       | 104 (13.2)        |                 |
| Visceral                              | 41 (59.4)       | 514 (65.3)        |                 |
| ECOG, No. (%)                         |                 |                   | .491            |
| 0                                     | 28 (58.3)       | 268 (1.0)         |                 |
| 1                                     | 12 (25.0)       | 200 (8.1)         |                 |
| 2                                     | 7 (14.6)        | 48 (9.1)          |                 |
| 3                                     | < 5             | 9 (1.7)           |                 |
| PD-L1 status, No. (%)                 |                 |                   | .946            |
| Negative                              | 13 (18.8)       | 169 (21.4)        |                 |
| Positive                              | < 5             | 46 (5.8)          |                 |
| Not documented                        | 52 (75.4)       | 573 (72.7)        |                 |
| Median TMB (muts/Mb), interquartile range | 11.3 (8.8-18.8) | 2.5 (1.3-3.8)     |                 |
| Microsatellite status, No. (%)        |                 |                   | .946            |
| MSI-H                                 | < 5             | < 5               |                 |
| MSI-I                                 | < 5             | < 5               |                 |
| MSS                                   | 68 (98.6)       | 728 (97.2)        |                 |
| Not documented                        | < 5             | 16 (2.1)          |                 |
| BRCA mutations, No. (%)               | 5 (7.2)         | 45 (5.7)          | .734            |
| PIK3CA mutations, No. (%)             | 46 (66.7)       | 341 (43.3)        | .001            |
| First-line therapy, No. (%)           |                 |                   | .005            |
| AI plus CDK4/6i                       | 31 (44.9)       | 510 (64.7)        |                 |
| Fulvestrant plus CDK4/6i              | 38 (55.1)       | 278 (35.3)        |                 |

(Continued on following page)
chemotherapy. Two patients with the longest TTD of 12.07 and 44.07 months were on fluorouracil/capecitabine in combination with ICIs.

**DISCUSSION**

Substantial progress has been made in the treatment of advanced HR+ HER2– BC in the past decade. The most notable advancement was an addition of CDK4/6i (palbociclib, ribociclib, abemaciclib) to ET in the first- and second-line settings, which significantly improved both progression-free survival and OS.18-22 Inevitably, nearly all patients eventually progress on ET and CDK4/6i. Several studies have evaluated potential genomic alterations conferring resistance to CDK4/6i using circulating tumor DNA.7,23-25 These studies demonstrated that the mechanisms of resistance to ET and CDK4/6i are highly complex, heterogeneous, and likely divergent among metastatic sites in the same patient. Subclonal mutations in several growth factor receptors and signal transduction pathways were reported in patients who developed resistance to CDK4/6i and ET. Owing to the mutation patterns, these studies suggested that APOBEC mutagenesis underlies these highly complex subclonal evolutions upon disease progression.24 Nevertheless, while several preclinical and clinical studies demonstrated that APOBEC mutagenesis potentially underlies clonal evolution and resistance to CDK4/6i, clinical outcomes of patients with APOBEC mutational signatures in HR+ HER2– treated with ET and CDK4/6i are limited.

To our knowledge, our analysis on 857 patients with HR+/HER2– BC treated with first-line ET and CDK4/6i is the first

| Characteristics | APOBEC+ (n = 69) | APOBEC– (n = 788) | P Adjusted (FDR) |
|-----------------|------------------|-------------------|------------------|
| Deceased, No. (%) | 27 (39.1)        | 239 (30.3)        | .26              |

TABLE 1. Pathological and Clinical Characteristics of APOBEC + Versus APOBEC– HR+ HER2– Breast Cancer in Clinico-Genomic Database (Continued)
to show that patients with APOBEC mutational signatures have an inferior outcome with shorter TTD and trend toward worsening OS with the standard-of-care CDK4/6i and ET compared with APOBEC patients. In the CGDB cohort, APOBEC+ patients had TTD of merely 7.8 (95% CI, 4.1 to 14.6) months with ET + CDK4/6i. Our data are in line with the previous preclinical data, which showed that APOBEC3B levels in HR+ breast tumors are associated with tamoxifen resistance. Our findings are also in line with the findings of Kingston et al who examined circulating tumor DNA in peripheral blood of patients with advanced BC, revealing that subclonal mutations were enriched in patients with APOBEC mutational signatures specifically PIK3CA mutations.

HR+ HER2− BC is generally considered immunologically unresponsive tumors, and unselected populations have not responded well to ICIs in early trials. In the previous phase IB JAVELIN trial with single-agent anti–PD-L1 avelumab, the

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### Table A

| ID | ILC | PIK3CA | BRCA | TMB | Line No. | Treatment                                      |
|----|-----|--------|------|-----|----------|------------------------------------------------|
| 1  |     |        |      | 25  | 3+       | Fulvestrant, pembrolizumab                      |
| 2  |     | 28.8   |      |     | 3+       | Pembrolizum                                     |
| 3  |     | 13.8   |      |     | 3+       | Atezolizum, paclitaxel-bound protein            |
| 4  |     | 13.8   |      |     | 3+       | Pembrolizum                                     |
| 5  |     | 26.3   | 1-2  |     |          | Cepacitabine, goserelin, pembrolizum            |
| 6  |     | 20     |      |     | 3+       | Atezolizum, paclitaxel-bound protein            |
| 7  |     | 23.5   |      |     | 3+       | Pembrolizum                                     |
| 8  |     | 20     |      |     | 3+       | Pembrolizum                                     |
| 9  |     | 11.3   |      |     | 3+       | Cepacitabine, pembrolizum                       |
| 10 |     | 10     | 1-2  |     | 3+       | Nivolumab                                       |
| 11 |     | 10     |      |     | 1-2      | Pembrolizum                                     |
| 12 |     | 11.3   |      |     | 3+       | Alpelisib, pembrolizum                          |
| 13 |     | 16.3   |      |     | 3+       | Pembrolizum                                     |
| 14 |     | 135    |      |     | 3+       | Paclitaxel, pembrolizum                         |

### Table B

| ID | ILC | PIK3CA | BRCA | TMB | Line No. | Treatment                                      |
|----|-----|--------|------|-----|----------|------------------------------------------------|
| 1  |     | 40     | 1-2  |     |          | Nivolumab, capecitabine                        |
| 2  |     | 74     | 1-2  |     |          | Nivolumab                                       |
| 3  |     | 61     | 1-2  |     |          | Pembrolizum, fluorouracil                       |
| 4  |     | 15     | 3+   |     |          | Pembrolizum, eribulin                          |
| 5  |     | 43     | 3+   |     |          | Atezolizum, cisplatin, paclitaxel               |
| 6  |     | 24     | 3+   |     |          | Pembrolizum, eribulin                          |
| 7  |     | 21     | 3+   |     |          | Pembrolizum                                     |
| 8  |     | 25     | 3+   |     |          | Pembrolizum                                     |

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**FIG 3.** Swimmer plots of time-to-treatment discontinuation on ICIs in patients with TMB-H MSS HR+ HER2− BC in (A) the CGDB cohort and (B) Mayo Clinic and Duke University cohort. PIK3CA status, BRCA 1 and BRCA 2 mutational status, ILC pathology, APOBEC signature status, TMB, and line number are indicated. BC, breast cancer; CGDB, Clinico-Genomic Database; ICI, immune checkpoint inhibitor; ILC, invasive lobular carcinoma; MSS, microsatellite stable; TMB, tumor mutational burden; TMB-H, high TMB.
ORR of patients with HR+ HER2– BC was merely 2.8%. The KEYNOTE-158 trial led to the approval of pembrolizumab in TMB-H advanced solid tumors. However, there were only five patients with BC included in that trial, and the response to ICIs in HR+ HER2 BC is largely unknown. In this study, we identified 16 MSS TMB-H patients in the CGDB who were treated with ICIs either single agent or combination. While the median TTD was relatively short of 2.2 months in this group, a subset of patients exhibited (n = 5) clinical benefit with TTD ≥ 3 months with two of five responses ongoing. Notably, in the Duke and Mayo cohort, all patients with TTD ≥ 12 months had very high TMB (40, 61, and 74 muts/Mb), indicating potential need for higher cutoff in BC. The NIMBUS trial, a phase II trial of nivolumab ≥10 muts/Mb, and 74 muts/Mb), indicating potential need for higher cutoff in BC. The NIMBUS trial, a phase II trial of nivolumab in combination with ipilimumab in HER2– metastatic BC with TMB-H, also reported the ORR of 16.7% with the TMB cutoff of ≥ 10 muts/Mb. In an exploratory analysis, they demonstrated that increasing the cutoff to 14 muts/Mb had a substantial impact on response rates with an ORR of 60%.

APOBEC mutagenesis is the dominant underlying mechanism of hypermutation in BC, followed by mismatch repair deficiency. However, it remains unclear whether the underlying mechanism of hypermutation within TMB-H tumors affects ICI response in BC. In our cohort, there is a strong correlation between APOBEC+ and TMB-H tumors (Appendix Fig A2). Larger cohorts of TMB-H patients treated with ICI would be needed to determine if APOBEC positivity within TMB-H tumors further predicts ICI response. Our group previously described a HR+ HER2– patient with APOBEC+ MSS with exceptional responses to ICIs last > 26 months. Similar results were also reported in the TAPUR study, which is a phase II basket trial of single-agent pembrolizumab in patients with metastatic BC with TMB ≥ 9 muts/Mb. Among 28 patients enrolled, 12 patients had HR+ HER2– BC. The ORR in all patients was 21% (95% CI, 8 to 41) with 4 HR+ HER2– patients with time on treatment of ≥ 6 months. In our analysis of 24 patients with TMB-H HR+ HER2– BC receiving ICIs, both of our RWD cohorts also showed that several patients with TMB-H APOBEC+ MSS tumors had clinically meaningful responses to ICIs lasting up to 44 months. Interestingly, two of the longest responders were treated with fluorouracil-based chemotherapy in combination with ICIs, raising the possibility that combination chemotherapy or ICI approaches may be beneficial in this population.

To the best of our knowledge, our study represents the largest cohort of patients with BC evaluated for APOBEC mutational signatures by NGS in tumor tissue. Similar to the study by Pareja et al, our study demonstrated an enrichment of APOBEC mutational signatures in ILC, especially in metastatic ILC. In this study, 309 BC samples with special histologic types were evaluated. The frequency of APOBEC+ in ILC was reported at a much high frequency of 45% compared with 17% in our overall ILC cohort and 22% in metastatic ILC. Differences may be attributable to signature calling methods or cutoffs. In our study, for example, the median TMB for APOBEC+ samples was 11 muts/mb versus eight in their study, suggesting that they may be calling samples with weaker APOBEC signatures.

We found that PIK3CA mutations are notably more frequent in the APOBEC+ group (67% prevalence), particularly PIK3CA E542K mutation, which is the potential site characteristic of APOBEC mutagenesis. On the basis of the SOLAR-1 trial, alpelisib plus fulvestrant was approved for PIK3CA-mutated HR+/HER2– BC. This finding raises the possibility of combination or serial use of PI3K-targeting and ICI agents in this population. Given that the APOBEC+ population demonstrated such poor outcomes on first-line CDK4/6i and ET in our study, this also raises the question of whether APOBEC+ PIK3CA-mutant patients would benefit from first-line PI3K inhibitor added to ET or even triplet therapy with PI3K inhibitor, CDK4/6i, and ET.

One limitation of our study is that patients in the APOBEC+ group were more likely to receive fulvestrant as their ET backbone than APOBEC– patients, possibly suggesting upfront endocrine resistance. However, data on ET use and disease-free interval in the adjuvant setting were not available. Nevertheless, after adjusting for treatment group, TTD for APOBEC+ patients is still significantly worse compared with the APOBEC– group. In summary, the results from our study suggest that APOBEC mutational signatures are a relatively common biomarker in BC with potential prognostic implications for HR+ HER2– patients with BC receiving ET and CDK4/6i. Given that this is a single retrospective study with the inability to control for patient heterogeneity and non-reported variables, this work is considered hypothesis-generating and must be validated in future studies, particularly from established tissue sets in landmark CDK4/6i inhibitor trials. Furthermore, since patients harboring APOBEC mutational signatures may have poor outcomes with the current standard-of-care ET in combination with CDK4/6i, future clinical trials are needed to identify potential novel therapies for this group of patients. Given the frequent co-occurrence of PIK3CA mutations in APOBEC+ tumors, alpelisib or new generation of mutant selective PI3K inhibitor could be considered. In particular, the SOLAR1 trial demonstrated no significant difference in outcomes among patients with high versus low TMB when treated with alpelisib. Moreover, since a subset of patients with very high TMB also had long-term disease control with ICIs, in this group of patients, pembrolizumab can be considered an indication in patients with TMB ≥ 10 muts/Mb. Further evaluation of novel immunotherapeutic strategies in APOBEC+, TMB-H, and HR+/HER2– advanced BC was warranted.
AFFILIATIONS
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S.S. and K.R. contributed equally to this work. E.S. and S.C. contributed equally to this work.

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DATA SHARING STATEMENT
All relevant data are provided in the main text or as supplementary information within the manuscript. Due to HIPAA requirements, we are not consented to share individualized patient genomic data, which contains potentially identifying or sensitive patient information. FMI is committed to collaborative data analysis, and we have well-established, and widely utilized mechanisms by which investigators can query our core genomic database of > 600,000 deidentified sequenced cancers to obtain aggregated data sets.

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FIG A1. Venn diagram describing the overlap between TMB-H and APOBEC+ tumors. TMB, tumor mutational burden; TMB-H, high TMB.

FIG A2. Box and Whisker plot of interaction between APOBEC and TMB. TMB, tumor mutational burden.