PEARL: A Phase Ib/II Biomarker Study of Adding Radiation Therapy to Pembrolizumab Before Neoadjuvant Chemotherapy in Human Epidermal Growth Factor Receptor 2–Negative Breast Cancer

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ABSTRACT

PURPOSE To assess safety and immune biomarkers after preoperative radiation therapy (RT) and anti-PD1 therapy in breast cancer.

MATERIALS AND METHODS A phase I/IIb trial of pembrolizumab with RT was conducted in patients with triple-negative breast cancer (TNBC) and hormone receptor–positive/human epidermal growth factor receptor 2–negative (HR+/HER2−) breast cancer. All received pembrolizumab followed by a second cycle + RT (anti-PD1/RT) of 24 Gy/three daily fractions delivered to the breast tumor and then neoadjuvant chemotherapy (NAC). Blood and tumor biopsies were obtained at baseline, after anti-PD1, and after anti–PD-RT. Coprimary end points were safety and change in tumor-infiltrating lymphocytes (TILs). Secondary end points were pathologic complete response (pCR), residual cancer burden (RCB) rates, and event-free survival (EFS).

RESULTS Sixty-six patients with stage I-III breast cancer (54 TNBC, 12 HR+/HER2−) were enrolled. The median follow-up was 32 months. Safety end point was met. Incidence of grade ≥3 toxicities was 41%. The pCR rate was 59.2%, 33.3%, and 54.5% for the TNBC, HR+/HER2−, and entire cohort, respectively. A total of 77.8% of TNBC and 41.6% of HR+/HER2− had a near pCR (RCB 0-1). The 3-year EFS was 80%. In the entire cohort, PD-L1 expression increased after anti-PD1 (median Combined Positive Score [CPS], 7.49-23.20; 95% CI, −41.88 to −6.30; $P = .044$) and anti-PD1/RT (median CPS, 7.49-23.41; 95% CI, −41.88 to −6.30; $P = .009$), compared with baseline. In TNBC, adding RT to anti-PD1 significantly decreased TILs (28.9%-17.1%; 95% CI, 2.46 to 21.09; $P = .014$). Baseline TILs correlated with PD-L1 expression and TNF-a.

CONCLUSION Preoperative RT with pembrolizumab is safe and results in high pCR rates and 3-year EFS, despite the lack of pembrolizumab during NAC. PD-L1 and TILs may be predictive biomarkers for preoperative anti-PD1/RT response. Reduction in TILs after adding RT to anti-PD1 highlights the importance of treatment sequencing.

ACCOMPANYING CONTENT

- Appendix
- Data Sharing Statement
- Data Supplement
- Protocol

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INTRODUCTION

The phase III study KEYNOTE-522 demonstrated higher pathologic complete response (pCR) rates and 3-year event-free survival (EFS) when adding the anti–PD-1 antibody pembrolizumab to neoadjuvant chemotherapy (NAC) in early stage triple-negative breast cancer (TNBC), establishing neoadjuvant immuno-chemotherapy as the current standard of care.[1,2] However, 78% of patients experienced grade 3/4 adverse events (AEs) with this regimen.[2] Thus, effective treatments with less toxicity are needed.

Adding radiation therapy (RT) to immune checkpoint inhibitor (ICI) therapy can invigorate immune responses.[3-5] RT induces immunogenic cell death and increases the release of tumor-specific antigens that attract immune cells to the tumor microenvironment (TME) and upregulate PD-L1 expression.[6-11] Induction of immune costimulatory molecules with RT can further strengthen antitumor immunity.[12,13]

A previous study showed that combining RT with pembrolizumab in patients with pretreated, metastatic TNBC unselected for PD-L1 status was promising.[14] We decided to

CONTEXT

Key Objective

Is the combination of radiation therapy (RT) and immune checkpoint blockade safe and effective in patients with breast cancer receiving neoadjuvant chemotherapy (NAC)?

Knowledge Generated

Preoperative pembrolizumab and RT administered before NAC was associated with low toxicities and high rates of pathologic response that approach those of current, standard-of-care regimens where immunotherapy is administered throughout chemotherapy for triple-negative breast cancer.

Relevance *(J.P.S. Knisely)*

Clinical trials comparing immune checkpoint blockade during neoadjuvant RT or NAC appear warranted in women with high-risk triple-negative breast cancer, and may be appropriate in other high-risk cohorts as well.*

*Relevance section written by *JCO* Associate Editor Jonathan P.S. Knisely, MD.

test this concept in the curative-intent setting in the PEARL (preoperative pembrolizumab with RT) study, designed as a phase Ib/II trial in patients with breast cancer in whom NAC was the standard of care. We hypothesized that the window regimen of preoperative pembrolizumab and RT to the primary tumor could enhance responses to NAC without substantive delay. Serial tumor and blood samples were collected to elucidate biomarkers and interrogate changes after anti-PD1 therapy alone and anti–PD1-RT.

MATERIALS AND METHODS

Patients

Eligibility criteria included age ≥18 years, an Eastern Cooperative Oncology Group status of 0-1, and biopsy-proven, stage I-III TNBC (estrogen receptor [ER] and progesterone receptor [PR] <10% by immunohistochemistry [IHC] and human epidermal growth factor receptor 2 [HER2] negativity per ASCO-CAP guidelines[15]) or high-risk, hormone receptor–positive/HER2-negative (HR+/HER2−) breast cancer, defined as ER and/or PR >10% by IHC, and required at least two of the following criteria: histologic grade II-III, Ki-67 >20%, and ER <75% by IHC. The minimal tumor size was 2 cm. All patients were NAC candidates. Key exclusion factors included HER2+,T4d, metastatic disease, and factors precluding immunotherapy receipt.

Trial Design

PEARL was a prospective, single-arm, phase Ib/II trial evaluating the safety of pembrolizumab and preoperative RT in patients with operable, TNBC or high-risk, HR+/HER2− breast cancer with NAC planned. The clinical trial (ClinicalTrials.gov identifier: NCT03366844) and consent forms were approved by the Institutional Review Board of the Cedars-Sinai Medical Center (Los Angeles, CA).

Enrolled patients received two cycles of intravenous pembrolizumab (200 mg) once every 3 weeks. RT was administered within the first 3 days of cycle 2 (C2) of pembrolizumab. Patients received 24 Gy in three daily fractions to the primary breast tumor. Radiation treatment planning guidelines are outlined in the protocol.

NAC commenced 3 weeks after C2 of pembrolizumab. NAC regimens were selected by the treating physician. Preoperative MR was not required and performed as clinically indicated. Surgery consisted of lumpectomy or mastectomy with axillary surgery. Eligibility for breast-conserving therapy followed 2016 National Comprehensive Cancer Network guidelines.[16] Radiation consisted of whole-breast (42.4 Gy/16 fractions) with or without regional nodal irradiation (RNI). The dose for postmastectomy radiation or RNI was 50 Gy/1.8-2 Gy fractions. No patients received a postoperative boost.

Paired peripheral blood for circulating biomarker analyses and fresh breast tumor biopsies were obtained at three serial timepoints: baseline, after C1D1 of pembrolizumab (anti-PD1), and after C2D1 of pembrolizumab + RT/before initiation of NAC (anti-PD1/RT; Fig 1). Research biopsies were not performed if tumor was not visible on imaging.

Assessments

Response to treatment was quantified by residual cancer burden (RCB).[17,18] Patients who achieved pCR (ypTis/T0ypN0) were considered responders. Patients with documented clinical progression or inoperability were considered RCB-III. All AEs were scored using Common Terminology Criteria for Adverse Events version 4.03 and monitored at baseline, once every 3 weeks during NAC, and once every 6 months after surgery until 1 year poststudy. Tumor-infiltrating lymphocytes (TILs) were evaluated on fresh

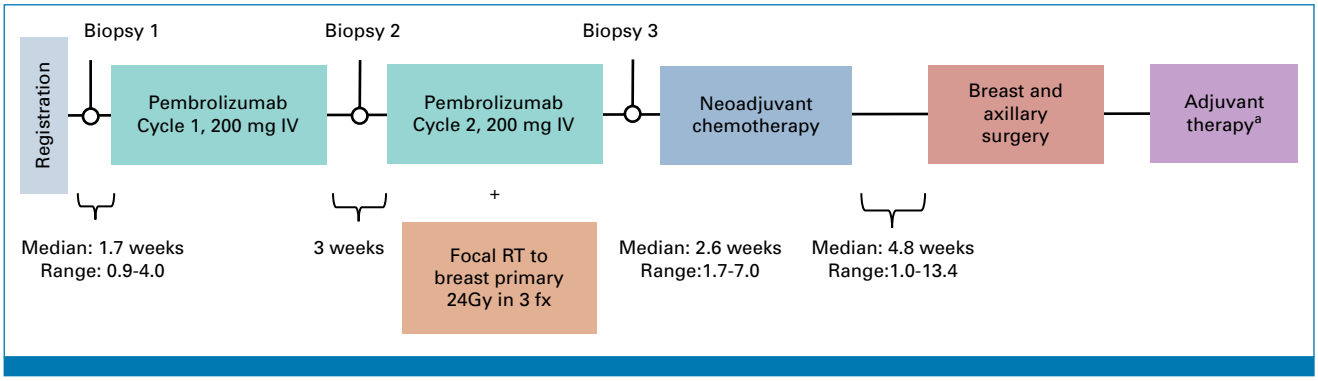

FIG 1. Study treatment and biopsy schema. [a]Adjuvant therapy included RT to the breast/chest wall and regional nodes, capecitabine for patients with TNBC, or endocrine therapy for patients with HR+/HER2− as per the standard of care. HER2−, human epidermal growth factor receptor 2−negative; HR+, hormone receptor−positive; RT, radiation therapy; TNBC, triple-negative breast cancer.

frozen tumor specimens according to the Salgado and International TIL Working Group criteria.[19,20]

The US Food and Drug Administration−approved 22C2 pharmDx companion diagnostic assay was used to determine the Combined Positive Score (CPS). A CPS of ≥1 was defined as PD-L1−positive.[1] Serum biomarkers were measured using multiplexed protein array kits (MesoScale Discovery). The panel included angiogenesis biomarkers, proinflammatory cytokines, and chemokines previously measured in a Clinical Laboratory Improvement Amendments−certified core facility at Massachusetts General Hospital.[21-30]

Statistical Analysis

The coprimary end points of phase Ib were (1) safety, defined by the number of patients who initiated NAC >4 weeks from anti-PD1/RT, and (2) change in mean % TILs from baseline to treatment(s). An intention-to-treat approach was used. A total of 10 patients with TNBC were enrolled in phase Ib to establish safety and immune efficacy. Using a Bayesian sequential design, the trial monitored the true probability (P_d) of delays to NAC. If P_d exceeded 20%, the trial would have been halted. The type I error probability was .05.

The sample size for the phase II component was powered for the end point of pCR in TNBC.[31] Data from 50 evaluable patients with TNBC were estimated to achieve an 80% power to detect an effect size of 1.20, using a two-sided paired *t*-test at the 0.05 significance level, compared with the historical pCR rate of 33% with NAC.[32,33]

The median follow-up was calculated from the start of anti-PD1 to the last clinical follow-up or the date of the event. The Kaplan-Meier method estimated EFS. Changes in PD-L1 expression, TILs, and circulating biomarkers were evaluated using *t*-tests. Comparison between treatment response groups and subtypes was evaluated using the Kruskal-Wallis test. Cox proportional hazard models assessed these variables with time-to-event data. A logistic regression model evaluated biomarkers (at the univariate level) and pCR status. All *P* values are two-sided and were considered significant if <.05. Because of the exploratory nature of this analysis, we did not correct the *P* values for multiple comparisons for the primary end points. For the exploratory circulating biomarker analyses, Benjamini-Hochberg false discovery rate-adjusted *P* values were reported. Spearman's correlation tests assessed the magnitude of associations between biomarker expression at each biopsy timepoint.

RESULTS

Baseline Characteristics

Between December 22, 2017, and December 6, 2021, 85 patients were screened and 66 (54 TNBC, 12 HR+/HER2−) were enrolled (Fig 2). Eight patients had research biopsies that were insufficient for analysis. One patient with TNBC had biopsies of two different tumors, leaving 57 patients and 58 tumors (49 triple negative, nine HR+/HER2−) for analysis. The median age was 53 years (range, 26-94). The majority (84%) presented with primary breast tumors ≤5 cm. Approximately 42.4% was cN+, and 96.9%% had anatomic stage II-III disease. Most (79%) of the patients received an anthracycline and taxane−containing regimen. Thirty-five percent of TNBC received the KEYNOTE-522 chemotherapy regimen. Fifty-one received mastectomy, and 49% received breast-conserving surgery. A total of 42.4% had immediate reconstruction (27 implant-based, one autologous). Nearly all patients received axillary staging. Of the 57 patients with evaluable biopsies, 78.9% was PD-L1+ (81.3% TNBC, 66.7% HR+/HER2−). Nearly half (47%) of the TNBC cohort received RNI or PMRT. Approximately 34% received adjuvant systemic therapy. Further details are given in Table 1.

Efficacy and Clinical Outcomes

The median follow-up in the entire cohort was 32 months (range, 11-60). The median time interval (range) between treatments is shown in Figure 1. In the entire cohort, 54.5% (36 of 66) achieved a pCR. At the time of data lock (6/30/

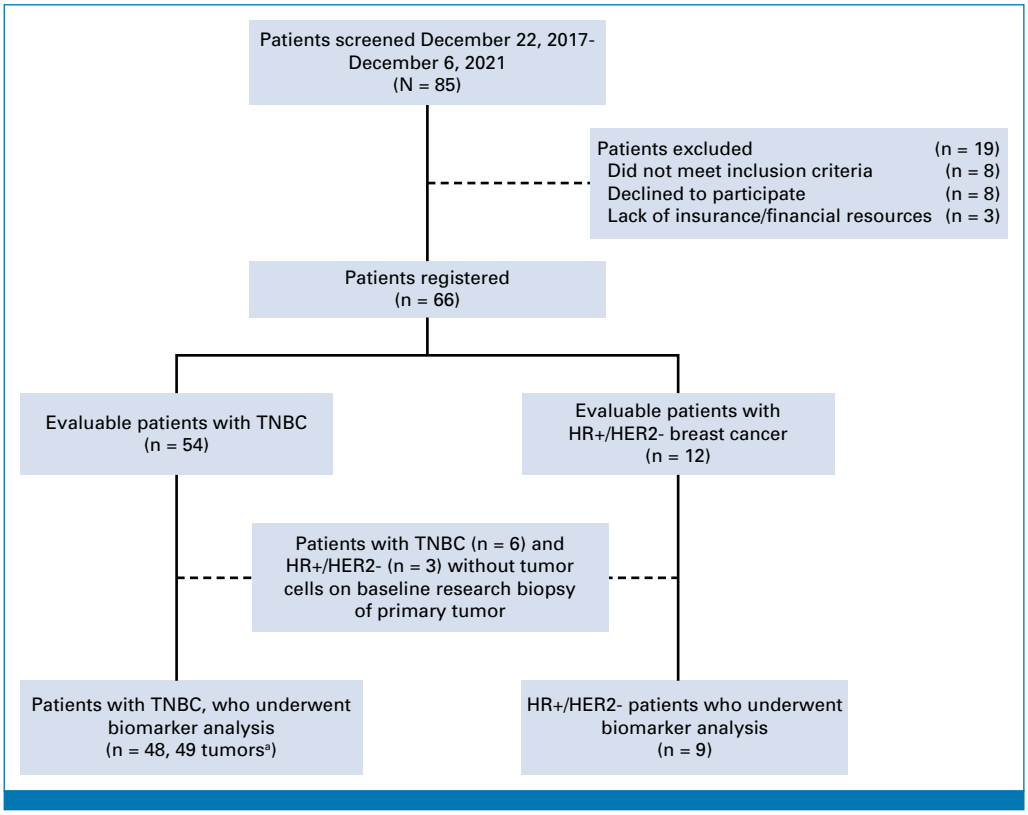

FIG 2. CONSORT diagram. [a]One patient with TNBC had two separate tumors biopsied. HER2–, human epidermal growth factor receptor 2–negative; HR+, hormone receptor–positive; TNBC, triple-negative breast cancer.

2023), eight patients (12.3%, all TNBC) had disease recurrence (four isolated local, four distant). Among them, six had residual disease (one RCB-II, five RCB-III). While one of the four patients with distant recurrences had achieved pCR, none with local recurrences had achieved pCR. The 3-year EFS was 80% for the entire cohort (Fig 3).

In the TNBC cohort, 59.2% (32 of 54) achieved pCR. Ten patients had RCB-I, of whom only one experienced a distant recurrence event. A total of 77.8% of patients with TNBC had a near pCR defined as RCB 0 or I. In the TNBC group, 18 had clinically node-positive disease (17 cN1, 1cN3); among them, 15 became ypN0, whereas the three who remained node-positive after NAC all had ypN1 disease. Given our broad definition of TNBC of ER <1%-10%, the association between pCR and ER levels, <1% (n = 20) versus 1%-10% (n = 34), was evaluated but was not significant (P = .78). In the HR+/HER2– cohort, four (33.3%) achieved pCR. Eight patients did not achieve pCR (one RCB-I, one RCB-II, six RCB-III). Only one patient presented with cN0 disease, and seven were cN+ (six cN1, one cN2).

Safety and Toxicities

The primary feasibility end point was met, with only two (3%) patients experiencing delays >4 weeks in initiating NAC (95% CI 0.37%, 10.52%). The incidence, severity, and types of AEs were comparable or lower, relative to those reported in KEYNOTE-522. Twenty-seven patients (40.9%) had grade ≥3 toxicities. Among them, nine (13.6%) had grade 3 toxicities probably or related to pembrolizumab and/or preoperative RT (Table 2). Three patients had grade 4 toxicities, but they were not attributed to either pembrolizumab or RT. Among the 44% who underwent immediate reconstruction, none experienced a reconstruction failure.

Changes in PD-L1 Expression in the Entire Cohort and by Subtype

Changes in PD-L1 expression across the biopsy timepoints are demonstrated in the entire cohort, by subtype and treatment response (Fig 4). Compared with baseline, PD-L1 expression increased after anti-PD1 (median CPS, 7.49-23.20; 95% CI, −26.56 to −0.61; P = .040) and after anti-PD1/RT (median CPS, 7.49-23.41; 95% CI, −41.88 to −6.30; P = .009; Fig 4A). Between anti-PD1 and anti-PD1/RT, there was a nonsignificant change in PD-L1 expression (median CPS, 23.2-20.67; 95% CI, −29.88 to 8.87; P = .28).

In TNBC, PD-L1 expression increased after both anti-PD1and anti-PD1/RT (median CPS, 8.46-23.39; 95% CI, −32.85 to −2.37; P = .024 and median CPS, 8.65-26.15; 95% CI, −46.61

TABLE 1. Patient Characteristics

Baseline Characteristic	Total Cohort (n = 66)	TNBC (n = 54)	HR+/HER2− (n = 12)	P[a]
Age at diagnosis, years				
Median (range)	53 (26-94)	56 (26-94)	42.5 (28-66)	.0103 (2.73-19.62)
<65 years, No. (%)	52 (78.8)	40 (74.1)	11 (91.7)	.270
Race, No. (%)				.915
White	48 (72.7)	39 (72.2)	9 (75.0)	
Black	8 (12.1)	7 (13.0)	1 (8.3)	
Asian	8 (12.1)	6 (11.1)	2 (16.7)	
Other	2 (3.0)	2 (3.7)	0	
Ethnicity, No. (%)				.074
Non-Hispanic	56 (84.8)	48 (88.9)	8 (66.7)	
Hispanic or Latino	10 (15.2)	6 (11.1)	4 (33.3)	
ECOG performance status, No. (%)				1.000
0	60 (90.9)	49 (90.7)	11 (91.7)	
1	6 (9.1)	5 (9.3)	1 (8.3)	
Tumor histology, No. (%)				1.000
Invasive ductal	61 (92.4)	49 (90.7)	12 (100.0)	
Invasive lobular	3 (4.5)	3 (5.6)	0	
Other[b]	2 (3.0)	2 (3.7)	0	
Clinical T-stage, No. (%)				**.025**
T1-T2	55 (83.3)	48 (88.9)	7 (58.3)	
T3-T4	11 (16.7)	6 (11.1)	5 (41.7)	
Clinical N-stage, No. (%)				**.011**
N0	38 (57.6)	35 (64.8)	3 (25.0)	
N1-N2	25 (37.9)	18 (33.3)	7 (58.3)	
N3-N4	3 (4.5)	1 (1.9)	2 (16.7)	
Clinical stage, No. (%)				**.009**
Stage I	2 (3.0)	1 (1.9)	1 (8.3)	
Stage II	56 (84.8)	49 (90.7)	7 (58.3)	
Stage III	8 (12.1)	4 (7.4)	4 (33.3)	
RCB, No. (%)				.0164
0	36 (54.5)	32 (59.3)	4 (33.3)	
1	11 (16.7)	10 (18.5)	1 (8.3)	
2	8 (12.1)	7 (13.0)	1 (8.3)	
3	11 (16.7)	5 (9.3)	6 (50.0)	
Ki-67 score				.2581
Median (%)	51.5 (5-98)	54 (5-98)	42.5 (10-90)	−26.8 to 7.14
≤15%, No. (%)	5 (7.6)	3 (5.6)	2 (16.7)	.2735
>15%, No. (%)	53 (80.3)	43 (79.6)	10 (83.3)	
Baseline PD-L1 status,[c] No. (%)				.3796
Positive	45 (68.2)	39 (72.2)	6 (50.0)	
Negative	12 (18.2)	9 (16.7)	3 (25.0)	
Unknown	9 (13.6)	6 (11.1)	3 (25.0)	

Treatment Characteristic	Total Cohort (n = 66)	TNBC (n = 54)	HR+/HER2− (n = 12)	P
Neoadjuvant chemotherapy, No. (%)				**.014**
T	1 (1.5)	1 (1.9)	0	
TP	9 (13.6)	9 (16.7)	0	
TC	2 (3.0)	0	2 (16.7)	
AC	1 (1.5)	1 (1.9)	0	

(continued on following page)

TABLE 1. Patient Characteristics (continued)

Treatment Characteristic	Total Cohort (n = 66)	TNBC (n = 54)	HR+/HER2− (n = 12)	*P*
Doxorubicin + taxane + cyclophosphamide[c]	32 (48.5)	23 (42.6)	9 (75.0)	
Doxorubicin + taxane + cyclophosphamide + carboplatin[d]	20 (30.3)	19 (35.2)	1 (8.3)	
Other (CMF)	1 (1.5)	1 (1.9)	0	
Surgery, No. (%)				1.000
Mastectomy	34 (51.5)	28 (51.9)	6 (50.0)	
Lumpectomy	32 (48.5)	26 (48.1)	6 (50.0)	
Axillary surgery, No. (%)				**.012**
SLNB	42 (63.6)	38 (70.4)	4 (33.3)	
ALND	23 (34.8)	16 (29.6)	7 (58.3)	
Neither	1 (1.5)	0	1 (8.3)	
Adjuvant systemic therapy, No. (%)				
Immunotherapy[e]	2 (3.0)	2 (3.7)	0	**<.0001**
Chemotherapy[f]	4 (6.1)	4 (7.4)	1 (8.3)	
Capecitabine	15 (22.7)	12 (22.2)	3 (25.0)	
AR inhibitor	1 (1.5)	1 (1.8)	0	
Hormone therapy[g]	12 (18.1)	4 (7.4)	9 (75.0)	
CDK 4/6 inhibitor[h]	1 (1.5)	0	1 (8.3)	
Adjuvant radiation therapy, No. (%)				**.025**
Postmastectomy + RNI	19 (28.8)			
Breast + RNI	12 (18.2)			
Breast only	23 (34.8)			
None	12 (18.2)			

NOTE. Bold entries indicate statistical significance ($P \leq .05$).
Abbreviations: AC, doxorubicin + cyclophosphamide; ALND, axillary lymph node dissection; AR, androgen receptor inhibitor enzalutamide; CMF, cyclophosphamide, methotrexate, and fluorouracil; CPS, Combined Positive Score; ECOG, Eastern Cooperative Oncology Group; HER2−, human epidermal growth factor receptor 2–negative; HR+, hormone receptor–positive; IHC, immunohistochemistry; MF, methotrexate and fluorouracil; RCB, residual cancer burden; RNI, regional nodal irradiation; SLNB, sentinel lymph node biopsy; T, taxane only; TC, taxane + cyclophosphamide; TNBC, triple-negative breast cancer; TP, taxane + carboplatin only.
[a]The nonparametric *P* value is calculated using the Kruskal-Wallis test for numerical covariates and Fisher's exact test for categorical covariates.
[b]Other includes invasive metaplastic features (n = 2).
[c]PD-L1–positive status defined as CPS ≥1 using the PD-L1 IHC 22C3 pharmDx assay (Agilent Technologies, 2020). ACT or TAC or TC-T-AC or AC-TC or TC-AC.
[d]AC-TP, TP-AC, or TC-AP.
[e]Pembrolizumab or ipilimumab + nivolumab.
[f]Carboplatin, CMF, MF or dose-dense AC.
[g]Palbociclib.
[h]Chi^2 test.

to −4.04; $P = .021$, respectively; Fig 4B), compared with baseline. By contrast, there was no significant change with the addition of RT to anti-PD1 in TNBC (median CPS, 23.39-26.15; 95% CI, −30.91 to 15.47; $P = .51$).

In HR+/HER2−, no changes between biopsy timepoints reached significance (median CPS, 1.72-5.82 from baseline to anti-PD1 and median CPS, 5.82-20.67 from baseline to anti-PD1/RT).

Changes in PD-L1 Expression by Treatment Response

In the entire cohort with baseline biopsies (n = 57), 83.9% (26 of 31) of responders were PD-L1+, relative to non-responders (73.1%, 19 of 26). There was no change in either responders or nonresponders between the biopsy timepoints. In responders, there was a significant increase in PD-L1 expression between baseline and anti-PD1/RT (median CPS, 9.31-34.78; 95% CI, −57.64 to −3.16; $P = .031$; Fig 4C). PD-L1 expression was significantly associated with TILs at baseline and after anti-PD1 (both $P < .01$).

Longitudinal Assessment of TILs

In cases where TILs were evaluable with baseline biopsies (n = 57), the change in mean TILs after anti-PD1 was not significant (20.5%-24.4%; 95% CI, −12.95 to 5.16; $P = .40$; Fig 5A). However, after the addition of RT to anti-PD1, the mean TILs decreased significantly (24.4%-15.6%; 95% CI, 0.93 to 16.79; $P = .029$), compared with anti-PD1 alone.

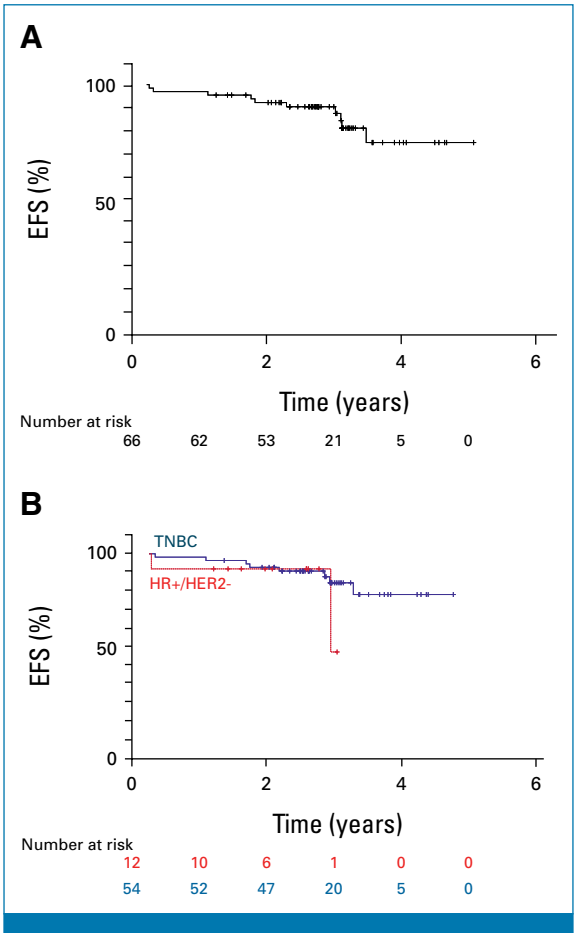

FIG 3. Kaplan-Meier curve for EFS for (A) the entire cohort (n = 66) and (B) by subtype (TNBC *v* HR+/HER2−). EFS, event-free survival; HER2−, human epidermal growth factor receptor 2−negative; HR+, hormone receptor−positive; TNBC, triple-negative breast cancer.

TABLE 2. Treatment-Related AEs

AE	n = 66, No. (%)		
	Any Grade	Grade 3	Grade 4
Any AE	57 (86.4)	24 (36.4)	3 (4.5)
Treatment-related AE[a]	53 (80.3)	9 (13.6)	0
Fatigue	26 (39.4)	0	0
Rash/pruritis	26 (39.4)	0	0
Diarrhea	13 (19.7)	1 (1.5)	0
Immune-related AE	19 (28.7)	7 (10.6)	0
Hypothyroidism/elevated TSH	8 (12.1)	1 (1.5)	0
Adrenal insufficiency	4 (6.1)	3 (4.5)	0
Transaminitis	4 (6.1)	2 (3.0)	0
Generalized muscle weakness, myalgia	4 (6.1)	0	0
Pneumonitis	3 (4.5)	2 (3.0)	0
Arthralgia	3 (4.5)	0	0
Colitis	2 (3.0)	1 (1.5)	0
SIADH/hyponatremia	2 (3.0)	1 (1.5)	0
Hyperthyroidism	2 (3.0)	0	0
Infusion-related reaction	1 (1.5)	0	0

Abbreviations: AE, adverse event; SIADH, syndrome of inappropriate antidiuretic hormone secretion; TSH, thyroid-stimulating hormone.
[a]Treatment-related AEs experienced by ≥10% of participants are reported in the table.

Compared with baseline, the mean TIL score in TNBC did not significantly increase after anti-PD1 (22.6%-28.9%; 95% CI, −16.67 to 4.09; $P = .23$). However, the addition of RT to anti-PD1 led to a significant decrease in mean TILs in TNBC, compared with anti-PD1 alone (28.9%-17.1%; 95% CI, 2.46 to 21.09; $P = .014$). In the HR+/HER2− cohort, TIL changes were not significant.

Impact of Biomarker Analyses With Paired Samples

The number of tumors that could be biopsied decreased at each subsequent timepoint after treatment. To address this, paired analyses between timepoints were performed to complement the unpaired analyses above. The results were confirmatory: (1) expression of PD-L1 increased after anti−PD-1 and anti-PD1/RT, compared with baseline ($P < .05$; Appendix Fig A4, online only) and (2) TILs decreased after the addition of RT to anti-PD1 ($P = .004$; Appendix Fig A5), but otherwise remained unchanged between timepoints.

Dynamics of Circulating Blood Biomarkers

The patterns of circulating proinflammatory markers, angiogenesis markers, and chemokines after anti-PD1 treatment alone and anti-PD1/RT treatment in the entire cohort are shown and reported in Appendix Figures A1-A3. Only vascular endothelial growth factor was significantly higher in the HR+/HER2− cohort, compared with the TNBC cohort across all time points (baseline $P = .0$; anti-PD1 $P < .001$ and anti-PD1/RT $P = .003$). No circulating blood biomarker was associated with TNBC response. Finally, TILs were significantly associated with baseline serum proinflammatory markers TNF-α and IFN-γ ($P_{adj} = .0043$ and $P_{adj} = .041$). PD-L1 expression was also associated with serum TNF-α and IFN-γ at baseline and after anti-PD1 and changes at all timepoints (all $P_{adj} < .05$). There was no association with neutrophil versus lymphocyte ratio at any biopsy timepoints and pCR ($P > .05$).

DISCUSSION

The PEARL study tested the effects of adding RT delivered preoperatively when the breast tumor was still intact, allowing synergy with pembrolizumab and direct exposure of tumor antigens and immunogenic mutations on the surface of cancer cells to CD8 T cells.[34-36] The study was designed to determine whether this approach can improve upon clinical responses to NAC relative to historical control rates, on the basis of preclinical evidence that RT can

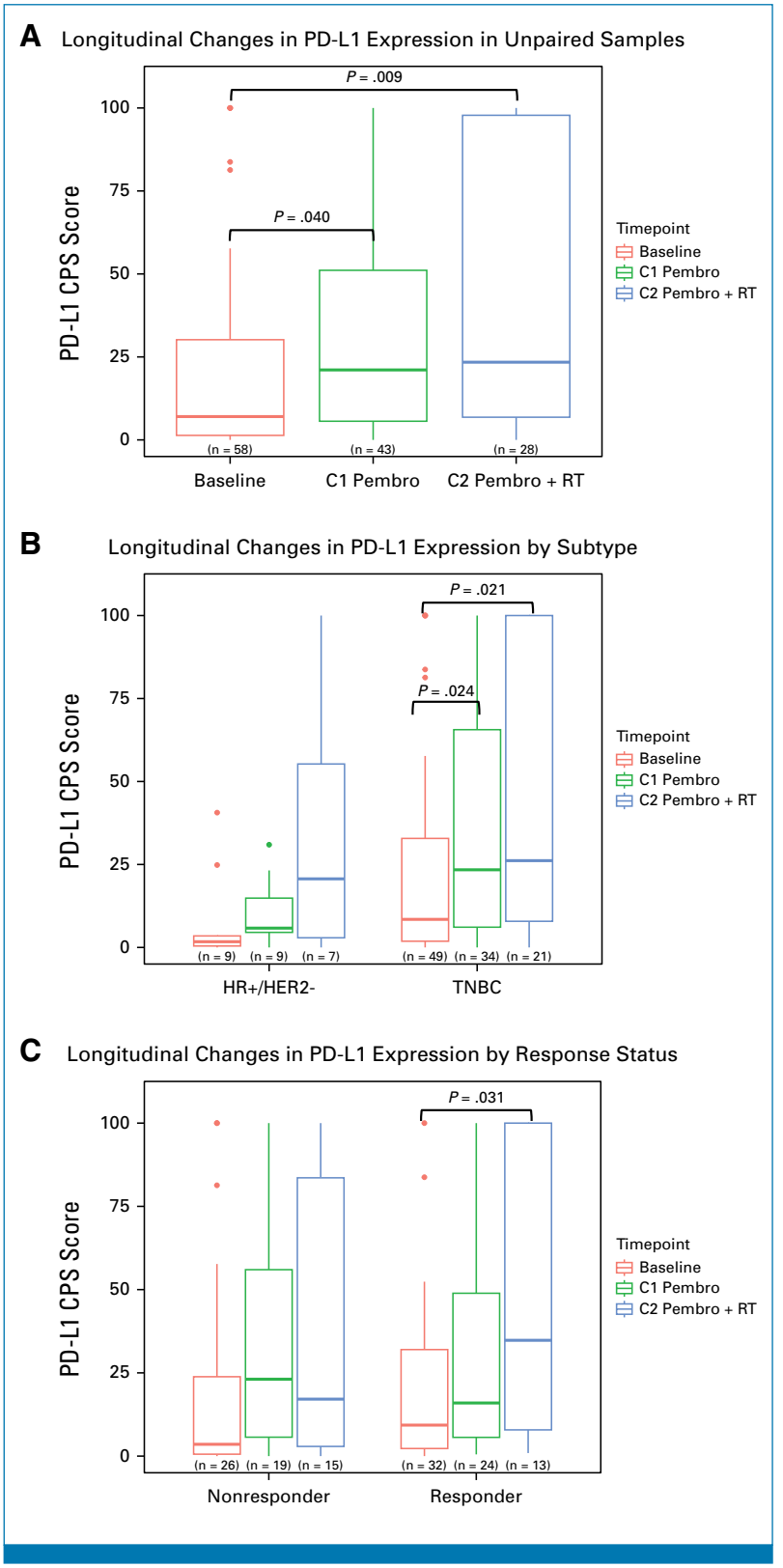

FIG 4. Longitudinal changes in PD-L1 expression. The PD-L1 IHC 22C2 pharmDx assay was performed to assess PD-L1 CPS ranging from 0 to 100, with changes in expression across all three timepoints (baseline, anti-PD1, anti-PD1 + RT) evaluated using the *t*-test. The box plot depicts of PD-L1 expression across three timepoints in unpaired samples in (A) the entire cohort, (B) by subtype, and (C) by treatment response groups. Longitudinal changes in PD-L1 expression (A) in unpaired samples, (B) by subtype, and (C) by response status. CPS, (continued on following page)

FIG 4. (Continued). Combined Positive Score; HER2−, human epidermal growth factor receptor 2−negative; HR+, hormone receptor−positive; IHC, immunohistochemistry; RT, radiation therapy; TNBC, triple-negative breast cancer.

transiently eliminate intratumoral immune suppression and intensify the inflammatory response generated by RT-mediated cell damage.[37-42] Adding RT to pembrolizumab did not delay NAC, was well tolerated, and resulted in high pCR rates in early-stage TNBC.

In our trial, the pCR rate of 59.2% in TNBC exceeded the historical 40% rates for NAC alone[43-45] and approached the 64.8% achieved in the pembrolizumab arm of KEYNOTE-522, which included pembrolizumab throughout NAC.[46] When we combined patients with TNBC with a near pCR (combined RCB-0-1) of 72.2%, this was comparable with 73.6% of patients in KEYNOTE-522 with combined RCB 0-1. We regard these results as promising, given that only 35% of PEARL patients received the KEYNOTE-522 regimen. The preoperative RT pembrolizumab and RT were well tolerated, with a much lower incidence of grade 3/4 immune-related toxicities, albeit with a higher rate of adrenal insufficiency than that in KEYNOTE-522.[2] The significance of this is unclear, given small sample size. The absence of reconstruction failure among patients was reassuring.

Both unpaired and paired analyses confirmed that PD-L1 increased from baseline after anti-PD1 or anti-PD1/RT. There was no meaningful conversion of PD-L1−negative to PD-L1−positive tumors by adding RT to pembrolizumab. It is possible that RT administration after one cycle of pembrolizumab might have weakened antitumor responses. A recent preclinical study demonstrated that administering anti-PD1 before RT nearly abolished systemic antitumor immunity, whereas administering anti-PD1 concurrently or immediately after RT stimulated abscopal responses.[47] Thus, conversion to PD-L1 positivity might have been higher if RT had been administered before or concurrently with pembrolizumab. We acknowledge conflicting data about the predictive value of PD-L1 assessment in the neoadjuvant setting from large trials such as IMpassion031 and KEYNOTE-522, which have demonstrated the benefit of the addition of ICI to chemotherapy irrespective of PD-L1 status.[1,48]

Our findings of reduced TILs after RT enforced the notion that RT should be administered before or concurrently with ICI, not afterward.[49] The increase in TILs after pembrolizumab alone confirmed other reports in breast cancer where response to ICI peaks within the first several weeks.[50,51] It is possible that the biopsy obtained after anti-PD1/RT captured RT-mediated cell death and limited assessment of the changes in the TME. In retrospect, measuring TILs in the lymph nodes and tertiary lymphoid structures (TLS) than in the primary tumor might have been more informative. In addition, data on other promising RT/immunotherapy (IT) response markers, such as pre-existing TLS, are emerging.[52] Our companion manuscript highlights additional changes seen at the single-cell level and identified phenotypes responsive to anti-PD1 alone and anti-PD1 with RT.[53]

Yet, despite the decrease in TILs after RT, pCR rates were substantive, and 3Y-EFS was high. A plausible explanation for this seeming discrepancy is that TIL changes post-treatment may not be the most accurate assessment of immune response. We could not examine specimens from breast surgeries after NAC, precluding the ability to examine the prognostic value of TILs in residual disease—a missed opportunity to identify immune evasion mechanisms in nonresponders.[54,55] Moreover, decreased TILs after RT in early TNBC may represent transient immunosuppression, and stromal TILs may increase by surgery and be associated with improved outcomes.[54,55] Thus, RT can still function as a potent systemic immunostimulant despite its potential to transiently deplete immune cells.[56]

Notably, TILs were closely associated with tissue PD-L1 expression at baseline and after anti-PD1, and both parameters correlated with the circulating levels of TNF-α and IFN-γ. Select circulating biomarkers increased after pembrolizumab treatment and were also associated with treatment resistance by subtype, suggesting that these circulating biomarkers may play complementary roles to tissue-based biomarkers. The confounding factor is that only patients with anti−PD1-resistant disease had residual tumors amenable to biopsy. Newer platforms that include numerous other biomarkers, including type I interferons, could demonstrate additional associations with treatment response.

The single-arm design of PEARL hindered the ability to quantify the contribution of each modality to the antitumor effect. PEARL was designed in the pre−KEYNOTE-522 era where there was no standard neoadjuvant regimen for TNBC and the results of Brightness trials that show the value of carboplatin had not been reported.[1,57] The exceptional results with preoperative RT/IT provide preliminary data for novel treatment approaches that may afford de-escalation of chemotherapy in select patients with TNBC, as is the subject of the SWOG 2212 trial (ClinicalTrials.gov identifier: NCT05929768). RT may induce systemic antitumor immune responses,[58,59] but the PEARL study was not designed to assess the abscopal effect. Trials such as P-RAD (ClinicalTrials.gov identifier: NCT04443348) will define the optimal dose of RT to combine with pembrolizumab in TNBC and high-risk HR+/HER2− breast cancer with node-positive disease, by evaluating lymph node response as a surrogate for the abscopal effect.[60]

With sample size notwithstanding, the pCR of 33.3% in HR+/HER2− patients mirrors the pCR rates of 34.2% and

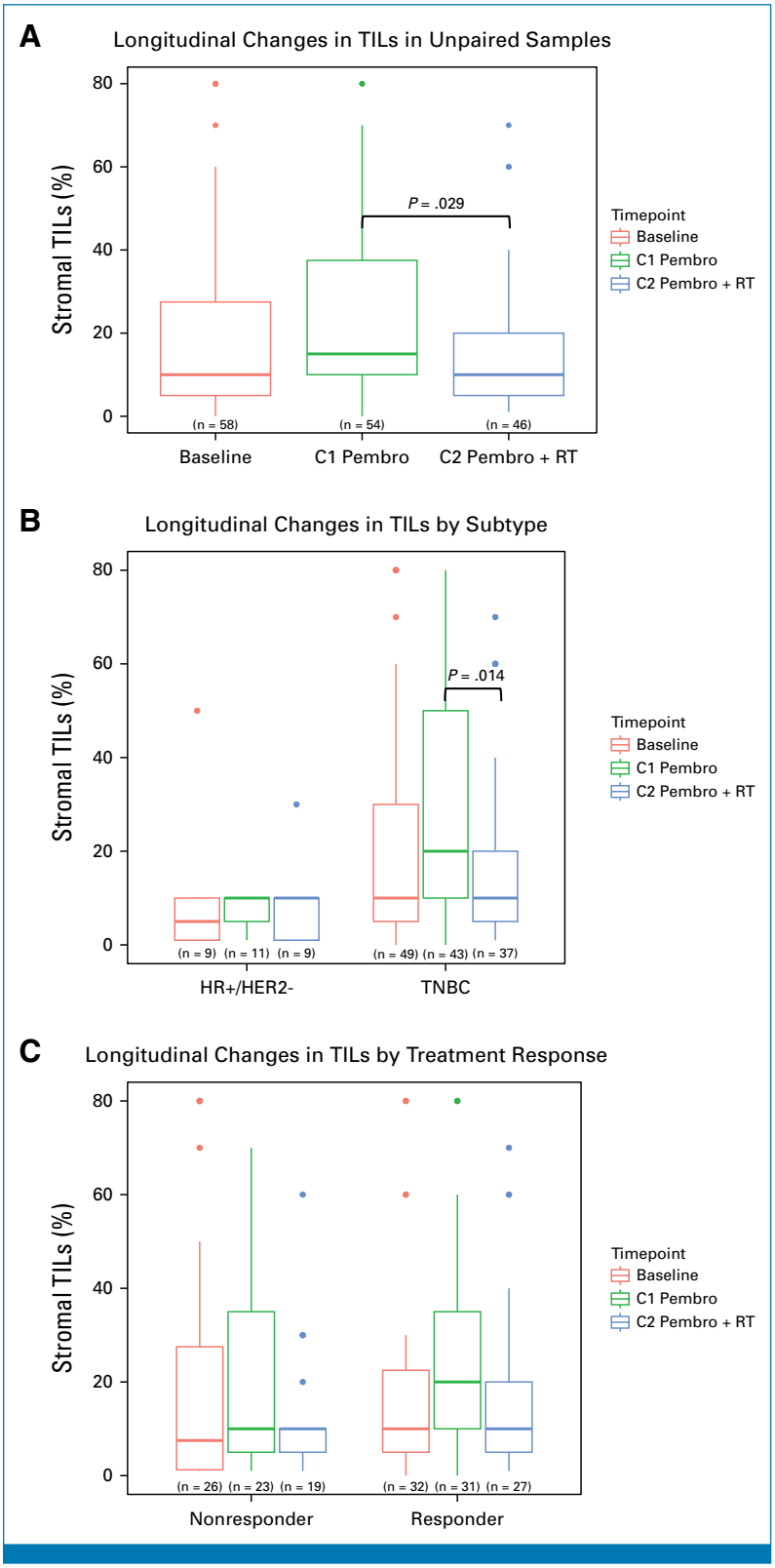

FIG 5. Longitudinal changes in TILs. TILs were measured and scored according to the Salgado criteria. The box plot depicts TIL score across all three timepoints (baseline, anti-PD1, anti-PD1 + RT) evaluated using the *t*-test. The box plot depicts PD-L1 expression across three timepoints in unpaired samples (A) in the entire cohort, (B) by subtype (B), and (C) by treatment response groups. Longitudinal changes in TILs in (A) unpaired samples, (B) by subtype, and (C) by treatment response. HER2–, human epidermal growth factor receptor 2–negative; HR+, hormone receptor–positive; RT, radiation therapy; TILs, tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancer.

28%, respectively, observed in the I-SPY trials evaluating pembrolizumab with NAC and durvalumab, olaparib, and once weekly paclitaxel in MammaPrint high-risk HR+ HER2− patients.[61,62] In I-SPY, adding pembrolizumab to NAC modestly improved pCR from 15.6% with NAC alone to 24.3%, regardless of PD-L1 status.[63] A similar pCR benefit for women with ER+/HER2− grade 2-3 breast cancer was reported in another trial, CheckMate 7FL, in which pCR rates were significantly improved by adding nivolumab to NAC (24.5% *v* 13.8% control).[64] Collectively, these data support the potential of immunotherapy combinations in HR+/HER2− breast cancer.

In summary, to our knowledge, this first phase I/IIb study demonstrated the safety of combined preoperative RT with pembrolizumab in patients with breast cancer receiving NAC. Efficacy was high despite the decline in TILs after RT and withholding pembrolizumab during NAC. This novel combination resulted in potent immunogenic responses, paving the way for future opportunities for this window regimen.

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REFERENCES

1. Schmid P, Cortes J, Pusztai L, et al: Pembrolizumab for early triple-negative breast cancer. N Engl J Med 382:810-821, 2020
2. Schmid P, Cortes J, Dent R, et al: Event-free survival with pembrolizumab in early triple-negative breast cancer. N Engl J Med 386:556-567, 2022
3. Deng L, Liang H, Burnette B, et al: Irradiation and anti–PD-L1 treatment synergistically promote antitumor immunity in mice. J Clin Invest 124:687-695, 2014
4. Postow MA, Callahan MK, Barker CA, et al: Immunologic correlates of the abscopal effect in a patient with melanoma. N Engl J Med 366:925-931, 2012
5. Burnette BC, Liang H, Lee Y, et al: The efficacy of radiotherapy relies upon induction of type I interferon–dependent innate and adaptive immunity. Cancer Res 71:2488-2496, 2011
6. Demaria S, Kawashima N, Yang AM, et al: Immune-mediated inhibition of metastases after treatment with local radiation and CTLA-4 blockade in a mouse model of breast cancer. Clin Cancer Res 11:728-734, 2005
7. Dewan MZ, Galloway AE, Kawashima N, et al: Fractionated but not single-dose radiotherapy induces an immune-mediated abscopal effect when combined with anti–CTLA-4 antibody. Clin Cancer Res 15:5379-5388, 2009
8. Verbrugge I, Hagekyriakou J, Sharp LL, et al: Radiotherapy increases the permissiveness of established Mammary tumors to rejection by immunomodulatory antibodies. Cancer Res 72:3163-3174, 2012
9. Ko EC, Formenti SC: Radiation therapy to enhance tumor immunotherapy: A novel application for an established modality. Int J Radiat Biol 95:936-939, 2019
10. Zeng J, See AP, Phallen J, et al: Anti-PD-1 blockade and stereotactic radiation produce long-term survival in mice with intracranial gliomas. Int J Radiat Oncol Biol Phys 86:343-349, 2013
11. Demaria S, Bhardwaj N, McBride WH, et al: Combining radiotherapy and immunotherapy: A revived partnership. Int J Radiat Oncol Biol Phys 63:655-666, 2005
12. Jobling MF, Mott JD, Finnegan MT, et al: Isoform-specific activation of latent transforming growth factor beta (LTGF-beta) by reactive oxygen species. Radiat Res 166:839-848, 2006
13. Kachikwu EL, Iwamoto KS, Liao YP, et al: Radiation enhances regulatory T cell representation. Int J Radiat Oncol Biol Phys 81:1128-1135, 2011
14. Ho AY, Barker CA, Arnold BB, et al: A phase 2 clinical trial assessing the efficacy and safety of pembrolizumab and radiotherapy in patients with metastatic triple-negative breast cancer. Cancer 126:850-860, 2020

15. Allison KH, Hammond MEH, Dowsett M, et al: Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. J Clin Oncol 38:1346-1366, 2020
16. Gradishar WJ, Anderson BO, Balassanian R, et al: Invasive breast cancer version 1.2016, NCCN Clinical Practice Guidelines in Oncology. J Natl Compr Cancer Netw 14:324-354, 2016
17. Symmans WF, Wei C, Gould R, et al: Long-term prognostic risk after neoadjuvant chemotherapy associated with residual cancer burden and breast cancer subtype. J Clin Oncol 35:1049-1060, 2017
18. Pusztai L, Denkert C, O'Shaughnessy J, et al: Event-free survival by residual cancer burden after neoadjuvant pembrolizumab + chemotherapy versus placebo + chemotherapy for early TNBC: Exploratory analysis from KEYNOTE-522. J Clin Oncol 40, 2022 (suppl 16; abstr 503)
19. Salgado R, Denkert C, Demaria S, et al: The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: Recommendations by an International TILs Working Group 2014. Ann Oncol 26: 259-271, 2015
20. Loi S, Michiels S, Adams S, et al: The journey of tumor-infiltrating lymphocytes as a biomarker in breast cancer: Clinical utility in an era of checkpoint inhibition. Ann Oncol 32:1236-1244, 2021
21. Tolaney SM, Boucher Y, Duda DG, et al: Role of vascular density and normalization in response to neoadjuvant bevacizumab and chemotherapy in breast cancer patients. Proc Natl Acad Sci USA 112:14325-14330, 2015
22. Tolaney SM, Ziehr DR, Guo H, et al: Phase II and biomarker study of cabozantinib in metastatic triple-negative breast cancer patients. Oncologist 26:e1483, 2021
23. Tolaney SM, Ziehr DR, Guo H, et al: Phase II and biomarker study of cabozantinib in metastatic triple-negative breast cancer patients. Oncologist 22:25-32, 2017
24. Boucher Y, Kumar AS, Posada JM, et al: Bevacizumab improves tumor infiltration of mature dendritic cells and effector T-cells in triple-negative breast cancer patients. NPJ Precis Oncol 5:62, 2021
25. Willett CG, Duda DG, di Tomaso E, et al: Efficacy, safety, and biomarkers of neoadjuvant bevacizumab, radiation therapy, and fluorouracil in rectal cancer: A multidisciplinary phase II study. J Clin Oncol 27:3020-3026, 2009
26. Willett CG, Boucher Y, di Tomaso E, et al: Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 10:145-147, 2004
27. Batchelor TT, Sorensen AG, di Tomaso E, et al: AZD2171, a pan-VEGF receptor tyrosine kinase inhibitor, normalizes tumor vasculature and alleviates edema in glioblastoma patients. Cancer Cell 11:83-95, 2007
28. Zhu C-Q, Ding K, Strumpf D, et al: Prognostic and predictive gene signature for adjuvant chemotherapy in resected non–small-cell lung cancer. J Clin Oncol 28:4417-4424, 2010
29. Blakeley JO, Ye X, Duda DG, et al: Efficacy and biomarker study of bevacizumab for hearing loss resulting from neurofibromatosis type 2–associated vestibular schwannomas. J Clin Oncol 34: 1669-1675, 2016
30. Plotkin SR, Duda DG, Muzikansky A, et al: Multicenter, prospective, phase II and biomarker study of high-dose bevacizumab as induction therapy in patients with neurofibromatosis type 2 and progressive vestibular schwannoma. J Clin Oncol 37:3446-3454, 2019
31. Yau C, Osdoit M, van der Noordaa M, et al: Residual cancer burden after neoadjuvant chemotherapy and long-term survival outcomes in breast cancer: A multicentre pooled analysis of 5161 patients. Lancet Oncol 23:149-160, 2022
32. Cortazar P, Zhang L, Untch M, et al: Pathological complete response and long-term clinical benefit in breast cancer: The CTNeoBC pooled analysis. Lancet (London, England) 384:164-172, 2014
33. Santonja A, Sánchez-Muñoz A, Lluch A, et al: Triple negative breast cancer subtypes and pathologic complete response rate to neoadjuvant chemotherapy. Oncotarget 9:26406-26416, 2018
34. Lin W, Xu Y, Chen X, et al: Radiation-induced small extracellular vesicles as "carriages" promote tumor antigen release and trigger antitumor immunity. Theranostics 10:4871-4884, 2020
35. Kwilas AR, Donahue RN, Bernstein MB, et al: In the field: Exploiting the untapped potential of immunogenic modulation by radiation in combination with immunotherapy for the treatment of cancer. Front Oncol 2:104, 2012
36. Liu J, Blake SJ, Yong MCR, et al: Improved efficacy of neoadjuvant compared to adjuvant immunotherapy to eradicate metastatic disease. Cancer Discov 6:1382-1399, 2016
37. Cytlak UM, Dyer DP, Honeychurch J, et al: Immunomodulation by radiotherapy in tumour control and normal tissue toxicity. Nat Rev Immunol 22:124-138, 2022
38. McLaughlin M, Patin EC, Pedersen M, et al: Inflammatory microenvironment remodelling by tumour cells after radiotherapy. Nat Rev Cancer 20:203-217, 2020
39. Rodriguez-Ruiz ME, Vanpouille-Box C, Melero I, et al: Immunological mechanisms responsible for radiation-induced abscopal effect. Trends Immunol 39:644-655, 2018
40. Shiao SL, Ruffell B, DeNardo DG, et al: TH2-polarized CD4+ T cells and macrophages limit efficacy of radiotherapy. Cancer Immunol Res 3:518-525, 2015
41. Yin L, Xue J, Li R, et al: Effect of low-dose radiation therapy on abscopal responses to hypofractionated radiation therapy and anti-PD1 in mice and patients with non-small cell lung cancer. Int J Radiat Oncol Biol Phys 108:212-224, 2020
42. Vanpouille-Box C, Formenti SC, Demaria S: TREX1 dictates the immune fate of irradiated cancer cells. Oncoimmunology 6:e1339857, 2017
43. van den Ende NS, Nguyen AH, Jager A, et al: Triple-negative breast cancer and predictive markers of response to neoadjuvant chemotherapy: A systematic Review. Int J Mol Sci 24:2969, 2023
44. Santa-Maria CA, Camp M, Cimino-Mathews A, et al: Neoadjuvant therapy for early-stage breast cancer: Current practice, controversies, and future directions. Oncology (Williston Park) 29:828-838, 2015
45. Hensing W, Santa-Maria CA, Peterson LL, et al: Landmark trials in the medical oncology management of early stage breast cancer. Semin Oncol 47:278-292, 2020
46. Reference deleted
47. Wei J, Montalvo-Ortiz W, Yu L, et al: Sequence of αPD-1 relative to local tumor irradiation determines the induction of abscopal antitumor immune responses. Sci Immunol 6:eabg0117, 2021
48. Mittendorf EA, Zhang H, Barrios CH, et al: Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): A randomised, double-blind, phase 3 trial. Lancet (London, England) 396:1090-1100, 2020
49. Monjazeb AM, Schalper KA, Villarroel-Espindola F, et al: Effects of radiation on the tumor microenvironment. Semin Radiat Oncol 30:145-157, 2020
50. Vanderlugt CL, Miller SD: Epitope spreading in immune-mediated diseases: Implications for immunotherapy. Nat Rev Immunol 2:85-95, 2002
51. Yost KE, Satpathy AT, Wells DK, et al: Clonal replacement of tumor-specific T cells following PD-1 blockade. Nat Med 25:1251-1259, 2019
52. Rodriguez AB, Peske JD, Woods AN, et al: Immune mechanisms orchestrate tertiary lymphoid structures in tumors via cancer-associated fibroblasts. Cell Rep 36:109422, 2021
53. Shiao SL, Gouin KH 3rd, Ing N, et al: Single-cell and spatial profiling identify three response trajectories to pembrolizumab and radiation therapy in triple negative breast cancer. Cancer Cell 42: 70-84, 2024
54. Luen SL, Salgado R, Loi S: Residual disease and immune infiltration as a new surrogate endpoint for TNBC post neoadjuvant chemotherapy. Oncotarget 10:4612-4614, 2019
55. Luen SJ, Salgado R, Dieci MV, et al: Prognostic implications of residual disease tumor-infiltrating lymphocytes and residual cancer burden in triple-negative breast cancer patients after neoadjuvant chemotherapy. Ann Oncol 30:236-242, 2019
56. Ngwa W, Irabor OC, Schoenfeld JD, et al: Using immunotherapy to boost the abscopal effect. Nat Rev Cancer 18:313-322, 2018
57. Geyer CE, Sikov WM, Huober J, et al: Long-term efficacy and safety of addition of carboplatin with or without veliparib to standard neoadjuvant chemotherapy in triple-negative breast cancer: 4-year follow-up data from BrighTNess, a randomized phase III trial. Ann Oncol 33:384-394, 2022
58. Lugade AA, Moran JP, Gerber SA, et al: Local radiation therapy of B16 melanoma tumors increases the generation of tumor antigen-specific effector cells that traffic to the tumor. J Immunol 174: 7516-7523, 2005
59. Matsumura S, Wang B, Kawashima N, et al: Radiation-induced CXCL16 release by breast cancer cells attracts effector T cells. J Immunol 181:3099-3107, 2008
60. National Library of Medicine (U.S.): Pre-op pembro + radiation therapy in breast cancer (P-RAD). Identifier NCT00103181, 2020. https://clinicaltrials.gov/study/NCT04443348
61. Nanda R, Liu MC, Yau C, et al: Pembrolizumab plus standard neoadjuvant therapy for high-risk breast cancer (BC): Results from I-SPY 2. J Clin Oncol 35, 2017 (suppl 15; abstr 506)
62. Pusztai L, Yau C, Wolf DM, et al: Durvalumab with olaparib and paclitaxel for high-risk HER2-negative stage II/III breast cancer: Results from the adaptively randomized I-SPY2 trial. Cancer Cell 39: 989-998.e5, 2021
63. Cardoso F, McArthur H, Schmid P, et al: LBA21 KEYNOTE-756: Phase III study of neoadjuvant pembrolizumab (pembro) or placebo (pbo) + chemotherapy (chemo), followed by adjuvant pembro or pbo + endocrine therapy (ET) for early-stage high-risk ER+/HER2− breast cancer. Ann Oncol 34:S1260-S1261, 2023
64. Loi S, Curigliano G, Salgado R, et al: LBA20: A randomized, double-blind trial of nivolumab (NIVO) vs placebo (PBO) with neoadjuvant chemotherapy (NACT) followed by adjuvant endocrine therapy (ET) ± NIVO in patients (pts) with high-risk, ER+ HER2− primary breast cancer (BC). Ann Oncol 34:S1259-S1260, 2023

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

PEARL: A Phase Ib/II Biomarker Study of Adding Radiation Therapy to Pembrolizumab Before Neoadjuvant Chemotherapy in Human Epidermal Growth Factor Receptor 2–Negative Breast Cancer

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APPENDIX

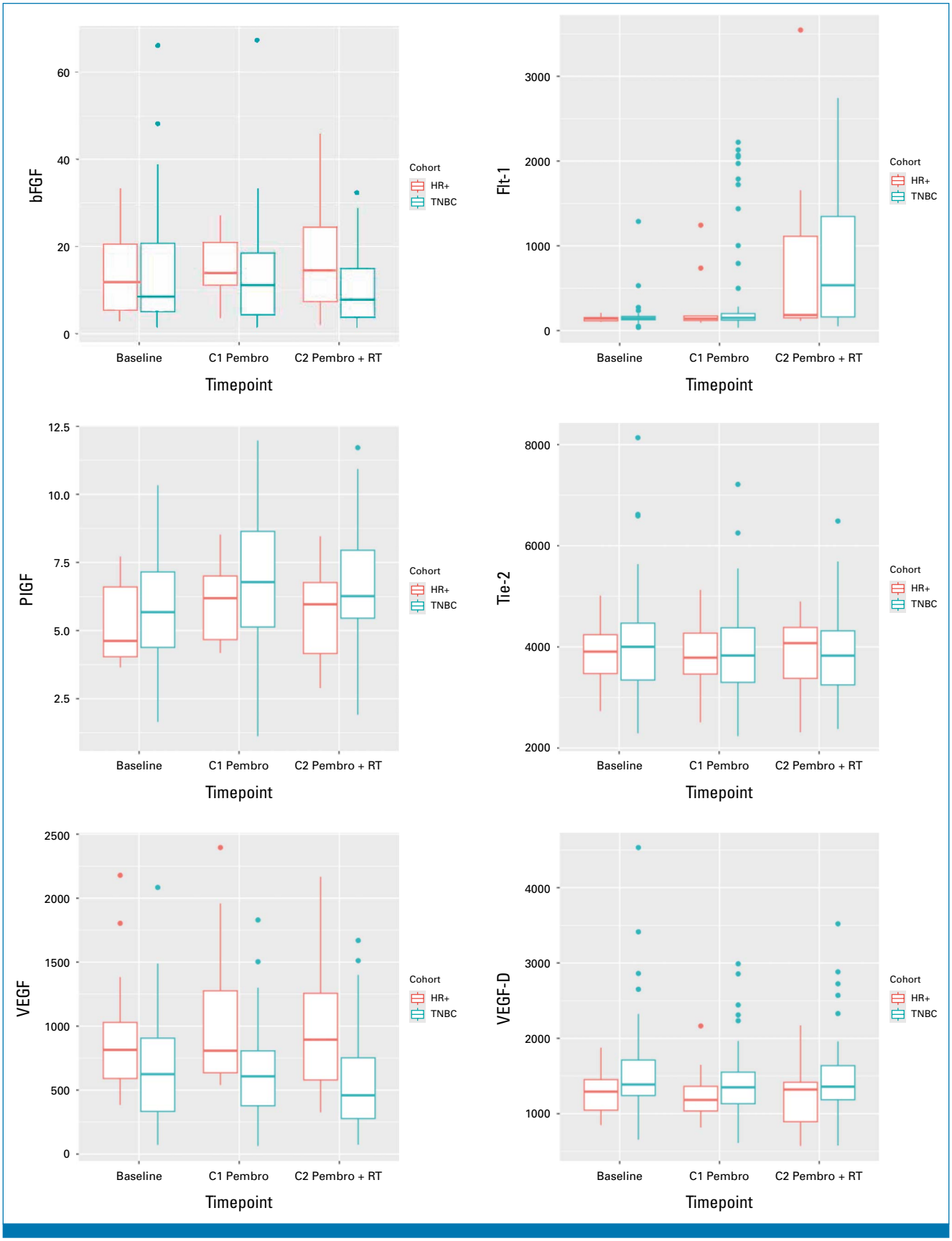

FIG A1. Dynamics of circulating blood biomarkers: angiogenesis markers. HR+, hormone receptor–positive; RT, radiation therapy; TNBC, triple-negative breast cancer; VEGF, vascular endothelial growth factor.

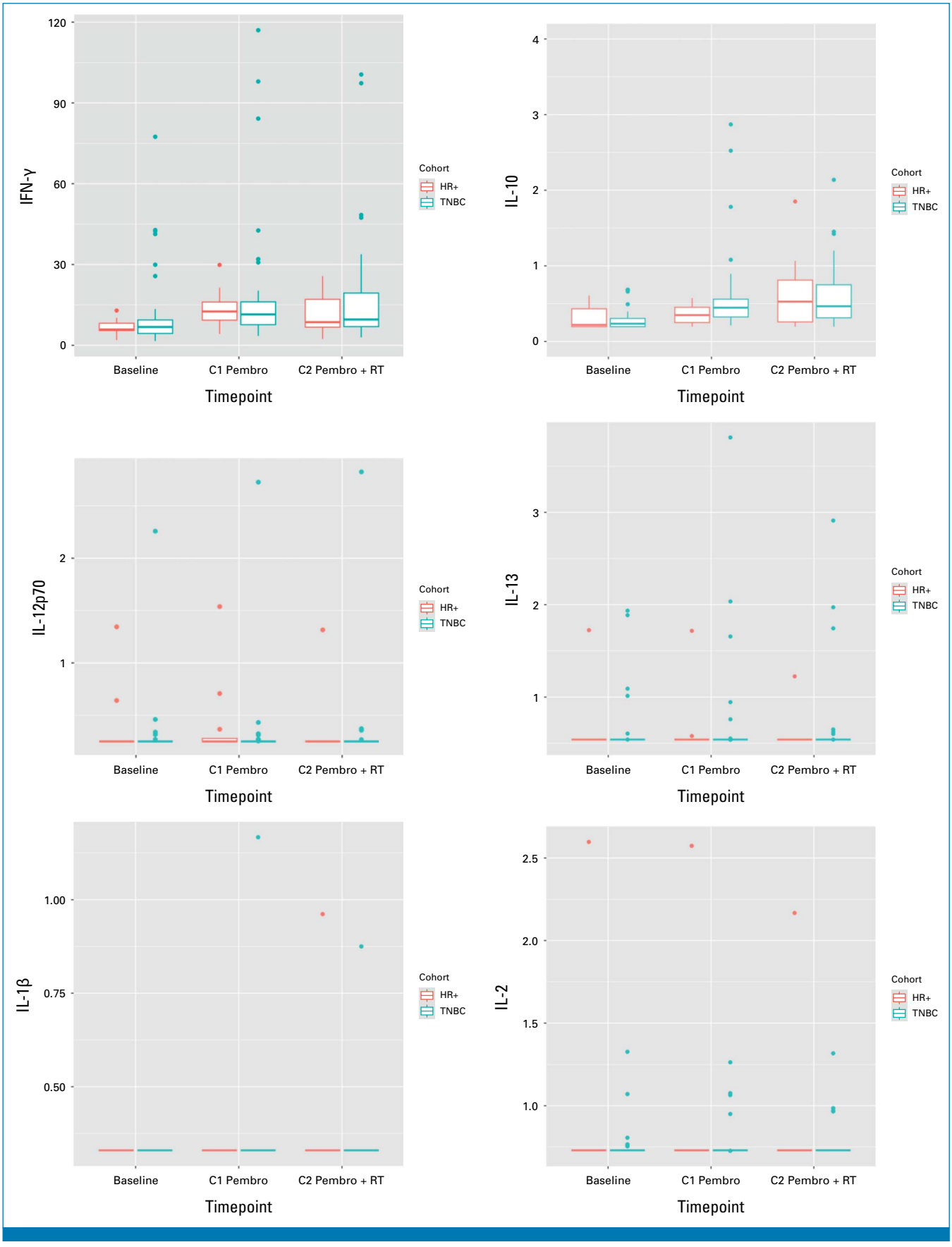

FIG A2. Dynamics of circulating blood biomarkers: proinflammatory markers. HR+, hormone receptor–positive; RT, radiation therapy; TNBC, triple-negative breast cancer. (continued on following page)

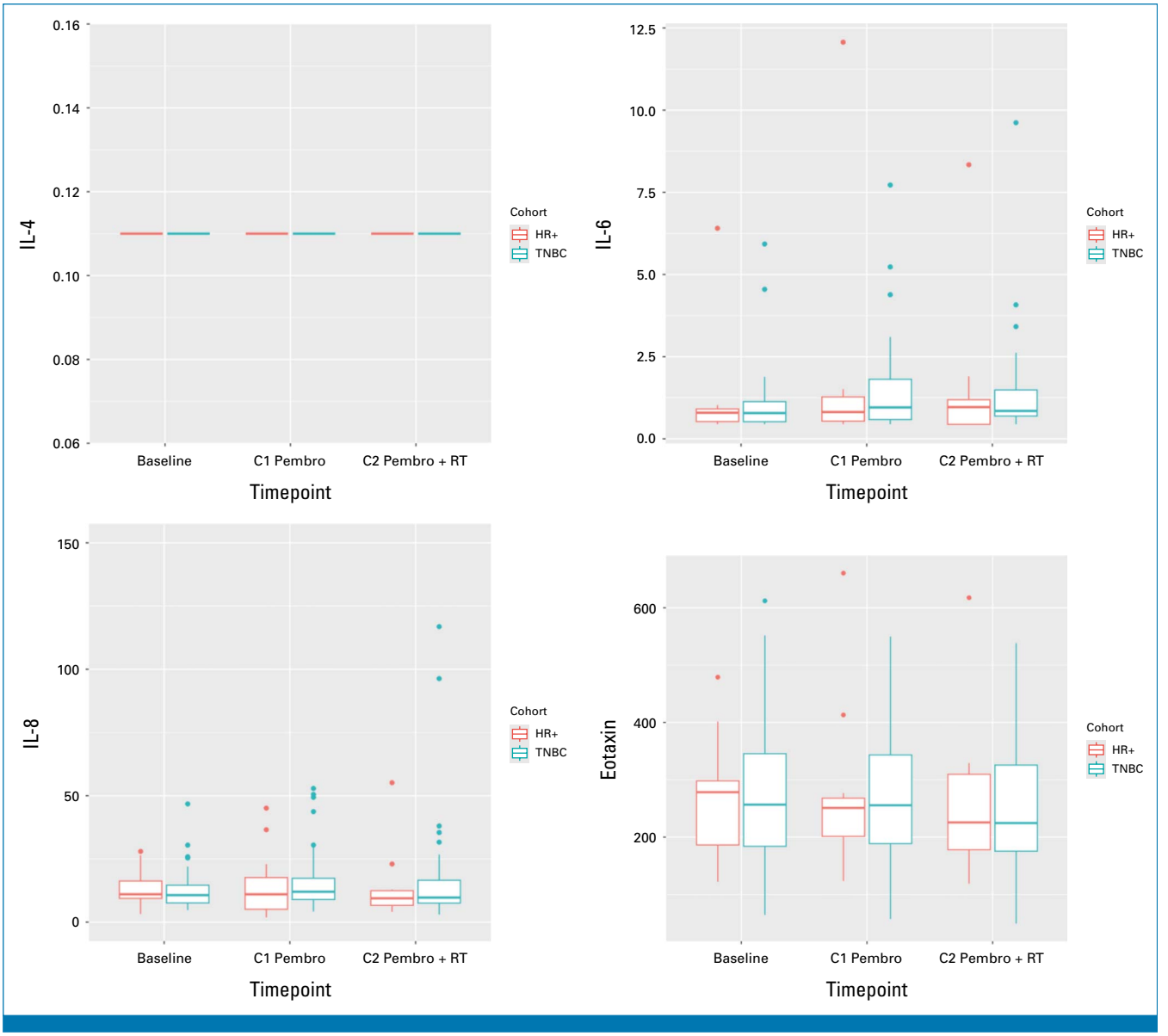

FIG A2. (Continued).

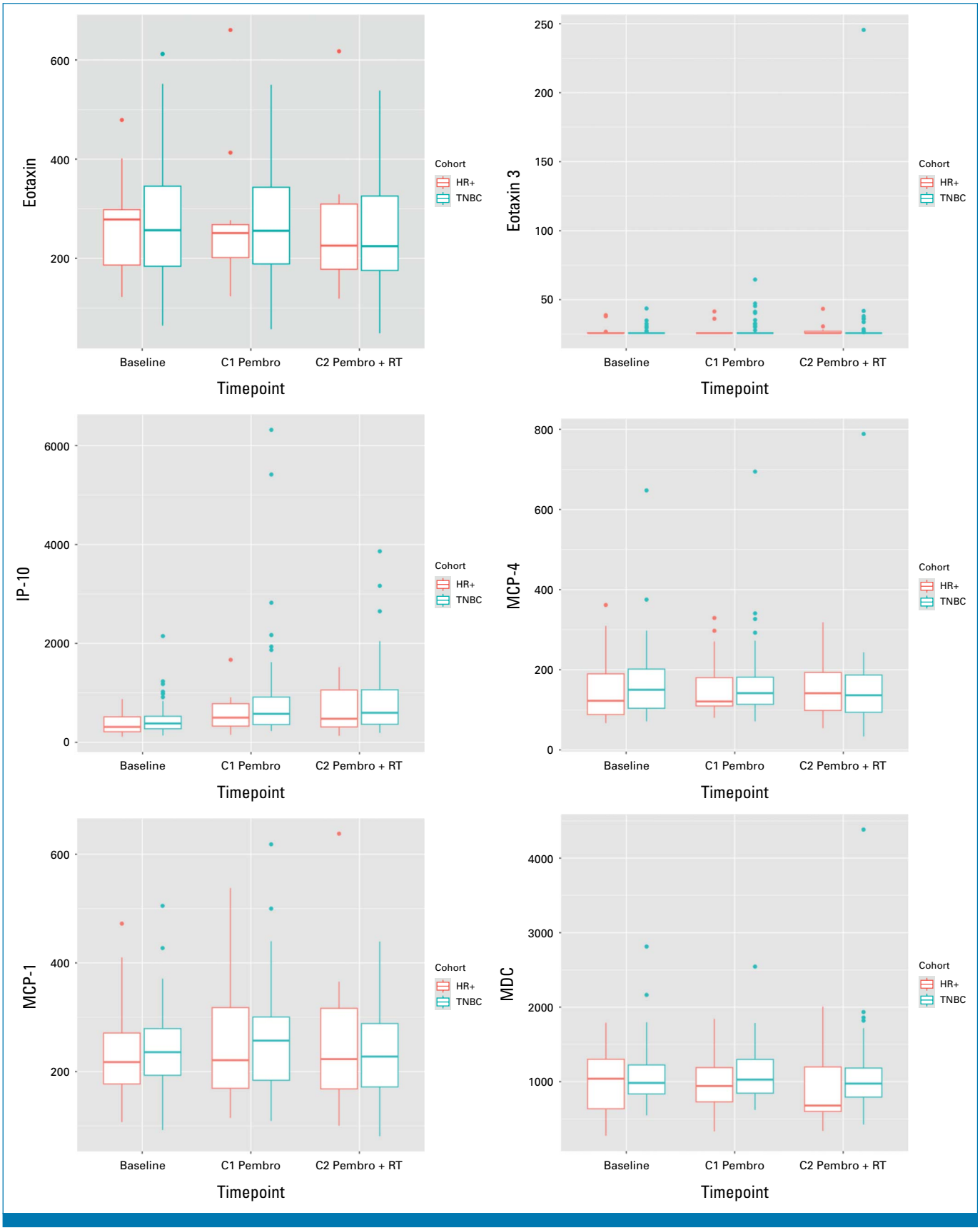

FIG A3. Dynamics of circulating blood biomarkers: chemokine markers. HR+, hormone receptor–positive; RT, radiation therapy; TNBC, triple-negative breast cancer. (continued on following page)

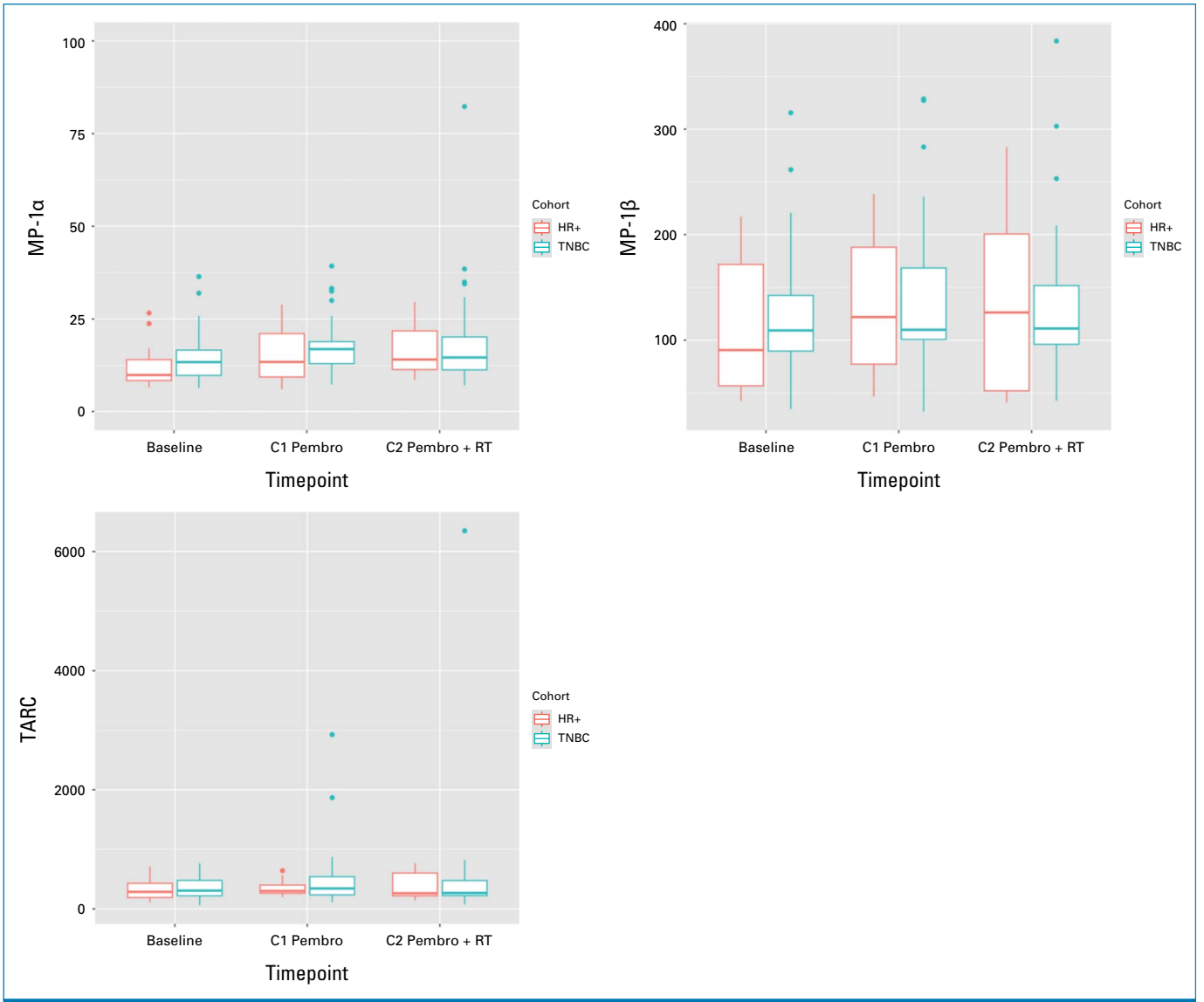

FIG A3. (Continued).

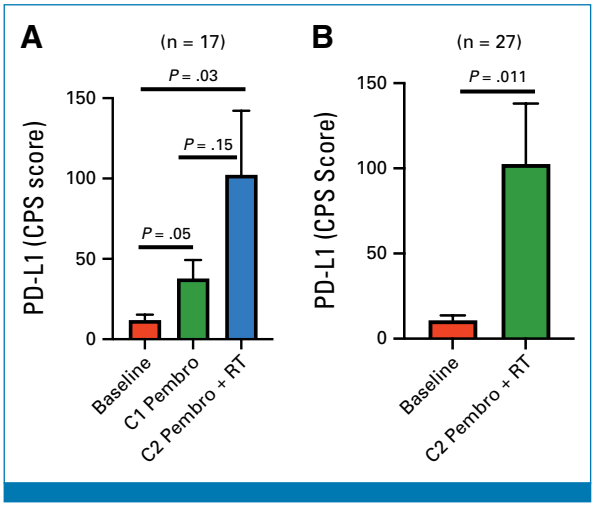

FIG A4. Changes in PD-L1 across (A) three biopsy timepoints and (B) two biopsy timepoints using paired samples. CPS, Combined Positive Score; RT, radiation therapy.

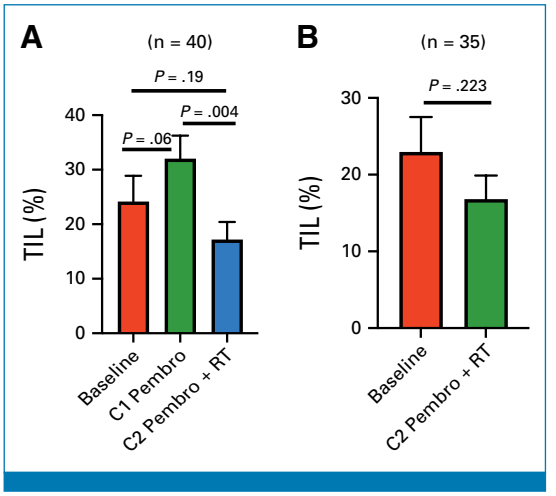

FIG A5. Changes in proportion of TILs across (A) three biopsy timepoints and (B) two biopsy timepoints using paired samples. RT, radiation therapy; TILs, tumor-infiltrating lymphocytes.