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Phase Ib study of pembrolizumab in combination with trastuzumab emtansine for metastatic HER2-positive breast cancer

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ABSTRACT

Background Preclinical and clinical data support potential synergy between anti-HER2 therapy plus immune checkpoint blockade. The safety and tolerability of trastuzumab emtansine (T-DM1) combined with pembrolizumab is unknown.

Methods This was a single-arm phase Ib trial (registration date January 26, 2017) of T-DM1 plus pembrolizumab in metastatic, human epidermal growth factor receptor 2 (HER2)-positive breast cancer. Eligible patients had HER2-positive, metastatic breast cancer previously treated with taxane, trastuzumab, and pertuzumab, and were T-DM1-naïve. A dose de-escalation design was used, with a dose-finding cohort followed by an expansion cohort at the recommended phase 2 dose (RP2D), with mandatory baseline biopsies. The primary endpoint was safety and tolerability. Secondary endpoints included objective response rate (ORR) and progression-free survival (PFS). Immune biomarkers were assessed using histology, protein/RNA expression, and whole exome sequencing. Associations between immune biomarkers and treatment response, and biomarker changes before and during treatment, were explored.

Results 20 patients received protocol therapy. There were no dose-limiting toxicities. The RP2D was 3.6 mg/kg T-DM1 every 21 days plus 200 mg pembrolizumab every 21 days. 85% of patients experienced treatment-related adverse events (AEs) ≥grade 2, 20% of patients experienced grade 3 AEs, and no patients experienced grade >4 AEs. Four patients (20%) experienced pneumonitis (three grade 2 events; one grade 3 event). ORR was 20% (95% CI 5.7% to 43.7%), and median PFS was 9.6 months (95% CI 2.8 to 16.0 months). Programmed cell death ligand-1 and tumor infiltrating lymphocytes did not correlate with response in this small cohort.

Conclusions T-DM1 plus pembrolizumab was a safe and tolerable regimen. Ongoing trials will define if there is a role for checkpoint inhibition in the management of HER2-positive metastatic breast cancer.

Trial registration number NCT03032107.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Phase 2 clinical trial data suggests possible efficacy of trastuzumab emtansine (T-DM1) plus immune checkpoint inhibitor in programmed cell death ligand 1 (PD-L1)-positive human epidermal growth factor receptor 2 (HER2)-positive metastatic breast cancer (MBC).

WHAT THIS STUDY ADDS

⇒ This phase Ib trial represents the first demonstration that T-DM1 plus pembrolizumab is a safe and tolerable regimen, with objective response rate of 20% and median progression-free survival of 9.6 months in this small, single-arm cohort. Analysis of immune biomarkers suggested that the microenvironment of HER2-positive breast tumors becomes less immunologically active as disease progresses over time, underscoring the importance of re-evaluating clinically relevant biomarkers as disease progresses. PD-L1 and tumor infiltrating lymphocytes did not correlate with response, potentially due to limited statistical power.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study supports that T-DM1 plus pembrolizumab is well-tolerated and has clinical activity in patients with HER2-positive MBC; ongoing trials will define if there is a role for checkpoint inhibition in the management of HER2-positive MBC.

INTRODUCTION

Human epidermal growth factor receptor 2 (HER2; also called ErbB2) is overexpressed in approximately 20% of invasive breast cancers.[1 2] In breast cancer, HER2 overexpression is an independent predictor of time to relapse and overall survival (OS) in multivariable models, and is a marker of poor prognosis.[3–6] Fortunately, several targeted therapies are available for HER2-positive breast cancer. The antibody-drug conjugate trastuzumab emtansine (T-DM1) consists of trastuzumab conjugated to emtansine (DM1), an anti-microtubule agent derived from maytansine. Several phase II[7–9] and phase III[10–12] trials have demonstrated that

T-DM1 is an effective treatment option for patients with HER2-positive metastatic breast cancer (MBC) whose disease has progressed on one or more lines of HER2-directed therapy.

However, acquired resistance to T-DM1 occurs in virtually all patients. Several studies have revealed a positive correlation between immune biomarkers in the tumor microenvironment (TME) and prognosis, as well as benefit from HER2-directed therapies, in HER2-positive breast cancer.[13 14] Programmed cell death protein 1 (PD-1) is an inhibitory receptor that is expressed on T cells and other cytotoxic lymphocytes. Antibodies against PD-1 and its ligand PD-L1, known as immune checkpoint inhibitors (ICIs), can enhance the T-cell cytotoxic response to cancer cells. Pembrolizumab is a humanized monoclonal antibody that blocks the interaction between PD-1 and PD-L1/PD-L2. Preclinical studies have shown that the combination of T-DM1 with ICIs targeting PD-1 and cytotoxic T-lymphocyte-associated antigen 4 can result in tumor regression.[15 16] These findings suggest that HER2-positive breast cancer might be amenable to combined treatment with trastuzumab-based and immunotherapy-based approaches. The randomized KATE2 trial examined T-DM1 plus or minus atezolizumab in HER2-positive MBC, and suggested possible benefit of combination therapy only among patients with PD-L1-positive tumors, though the specific immunological mechanisms of benefit remain unclear.[17]

To build on these clinical and preclinical data supporting potential synergy between anti-HER2 therapy plus immune checkpoint inhibition, we conducted a phase Ib study of the safety and efficacy of a combination regimen of pembrolizumab plus T-DM1 in patients with HER2-positive MBC. On-treatment tumor biopsies were performed to explore immunologic correlates of benefit from the regimen.

MATERIALS AND METHODS

Study design and patient population

This was a single-arm phase Ib trial performed at Dana-Farber Cancer Institute. The complete study protocol is provided as an online supplemental appendix.

Eligible patients were required to have stage IV invasive breast cancer that was HER2-positive according to American Society of Clinical Oncology-College of American Pathologists 2013 guidelines.[18] Patients in the dose de-escalation cohort could have evaluable or measurable disease; patients in the expansion cohort were required to have measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST; V.1.1).[19] Patients must have had prior therapy with trastuzumab and a taxane, either separately or in combination. In addition, patients must have received one line of prior therapy for MBC, or have developed disease recurrence within 6 months after completing adjuvant therapy. Patients were required to be at least 18 years old with an Eastern Cooperative Oncology Group performance status ≤2 and left ventricular ejection fraction within normal institutional limits. Patients who received prior T-DM1, pembrolizumab, or any other anti-PD-1/anti-PD-L1 therapies were ineligible. Patients with any autoimmune disease that was active and/or requiring immunosuppressive therapy were also excluded.

The study was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Standards and the Declaration of Helsinki.

Treatment protocol

The study consisted of a dose de-escalation (dose-finding) cohort, followed by an expansion cohort at the recommended phase II dose (RP2D). The primary objective was to evaluate the safety and tolerability of T-DM1 plus pembrolizumab in patients with HER2-positive MBC. The initial doses used were T-DM1 3.6 mg/kg intravenously every 21 days, and pembrolizumab 200 mg intravenously every 21 days. An initial six patients were planned for treatment at the starting dose level, and if one or fewer dose-limiting toxicities (DLTs) were noted in the first six patients, that dose level was to be declared the RP2D. If two or more DLTs were noted in the first six patients, the study would proceed to a lower dose level (T-DM1 3.0 mg/kg intravenously every 21 days, with the starting dose of pembrolizumab), and if one or fewer DLTs were noted in the first six patients at that dose level, it would be declared the RP2D. If two or more DLTs were noted in the first six patients at the reduced dose level, study enrollment would be stopped. DLTs were generally prespecified clinically significant symptomatic or laboratory-based adverse events (AEs) of grade 3 or higher (see clinical trial protocol). DLTs were assessed for a 21-day period from the first dose of study treatment.

Following registration, all patients underwent restaging studies every 6 weeks (or every 9 weeks, after being on study for >24 weeks total). Response and progression were evaluated using RECIST V.1.1.[19] Patients were also evaluated using the immune-related response criteria (irRECIST).[20] Patients remained on study until disease progression (with ongoing treatment allowed for up to 4 weeks following radiographic progression for any clinically stable patient), unacceptable AE(s), or if any other reason arose to make a patient unable/unwilling to comply with study therapy. Cessation of treatment was also allowed in patients who experienced complete response (CR) following >24 weeks on study. After study completion, patients were followed indefinitely for OS events.

All patients were asked to provide archival tumor tissue, if available. Patients in the expansion cohort were required to undergo a research biopsy at baseline (baseline biopsy was optional for patients in the dose de-escalation cohort). In addition, an optional research biopsy was performed after 6 weeks on protocol therapy.

Statistical design and methods

The primary endpoint of the study was the safety and tolerability profile of the regimen, as assessed by DLTs

during the first 21 days of treatment, determination of the RP2D, and maximum grade of all treatment-related AEs using Common Terminology for Adverse Events, V.4.0. Sample size for the study was determined by the results of the dose de-escalation phase. Depending on dose de-escalation results, a total of 6 or 12 patients were planned for enrollment onto the dose de-escalation cohort, and once the RP2D dose was declared, an additional 15 patients were planned for enrollment onto an expansion cohort at the RP2D. All statistical analyses were performed in SAS V.9.3 and R V.4.0.

Genomic analysis methods

Whole exome and transcriptome sequencing was performed and analyzed using established methods (see online supplemental methods document). To identify candidate genomic features associated with response, we compared patients with clinical benefit to those with no clinical benefit. Clinical benefit was defined as CR or partial response (PR) by RECIST V.1.1 or stable disease (SD) ≥24 weeks, and no clinical benefit was defined as progressive disease or SD <24 weeks. Continuous molecular variables were compared between clinical benefit versus no clinical benefit groups using the non-parametric Mann-Whitney Wilcoxon (MWW) test with the *wilcox.test*() or *stat_compare_means(method = 'wilcox')* R function. The proportion of tumors with mutation and copy number alterations were compared with two-sided Fisher's exact tests (*fisher.test*() R function). We controlled the false discovery rate for gene set enrichment analysis (GSEA) with the Benjamini-Hochberg method using a threshold of q<0.05 and used R studio V.1.2.5001 to run statistical analyses.

Protein expression and histology analysis methods

Stromal tumor-infiltrating lymphocytes (sTILs) were assessed in tumor tissue according to the guidelines established by the International TILs Working Group 2014.[21] Individual immunohistochemical staining for PD-L1 (22C3 antibody, quantified by Combined Prognostic Score (CPS)), CD8 (scored as the proportion of stromal interface area occupied by CD8+ cells), and HLA-ABC (scored in the tumor cell compartment according to published methods[22 23]) was performed on formalin-fixed paraffin-embedded tumor tissue from archival, trial baseline, and trial on-treatment time points, and scored by a breast pathologist.

RESULTS

Patient characteristics

Twenty patients were enrolled between February 2, 2017 and August 13, 2019 (6 patients in the dose de-escalation cohort, and 14 patients in the expansion cohort). The trial was closed early (one patient short of goal accrual) due to slow accrual. Patient and tumor characteristics are described in table 1. The median age was 54 (range 37–74), and all patients were women. Patients received a median of one line of prior therapy for metastatic disease. All patients had received prior trastuzumab, taxane, and pertuzumab in either the adjuvant or metastatic setting.

Table 1 Patient and tumor characteristics

Characteristic	No. of patients (%)
Age, years	
Median (range)	54 (37–74)
Sex	
Female	20 (100)
Race	
White	16 (80)
Black	3 (15)
Asian	1 (5)
Ethnicity	
Non-Hispanic	20 (100)
ECOG PS at baseline	
0	14 (70)
1	5 (25)
2	1 (5)
ER/PR status at initial diagnosis	
ER and/or PR positive	14 (70)
ER and PR negative	6 (30)
Disease sites at trial enrollment	
Lymph node	13 (65)
Breast/chest wall	10 (50)
Lung/pleura	9 (45)
Bone	9 (45)
Liver	4 (20)
Other soft tissue	3 (15)
Central nervous system*	1 (5)
Disease-free interval† (from primary diagnosis to metastatic diagnosis)	
≤2 years	5 (25)
>2 years	6 (30)
Dates missing	4 (20)
Lines of chemotherapy for metastatic disease	
Median (range)	1 (0–2)
None	2 (10)
One line	14 (70)
Two lines	4 (20)
Prior therapy in any setting	
Anthracycline chemotherapy	5 (25)
Taxane chemotherapy	20 (100)
Trastuzumab	20 (100)
Pertuzumab	20 (100)
T-DM1	0

*Patients with treated, asymptomatic brain metastases were eligible for trial participation.
†Five patients diagnosed with de novo metastatic disease are not included in this portion of the table.
ECOG PS, Eastern Cooperative Oncology Group performance status; ER, estrogen receptor; PR, progesterone receptor; T-DM1, trastuzumab emtansine .

Safety

There were no DLTs in the dose de-escalation cohort. Thus, 3.6 mg/kg T-DM1 every 21 days plus 200 mg pembrolizumab every 21 days was chosen as the RP2D. Overall, 85% of patients experienced treatment-related AEs of grade 2 or higher (table 2; grade 1 AEs were not

Table 2 Treatment-related adverse events

	All grades N (%)	Grade 2 N (%)	Grade 3 N (%)
Total	17 (85)	13 (65)	4 (20)
A. Possibly immune-related adverse events of clinical interest			
AST increased	4 (20)	3 (15)	1 (5)
Pneumonitis	4 (20)	3 (15)	1 (5)
Arthralgia	3 (15)	3 (15)	–
ALT increased	2 (10)	1 (5)	1 (5)
Influenza-like symptoms	2 (10)	2 (10)	–
Hypothyroidism	2 (10)	2 (10)	–
Hyperthyroidism	1 (5)	1 (5)	–
B. All other adverse events			
Fatigue	8 (40)	7 (35)	1 (5)
Anemia	5 (25)	5 (25)	–
Constipation	4 (20)	4 (20)	–
Nausea	4 (20)	4 (20)	–
Myalgia	3 (15)	3 (15)	–
Peripheral sensory neuropathy	3 (15)	3 (15)	–
Anorexia	2 (10)	2 (10)	–
Dry mouth	2 (10)	2 (10)	–
Neutrophil count decreased	2 (10)	2 (10)	–
Platelet count decreased	2 (10)	2 (10)	–

Grade 1 toxicities were not collected. There were no grade 4–5 toxicities observed on trial. Only events with incidence >10% are shown in the table.
ALT, alanine aminotrasnerase; AST, aspartate aminotransferase.

collected). Twenty per cent of patients experienced grade 3 AEs, and no patients experienced grade >4 AEs. The most common all-grade AEs were fatigue (40%), anemia (25%), elevated aspartate aminotransferase (20%), constipation (20%), nausea (20%), and pneumonitis (20%). AEs with any grade 3 occurrence were fatigue, elevated aspartate aminotransferase, elevated alanine aminotransferase, pneumonitis, lung infection, oral mucositis, and vomiting, each of which occurred in one patient (5%). Two of these grade 3 toxicities were classified as serious AEs: lung infection and vomiting. Possibly immune-related AEs of clinical interest included elevated aspartate aminotransferase (20% of patients) and alanine aminotransferase (10%), hypothyroidism (10%), influenza-like symptoms (10%), arthralgias (15%), and pneumonitis (20%). The four cases of pneumonitis occurred after 2, 3, 4, and 12 cycles of trial therapy. All patients were treated with steroids for presumed ICI pneumonitis, with improvement; in three of four cases, pembrolizumab was permanently stopped but T-DM1 was resumed without any apparent increase in pulmonary abnormalities. In two of four cases, a second diagnostic explanation for pulmonary disease was also strongly considered and treated (congestive heart failure in one case; progressive lymphangitic carcinomatosis in one case).

Six patients (30%) required pembrolizumab dose holds (median hold duration: 21 days (range 7–28 days)), all either for toxicity or per physician discretion. Pembrolizumab dose reductions were not allowed on trial. Seven patients (35%) required T-DM1 dose holds (median hold duration: 20 days (range 7–21 days), all either for toxicity or per physician discretion; five patients (25%) received a T-DM1 dose reduction.

Efficacy

Median follow-up was 32.7 months. Four patients had a PR and no patients had a CR, consistent with an overall response rate (ORR) of 20% (95% CI 5.7% to 43.7%). Six patients (30%) had SD for >24 weeks, therefore the clinical benefit rate (CBR; CR+PR+SD ≥24 weeks) was 50% (95% CI 27.2% to 72.8%; figure 1A). Median progression-free survival (PFS) was 9.6 months (95% CI 2.8 to 16.0 months), and the median duration of response was 10.1 months (95% CI 3.1 to NA months; figure 1B; online supplemental table S1).

Immune microenvironment architecture and changes over time

The immune microenvironment was characterized in pretreatment tumor biopsies performed on trial, and compared with both a prior time point (if previous archival tissue was available) and an on-treatment time point (after 6 weeks on protocol therapy). At each time point, immune biomarkers were evaluated by protein expression (immunohistochemical staining), histology (sTILs), measured by international consensus guidelines[21], and genomics (whole exome sequencing (WES) and bulk RNA sequencing (RNA-seq)). Of the 20 enrolled patients, 12 (60%) had tumor tissue that passed quality control for WES, including 8 patients with baseline samples, 3 patients with baseline and on-treatment samples, and 1 patient with only an on-treatment sample. WES was not performed on archival specimens. Across all 15 tumor samples, the median non-synonymous tumor mutational burden (TMB) was 3.0 mutations/Mb (range 1.5–7.6), and no patient had TMB >10 mutations/Mb (figure 2). The median tumor purity (the proportion of sample DNA from tumor cells) was 0.48 (IQR 0.31–0.65), and the median tumor heterogeneity (the proportion of subclonal mutations) was 0.33 (IQR 0.31–0.44). The median purity-corrected tumor ploidy (the number of chromosome pairs) was 2.05 (IQR 1.95–3.22). The most frequently mutated breast cancer genes were *TP53* and *PIK3CA* in 67% and 33% of patients with sequenced tumors, respectively (figure 2). Individual tumor genomic features are shown in online supplemental table S2.

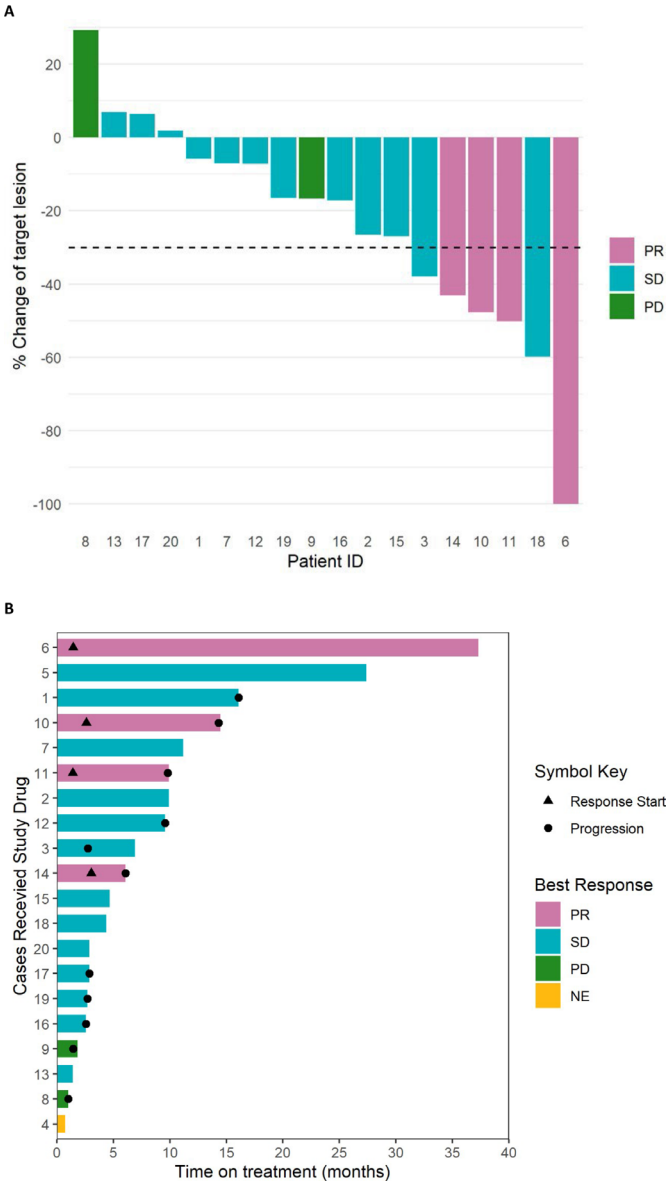

Figure 1 Changes in tumor burden and response duration on study. (A) Waterfall plot showing responses in target lesions during trial therapy. Patients 4 and 5 are not shown: patient 4 was unevaluable; patient 5 had no target lesion at baseline, and overall response (SD) was from a non-target lesion. Patient 18 had two scans showing SD, and one scan showing PR, however PR was not confirmed so this patient was categorized as SD. Patient 3 had one scan showing PR followed by another scan showing SD, so PR was not confirmed and this patient was categorized as SD. (B) Swimmer's plot showing response duration on trial therapy. Patient 3 had a new non-target lesion at cycle 4, but target lesion was read as SD, and the patient stayed on treatment. NE, not evaluable; PD, progressive disease; PR, partial response; SD, stable disease.

Levels of CD8, PD-L1, HLA-ABC (by immunohistochemistry (IHC)), and sTILs (by histology) at the baseline time point on trial are shown in figure 3A. Median PD-L1 CPS was 1 (range 0–40), with median sTILs of 7.5% (range 1–30%). For all four biomarkers, there was a visual trend showing decreasing levels at the time of baseline biopsy on trial, compared with a prior time point (ie, the immune microenvironment appeared to become less 'hot' over time; figure 3B), and then a visual trend toward increasing biomarker levels over the course of trial therapy (figure 3C), indicating an immune-activating effect of the regimen. Similarly, gene expression analysis showed a trend towards higher TILs, CD8 T cells, and PD-L1 at on-treatment as opposed to baseline trial biopsy time points (figure 4A).

Response predictors in the immune microenvironment

Immune biomarkers predicting clinical benefit from the treatment regimen were evaluated based on gene expression, WES, and protein staining/histology. Of the 20 enrolled patients, 14 (70%) had tumor tissue that passed quality control for whole transcriptome sequencing (RNA-seq; online supplemental table S3), including 8 patients with baseline samples, 3 patients with baseline and on-treatment samples, and 3 patients with only on-treatment samples. RNA-seq was not performed on archival specimens. GSEA using the 50 Molecular Signature Database hallmark gene sets[24] revealed that three of the top six most enriched gene sets in baseline tumors from patients with clinical benefit were immune gene sets (figure 4B, online supplemental table S4), specifically the interferon-alpha, interferon-gamma, and allograft rejection pathways, while the epithelial to mesenchymal gene set was the most enriched in tumors from patients with no clinical benefit (figure 4B, online supplemental table S5). These three immune gene sets were also enriched in on-treatment versus baseline tumors (figure 4B, online supplemental table S4). Single sample GSEA (ssGSEA) showed a similar trend towards enrichment of these immune gene sets in baseline and on-treatment biopsies from patients with clinical benefit (figure 4C, online supplemental table S5). Immune cell deconvolution analyses (CIBERSORTx) of bulk RNA-seq data showed higher TILs in baseline biopsies from patients with above median PFS (online supplemental Figure S1A), although CD8 T cells and PD-L1 gene expression were not different (online supplemental figures S1B,C). These patients with above median PFS also had higher resting memory CD4 T cells and trended towards having higher plasma cells (online supplemental figure S1D,E).

Using WES data, we examined whether TMB, tumor ploidy, tumor heterogeneity, and tumor purity were related to clinical benefit from T-DM1 and pembrolizumab benefit based on prior work showing that these genomic features associated with PD-1 response in other solid tumors.[25 26] However, none of these aggregate genomic features associated with clinical benefit in our cohort (online supplemental figures S2A-D), potentially due to the limited power of our small sample size. We then performed an unbiased analysis for single-gene predictors of response to T-DM1 and pembrolizumab across all mutated genes detected in this cohort. Before correcting for multiple hypothesis testing, no single-gene non-synonymous somatic variant was associated with clinical

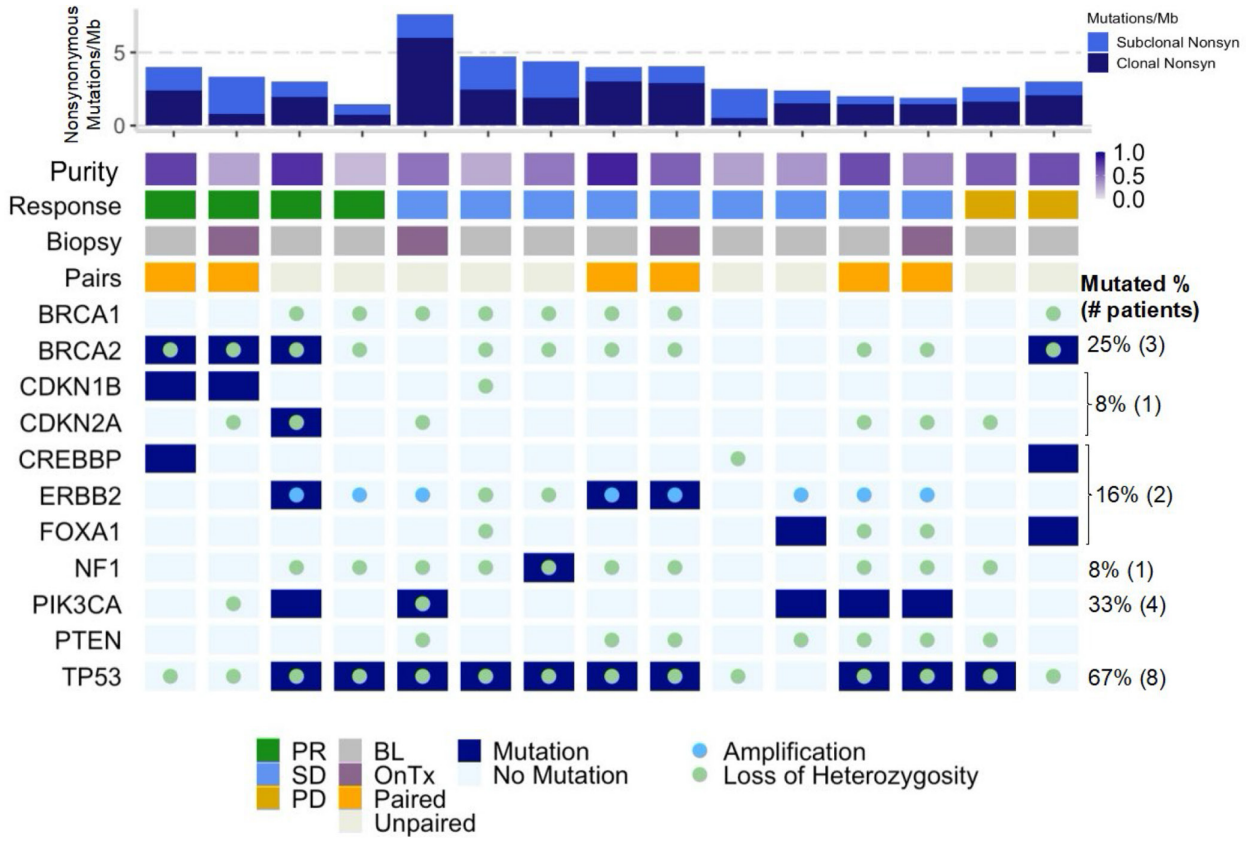

Figure 2 Genomic cohort characteristics. Each column of this co-mutation plot represents a tumor biopsy. Tumor biopsies are ordered by Response Evaluation Criteria in Solid Tumors response, and within each response subgroup by decreasing non-synonymous (Non-syn) mutational load (top row). Non-synonymous mutational burden is further subdivided into clonal (dark blue) and subclonal (light blue) mutational load. Tumor purity is the inferred proportion of the tumor sample that is from cancer cells compared with other cell types with dark purple corresponding to a purity of 1. The biopsy timing (baseline in gray vs on-treatment in purple) is indicated, and paired biopsies from the same patient are shown in orange. Mutations and copy number alterations in genes commonly mutated in breast cancer are shown for each tumor. BL, baseline; OnTx, on-treatment; PD, progressive disease; PR, partial response; SD, stable disease.

benefit at unadjusted Fisher's exact p<0.05 (online supplemental figure S3A), underscoring the large sample sizes needed for sufficient power to detect these associations.[27] Similarly, no single-gene amplification or homozygous deletion was associated with clinical benefit at unadjusted Fisher's exact p<0.05 prior to multiple hypothesis testing correction (online supplemental figure S3B).

PD-L1 status by IHC and sTILs by histology were evaluable in baseline tumor biopsy for 14 patients. Overall there were no evident associations between PD-L1 status, sTILs, and various parameters of clinical benefit from T-DM1 plus pembrolizumab (online supplemental table S6). ORR was numerically lower in PD-L1-positive patients (measured by CPS cut-off of >1 or ≥10) and in patients with high TILs (≥10%) compared with PD-L1-negative patients or patients with low TILs (<10%), respectively. As with the genomic assays, these analyses were limited by small patient numbers in each subgroup.

Antigen presentation biomarkers

Based on prior work showing that downregulation of antigen presentation machinery facilitates resistance to checkpoint inhibitors,[23 28 29] we specifically investigated antigen presentation genes in WES data (online supplemental figure S4). Only one patient had a mutation in an antigen presentation gene, specifically a clonal nonsense mutation (p.S72*) in *B2M*, and this patient had SD with PFS of 9.6 months. Analysis of antigen presentation gene expression (online supplemental table S7) focused on genes previously found to be associated with ICI response in melanoma.[25] MHC-II-associated HLA genes and NLRC5, which is the master regulator and positively induces MHC-I antigen presentation, demonstrated higher expression in on-treatment versus baseline tumors (MWW p<0.05; online supplemental figure S5A). In baseline tumor biopsies, antigen presentation ssGSEA scores did not differ by clinical benefit (online supplemental figure S5B). Comparing on-treatment to baseline tumor biopsies, patients with versus without clinical benefit had numerically higher median fold changes in antigen presentation (4% vs 1%) and HLA-II (11% vs −3%) ssGSEA scores (online supplemental figure 5C-D, supplemental table 8). However, these results must be interpreted with caution, given the small number of on-treatment samples (n=4 with clinical benefit; n=2 without clinical benefit).

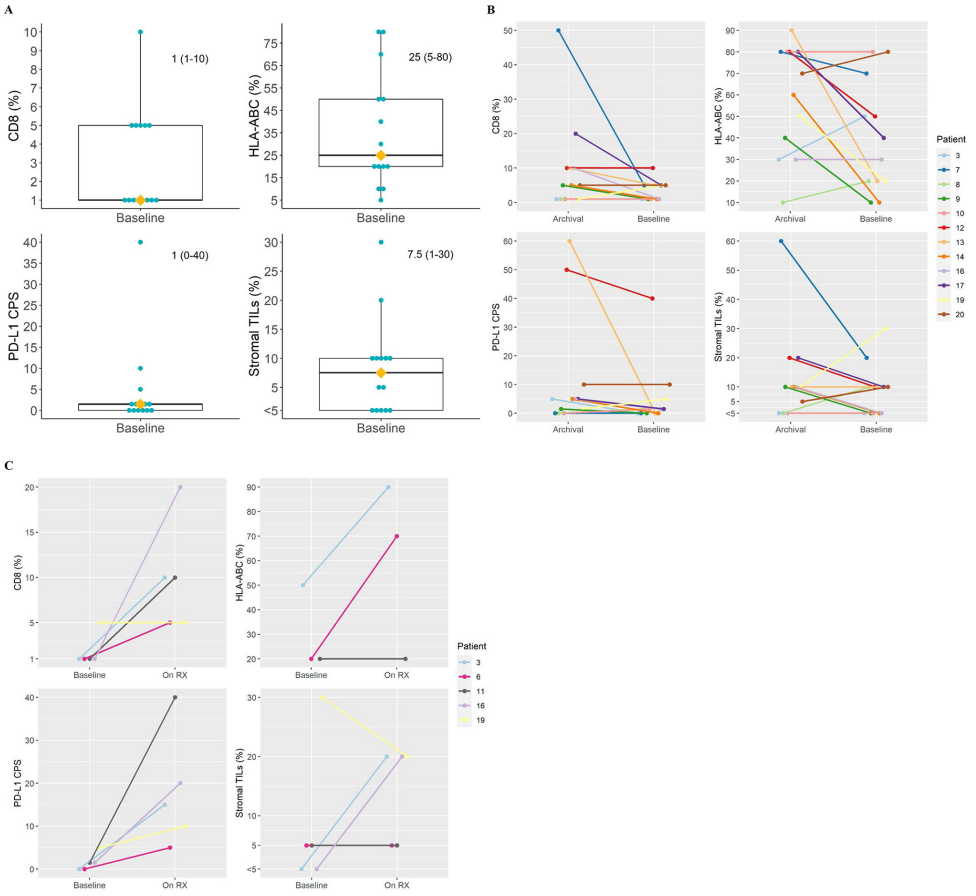

Figure 3 Immune microenvironment architecture over time by protein expression and histology. (A) Levels of immune biomarkers at baseline time point on trial. CD8, PD-L1, and HLA-ABC were measured by immunohistochemical staining. Stromal TILs were measured by histology. Each blue dot represents an individual patient; yellow diamonds indicate median values. The median and range (in parentheses) of values are indicated in the upper right corner of each plot. (B) Change in level of each biomarker from various pre-trial time points ('archival' on X-axis) to baseline on-trial time point ('baseline' on X-axis). Each line represents an individual patient. Only patients with evaluable biomarker data at both of the indicated time points are shown on each plot. (C) Change in level of each biomarker from baseline on trial time point ('baseline' on X-axis) to on-treatment trial time point following 6 weeks of therapy ('on RX' on X-axis). Each line represents an individual patient. Only patients with evaluable biomarker data at both of the indicated time points are shown on each plot. CPS, Combined Prognostic Score; PD-L1, programmed death ligand-1; TILs, tumor infiltrating lymphocytes.

DISCUSSION

The results of this phase Ib trial show that a combination regimen of pembrolizumab plus T-DM1 is well-tolerated and has clinical activity in patients with HER2-positive MBC. Biomarker analyses were limited by the small sample size of the cohort, however analysis of immune-related biomarkers based on protein expression, gene expression, and exome sequencing suggested that the microenvironment of HER2-positive breast tumors becomes less immunologically active as disease progresses over time, and tends to become re-inflamed early during treatment with T-DM1 and pembrolizumab. Antigen presentation-related gene expression tended to increase on treatment. While some immune signatures in baseline biopsies correlated with clinical benefit from the regimen, there was no clear association with benefit for patients with higher PD-L1 protein expression or higher sTILs, likely due to the small sample size of the cohort.

The safety profile of T-DM1 plus pembrolizumab in this trial was acceptable and consistent with the previously known toxicity profiles of each drug, with no grade 4 or 5 events. AEs with any grade 3 occurrence were fatigue, elevated aspartate aminotransferase, elevated alanine aminotransferase, pneumonitis, lung infection, oral mucositis, and vomiting, each of which occurred in one patient (5%). In the KATE2 trial, which explored T-DM1 with or without atezolizumab, a higher rate of thrombocytopenia was observed in the T-DM1/atezolizumab combination arm compared with the T-DM1 alone arm, raising some concern for possible exacerbation of thrombocytopenia with the combination of T-DM1 and checkpoint inhibitor. However, our finding of no episodes of grade 3 or higher thrombocytopenia, and a 10% rate (two patients) of lower grade thrombocytopenia, is somewhat reassuring, though our cohort size is small.

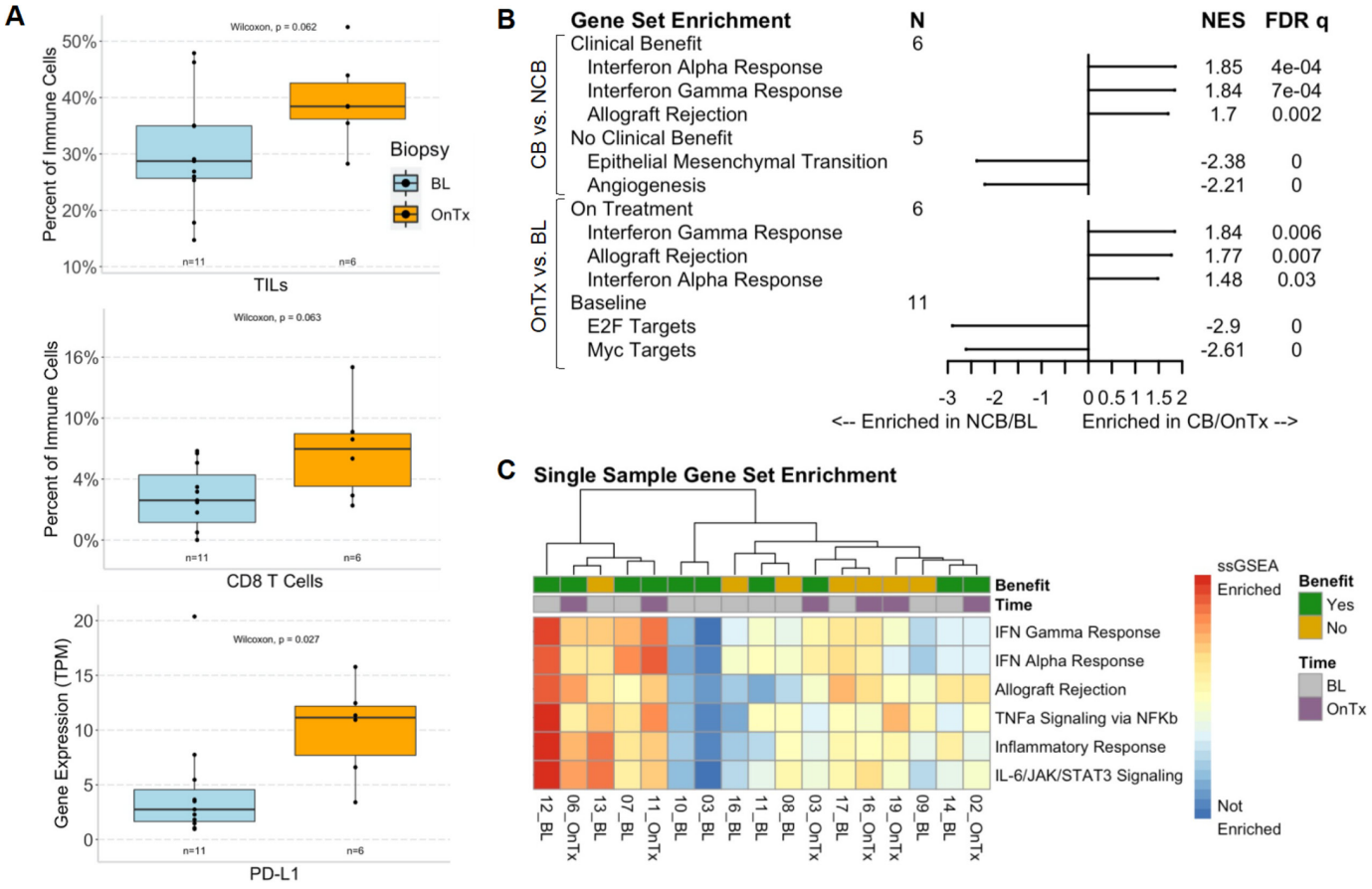

Figure 4 Immune gene enrichment in clinical benefit and on treatment. (A) Tumor-infiltrating lymphocytes and CD8 T cells inferred by RNA sequencing trended towards being higher in on-treatment (orange) versus baseline (blue) tumor biopsies, and PD-L1 gene expression was significantly higher in on-treatment biopsies. Unadjusted Mann-Whitney Wilcoxon p values are shown. Boxplot limits indicate the (IQR; 25th–75th percentile), with a center line indicating the median. Whiskers show the value ranges up to 1.5×IQR above the 75th or below the 25th percentile with outliers beyond those ranges shown as individual points. (B) Molecular Signature Database hallmark gene set enrichment analysis (GSEA) revealed immune gene set enrichment in patients with clinical benefit (CB) versus no clinical benefit (NCB) and in on-treatment (OnTx) versus baseline (BL) biopsies. (C) A heatmap of single sample GSEA (ssGSEA) scores, where each column is a tumor labeled by CB (CB in green vs NCB in yellow) and biopsy time (OnTx in purple vs BL in gray), showed a similar trend towards enrichment of immune gene sets in patients with CB. Color indicates the ssGSEA score from least enriched (blue) to most enriched (red). FDR, false discovery rate; NES, normalized enrichment score; PD-L1, programmed death ligand-1.

The 20% rate of all-grade pneumonitis observed in our cohort bears watching if future studies of this combination are pursued, though only one episode (5%) of pneumonitis was grade 3. Pneumonitis is a well-described side effect of both T-DM1 alone and all checkpoint inhibitor-based regimens in HER2-positive breast cancer, however incidence is usually in the low single digits. Pneumonitis occurred in 1.9% and 4% of patients treated with T-DM1 alone in the DESTINY-Breast03 and KATE2 trials, respectively, and pneumonitis incidence was identical (4%) among KATE2 patients who received T-DM1 plus atezolizumab.[17 30] It is possible that the increased rate of pneumonitis in this cohort reflects a true different biology for combined T-DM1/pembrolizumab compared with combined T-DM1/atezolizumab. Indeed, it has been noted in non-small cell lung cancer that regimens incorporating PD-1 blockade produce more pneumonitis than those incorporating PD-L1 blockade,[31] though this has not been seen in breast cancer.[32 33] However, it is likely that the increased rate of pneumonitis we observed in this cohort was due to chance and small sample size, compounded by the fact that pneumonitis is a clinical diagnosis and thus always associated with some uncertainty. A larger randomized trial of T-DM1 plus or minus pembrolizumab would likely be necessary to determine whether the cases of pneumonitis seen here were due to biology or to chance.

Clinical activity of T-DM1 plus pembrolizumab in this trial was comparable to that seen with similar regimens and similar cohorts in the past. Given the non-randomized nature of the study design, it is not possible to say whether pembrolizumab added to the activity of T-DM1 based on our results. The presented data show an ORR of 20%, 24-week clinical benefit rate of 50%, and median PFS of 9.6 months for the T-DM1/pembrolizumab doublet. EMILIA, the original landmark study of single-agent

T-DM1 as second-line therapy for HER2-positive MBC (following taxane and trastuzumab), showed an ORR of 43.6% and median PFS of 9.6 months.[10] However, none of the patients in EMILIA had received prior pertuzumab, whereas all of the patients in our cohort had received prior pertuzumab. A smaller retrospective cohort study of T-DM1 in patients previously treated with pertuzumab demonstrated a tumor response rate of 17.9% and median duration on therapy of 4.0 months.[34] In the more recent KATE2 trial, which evaluated T-DM1 plus or minus the anti-PD-L1 checkpoint inhibitor atezolizumab, 48% of KATE2 trial participants received prior pertuzumab, and all had received prior taxane and trastuzumab; in the control arm of patients who received T-DM1 alone, the median PFS was 6.8 months.[17] In the T-DM1 arm of the second-line DESTINY-Breast03 trial, in which approximately 60% of participants had received prior pertuzumab, median PFS was also 6.8 months.[35]

At this point, the role of checkpoint inhibition in combination with HER2-directed therapy in HER2-positive MBC remains unclear, and trials are ongoing to better define it. The randomized KATE2 trial of T-DM1 plus or minus atezolizumab did not meet its primary endpoint, failing to demonstrate improved PFS with the addition of atezolizumab to T-DM1 in the overall trial population. However, among the prespecified patient subgroup with tumors positive for PD-L1 staining (approximately 40% of patients), there was a suggestion of benefit with the addition of atezolizumab in terms of both PFS (median PFS 8.5 months vs 4.1 months for patients receiving atezolizumab vs placebo, respectively)[17] and OS.[36] There was no suggestion of benefit for patients with PD-L1-negative tumors.[17] Similarly, in the single-arm PANACEA trial, patients with trastuzumab-refractory HER2-positive MBC received treatment with trastuzumab plus pembrolizumab. Among patients with PD-L1-negative tumors, no patients had an objective response, compared with a 15% ORR in the PD-L1-positive subpopulation (N=46).[37] In a small cohort of patients with heavily pretreated HER2-positive breast cancer with PD-L1-negative tumors, the combination of trastuzumab and durvalumab (anti-PD-L1 antibody) produced a 0% ORR.[38] Overall, given some suggestion of clinical activity for checkpoint inhibition plus HER2-directed therapy in PD-L1-positive, pretreated HER2-positive MBC, further evaluation of T-DM1 plus atezolizumab is planned in this patient subset (KATE3 trial; NCT04740918). In the first-line metastatic setting, a large ongoing randomized phase III trial is investigating the addition of atezolizumab to the current standard of taxane plus trastuzumab and pertuzumab (NCT03199885). Though the combination of immune checkpoint blockade and HER2-directed therapy remains an area of great interest in breast cancer, to our knowledge, no further studies specifically of pembrolizumab plus T-DM1 are currently planned.

While these previous trials have suggested that PD-L1 may be an important biomarker of response to combined therapy with ICI and a HER2-targeted agent, exploratory analyses in the small cohort from this trial did not identify such an association: ORR was numerically lower in PD-L1-positive patients from this trial cohort. Other trials have similarly suggested that higher TILs correlate with more favorable response to HER2-targeted regimens with or without checkpoint blockade for metastatic HER2-positive tumors. In the CLEOPATRA trial of docetaxel/trastuzumab plus or minus pertuzumab as first-line therapy for HER2-positive MBC, each 10% increase in sTILs (measured on archival or fresh pretreatment tumor samples) was associated with a significant increase in OS.[14] In the KATE2 trial, sTILs (assessed histologically) and CD8+ cells (assessed by IHC) both correlated with improved PFS.[17] By contrast, our data demonstrate no evident association between sTILs and various parameters of clinical benefit from the T-DM1 plus pembrolizumab regimen. Since the CLEOPATRA trial and KATE2 trial data sets are larger than the data set presented here, it seems likely that our inability to replicate these biomarker associations may be due to underpowering.

Our genomic results support established ICI response correlates and indicate new directions for future research. The absence of patients with high TMB underscores the known low prevalence of high TMB in breast cancer.[39] Of note in the KATE2 trial, many immune-related biomarkers correlated with longer PFS, but TMB did not.[17] Our transcriptomic analyses showed that immune infiltration and antigen presentation associated with treatment and response, consistent with prior studies in solid tumors.[40–45] The role of antigen presentation in ICI response in HER2-positive breast cancer is still being elucidated. Our finding of a higher on-treatment rise in HLA-II gene expression in patients with longer PFS points to the need to investigate whether professional antigen presenting cells, such as dendritic cells, contribute to ICI responses in HER2-positive breast cancer and calls for future single-cell studies to answer this question. It is potentially notable that two of four patients with PR on the trial regimen were found to have mutations (and loss of heterozygosity) in *BRCA2*. However given the very small size of our cohort, this finding should be interpreted in the context of larger clinico-genomic data sets that have not consistently demonstrated a correlation between *BRCA1/2* mutation and response to checkpoint blockade. This is an area of controversy and active investigation. While some analyses have suggested a relationship between *BRCA2* alterations and ICI responsiveness,[46] others have pointed out that many *BRCA1/2* alterations do not contribute to tumor pathogenesis,[47] and have not identified *BRCA1/2* as predictors of ICI response.[48]

Our data support prior findings across breast cancer subtypes suggesting that the immune microenvironment tends to become less inflamed as disease progresses over time. In this cohort's metastatic population on second-line therapy, all baseline biomarkers were assessed in a biopsy immediately prior to initiation of trial therapy, and compared in pairwise fashion to a previous disease time point, often either the time of original diagnosis or

the time of metastatic diagnosis. Though sample size was not sufficient for formal statistical analysis, there was a visual trend showing generally decreased levels of each immune biomarker (sTILs by histology; CD8, PD-L1, and HLA-ABC by IHC) over time. Evidence suggests that triple-negative MBC is less responsive to immune checkpoint inhibition in later lines of therapy compared with earlier lines, though a similar comparison has not been performed in HER2-positive tumors.[49] The suggestion in our data set of a less 'hot' and more immunosuppressed TME over time supports the development of combined immune checkpoint blockade plus HER2-targeted regimens earlier in the course of disease. Conversely, the IMpassion050 trial examined standard chemotherapy plus or minus atezolizumab as neoadjuvant therapy for high-risk treatment-naïve, non-metastatic HER2-positive tumors, and found no improvement in pathologic CR rates with the addition of the immunotherapy agent, though event-free survival data are immature and underpowered.[50] The results of the ongoing phase III trial investigating addition of atezolizumab to first-line therapy in HER2-positive MBC (NCT03199885) will be of particular interest. Of additional note, the use of T-DM1 is likely to be pushed later in the typical treatment course of HER2-positive MBC, based on the recent results of the DESTINY-Breast03 trial demonstrating superiority of famtrastuzumab deruxtecan-nxki over T-DM1 in the second line.[35]

There are notable limitations to these data. As discussed, the small sample size of this phase 1b trial means that definitive conclusions about the efficacy of the regimen are not possible. Likewise, biomarker analyses are underpowered to identify correlations with response. Nonetheless, the small single-institution nature of the trial meant that we were able to collect a unique set of serial biospecimens from standardized time points, allowing us to examine biomarker changes over time within each individual patient, both prior to and during treatment with T-DM1 plus pembrolizumab.

In conclusion, the landscape of treatment options for HER2-positive MBC is expanding rapidly. We demonstrate here that T-DM1 plus pembrolizumab is a safe and effective regimen. This small cohort did not suggest a biomarker predictive of response, though studies of similar regimens in larger cohorts have suggested that PD-L1 may be a useful marker of responsiveness to immune checkpoint blockade combined with HER2-targeted therapies. Ongoing trials will define if there is a role for checkpoint inhibition in the management of HER2-positive MBC.

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