Current Oncology Reports (2018) 20: 51
https://doi.org/10.1007/s11912-018-0701-2

BREAST CANCER (B OVERMOYER, SECTION EDITOR)

Checkpoint Inhibitors in the Treatment of Breast Cancer

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Published online: 30 April 2018

Abstract

Purpose of Review The treatment landscape for many cancers has dramatically changed with the development of checkpoint inhibitors. This article will review the literature concerning the use of checkpoint inhibitors in breast cancer.

Recent Findings The histological subtype of BC with the strongest signal of efficacy has been triple-negative breast cancer (TNBC). Early trials of single-agent checkpoint inhibitors did not demonstrate a uniformly positive signal. Clinical studies suggest response rates between 5 and 10% in pretreated patients and roughly 20–25% for untreated advanced TNBC. However, in the small subset of patients who do respond, the response is often durable. More encouraging results have been reported with their use in combination with chemotherapy in the neoadjuvant setting. Larger phase III studies are underway to confirm these earlier findings.

Summary An immune-directed therapeutic approach for the management of BC is underway, and it is likely that combination therapy will be required to achieve a level of efficacy worthy of use in the BC treatment paradigm. These agents are not without both economic and clinical toxicity; therefore, it is imperative that we identify patients most likely to benefit from these therapies through well-designed biologically plausible clinical studies and by evaluating novel combinatorial approaches with informative biomarker driven correlative studies.

Keywords Breast cancer · Triple-negative breast cancer · Immunotherapy · Checkpoint inhibitors · PD-1 · PD-L1

Introduction

In the USA, breast cancer is the most common cancer diagnosed in women and is the second most frequent cause of cancer death after lung cancer. In 2017, an estimated 252,710 women will be diagnosed with BC, with an estimated 40,610 deaths [1]. Over the past 2 decades, the mortality from breast cancer has steadily decreased due in part to early detection and advances in therapy [2]. However, for the patients who present with advanced disease or develop metastatic disease despite adjuvant therapy, their disease is generally incurable, treatments are palliative, and most patients will ultimately succumb to their disease. The treatment options for BC vary considerably depending on the histological subtype. There are a number of very effective targeted therapies available for estrogen receptor (ER)-positive disease and for human epidermal growth factor receptor 2 (HER2)-positive disease. However, triple-negative breast cancer (TNBC) is a particularly aggressive subtype accounting for 15–20% of all breast cancers. While TNBC may respond briefly to conventional chemotherapy, the clinical outcome for these patients is poor. This subtype represents an unmet need for improved therapies, as molecular drivers of the disease remain unclear and we lack an accepted standard of care approach guided by tumor biology.

In the last several decades, treatment for cancer has largely focused on the inherent biology of the cancer cell, with ongoing efforts to identify mutations within cancer cells. More recently, the importance of the tumor microenvironment and in particular immune cells has shed new light on the interplay between the immune system and tumor progression. An evolving understanding of this relationship has dramatically changed the treatment landscape for many cancers with the development of checkpoint inhibitors. The anti-cytotoxic T

This article is part of the Topical Collection on *Breast Cancer*

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lymphocyte antigen-4 (CTLA-4) antibody ipilimumab was the first checkpoint inhibitor to be FDA-approved, for use in advanced melanoma. Subsequently, the FDA has approved inhibitors of programmed death-1 (PD-1) and/or programmed death-ligand 1 (PD-L1) in melanoma, non-small cell lung cancer (NSCLC), urothelial carcinoma, renal cell carcinoma, squamous cell carcinoma of the head and neck, Merkel cell carcinoma, Hodgkins lymphoma, microsatellite instability high (MSI-H) tumors, and gastric cancer. There is much excitement and enthusiasm among the breast cancer community for the use of checkpoint inhibitors in BC. Early phase studies of checkpoint inhibitors in BC have demonstrated evidence of activity, specifically in TNBC, with many confirmatory phase III studies underway.

This article will review the literature supporting the use of checkpoint inhibitors in BC, their efficacy, safety, and biomarker development to improve patient selection for treatment with these agents.

Immunogenicity of Breast Cancer

The treatment of BC has largely been driven by identifying cellular pathway alterations within tumors, e.g., overexpression of ER in ER-positive BC and HER2 in HER2-positive disease, which drive tumor cell growth. Inhibition of these pathways with targeted therapies can lead to tumor cell apoptosis. In contrast, whether the immune system can recognize a tumor (immunogenic vs non immunogenic) reflects the interplay between the tumor and surrounding microenvironment. The tumor microenvironment includes many different immune cells, each playing their own role in the interplay between immune activation/tumor kill and immune anergy/tumor survival [3]. The challenge to harnessing the immune system to target cancer, and in particular with breast cancer, has been to recognize which cellular features stimulate an immune response.

The wide range in immunogenicity of tumors has in part been attributed to the mutational burden present in the cancer. Traditionally, BC has not been regarded as a typical immunogenic tumor, unlike other tumors such as melanoma, renal cell cancer, and lung cancer. Tumors with more mutations express more neo-antigens and appear "hot" to the immune system, while tumors with fewer mutations appear "cold" [4]. Alexandrov and colleagues analyzed 4,938,362 mutations from 7042 cancers and demonstrated that melanoma, NSCLC, and bladder cancers contained the highest number of somatic mutations (median of 10 mutations per megabase) [5]. Breast cancer was in the lower end of the spectrum, with a median of 1 mutation per megabase [5]. However, accumulating data has shown tumor-infiltrating lymphocytes (TILs) to be present in BC tissues, with a positive association in outcome in both the early-stage and the advanced disease setting in HER2-positive BC [6–9] and TNBC [7–13], but not in smaller luminal–HER2-negative disease [9]. TILs have also be shown to predict response to immune checkpoint inhibition [14]. The role of TIL evaluation in BC remains under clinical investigation; it is currently not routinely incorporated as a prognostic marker in clinical practice and its definitive role as a predictive marker is still as of yet unproven.

Checkpoint Inhibition in Cancer: the Biology of CTLA-4 and PD-1/PD-L1 Pathways

Tumor antigen presentation to T cells is mediated by the peptide-major histocompatibility complex (MHC) molecule, which is recognized by the T cell receptor (TCR) on antigen-presenting cells (APC), such as dendritic cells, macrophages, and tumor cells [15]. CD28, which is a co-stimulatory molecule, is required for T-cell activation. CTLA-4, which is an immune checkpoint, is expressed exclusively on T cells where it counteracts the activity of CD28. CD28 and CTLA-4 share identical ligands B7.1 and B7.2. However, CTLA-4 has a greater affinity for both ligands, and directly competes with CD28 [15]. Therefore, CTLA-4 expression on the surface of T cells restricts the activation of T cells by reducing CD28 ligand binding (see Fig. 1).

CTLA-4 expression is increased upon T cell activation and acts to inhibit T cell function, thereby keeping T cell activation in balance [16]. This is a normal process, which prevents excessive immune activation and damage to normal tissues. Therefore, inhibition of CTLA-4 by a monoclonal antibody may result in a positive anti-tumor effect.

PD-1 is another immune checkpoint that has been identified as a target for immune-directed cancer therapy [15]. PD-1 has two ligands, PD-L1 and -L2. Tumors can express PD-L1 on their cell surface, which binds to PD-1 on activated T cells. This results in downmodulating of T cells, and therefore inhibition of T-cell cytotoxicity on tumor cells. Similar in principle to inhibition of CTLA-4, an antagonistic monoclonal antibody directed to PD-1 and/or PD-L1 may lead to immune induced tumor apoptosis (see Fig. 1).

PD-1 and PD-L1 Checkpoint Inhibitors in Breast Cancer

Targeting the PD-1 and PD-L1 pathway has resulted in the approval of 5 agents in the treatment of various cancers. A number of these PD-1 and PD-L1 antagonistic monoclonal antibodies have been evaluated in BC, including pembrolizumab, atezolizumab, durvalumab, and avelumab. The observed clinical efficacy to date from these agents varies considerably depending on the molecular subtype of BC, with the strongest signal of efficacy

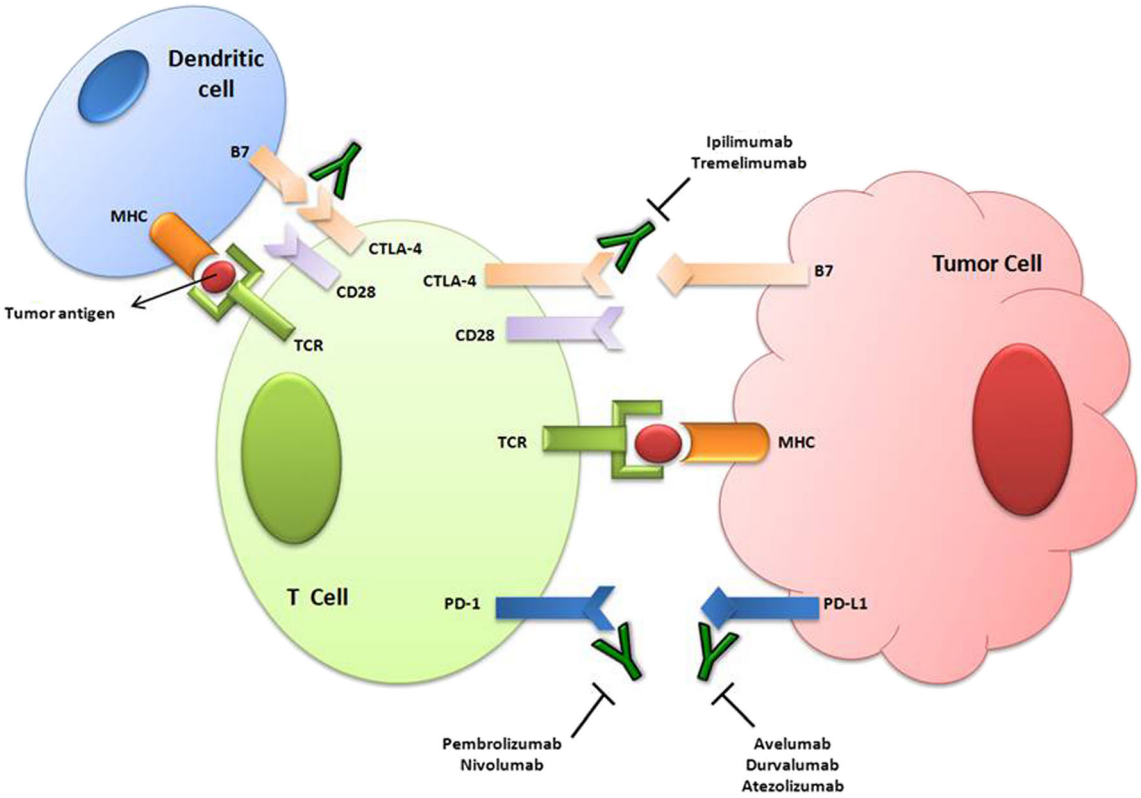

Fig. 1 CTLA-4 and PD-1/PD-L1 checkpoint interaction between T cell and tumor. TCR T cell receptor, MHC major histocompatibility complex, CTLA-4 cytotoxic T lymphocyte antigen-4, PD1 programmed cell death protein 1, PD-L1 PD1 ligand

being in metastatic TNBC. The following section along with Table 1 provides a summary of the clinical trial data involving PD-1 and PD-L1 inhibitors in BC.

Pembrolizumab (PD-1 Inhibitor) as a Single Agent in Metastatic TNBC

The KEYNOTE-012 study was a nonrandomized, phase Ib trial evaluating single-agent pembrolizumab (10 mg/kg every 2 weeks) in patients with PD-L1-positive recurrent or metastatic cancers, including a TNBC cohort [17]. PD-L1 positivity was defined as ≥ 1% membrane staining. Of 111 screened breast tumors, 65 (58.6%) were noted to be PD-L1 positive, 32 of which enrolled on the study. Patients were heavily pretreated and two thirds had received ≥ 3 prior therapies. Of the 32 patients enrolled, 27 had evaluable disease by RECIST v1.1. The confirmed overall response rate (ORR) was 18.5% for all patients. One patient had a complete response (CR), four patients had a partial response (PR), and seven patients had stable disease [17]. The median duration of response (DOR) was not yet reached (range, 15.0 to ≥47.3 weeks). The most commonly reported adverse events (AEs) included fatigue, nausea, and arthralgia. Grade ≥ 3 toxicity was reported in 5 patients.

Pembrolizumab was subsequently evaluated in the phase II KEYNOTE-086, single-arm study, in advanced TNBC [18•]. Cohort A of KEYNOTE-086 evaluated the efficacy and safety of pembrolizumab (200 mg IV every 3 weeks) in 170 patients with previously treated TNBC, regardless of PD-L1 expression. Forty-four percent of patients had three prior lines of chemotherapy in the advanced setting. Sixty-two percent had PD-L1-positive tumors ($n = 105$). The ORR was low at only 4.7%, with 1 patient achieving a CR and 7 patients a PR, in addition to 35 patients having SD. There was no difference in response between patients who were PD-L1 positive or negative (ORR 4.8 and 4.7%, respectively). The median DOR was 6.3 months (1.2 to 10.3+). The PFS was similar in both the PD-L1 positive and negative cohorts (2.7 and 1.9 months, respectively). There was no significant difference in OS, being 8.9 months in all patients and 8.3 vs 10 months in the PD-L1 positive and negative cohorts, respectively. It is worth noting that the median OS had not been reached for the 8 patients who achieved a clinical response. No new safety signals were observed.

Cohort B of KEYNOTE-086 evaluated pembrolizumab (200 mg IV every 3 weeks) as first-line therapy for patients with PD-L1-positive TNBC [19•]. The study enrolled 84 patients, 73 (87%) of which had received prior neoadjuvant or adjuvant chemotherapy. The ORR was 23.1%, with 3 patients

Table 1 Study results of anti PD-1 and PD-L1 monoclonal antibodies investigated in breast cancer

Drug	Trial	Phase	BC subtype	Disease setting	Prior lines of therapy	Patients *N* = evaluable/ enrolled	ORR	mPFS, months	mOS, months
Pembrolizumab	KEYNOTE-012 [17]	Ib	TNBC	Metastatic	≥ 2	*n* = 27/32 PD-L1+ *n* = 27/32	18.5% 18.5%	NR	NR
	KEYNOTE-086 [18•] Cohort A	II	TNBC	Metastatic	≥ 1	*n* = 170 PD-L1+ *n* = 105 PD-L1− *n* = 64*	4.7% 4.8% 4.7%	2.0	8.9
	KEYNOTE-086 [19•] Cohort B	II	TNBC	Metastatic	0	*n* = 84 PD-L1+ *n* = 84	23.1%	2.1	16.1
	KEYNOTE-028 [20]	Ib	ER+	Metastatic	≥ 2	*n* = 25 PD-L1+ *n* = 25	12%	NR	NR
	KEYNOTE-014 [21]	Ib/II	HER2+	Metastatic	≥ 2	*n* = 58 PD-L1+ *n* = 46 PD-L1− *n* = 12	 15.2% 0%	 2.7 2.5	 16.1 7
	ENHANCE-1 [22•] Pembro + Eribulin	Ib/II	TNBC	Metastatic	≤ 2	*n* = 106/107 PD-L1+ n = 49 PD-L1− *n* = 49 1st line *n* = 65 1–2 prior lines *n* = 41	26.4% 30.6% 22.4% 29.2% 22%	4.2	17.7
	KEYNOTE-173 [23] Cohort A Pembro + NP-AC	Ib	TNBC	Neoadjuvant	0	*n* = 10	pCR 60%	NE	NE
	KEYNOTE-173 [23] Cohort B Pembro + NP + C-AC	Ib	TNBC	Neoadjuvant	0	*n* = 10	pCR 90%	NE	NE
	I SPY-2 [24•] Pembro + P-AC vs P-AC	II	TNBC	Neoadjuvant	0	Pembro + P-AC *n* = 29 P-AC *n* = 85	pCR 60% 20%	NE	NE
	I SPY-2 [24•] Pembro + P-AC vs P-AC	II	ER+	Neoadjuvant	0	Pembro + P-AC *n* = 40 P-AC *n* = 95	pCR 34% 13%	NE	NE
Atezolizumab	Atezolizumab [25•]	I	TNBC	Metastatic	≤ 3	*n* = 112 PD-L1+ *n* = 71 PD-L1− *n* = 37 1st line ≥ 2nd line	10% 13% 5% 26% 12%	NR	9.3
	Atezolizumab + nab-paclitaxel [26]	Ib	TNBC	Metastatic	≤ 2	*n* = 32 1st line *n* = 13	38% 46%	NR	NR
Avelumab	JAVELIN Solid Tumor [27]	Ib	TNBC ER+ HER2+	Metastatic	≤ 3	*N* = 168 TNBC n = 58 ER+ *n* = 72 HER2+ *n* = 26	4.8% 8.6% 2.8% 3.8%	NR	NR
Durvalumab	MEDIOLA [35] Durvalumab + Olaparib	I/II	gBRCA BC	Metastatic	Any#	*n* = 25 1st line *n* = 9 2nd line *n* = 9 3rd line *n* = 5 ≥ 4th line *n* = 2	 67% 67% 20% 0%	NR	NR

Pembro, pembrolizumab; *BC*, breast cancer; *NR*, not reported; *NE*, not evaluable; *TNBC*, triple-negative breast cancer; *ER+*, estrogen receptor positive; *HER2+*, human epidermal receptor positive; *pCR*, pathological complete response; *PD-L1*, programmed death 1 ligand; *ORR*, overall response rate; *mPFS*, median progression free survival; *mOS*, median overall survival; *OS*, overall survival; *NP-AC*; nab-paclitaxel followed by doxorubicin and cyclophosphamide, *NP+C-AC*; nab-paclitaxel and carboplatin followed by doxorubicin and cyclophosphamide, *P-AC*, paclitaxel followed by doxorubicin and cyclophosphamide; *gBRCA*, germline BRCA mutated

*Denotes 1 pt. with unknown PD-L1 status

# Denotes patients must have received prior anthracycline and taxane

achieving a CR and 16 a PR. Twelve of the 19 responses were ongoing at data cutoff, and the median DOR was 8.4 months (range 2.1+ to 13.9+). Median PFS was 2.1 months and median OS was 16.1 months. Again, there was no new safety

signals observed and the most common immune AE was hypothyroidism (10%).

Pembrolizumab in ER-Positive Metastatic Breast Cancer

The KEYNOTE-028 trial evaluated pembrolizumab in metastatic ER-positive/HER2-negative breast cancer [20]. The cutoff for positivity was similar to the KEYNOTE-012 study being ≥ 1% membrane staining. Of 261 screened tumors, 48 were PD-L1 positive (19%) and 25 patients enrolled. The ORR was 12%, with 3 patients achieving a PR and 4 with SD. All 3 responders remained on study treatment for ≥ 26 weeks at the time of abstract presentation.

Pembrolizumab Combined with Trastuzumab in HER2-Positive Metastatic Breast Cancer

KEYNOTE-014 is the first reported study of pembrolizumab in combination with trastuzumab in HER2-positive advanced BC [21]. This is a phase Ib/II study evaluating the safety and efficacy of pembrolizumab and trastuzumab in trastuzumab-resistant HER2-positive BC. Of 146 screened patients, 68 (53.5%) were PD-L1 positive. Ten percent of patients were excluded as they were centrally confirmed HER2-negative. The study enrolled 58 patients, 46 to the PD-L1-positive cohort and 12 to the PD-L1-negative cohort. These patients were heavily pretreated, with all patients having received prior chemotherapy (anthracycline/taxane regimen) and 87.9% having received additional anti-HER2 therapy post progression on trastuzumab (adotrastuzumab emantansine 72%, pertuzumab 30%, and other agent 44%). The ORR in the PD-L1-positive cohort was 15.2%, with 7 responses (2 CR and 5 PR). There were no responses in the PD-L1-negative cohort. The median duration of response was 11.2 months, with 5 patients (10.8%) continuing on therapy at time of reporting. The PFS was similar in both the PD-L1 positive and negative cohorts (2.7 and 2.5 months, respectively). The OS was significantly longer in the PD-L1-positive cohort 16.1 vs 7 months (90% CI 13.1-NR, $p = 0.0006$). However, the difference in OS could not be easily explained. Analysis of TILs found the majority of tumors had low levels in these metastatic tumor biopsy samples. Stromal TIL (sTIL) levels were associated with response (sTIL > 5% ORR 39 vs 5% if sTIL < 5%). The combination was well tolerated, with grade 3/4 dyspnea reported in 2 patients. There were no cardiac events. The grade 3/4 immune AE rate was 10.3%, including thyroid dysfunction ($n = 4$) and pneumonitis ($n = 2$).

Pembrolizumab Combined with Chemotherapy in Metastatic TNBC

The phase Ib/II ENHANCE-1 trial of pembrolizumab and the chemotherapy agent eribulin mesylate enrolled 107 patients with metastatic TNBC, for which 66 (61.7%) patients had received no prior chemotherapy in the metastatic setting and 41 (38.3%) patients had received 1–2 prior lines of chemotherapy [22•]. The PD-L1 positivity rate was 45.8% (49/107). Patients received pembrolizumab at 200 mg IV every 3 weeks and eribulin 1.4 mg/m^2 on days 1 and 8 of a 21-day cycle. Of the 106 evaluable patients, the ORR for all patients was 26.4% (3 CR and 25 PR). In PD-L1-positive tumors, ORR was 30.6 vs 22.4% for PD-L1-negative disease. The ORR was 29.2% in patients treated in the first-line setting and 22% in patients with 1–2 prior lines of therapy. The median DOR was 8.3 months with a PFS of 4.2 months and OS of 17.7 months. Grade 3 or 4 AEs were reported in 47.7 and 18.7%, respectively. The most common grade 3/4 AEs were neutropenia (30.8%), peripheral neuropathy (9.3%) and anemia, fatigue, and hypokalemia (5.6% each). Immune-related adverse events occurred in 67%, of which 12.8% were grade 3 and 4 events and included rash (5.1%), hyperglycemia (2.6%), and pneumonitis (2.6%).

Pembrolizumab Combined with Chemotherapy in the Neoadjuvant Setting

Pembrolizumab has also been investigated in the neoadjuvant setting. The KEYNOTE-173 is a phase Ib study of pembrolizumab (200 mg IV every 3 weeks) plus chemotherapy as neoadjuvant therapy for locally advanced TNBC [23]. Patients were enrolled into 1 of 2 cohorts; cohort A—pembrolizumab plus weekly nab-paclitaxel (125 mg/m^2) followed by pembrolizumab plus doxorubicin and cyclophosphamide (AC) every 3 weeks—and cohort B—pembrolizumab plus weekly nab-paclitaxel (100 mg/m^2) and carboplatin (AUC 6) followed by pembrolizumab plus AC. The pathological complete response (pCR) rate (defined as no invasive residual disease in the breast and lymph nodes; ypT0Tis and ypN0) was 60% (90% CI, 30–85) in cohort A ($n = 10$) and 90% (90% CI, 61–100) in cohort B ($n = 10$). There were no new safety signals observed with the combination of pembrolizumab and chemotherapy.

Pembrolizumab was also evaluated in the phase II, neoadjuvant, adaptively randomized, multicenter I-SPY2 trial [24•]. The goal of this trial design is to efficiently identify promising agents to take to phase III with a high probability of success. A total of 249 patients were randomized; 69 to receive pembrolizumab (200 mg IV every 3 weeks)

in combination with weekly paclitaxel, 180 patients to weekly paclitaxel alone in the control arm, and all patients then continued to receive neoadjuvant AC, followed by surgery. Pembrolizumab was not continued in the adjuvant setting. Forty patients in the pembrolizumab arm had ER+ disease and 29 had TNBC. It is worth noting that the results are estimated pCR rates, as raw pCR rates are biased due to the adaptive design of the trial. If the predicted probability of success in a phase III trial of 300 patients was > 85%, then the drug would graduate from the trial. Findings showed that the estimated pCR rate (ypT0/Tis and ypN0) was significantly higher with the addition of pembrolizumab in patients with TNBC than in the control arm; (60 vs 20%; HR 0.6; 95% CI 0.43–0.78), with a > 99% probability of success in a phase III study. Interestingly, the pCR rate was also increased in ER-positive/HER2-negative patients (34 vs 13%; HR 0.34; 95% CI 0.19–0.48), with an 88% probability of success in a phase III study. Toxicity was similar to other studies with pembrolizumab, except the rate of adrenal insufficiency (AI) was higher than previously reported in other studies of pembrolizumab across different cancer types, both as single agent [29, 30] and in combination with chemotherapy [31]. The rate of AI was 8.7%, with 6 out of the 69 patients in the pembrolizumab arm experiencing AI, 5 of which were grade 3–5 in severity. Three of the cases were related to hypophitis and therefore secondary AI, while 3 were primary AI. All patients were commenced on long-term hormone replacement.

Atezolizumab (PD-L1 Inhibitor) as a Single Agent in Metastatic TNBC

A phase I trial evaluated the use of atezolizumab across many cancer types. Results from the dose expansion cohort of TNBC in this study were recently reported [25•]. Of the evaluable 112 patients, 17% were treated in the first-line setting, 24% as second-line, and 58% had received ≥ 2 prior therapies. PD-L1 expression was positive (defined as ≥ 5% positive staining) in 63% of tumors. The ORR was 10% in the total population, 13% for PD-L1-positive disease and 5% in PD-L1-negative tumors. In addition, patients treated in the first-line setting had a higher response rate in comparison to those treated with ≥ 1 lines of therapy (26 vs 12%, respectively). The median OS was 9.3 months (95% CI 7–12.6) and the median duration of response was durable at 21.1 months. Of note, among the 11 responders (CR and PR), all were alive at 2 years.

An important observation to note from both this study with atezolizumab and from the KEYNOTE-086 cohort A is that while the ORR is low in this heavily pretreated population of TNBC, if patients do achieve a response, it is often durable. This would suggest that a small number of patients will achieve an excellent response to single-agent PD-1 or PDL-1 blockade. However, the difficulty is how to identify these patients.

Atezolizumab Combined with Chemotherapy in Metastatic TNBC

Atezolizumab in combination with nab-paclitaxel was evaluated in a phase Ib study that enrolled 32 patients with advanced TNBC [26]. Patients could have received 0–2 prior lines of therapy and PD-L1 positivity was not a required for enrollment. The ORR was 38% (95% CI, 21–56) in the total population and 46% (95% CI, 19–75) in patients treated as first-line. Historically, the reported response rate from first-line nab-paclitaxel in TNBC is in the range of 30–35% [32]. Responses were observed in both PD-L1-positive and PD-L1-negative tumors. Similar to other studies, durable responses were observed. The most common treatment-related AE was decreased neutrophil count (53% all grade; 41% grade 3 to 4).

Avelumab (PD-L1 Inhibitor) as a Single Agent in Metastatic TNBC

Avelumab is another PD-L1 inhibitor in clinical development across many cancer types. The phase Ib trial JAVELIN study enrolled 168 patients from different breast cancer subtypes: 58 triple-negative, 72 hormone-receptor positive and HER2 negative, and 26 HER2 positive [27]. Patients had to have progressed on standard therapy but could not have received > 3 lines of therapy in the advanced setting. PD-L1 positivity was not required for eligibility. The ORR in the total population was low, at 4.8%; (2.8% in ER+ cohort, 3.8% in HER2+ cohort and 8.6% in TNBC cohort). One patient had a CR, 7 patients had a PR, and 40 patients had SD. Five of the eight responders were in the TNBC subgroup. Of the 9 TNBC patients with PD-L1 expression within immune cells in the tumor, the ORR was 44.4 vs 2.6% in the 39 TNBC patients with negative PD-L1 expression. In the total population, median time to response was 11.4 weeks, and median DOR was 28.7 weeks. Avelumab was found to have an acceptable safety profile. Potential immune-related toxicities occurred in approximately 10% of patients, including hypothyroidism, pneumonitis, thrombocytopenia, and autoimmune hepatitis, four of which were grade 3 or grade 4. Treatment-related death occurred in two patients (liver failure and respiratory disease).

Durvalumab (PD-L1 Inhibitor) Combined with PARP Inhibition in Metastatic Breast Cancer

The poly(ADP-ribose) polymerase (PARP) inhibitors olaparib and talazoparib have shown clinical benefit over chemotherapy in HER2-negative advanced BC with a deleterious germline BRCA (gBRCA) mutation in the phase III OlympiAD [33] and EMBRACA [28] studies, respectively. An exciting therapeutic approach would be to combine checkpoint inhibitors with PARP inhibition. The clinical rationale for combining a checkpoint inhibitor in BRCA mutated cancers is due to their inherent defect in homologous repair [34]. These cancers accumulate DNA damage and are genomically unstable and therefore may be more immunogenic.

The MEDIOLA trial is a phase I/II open-label basket study of olaparib and durvalumab in patients with advanced solid tumors. The cohort with HER2-negative and gBRCA mutation positive-advanced BC was recently presented in abstract form [35]. Patients could not have received a PARP inhibitor or immunotherapy, prior anthracycline and taxane was required and prior platinum therapy was allowed. Patients received single-agent olaparib 300 mg OD for 4 weeks, then durvalumab 1.5 g IV every 4 weeks was added from week 4 onwards. A total of 25 patients were enrolled, 12 (48%) having ER-positive disease and 13 (52%) having TNBC. The ORR was 67% in patients with no prior therapy ($n = 6/9$), 67% in patients with 1 prior therapy ($n = 6/9$), 20% in patients with 2 prior therapies ($n = 1/5$), and 0% patients with 3+ prior therapies ($n = 0/2$). The median PFS had not been reached, with data cutoff at 6 months. Note the median PFS in the OlimpiAD study was 7 months [33]. The combination was generally well tolerated with no unexpected toxicity observed. Grade 3 or higher events included anemia (8%), neutropenia (8%), and fatigue (4%), all attributed to olaparib. A single case of grade 3 or higher of both hemolysis and pancreatitis was reported and thought to be related to durvalumab therapy.

Biomarkers to Predict Response to Checkpoint Inhibitors

It is clear from the studies reported to date that less than 20% of pretreated BC patients benefit from single-agent checkpoint inhibition. The observed PFS is between 2 and 3 months, which suggests that most patients treated with these agents are progressing. However, what has been consistent across these studies is that a small subset of patients do respond, and the response is often durable. However, identification of these patients has proved a challenge. It is therefore imperative that biomarkers are developed to help identify patients most likely to benefit from these agents.

The obvious biomarker evaluated in early trials of checkpoint inhibitors was PD-L1 expression. However, the inconsistent predictive value of PD-L1 has been demonstrated across BC and many other cancers. In a number of the BC studies, the response rate in PD-L1-positive tumors has been shown to be higher; however, responses were also seen for patients with PD-L1-negative tumors [18•, 22•, 25•, 26, 27].

Microsatellite instability (MSI) is a hypermutated phenotype that occurs in tumors with impaired DNA mismatch repair (MMR), which is a repair pathway responsible for correcting errors during DNA replication [36]. The incidence of MSI in BC is very low, with reports in the literature of 1–2% by traditional testing methods [37–39]. A recent study utilizing next-generation sequencing (NGS) to identify cancers that are MSI found that of 11,553 samples from 10,900 patients, 204 from193 patients were MSI-H [40]. Of 1049 breast cancer samples, there was no case of MSI indentified by NGS. While another study analyzed whole exome data from 11,139 tumor-normal pairs from The Cancer Genome Atlas across 39 cancer types and identified MSI in 3.8% of all cancers assessed [41]. Of 1044 breast cancer cases, 16 (1.53%) were identified to be MSI.

The FDA approved pembrolizumab for microsatellite instability-high (MSI-H) or mismatch repair-deficient (dMMR) solid tumors, based on data from 149 patients with MSI-H or dMMR cancers enrolled across five single-arm clinical trials [42•]. Of the 149 patients, 90 had colon cancer, while the remaining 59 patients were diagnosed with 14 other cancer types, of which 2 patients had BC. In the total population, the ORR was 39.6% with a DOR of ≥ 6 months being 78%. Of the 2 BC patients, ORR was 100% with both patients achieving a PR, lasting 7.6 and 15.9 months, respectively.

It is important that the possibility of a BC tumor being MSI-H is not overlooked. Routine testing for MSI is not feasible due to the very low incidence in BC. However, given that next-generation sequencing is becoming a component of routine clinical practice, especially in the advanced setting, it may identify patients with MSI-H tumors that would be candidates for checkpoint inhibitor therapy.

Future Directions

Results from the checkpoint inhibitor studies outlined above offer some encouragement that targeting the PD-1/PD-L1 axis may well prove to be a successful therapeutic option for selected BC patients in the future. In order to confirm this, numerous phase III studies both in advanced and early stage disease are ongoing. In an attempt to enhance activity, combinatorial approaches are being evaluated, with a number of studies planned or underway that will combine checkpoint inhibitors with (1) newer immunotherapy drugs, which are in early phase development, (2) chemotherapies, (3) targeted

Table 2 Selection of ongoing phase II/III studies of anti PD-1 and PD-L1 monoclonal antibodies in breast cancer

Drug	Trial identifier	Phase	BC subtype	Neoadjuvant	Adjuvant	1st line metastatic	≥ 2nd line metastatic	Target accrual status
Pembrolizumab	KEYNOTE-119 (NCT02555657)	III	TNBC				Pembrolizumab vs single-agent chemotherapy of physicians choice*	600 ongoing
	KEYNOTE-355 (NCT02819518)	III	TNBC			Pembrolizumab + P or NP or GC vs placebo + P or NP or GC		858 recruiting
	KEYNOTE-522 (NCT03036488)	III	TNBC	Pembrolizumab + PC-AC or EC vs Placebo + PC-AC or EC				855 recruiting
	(NCT02954874)	III	TNBC		Pembrolizumab in patients with residual disease (> 1 cm and/or positive nodes) post standard NACT			1000 recruiting
Atezolizumab	IMpassion131 (NCT03125902)	III	TNBC			Atezolizumab + P vs placebo + P		540 recruiting
	IMpassion130 (NCT02425891)	III	TNBC			Atezolizumab + NP vs placebo + NP		900 ongoing
	Impassion031 (NCT03197935)	III	TNBC	Atezolizumab + NP-AC vs Placebo + NP-AC				204 recruiting
	NCT03281954	III	TNBC	Atezolizumab + PC-AC or EC vs placebo + PC-AC or EC				1520 recruiting
	NCT02924883	II	HER2+				Atezolizumab + T-DM1 vs placebo + T-DM1	200 ongoing
Avelumab	A-Brave (NCT02926196)	III	TNBC		Avelumab in patients with N2 disease post adjuvant chemotherapy or with residual disease post NACT			335 recruiting
Durvalumab	DORA (NCT03167619)	II	TNBC				Durvalumab + olaparib vs olaparib in TNBC patients following response to platinum	60 planned

Pembro, pembrolizumab; *BC*, breast cancer; *TNBC*, triple-negative breast cancer; *ER+*, estrogen receptor positive; *HER2+*, human epidermal receptor positive; *pCR*, pathological complete response; *NP*, nab-paclitaxel; *P*, paclitaxel; *AC*, doxorubicin and cyclophosphamide; *PC*, paclitaxel and carboplatin; *EC*, epirubicin and cyclophosphamide; *GC*, gemcitabine and carboplatin; *T-DM1*, trastuzumab emantasine; *NACT*, neoadjuvant chemotherapy

*Choice of chemotherapy incudes capecitabine, eribulin, gemcitabine, and vinorelbine

agents, and (4) locoregional modalities (such as radiotherapy). Table 2 provides a summary of some of the ongoing phase II/III checkpoint inhibitor studies in BC.

Conclusions

Tumor targeting with immune checkpoint inhibitors has resulted in a paradigm shift for the treatment of a number of cancers. However, their use in BC is still experimental. Due to impressive results in other cancer types, there has been eagerness by both physician and patient to enroll to studies of these agents in BC. Unfortunately, response rates have been underwhelming compared to other disease types. Single-agent checkpoint inhibitor therapy is unlikely to be sufficient in BC unlike other cancers. Pretreated patients can expect a response rate of between 5 and 10% while response rates for untreated advanced TNBC is approx. 20–25%. Responses do not appear to be superior to standard chemotherapy agents administered in the first-line setting. However, what has been consistently demonstrated across the reported studies is that in the small subset of patients who do respond, the response is often durable. The overarching difficulty is in identifying these patients. No successful biomarker has currently been developed to inform better patient selection for treatment with checkpoint inhibition.

Use of these agents at an earlier stage of the disease does show promise, with very encouraging rates of pCR in both TNBC and unexpectedly in ER-positive disease. Larger phase III studies will be required to confirm these earlier findings and enrollment to these studies is underway. However, caution needs to be exerted with the use of these drugs in unselected and/or lower-risk patients in the (neo)adjuvant settings as toxicity is not insignificant, with patients exposed to potential lifelong toxicities.

In order to inform the future role of immunotherapy in the management of BC, it is imperative that clinical investigation of immune-directed therapies in BC going forward should be in a well-designed biologically plausible clinical study, evaluating novel combinatorial approaches with informative biomarker-driven correlative studies.

Compliance with Ethical Standards

Conflict of Interest Tomas G. Lyons declares that he has no conflict of interest.

Maura N. Dickler has received compensation from Genentech/Roche, Novartis, Eli Lilly, TapImmune, G1 Therapeutics, Pfizer, AstraZeneca, CTI BioPharma (Cell Therapeutics), Hengrui Therapeutics, Driver, and Flatiron Health for service as a consultant.

Elizabeth E. Comen declares that she has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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