Review Article

Checkpoint Inhibitors in Triple-Negative Breast Cancer (TNBC): Where to Go From Here

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Advances in cancer immunotherapy and a growing body of research have focused on the role of the antitumor response in breast cancer. Triple-negative breast cancer (TNBC) is the most immunogenic breast cancer subtype, and there is strong evidence that tumor-infiltrating lymphocytes in TNBC have prognostic value and are associated with clinical outcome and improved survival. Evading antitumor immunity is a hallmark for the development and progression of cancer. Immunotherapy studies have focused on the role of the programmed cell death-1 (PD-1) receptor/programmed death-ligand 1 (PD-L1) pathway in maintaining immunosuppression in the tumor microenvironment. Blockade of the PD-1/PD-L1 axis has emerged as a promising therapeutic option to enhance antitumor immunity and is actively being investigated in TNBC, with encouraging results. In this article, the authors review the current literature on checkpoint inhibitors in TNBC with a focus on PD-1/PD-L1 antibodies and discuss combination strategies and novel approaches for improving antitumor immunity and clinical outcome. ***Cancer* 2018;124:2086-103**. © *2018 American Cancer Society*.

KEYWORDS: checkpoint inhibitors, immunotherapy, programmed cell death-1 (PD-1) receptor, programmed death-ligand 1 (PD-L1), triple-negative breast cancer.

INTRODUCTION

Significant developments in cancer immunotherapy have led to important breakthroughs and paradigm shifts in the treatment of malignancy. Although breast cancer traditionally has been considered less immunogenic, immunogenic tumor antigens, such as the human epidermal growth factor receptor 2 (HER2), have been described in breast cancer, and recent data from large clinical trials demonstrate that subsets of breast cancers contain tumor-infiltrating lymphocytes (TILs), which are indicative of an adaptive immune response.[1-3] The demonstration of the impact of the tumor immune microenvironment on patient survival and response to therapy has emerged as an important development in breast cancer. In particular, triple-negative breast cancer (TNBC) has a clear association with TILs (host immunity), with improved survival in early stage TNBC, and has been identified as a robust and independent prognostic biomarker across studies.[4-9] These findings have attracted increased attention and have led to the study of immune-checkpoint blockade (ICB) therapeutics in TNBC.

TNBC represents 20% of all breast cancers and is characterized by the lack of estrogen receptor (ER) and progesterone receptor expression and the absence of HER2 amplification. TNBCs tend to behave more aggressively and frequently are histologic high-grade tumors associated with higher rates of relapse and mortality. Unlike the other breast cancer subtypes, which harbor targets like ER or HER2, there are no approved targeted therapies for TNBC, and systemic chemotherapy remains the standard of care. The responses to chemotherapy are frequently short-lasting, making this disease subtype a clinical challenge to treat with the need for novel and more effective targeted therapies or combinations.

Lymphocytic infiltrates in the stroma are observed across breast cancer subtypes, but TNBC in particular has more prominent immune infiltration.[10] TNBC is also characterized by genomic instability and higher rates of mutation, which can lead to the production of neoantigens and increased immunogenicity.[11,12] TNBC is also more likely to have increased expression of the programmed death-ligand 1 (PD-L1) in the tumor microenvironment, making it an ideal candidate for targeted therapy with ICB, which is generally well tolerated and less toxic than cytotoxic chemotherapy and has the potential to produce durable responses in patients with advanced cancer. Although initial trials with ICB were conducted as monotherapy, combination trials now outnumber single-agent trials. Most trials have focused on TNBC and metastatic disease; however, the spectrum spans all breast cancer subtypes and includes less common populations (such as lobular cancers, inflammatory breast cancer, and brain metastases), and studies have been expanded into the neoadjuvant and adjuvant settings.

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COMPOSITION OF TILS IN PRIMARY BREAST CANCERS

The immune cell composition of the tumor microenvironment is diverse and is made up of leukocytes, including T and B lymphocytes, natural killer (NK) cells, macrophages, and dendritic cells,[13-15] with T lymphocytes comprising most of the tumor infiltrate.[16] A study of the immune infiltrate in breast cancer by flow cytometry and microarray of freshly obtained primary tumors revealed that T lymphocytes represented 75% of TILs, B lymphocytes comprised <20%, monocytes/macrophages were <10%, and NK cells or NK T cells made up <5%.[16] Cluster of differentiation 8 (CD8)-positive T lymphocytes are the predominant component of TILs and can exhibit cytotoxic activity toward tumor cells that express tumor-associated antigens presented by major histocompatibility (MHC) class I molecules. CD4-positive T lymphocytes, also known as T-helper cells (Th cells), play an integral role in the adaptive immune system through the recruitment, activation, and regulation of CD8-positive T cells as well as B cells.[17] Th cells are activated when they are exposed to antigens expressed by MHC class II molecules and produce cytokines like interferon γ, which is important for the function of CD8-positive cells. Th cells also help shape innate immunity through the recruitment and activation of macrophages and NK cells.

PREVALENCE AND PROGNOSTIC IMPLICATIONS OF TILS IN EARLY TNBC

We recently summarized the individual trial data reporting associations of TILs with clinical outcome in TNBC.[9] A large pooled analysis was performed of these clinical trials, which evaluated TILs in patients with TNBC who received treatment with anthracycline or anthracycline plus taxane chemotherapy.[8] Individual data were collected from 991 patients with TNBC in 6 randomized trials (BIG2-98, Eastern Cooperative Oncology Group 2197 [ECOG 2197], ECOG 1199, FinHER, and 2 trials from the Gustave Roussy Institute). Each 10% increase in stromal TILs was associated with a 14% relative reduction in invasive disease-free survival (IDFS) events (hazard ratio, 0.86;, 95% confidence interval [CI], 0.80-0.93; $P < .0001$) and a 17% relative reduction in deaths (hazard ratio, 0.83; 95% CI, 0.76-0.91; $P = .0001$). In multivariable analysis adjusted for age, tumor size, lymph node status, and chemotherapy regimen, stromal TILs provided significant independent prognostic information for IDFS and overall survival (OS). That large pooled analysis confirmed the strong prognostic role of stromal TILs in patients with early stage TNBC who receive chemotherapy.

A standardized methodology for TIL evaluation in breast cancer is an important step in improving the consistency and reproducibility of TIL measurement. A consensus guideline by the International TILs Working Group is now available with methods recommended for use in future prospective breast cancer studies.[18]

PROGRAMMED CELL DEATH-1 RECEPTOR/ PD-L1 EXPRESSION IN BREAST CANCER

Evading antitumor immunity is a hallmark for the development and progression of cancer.[19] Recent studies have focused on the role of the programmed cell death-1 (PD-1) receptor pathway in maintaining immunosuppression in the tumor environment. PD-1 is a member of the T-cell coregulatory receptor family; and, when it binds to its ligands, PD-L1 and PD-L2, it attenuates T-cell function, survival, and expansion, thereby mediating immune tolerance. PD-L1 is expressed on activated T cells within the tumor microenvironment; however, tumors can also express PD-L1, which has been demonstrated in breast cancer, melanoma, lung cancer, and renal cell cancer, among other malignancies.[20-23] This expression of PD-L1 enables inhibition of the local immune response.

TNBC is the most immunogenic breast cancer subtype, with higher PD-L1 expression levels and more TILs. In general, leukocytes in the stroma are usually PD-L1–positive compared with the much lower expression in tumor cells.[24] Results of PD-L1 expression studies from institutional series and clinical trial data sets are summarized in Table 1.[25-33]

Many trials using PD-L/PD-L1 antibodies in breast cancer and other malignancies, such as lung cancer, have required positive PD-L1 status for eligibility to enrich for study participants who are likely to respond to therapy. A challenge of this strategy is that there is currently no standardized test for the PD-L1 biomarker. For instance, there are differences in technology (immunohistochemistry [IHC], DNA microarray, and in situ hybridization), analysis at the protein or messenger RNA level, different antibodies for IHC with variable scoring systems using different cutoff values for interpretation of positivity, different analyses based on expressing cell types (tumor cells, tumor-infiltrating immune cells, or both), as well different scoring methodology for messenger RNA analysis. Aside from the US Food and Drug Administration-approved companion diagnostic kit for use in nonsmall cell lung cancer and gastric or gastroesophageal junction adenocarcinomas with pembrolizumab (PD-L1 IHC

TABLE 1. Programmed Death Ligand Expression in Selected Studies of Breast Cancer

Study	Population	Method (Antibody and Definition of Positivity)	No. of Patients/ Total No. With PD-L1 Expression (%)	Comments	References
Institutional series	Ductal carcinoma in situ (DCIS)	IHC (5H1, ≥5% positive immune-infiltrating cells)	22/27 (81)	PD-L1 expression associated with TILs and HR-negative DCIS; none of the DCIS cells expressed PD-L1	Thompson 2016[25]
Institutional series	Early breast cancers	IHC (B7-H1, MIH1 clone [eBioscience; ThermoFisher Scientific, Springfield Township, NJ], ≥5% positive cells)	22/44 (50)	41% Expression in TILs, 35% expression in tumor cells; PD-L1 expression associated with higher grade, HR-negative disease	Ghebeh 2008[26]
Phase 1: Atezolizumab trial	Metastatic TNBC; first, second, and third line	IHC (SP142 [Ventana Medical Systems, Tucson, AZ], ≥5% positive immune-infiltrating cells = IC2/IC3)	71/111 (63)	Higher response rate to atezolizumab with higher PD-L1 expression (13% vs 5%)	Schmid 2017[27]
Phase 2: Pembrolizumab trial	Metastatic TNBC, second line and greater (cohort A)	IHC (22C3 [pharmDX; Dako/ Agilent Technologies, Santa Clara, CA], combined positive score ≥1%)	183/306 (62)	No difference in response rate to pembrolizumab by PD-L1 expression (4.8 vs 4.7%)	Adams 2017[28]
	Metastatic TNBC, first-line setting (cohort B)	IHC (22C3 [pharmDx; Dako/ Agilent Technologies], combined positive score ≥1%)	79/137 (58)		Adams 2017[29]
Phase 1: Atezolizumab plus nab-paclitaxel	Metastatic TNBC, first, second, third line	IHC (SP142 [Ventana Medical Systems], ≥1% positive immune-infiltrating cells = IC1/IC2/IC3)	12/24 (50)	12.5% Expression in tumor cells, 50% in infiltrating immune cells	Adams 2016[30]
Institutional series	Stage I-III TNBC	IHC (9A11 mouse monoclonal antibody, ≥1% positive cells)	51/193 (26) In cancer cells, 163/177 (92) in immune cells	No difference in expression between BRCA1-associated and sporadic TNBC, PD-L1 expression in cancer cells associated with AR expression	Tung 2016[31]
Institutional series	Inflammatory breast cancer	mRNA	42/112 (38)	PD-L1 expression associated with ER-negative status, basal and HER2-enriched subtypes, as well as CD8-positive T-cell–specific gene signatures	Bertucci 2015[32]
Institutional series	Metaplastic breast cancer (MPBC)	IHC (SP142 [Spring Bioscience; Ventana Medical Systems], ≥5% tumor cells with ≥2+ intensity)	33/72 (46)	PD-L1 expression in MPBC greater than in TNBC (46% vs 9%)	Joneja 2017[33]

Abbreviations: AR, androgen receptor; BRCA1, BReast CAncer susceptibility gene 1; CD8, cluster of differentiation 8 (a transmembrane glycoprotein that serves as a co-receptor for the T-cell receptor); ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; IC, immune cells; IHC, immunohistochemistry; mRNA, messenger RNA; PD-L1, programmed death-ligand 1; TILs, tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancer.

22C3; pharmDx; Dako North America, Inc, Santa Clara, CA), the use of different analysis platforms has subsequently contributed to variability in PD-L1 expression and interpretation reported across different studies in the literature.

MONOTHERAPY WITH ANTI–PD-1/PD-L1 THERAPY IN METASTATIC TNBC

Most of the initial ICB trials focused on metastatic TNBC (mTNBC) with the use of single-agent immune-checkpoint inhibitors targeting PD-1 or PD-L1 (Table 2).[27-30,34-37] In the first proof-of-principle study, the phase 1b KEYNOTE-012 trial, patients with advanced, PD-L1–positive TNBC, urothelial cancer, gastric cancer, and head and neck cancer received the PD-1 inhibitor pembrolizumab (clinicaltrials.gov identifier NCT01848834). Positive PD-L1 status was defined as expression in the stroma or in ≥1% of tumor cells by IHC. In total, 111 patients with TNBC were screened for PD-L1 expression; of these, 58.6% were positive for PD-L1, and 32 women were enrolled (median age, 50.5 years; age range, 29-72 years), and most were heavily pretreated.[30] The median number of prior lines of systemic therapy for metastatic disease was 2; 46.9% of patients had received at least 3 prior lines of therapy, and 25% had received at least 5. The median number of doses administered was 5, and the median duration of treatment was 59.5 days (range, 1-530 days). Common adverse events (AEs) were mild (eg, fatigue, nausea, arthralgia, and myalgia), 5 patients had grade ≥3 toxicity, and there was 1 treatment-related death from disseminated intravascular coagulation. Of the 27 patients who were evaluable for response, the overall response rate (ORR) was 18.5% (95% CI, 6.3-38.1), and the median time to response was 17.9 weeks.[30] One patient (3.7%) had a complete response (CR), 4 (14.8%) had a partial response (PR), and 7 (25.9%) had stable disease.

Likewise, another phase 1 trial evaluated the use of atezolizumab, a monoclonal antibody that binds to PD-L1, in patients (n = 27) who had mTNBC with PD-L1 expression (NCT01375842).[35] Overall, atezolizumab was well tolerated, and the most common AEs included fatigue, fever, nausea, decreased appetite, and asthenia. Eleven percent of patients had grade ≥3 AEs. Among the 21 patients who were evaluable for efficacy, the ORR was 19%, with 2 CRs and 3 PRs. Results from the expansion cohort of mTNBC in that phase 1 study were recently reported for 115 patients.[27] Seventeen percent of patients received treatment in the first-line setting, 24% had received 1 prior line, and 58% had received ≥2 prior treatments in the advanced setting. Two-thirds of tumors had high levels of PD-L1 expression, defined as ≥5% positive immune-infiltrating cells. The trial initially selected patients who had ≥5% PD-L1–expressing immune cells (Ventana SP142 assay; Ventana Medical Systems, Tucson, AZ) but later included patients who had lower or absent PD-L1 expression. Overall, the ORR was 10% (n = 115), with a higher ORR in PD-L1–positive (13%) versus PD-L1–negative (5%) patients. The results also indicated that the response rate was higher in patients who received treatment in the first-line setting (26% vs <10% in subsequent lines). Among responders, the median duration of response was durable at 21.1 months.[27] Overall, several of the observed responses in both of the phase 1 monotherapy trials described above were durable, which is rare with standard chemotherapy in patients with mTNBC.

The recently reported phase 2 multicohort, single-arm KEYNOTE-086 trial is the largest ICB study reported in mTNBC to date (NCT02447003). Cohort A of KEYNOTE-086 evaluated the efficacy and safety of pembrolizumab in patients with previously treated mTNBC, regardless of PD-L1 expression.[28] Of 386 patients who were screened, 170 were identified as eligible and were enrolled (median age, 54 years). Forty-four percent of patients had received 3 prior lines of chemotherapy in the advanced setting, 51% had elevated levels of lactate dehydrogenase (but <2.5 times the upper normal limit, according to eligibility criterion), and 74% had visceral metastases. Sixty-two percent of patients had PD-L1-positive tumors based on the PD-L1 IHC 22C3 pharmDx assay, with positivity defined as a combined positive score ≥1% (the number of PD-L1–positive cells of the total number of tumor cells multiplied by 100). After a median follow-up of 10.9 months, 5% of patients remained on treatment. The ORR was 4.7%. In this cohort, similar responses were observed both in patients with PD-L1–positive tumors and in those with PD-L1–negative tumors (ORR, 4.8% and 4.7%, respectively). It is noteworthy that no responses were observed in patients who had liver metastases. The median duration of response was 6.3 months (range, 1.2-10.3 months). Treatment-related AEs of any grade occurred in 60% of patients, and grade 3 or 4 AEs occurred in 12%. Immune-related AEs of any grade occurred in 19% of patients, of which 1.2% were grade 3 or 4, and the most common were hypothyroidism, hyperthyroidism, and pneumonitis. Four percent of patients discontinued treatment because of AEs, and there were no AE-related deaths.

TABLE 2. Reported Trials of Programmed Death 1-Receptor/Programmed Death-Ligand 1 Therapeutics in Metastatic Breast Cancer

Study Phase and Agents	Population and No. (Screened, Enrolled, Evaluable)	Primary Endpoint	Proportion With No Prior Chemotherapy in a Metastatic Setting, %	Safety	ORR, %	Median PFS, mo	Median OS, mo	References
Monotherapy								
Phase 1b: Pembrolizumab	mTNBC, PD-L1+ (111 screened, 65 PD-L1+, 32 enrolled, 27 evaluable for efficacy)	Safety	15.6	At least 1 grade 3-5 AE in 5 patients (15.6%); 5 grade 3 AEs (anemia, aseptic meningitis, lymphopenia, headache, pyrexia); 1 patient died from DIC accompanied by grade 4 decreased blood fibrinogen	18.5	1.9	11.2	Nanda 2016[34] (KEYNOTE-012; JCO)
Phase 1a: Atezolizumab	mTNBC, PD-L1+, later expanded to include PD-L1− (enrolled 115)	Safety	17	Grade 3-5 AEs in 11% (5 grade 3 events [adrenal insufficiency, neutropenia, nausea, vomiting, decreased WBC count], 1 grade 5 pulmonary hypertension)	10 (13% PD-L1+, 5% PD-L1−)	1.4	9.3	Emens 2015[35] (AACR); Schmid 2017[27] (AACR)
Phase 1b: Avelumab	mBC, PD-L1+/PD-L1− (168 enrolled, including 58 with TNBC)	Safety	0 (for TNBC)	At least 1 grade 3-5 AE in 24 patients (14.3%), including fatigue, anemia, increased GGT, arthralgia, and irAEs with autoimmune hepatitis (1.8%); 2 treatment-related deaths (acute liver failure, respiratory distress)	4.8 (33% PD-L1+, 2.4% PD-L1−)			Dirix 2015[36] (JAVELIN, SABCS)
Phase 2: Pembrolizumab	mTNBC, PD-L1+/PD-L1− (386 screened, 170 enrolled)	Safety and efficacy	0	Grade 3-4 AEs in 12%; 19%, irAEs of any grade, of which 1.2% were grade 3-4 (most common were hypothyroidism, hyperthyroidism,	4.7 (4.8% PD-L1+, 4.7% PD-L1−)	2	8.9	Adams 2017[28] (KEYNOTE-086, cohort A; ASCO)

TABLE 2. *Continued*

Study Phase and Agents	Population and No. (Screened, Enrolled, Evaluable)	Primary Endpoint	Proportion With No Prior Chemotherapy in a Metastatic Setting, %	Safety	ORR, %	Median PFS, mo	Median OS, mo	References
				and pneumonitis); no grade 5 AEs				
	mTNBC, PD-L1+ (167 screened, 79 PD-L1+, 52 enrolled)	Safety	100	Grade 4 (8%) with grade 3-4 AEs (back pain, fatigue, hyponatremia, hypotension, and migraine); no grade 5 AEs	23.1	2.1	NA	Adams 2017[29] (KEYNOTE-086, cohort B; ASCO)
Combination								
Phase 1b: Atezolizumab plus nab-paclitaxel	mTNBC, PD-L1+/ PD-L1−	Safety	40.6	Most common AE was decreased neutrophil count (53% all grades; 41% grade 3-4); no grade 5 AEs	38	NA	NA	Adams 2016[30] (ASCO)
Phase 1b/2: Pembrolizumab plus eribulin mesylate	mTNBC, PD-L1+/ PD-L1− (39 enrolled)	Safety and efficacy	43.6	Most common grade 3-4 AEs were neutropenia (31%) and fatigue (8%); SAEs in 36% of patients (no grade 5)	33.3	NA	NA	Tolaney 2016[37] (SABCS)

Abbreviations: −, negative; +, positive; AACR, American Association for Cancer Research abstract; AEs, adverse events; ASCO, American Society of Clinical Oncology abstract; DIC, disseminated intravascular coagulation; GGT, γ-glutamyl transferase; JCO, the *Journal of Clinical Oncology*; mBC, metastatic breast cancer; mTNBC, metastatic triple-negative breast cancer; NA, not applicable; ORR, overall response rate; PFS, progression-free survival; OS, overall survival; PD-L1, programmed death-ligand 1; SABCS, San Antonio Breast Cancer Symposium abstract; SAEs, serious adverse events; TNBC, triple-negative breast cancer; TRAE, treatment-related adverse events; WBC, white blood cell.

Cohort B of the KEYNOTE-086 trial evaluated pembrolizumab as first-line therapy for patients who had PD-L1–positive (defined as ≥1% PD-L1–positive cells, whether tumor or immune cells) mTNBC.[29] Of the first 52 patients enrolled, the median age was 53 years, 40% had elevated lactate dehydrogenase levels, 69% had visceral metastases, and 87% had received prior neoadjuvant or adjuvant chemotherapy. After a median follow-up of 7.0 months (range, 4.4-12.5 months), 29% of patients remained on treatment. The preliminary ORR was 23.1% (95% CI, 14%-36%), and the median duration of response was 8.4 months (range, 2.1-8.4 months). These results add to the observed trend of improved ICB responsiveness with earlier treatment in mTNBC.

The phase 1b trial JAVELIN study (Avelumab in Metastatic or Locally Advanced Solid Tumors; NCT01 7724) included metastatic breast cancer, and patients did not require tumors PD-L1–positive tumors for eligibility.[36] Avelumab is a monoclonal antibody that binds to PD-L1. In this trial, the ORR was 8.6% (95% CI, 2.9-19) for patients with TNBC (n = 58). Five patients had a PR, and 13 had stable disease. PD-L1 expression determined by IHC on tumor cells was not predictive of a clinical response to avelumab; however, tumors that contained *hotspots* of PD-L1 immune cells (ie, PD-L1 expression in ≥10% of immune cells within the tumor) had response rates of 44% (4 of 9 patients). Among the 5 responders with TNBC, 4 (80%) had immune cell hotspots in their tumors.

None of the reported ICB monotherapy trials in patients with mTNBC had a median progression-free survival (PFS) longer than that in historic chemotherapy controls, indicating that therapeutic benefit is limited to a minority of patients, predictive biomarkers for patient selection are needed, and combination therapies should be investigated. However, preliminary data across these studies do suggest that survival is promising for those patients who achieve responses to ICB monotherapy.[27-29]

COMBINATION WITH ANTI–PD-1/PD-L1–CONTAINING THERAPY IN MTNBC

Although the use of immune-checkpoint inhibitors has exhibited activity as a single agent in TNBC, only a small group of patients derive a benefit. An important challenge is to reliably identify the responders. Efforts are now focusing on combination strategies with potential synergistic effects, primarily with cytotoxic chemotherapy, to improve ORR and clinical outcomes. Combining immunotherapy with chemotherapy may enhance tumor-specific T-cell immunity by exposing the immune system to high levels of tumor antigens (immunogenic cell death), lowering tumor burden and depleting suppressive immune cell populations (eg, regulatory T cells), and modulating T-cell and NK-cell function.[38] Preclinical evidence in several tumor types suggests the synergy of chemoimmunotherapy and sensitization of tumors to ICB by chemotherapy.[39]

To date, results from 2 trials in mTNBC have been presented in which atezolizumab was combined with albumin-bound paclitaxel (nab-paclitaxel)[29] and pembrolizumab combined with eribulin mesylate[37] respectively (Table 2). The phase 1b study of atezolizumab and nab-paclitaxel was performed in patients (n = 32) who had received from 0 to 2 prior lines of therapy in the metastatic setting and were unselected for PD-L1 status (NCT01633970).[29] Of the 32 patients who were evaluable for efficacy, the ORR was 38% (95% CI, 21%-56%), and it is noteworthy that the ORR in the first-line setting was 46% (95% CI, 19%-75%). The median follow-up was 5.21 months (range, 0.6-12.6 months), the median patient age was 56 years (range, 32-84 years), and 87% of patients had received prior taxane chemotherapy. Responses were observed both in patients with tumors that expressed PD-L1 and in those with tumors that had little or no PD-L1 expression and appeared to be more frequent in the first-line compared with subsequent line settings. Although PD-L1 was not identified as a predictive biomarker in that trial (which is not unexpected in a combination trial with chemotherapy), a trend linking baseline TIL levels to responses was observed and warrants additional study. It is encouraging to note that the authors observed durable responses, which persisted after nab-paclitaxel was discontinued. The most common treatment-related AE was decreased neutrophil count (53% all grade; 41% grades 3-4). No dose-limiting toxicity or related deaths occurred.

The phase 1b/2 trial of pembrolizumab and eribulin mesylate involved 89 patients with mTNBC. The median patient age was 53 years (range, 32-80 years), 43.6% had received no prior chemotherapy, and 56.4% had received 1 or 2 prior lines of chemotherapy (NCT03051659).[37] Forty-four percent of patients had tumors that were PD-L1–positive, which was defined as staining in the stroma or in ≥1% tumor cells. Of the 39 patients who were evaluable at the interim analysis, the ORR in the combination arm was 33.3% (95% CI, 19.5%-48.1%). The ORR for patients who had untreated mTNBC (n = 17) increased to 41.2% (95% CI, 19.3-62.8); and for those who had received 1 or 2 previous therapies, the ORR was lower at 27.3% (95% CI, 11.3%-46.4%). There were no

differences in responses among patients who had PD-L1–positive tumors (n = 17; ORR, 29.4%) or PD-L1–negative tumors (n = 18; ORR, 33.3%). Overall, 66.7% of patients had grade 3 or 4 treatment-related AEs. The most common grade 3 and 4 AEs were neutropenia (30.8%) and fatigue (7.7%). Sixty-seven percent of patients experienced possible immune-related AEs, of which 12.8% were grade 3 and 4 events and included hypothyroidism, rash, hyperglycemia, hyperthyroidism, and pneumonitis. In summary, in the reported chemoimmunotherapy trials for mTNBC, no new or additive safety signals were identified.

NEOADJUVANT THERAPY WITH ANTI–PD-1/PD-L1 ANTIBODIES IN TNBC

It has been observed that patients with TNBC who achieve a pathologic CR (pCR) after neoadjuvant chemotherapy have improved survival, whereas those who have residual disease are at higher risk for recurrence.[40] The recently reported phase 2 randomized, multicenter I-SPY2 study (an adaptive trial platform to test novel agents) achieved an increase in pCR in patients who had locally advanced (stage II/III) TNBC or hormone receptor (HR [ie, ER and PR])-positive/HER2-negative breast cancer when pembrolizumab was added to the taxane segment of the standard neoadjuvant regimen, followed by doxorubicin and cyclophosphamide (NCT01042379) (Table 3).[41-44] In total, 249 patients were randomized, of whom 69 were to receive pembrolizumab in combination with paclitaxel, 180 were to receive paclitaxel alone in the control arm, and all patients then continued to receive neoadjuvant doxorubicin and cyclophosphamide. The findings indicated that the estimated pCR rate (negative pathologic post-treatment tumor classification/tumor in situ and negative pathologic post-treatment lymph node status [ypT0/Tis ypN0]) was significantly higher with the addition of pembrolizumab in patients who had TNBC (60% vs 20%, respectively) and in those who had HR-positive/HER2-negative breast cancer (34% vs 13%) compared with standard therapy, and the predictive probability of success in a phase 3 trial was 99%. No additive toxicities were observed except for an excess of adrenal insufficiency in the pembrolizumab arm, which appeared to be greater than that reported from chemoimmunotherapy trials conducted in other cancer types. This finding is significant, because management requires long-term steroid replacement for patients who are treated in the curative setting.

Preliminary results from additional randomized neoadjuvant trials in TNBC combining standard cytotoxic regimens with ICB also recently were reported (Table 3). The phase 1b KEYNOTE-173 study evaluated pembrolizumab plus chemotherapy as neoadjuvant therapy for locally advanced TNBC in 2 cohorts (NCT02622074).[42] Preliminary data were reported from 10 patients in cohort A (single-dose pembrolizumab, followed by 4 cycles of pembrolizumab every 3 weeks in combination with weekly nab-paclitaxel, followed by 4 cycles of pembrolizumab in combination with doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 every 3 weeks) and 10 patients in cohort B (the same treatment as in cohort A plus carboplatin at an area under curve of 6 every 3 weeks was added to pembrolizumab and nab-paclitaxel). The ypT0/Tis ypN0 pCR rate (no invasive residual disease in the breast and lymph nodes) was 60% (90% CI, 30%-85%) in cohort A and 90% (90% CI, 61%-100%) in cohort B.

In the randomized phase 2 GeparNuevo study, durvalumab, a monoclonal antibody that binds to PD-L1, was added to an anthracycline-containing and taxane-containing neoadjuvant regimen for patients with TNBC (NCT02685059).[43] The patients received durvalumab or placebo monotherapy for the first 2 weeks (window phase), followed by durvalumab or placebo plus nab-paclitaxel weekly for 12 weeks, followed by durvalumab or placebo plus epirubicin and cyclophosphamide every 2 weeks for 4 cycles. Of the 50 patients enrolled, the addition of ICB to standard neoadjuvant chemotherapy did not identify unexpected toxicity, and most AEs included chemotherapy-related toxicities. In addition, the safety of administering durvalumab concomitant with sequential taxane and anthracycline neoadjuvant chemotherapy is being studied in a phase 1/2 single-arm trial of stage I through III TNBC (NCT02489448). Patients received durvalumab in combination with weekly nab-paclitaxel, followed by dose-dense doxorubicin and cyclophosphamide with durvalumab. The phase 1 portion established the safety of the combination,[44] and the ongoing phase 2 portion of the trial will assess the efficacy of the combination (n = 50).

ONGOING TRIALS WITH ICB

Tables 4 and 5 list select, ongoing clinical trials involving ICB in the metastatic and neoadjuvant/adjuvant settings, respectively.

Ongoing Monotherapy Trials in mTNBC

The randomized, global phase 3 KEYNOTE-119 trial will compare the efficacy and safety of pembrolizumab monotherapy versus single-agent chemotherapy per

TABLE 3. Reported Trials of Programmed Death 1-Receptor/Programmed Death-Ligand 1 Therapeutics in Neoadjuvant Triple-Negative Breast Cancer

Study Phase and Agents	Population	Safety	pCR Rate	References
Randomized phase 2: Paclitaxel ± pembrolizumab → AC every 2-3 wk	Stage II/III TNBC (n = 29) or HR-positive/HER2-negative (n = 40)	AI in 6 patients, at least 3 related to hypophysitis (secondary AI), 5 late (after completion of AC), and 1 during pembrolizumab; variable presentation (nausea, vomiting, fatigue, weakness)	yT0/Tis ypN0 60% (with pembrolizumab) vs 20% in TNBC	Nanda 2017[41] (I-SPY2; ASCO)
Randomized phase 1b: Pembrolizumab → pembrolizumab plus nab-paclitaxel (± carboplatin) → pembrolizumab plus AC	Locally advanced TNBC: Cohort A, without carboplatin (n = 10); cohort B, with carboplatin (n = 10)	Grade 3-4 AEs reported for 8 patients in cohort A and 10 in cohort B; 1 patient in cohort A and 2 in cohort B discontinued treatment for AEs (2 had ALT elevations with pembrolizumab, 1 had DVT with chemotherapy)	yT0/Tis ypN0 60% in cohort A and 90% in cohort B	Schmid 2017[42] (KEYNOTE-173 ASCO)
Randomized phase 2: Durvalumab/PLA → durvalumab/PLA plus nab-paclitaxel → durvalumab/PLA plus EC	cT1b-c to T4a-d TNBC (n = 50)	Ten patients (20%) with at least 1 grade 3-4 AE, 4 SAEs, and 5 irAEs	Not yet reported	Loibl 2017[43] (GeparNuevo; ASCO)
Phase 1/2: Durvalumab concomitant with weekly nab-paclitaxel and dose-dense AC	Stage I-III TNBC: phase 1 (n = 7), phase 2 (n = 50)	Grade 2 fatigue (n = 1) and grade 3 dehydration and dyspnea (n = 1); no grade 4/5 AEs	Not yet reported	Pusztai 2017[44] (ASCO)

Abbreviations: ±, with or without; AC, doxorubicin plus cyclophosphamide; AEs, adverse events; AI, adrenal insufficiency; ALT, alanine aminotransferase; ASCO, American Society of Clinical Oncology; cT, clinical tumor classification; DVT, deep vein thrombosis; EC, epirubicin plus cyclophosphamide; HR, hormone receptor; HER2, human epidermal growth factor receptor 2; irAEs, immune-related adverse events; pCR, pathologic complete response; PLA, placebo; SAEs, serious adverse events; Tis, tumor in situ; TNBC, triple-negative breast cancer; TRAE, treatment-relate adverse event; ypN, pathologic post-treatment lymph node status; ypT, pathologic post-treatment tumor classification; yT, post-treatment tumor classification.

TABLE 4. Select Ongoing Trials of Programmed Death 1-Receptor/Programmed Death-Ligand 1 Therapeutics in Metastatic Triple-Negative Breast Cancer (None Selected by Programmed Death-Ligand 1 Status)

Study Phase and Title	Study Agents	Population	Target/Enrollment	Primary Endpoint	NCT ID No.
Monotherapy					
Randomized phase 3, KEYNOTE-119: Study of single-agent pembrolizumab vs single-agent chemotherapy for mTNBC	Pembrolizumab or single-agent chemotherapy as chosen by treating physician (capecitabine, gemcitabine, eribulin, or vinorelbine)	mTNBC, previously treated with anthracycline and/or taxane in neoadjuvant/adjuvant/metastatic setting and received 1-2 prior systemic treatments in metastatic setting	600/Completed	PFS, OS	NCT0255565
Combination					
Randomized phase 3, IMPASSION-130: Study of atezolizumab in combination with nab-paclitaxel vs placebo with nab-paclitaxel for participants with previously untreated mTNBC	Nab-paclitaxel ± atezolizumab	mTNBC, no prior systemic therapy	900/Completed	PFS, OS	NCT02425891

Abbreviations: ±, with or without; mTNBC, metastatic triple-negative breast cancer; nab-paclitaxel, albumin-bound paclitaxel; NCT ID, National Clinical Trials identifier (www.clinicaltrials.gov); OS, overall survival; PD-L1, programmed death-ligand 1; PFS, progression-free survival.

investigator's choice in patients receiving second-line or third-line treatment for mTNBC (NCT02555657).[45] Patients must have received previous treatment with an anthracycline and/or a taxane in the neoadjuvant, adjuvant, or metastatic setting. The single-agent chemotherapy per investigator's choice is capecitabine, gemcitabine, eribulin, or vinorelbine. Randomization is stratified by PD-L1 tumor status and history of prior neoadjuvant or adjuvant chemotherapy versus de novo metastatic disease. Primary endpoints are PFS and OS based on Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1). Secondary endpoints include safety, ORR, response duration, and the disease-control rate. The study has completed recruitment of 600 patients, and results are pending.

Ongoing Combination Trials of Chemoimmunotherapy in mTNBC

The phase 3, randomized IMPASSION-130 trial is investigating the combination of nab-paclitaxel with or without atezolizumab in the first-line setting in TNBC (NCT02425891) and has completed accrual.[46] Stratification factors include the presence of liver metastases and receipt of prior taxane therapy. Eligibility included histologically documented, locally advanced TNBC or mTNBC; no prior systemic therapy for advanced TNBC (adjuvant/neoadjuvant chemotherapy was allowed); a washout period of at least 12 months from prior taxanes; an ECOG performance of 0 or 1; and measurable disease according to RECIST v1.1. Coprimary endpoints include PFS and OS, and the total sample size is 900. Other endpoints include safety, response rate, and response duration. Tumor biopsies will be obtained at baseline and at the time of disease progression to assess biomarkers of response and immune escape.

Smaller trials, such as the phase 2 study of pembrolizumab and nab-paclitaxel in patients who received $\leq$2 prior lines of therapy for metastatic, HER2-negative breast cancer (n = 50; NCT02752685), also incorporate serial tumor biopsies during treatment to assess changes in the tumor immune microenvironment from baseline with chemotherapy alone (cycle 1) and then with chemoimmunotherapy (cycle 2 and subsequent cycles).[47]

Neoadjuvant Trials

The phase 3 KEYNOTE-522 clinical trial will evaluate pembrolizumab in combination with neoadjuvant chemotherapy for TNBC (NCT03036488). The target accrual will be >800 patients, with the primary endpoint of pCR. This is a randomized, placebo-controlled trial enrolling patients with clinical stage IIB through IIIB TNBC who first will be randomized to receive either pembrolizumab or placebo with weekly paclitaxel and carboplatin (weekly or every 3 weeks) for 4 cycles. This will be followed by treatment with pembrolizumab or placebo

TABLE 5. Select Ongoing Trials of Programmed Death 1-Receptor/Programmed Death-Ligand 1 Therapeutics in Neoadjuvant and Adjuvant Therapy for Triple-Negative Breast Cancer

Study Phase and Title	Study Agents	Population	Target/Enrollment	Primary Endpoint	NCT ID No.
Neoadjuvant					
Randomized phase 3. IMPASSION-031: Study of atezolizumab plus chemotherapy vs placebo plus chemotherapy as neoadjuvant therapy and atezolizumab vs placebo as adjuvant therapy in participants with TNBC	Atezolizumab (or placebo) plus nab-paclitaxel → AC → surgery → complete 1 y of atezolizumab (or placebo)	cII-cIII TNBC	204/Ongoing	pCR (ypT0/Tis ypN0)	NCT03197935
Randomized phase 3. KEYNOTE-522: Study of pembrolizumab plus chemotherapy vs placebo plus chemotherapy as neoadjuvant therapy and pembrolizumab vs placebo as adjuvant therapy in participants with TNBC	Pembrolizumab (or placebo) plus paclitaxel plus carboplatin → pembrolizumab (or placebo) plus AC → surgery → pembrolizumab (or placebo) for 9 cycles	cIIB-cIIIB TNBC	800/Ongoing	pCR (ypT0/Tis ypN0)	NCT03036488
Randomized phase 3. Neoadjuvant therapy in TNBC with anti–PD-L1 (MPDL3280A)	Experimental: Atezolizumab plus carboplatin plus nab-paclitaxel → surgery → AC or EC or FEC; Control: carboplatin plus nab-paclitaxel → surgery → AC or EC or FEC	Locally advanced TNBC	272/Ongoing	EFS	NCT02620280
Adjuvant					
Randomized phase 3. Efficacy and safety of pembrolizumab as adjuvant therapy for TNBC with ≥1 cm residual invasive cancer or positive lymph nodes after neoadjuvant chemotherapy	Pembrolizumab × 1 y vs no intervention (observation)	Residual disease after neoadjuvant chemotherapy (residual tumor 1 cm and/or axillary lymph node-positive)	1000/Ongoing	DFS	NCT02954874
Randomized phase 3. A-BRAVE: Adjuvant treatment for patients who have high-risk TNBC with the anti–PD-L1 antibody avelumab	Avelumab × 1 y vs no intervention (observation)	Four or more axillary lymph nodes and adjuvant chemotherapy, including anthracycline and taxane OR evidence of residual disease after neoadjuvant chemotherapy	335/Ongoing	DFS	NCT02926196

Abbreviations: AC, doxorubicin plus cyclophosphamide; cII-III, clinical stage II and III; DFS, disease-free survival; EC, epirubicin plus cyclophosphamide; EFS, event-free survival; FEC, 5-fluorouracil plus epirubicin plus cyclophosphamide; nab-paclitaxel, albumin-bound paclitaxel; NCT ID, National Clinical Trials identifier (www.clinicaltrials.gov); pCR, pathologic complete response; PD-L1, programmed death-ligand 1; Tis, tumor in situ; TNBC, triple-negative breast cancer; ypN, pathologic post-treatment lymph node status; ypT, pathologic post-treatment tumor classification.

for 4 cycles in combination with doxorubicin and cyclophosphamide. Surgery will then be followed by adjuvant pembrolizumab or placebo for 9 cycles.

There are other ongoing neoadjuvant studies, including the phase 2 portion of concomitant durvalumab with weekly nab-paclitaxel followed by doxorubicin and

cyclophosphamide in stage I through III TNBC (NCT02489448), a phase 2 neoadjuvant study evaluating atezolizumab and nab-paclitaxel in TNBC (NCT02530489), a phase 3 neoadjuvant trial of the addition of atezolizumab to carboplatin and nab-paclitaxel versus a control arm of carboplatin and nab-paclitaxel in patients with locally advanced TNBC (NCT02620280), and a phase 3 neoadjuvant trial evaluating the addition of atezolizumab to nab-paclitaxel followed by doxorubicin and cyclophosphamide in stage II and III TNBC (IMpassion031; NCT03197935).

Adjuvant Trials

Several clinical trials have been designed to evaluate the role of ICB as adjuvant therapy in patients with TNBC who have residual disease, because the presence of residual disease after neoadjuvant treatment predicts a poor prognosis. The phase 3 trial (Southwest Oncology Group [SWOG]-S1418, BR006) will evaluate adjuvant treatment with pembrolizumab in patients with TNBC (n = 1000) who have completed definitive local therapy (NCT02954874). This is a collaborative effort led by SWOG and NRG Oncology and is sponsored by the National Cancer Institute. Eligible patients are those who did not achieve a pCR after at least 16 to 24 weeks of neoadjuvant chemotherapy followed by surgery, with residual tumor $\geq$1 cm and/or axillary lymph node-positive disease. Randomization is to either 12 months of treatment with pembrolizumab or observation. The primary endpoint is invasive disease-free survival. Tissues will be collected to enable correlative analyses of predictive markers.

The A-BRAVE trial is a phase 3 randomized study investigating adjuvant treatment with avelumab in 335 patients with TNBC (NCT02926196). Patients who have completed definitive curative therapy, including surgery, adjuvant chemotherapy, and radiotherapy, are eligible if they have more than 4 involved axillary lymph nodes and their adjuvant chemotherapy included at least 3 courses of an anthracycline and 3 courses of a taxane. Patients who undergo neoadjuvant chemotherapy must have pathologic evidence of residual, invasive carcinoma in the breast and/or axillary lymph nodes. Patients will be randomized to receive either adjuvant avelumab for 1 year or no intervention. The primary outcome measure is disease-free survival.

ICB and Vaccine Strategies

The role of therapeutic vaccines for breast cancer has been studied extensively and has demonstrated the generation of T-cell immunity; however, clinical efficacy has been less robust. Breast cancer expresses different tumor-associated antigens, such as HER2, mucin 1 (MUC1), telomerase, and mammoglobin, among others, which have been the target of vaccine development.[48] Many early breast cancer vaccine studies focused on targeting HER2, which had demonstrated promise in early phase trials but less a clear of benefit in larger trials.[49,50] Similar results were observed with another antigen target, MUC1 (with its epitope sialyl-Tn), which is a glycoprotein highly expressed in breast cancer and contributes to tumor growth and metastases.[51,52]

With methods using vaccines alone, the T-cell response may be weak or may be hindered by immune suppression from the tumor microenvironment. With the rapid development and recent clinical successes observed using ICB, strategies that combine vaccines to accelerate T-cell priming and activation with checkpoint inhibitors to block immune-suppressive pathways have emerged as attractive options to generate more effective T-cell responses. Several pilot studies combining breast cancer vaccines with PD-1/PD-L1 antibodies are now under investigation in clinical trials, including in the metastatic setting (durvalumab in combination with the Vigil vaccine, which is comprised of granulocyte macrophage-colony–stimulating factor [GM-CSF] and bifunctional short-hairpin RNA silencer furin vector-transfected, autologous tumor cells; NCT02725489) and the adjuvant setting for stage II and III TNBC (durvalumab and the peptide vaccine PVX-410; NCT02826434).[53]

Combination Dual-Checkpoint Blockade Therapy

The combination of nivolumab, an anti–PD-1 antibody, and ipilimumab, an anticytotoxic T-lymphocyte antigen-4 (CTLA-4) antibody, has demonstrated durable responses compared with monotherapy with ipilimumab and nivolumab alone in patients with metastatic melanoma; however, the combination is associated with greater toxicity.[54,55] In breast cancer, CTLA-4 blockade alone appears to be minimally active, at least in HR-positive breast cancer (in combination with endocrine therapy). The CTLA-4–blocking antibody, tremelimumab, was studied with exemestane in patients (n = 26) with metastatic, ER-positive breast cancer.[56] Results indicated limited clinical activity, and the best overall response was stable disease for at least 12 weeks in 42% of patients. Combinations of PD-L1 with CTLA-4 antibodies currently are being studied in breast cancer, such as the ongoing, randomized phase 1/2 open-label trial of nivolumab plus ipilimumab versus nivolumab alone for patients with

metastatic solid tumors, which includes those with TNBC (NCT01928394), as well as the single-arm phase 2 study of durvalumab in combination with tremelimumab for patients with metastatic, HER2-negative breast cancer (NCT02536794).

Other trials focused on early efforts with novel antibody combinations to help generate and amplify T-cell responses include the study of stimulatory agonist antibodies such as OX40 (cluster of differentiation 134 [CD134], also known as tumor necrosis factor superfamily, member 4), which is expressed in TILs and activated immune cells (NCT01862900); histone deacetylase (HDAC) inhibitors, such as the randomized phase 2 trial of atezolizumab with or without entinostat in patients with mTNBC (NCT02708680); monoclonal antibodies that target the inducible T-cell costimulator (ICOS) protein on the surface of T cells, stimulate effector T cells, and reduce regulatory T cells, such as JTX-2011 (ICONIC study; NCT02904226); and antibodies against T-cell immunoreceptor with immunoglobulin and immunoreceptor tyrosine-based inhibition motif (anti-TIGIT), such as OMP-31M32 (NCT03119428).

Combined ICB and Cryotherapy or Radiotherapy

Thermal ablation treatment, or cryoablation, involves the insertion of a thermal probe into tumor tissue with the administration of freezing temperatures, leading to tumor lysis. This mechanism may facilitate an antitumor immune response through the enhanced release of tumor antigens. In a preclinical murine model of prostate cancer that combined tumor cryoablation with ipilimumab, the combination led to control of the growth of metastases.[57] Limited data exist in TNBC, and a presurgical study was performed of single-dose ipilimumab received either alone or in combination with cryoablation by patients with early breast cancer.[58] Of 19 patients who received either cryoablation, ipilimumab, or both, only 3 had TNBC. The combination was identified as safe and well tolerated, and favorable immunologic effects were observed, including increased Th1-cytokine production, intratumoral proliferation of effector T cells compared with regulatory T cells, and peripheral T-cell proliferation and activation. An ongoing trial is now exploring preoperative cryoablation combined with both ipilimumab and nivolumab in early stage breast cancer (NCT02833233).

Radiotherapy is another treatment modality that may induce and/or enhance immune responses and work synergistically with ICB. Radiation can increase tumor mutation burden and induce the release of tumor antigens during tumor cell death, in addition to proinflammatory signals that trigger the innate immune system to activate antitumor T-cell responses.[59,60] In the initial pilot study using a poorly immunogenic murine model of metastatic breast cancer refractory to anti–CTLA-4 therapy, the combination of local radiation to the primary tumor with CTLA-4 blockade demonstrated synergistic inhibition of tumor growth and metastases as well as improved survival.[61] This synergy also has been observed in combination with PD-1/PD-L1 blockade.[62-64] In the clinical setting, the combination of radiotherapy with ICB has been studied in trials of other tumor types, such as melanoma, lung cancer, and prostate cancer, with various responses.[65-67]

There are several ongoing clinical studies in breast cancer evaluating radiotherapy with ICB. These include a phase 2 trial of pembrolizumab plus radiotherapy in mTNBC (NCT02730130); a phase 1 trial of stereotactic radiosurgery for oligometastatic disease in combination with pembrolizumab (NCT02303366); a phase 1 trial of pembrolizumab with hypofractionated radiotherapy in patients with advanced cancers, including breast cancer, who have progressed after at least 1 regimen of systemic therapy (NCT02303990); and a phase 2 trial of nivolumab after induction therapy with radiation or chemotherapy (low-dose doxorubicin, cyclophosphamide, or cisplatin) in patients with mTNBC (NCT02499367). Although the advantages of radiotherapy as a combination partner are many, such as individualized treatment (in situ auto vaccine), universal applicability as a standard modality, and the ability to radiate single progressing lesions, ultimately, a randomized trial of ICB with and without radiotherapy is needed to establish additive/synergistic immune effects in the setting of systemic ICB.

CHALLENGES, NOVEL APPROACHES, AND FUTURE OPPORTUNITIES IN TNBC: WHERE DO WE GO FROM HERE?

Currently, as heightened awareness of the role of immunobiology in cancer grows and the number of clinical trials evaluating immunotherapy in breast cancer continues to expand rapidly, a limitation of this approach is that overall response rates in the advanced setting are relatively modest compared with the rates achieved in other immunogenic cancer types like melanoma and lung cancer. Although some patients with breast cancer may experience a dramatic response to ICB, which can be durable for years, only a few appear to benefit.

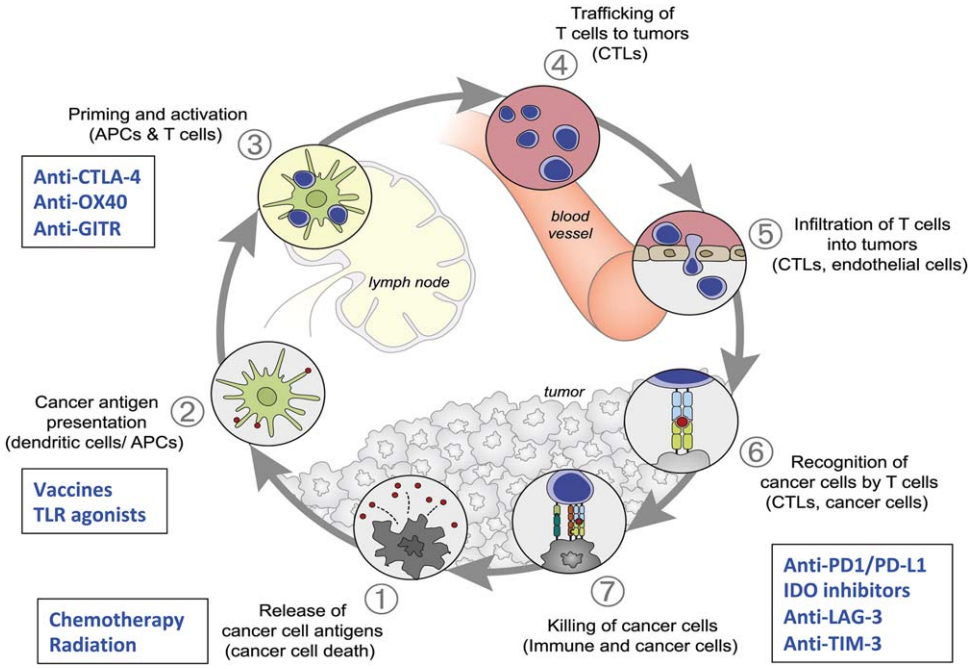

Figure 1. The cancer-immunity cycle and immunotherapy targets are illustrated. The development of an effective antitumor immune response depends on several steps, as depicted here. Tumors may escape immune detection by a variety of mechanisms, including loss of antigen and major histocompatibility complex (MHC) expression, expression of immunosuppressive cytokines, inhibition by suppressive T cells, and expression of inhibitory checkpoints (cytotoxic T-lymphocyte antigen-4 [CTLA-4] and programmed death-ligand 1 [PD-L1]). There is a wide range of potential therapeutic targets, for instance, antibody blockade of CTLA-4 or programmed death-1 (PD-1) receptor/PD-L1 axis results in activation of T cells and enhanced antitumor immune response. APCs indicates antigen-presenting cells; CTLs, cytotoxic T lymphocytes; GITR, glucocorticoid-induced tumor necrosis factor receptor family-related gene; IDO, indoleamine 2,3-dioxygenase; LAG-3, lymphocyte-activation gene 3 protein; OX40, cluster of differentiation 134 (CD134), also known as tumor necrosis factor superfamily, member 4; TIM-3, T-cell immunoglobulin domain and mucin domain-3; TLR, toll-like receptor. Adapted from: Chen DS, Mellman I. Oncology meets immunology: the cancer-immunity cycle. Immunity. 2013;39:1-10.[68]

Disease Setting

Approaches to improve outcomes in breast cancer include moving immunotherapy into an earlier disease setting. Studies of neoadjuvant ICB demonstrate promising preliminary efficacy. However, potential safety concerns must be addressed when combining ICB with multiagent chemotherapies and exposing potentially curable patients. Adjuvant trials, in contrast, can select patients who have residual disease after neoadjuvant treatment, which is indicative of chemotherapy-resistant disease and a poorer prognosis. Which approach is more beneficial remains to be studied, because the use of ICB may have different efficacy in microscopic disease in the adjuvant setting versus macroscopic disease in the neoadjuvant setting, in treatment-naive versus chemotherapy-refractory cancers, and with or without concurrent chemotherapies. In the metastatic setting, studies have suggested greater response rates in first-line versus later line settings for ICB monotherapy, which should be taken into consideration for trial development and for patients who are considered for compassionate use of ICB outside of clinical trials.

Combinations

The use of ICB alone is likely an insufficient therapeutic approach in breast cancer; therefore, combining ICB with antigen-directed therapies like chemotherapy, radiotherapy, targeted therapies, or other novel immunotherapies and combinations may enhance the development of immunogenic tumors through both generation and amplification of T-cell response and inhibition of immune checkpoints (Fig. 1).[68] In addition, several other promising, novel checkpoint molecules are actively being investigated in malignancy, including breast cancer. These include lymphocyte-activation gene 3 (LAG-3) which is coexpressed with PD-1 on T cells and inhibits T-cells activation and proliferation; and T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3), which also is coexpressed frequently with PD-1 and suppresses the

activation of effector T cells. Antibodies targeting LAG-3 and TIM-3 are being studied in the preclinical and clinical settings and have demonstrated evidence of efficacy.[69,70] Furthermore, other immunoregulatory molecules that are being explored as therapeutic agents include stimulatory molecules, such as OX40 and glucocorticoid-induced tumor necrosis factor receptor family-related (GITR), and inhibitory molecules, such as ICOS, TIGIT, and indoleamine 2,3-dioxygenase (IDO1).[71]

Biomarkers

Selecting patients for PD-L1 expression within the tumor microenvironment may help enrich for responses, as demonstrated recently in a patient with metaplastic breast cancer who had an unprecedented response to ICB.[72] However, assay variability remains an issue, and 2 recent TNBC studies with greater than 100 patients each demonstrated little or no differences in response according to PD-L1 status. Therefore, identifying other biomarkers of therapeutic response or resistance will be vital for using ICB most effectively. TILs have emerged as a predictive biomarker, and histopathologic enumeration has been standardized successfully by a global working group[18] (the International TILs Working Group; available at: https://www.tilsinbreastcancer.org, Accessed November 22, 2017). In a recent study that analyzed data from the phase 2 KEYNOTE-086 trial (NCT02447003), in which patients with mTNBC received pembrolizumab monotherapy whether they had tumors with PD-L1 expression (cohort A, previously treated) or tumors that were PD-L1–positive (cohort B, previously untreated), patients who had high levels of TILs were more likely to respond to treatment.[73] Of the 223 patients enrolled, 193 had evaluable tumor samples, including 147 from cohort A and 46 from cohort B. Stromal TILs were quantified on hematoxylin and eosin-stained tumor sections. Cohort B had higher median TIL levels compared with cohort A in lymph node versus nonlymph node samples. The median TIL levels in responders and nonresponders in cohort A were 10% (range, 7.5%-25%) and 5% (range, 1%-10%), respectively, and 50% (range, 5%-70%) and 15% (range, 5%-37.5%), respectively, in cohort B. The ORR in cohort A for patients who had greater than median TIL levels was 6% versus 2% for those who had less than median TIL levels, and the ORR was 39% versus 9%, respectively, for the same groups in cohort B. When the cohorts were combined, patients with higher TIL levels had significantly improved ORRs (odds ratio, 1.26; 95% CI, 1.03-1.55; $P = .01$) and disease control rates (odds ratio, 1.22; 95% CI, 1.02-1.46; $P = .01$).

The mutation burden in breast cancer is relatively low compared with that in tumor types like lung cancer, bladder cancer, and melanoma.[74,75] In these other tumor types, the extent of nonsynonymous mutations correlates with the response to ICB.[76] There is heterogeneity in the mutation burden in breast cancer: One study examined mutation load distribution using whole-exome sequencing data available for 762 invasive breast tumors from The Cancer Genome Atlas.[74] Tumors that were ER-negative were characterized by significantly higher nonsynonymous mutations compared with tumors that were ER-positive. It would be worthwhile to explore whether the incorporation of therapies like platinum agents, poly (ADP-ribose) polymerase (PARP) inhibitors, and radiotherapy can help potentiate mutational load in these tumor subtypes.

Microsatellite instability status

Tumors with genetic defects in mismatch-repair pathways are known to harbor hundreds to thousands of somatic mutations, especially in regions of repetitive DNA known as microsatellites. The accumulation of mutations in these coding and noncoding regions of the genome is termed *microsatellite instability* (MSI). Tumors with high MSI (MSI-H) have significant up-regulation of immune-checkpoint proteins, including PD-1 and PD-L1,[77] and exhibit sensitivity to ICB. Recently, the first ICB received accelerated approval by the US Food and Drug Administration for the treatment of MSI-H or mismatch repair-deficient solid tumors. Although 2 patients with breast cancer were included in the approval data from 149 patients with MSI-H or mismatch repair-deficient cancers enrolled across 5 single-arm clinical trials, only a very small subset of tumors in breast cancer is MSI-H, with reported frequencies of less than 2%.[78-81]

Environmental Modifiers of Immunity Like the Microbiome

The gut microbiome may play an important role in breast cancer development and response to therapy.[82-84] In particular, commensal bacterial microbiota may influence the antitumor effects of checkpoint blockade with anti–PD-1/anti–PDL-1 and anti–CTLA-4 antibodies.[85,86] The gut bacterial microbiome was studied in a murine model of melanoma, and differences were observed in antitumor immunity among mice that had distinct commensal microbiota.[85] An antitumor effect was associated with the presence of *Bifidobacterium*. When a cocktail of *Bifidobacterium* species was administered orally to mice with tumors, these mice displayed improved antitumor

control compared with those not treated with *Bifidobacterium*. It is noteworthy that the mice receiving *Bifidobacterium* achieved control of tumor growth to the same degree as those that received PD-L1 checkpoint blockade; however, the mice that received the combination of both *Bifidobacterium* and PD-L1 had nearly complete resolution of tumor growth. This effect was mediated by the modulation of dendritic cell function, leading to improved effector function of CD8-positive T cells and accumulation in the tumor microenvironment. Similarly, another study demonstrated that, in both murine and human melanoma, *Bacteroides* played a role in antibody efficacy against CTLA-4.[86] These preliminary results demonstrated that gut bacterial microbial composition potentially may influence antitumor immunity as well as responses to ICB.

Several ongoing studies are evaluating the association of the gut microbiome with response to ICB, including in breast cancer (NCT02752685).[47]

CONCLUSION

TNBC is the most immunogenic breast cancer subtype, with higher PD-L1 expression and more TILs. In cancer, the PD-1/PD-L1 pathway is a common mechanism of immune escape used by tumor cells, and blockade of the PD-1/PD-L1 axis has emerged as a therapeutic target to enhance antitumor immunity. Initial clinical trials in TNBC have demonstrated promising activity with ICB. However, despite the encouraging results already described in reported studies, challenges remain to improve response rates, durability of responses, and OS. Novel combination immunotherapy approaches are actively being studied with ICB and chemotherapy, targeted therapies, other immune therapies, as well as radiotherapy in clinical trials in the metastatic, neoadjuvant, and adjuvant settings. The identification of improved biomarkers to predict for clinical benefit of patients who received treatment with PD-1/PD-L1 inhibitors and other immunotherapeutic agents is also an important area of investigation and is crucial for the optimal implementation of immunotherapy in women with TNBC.

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The authors made no disclosures.

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