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BREAST CANCER (RA LEON FERRE, SECTION EDITOR)

Immune Checkpoint Inhibitors and Novel Immunotherapy Approaches for Breast Cancer

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Abstract

Purpose of Review To critically review the existing evidence on immune checkpoint inhibitors (ICIs) in early-stage and metastatic breast cancer and discuss emerging strategies in the different breast cancer subtypes.

Recent Findings Immunotherapy has become one of the major milestones in contemporary oncology, revolutionizing the treatment of multiple solid tumors. ICI agents combined with chemotherapy have demonstrated significant efficacy in both early-stage and metastatic triple-negative breast cancer. However, only a subgroup of patients responds to those agents and some associated toxicities, although infrequent, can be life-disabling. Emerging data from immunotherapy studies in advanced hormone receptor–positive (HR +) breast cancer as well as HER2-positive disease are arising with mixed results.

Summary Although breast cancer has not classically been considered a hot tumor, ICIs have proven to be effective in a subset of breast cancer patients. However, much remains to be learned, and the identification of new biomarkers beyond PD-L1 expression is essential not only to improve the efficacy of ICI but also to identify patients who can avoid them, together with their toxicities and costs.

Keywords Immune checkpoint inhibitors · Immunotherapy · Breast cancer · Biomarkers · Triple-negative breast cancer · Hormone receptor–positive breast cancer · HER2-positive breast cancer · PD-L1 · PD-1 · Tumor mutational burden · Tumor-infiltrating lymphocytes · Immune response · Immune checkpoint blockade · Tumor subtype · Early stage · Metastatic breast cancer

Introduction

The host immune system plays a crucial role not only in tumor development but also in tumor progression [1]. In recent years, exploiting intrinsic mechanisms of the host immune system to eradicate cancer cells has achieved impressive success in some solid tumors. Any strategy based on enhancing both innate and adaptive immune responses for treating cancer is considered immunotherapy. This includes immune checkpoint inhibitors (ICIs), cancer vaccines, antibody–drug conjugates (ADCs), oncolytic viruses, and adoptive immune cell therapies. Traditionally, breast cancer has not been considered a highly immunogenic tumor since it is characterized by a low tumor mutation burden in comparison to other tumors. Nevertheless, breast cancer is a very heterogeneous disease, with different treatment sensitivities and clinical outcomes [2].

James P. Allison and Tasuku Honjo were awarded the Nobel Prize in Medicine for their respective discoveries of ICI in 2018. Since these discoveries, ICIs have dramatically

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changed the treatment landscape of multiple neoplasms including lung cancer and melanoma, among others [3, 4]. Under normal conditions, the immune system uses an inhibitory checkpoint pathway to regulate the immune response against pathogens to prevent exaggerated responses, and limit tissue damage and the autoimmune activity. This mechanism is conducted by several immune checkpoint molecules like the programmed cell death protein 1 (PD-1) and the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). They downregulate and inhibit T cells by binding to their ligands: the programmed death ligand 1 (PD-L1), the programmed death-ligand 2 (PD-L2), and CD80/CD86 [5]. Tumor cells take advantage of this mechanism to create an immunosuppressive microenvironment [6]. The anti-PD-1, anti-PD-L1, and anti-CTLA-4 monoclonal antibodies circumvent this immune downregulation, providing cell-mediated antitumor activity [2].

The aim of this review is to summarize the current status of ICI in the treatment of both early-stage and metastatic breast cancer, revise predictive biomarkers of response, and analyze new potential approaches.

ICIs in Triple-Negative Breast Cancer

Triple-negative breast cancer (TNBC) is considered the most immunogenic subtype of breast cancer with higher rates of PD-L1 expression on both tumor and immune cells, and higher tumor infiltrating lymphocyte (TIL) density [7]. Moreover, whole-exome sequences of DNA breast cancer cells demonstrated that basal-like and high-grade tumors were associated with the highest rate of tumor mutational burden (TMB) [8]. For all these reasons, the majority of studies evaluating immune checkpoint blockade have been conducted in TNBC. The use of anti-PD1 and anti-PDL1 monoclonal antibodies as single agent has shown sparse response rates [9–12]. Chemotheraphy increases PD-L1 expression and promotes antitumor immunogenicity. Likewise, cell death caused by cytotoxic agents can increase the expression of neoantigens, decrease the number of immunosuppressive cells, upregulate pro-inflammatory cytokines in the tumor environment, and lead to immunogenic death [13]. Therefore, the majority of trials have focused on immune-compatible cytotoxic drugs combined with anti-PD-1 or anti-PD-L1 antibodies. Table 1 summarizes key phase II and III trials assessing the efficacy of ICI in TNBC.

Advanced TNBC

The first trial that reported clinical benefit of ICIs in advanced TNBC was the KEYNOTE-012 trial. This phase I evaluated the safety of pembrolizumab in patients with metastatic TNBC with at least 1% of PD-L1 expression. Most patients had received ≥ 3 lines of treatment. Despite this, the trial showed promising results with an ORR of 18.5%, similar to that reported with chemotherapy in this setting [9]. From the subsequent studies evaluating ICI as single-agent, we learned that patients who benefit the most are those who receive it as first-line treatment and those whose tumors express PD-L1. However, even this population experienced little or no benefit in terms of PFS and OS [10, 20]. Low response rates observed with ICI as single-agent led research focus on the combination of chemotherapy plus immunotherapy.

The first trial that studied the combination of atezolizumab plus nab-paclitaxel ($N = 33$) reported an ORR of 39.4%, and a PFS and OS of 5.5 months and 14.7 months, respectively [26]. Based on these data, the IMpassion130 was conducted in patients with TNBC, regardless of PD-L1 status, and no prior treatment. PD-L1 expression was assessed by VENTANA SP142 PD-L1 assay on immune cells, and levels greater than or equal to 1% were considered positive. It was the first phase III trial to report positive results with ICI and chemotherapy in breast cancer, with significant improvement in both PFS and OS in patients with PD-L1-positive disease. In March 2019, atezolizumab was granted accelerated approval by the FDA for patients with advanced TNBC and PD-L1 + expression (≥ 1% by Ventana SP142), making it the first immunotherapeutic agent to be approved in this setting [22••, 23•].

In the subsequent IMpassion131 trial, the addition of atezolizumab to paclitaxel did not improve survival outcomes in the PD-L1-positive population, despite having a similar design to IMpassion131.(18) Several hypotheses have been proposed to explain the inconsistent results between IMpassion130 and IMpassion13, mostly centered around differences in the study populations and in the choice of chemotherapy partner for each study. Small differences in patient selection could have influenced the results, which is suggested by the excellent outcome observed in the placebo arm of IMpassion131 compared to the placebo arm of IMpassion130. In terms of chemotherapy partner, IMpassion131 used paclitaxel, while IMpassion130 used nab-paclitaxel. It has been proposed that these two drugs may have differential immunologic effects on the TME. Additionally, steroids are often administered as premedication for paclitaxel (but not for nab-paclitaxel), which could blunt the immune response. However, KEYNOTE-355 (described below) allowed either paclitaxel or nab-paclitaxel, and no differences in outcome were seen between patients who received either regimen [22••, 23•, 24•, 25••, 27]. US approval for atezolizumab was withdrawn by Genentech in September 2021. In Europe, atezolizumab plus nab-paclitaxel is still approved as first-line treatment for TNBC with positive PD-L1 expression.

The phase III KEYNOTE-355 compared the efficacy of several chemotherapy agents (paclitaxel nab-paclitaxel, or gemcitabine and carboplatin) in combination with

Table 1 Main phase II and III trials with published results studying immune checkpoint inhibitors in early and metastatic TNBC

Trial designation phase/*N*	Study population	Study treatment	Outcomes
Early-stage TNBC			
GeparNuevo [14] NCT02685059 Phase II 174 patients	Stages I and III TNBC	Neoadjuvant treatment (weekly nab-paclitaxel followed by AC) plus atezolizumab/pbo	pCR: 53.4% vs 44.2% with atezolizumab and pbo, respectively ($p=0.224$) At 3-year of follow up (atezolizumab vs pbo, respectively): iDFS: 84.9% vs 76.9% (HR 0.54, $p=0.0559$) DDFS: 91.4% vs 79.5% (HR 0.37 $p=0.0148$) OS: 95.1% vs 83.1% (HR 0.26 $p=0.0076$)
I-SPY [15] NCT01042379 Phase II 29 patients	Stages II and III TNBC	Neoadjuvant treatment (weekly paclitaxel followed by AC) ± pembrolizumab	pCR 60% vs 22% in the pembrolizumab and control arms, respectively
KEYNOTE-522 [16••, 17•] (NCT03036488) Phase III 602 patients	Stage II and III TNBC	Neoadjuvant treatment (weekly carboplatin + paclitaxel followed by AC/EC) plus pembrolizumab/pbo. Subsequent Adjuvant treatment with pembrolizumab/pbo	pCR: 64.8% in pembrolizumab arm vs. 51.2% in pbo ($p=0.00055$) At 3-year: EFS: 84.5% in pembrolizumab arm vs 76.8% pbo (HR = 0.63, $p=0.0003$) DRFS 87% in pembrolizumab arm vs 80.7% pbo (HR = 0.61)
IMPASSION 031 [18••] (NCT03197935) Phase III 333 patients	Stages II and III TNBC	Neoadjuvant treatment (weekly nab-paclitaxel + followed by AC) plus atezolizumab/pbo. Subsequent adjuvant atezolizumab/pbo	PCR 57.6% in atezolizumab arm vs 41.1% pbo ($p=0.0044$)
NeoTRIP [19] (NCT02620280) Phase III 280 patients	Stage II and III (allowed N3 +) TNBC	Neoadjuvant weekly carboplatin + nab-paclitaxel ± atezolizumab. Subsequent 4 cycles of adjuvant AC/EC	pCR: 48.6% in atezolizumab arm vs. 44.4% control ($p=0.48$)
Metastatic TNBC			
KEYNOTE-086: cohort A [10] (NCT02447003) phase II 170 patients	Previously treated (> 1 line) metastatic TNBC	Pembrolizumab as single agent	ORR 5.3% (CI 95%, 2.7–9.9) ORR PD-L1 + population: 5.7% (CI 95%, 2.4–12.2) DCR: 7.6% (CI 95%, 4.4–12.7) DCR PD-L1 + population 9.5% (C I 95%, 5.1–16.8) PFS: 2.0 months (CI 95%, 1.9–2.0) OS: 9.0 months (CI 95%, 7.6–11.2)
KEYNOTE-086: cohort B [20] (NCT02447003) phase II 84 patients	First line in metastatic TNBC	Pembrolizumab as single agent	ORR 21.4% (CI 95%, 13.9–31.4) DCR: 10.4 months (CI 95% 4.2 to 19.2) PFS: 2.1 months (CI 95% 2.0–2.2) OS:18.0 months (CI 95% 12.9–23.0)
ENHACE-1 [21] NCT02513472 phase Ib/II 167 patients	1st and 2nd line in metastatic TNBC	Eribulin plus pembrolizumab	ORR:23.4% (CI 95%: 17.2–30.5) There was a trend toward more robust activity for the combination among patients with PD-L1 + tumors compared to PD-L1 − tumors in the first-line setting

Table 1 (continued)

Trial designation phase/*N*	Study population	Study treatment	Outcomes
IMpassion130 [22••, 23•] NCT02425891 phase III 902 patients	1st line in metastatic TNBC	Nab-paclitaxel plus atezolizumab/pbo	ITT population: OS: 21.0 months (CI 95% 19.0–22.6) with nab-paclitaxel and atezolizumab vs 18.7 months (16.9–20.3) with pb.HR: 0.86; 95% CI 0.72–1.02 PFS: 7.2 months (CI 95% CI 5.6–7.4) with nab-paclitaxel and atezolizumab l vs 5.5 months (5.3–5.6) with pbo. HR: 0.80; 95% CI 0.69–0.92 PD-L1 + population: OS: 25 months (95% CI 19.6–30.7) with nab-paclitaxel and atezolizumab vs 18.0 months (13.6–20.1) with pbo. HR: 0.71; CI 95% CI 0.54 – 0.9 PFS: 7.5 months (95% CI 6.7–9.2) with nab-paclitaxel and atezolizumab and 5.3 months (3.8–5.6) with pbo. HR: 0.63; CI 95% 0.50–0.80
IMpassion131 [24•] NCT03125902 phase III 651 patients	1st line in metastatic TNBC	Paclitaxel plus atezolizumab/pbo	ITT population: PFS: 5.7 months with paclitaxel and atezolizumab vs 5.6 months with pbo. HR: 0.86; 95% CI 0.70–1.05 OS: 19.2 months (95% CI 16.6–22.1) with paclitaxel and atezolizumab l vs 22.8 months (95% CI 17.1–28.3) with pbo. HR: 1.12; CI 95% CI 0.88–1.43 PD-L1 + population: PFS: 6.0 months with paclitaxel and atezolizumab vs 5.7 months with pbo. HR 0.82, 95% CI 0.60–1.12 OS: 22.1 months (95% CI 19.2–30.5) with paclitaxel and atezolizumab vs 28.3 months (95% CI 19.1-not estimable) with pbo. HR: 1.11; CI 95% 0.76–1.64
KEYNOTE-355 [25••] NCT02819518 phase IIII 882 patients	1st line in metastatic TNBC	Chemotherapy (paclitaxel, nab-paclitaxel, carboplatin-gemcitabine,	ITT population PFS: 7.5 months with Cht and pembrolizumab vs 5.6 months with pbo. HR: 0.82, CI 95% 0.69–0.97 CPS ≥ 10: 9.7 months with Cht and pembrolizumab vs 5.6 months with pbo. HR: 0.65; CI 95% 0.49–0.86 CPS ≥ 1: 7.6 with Cht and pembrolizumab vs 5.6 months with pbo. HR: 0.74; CI 95% 0.61–0.90 PD-L1 < 1: 6.3 months with Cht and pembrolizumab vs 6.2 months with pbo. HR: 1·08, CI 95% 0·77–1.53

TNBC triple negative breast cancer, *pbo* placebo, *AC* doxorrubicin and cyclophosphamide, *pCR* pathologic complete response, *EFS* event-free survival. *iDFS* invasive disease-free survival, *DDFS* distant disease-free survival, *HR* hazard ratio, *OS* overall survival, *CI*: confidence interval, *AEs* adverse events, *ORR* overall response rate, *DCR* disease control rate, *Cht*. chemotherapy

pembrolizumab or placebo as first-line treatment in patients with advanced TNBC. PD-L1 expression was tested by Dako 22C3 pharmDx assay. Patients with CPS ≥ 10 (about 38%) treated with pembrolizumab presented better PFS (9.7 *vs* 5.6 months, HR 0.65; $p = 0.0012$) and OS (23 *vs* 16 months, HR 0.73, $p = 0.0093$) compared to placebo. Following these results, the FDA fully approved pembrolizumab with chemotherapy in advanced TNBC with CPS ≥ 10. The approval does not specify the line of therapy nor the chemotherapy backbone [25••].

The phase II NEWBEAT study evaluated the addition of nivolumab to the first-line combination of paclitaxel and bevacizumab in patients with HER2-negative metastatic breast cancer. Twelve-month OS and PFS were 87.1% and 75.8%, respectively. ORR was 83.3% in patients with TNBC [28]. Several other combinations of antiangiogenics with ICI have been tested in metastatic TNBC, such as cabozantinib and nivolumab reporting small ORR [29]. The phase II ATR ACTIB (NCT04408118) trial is evaluating the efficacy and safety of first-line atezolizumab plus paclitaxel and bevacizumab in patients with advanced TNBC.

Early-Stage TNBC

In the neoadjuvant setting, two early trials tested the combination of pembrolizumab and chemotherapy in high risk TNBC. The phase II I-SPY2 trial studied pembrolizumab with weekly paclitaxel and anthracyclines in early HER2-negative breast cancer (with TNBC and HR + cohorts), regardless of PD-L1 status. In the TNBC cohort, pembrolizumab plus chemotherapy triplicated the estimated pCR rate with respect to the control arm [15]. The single-arm phase Ib trial KEYNOTE-173 studied pembrolizumab in combination with six different chemotherapy regimens. Interestingly, this trial showed that higher rates of PD-L1 expression and stromal TIL levels were associated with higher probability of achieving a pCR [30].

The largest trial in early TNBC is the KEYNOTE-522, which led to the first regulatory approval of an ICI in early-stage breast cancer. This phase III study assessed neoadjuvant pembrolizumab in combination with carboplatin and paclitaxel followed by AC. After surgery, the patients continued blinded treatment with pembrolizumab/placebo to complete 1 year. In the first interim analysis including 602 patients, the addition of pembrolizumab significantly improved the pCR rate regardless of PD-L1 expression [16••]. The highest pCR though was observed in patients whose tumors had an increased PDL-1 positivity. Importantly, after a median follow-up of 37 months, the 3-year event-free survival (EFS) rate was 84.5% in the experimental arm *vs* 76.8% in the control arm (HR = 0.63, $p = 0.0003$). In addition, pembrolizumab improved distant recurrence-free survival (DRFS) by 7.5% and showed a favorable trend in overall survival (OS) (HR 0.72 [95% CI, 0.51–1.02]). Pembrolizumab was also associated with improved EFS among patients who had residual disease, and not only among those with pCR [17•].

The IMpassion031 is another phase III trial in which patients were randomized to receive either atezolizumab or placebo with nab-paclitaxel for 12 weeks, followed by 4 cycles of AC. Although atezolizumab provided higher pCR rate in the PD-L1-positive population (69% vs. 49%), patients without PD-L1 expression also showed a higher pCR rate with atezolizumab (48% vs. 34%) [18••].

Atezolizumab was also studied in combination with neoadjuvant chemotherapy in the phase III NeoTRIP trial that compared carboplatin plus nab-paclitaxel with atezolizumab/placebo in high-risk TNBC. Following surgery, 4 cycles of anthracycline-based regimen were administered as per the investigator's choice. The addition of atezolizumab did not significantly increase the pCR rate, but [19] results from other primary endpoints like EFS are still pending. The lack of benefit in terms of pCR could be related to the decision to use anthracyclines post-operatively. Early modulation of biological pathways and immune microenvironment by anthracyclines and taxanes might be different, with anthracyclines eliciting a stronger immune-modulatory effect compared to nab-paclitaxel/carboplatin, which could be particularly evident in "immune low" tumors [31].

The phase II GeparNuevo trial investigated the addition of durvalumab to neoadjuvant chemotherapy. Interestingly, although the pCR rate with durvalumab was not significantly increased, after 42.2 months of follow-up, the addition of durvalumab showed significant differences in invasive disease-free survival, distant disease-free survival, and OS. These results raise the hypothesis that immunotherapy in early breast cancer may develop immunological memory with a long-term protective effect [14, 32].

A systematic review and meta-analysis of all randomized chemo-immunotherapy trials for early-stage TNBC confirmed a significant benefit in terms of pCR with the addition of PD-1 or PD-L1 blockade to neoadjuvant chemotherapy [33]. The evaluation of ICI as adjuvant therapy in early-stage TNBC is being assessed in the ongoing IMpassion030 (NCT03498716) trial, which is testing atezolizumab in combination with adjuvant chemotherapy. Several trials are also evaluating the use of ICI for patients with invasive residual disease following neoadjuvant treatment, including its combination with capecitabine (NCT03756298).

ICIs in Patients with gBRCA1/2 Mutated TNBC

TNBC has the highest incidence of germline BRCA1/2 (gBRCA1/2) mutations, with prevalence rates of 10–15% [34]. PARP inhibitors have demonstrated antitumor efficacy in HER2-negative breast cancer harboring gBRCA1/2

mutations [35, 36]. Interestingly, PARP blockade appears to increase PD-L1 expression in TNBC cells [37], making them potentially targetable by ICI. The phase II TOPACIO trial evaluated the safety and efficacy of niraparib and pembrolizumab in patients with advanced TNBC irrespective of BRCA mutation and PD-L1 status. Among the 45 evaluable patients, ORR was 29%, with a DCR of 49%. Higher clinical effectiveness was found in patients with gBRCA1/2 mutations [38]. The phase Ib/II MEDIOLA trial tested olaparib in combination with durvalumab in patients with solid tumors, including a cohort of patients with gBRCA1/2 mutated HER2-negative metastatic breast cancer. Median PFS was longer in treatment-naïve patients and in the TNBC subgroup, with a median PFS of 4.9 months and OS of 20.5 months [39]. Given the encouraging preliminary results obtained so far, several studies are assessing PARP inhibitors in combination with ICI in breast cancer (NCT02849496, NCT03801369).

ICIs in Hormone Receptor–Positive, HER2-Negative Breast Cancer

Hormone receptor–positive (ER +), HER2-negative breast is characterized by an immunologically cold nature, with lower rates of PD-L1 expression, lower TILs levels, and less genomic instability and TMB [40]. ICIs have also been tested in this subtype, with less encouraging results than those observed in TNBC. The main trials in ER + HER2-negative breast cancer are listed in Table 2.

In the phase I JAVELIN trial evaluating avelumab monotherapy in pretreated patients with all subtypes of metastatic breast cancer, the ORR was only 3%, and the response was not correlated with tumor PD-L1 expression [11]. The phase Ib KEYNOTE-028 trial tested pembrolizumab as single-agent in heavily pretreated patients with ER + HER2-negative breast cancer. The ORR was 12%, but a promising median DOR of 12 months was observed [41]. Evidence for the combination of chemotherapy and immunotherapy in ER +, HER2-negative breast cancer is scarce. A randomized phase II study assessing the addition of pembrolizumab to eribulin in patients that had received endocrine therapy and up to 2 lines of chemotherapy failed to demonstrate any benefit in terms of PFS, ORR, or OS over eribulin alone in either the intention-to-treat or PD-L1 + population. A 65% of grade ≥ 3 toxicity was reported, including two treatment-related deaths in the combination group [42].

CDK4/6 inhibitors in combination with endocrine therapy have become the gold standard first-line treatment for advanced ER +, HER2-negative breast cancer. CDK4/6 inhibitors suppress retinoblastoma phosphorylation in cancer cells, which induces cell cycle arrest and inhibits cell proliferation. In addition, they inhibit immunosuppressive regulatory T cell proliferation, increase neoantigen presentation, and induce the release of proinflammatory cytokines [40]. These findings suggested that it could exist a synergic mechanism between CDK4/6 inhibitors and ICI [48–50]. The NEWFLAME trial evaluated the combination of abemaciclib, endocrine therapy, and nivolumab in patients with ER + HER2-negative metastatic breast cancer, but it had to prematurely stop due to safety concerns [51]. The combination of palbociclib and endocrine therapy with pembrolizumab as first-line therapy in metastatic breast cancer was tested in a phase II single-arm study. The investigational combination was well tolerated and demonstrated an ORR of 56% and PFS of 25.2 months. Grade III–IV adverse events were neutropenia (83%), leucopenia (65%), thrombocytopenia (17%), and elevated liver enzymes (17%) [52].

In the early stage, the combination of neoadjuvant palbociclib and anastrozole with nivolumab in postmenopausal patients with ER + /HER2-negative breast cancer was evaluated in the phase II Checkmate 7A8 study. The study was closed after the safety run-in phase ended, due to higher incidence of grade 3 hepatic toxicity than historical single-agent profiles [53].

Findings from the cohort of ER + /HER2-negative breast cancer from I-SPY 2 trial showed an estimated pCR of 30% in the chemotherapy plus pembrolizumab arm, which doubled the estimated pCR of 13% observed in the chemotherapy arm alone. Moreover, patients who reached pCR following pembrolizumab plus chemotherapy had a 3-year EFS of 93% at a median follow-up of 2.8 years.(14) Two large ongoing phase III studies are evaluating the role of ICI as neoadjuvant treatment in early-stage ER + breast cancer (see Table 3).

ICIs in HER2-Positive Breast Cancer

HER2-positive breast cancers are generally considered more immunogenic than ER +, HER2-negative tumors, but less immunogenic than TNBC. Immune TME has emerged not only as a potential prognostic factor in HER2-positive breast cancer but seems also to modulate the response to anti-HER2 therapies [54]. Importantly, ERBB family members seem to play an important role in evading the antitumor response by modulating the immunological landscape of the TME [55]. Differences in immunogenicity exist also among intrinsic molecular subtypes. HER2-enriched subtype has higher levels of TILs and higher expression of immune activation genes. Additionally, some HER2-targeted therapies have immunogenic properties, activating the antibody-dependent cellular cytotoxicity. In this context, ICIs have mainly been tested in combination with anti-HER2-directed therapy, where preclinical studies showed promising results.

Table 2 Main clinical trials with published results studying immune checkpoint inhibitors in metastatic ER positive/HER2-negative, and metastatic HER2-positive breast cancer, regardless of hormone receptor status

Trial designation phase/*N*	Study population and setting	Study treatment	Outcome
JAVELIN [11] NCT01772004 Phase I 168 patients 72 patients in ER + HER- cohort	Metastatic breast cancer previously treated with taxanes and anthracyclines	Avelumab	ORR: 3.0% (95% CI 1.0–6.8) ORR ER + /HER2 − : 2.8% AEs of any grade: 68.5%, including a grade ≥ 3 event in 13.7%
KEYNOTE-028 [41] NCT02054806 Phase Ib 25 patients	Metastatic ER-positive, HER2 negative breast cancer with PD-L1 + expression and progression to standard therapy	Pembrolizumab	ORR: 12.0% (95% CI, 2.5–31.2%) Clinical benefit rate (CR + PR + [SD for ≥ 24 weeks]): 20% (95% CI, 7–41) Median duration of response: 12.0 months (7.4–15.9) AEs: 64%
NCT03051659 [42] Randomized phase II 88 patients	Metastatic ER-positive, HER2-negative breast cancer	Eribulin with or without pembrolizumab	PFS, 4.1 vs 4.2 months; HR, 0.80; 95% CI, 0.50–1.26; $p = 0.33$ ORR, 27% vs 34%, respectively; $p = 0.49$) Grade ≥ 3 AEs, 65%,
KELLY [43] NCT03222856 Phase II trial 44 patients	Metastatic ER-positive HER2-negative breast cancer	Pembrolizumab plus eribulin	Clinical benefit rate 56.8% ORR 40.9% 95% CI: 26.3–56.8 Median PFS 6 months (95% CI: 3.7–8.4)
NCT03523572 [44] phase Ib/II 52 patients	Metastatic HER2-positive breast cancer progressing on TDM1 and HER2 low metastatic breast cancer progressing to prior standard chemotherapy	T-Dxd plus nivolumab	ORR: 59.4%in HER2 + and 37.5% in the HER2 low cohort DCR: 90.6% and 75.0% in the HER2 + and HER2 low cohort PFS: 8.6 months (95% CI, 5.4-NE) in the HER2 + cohort and 6.3 months (95% CI, 2.3-NE) in the HER2 low cohort AEs grade ≥ 3 occurred in 43.8%. 10.4% of all patients had treatment-related interstitial lung disease
PANACEA [45] NCT02129556 phase Ib/II 58 patients	Metastatic HER2-positive breast cancer progressing on trastuzumab treatment	Trastuzumab plus pembrolizumab	ORR PD-L1 + population: 15% No ORR benefit in PD-L1 negative population AEs:71%, including a grade ≥ 3 in 29%
KATE2 [46] NCT02924883 phase II 202 patients	Metastatic HER2-positive breast cancer progressing to trastuzumab and taxanes	TDM1 plus atezolizumab/pbo	PFS: 8.2 months (95% CI.5·8–10.7) in atezolizumab arm vs 6·8 months (4.0–11.1) in pbo (HR: 0.82, 95% CI 0.55–1.23; $p = 0.33$) AEs grade ≥ 3: 33% among patients who received atezolizumab vs 13% in the pbo arm
CCTG IND.229 [47] NCT02649686 Phase Ib 15 patients	Metastatic HER2-positive breast cancer progressing to standard therapy	Trastuzumab + durvalumab	No responses by RECIST 29% SD All pts < 1% PD-L1 tumor expression
NCT03523572 [44] Phase Ib 48 patients	Metastatic HER2-positive and HER2-low breast cancer progressing to standard therapy	Trastuzumab-deruxtecan + nivolumab	HER2-positive cohort: ORR 59%

Nevertheless, these data have been inconsistent with the clinical evidence.

Trastuzumab has shown to induce both local and systemic immunomodulation, which have been correlated with therapeutic outcomes [56]. In addition, trastuzumab-resistant tumors exhibit an upregulated expression of PD-1/PD-L1 [57]. In the advanced setting, none of the trials evaluating the combination of trastuzumab plus durvalumab (NCT02649686) [47], or avelumab (NCT01772004) [58], observed a significant clinical benefit. The single-arm phase I/Ib PANACEA trial studied the combination of pembrolizumab plus trastuzumab in trastuzumab-resistant patients, resulting in an ORR of 15% in the PD-L1 + population, but no responses were observed in patients with PD-L1 − tumors [45]. A phase II (NCT03125928) and a phase III (NCT03199885) trials are currently assessing the combination of paclitaxel, trastuzumab, pertuzumab, and atezolizumab as first-line treatment in metastatic breast cancer. The phase II KATE2 study randomized patients to either T-DM1 plus atezolizumab or placebo irrespective PD-L1 status, as second-line treatment. No improvement in PFS was observed; however, a prespecified exploratory analysis of the PD-L1 + population showed a trend in favor of the combination (8.5 vs 4.1; HR 0.60 95% CI 0.32–1.11) [46]. The phase III KATE3 study is evaluating T-DM1 plus atezolizumab or placebo in HER2-positive and PD-L1-positive population. Other ICIs, such as pembrolizumab, are currently being studied in combination with T-DM1 (NCT03032107). There are also ongoing trials evaluating ICIs with the novel anti-HER2 agents. Trastuzumab-deruxtecan (T-DXd) is an ADC that has recently appeared in the battery of treatments for HER2-positive disease with robust and practice-changing results [59]. The combination of T-DXd with nivolumab showed synergic antitumor efficacy in mice [60, 61]. A phase I trial (NCT03523572) studied the combination in HER-2 positive and HER-2 low breast cancer. Preliminary outcomes indicate antitumor activity in both HER2-positive and HER2-low breast cancer patients (ORR of 59.4% and 37.5%, respectively). The safety profile was generally manageable with interstitial lung disease reported in 10.4% of the patients (grade 2 in 4 and grade 5 in 1 patient) [44]. The combination of tucatinib and pembrolizumab is also under study in a phase II trial (NCT04789096).

In the early stage, the combination of pertuzumab, trastuzumab, and chemotherapy is the standard neoadjuvant treatment in high-risk, HER2-positive early breast cancer. The IMpassion050 (NCT03726879) is a phase III study evaluating the efficacy and safety of pertuzumab, trastuzumab, chemotherapy, and atezolizumab. The first analysis did not show any benefit in terms of pCR in the ITT or PD-L1-positive populations, so the trial was stopped prematurely [62]. However, longer follow-up is needed for secondary endpoints, which include EFS and OS. There are several clinical trials testing the combination of ICIs, HER2-blockade, and chemotherapy in early breast cancer, such as the neoadjuvant randomized, open-label phase III APTneo study (NCT03595592), and the ASTEFANIA study (NCT04873362), which evaluates adjuvant atezolizumab/placebo plus T-DM1 in patients with residual disease following preoperative treatment (see Table 3).

Predictive Biomarkers

One of the key issues in ICI research is to find and integrate robust biomarkers to improve the efficacy of ICI by identifying which patients with breast cancer are more likely to respond to these therapies and those who will not. Most of these biomarkers have been extrapolated from other solid tumors.

PD-L1/PD-1 Expression

PD-1 is an inhibitory surface receptor mainly expressed by cytotoxic effector T cells, and also by B-cells, natural killer, activated monocytes, and dendritic cells. The ligands of PD-1 are PD-L1 and PD-L2, which can be expressed by tumor cells, but also by other cells in the TME, such as TILs, macrophages, and fibroblasts [63]. Although PD-L1 expression has shown to have good predictive value for ICI's efficacy in metastatic TNBC [22••, 25••], several challenges have hampered the generalization of PD-L1 as a biomarker. One important issue is its determination, as each sponsor implemented a different IHC-based assay for PD-L1 evaluation using different antibodies, different quantification technics, and different cutoffs to define PD-L1 positivity. In early-stage TNBC, the benefit of ICI in combination with chemotherapy is obtained, regardless of PD-L1 expression. Nevertheless, PD-L1 expression was associated with higher rates of pCR regardless of treatment, suggesting it is a marker of immune activation and therefore a prognostic marker in this setting [16••, 18••]. Other challenges include variability and dynamic changes of PD-L1 expression among tumors, and between primary tumors and metastases.(62) This raises the question whether an archival tumor can be representative of what is happening in the current TME. An appealing and quick alternative is the detection of soluble PD-L1, since it is minimally invasive and can be repeated over the course of the disease. However, different physiological conditions such as pregnancy or autoimmune diseases can increase it [64, 65]. The utility of soluble PD-L1 as a predictive biomarker for ICI is under active investigation.

Table 3 Ongoing phase III trials with checkpoint inhibitors in early and metastatic breast cancer (by March 31, 2022)

Trial *N*	Setting	Study treatment	Principal endpoint
TNBC			
GeparDouze NCT03281954 1520 patients	Early TNBC	Neoadjuvant chemotherapy (paclitaxel + carboplatin + followed by AC) plus atezolizumab/pbo and adjuvant atezolizumab/pbo	pCR 5 year-EFS
A-BRAVE NCT02926196 474 patients	Early TNBC	1 year of adjuvant avelumab vs observation	DFS
NCT02954874 1050 patients	Adjuvant treatment in early TNBC, patients with residual disease after neoadjuvant chemotherapy	Pembrolizumab/observation	iDFS
IMpassion132 NCT03371017 572 patients	1st line in metastatic TNBC with early recurrence (12 months)	Chemotherapy (carboplatin, carboplatin-gemcitabine or capecitabine) plus atezolizumab/pbo	OS in PD-L1 positive OS in ITT population
NCT04177108 242 patients	1st line in metastatic TNBC	Paclitaxel plus ipatasertib/pbo plus atezolizumab/pbo	PFS
KEYLYNK-009 NCT04191135 932 patients	1st line in metastatic TNBC	Induction chemotherapy with carboplatin-gemcitabine plus pembrolizumab followed by pembrolizumab ± olaparib maintenance	PFS OS
KEYNOTE-119 NCT02555657 692 patients	2nd or 3rd line in metastatic TNBC	Pembrolizumab vs chemotherapy (capecitabine, eribulin, gemcitabine, or vinorelbine)	OS in CPS ≥ 1 OS in CPS) ≥ 1 OS ITT population
ER positive HER2 negative breast cancer			
The Checkmate 7FL NCT04109066 1200 patients	Neoadjuvant treatment in ER + breast cancer	Chemotherapy (weekly paclitaxel followed by AC/EC) plus nivolumab/pbo and adjuvant ET plus nivolumab/pbo	pCR EFS
KEYNOTE-756 NCT03725059 1140 patients	Neoadjuvant treatment in ER + breast cancer	Chemotherapy (weekly paclitaxel followed by AC/EC) plus pembrolizumab/pbo and adjuvant ET plus pembrolizumab/pbo	pCR EFS
AMBITION NCT04732598 280 patients	1st line in metastatic ER-positive HER2-negative breast cancer	Paclitaxel plus bevacizumab ± atezolizumab	PFS
KEYNOTE-B49 NCT04895358 800 patients	Endocrine resistant, ER-positive metastatic breast cancer with PD-L1 + expression	Chemotherapy (nab-paclitaxel, liposomal doxorubicin or capecitabine) plus pembrolizumab/pbo	PFS and OS in CPS ≥ 10 and ≥ 1
HER2 positive breast cancer			
APTneo NCT03595592 650 patients	Neoadjuvant treatment in early HER2-positive breast cancer	HTCP AC plus atezolizumab followed by HTCP plus atezolizumab HTCP plus atezolizumab	pCR
ASTEFANIA NCT04873362 1700patients	Early breast cancer patients with residual invasive disease following preoperative therapy	Atezolizumab/pbo with T-DM1	IDFS
NCT03199885 600 patients	1st line in HER2 positive metastatic breast cancer	Pertuzumab, trastuzumab, and taxanes plus atezolizumab/pbo	PFS
KATE3 NCT04740918 350 patients	Up to 3rd line HER2-positive and PD-L1 + breast cancer	TDM1 plus atezolizumab/pbo	PFS OS

TNBC, triple-negative breast cancer; *AC*, adriamycin and cyclophosphamide; *pbo*, placebo; *pCR*, pathologic complete response; *EFS*, event-free survival; *DFS*, disease-free survival; *iDFS*, invasive disease-free survival; *OS*, overall survival; *PFS*, progression-free survival; *ITT*, intention to treat; *EC*, epirubicin and cyclophosphamide; *HTCP*, docetaxel, carboplatin, and trastuzumab

Tumor-Infiltrating Lymphocytes

TILs have emerged as an attractive biomarker due to its easy and feasible determination. In early breast cancer, the increase in stromal TILs predicts response to neoadjuvant chemotherapy in all breast cancer subtypes. Moreover, increased TIL levels are associated with a survival benefit in HER2-positive and especially TNBC, but not in luminal-HER2-negative tumors [66]. In luminal breast cancer, the value of TILs is controversial [67]; nevertheless, TILs are an established prognostic biomarker in TNBC and several international consensuses for early-stage breast cancer already recommend its systematic determination in clinical practice [68, 69].

The value of TILs as a biomarker of response to immunotherapy is under development. A retrospective analysis performed on tumor samples from the IMpassion130 trial showed that stromal TILs were associated with PD-L1 positivity. In the control arm, TILs ≥ 10% (pre-specified threshold) were not associated with a benefit in PFS or OS; however, patients with increased stromal TILs had longer PFS and OS when treated with atezolizumab plus nab-paclitaxel [27]. In HER2-positive breast cancer breast, the KATE2 trial showed that higher TILs levels were associated with PD-L1 positivity and in patients with higher TILs, the addition of atezolizumab to T-DM1 showed a greater benefit in PFS [46]. In the PANACEA trial, patients with higher TILs had higher ORR to pembrolizumab plus trastuzumab compared to patients with low TIL tumor samples [45]. In a recent meta-analysis, evaluating 2500 patients with 33 different tumor types and treated with immunotherapy, CD8 + TIL levels were associated with improved patient's outcomes, regardless of ICI agent [70].

Tumor Mutational Burden

TMB is the number of mutations within the coding region of a tumor genome, and it is commonly reported as the number of non-synonymous mutations per megabase (Mut/Mb). Highly mutated tumors can produce many neoantigens, and these might increase T cell reactivity. In June 2020, the FDA approved pembrolizumab monotherapy for the treatment of previously treated patients with unresectable or metastatic TMB-high (TMB-H; ≥ 10 Mut/Mb) solid tumors, based upon the results of KEYNOTE-158 trial. In this non-randomized, multi-cohort phase II trial, patients with TMB-H tumors had an ORR of 29% *vs* 6% in non-TMB-H tumors [71]. However, high TMB is uncommon in breast cancer, although it is more frequently observed in invasive lobular cancer (8%). Barroso-Sousa et al. analyzed 3969 breast cancer samples by either whole-exome or gene panel sequencing, and around 5% had a TMB ≥ 10. Median TMB significantly varied according to tumor subtype (TNBC > HER2-positive > HR + /HER2-negative, $p < 0.05$) and tumor sample type (metastatic > primary, $p = 2.2 \times 10–16$). In this series, anecdotal responses to ICI were noted in TMB-H tumors [72]. The TAPUR study is a phase II basket trial assessing the safety and efficacy in real-world practice of commercially available, targeted anticancer drugs in patients with advanced cancer whose tumor harbors a potentially actionable genomic variant. Data from 28 patients with high TMB (9–37 Mut/Mb) revealed a disease control rate and ORR of 37% (95% CI 21–50) and 21% (95% CI 8–41), respectively. Median PFS was 10.6 weeks [73].

Dual checkpoint inhibition with nivolumab plus ipilimumab may be a promising treatment option for patients with HER2-negative metastatic breast cancer and high TMB, according to results of the NIMBUS trial. This phase II study ($N = 30$) assessed this immunotherapy doublet in patients with metastatic HER2-negative breast cancer (70% ER + disease) and high TMB (≥ 9 mutations/Mb). There were five (16.7%) confirmed ORR, meeting the study's primary endpoint. Interestingly, the ORR among patients with a TMB of ≥ 14 Mut/Mb was 60%, with a median PFS of 9.5 months compared to 4% and 1.4 months, respectively, in the lower TMB group [74]. Approximately 10% of patients with metastatic breast cancer have high TMB and could potentially benefit from this approach; however, the optimal TMB cutoff for selecting suitable patients remains unclear.

Next-Generation Sequencing

The gain of function mutation (amplification) in CD274 gene (which encodes for PD-L1) predicted better outcomes with durvalumab in metastatic TNBC [75]. One study examined the genomic mutational landscape of more than 3000 metastatic breast cancers and found that most frequent mutations occurred in PIK3CA, FGFR1, and PTEN; however, these mutations were not associated with response to ICI. Mutations in BRAF, STK11, and MDM2 have been associated to immunotherapy response but in breast cancer, those are rare mutations (< 1%) [76]. Microsatellite instability (MSI) is a pattern of hypermutation that occurs in genomic microsatellites, and it is caused by defects in the mismatch repair system (MMR). Increased mutational load caused by MMR deficit (dMMR) or MSI-high has been shown to predict response to anti-PD-1/PD-L1 in different tumors [77]. FDA approved pembrolizumab for patients with advanced MSI-H/dMMR solid tumors, and also dostarlimab in the same setting [78, 79]. Using large breast cancer datasets, an analysis of 2195 breast cancer patients showed an association between ICI benefit and tumors with somatic mutations in the homologous recombination pathway, with improved OS (HR 0.55, $p = 0.005$, FDR < 0.10) [80]. The APOBEC family of zinc-coordinating enzymes converts cytosine to uracil in single-strand DNA. Dysregulated activity is a

major source of mutations in various cancer types. Different mutational signatures are present in this population, with APOBEC activity being the most common dominant process. The hypermutation occurs in 5% of all breast cancers with the highest enrichment in metastatic tumors. This signature could be used as a potential ICI biomarker but there is still a lack of research in this field [72, 81, 82].

Current Challenges

Given the statistically significant improvement in pCR and EFS observed in the KEYNOTE-522 study, pembrolizumab in combination with neoadjuvant chemotherapy is now considered the new standard of care for patients with high-risk TNBC [17•]. However, new questions arise in this scenario. In this study, patients who achieved a pCR had excellent EFS, regardless of the use of pembrolizumab. Therefore, do those patients really need the adjuvant pembrolizumab? On the other hand, management of patients with TNBC and residual disease following KEYNOTE-522 neoadjuvant treatment is unclear. In patients with residual disease, the current standard of treatment is adjuvant capecitabine based upon the CREATE-X trial, in which significant survival benefits were observed [83]. Importantly, in the KEYNOTE-522, adjuvant capecitabine was not allowed. Nevertheless, patients with residual disease treated with adjuvant pembrolizumab had a significant EFS benefit compared to placebo. However, it remains unknown whether this EFS benefit is derived from the neoadjuvant portion or the adjuvant portion of pembrolizumab. Immunotherapy may lead to immunologic memory, and response may not tell the whole story of how these drugs work. Remarkably, in the GEPARNUEVO clinical trial, there was a similar EFS benefit observed with ICI, even though ICI was only administered in combination with neoadjuvant chemotherapy and not continued adjuvantly (XX). Combination therapy of adjuvant pembrolizumab plus capecitabine in this setting is an appealing approach and, based upon studies on other malignancies, could be safely combined; however, there is no data to support this strategy. Similarly, in patients with gBRCA1/2-mutated TNBC and residual disease following neoadjuvant treatment, adjuvant olaparib demonstrated a significant improvement not only in DFS, but also in OS.(82) Combination of adjuvant pembrolizumab and olaparib in patients with residual disease is also unexplored. Another challenge is patients who relapse after having received an ICI-based treatment in the early setting. Those patients will probably not be sensitive to PD-1/PD-L1 blockade anymore, highlighting the need for emergent strategies beyond conventional chemotherapies and ICI [84].

Response rates have classically been used as a marker of effectiveness. However, we have learned that immunotherapy can generate a pattern of response that is maintained even after treatment cessation [85]. Future studies may need to consider not only the depth of response, but also its duration. On the other hand, most trials in metastatic breast cancer have studied the simultaneous combination of chemotherapy and immunotherapy. It is possible that other schemes such as induction with chemotherapy and ulterior maintenance with immunotherapy may induce even stronger responses and delay tumor progression.

Novel Approaches

New approaches are moving toward environmental modifiers of immunity including the microbiome, and metabolic and hormonal parameters. Combination strategies do not only focus on chemotherapy, but also on other therapies like cryoablation, radiotherapy, oncolytic viruses, antibody associations, or other targeted therapies [86]. Table 3 synthetizes a selection of ongoing phase III trials that evaluate ICI and novel combinations, and Table 4 shows new treatments beyond ICI such as cancer vaccines, oncolytic viruses, cell therapies, and modulation of cytokines.

As we are moving toward a more personalized medicine, targeted therapies are object of growing interest. The PI3K/AKT/mTOR pathway has shown to play an important role in endocrine resistance [97]. Emerging evidence supports its importance in creating an immunosuppressive TME [98], providing an interesting rational for combination strategies. In a phase I clinical trial, ipatasertib plus taxane and atezolizumab (NCT03800836) showed benefit in terms of ORR [99, 100]. An ongoing phase III trial will provide further results of the combination of these three agents (NCT04177108). Likewise, alterations in the mitogen-activated protein kinase signaling haven been associated with negative immunity regulation, and resistance to taxanes in cell cultures [101]. Based on this, the phase II COLET trial tested the combination of cobimetinib, taxanes (nab-paclitaxel or paclitaxel), and atezolizumab as first-line in TNBC, showing clinical benefit with an acceptable safety profile. Noteworthily, patients receiving paclitaxel experienced better ORR and PFS than those with nab-paclitaxel.

The combination of PD-1/PD-L1 inhibitors with antibodies targeting other co-inhibitory molecules, such as anti-LAG3, anti-TIM3, and anti-TIGIT (NCT02913313; NCT03099109), or with antibodies targeting co-stimulatory molecules like OX40 (NCT03971409) or 4-1BB (NCT03364348, NCT03414658), has triggered research interest in breast cancer. The use of anti-RANKL has shown activation also of the immune system in breast cancer [102]. The combination of the PD-L1 inhibitor durvalumab and the anti-CTLA4 tremelimumab was studied in metastatic breast cancer with clinical benefit in TNBC

Table 4 New immunotherapy approaches beyond immune checkpoint inhibitors

Immunotherapy approach	State of art in breast cancer	Main clinical trials (by August 30, 2022)
ONCOLYTIC VIRUSES constitute an anti-tumor tool through two mechanisms, either through non-replicative viruses that infect the tumor cell causing its lysis, destroying tumor vasculature and activating the immune response against tumor cells, or by acting as vectors that generate cytotoxic proteins, insertion of therapeutic genes, or sensitization of cancer cells to chemo-/radiotherapy among others	A wide variety of viruses have been studied, but only talimogene laherparepvec (T-VEC) in melanoma is FDA approvedl. [87] A large number of preclinical studies have been reported in breast cancer, and some of them have also been evaluated in early clinical trials. However, to date, oncolytic viruses have not been as clinically successful as other immunotherapeutic approaches. [88–91]	● NCT03004183 Phase II. Stereotactic Body Radiation Therapy and in situ oncolytic virus therapy in metastatic TNBC ● NCT04445844 Phase II. INCMGA00012 and the oncolytic virus pelareorep in metastatic TNBC ● NCT02779855 Phase I/II. T-VEC in combination with neoadjuvant chemotherapy in TNBC ● NCT04215146 Phase I/II. Overall response rate by inducing an inflammatory phenotype in metastatic BC with the oncolytic reovirus pelareorep in combination with avelumab and paclitaxel
CHIMERIC ANTIGEN RECEPTOR cell (CAR) cell therapy relies on modifying T cell receptors to express chimeric antigen receptors that target a specific tumor antigen. Several efforts have been made to enhance the activation and the specificity of CAR-T cells which have resulted in 4 generations of CARs classified depending on the number of costimulatory domains	In contrast to the remarkable clinical success of CAR-T cells in hematology, these therapies have more limitations in solid tumors, such as greater difficulty in reaching tumor cells and a hostile tumor microenvironment. Several CAR-T cells have been engineered to target different breast cancer antigens in the metastatic setting. However, they are still at an early stage and require further validation in clinical trials. Their combination with ICIs and other immunotherapies is expected to ameliorate cancer patient outcomes. [89, 90, 92–94]	● NCT04430595 Phase I/II. Multi-4SCAR-T Therapy Targeting Breast Cancer ● NCT04650451 Phase I/II. safety and activity of HER2-targeted dual switch CAR-T cells (BPX-603) in subjects with previously treated advanced HER2-positive solid tumors ● NCT04348643 Phase I/II. CEA-targeted CAR-T therapy in patients with relapsed and refractory CEA + cancer
CANCER VACCINES aim to induce the recruitment of T-cells into the tumor environment and thereby trigger an immune response. Cancer vaccines can target tumor-associated antigens such as HER2 or mucin (MUC), but they can also be viral vaccines, DNA vaccines or even whole cell-based vaccines. In addition, there is the option of using viral vectors or bacterial plasmids to function as a shuttle system that delivers and expresses a tumor antigen to help activate targeted cellular and humoral immunity	Active immunotherapy in breast cancer has been broadly studied. Although encouraging results were obtained in preclinical analyses, most clinical trials with vaccines failed to show significant clinical improvement in breast cancer [89, 95].	● NCT00003638 Phase III. Theratope vaccine for metastatic breast cancer ● NCT03562637 Phase III. Anti-Globo H Vaccine Adagloxad Simolenin (OBI-822)/OBI-821 in the adjuvant treatment of high-risk early stage Globo H-Positive TNBC ● NCT01479244 Phase III. NeuVax (Nelipepimut-S or E75) vaccine to prevent breast cancer recurrence
Modulation of CYTOKINES existing in the tumor microenvironment can be done pharmacologically to increase those that promote the immune response or inhibit those that promote tumor progression	IL-2 is one of the most studied, as it enhances the activation of cytotoxic T and NK cells, boosting the antitumor response. Conversely, inhibition of immunosuppressive cytokines, such as TGF-β, IL-6, and IL-8 (associated with advanced disease, increased risk of recurrence, therapeutic resistance, and immune suppression) is another promising area of research. However, the evidence in clinical trials of these approaches is very limited	● NCT02491697 Phase II. Dendritic Cells Co-cultured With Cytokine-induced Killer Cells immunotherapy combined with capecitabine versus capecitabine monotherapy in advanced breast cancer ● NCT04373031 Phase II. Pembrolizumab, IRX-2, and chemotherapy in early TNBC

Table 4 (continued)

Immunotherapy approach	State of art in breast cancer	Main clinical trials (by August 30, 2022)
ANTIBODY DRUG CONJUGATES (ADC) consist of a monoclonal antibody covalently linked to a cytotoxic drug by a chemical linker. The aim of this pharmacological structure is to achieve pharmacological activity mainly in the cells targeted by the antibody	In recent years, the emergence of several antibody–drug conjugates like trastuzumab deruxtecan or sacituzumab have dramatically changed the paradigm of HER-positive disease, TNBC, and HER2-low. Current studies focus on their positioning within the therapeutic battery for the different types of breast cancer [96].	● NCT03734029 Phase III. DS-8201a, an Anti-HER2-antibody Drug Conjugate versus treatment of physician's choice for HER2-low, unresectable and/or metastatic breast cancer ● NCT04595565, Phase II. Postneoadjuvant Sacituzumab Govitecan in HER2-negative breast cancer patients with high relapse risk after standard neoadjuvant treatment ● NCT05104866 Phase III. Dato-DXd versus investigator's choice of chemotherapy in metastatic ER positive, HER2-negative breast cancer who have been treated with one or two prior lines of systemic chemotherapy ● NCT05374512 Phase III. Dato-DXd versus investigator's choice chemotherapy in patients with locally recurrent inoperable or metastatic TNBC, who are not candidates for PD-1/PD-L1 inhibitor therapy ● NCT05426486 Phase III. ARX788 combined with pyrotinib maleate versus trastuzumab plus pertuzumab with docetaxel and carboplatin as neoadjuvant treatment in HER2 + breast cancer

TNBC triple-negative breast cancer, *CEA* carcinoembryonic antigen, *CAR-T* chimeric antigen receptor T cell, *HER-2* Human Epidermal Growth Factor Receptor 2, *ER* Hormone receptor–positive, *IL* Interleukin, *TGF-β* Transforming growth factor beta, *NK* natural killers, *BC* breast cancer

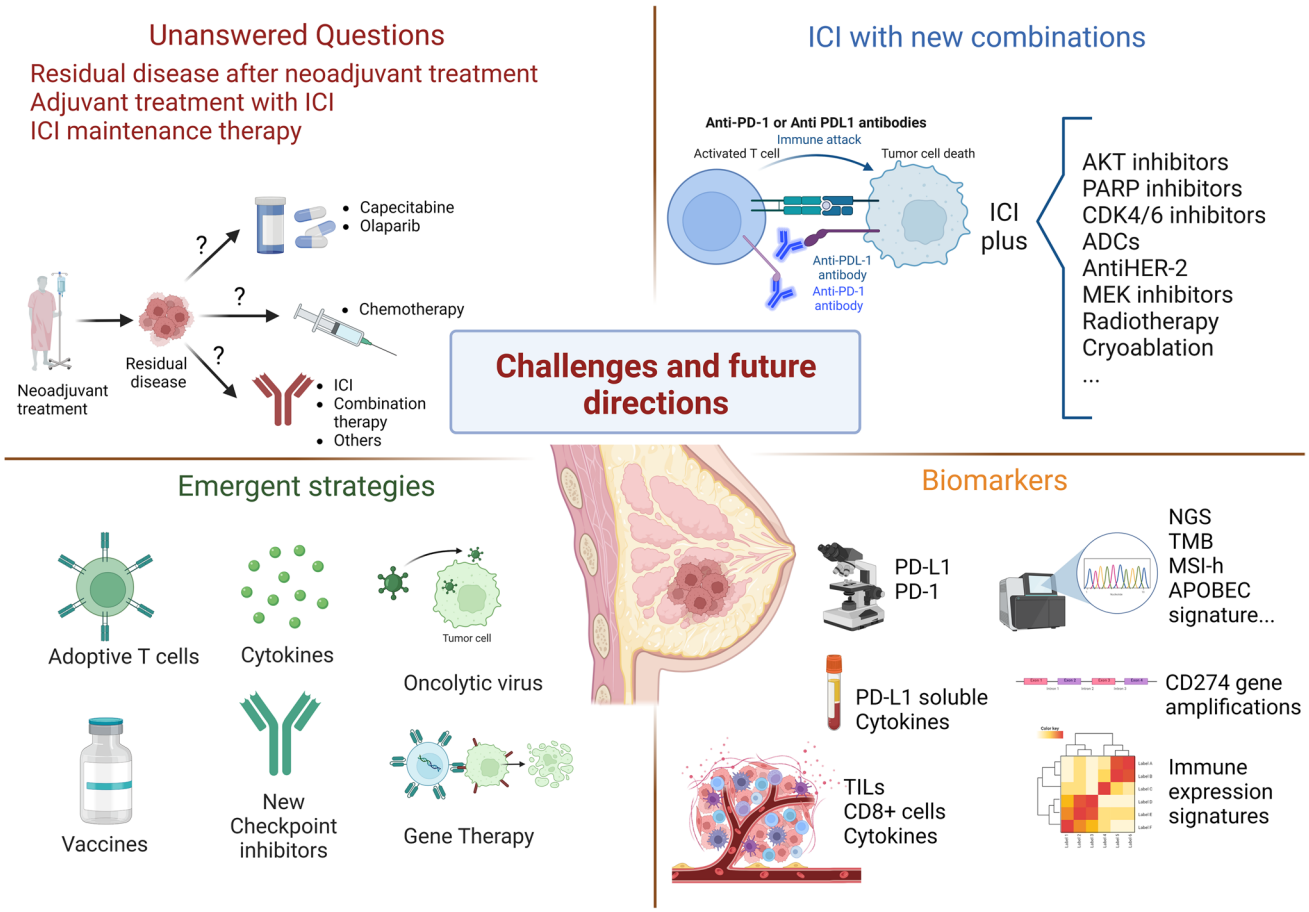

Fig. 1 Current challenges and novel immunotherapy approaches in breast cancer. There are still many questions to be resolved, like which is the best adjuvant treatment for patients with residual disease following neoadjuvant treatment with ICI in TNBC. In metastatic disease, after a combined therapy of ICI and chemotherapy (Ch.), could ICI be used as maintenance treatment? Another important point on the research agenda is which combinations will increase the efficacy of ICI not only in TNBC but also in other breast cancer subtypes: combinations of ICI plus AKT inhibitors, PARP inhibitors, antiangiogenic, or with HER2-targeted therapies, are under investigation. Novel immune approaches beyond antiPD-1/PDL-1, such as vaccines, gene therapy, and oncolytic viruses, are being developed. Finally, identification and integration of robust biomarkers to select optimal patients for immunotherapy is crucial. Abbreviations: ICI, immune checkpoint inhibitors; PARP, diphosphate-ribose polymerase; CDK, cyclin-dependent kinase; ADC, antibody–drug conjugate; PD-L1, programmed death-ligand 1; PD-1, programmed cell death protein, NGS, next-generation sequencing; TMB, tumor mutational burden; MSI-h, microsatellite instability high; TILs, tumor infiltrating lymphocytes. Figure created with biorender.com

[103]. Bempegaldesleukin is a selective agonist of the βγ-receptor complex of IL-2. Studies supported the ability of this drug to turn PD-L1-negative tumors into positive ones. It is currently being tested in combination with nivolumab in metastatic solid tumors (NCT02983045). Preliminary results from the metastatic TNBC cohort indicate an acceptable safety profile with similar disease control rates (50%) both in the PD-L1-negative and in the PD-L1-positive population.

Finally, the combination of ICI with local ablative therapies is another immunotherapy approach. Irradiating tumor lesions may boost the systemic immune response and eventually impact in distant metastatic sites, which is known as the abscopal effect. Although the bulk of the evidence is preclinical so far, several trials have evaluated the integration of radiotherapy with checkpoint inhibition in breast cancer, with contradictory results. Many trials evaluating different drugs, radiation dosing, and fractioning are ongoing (NCT04990921, NCT02730130, NCT0523369). Figure 1 illustrates some of the current challenges and novel immunotherapy approaches for breast cancer.

Conclusions

Immunotherapy is the fourth pillar of modern oncology and although the most robust evidence of its use is for TNBC, more and more data are arising on its use in other breast cancer subtypes. Ongoing clinical trials will further refine immunotherapy strategies in breast cancer. This must be accompanied by the identification and integration of robust biomarkers to

improve the efficacy of immunotherapy and also to identify patients who can avoid them, and therefore, their associated toxicities and costs.

Author Contribution Monica Cejuela and Andrea C. Vethencourt performed the literature search and drafted the first manuscript, and Sonia Pernas critically revised the work.

Declarations

Conflict of Interest S.P. has served as an advisor/consultant for AstraZeneca, Daiichi Sankyo, Eisai, Novartis, Polyphor, Roche, and SeattleGenetics.

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.

Ethics Approval Not applicable.

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• Of importance
•• Of major importance

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