Pembrolizumab in Combination with Binimetinib in Patients with Unresectable Locally Advanced or Metastatic Triple-Negative Breast Cancer

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ABSTRACT

Purpose: Activation of the RAS/MAPK pathway is associated with reduced tumor-infiltrating lymphocytes and poor outcomes in triple-negative breast cancer. Previous studies demonstrated that inhibition of the MAPK pathway with a MEK inhibitor is synergistic with immune checkpoint inhibitors.

Patients and Methods: We conducted a phase I/II trial of pembrolizumab and binimetinib in patients with metastatic triple-negative breast cancer with ≤3 prior lines of therapy. There were two dose levels (DL) with binimetinib at 45 mg at DL 0 and 30 mg at DL −1.

Results: The recommended phase II dose was the standard dose of pembrolizumab with binimetinib 30 mg twice daily. The objective response rate (ORR) was 30.4%, with a numerically higher ORR in patients without liver metastasis at 45.5%. Among patients who achieved objective responses, 80% had a duration of response >12 months and ongoing even after stopping treatment (5.4–69.0 months). Patients with PD-L1–positive tumors (modified proportion score ≥10) were more likely to respond with an ORR of 66.7%. However, clinical benefit was observed in 25% of patients with PD-L1–negative tumors. Consistent with preclinical studies, four of six patients with clinical benefit had either increased PD-L1 or decreased p-ERK expressions in serial circulating cancer-associated macrophage-like cells after starting binimetinib.

Conclusions: Pembrolizumab and binimetinib at 30 mg are safe with manageable toxicities. Promising activity was observed in patients without liver metastases. Future larger clinical trials are warranted to further evaluate the efficacy of this chemotherapy-free combination.

Introduction

Immune checkpoint inhibitors (ICI) have recently expanded our treatment armamentarium for triple-negative breast cancer (TNBC). Pembrolizumab is a humanized monoclonal antibody against PD-1 designed to inhibit the interaction of PD-1 with PD-L1 and PD-L2 and allow a more effective antitumor immune response. The combination of chemotherapy and pembrolizumab became the standard-of-care therapy in patients with TNBC in both early-stage and metastatic settings (1, 2). However, the benefit of ICI in patients with metastatic TNBC seems to be limited to patients with PD-L1–positive disease (3). Although the combinations of chemotherapy and ICIs are quite effective with a high objective response rate (ORR; ref. 3), single-agent ICIs have rather modest efficacy in TNBC, especially in previously treated patients with metastatic TNBC (4–6).

Multiple studies have established the importance of host immune response measured by the number of tumor-infiltrating lymphocytes (TIL) and PD-L1 expression with ICI response. Previous studies demonstrated that activation of the RAS/MAPK pathway is associated with reduced TILs and poor response to neoadjuvant chemotherapy in TNBC (7). Further study showed that inhibition of the MAPK signaling pathway with an MEK inhibitor upregulates MHC and PD-L1 expression in both *in vivo* and *in vitro* TNBC models via STAT activation (7, 8). Moreover, the combination of anti–PD-1 or anti–PD-L1 with MEK inhibitors was synergistic and resulted in significantly improved tumor control in syngeneic TNBC mouse models (7). Similar synergy was also observed in other syngeneic mouse models with colon cancer and melanoma, in which MEK inhibition increased tumor antigen–specific $CD8^+$ T-cell infiltration and protected them from chronic T-cell receptor stimulation–inducing apoptosis (9).

Binimetinib is an oral, ATP-uncompetitive, selective allosteric small-molecule inhibitor of MEK 1/2 (10, 11). Binimetinib was approved by the US FDA in 2018 for patients with metastatic melanoma in combination with a BRAF inhibitor, encorafenib. In this study, we evaluated the combination of pembrolizumab and binimetinib in a single-arm phase I/II trial (NCT03106415) in patients with locally advanced or metastatic TNBC.

Patients and Methods

Patient population

This trial included patients ≥18 years of age with histologically confirmed unresectable locally advanced or metastatic adenocarcinoma

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Translational Relevance

Pembrolizumab, in combination with a MEK inhibitor, binimetinib, has promising activity in metastatic triple-negative breast cancer, particularly in patients without liver metastases, with manageable toxicities. Responses were observed in both PD-L1–positive and PD-L1–negative diseases, with durable responses observed even after stopping therapy. Serial circulating cancer-associated macrophage-like cells hold potential as a blood-based noninvasive approach to evaluate the biological effects and pharmacodynamic markers of MEK inhibitors.

of the breast, with estrogen receptor ≤10%, progesterone receptor ≤10%, and HER2 negativity defined as IHC zero or HER2 IHC 1+ or 2+ without HER2 gene amplification (ratio <2.0 or copy number <4.0 signals/cells), and Eastern Cooperative Oncology Group performance status ≤1. Moreover, patients must have had measurable disease, adequate organ functions, and adequate cardiac function defined as left ventricular ejection fraction ≥50% and QTc interval ≤480 mcs. In the phase I portion, there was no limit on the number of prior lines of therapy. However, only patients with ≤3 prior lines of therapy were included in the phase II portion. Patients with immunocompromised condition, active infection, hypersensitivity to pembrolizumab or binimetinib, active brain metastases, autoimmune disease, history of pancreatitis, history of retinal vein occlusion, retinal degenerative disease, Gilbert syndrome, other active malignancy within 3 years, uncontrolled cardiovascular disease, neuromuscular disorder, impaired gastrointestinal function, and major surgery within 3 weeks were excluded.

Study design

This trial is a phase I/II, single-arm, open-label study of pembrolizumab in combination with binimetinib in patients with unresectable locally advanced or metastatic TNBC. The phase I portion was a dose-finding study, utilizing the classic 3 + 3 study design. The objective of the phase I portion was to estimate the MTD or the recommended phase II dose (RP2D) and to determine the dose-limiting toxicities (DLT). The MTD was defined as the dose level (DL) below the lowest dose that induced DLT in at least one-third of patients (at least two of a maximum of six new patients). For phase II, the primary endpoint was the ORR by RECIST criteria version 1.1. The Simon two-stage optimal design (12) was used to test the null hypothesis that this two-drug combination has an ORR of at most 15% versus the alternative hypothesis that it has an ORR of at least 35%. Patients in phase I who were treated with the RP2D and were evaluable for response would be included in the phase II analysis.

This study was approved by the Mayo Clinic Institutional Review Board and was conducted in compliance with the Declaration of Helsinki, the International Conference on Harmonization, and Good Clinical Practice. This study followed the reporting guidelines under the CONSORT guidelines. Voluntary written informed consent was obtained from all patients.

Treatment

Patients received single-agent binimetinib for 2 weeks, followed by a research blood draw and optional tumor biopsy to evaluate the effects of binimetinib prior to starting pembrolizumab. There were two DLs in the phase I portion. Patients in DL 0 received binimetinib at the dosage of 45 mg oral twice daily continuously, and DL −1 received 30 mg oral twice daily continuously. All patients received pembrolizumab as an intravenous infusion at the dosage of 200 mg every 3 weeks. Treatment was continued until disease progression, unacceptable toxicities, patient withdrawal, or the treating physician's decision.

TILs and PD-L1

Patients must undergo mandatory research biopsy prior to starting on the treatment protocol unless adequate tissue was available from the previous biopsy obtained ≤90 days prior to registration. Stromal TIL (sTIL) quantification and PD-L1 expression on research biopsy samples were assessed centrally. sTILs were categorized into 0, 1+, 2+, or 3+. PD-L1 was evaluated using PD-L1 IHC 22C3 pharmDx (Agilent Technologies) and quantified according to the modified proportion score (MPS). PD-L1 positive was defined as MPS ≥10.

CellSieve microfiltration for cancer-associated macrophage-like cell isolation, circulating tumor cell isolation, and PD-L1 scoring

Peripheral whole blood (7.5 mL) was collected in CellSave preservative tubes, maintained at room temperature, and processed within 96 hours after collection. A CellSieve Microfiltration Assay uses a low-pressure vacuum system to filter peripheral blood based on size exclusion of >7 μm. Cancer-associated macrophage-like cells (CAML) and circulating tumor cells (CTC) were identified based on morphologic features and the phenotypic expression of CD45, PD-L1 (Creatv MicroTech, clone #q9nzq7), cytokeratins 8, 18, and 19 (catalog. #CellSieve Enumeration Kit), and Fluoromount-G with DAPI (Thermo Fisher Scientific, catalog #0100-20) using pre-established cytologic features by a trained cytologist (13–17). An Olympus BX54WI Fluorescent microscope with Carl Zeiss AxioCam and Zen2011 Blue (Carl Zeiss) was used for all imaging. Objects imaged via a fluorescent microscope were analyzed and quantified for CTCs and CAMLs found in peripheral whole blood samples. CTCs were characterized by CD45 negativity with a filamented cytokeratin signal and a DAPI-positive nucleus with pleomorphic criteria, as previously defined (13–17). CAMLs were characterized by CD45 positivity or negativity, a cell surface or intracellular cytokeratin signal, and a polynucleated DAPI-positive nucleus, as previously defined (13–17). PD-L1 expression in isolated cells was quantified by pixel intensity using the Zen2011 Blue software system and scored as previously described (14–16). For this study, PD-L1 expression was converted from a quartile score into a binary scoring system (1 = low expression, 2 = medium expression, and 3 = high expression).

Statistical analysis

Patient demographics, including age, follow-up time, and tumor size, were summarized using the median and range, whereas the remaining categorical variables were summarized with frequency and percentage. The overall ORR by RECIST was estimated using the approach of Jung and Kim (On the estimation of the binomial probability in multistage clinical trials. Stat Med 23:881–96, 2004). The 90% lower confidence bound was calculated using the approach of Koyama and Chen (Proper inference from Simon's two-stage designs. Stat Med 27:3145–54, 2008). The clinical benefit rate (CBR) was estimated via a binomial proportion with a two-sided 95% confidence interval (CI). Overall survival (OS) and progression-free survival (PFS) were summarized using Kaplan–Meier curves.

For PD-L1 and p-ERK, if a patient had zero CTCs, expression level was set to zero. If a patient had zero CAMLs, the largest CAML size was set to zero. Any CTC was defined as any true or apoptotic CTC count greater than one. Baseline and changes from baseline for CTC count, CTC expression, CAML count, CAML expression, CAML size, and TILs were assessed for associations with ORR, CBR, OS, and PFS using Wilcoxon rank-sum test, χ^2 tests, Kaplan–Meier curves, and Cox proportional hazards regressions, as appropriate. Spearman correlations between MPS CTC and CAML measures were also tabulated.

Unless otherwise noted, statistical tests were two-sided, with the α level set at 0.05 for statistical significance. No adjustments were made for multiple comparisons. The analysis was completed with R-studio based on version R-4.2.2.

Data availability

The data generated in this study are not publicly available because of potential patient privacy concerns but are available upon reasonable request from the corresponding author.

Results

Patient characteristics

A total of 23 patients were enrolled in this phase I/II trial, including 13 patients in the phase I portion, with five of these patients registered to DL 0 with binimetinib of 45 mg oral twice daily and eight patients registered at DL −1 with binimetinib of 30 mg oral twice daily. The CONSORT diagram for the phase I portion is shown in **Fig. 1**. The MTD or the RP2D was DL −1 with binimetinib of 30 mg oral twice daily and pembrolizumab 200 mg IV every 3 weeks. As prespecified in the protocol, the eight patients treated in RP2D were included in the phase II portion of the study, along with 10 additional patients. Patient characteristics are shown in **Table 1**. The median age was 58 years (range, 37–78 years), seven (31.8%) were African American, and 18 (81.8%) were postmenopausal. A total of 14 (63.6%) patients had no prior systemic therapy in the metastatic setting, and eight (36.4%) patients had 1 to 2 prior lines of therapy in the metastatic setting. The representativeness of study participants is summarized in Supplementary Table S1.

DLTs in the phase I portion

There were four patients treated in DL 0 as one patient withdrew from the study prior to starting study treatment. DLT was observed in two of four patients in DL 0, with grade 3 alanine aminotransferase (ALT) abnormality in one patient and grade 3 flank pain together with grade 3 nausea and vomiting >48 hours despite antiemetic therapy in the other patient. The subsequent binimetinib dosage was reduced to DL −1 with 30 mg twice daily. In the next six patients, one DLT was observed with grade 3 aspartate aminotransferase (AST)/ALT abnormality. However, that particular patient had existing liver metastasis prior to treatment. Thus, DL −1 was the MTD or RP2D, and an additional 12 patients were treated with DL −1 in phase II.

Outcomes

Overall, 18 patients were treated in DL −1 and were included in phase II efficacy evaluation. All 18 patients were evaluable for response. Confirmed objective responses were observed in five patients, with one complete response and four partial responses (PR). Using the approach of Jung and Kim to calculate the ORR and the approach from Koyama and Chen to calculate the 90% CI yields, an ORR of 30.4% (95% CI, 12.2%–47.3%) was observed. The confirmed CBR was 33.3% (95% CI, 13.3%–59.0%), with six of 18 having had complete response, PR, or stable disease (SD) ≥24 weeks. We further evaluated responses among patients with and without liver metastases. Among all five patients with liver metastases, no response was observed. The ORR in patients without liver metastases was 45.5% (95% CI, 16.7%–76.6%), and the CBR was 54.5% (95% CI, 23.4%–83.3%). The ORR by the number of prior lines of therapy is summarized in **Table 2**. All patients who achieved the ORR and CBR received the combination of pembrolizumab and binimetinib in the first-line metastatic setting.

The median PFS in DL 0 was 2.4 (95% CI, 0.5–NE) and in DL −1 was 7.9 months (95% CI, 3.9–NE). The median OS in DL 0 was 7.0 (95% CI, 0.5–NE) and in DL −1 was 33.2 months (95% CI, 11.2–NE). Among patients who achieved objective responses, three of five (60%) had a duration of response greater than 12 months, which was ongoing even after stopping treatment (range, 5.4–69.0 months with 32 months on active monitoring period followed by 69 months after), including one patient who discontinued the combination treatment because of toxicities but was subsequently treated with single-agent pembrolizumab. The patient with the longest duration of response at 69 months had 32 months in the active monitoring period and an additional 37 months after the active monitoring period. These four patients remained without evidence of disease even after stopping all treatment up to almost 6 years after initiation of the trial. One of these four patients developed disease progression 38 months after stopping the treatment, but her tumor responded again after starting back on pembrolizumab and binimetinib. The swimmer plot of tumor burden changes over time is shown in **Fig. 2A**. In addition, Kaplan–Meier curves for PFS and OS are shown in **Fig. 2B** and **C**, respectively.

Overall adverse events

Adverse events (AE) were generally manageable and consistent with previously reported AEs for binimetinib and pembrolizumab. The 12 most common AEs reported in the first cycle with single-agent binimetinib in all patients were as shown in **Table 3**, including peripheral sensory neuropathy, blood and lymph system disorder, fatigue, nausea, constipation, diarrhea, ALT/AST elevation, and hypertension. Moreover, rashes, including acneiform (31.8%) and papulopustular lesions (22.7%), were also common but were mainly grade 1 and 2. However, there was one patient with grade 3 papulopustular rash with binimetinib at the dose of 30 mg, requiring dose reduction. Asymptomatic cardiac troponin T increase was also observed in 22.7% of patients without significant cardiac events, and no ECG change was observed to suggest cardiac ischemia. Similar AEs continued to be observed in cycle two and beyond when pembrolizumab was added. These AEs are shown in **Table 4**, with fatigue, anemia, blood and lymphatic system disorders, and AST elevation being the most common AEs. A total of 72.7% of patients experienced grade ≥3 AEs with any relationship. When limiting to only those at least possibly related to the treatment, that rate fell to 54.5% with no grade 4 or 5 AEs documented.

Correlative studies

Tumor PD-L1 expression

PD-L1 expression in tumors was evaluated in mandatory biopsy or archival samples within 90 days prior to the enrollment using IHC 22C3. There were 22 subjects that were able to be assessed for PD-L1 MPS, and

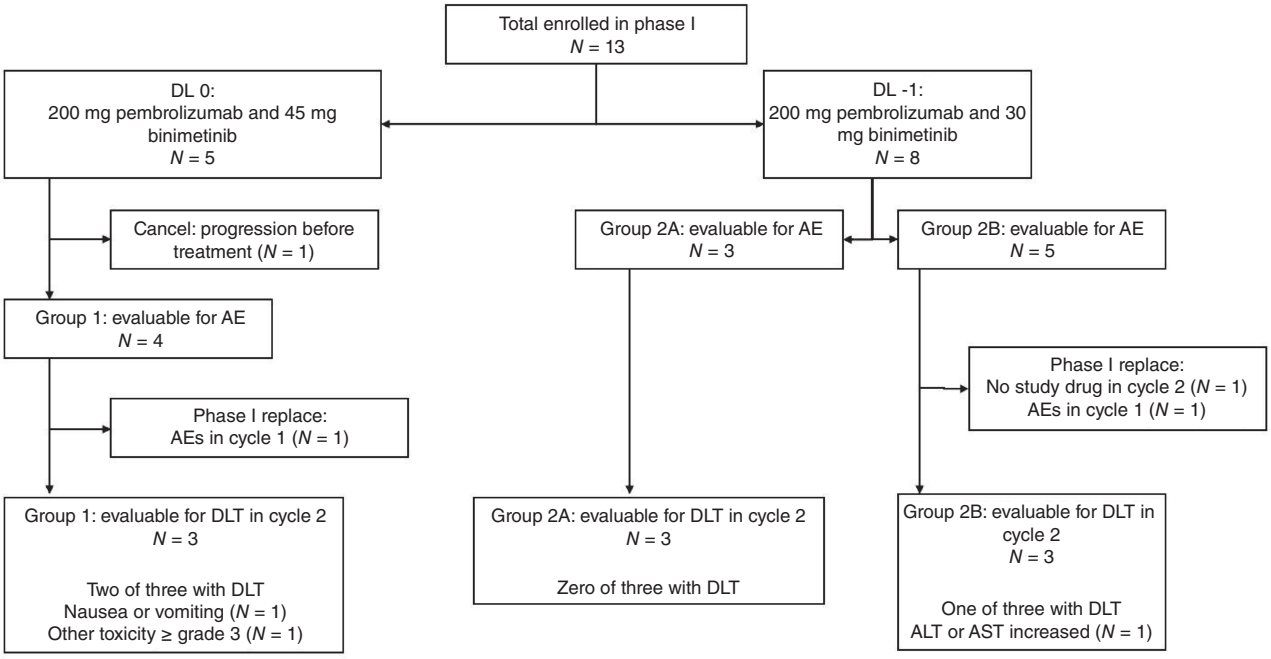

Figure 1.

Phase I CONSORT diagram showing the distribution of patients in the phase I portion of the trial. Thirteen patients were included in the phase I portion of this trial, including five patients treated at DL 0 and eight patients treated at DL 1. Four patients were evaluable for AE at DL 0, but one patient did not complete cycle 1 and was replaced. Two of three patients developed DLT. Therefore, eight subsequent patients were treated with DL −1. No DLT was observed in the first three-patient cohort (group 2A). Five additional patients were enrolled in DL −1 (group 2B). Two patients were not evaluable for DLT and were replaced. One of three patients in this group developed DLT.

18 patients were evaluated for the ORR. Overall, nine of the 22 patients (40.9%) had tumors with PD-L1 MPS ≥10, including three patients treated at DL 0 and six patients at DL −1. Among six patients treated in DL −1 and included in the phase II portion with PD-L1 positivity and evaluable ORR, both the ORR and CBR were 66.7%. However, one (8.3%) patient of 12 patients with PD-L1 MPS ≤10 tumors and evaluable ORR had an objective response, and two (16.7%) patients had clinical benefit ≥24 weeks. PD-L1 expression was significantly associated with ORR ($P = 0.009$) and CBR ($P = 0.03$) but not PFS ($P = 0.37$) and OS ($P = 0.18$). PD-L1 expression by ORR is shown in **Fig. 3A**.

TILs

sTILs were centrally assessed in mandatory biopsy or archival samples within 90 days prior to the enrollment. sTIL quantification was available in 19 patients with three missing cases in DL −1. sTILs were scored as 0, 1+, 2+, and 3+. Overall, sTILs were not significantly associated with any of the endpoints, including ORR ($P = 0.23$), CBR ($P = 0.17$), PFS ($P = 0.16$), and OS ($P = 0.60$). sTIL according to ORR is as shown in **Fig. 3B**. One of the patients with sTILs zero had progressive disease. In contrast, numerically, more ORR was observed in patients with sTILs 3+, with three of five patients with ORR and one additional patient with SD, which corresponds to four of six patients (66.7%) with CBR. Intriguingly, the patient with a PD-L1–negative tumor (MPS ≤10) who achieved an ORR had low TILs with 1+.

CTCs

Enumeration of CTCs and the measurement for both PD-L1 and p-ERK in CTCs were performed. However, the majority of samples (18 of 21 patients, 85.7%) had no detectable CTCs at baseline after subsequent treatment. Only two patients had no detectable CTCs at baseline, but they became detectable after treatment. One of the patients developed progressive disease, and the other patient had a PR. Due to a small number of detectable CTCs overall, we were unable to perform meaningful statistical analysis with CTC because of the inability to compare changes during the treatment.

CAMLs PD-L1 and p-ERK

In contrast to CTCs, circulating CAMLs are detectable in the majority of cases. A total of 19 of 21 patients (90.5%) had ≥1 CAMLs detected at baseline. We further evaluated blood-based biomarkers with mean PD-L1 expression in CAMLs as well as CAML count and size. Baseline mean CAML count ($P = 0.71$), baseline CAML size ($P = 0.52$), baseline PD-L1 expression in CAML ($P = 0.06$), decrease in CAML count after cycle one ($P = 0.40$), decrease in CAML size after cycle one ($P = 0.23$), and decrease in PD-L1 expression in CAML expression after cycle one ($P = 0.17$) were not significantly associated with CBR. Any decrease in CAML count ($P = 0.01$) and any decrease in CAML size ($P = 0.009$) were associated with longer OS. No significant differences were observed in PFS. PD-L1 expression in CAML was not associated with PD-L1 expression in archival tissue (Spearman $\rho = 0.13$).

For p-ERK in CAMLs, only a decrease in p-ERK expression in CAMLs ($P = 0.04$) was significantly associated with CBR. No association was identified between CBR and any of the following: decrease in CAML size ($P = 0.33$), decrease in CAML mean expression ($P = 0.09$), baseline CAML count ($P = 0.94$), baseline CAML size ($P = 0.94$), or baseline CAML mean expression ($P = 0.06$). No associations were also identified for PFS (decreased

Table 1. Patient characteristics.

Characteristic	DL 0 (*N* = 4)	DL −1/phase II (*N* = 18)	Total (*N* = 22)
Age			
N	4	18	22
Mean (standard deviation)	63.3 (6.5)	57.1 (11.3)	58.2 (10.8)
Median	63.4	57.6	58.4
Range	57.3–69.0	37.2–77.8	37.2–77.8
Race, *n* (%)			
Black or African American	3 (75.0%)	4 (22.2%)	7 (31.8%)
Not reported: patient refused or not available	0 (0.0%)	1 (5.6%)	1 (4.5%)
Unknown: patient unsure	0 (0.0%)	2 (11.1%)	2 (9.1%)
White	1 (25.0%)	11 (61.1%)	12 (54.5%)
Ethnicity, *n* (%)			
Hispanic or Latino	0 (0.0%)	1 (5.6%)	1 (4.5%)
Not Hispanic or Latino	4 (100.0%)	16 (88.9%)	20 (90.9%)
Unknown: patient is unsure of their ethnicity	0 (0.0%)	1 (5.6%)	1 (4.5%)
Gender, *n* (%)			
Female	4 (100.0%)	18 (100.0%)	22 (100.0%)
Menopausal status, *n* (%)			
Postmenopausal	4 (100.0%)	14 (77.8%)	18 (81.8%)
Premenopausal	0 (0.0%)	4 (22.2%)	4 (18.2%)
ECOG PS, *n* (%)			
0	3 (75.0%)	17 (94.4%)	20 (90.9%)
1	1 (25.0%)	1 (5.6%)	2 (9.1%)
Prior surgery, *n* (%)			
No	1 (25.0%)	3 (16.7%)	4 (18.2%)
Yes	3 (75.0%)	15 (83.3%)	18 (81.8%)
Prior RT, *n* (%)			
No	1 (25.0%)	5 (27.8%)	6 (27.3%)
Yes	3 (75.0%)	13 (72.2%)	16 (72.7%)
Prior neoadjuvant Rx, *n* (%)			
No	3 (75.0%)	10 (55.6%)	13 (59.1%)
Yes	1 (25.0%)	8 (44.4%)	9 (40.9%)
Prior metastatic Rx, *n* (%)			
No	4 (100.0%)	10 (55.6%)	14 (63.6%)
Yes	0 (0.0%)	8 (44.4%)	8 (36.4%)

Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status; RT, radiation; Rx, treatment.

CAML count $P = 0.48$, decreased CAML size $P = 0.46$, and decreased CAML mean expression $P = 0.70$) and OS (decreased CAML count $P = 0.10$ and decreased CAML mean expression $P = 0.60$), except reduction in CAML size after treatment is associated with significantly improved OS ($P = 0.04$).

Overall, among 21 patients with CAMLs in both pre- and post-treatment samples, 11 patients had increased PD-L1 expressions, and seven patients had decreased p-ERK in CAMLs after starting on binimetinib. Intriguingly, four of six patients with clinical benefit had either increased PD-L1 or decreased p-ERK expressions.

Discussion

ICIs have become an integral part of the treatment of TNBC. However, single-agent ICI only had modest activity in TNBC. The previous phase III KEYNOTE-119 trial (6) demonstrated no significant improvement in OS with single-agent pembrolizumab compared with chemotherapy. Furthermore, the median OS in the overall population was merely 9.9 months in the single-agent pembrolizumab group. Even among patients with a MPS score of ≥10, the median OS for pembrolizumab was 12.7 months.

Table 2. ORR and CBR by the number of prior lines of therapy.

	Number of patients	Number of patients with ORR	Number of patients with CBR
Treatment naïve	5	2 (40.0%)	2 (40.0%)
Prior neoadjuvant/adjuvant	8	2 (25.0%)	2 (25.0%)
Prior adjuvant	10	4 (40.0%)	5 (50.0%)
First line in the metastatic setting	10	5 (50.0%)	6 (60.0%)
≥1 line in the metastatic setting	8	0 (0.0%)	0 (0.0%)

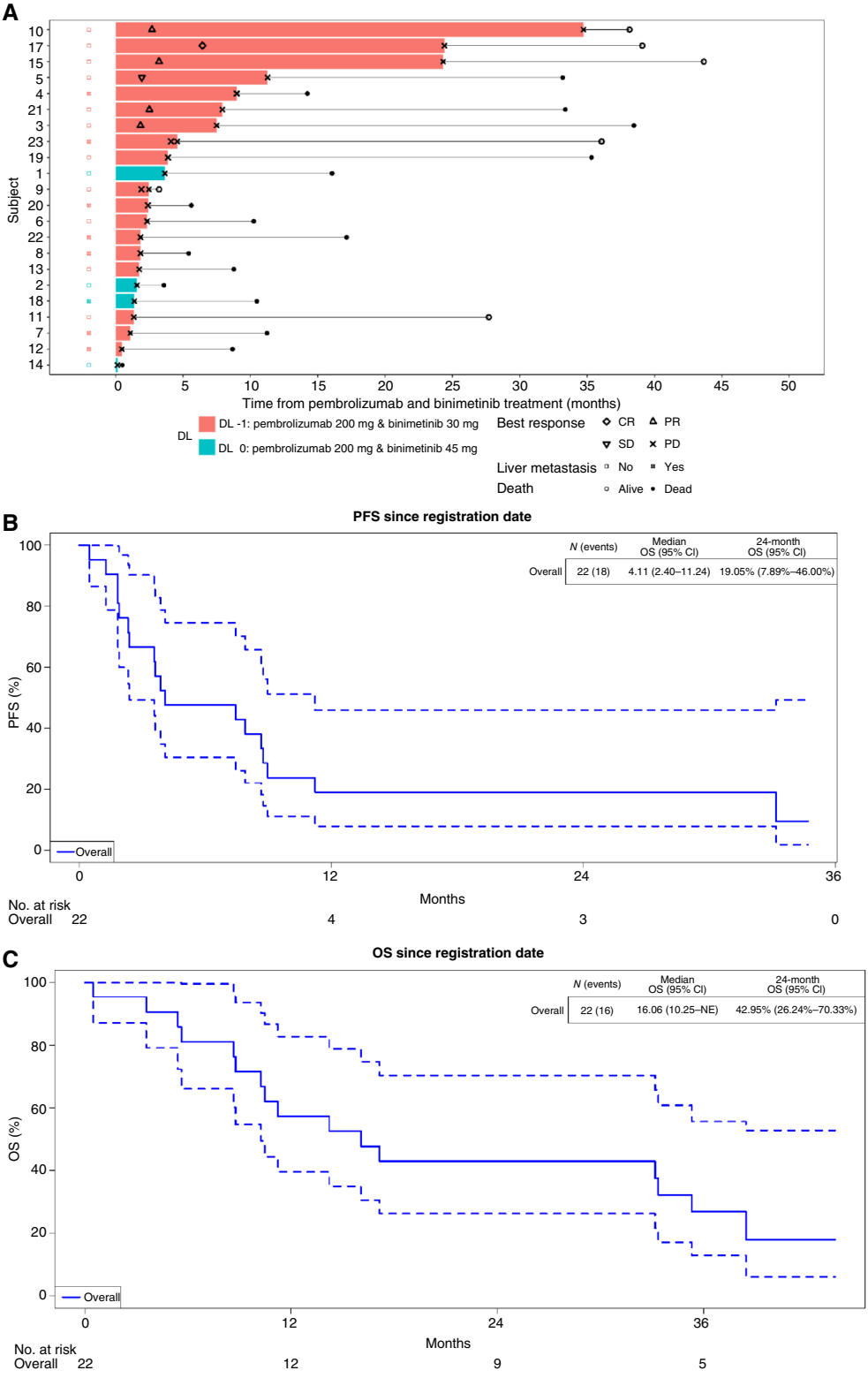

Figure 2.
Outcomes of patients treated with pembrolizumab in combination with binimetinib. **A,** Swimmer plot of tumor burden changes over time with pembrolizumab in combination with binimetinib. CR, complete response; PD, disease progression. **B,** Kaplan–Meier curve of PFS among evaluable patients. **C,** Kaplan–Meier curve of OS among evaluable patients.

Currently, the standard-of-care therapy for patients with metastatic PD-L1–positive TNBC is pembrolizumab in combination with chemotherapy. This is based on the phase III KEYNOTE-355 trial, which demonstrated that the pembrolizumab–chemotherapy group had significant improvements in both PFS (18) and OS (3) compared with the placebo–chemotherapy group. However, the median

Table 3. Twelve most common AEs in cycle 1 with single-agent binimetinib ($n = 22$).

	Grade 1		Grade 2		Grade 3		Grade 4		Total	
AE	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**
Peripheral sensory neuropathy	9	40.9	2	9.1					11	50.0
Blood and lymph system disorders	8	36.4							8	36.4
Fatigue	7	31.8	1	4.5					8	36.4
Nausea	6	27.3	1	4.5					7	31.8
Rash acneiform	7	31.8							7	31.8
Constipation	6	27.3							6	27.3
Diarrhea	6	27.3							6	27.3
Alkaline phosphatase increased	5	22.7							5	22.7
AST increased	4	18.2			1	4.5			5	22.7
Cardiac troponin T increased	5	22.7							5	22.7
Hypertension	1	4.5	1	4.5	3	13.6			5	22.7
Papulopustular rash	3	13.6	1	4.5	1	4.5			5	22.7

PFS of patients receiving pembrolizumab in combination with chemotherapy was merely 9.7 months (18) and the median OS was 23 months (3), even in patients with PD-L1 MPS ≥10 tumors.

Our study demonstrated the promising efficacy of pembrolizumab in combination with binimetinib with a median OS of 33.2 months (95% CI, 11.2–NE) in patients who received the RP2D. More remarkably, the responses are very durable, with four of five patients with ORR, including a patient with brain metastasis, having no evidence of disease lasting up to 6 years even after stopping all treatments. Furthermore, the combination of binimetinib and pembrolizumab seemed to have more favorable side effect profiles. Compared with chemotherapy in combination with pembrolizumab, binimetinib and pembrolizumab have relatively less frequent severe toxicity with grade ≥3 AEs at least possibly related occurring in 54.5% of patients compared with 78% in the KEYNOTE-355 trial (3). Furthermore, no grade 4 or 5 treatment-related AE was observed in this study compared with the 0.4% treatment-related deaths seen in the KEYNOTE-355 trial (3).

In line with observations from other solid malignancies, in which ICI therapies demonstrate higher response rates in the first-line setting, our findings underscore the potential benefits of this combination therapy in the first-line metastatic setting. All patients in our study who achieved ORR or SD received the combination of pembrolizumab and binimetinib as first-line treatment. The observed ORR of 50% and CBR of 60% in this trial compare favorably with the outcomes reported for pembrolizumab monotherapy in the KEYNOTE-086 cohort B trial (5), in which previously untreated patients with TNBC with PD-L1–positive disease demonstrated an ORR of 21.4% and a CBR of 23.8%. These results suggest that adding binimetinib to pembrolizumab may enhance therapeutic efficacy, supporting further investigation of this combination in the first-line metastatic setting.

Based on several previous studies showing poor responses to ICIs in patients with advanced solid malignancies with liver metastases (19–22), we further evaluated the ORR in patients with and without liver metastases. The preclinical study demonstrated that liver metastases sequestered systemic $CD8^+$ T cells, resulting in systemic immune desert and diminishing immunotherapy efficacy (19). In accordance with prior studies, our analysis reveals a lack of ORR among patients harboring liver metastases ($n = 5$) with the combination of pembrolizumab and binimetinib. In contrast, individuals without liver metastases had an ORR of 45.5%, with a CBR of 54.5%. However, our study has limitations, particularly with the small sample size and being a single-arm trial. Nevertheless, our study underscores the promise of the proposed therapeutic approach, particularly in patients lacking liver metastases, warranting

Table 4. Ten most common AEs in cycle 2 and beyond with the combination of pembrolizumab and binimetinib ($n = 22$).

	Grade 1		Grade 2		Grade 3		Grade 4		Total	
AE	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**
Fatigue	10	45.5	6	27.3	1	4.5			17	77.3
Anemia	9	40.9	6	27.3					15	68.2
Blood and lymph system disorders	13	59.1	1	4.5	1	4.5			15	68.2
AST increased	11	50.0	1	4.5	1	4.5			13	59.1
Nausea	11	50.0			2	9.1			13	59.1
Diarrhea	7	31.8	5	22.7					12	54.5
Cardiac troponin T increased	10	45.5			1	4.5			11	50.0
CPK increased	7	31.8	4	18.2					11	50.0
Peripheral sensory neuropathy	8	36.4	1	4.5	1	4.5			10	45.5
Fatigue	10	45.5	6	27.3	1	4.5			17	77.3
Anemia	9	40.9	6	27.3					15	68.2

Abbreviation: CPK, creatine phosphokinase.

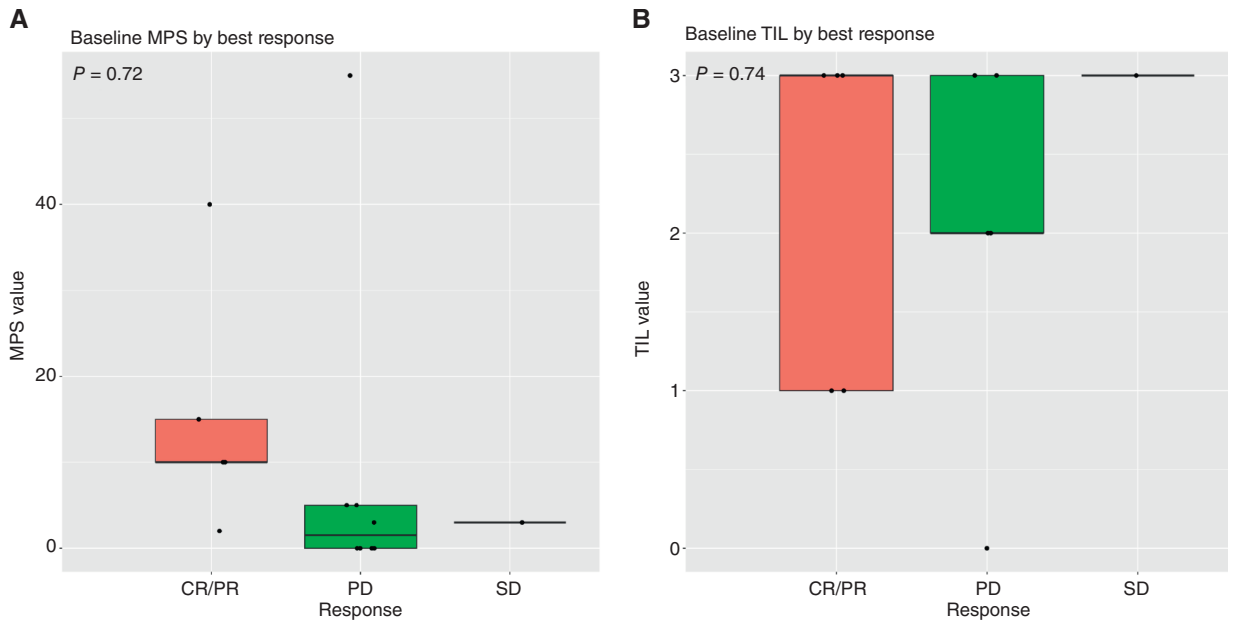

Figure 3.

A, Box plot of PD-L1 expression in the baseline tumor tissue according to ORR showing higher PD-L1 expression in patients who achieved complete response or PR. **B,** Box plot of sTILs in the baseline tumor tissue according to ORR showing no significant association between sTILs and ORR. CR, complete response; PD, disease progression.

further investigation and consideration in clinical management strategies.

As observed in the KEYNOTE-119 trial (6) and KEYNOTE-355 trials (18), patients with PD-L1–positive disease are more likely to have ORR or clinical benefit. However, consistent with the preclinical studies, which showed that MEK inhibitor upregulates PD-L1 expression (7, 8), clinical benefits were observed with this combination in patients with PD-L1–negative disease, with one (8.3%) patient of 12 patients with PD-L1–negative tumors and evaluable ORR having an objective response and two (16.7%) patients having clinical benefits ≥ 24 weeks. Furthermore, among 21 patients with CAMLs in both pre- and posttreatment samples, 11 patients had increased PD-L1 expressions in CAMLs after starting on binimetinib. A reduction in p-ERK expression in CAML was also significantly associated with CBR. Moreover, four of six patients with clinical benefit had either increased PD-L1 or decreased p-ERK expressions. These results highlight the possibility of using this blood-based assay as a pharmacodynamic marker to evaluate the biological effects of MEK inhibitors in tumor cells without requiring invasive biopsies. However, future studies are needed to further evaluate this assay as a predictive biomarker.

In summary, pembrolizumab in combination with binimetinib at 30 mg is safe with manageable toxicities. Promising activity with durable responses was observed, particularly in patients without liver metastases. Consistent with the preclinical data, increased PD-L1 and decreased p-ERK expressions were observed in peripheral blood CAML. Larger clinical trials are warranted to further evaluate the efficacy of this chemotherapy-free combination, particularly in patients without liver metastases.

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Authors' Contributions

S. Chumsri: Conceptualization, resources, data curation, supervision, funding acquisition, validation, investigation, methodology, writing–original draft, project administration, writing–review and editing. **J.J. Larson:** Data curation, software, formal analysis. **E. Liu:** Data curation, software, formal analysis. **K.S. Tenner:** Data curation, software, formal analysis. **D. Adams:** Resources, methodology, project administration. **M.T. Weidner:** Investigation, project administration. **A.N. Arnold:** Investigation, project administration. **D.L. Haley:** Investigation, project administration. **P. Advani:** Investigation, project administration. **K. Sideras:** Investigation, methodology, writing–review and editing. **A. Moreno-Aspitia:** Investigation, project administration, writing–review and editing. **E.A. Thompson:** Visualization, writing–review and editing. **E.A. Perez:** Conceptualization, funding acquisition, visualization, writing–review and editing. **K.L. Knutson:** Conceptualization, writing–review and editing.

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Note

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