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A review of immune checkpoint blockade in breast cancer

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ABSTRACT

In the recent years characterized by the cancer immunotherapy revolution, attention has turned to how to potentially boost and/or generate an efficient anti-tumor immune response in breast cancer (BC). Clinical activity of immune checkpoint blockade (ICB) targeting PD-1 or PD-L1 in BC has been more evident in the triple negative subtype and in earlier lines of the treatment. Remarkably, some responders to single agent ICB have achieved durable responses with metastatic disease, possibly as a result of treatment-induced immunological memory. However, most BC are immunologically quiescent and current research efforts developing ICB combinations are attempting to convert "cold" into "hot" tumors by manipulating the tumor microenvironment, expanding anti-tumor T cells improving efficient antigen presentation, and suppressing pro-tumor inhibitory cells. The aim of this review is to summarize existing data on the efficacy of immune checkpoint blockers as single agents and combination strategies in all BC subtypes, highlighting the BC subgroups that benefit most from ICB.

Introduction

Breast cancer (BC) has traditionally been considered a poorly immunogenic tumor [1] with negative results obtained in early and phase III trials of vaccines in both the early and advanced settings [2] and with other forms of immunotherapy such as Lymphocyte Activation Gene-3 (LAG3) agonists [3]. However, the triple negative (TNBC) [4] and HER2-positive [5] subtypes are characterized by a more extensive immune infiltration that can impact prognosis and may mediate response to treatment. Thus, patients with either TNBC or HER2-positive BC may be ideal candidates for boosting a pre-existing immune response in a cancer immunotherapy strategy. In contrast, high immune infiltration in the luminal subtypes [6] and in lobular BC [7] is associated with a bad prognosis, highlighting the possible dual role played by the natural/spontaneous immune response in BC, differing by subtype and by histotype. In this regard, a recent study showed that transcriptional regulation can positively impact the immune exclusion in luminal BC [8].

Increasing evidence on the clinical relevance of tumor-infiltrating lymphocytes (TIL) [9-12] will hopefully lead to their future incorporation as a biomarker of routine clinical use. To this end, a main step has been the recent introduction of TIL in the new World Health Organization classification of BC [13]. Further, numerous trials of immunotherapy are now incorporating TIL as a biomarker for patient stratification, reflecting a growing clinical need to optimize patient selection, considering the costs and toxicities linked to autoimmune-like adverse events (AEs) that can occur with these new treatments [14]. Targetable inhibitory immune checkpoint molecules, including the Cytotoxic T

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Lymphocyte Antigen (CTLA-4), the Programmed-Cell Death-1 (PD-1) receptor [15] and its ligands PD-L1 [16] and PD-L2 [16] have been characterized in BC, and represent potential targets for cancer immunotherapy (particularly PD-1 and PD-L1) [17].

Results from trials investigating immune checkpoint blockade (ICB) in BC suggest efficacy as monotherapy may be higher in 1) the TNBC subtype; 2) earlier lines of treatment (eg, first line in the metastatic setting and in the neoadjuvant approach); and 3) the PD-L1$^+$ subgroups (either in TNBC, HER2-positive and in a smaller proportion of patients diagnosed with tumors of luminal subtypes). With the anti-PD-L1 antibody (Ab) atezolizumab administered as a single agent, the tumors of 11 women, 9.6% of all those treated, responded to therapy. All those whose tumors responded were alive 2 years after treatment began with a median duration of response (DOR) for these 11 of 19-21 months, but a median progression-free survival (PFS) of only 1.4 (95%CI, 1.3-1.6) months for all 116 evaluable patients [18,19]. Further, in TNBC, improved efficacy from PD-1/PD-L1 ICB may be achieved if administered with chemotherapy [20] with higher objective response rates (ORR) obtained with cisplatin or doxorubicin [21]. The latter results in the advanced setting were subsequently addressed in early-stage TNBC, by adding atezolizumab to either nab-paclitaxel or paclitaxel [22-24]. While the addition of atezolizumab to nab-paclitaxel but not to paclitaxel led to an improved PFS in the metastatic setting, to date neither trial has reported an overall survival (OS) benefit. The value, if any, of nab-paclitaxel will need confirmation, given the nearly identical ORRs and OS in both the intention-to-treat (ITT) and the PD-L1$^+$ subgroups with a difference only in PFS. Thus, the Food and Drug Administration (FDA) approval for atezolizumab was restricted to "combination with paclitaxel protein-bound for unresectable locally advanced or metastatic TNBC whose tumors express PD-L1 as determined by an FDA approved test" and notes the "accelerated approval (is) based on PFS" and was withdrawn by FDA (https://www.gene.com/media/press-releases/14927/2021-08-27/genentech-provides-update-on-tecentriq-u).

Further, other combinations including the use of Poly (ADP-ribose) polymerase inhibitors (PARPi) will need to be further investigated to determine if the addition of ICB accrues benefit [25,26]. The uncertainty arises from published data including the addition of pembrolizumab to niraparib [25] that achieved an ORR of 21% (10/55 patients) but included 7 whose tumors harbored *BRCA* mutations where single agent niraparib has similar modest activity. For example, a higher ORR of 63% in women with germline *BRCA1/2* mutations who were treated with durvalumab plus olaparib [26] was similar to the 59.9% ORR achieved with olaparib alone, and resulted in median DOR and PFS values of 9.2 and 8.2 months, respectively, with the addition of durvalumab, values also similar to the median DOR and PFS of 6.4 and 7.0 months, respectively, with olaparib monotherapy [27,28].

Additionally, uncertainties still exist about the biomarkers that could reliably allow for appropriate patient selection. Among them, PD-L1 assessment on immune cells (ICs) by immunohistochemistry (IHC) was the first United States Food and Drug Administration approved test (first line of treatment) for patient selection in metastatic TNBC. Reproducibility of PD-L1 assessment on ICs is still under debate [29].

Below we discuss the actual state of the art of ICB in BC among the various subtypes including TNBC, HER2-positive and luminal BC.

Results from early phase trials

Tables 1–3 summarize the results from clinical trials that have evaluated ICB in BC. *Early phase trials in metastatic BC* revealed that immune checkpoint blockers as single-agents targeting the PD-1/PD-L1 pathway appeared to achieve higher ORR in: TNBC (range: 3%–44%); PD-L1$^+$ tumors (range: 5%–44% 39% in first line, PD-L1$^+$ TNBC); first line therapy (23%–26%) [18,26]; tumors with a high extent of stromal TIL (6% in second or subsequent lines of treatment, TIL cut-off 5%); patients with low levels of lactate dehydrogenase [30]; the presence of lymph node (LN) metastases and the absence of liver metastases [31]. Of note, in the IMpassion130 phase III trial, similar results were observed with germline *BRCA1* and *BRCA2* status [32]. Finally, as noted above, combinations of PARP-inhibitors plus anti-PD-(L)1 agents achieved ORRs that were similar to results obtained in the EMBRACA [33] and OLYMPIAD trials [27,28] that did not include ICB.

In the HER2-positive subtype (Table 2), clinical trials in the trastuzumab resistant-setting combining therapies targeting HER2 - trastuzumab and trastuzumab-emtasine (T-DM1) - with an anti-PD-(L)1 agent, has reported a suggestion of benefit in the PD-L1$^+$ subgroups (expression evaluated by combined positive score (CPS) and on ICs) [34,35]. Specifically, the combination of durvalumab and trastuzumab administered at standard full doses of both agents found no significant clinical activity in patients with heavily pretreated HER2-positive PD-L1$^-$ metastatic BC (MBC) [34]. While the addition of pembrolizumab to trastuzumab achieved an objective response in 6 of 40 patients (15%, 90% CI 7-29) whose tumors were PD-L1$^+$; but reported no objective responders among patients with PD-L1$^-$ tumors [35]. Finally, using the anti-PD-L1 avelumab as a single agent in patients with MBC led to the conclusion that PD-L1 expression on tumor-associated ICs may be associated with a higher probability of clinical response to avelumab in MBC [36].

In the luminal subtype (Table 3), the use of pembrolizumab in the metastatic setting, in the PD-L1$^+$ subgroup (scored on either tumor cells (TC) and on stromal cells (SC)) achieved a modest ORR of 12% [37]. Combinations including the anti-CTLA-4 tremelimumab, employed as an immune attractant, and hormone therapy (HT) have not fared any better with no responses reported amongst 26 patients [38]. Additionally, despite pre-clinical evidence that purports to show not only an anti-tumor effect, but also an augmentation of T cell activation by the CDK4/6 inhibitor abemaciclib [39–41], clinical trials supporting this strategy have not emerged. A trial begun in 2016 and as of July 2021 not yet reported, enrolled 28 patients with hormone receptor positive (HR+), HER2- MBC who had received 1 to 2 prior lines of chemotherapy to a regimen of pembrolizumab plus abemaciclib. While no new safety signals were detected, the ORR was a modest 14.3% with an uncertain contribution from the addition of pembrolizumab to abemaciclib [42].

What emerges from these studies is unclear benefit of ICB in many subtypes of BC although as with other cancers, patients who benefit from regimens that include an ICB can have lasting responses. Further, pseudoprogression occurs rarely in BC trials and abscopal effects have not been described (43).

ICB in TNBC

Results from the trials employing ICB in TNBC are summarized in Table 1 and Figure 1. Lacking expression of HR and amplification or overexpression of the HER2 receptor, TNBC has been considered as an orphan disease, with chemotherapy the main systemic therapeutic option. The subgroup of patients harboring germline *BRCA1/2* mutations can benefit from treatment with a PARPi, as demonstrated by the EMBRACA [33] and OLYMPIAD [27] studies. Interestingly, in TNBC, increased levels of TIL are associated with improved outcomes, whereas low TIL infiltration has been linked with bad prognosis [27,28], rendering the objective of increasing immune infiltration in "cold" tumors a potential means to improve outcomes.

It is well known that TNBC is a heterogeneous disease and from an immunophenotypical profiling point of view, tumors with poor infiltration of CD8$^+$ TIL (cytotoxic T lymphocytes) exhibit the

Table 1
Immune Checkpoint Blockade (ICB) in Triple Negative Breast Cancer (TNBC).

Trial	• Setting • Phase of Trial • Subtype	Agents	Number of pts (%)	Results, n (%)		Grade 3/4 AEs
KEYNOTE-522 [NCT03036488] Schmid et al, New Eng J Med 2020	• Neoadjuvant • Phase III • TNBC	Pembrolizumab 200 mg + paclitaxel 80 mg/m^2 + carboplatin AUC5 d1 q3w for 4 cycles → pembrolizumab 200 mg + epirubicin 90 mg/m^2 + cyclophosphamide 600 mg/m^2 d1 q3w for 4 cycles	**Number of pts (%)** 784 pts PD-L1+:656 (83.7%) PD-L1-:127 (16.2%)	**pCR** 260 (64.8%)	**36 m EFS** 84.5%	All = 76.8% • Febrile neutropenia (14.6%) • Pyrexia (2.6%)
		Placebo + paclitaxel 80 mg/m^2 + carboplatin AUC5 d1 q3w for 4 cycles → placebo + epirubicin 90 mg/m^2 + cyclophosphamide 600 mg/m^2 d1 q3w for 4 cycles	390 pts PD-L1+:317 (81.3%) PD-L1-:69 (17.7%)	103 (51.2%)	76.8%	All = 72.2% • Febrile neutropenia (12.1%) • Pyrexia (0.3%)
NeoTRIPaPDL1 NCT002620280 Gianni et al, ASCO 2020	• Neoadjuvant • Phase III • TNBC	Atezolizumab 1200 mg d1 + nab-paclitaxel 125 mg/m^2 d1, 8 + carboplatin AUC2 d1, 8 q3w for 8 cycles	**Number of pts (%)** All PD-L1+ (57%)	**pCR** 43.5% 51.9%	**ORR** 76.1% N.A.	• 1.4% infusion-related reactions • Increased AST/ALT
		Placebo d1 + nab-paclitaxel 125 mg/m^2 d1, 8 + carboplatin AUC2 d1, 8 q3w for 8 cycles	All PD-L1+ (54%)	40.8% 48%	68.3% N.A.	• 0.7% infusion-related reactions • Increased AST/ALT
IMPASSION-031, Mittendorf, Lancet 2020	• Neoadjuvant • Phase III • TNBC	Atezolizumab 840 mg q2w + nab-paclitaxel 125 mg/m^2 qw → atezolizumab 840 mg q2w + doxorubicin 60 mg/m^2 and cyclophosphamide 600 mg/m^2 q2w	**Number of pts (%)** All (n = 165) PD-L1 + (n = 7)	**pCR** 95 (57.6%) 53 (68.8%)	–	All = 23% • Anemia • Increased AST/ALT • Neutropenia • Pruritus • Leucopenia • Febrile neutropenia
		Placebo + nab-paclitaxel 125 mg/m2 q1w → placebo + doxorubicin 60 mg/m2 and cyclophosphamide at 600 mg/m2 q2w	All (n = 68) PD-L1 + (n = 75)	69 (41,1%) 37 (49.3%)	–	All = 6% • Nausea • Anemia • Neutropenia • Increased AST/ALT
I-SPY 2 NCT01042379 Nanda et al, ASCO 2017	• Neoadjuvant • Phase II • TNBC and HR+/HER2- BC	Pembrolizumab 200 mg d1 q3w + paclitaxel 80 mg/m^2 d1 q1w for 12 weeks → doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 d1 q3w for 4 cycles	**Number of pts (%)** 69 pts TNBC: 29 (71.4%) HR+: 40 (28%)	**pCR** 62.4% 34.2%	–	–
		Placebo + paclitaxel 80 mg/m^2 qw for 12 weeks → doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 d1q3w for 4 cycles	188 pts TNBC: 89 (19.3%) HR+: 99 (14.8%)	 22.3% 13.6%	–	–
Gepar Nuevo NCT02685059 Loibl et al, Ann Oncol, 2019	• Neoadjuvant • Phase II • TNBC	Durvalumab 1.5 g q4w + nab-paclitaxel 125 mg/m² qw for 12 cycles → durvalumab 1.5 g q4w + epirubicin 90 mg/m² + cyclophosphamide 600 mg/m² q2w for 4 cycles	**Number of pts (%)** 88 pts PD-L1+: 69 (88.5%)	**pCR** 47 (53.4%) 37 (53.6%)	–	All = 3.3% • Leukopenia • Neutropenia • Fatigue • Diarrhea • Skin reactions • Myalgia • Neuropathy
		Placebo + Nab-paclitaxel 125 mg/m² qw for 12 cycles → placebo + epirubicin 90 mg/m² + cyclophosphamide 600 mg/m² q2w for 4 cycles	86 pts PD-L1+: 69 (86.2%)	38 (44.2%) 35 (50.7%)	–	All = 4.9% • Leukopenia • Neutropenia • Nausea • Hand-foot syndrome • Hot flashes

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Table 1 (*continued*)

Trial	• Setting • Phase of Trial • Subtype	Agents	Number of pts (%)	Results, n (%)		Grade 3/4 AEs
NCT02489448 **Foldi et al, NPJ Breast Cancer [Internet] Nature Research 2021**	• Neoadjuvant • Phase I/II • TNBC	Durvalumab 10 mg/kg + nab-paclitaxel q1w for 12 cycles → dose dense doxorubicin + cyclophosphamide (ddDC) q2w for 4 cycles	**Number of pts (%)** All: 55 PD-L1+: 33 (63%) PD-L1-: 22 (37%)	**pCR** 24 (44%) 55% 32%	–	All = 25% • Neutropenia • Neutropenic fever • Fatigue • Dyspnea • Transaminitis • Hypertension • Skin rash
KEYNOTE-173 **NCT020622074** **Schmid et al, Ann Oncol, 2020**	• Neoadjuvant • Phase Ib • TNBC	Chemotherapy: Six cohorts with different regimens Pembrolizumab: 200 mg d1 q3 weeks (w) for 9 cycles +	**Number of pts (%)**	**pCR**	**ORR**	
		A: Nab-paclitaxel 125 mg/m^2 d1, 8, 15 for 4 cycles → doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 d1 q3w for 4 cycles	10 (16.6%)	5 (50%)	80%	Neutropenia (73%) • Febrile neutropenia (22%) • Anemia (20%) • Thrombocytopenia (8%)
		B: Nab-paclitaxel 100 mg/m^2 + carboplatin AUC6 d1 q3w for 4 cycles → doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m2 d1 q3w for 4 cycles	10 (16.6%)	8 (80%)	100%	
		C: Nab-paclitaxel 125 mg/m^2 + carboplatin AUC5 d1 q3w for 4 cycles → doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 d1q3w for 4 cycles	10 (16.6%)	8 (80%)	100%	
		D: Nab-paclitaxel 125 mg/m^2 + carboplatin AUC2 d1, 8, 15 for 4 cycles → doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 d1 q3w for 4 cycles	10 (16.6%)	6 (60%)	90%	
		E: Paclitaxel 80mg/m^2 + Carboplatin AUC5 d1q3w for 4 cycles → doxorubicin 60 mg/m^2 + cyclophosphamide 600mg/m^{2}d1q3w for 4 cycles	10 (16.6%)	2 (20%)	90%	
		F: Paclitaxel 80 mg/m^2 + carboplatin AUC2 d1, 8, 15 for 4 cycles → doxorubicin 60 mg/m^2 + cyclophosphamide 600 mg/m^2 d1q 3w for 4 cycles	10 (16.6%)	5 (50%)	70%	
IMPASSION130 **NCT02425891** **Schmidt et al, NEJM 2018** **Emens et al, Ann Oncol 2021**	• 1st line (met) • Phase III • mTNBC	Atezolizumab 840 mg + nab-paclitaxel 100 mg/m^2 d1, 8, 15 q4w	**Number of pts (%)** All: 451 PD-L1+:185 (41%)	**ORR** 252 (56%) 109 (58.9%)	**PFS / OS** 7.2 m / 21 m 7.5 m / 25.4 m	All = 41.9 % • Potential irAEs = 6.5% • Neutropenia • Peripheral neuropathy • Fatigue • Anemia
		Placebo + Nab-Paclitaxel 100 mg/m^2 days 1, 8, 15 q4w	All: 451 PD-L1+:184 (41%)	206 (45.9%) 78 (42.4%)	5.5 m / 18.7 m 5.0 m / 17.9 m	All = 30.2% • Potential irAEs = 4.7% • Neutropenia • Peripheral neuropathy • Fatigue • Anemia.

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Table 1 (*continued*)

Trial	• Setting • Phase of Trial • Subtype	Agents	Number of pts (%)	Results, n (%)		Grade 3/4 AEs
KEYNOTE-355 **NCT02819518** **Cortes et al, Ann Oncol 2017**	• 1st line (met) • Phase III • mTNBC	Pembrolizumab 200 mg q3w + nab-paclitaxel 100 mg/m^2 d1, 8, 15 q4w OR paclitaxel 90 mg/m^2 d1, 8, 15 q4w OR gemcitabine 1000 mg/m^2 + carboplatin AUC2 d1, 8 q3w	**Number of pts (%)** All: 566 CPS ≥1 : 425 CPS ≥10: 220	– 	**PFS** 7.5 m 7.6 m 9.7 m	All = 68.1% • Neutropenia • Anemia • Fatigue • Increased ALT) • irAEs: 5.5% • Colitis • Pneumonitis • Hypothyroidism • Hyperthyroidism
		Placebo + nab-paclitaxel 100 mg/m^2 d1, 8, 15 q4w OR paclitaxel 90 mg/m^2 d1, 8, 15 q4w OR gemcitabine 1000 mg/m^2 + carboplatin AUC2 d1, 8 q3w	All: 281 CPS ≥1 : 211 CPS ≥10: 103	–	m 5.6 m 5.6 m	All = 66.9% • irAEs = 0% • Neutropenia • Anemia • Fatigue • Increased ALT)
IMPASSION131 **NCT03125902** **Miles et al, Ann Oncol 2021**	• 1st line (met) • Phase III • mTNBC	Atezolizumab 840 mg d1,15 q4w + Paclitaxel 90 mg/m^2 d1, 8, 15 q4w	**Number of pts (%)** All: 431 PD-L1+: 191	**ORR** 123 (53.6%) 121 (63.4%)	**PFS / OS** 5.7 m / 19.2 m 6 m / 22.1m	All = 49% • Peripheral neuropathy • Neutropenia • Anemia • Diiarrhea
		Placebo + Paclitaxel 90 mg/m^2 d1, 8, 15 q4w	All: 220 PD-L1+: 101	104 (47.1%) 56 (55.4%)	5.6 m / 22.8 m 5.7 m / 28.3 m	All = 43% • Peripheral neuropathy • Neutropenia • Anemia
NEWBEAT trial **Ozaki et al, SABCS 2019**	• 1st line (met) • Phase II • mTNBC and mHR+/HER2- BC	Nivolumab 240 mg/m^2 d1, 15 + paclitaxel 90 mg/m^2 d1, 8, 15 + bevacizumab 10 mg/kg on d1, 15 q4w	**Number of pts (%)** All: 57 TNBC: 18 HR+/HER2-: 39	**ORR** 39 (70%) 10 (59%) 29 (74%)	**PFS** 14.8 m 8.1 m 19.1 m	All = 65% • Diarrhea, • Increased AST and ALT • Liver dysfunction • Cholangitis
TONIC TRIAL **NCT02499367** **Kok et al, Ann Oncol 2017** **Voorwerk et al, Nat Med 2019**	• ≥ 1st line (met) • Phase II • mTNBC		**Number of pts (%)** All: 66 PD-L1+ T-:44 (67%) PD-L1- TC:21 (31%) PD-L1+ T-IC: 60 (91%) PD-L1- T-IC: 5 (8%)	**ORR** 13 (20%)	**PFS** 1.9 m	All: 3% • Nivolumab irAEs: 16 • Dyspnea • Increased γ-GT • Increased amylase • Anemia
		Control waiting period for 2 w → nivolumab Irradiation days 1-3 x 8 Gy → nivolumab Cyclophosphamide 50 mg daily orally for 2 w → nivolumab Cisplatin 40 mg/m^2 for 2 cycles → nivolumab Doxorubicin 15 mg for 2 cycles → nivolumab	12 (18%) 12 (18%) 12 (18%) 13 (20%) 17 (26%)	2 (17%) 1 (8%) 1 (8%) 3 (23%) 6 (35%)	– – – – –	

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Table 1 (*continued*)

Trial	• Setting • Phase of Trial • Subtype	Agents	Number of pts (%)	Results, n (%)		Grade 3/4 AEs
KEYNOTE-012 **NCT02447003** **Nanda et al, J Clin Oncol 2016**	• > 1st line (met) • Phase Ib • PD-L1+ mTNBC	Pembrolizumab 10 mg/kg q2w	**Number of pts (%)** All: 27	**ORR** 18.5%	**PFS / OS** 1.9 m / 11.2 m	• Anemia • Aseptic meningitis • Lymphopenia • Headache • Pyrexia
SAFIR-02 BREAST IMMUNO **NCT02299999** **Dalenc et al, SABC 2019** **Bachelot et al, Nat Med 2021**	• 1st/2nd line (met) • Phase II • mTNBC • mHER2+ • mHR+/HER2	Durvalumab 10 mg/kg q2w	**Number of pts (%)** All:131 TNBC: 47 (37.6%) HR+: 76 (60.8%) HER2+:2 (1.6%)	 – – – –	**PFS / OS** 2.7 m / 21.7 m NA / 21.2 m – –	All = 13,2% • Hypothyroidism • Hepatitis • Diarrhea • Pyelonephritis
		Maintenance chemotherapy in patients with CR/PR/SD after 6-8 cycles and not targetable molecular alteration BC	 All:68 TNBC: 35 (52.2%) HR+: 32 (47.8%) HER2: 0 (0 %)	 – – –	**PFS / OS** 4.6 m / 17.9 m OS: 14.0 m – –	All = 15,9%: • Neutropenia • Peripheral neuropath • Diarrhea • Thrombocytopenia
GELATO TRIAL **NCT03147040** **Adams, ESMO Breast 2021**	• 1st/2nd/3rd • Phase II • mILC	Carboplatin AUC 1.5 qw for 12 cycles + Atezolizumab 1200 mg q3w starting after two administrations of carboplatin	**Number of pts (%)** All: 23 TNBC: 5	**ORR** 4 (17%) 4 (80%)	–	
TOPACIO trial **NCT02657889,** **Vinayak et al, JAMA 2019**	• 2nd line (met) • Phase II • mTNBC	Niraparib 200 mg oral once daily + pembrolizumab 200 mg q3w	**Number of pts (%)** All: 47 Germline *BRCA*^{mut}: 15 (32%) Germline *BRCA*^{wt}: 27 (57%)	**ORR** 10 (21%) 7 (47%) 3 (11%)	**PFS** 8.3 m 2.1 m	All = 40% • Anemia • Thrombocytopenia • Fatigue
MEDIOLA trial **Domchek et al, Lancet 2020**	• ≥2nd line (met) • Phase II • HER2- germline *BRCA*^{mut}	Olaparib 300 mg twice daily + durvalumab 1.5 g q4w	**Number of pts (%)** All: 34 TNBC 18 (53%)	**ORR** 19 (63.3%) –	**PFS / OS** 8.2 m / 20.5 m 4.7 m / 20.5 m	All = 32% • Anemia • Neutropenia • Pancreatitis
KEYNOTE-119 **NCT02555657** **Winer et al, Lancet Oncol 2021**	• 2nd/3rd line (met) • Phase III • PD-L1+ mTNBC	Pembrolizumab 200 mg q3w	**Number of pts (%)** All: 312 CPS ≥1: 203 CPS ≥10: 96 CPS ≥20: 57	**ORR** 30 (9.6%) 12.3% 17.7% 26.3%	**PFS / OS** 2.1 m / 9.9 m 2.1 m / 10.7 m 2.1 m / 12.7 m 3.4 m / 14.9 m	Anemia (1%) • Increased AST (3%) • irAEs: • Myositis • Hypothyroidism • Pneumonitis
		Investigator-choice chemotherapy (capecitabine, eribulin, gemcitabine, or vinorelbine)	All: 310 CPS ≥1: 202 CPS ≥10: 98 CPS ≥20: 52	33 (10.6%) 9.4% 9.2% 11.5%	3.3 m / 10.8 m 3.1 m / 10.2 m 3.4 m / 11.6 m 2.4 m / 12.5 m	• Anemia (3%) • Neutropenia (10%)

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Table 1 (*continued*)

Trial	• Setting • Phase of Trial • Subtype	Agents	Number of pts (%)	Results, n (%)		Grade 3/4 AEs
KEYNOTE-086 NCT02447003 **Adams et al, Ann Oncol 2018**	• ≥ 2nd line (met) • Phase II • mTNBC	Pembrolizumab 200 mg q3w	All: 170 PD-L1+: 105 PD-L1-:64	**ORR** 9 (5.3%) 6 (5.7%) 3 (4.7%)	**PFS / OS** 2 m / 9 m 2 m / 8.8 m 1.9 m / 9.7 m	• 12.9% • Diarrhea • Hypothyroidism • Type I diabetes mellitus • Pneumonitis
NCT02730130 **Ho et al, ASCO 2018**	• >2nd line (met) • Phase II • mTNBC	Pembrolizumab 200 mg + RT d1 prior to dose 1 of pembrolizumab (palliative purpose)	**Number of pts (%)** All: 17	**ORR** 3 (17.6%)	**PFS / OS** 2.6 m / 7.6 m	
JAVELIN **NCT01772004** **Dirix et al, Breast Cancer Res Treat, 2017**	• >2nd line (met) • Phase Ib • mTNBC HR+/HER2- HER2+	Avelumab 10 mg/m^2 q2w	**Number of pts (%)** All: 168 mTNBC: 58 (34.5%) PD-L1+: ≥1% TC: 62.5% ≥5% TC: 16.9% ≥25% TC: 2.2% ≥10% T-IC: 8.8%	**ORR** 3% 3 (5.2%) 2.4% 4.4% 0% 16.7%	**PFS/OS** 5.9 wks/ 8.1 m 5.9 wks/ 9.2 m 5.9 m / 6.5 m 6 m / 6.5 m 6 m / 9.2 m 6.1 m / 11.3 m	All = 13.7% • Fatigue • Back pain, • Arthralgia • Pyrexia, • Abdominal pain • Anemia, • Dyspnea, pleural effusion, • AST increased • Autoimmune hepatitis.

Immune Checkpoint Blockade (ICB) in Triple Negative Breast Cancer (TNBC).

AEs = adverse events; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BR = breast cancer; d = day; CPS = combine positive score; CR = complete response; d = day; EFS = event free survival; γ-GT = gamma-glutamyl transferases; HR = hormone receptor; irAEs = immune-related adverse events; ITT = intention to treat population; m = months; mTNBC = metastatic triple negative breast cancer; N.A. = not available; nab = nanoparticle albumin bound; pts = patients; ORR = overall response rate; OS = median overall survival; pCR = pathological complete response; PD-L1 = programmed cell death 1 protein ligand 1; PFS = median progression free survival; PR = partial response; pts = patients; SD = stable disease; TC = tumor cells; T-IC = tumor-associated immune cells; wks = weeks.

Table 2
Immune Checkpoint Blockade (ICB) in HER2-positive Breast Cancer (HER2+ BC).

Trial	• Setting • Phase of Trial • Subtype	Agents	Number of pts (%)	Results, n (%)		Grade 3/4 AEs
KATE-2 trial **NCT02924883,** **Emens et al, Lancet 2020**	• >2nd line (met) • Phase II • mHER2+	Atezolizumab 1200 mg + trastuzumab emtansine 3.6 mg/kg q3w	**Number of pts (%)** All: 133 PD-L1+:57 (43%)	**ORR** 60 (45%) 30 (54%)	**PFS** 8.2 m 8.5 m	• Thrombocytopenia (13%), • increased AST/ALT (8-5%) • Anemia (5%) • Neutropenia (5%)
		Placebo + trastuzumab emtansine 3.6 mg/kg q3w	All: 69 PD-L1+:29 (39%)	30 (43%) 9 (33%)	6.8 m 4.1 m	• Thrombocytopenia (4%), • increased AST/ALT (4%) • Neutropenia (3 %)
PANACEA trial **NCT02129556,** **Loi et al, Lancet 2019**	• >2nd line (met) • Phase II • mHER2+	Pembrolizumab 200 mg q3w + trastuzumab 6 mg/kg q3w	**Number of pts (%)** All:52 PD-L1+: 40 (77%) PD-L1-: 12 (23%)	**ORR** 6 (15%) 0 (0)	**PFS / OS** 2.7 m / N.R. 2.5 m / 7 m	• 29% • Dyspnea, • Pneumonitis, p • Pericardial effusion • Upper respiratory infection
NCT02649686 **Chia et al, ASCO 2018**	• >2nd line (met) • Phase Ib • mHER2+	Durvalumab 1125 mg + trastuzumab 8 mg/kg loading then 6 mg/kg q3w	**Number of pts (%)** All: 14 PD-L1-: 100%	**ORR** 0%	**PFS / % 6 m OS / % 12 m OS** 1.45 m / 51.6% / 17.2%	All = 14% • Type 1 diabetes mellitus • Increased amylase
JAVELIN **NCT01772004** **Dirix et al, Breast Cancer Res Treat, 2017**	• >2nd line (met) • Phase Ib • mTNBC mHR+/HER2- mHER2+	Avelumab 10 mg/m^2 q2w	**Number of pts (%)** All: 168 HER2+: 26 (15.5%)	**ORR** 3% 0 (0%)	**PFS / OS** 5.9 wks / 8.1 m N.A.	• 13.7%: • Fatigue • Back pain, • Arthralgia • Pyrexia, • Abdominal pain • Anemia • Dyspnea • Pleural effusion, • Increased AST • Autoimmune hepatitis

Immune Checkpoint Blockade (ICB) in HER2-positive Breast Cancer (HER2+ BC).
AEs = adverse events; ALT = alanine aminotransferase; AST = aspartate aminotransferase; m = months; N.A. = not available; N.R. = not reached; ORR = overall response rate; OS = median overall survival; PD-L1 = programmed cell death 1 protein ligand 1; PFS = median progression free survival; wks = weeks.

Table 3
Immune Checkpoint Blockade (ICB) in Luminal Breast Cancer (LBC).

Trial	• Setting • Phase of Trial • Subtype	Agents	Number of pts (%)	Results, n (%)		Grade 3/4 AEs
GIADA trial, **Dieci et al, ESMO 2020**	• Neoadjuvant • Phase II • Luminal B BC	Epirubicin 90 mg/m^2 + cyclophosphamide 600mg/m^2 q3w + triptorelin 3,75 mg im q4w for 4 cycles → nivolumab 240 mg q2w for 12 cycles + triptorelin 3,75 mg im q4w + exemestane 25 mg daily	**Number of pts (%)** 43	**pCR** 7 (16.3%)	—	• 16-18% Increased γ-GT, AST and ALT
NEWFLAME TRIAL **Masuda et L, SABCS 2020**	• 1st/2nd line (met) • Phase II • mHR+ / HER2-	Fulvestrant 500 mg q4w + Abemaciclib 150 mg twice daily + Nivolumab Letrozole 2,5 mg daily + Abemaciclib 150 mg twice daily + Nivolumab	**Number of pts (%)** 11 5	**ORR** 54.4% 29%	—	All = 60-66% • Increased AST/ALT • Interstitial lung disease Note: Trial discontinued because of AEs)
GELATO TRIAL **NCT03147040** **Adams, ESMO Breast Cancer 2021**	• 1st/2nd/3rd (met) • Phase II • mILC	Carboplatin AUC1.5 qw for 12 cycles + Atezolizumab 1200 mg q3w starting after two administrations of carboplatin	**Number of pts (%)** All: 23 HR+: 18 (78%)	**ORR** 4 (19%) 2 (11.1%)	—	
NCT02752685 **Novik et al. ESMO Breast Cancer 2020**	• ≥2nd line (met) • Phase II • mHR+ / HER2-	Nab-paclitaxel 100 mg/m^2 1, 8 q3w + pembrolizumab 200 mg q3w (starting with cycle 2)	**Number of pts (%)** 20	**ORR** ORR: 5 (25%)	**DOR / PFS / OS** 3.9 m / 5.6 m / 15.7 m	All = 60% • Neutropenia • Pneumonitis • Hyponatremia
osNCT02779751 **Rugo et al, ASCO 2020**	• 2nd line (met) • Phase Ib • mHR+ / HER2-	Abemaciclib 120 mg orally twice daily + pembrolizumab 200 mg q3w	**Number of pts (%)** 28	**ORR** 8 (29%)	**PFS / OS** 8.9 m / 26.3 m	• Neutropenia (29%) • AST increase (18%) • Diarrhea (11%) • Increased ALT (11%)
MEDIOLA trial **Domchek et al, Lancet 2020**	• ≥2nd line (met) • Phase II • HER2- germline *BRCA*^{mut}	Olaparib 300 mg twice daily + durvalumab 1,5 g q4w	**Number of pts (%)** All: 34 HR+ 16 (47%)	**ORR** 19 (63.3%) —	**PFS / OS** 8.2 m / 20.5 m 9.9 m / 23.4 m	All = 32% • Anemia • Neutropenia • Pancreatitis
KELLY TRIAL **NCT0322285,** **Perez-Garcià et al, EJC 2021**	• ≥2nd line (met) • Phase II • HR+/ HER2-	Pembrolizumab 200 mg d1 + eribulin mesylate 1.4 mg/m^2 d 1, 8 q3w	**Number of pts (%)** All: 44 PD-L1+: 21 PD-L1-: 22	**ORR** 18 (40%) 8 (38.1%) 90 (40.9%)	**PFS / OS** 6 m / NR — —	All = 25% • Neutropenia • Fever • Peripheral neuropathy
NCT03051659 **Tolaney et al, JAMA 2020**	• >2nd line (met) • Phase II • HR+/ HER2-	Pmbrolizumab 200 mg d1 + eribulin mesylate 1.4 mg/m^2 d 1, 8 q3w	**Number of pts (%)** All: 44 PD-L1+: 13 (29.5%)	**ORR** 12 (27%) 3 (23%)	**PFS** 4.1 m 4.2 m	All = 68% • Neutropenia • Oral mucositis • Increased AST/ALT • Peripheral neuropathy • Fatigue
		Eribulin mesylate 1.4 mg/m^2 d 1, 8 q3w	All: 44 PD-L1+: 11 (25%)	15 (34%) 5 (45%)	m 4.3 m	All = 61% • Neutropenia • Oral mucositis • Increased AST/ALT • Peripheral neuropathy • Fatigue

(*continued on next page*)

Table 3
(*continued*)

Trial	• Setting • Phase of Trial • Subtype	Agents	Number of pts (%)	Results, n (%)		Grade 3/4 AEs
KEYNOTE-028 **NCT02054806** **Rugo et al, Clin Cancer Res 2018**	• >2nd line (met) • Phase Ib • HR+/ HER2- PD-L1+ BC	Pembrolizumab 10 mg/kg q2w	**Number of pts (%)** All: 25	**ORR** 3 (12%)	**DOR / PFS / OS** 12 m / 1.8 m / 8.6 m	All = 16%: • Nausea • Septic shock, • Increased γ-GT • Weakness • Autoimmune hepatitis
JAVELIN **NCT01772004** **Dirix et al, Breast Cancer Res Treat, 2017**	• >2nd line (met) • Phase Ib • mTNBC mHR+/HER2- mHER2+	Avelumab 10 mg/m^2 q2w	**Number of pts (%)** All: 168 HR+:72 (42.9%)	**ORR** 3% 2 (2.8%)	**PFS/OS** 5.9 wks / 8.1 m N.A.	All = 13.7% • Fatigue • Back pain • Arthralgia • Pyrexia • Abdominal pain • Anemia • Dyspnea, pleural effusion, • Increased AST • Autoimmune hepatitis.

Immune Checkpoint Blockade (ICB) in Luminal Breast Cancer (LBC).
AEs = adverse events; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BC = breast cancer; d = day; DOR = median duration of response; γ-GT = gamma-glutamyl transferases; HR = hormone receptor; ILC = invasive lobular breast cancer; m = months; Nab = nanoparticle albumin bound; N.A. = not available; ORR = overall response rate; OS = median overall survival; pCR = pathological complete response; PD-L1 = programmed cell death 1 protein ligand 1; PFS = median progression free survival; pts = patients; TNBC = metastatic triple negative breast cancer; wks = weeks.

poorest prognosis; with expression of signatures for fibrosis and fibrotic foci and the immunosuppressive B7-H4 implicated in the prevention of immune infiltration [44]. In contrast, tumors with high CD8$^+$ TIL infiltration in intratumoral areas have the best outcomes, with increased expression of immune checkpoint molecules, and a sustained infiltration by macrophages, as well as of FoxP3$^+$ CD4$^+$ T cells (regulatory T cells) in epithelial areas. Interestingly, when CD8$^+$ TIL localize in the stroma, PD-L1 expression is present in stromal cells and characterizes a tumor microenvironment (TME), with interleukin (IL)-17–producing $\gamma\delta$ T-cells that recruit pro-tumor neutrophils. These cells create an immunosuppressive stromal TME with high expression of stromal PD-L1, indoleamine 2,3-dioxygenase and the presence of stromal FoxP3$^+$ CD4$^+$ T cells [44]. These immunophenotypes will ideally help researchers to identify subgroups of patients that are candidates for ICB administered as a single agent, or in combination with other drugs that try to ameliorate an immunosuppressive TME.

In patients with previously untreated stage II or stage III TNBC regardless of PD-L1 status, the phase III KEYNOTE 522 trial administered four cycles of pembrolizumab (or placebo) with paclitaxel and carboplatin as the first neoadjuvant treatment followed by an anthracycline plus cyclophosphamide plus pembrolizumab (or placebo) as a second neoadjuvant treatment. This strategy achieved a significantly improved pathological complete response (pCR) rate in the entire cohort (65% vs 51%, $P < 0.001$) and in the PD-L1$^+$ subgroup (69% *v* 55% in PD-L1$^+$ compared with 45% *versus* 30%in PD-L1$^-$), as well as an improved event-free survival (EFS) (84.5% *v* 76.8% at the 36-month interim analysis 4 (IA4), HR 0.63 (0.48–0.82) [45]. These results are encouraging and might represent the first step for a change in treatment paradigms in early-stage TNBC, in the neoadjuvant setting [45]. Safety was manageable and consistent with the known toxicities associated with each regimen used. Surprisingly, in the NeoTRIPaPD-L1 [24] the neoadjuvant nab-paclitaxel and carboplatin with atezolizumab, followed by an anthracycline plus cyclophosphamide +/− fuorouracil with atezolizumab after surgery did not result in an increase in the pathologic complete response (pCR) rate in early-stage TNBC. The failure of the NeoTRIPaPD-L1 trial to confirm the results of KEYNOTE 522 could be partially due to a baseline imbalance in stromal and intratumoral TIL that might have resulted in the smaller pCR difference between arms, or possibly to the lack of anthracyclines in the neoadjuvant chemotherapy regimen. Indeed, atezolizumab increased pCR by more than 10% in “immune-rich” groups (PD-L1 IC$^+$, high stromal TIL/intratumoral TIL). Further, PD-L1 dynamic is strong and divergent by arm, with atezolizumab turning most PD-L1$^-$ to PD-L1$^+$.

In TNBC trials have been completed or are being conducted in the neo-adjuvant setting, and in first line and as second or more advanced lines of therapy in the metastatic setting and these are summarized below.

Trials performed in the neoadjuvant setting have explored the following:

1. Neoadjuvant chemotherapy consisting of a carboplatin-paclitaxel based regimen followed by an anthracycline-based treatment given with the anti-PD-1 Ab, pembrolizumab (KEYNOTE 173, phase I/II trial) [46,47], followed by +/- administration of carboplatin after surgery.
2. Paclitaxel +/- pembrolizumab followed by an anthracycline based neoadjuvant regimen without pembrolizumab, followed by +/- administration of pembrolizumab after surgery (I-SPY 2) [48]. I-SPY 2 demonstrated an absolute increase in the likelihood of pCR achievement in all BC subtypes, particularly in the TNBC when pembrolizumab was given in combination with paclitaxel.

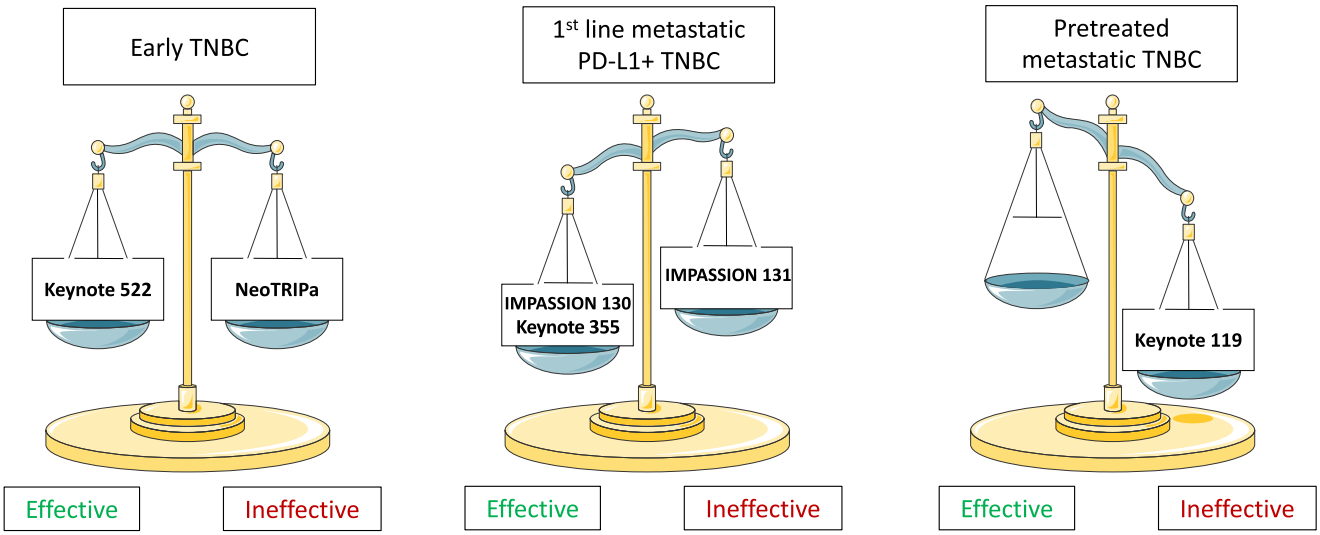

Fig. 1. *Triple Negative Breast Cancer (TNBC) and response to immune checkpoint blockade (ICB).* A) In the early setting, the efficacy of ICB in combination with chemotherapy is still under debate; B) in patients with advanced TNBC, the efficacy of ICB in combination with chemotherapy as a first-line therapy became the new standard of care; C) in patients with pretreated metastatic TNBC, thus far the evidence does not support the use of ICB in monotherapy or in combination with chemotherapy.

3. In GeparNUEVO [49], the anti-PD-L1 agent, durvalumab, was given every four weeks first with weekly nab-paclitaxel, followed by an anthracycline-based chemotherapy regimen, followed by +/- administration of durvalumab after surgery [49]. In the window subgroup where either durvalumab or placebo were given as single agents two weeks prior to the start of nab-paclitaxel, durvalumab increased the rate of pCR compared to placebo (pCR 61.0% *v* 41.4%, OR = 2.22, 95%CI 1.06–4.64, $P = 0.035$; interaction $P = 0.048$), suggesting that administration of the ICB before chemotherapy might result in an increase in tumor responsiveness to standard neoadjuvant treatments.
4. Concurrent administration of durvalumab with nab-paclitaxel for twelve cycles followed by four cycles of dose dense doxorubicin plus cyclophosphamide resulted in a pCR of 46% (59% in the PD-L1$^+$ population *versus* 32% in patients whose tumors were PD-L1) [50].
5. In the IMpassion031, trial the combination of 12-weekly paclitaxel with or without atezolizumab followed by doxorubicin and cyclophosphamide with and without atezolizumab was tested. The results showed an increase in terms of ORR in the ITT population (58% *v* 41%) with a manageable toxicity [51,52].
6. Pending trials are testing new treatment strategies such as 1) the efficacy of neoadjuvant atezolizumab with neoadjuvant chemotherapy followed by adjuvant atezolizumab in TNBC (GeparDouze trial); 2) the efficacy, safety and pharmacokinetic profile of adjuvant atezolizumab plus standard chemotherapy *versus* chemotherapy alone in early-stage TNBC (ALEXANDRA trial) [53]; 3) in the presence of residual cancer burden; the BRAVE protocol will randomize patients to receive placebo or radiotherapy or the anti-PD-L1 avelumab, with the aim to improve EFS in this high-risk group of patients [54].

In the first-line metastatic setting (Table 1) trials thus far have revealed:

1. Efficacy of the combination of the anti-PD-L1 atezolizumab plus chemotherapy with nab-paclitaxel, with improved PFS (observed in the ITT with a significant 10 months gain in the exploratory analysis of OS (HR 0.62, CI 0.45–0.86) conducted in the PD-L1$^+$ (IC) subgroup. Of note, a major benefit in PFS has been observed in previously untreated TNBC patients [22].
2. Lack of a statistically significant difference in terms of ORR, PFS and OS with the combination of atezolizumab plus paclitaxel compared to paclitaxel alone; that might possibly be due to steroid premedication before the administration of paclitaxel, since steroids may interfere with TIL recruitment and PD-L1 expression on IC which are key immune predictive factors of response in TNBC [23,55].
3. Efficacy in terms of PFS, of pembrolizumab versus placebo in combination with chemotherapy (nab-paclitaxel, paclitaxel, or gemcitabine plus carboplatin) in patients with a CPS higher than 10 (9.7 *v* 5.6 months). The study failed to demonstrate an improvement in the ITT population and for this reason is formally hostile [56]. The phase II NewBEAT trial showed an ORR of 59% and a PFS of 8.1 months with the combination of paclitaxel, nivolumab and bevacizumab in untreated metastatic TNBC patients [57].
4. Recently in advanced lobular infiltrating carcinoma the GELATO trial investigated the activity of atezolizumab in combination with carboplatin: the ORR was exiguous in the whole population but reached 80% in the TNBC subgroup [58].
5. Finally, in patients with advanced TNBC who have been previously treated, compared to physician choice chemotherapy (eg, capecitabine, eribulin, gemcitabine or vinorelbine) OS with pembrolizumab as a single-agent was not significantly different in either the primary analysis populations (overall and PD-L1$^+$: PD-L1 CPS≥1%) representing around 65% of cases nor in the 30% of cases with CPS≥10% (KEYNOTE 119) [59,60]. An unplanned exploratory analysis revealed a potential benefit in the 17% of patients with a PD-L1$^+$ CPS ≥20% with more durable responses to pembrolizumab than with chemotherapy (NCT02555657) [60]. Furthermore, in the pembrolizumab arm, but not in the chemotherapy arm, TIL levels were significantly higher in patients whose tumors responded *versus* those whose tumors did not respond and, as a continuous variable, were significantly ($P < 0.05$) associated with all clinical outcomes tested in the pembrolizumab but not in the chemotherapy arm. TIL median distribution was of 5%.

The ideal companion for ICB in TNBC is still under investigation. In the metastatic setting, the TONIC trial [21] showed that compared to placebo, the highest ORRs were seen with doxorubicin plus nivolumab, cyclophosphamide (50 mg po/d), cisplatin (40 mg/m^2 x 2) and radiotherapy (3 × 8 Gy). Despite that, in the NCT02730130 trial, the combination of pembrolizumab with radiotherapy induced a response in only 18% of patients with a modest PFS of 2.6 months in pre-treated advanced TNBC. In the early setting, it has been recently demonstrated that nab-paclitaxel is efficacious whereas paclitaxel may not be, an observation that must be confirmed [24,61].

The issue of biomarkers

Whether PD-L1 expression can be a reliable biomarker of response to ICB in BC, is still a matter of debate. First of all, PD-L1 expression has been assessed as a CPS on TC, TIL, and macrophages, taking into account the total viable tumor cells [62], or on macrophages and TIL with the SP142 Ab clone [63,64]. The best cut-point to discriminate between PD-L1$^+$ and PD-L1$^-$ tumors is still to be defined although in BC it usually corresponds to >1% positive cells. While the clinical activity of ICB has been observed even when PD-L1 assessment is performed in the primary *versus* the metastatic tumor tissue, >1% PD-L1 expression by ICs is heterogeneous across different sites of metastases [65]. It is lower in liver (13%), but higher in brain (44%), breast (43%), lung (43%), LN (51%) and skin (48%) [66].

Starting with PD-L1 assessment by IHC, an exploratory post-hoc biomarker study [65] evaluated PD-L1 expression using the FDA approved SP142 Ab, the 22C3 Ab and the SP263 Ab. PD-L1 was scored on ICs with two Abs (SP142 and SP263) whereas the CPS was employed for scoring PD-L1 staining performed with 22C3. PD-L1 prevalence ranged from 46% to 81%, being higher when using the CPS, with suboptimal overall percentage agreements (OPAs) of SSC3 and SP263 with SP142 of 64 and 69% respectively). When using the cut-off of 1% for PD-L1 positivity, SP142 was the Ab that predicted a major benefit from the combination of atezolizumab plus nab-paclitaxel in terms of either PFS or OS.

In patients whose tumors do not express PD-L1, SAFIR02, a randomized phase II trial comparing durvalumab *versus* chemotherapy as maintenance treatment in patients with MBC, durvalumab was associated with a better OS in the TNBC subgroup with gain/amplification (HR 0.18, 95%CI 0.05–0.71), compared to the neutral/loss TNBC subgroup (HR 1.1, 95%CI 0.47–2.6) (67).

Compared with patients with BC whose tumors have low extent of TIL those whose tumors have high TIL seem to benefit more from ICB. In the prespecifed analysis of KEYNOTE 119, the levels of TIL identified patients with metastatic TNBC with a greater chance of achieving a response to pembrolizumab monotherapy, particularly in the first-line setting [68]. The cut-off, based on the median expression, was 5% for pre-treated TNBC and 17.5% for untreated TNBC. According to the IMpassion 130 trial, tumors with higher than 0.5% CD8$^+$ TIL derived more significant benefit from the combination of atezolizumab and nab-paclitaxel compared to those tumors with low CD8$^+$ TIL [32]. Specifically, intratumoral CD8$^+$ cells, but not stromal TIL, were well correlated with PD-L1 in IC and were predictive of atezolizumab plus nab-paclitaxel efficacy for PFS/OS, while stromal TIL only predicted PFS benefit. It is worth noting that the majority of PD-L1$^+$ tumors present with higher extent of TIL infiltration, in particular CD8$^+$ TIL, so prospective trials with reliable multivariate analyses are needed to verify a predictive role for CD8$^+$ cells independent of the PD-L1 status [32].

The GeparNuevo trial investigated the role of several immune biomarkers as predictors of response to ICB [69]. According to the results of Karn *et al.*, in early TNBC tumor mutational burden (TMB), immune gene expression profiling (GEP) and TIL infiltration all add independent value for pCR prediction. Indeed, Luen *et al.*, have already demonstrated the lack of correlation between TMB and TIL in BC. Whether TMB also is associated with prolonged survival outcomes is still to be confirmed, as Samstein *et al.*, showed that TMB does not predict for OS in both HR+ and HR- tumors with results from the TNBC cohort still pending.

An association between the Homologous Recombination Deficiency (HRD), that occurs in almost 40% of early-stage TNBC [70], and an increase in TMB has been postulated but lacks solid evidence. Moreover, germline *BRCA1/2* mutations do not predict response to ICB in the IMpassion 130 trial [32]. Whether HRD signatures or HRD functional assays (eg, RAD51) may predict response to ICB is still under investigation [70,71].

Given the foregoing, we can conclude that we are still far from identifying the most accurate biomarker of response to ICB in TNBC and at the present time, probably the best strategy to select patients whose tumors might respond to ICB is to combine PD-L1 expression, some assessment of TIL and/or CD8$^+$ cells, TMB and immune signatures into a reliable predictive modeling [72] (Fig. 2).

In conclusion for patients with a diagnosis of TNBC we need better paradigms for selecting patients that might be candidates for ICB either as a single-agent or in combination with other therapies. Perhaps this might be achievable with the use of immunophenotype models employing ideally, easily assessable and standardized biomarkers such as TIL assessment on hematoxylin and eosin (H&E) stained slides, or with the use of IHC. It would be desirable to be able to turn “cold” tumors into “hot” tumors, by identifying ideal companions such as chemotherapy, targeted agents (eg, MEK-inhibitors, VEGF-inhibitors, etc) and antibody-drug conjugates. Also, an improvement in risk stratification will aid in pursuing personalized treatments, being aware that not all patients with a diagnosis of TNBC have a bad prognosis. Finally, safety might represent a concern if irreversible immune-related AEs (irAEs) might impact the quality of life of long-term survivors. The most common irAEs observed in trials of ICB in TNBC were rash or pruritus, hypothyroidism, and hepatitis [73–75]. Among the possible clinical advantages of using ICBs in early-stage TNBC, a decrease in the use of chemotherapy in patients with high TIL/PD-L1$^+$ TNBCs might be a goal that one can aspire to.

ICB in HER2-positive BC

Results from the trials employing ICB in HER2-positive BC are summarized in Table 2. The prognostic role of TIL in HER2-positive BC has been widely investigated in both the early and the advanced settings (see Introduction) [76,77]. Remarkably an extensive immune infiltration has also been associated with a better response to anti-HER2 agents [78–80], for the immune-related mechanisms that accompany the use of monoclonal Abs (mAb) such a trastuzumab [81] and pertuzumab, that can be potentiated with concurrent use of the tyrosine kinase inhibitor lapatinib. The prevalence of PD-L1 expression by IC was estimated at 42% both in the adjuvant setting in the APT trial (employing the E1L3N IHC assay) and in the advanced setting, in the KATE-2 trial (using the SP142 IHC assay) [63,82].

In the early setting, two trials are investigating the efficacy of anti-HER2 mAbs in combination with ICB. The APTneo trial is testing the combination of trastuzumab, pertuzumab, carboplatin and paclitaxel as neoadjuvant therapy for six cycles every three weeks in patients with HER2-positive BC (NCT03595592 [83]). Similarly, in the Impassion 050 trial participants will receive atezolizumab for four cycles during the neoadjuvant phase with dose dense doxorubicin, followed by atezolizumab for four cycles with paclitaxel for 12 continuous weeks, with trastuzumab and pertuzumab (NCT03726879 [84]).

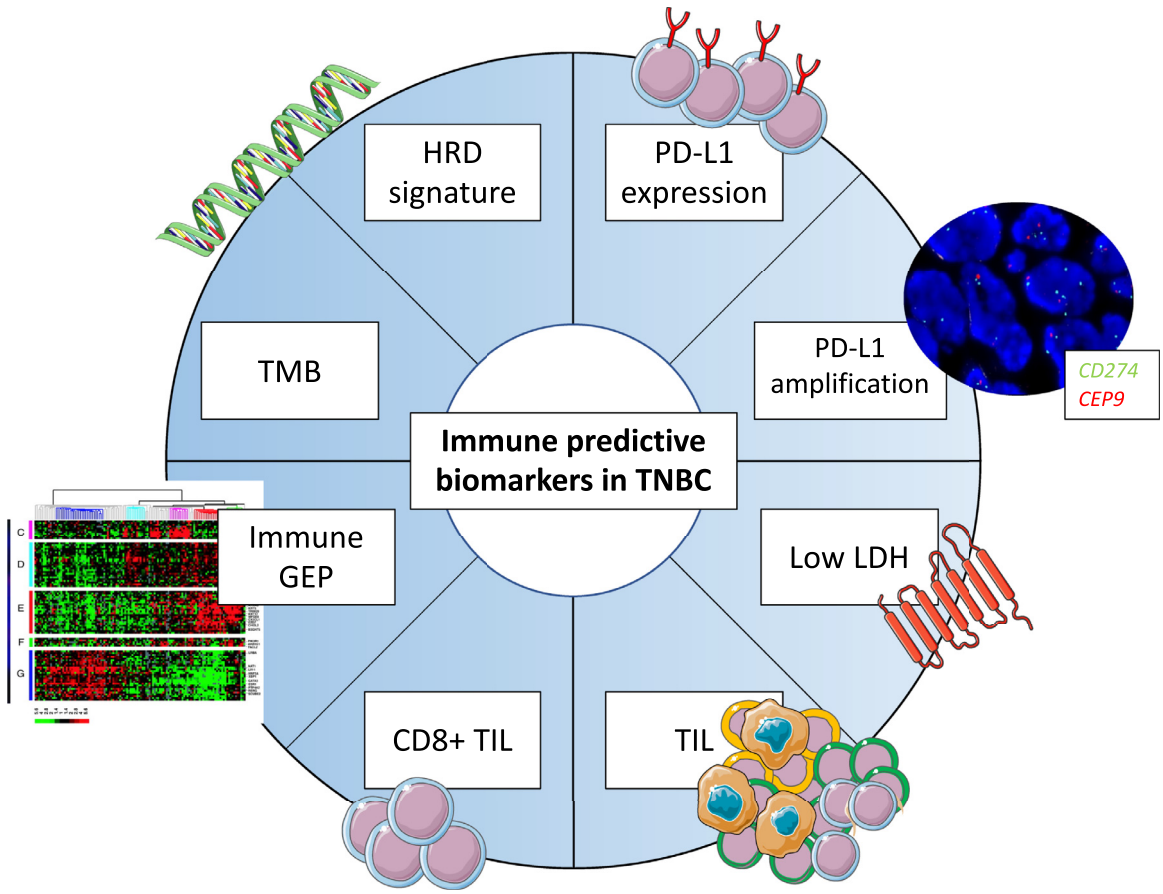

Fig. 2. *Predictive biomarkers of response to immune checkpoint blockade (ICB) in Triple Negative Breast Cancer (TNBC).* Several predictive factors of response to ICB have been identified in TNBC patients, such as PD-L1 expression and amplification, LDH level, extent and composition of tumor-infiltrating lymphocytes (TIL), immune gene expression profiling (GEP), tumor mutational burden (TMB) and Homologous Recombination Repair Deficiency (HRD) signature. None of these biomarkers is able to predict response to ICB accurately; indeed, their combination may more precisely select patients with TNBC who might respond to ICB.

In the advanced setting, in the HER2-positive trastuzumab resistant population ICB with pembrolizumab in association with trastuzumab gave rise to responses and stability of the disease only in the PD-L1$^+$ population and was higher in highly infiltrated tumors (cut-off for stromal TIL was 5%) in a phase II trial [35]. In this study, PD-L1 assessment was performed with a CPS and cut-off for positivity of 1%. The anti-PD-L1 agents avelumab [36] (given as a single agent) and durvalumab [34] (given in association with trastuzumab) did not generate any response in this patient population. Remarkably, the PANACEA trial revealed that in the PD-L1$^+$ cohort (44 patients), the median ORR was 20%; with median PFS (2.7 *v* 2.5 months) and a significant OS gain of 9 months (16 *v* 7 months) in the PD-L1$^+$ subgroup *versus* the PD-L1$^-$.

The phase II KATE-2 trial [63] tested the combination of the anti-PD-L1 atezolizumab plus trastuzumab emtasine (T-DM1) in patients with locally advanced or metastatic HER2-positive BC previously treated with trastuzumab and taxanes, who had experienced progression within 6 months from a previous adjuvant therapy or whose cancer progressed during therapy for metastatic disease. The primary endpoint of the study (investigator assessed PFS) was not reached in the ITT population, whereas the exploratory endpoint of PFS in the PD-L1$^+$ subgroup was reached with a gain of 4.4 months in PFS in the atezolizumab plus T-DM1 treated group (8.5 *v* 4.1 months). OS did not differ in a statistically significant way, although OS rate was numerically higher in the PD-L1$^+$ subgroup. The new drug combination was safe, although the discontinuation rate due to AEs was 29% *versus* 15%, with a major incidence of grade 3 thrombocytopenia, anemia, hepatotoxicity, immune-related rash, hypothyroidism and pancreatitis. Overall, the safety profile of this new combination was consistent with that of each drug.

In women with advanced incurable HER2-positive BC, irrespective of PD-L1 expression, the phase II DIAMOND study will test a 16-week induction phase of ICB combining the CTLA-4 targeting antibody, tremelimumab, with the anti-PD-L1 agent, durvalumab, plus trastuzumab and estrogen suppression for patients who tumors are HR+. This will be followed by the combination of durvalumab, trastuzumab and estrogen suppression in weeks 17–52. PD-L1 status will be assessed.

AVIATOR will explore combinations of trastuzumab, the anti-PD-L1 avelumab and vinorelbine; trastuzumab plus vinorelbine; and trastuzumab, avelumab, vinorelbine and PF-05082566 in women with PD-L1 unselected advanced HER2-positive BC previously treated with trastuzumab and pertuzumab, who have not received prior immunotherapy (NCT03414658 [85]).

Emerging data suggests the use of ICB in women with advanced HER2-positive BC should begin by enriching for PD-L1 expression by IC and for TIL if we are to see some benefit from the combination of PD-(L)1 ICB and anti-HER2 agents (trastuzumab and T-DM1). In early settings it is clear that HER2 amplification/over expression, high TIL and PD-L1 expression are present in patients characterized by a better prognosis and by a better response to standard treatments and here the question is whether these patients could be treated with only PD-(L)1 ICB [86]. The possible emergence of long term irAEs must be considered, in order to test their potential impact on patient reported outcomes.

Probably ICB might serve as a weapon to be used in-patients with residual disease after neoadjuvant therapy that we know are characterized by the worse prognosis [87]. The main question is how to handle TIL-low tumors. These patients should be treated with combination strategies aiming at inducing an immune infiltration that might turn pre-existing "cold" tumors "hot".

ICB in luminal BC

Results from trials employing ICB in luminal BC are summarized in Table 3. Luminal BC is considered a poorly immunogenic tumor, with lower baseline TIL infiltration than seen in TNBC and the HER2-positive subtypes [88,89]. However, TIL infiltration was meaningful in terms of response to neoadjuvant treatments and prognosis [6]. Rugo *et al.* [37], presented their work on the use of pembrolizumab in advanced PD-L1$^+$ cases (representing 19.4% of the screened population, PD-L1 expression was evaluated on either SC or TC, cut-off >1%).

In the early setting, in the GIADA trial, premenopausal patients with Luminal B BC were treated with three cycles neoadjuvant epirubicin plus cyclophosphamide (EC) followed by eight cycles of nivolumab (anti-PD-1) with triptorelin started concomitantly with chemotherapy and exemestane started concomitantly with nivolumab. The study was formally negative with a pCR rate of 16.3% (primary endpoint not met: pre-planned pCR 8/43) [90].

In the advanced setting, the NewBEAT trial tested nivolumab in combination with paclitaxel and bevacizumab as first line therapy in HER2- MBC. In the HR+ subgroup, the ORR was of 74%, with a median PFS of 19.1 months [57]. While a phase II trial investigating the role of nab-paclitaxel and pembrolizumab in patients with HR+/HER2- MBC reported a partial response (PR) rate of 25% and a stable disease (SD) rate of 35%. Median PFS was 5.6 months with a median OS of 15.7 months [91]. Additionally, the combined administration of pembrolizumab and eribulin showed an ORR of 27-40% independent of the PD-L1 expression; a range similar to the ORR of eribulin alone in the same subset of patients (34%) [92,93]. Finally in the GELATO trial, the combination of carboplatin and atezolizumab achieved a modest ORR of 11% in women with HR+ BC with lobular histotype [58].

Luminal tumors represent the ideal *scenario* for turning a "cold" into a "hot" tumor [94]. Administering the immune attractant tremelimumab (anti-CTLA-4) in combination with exemestane in patients with advanced luminal BC, in a phase Ib trial achieved an ORR of 42% and SD >12 weeks [38].

Preclinical data reveal not only anti-tumoral but also inflammatory effects (stimulating T cells) for the CD4K/CD6K inhibitor, abemaciclib, offering a potential for synergistic activity with anti-PD-L1 ICB [39–41]. However, combining pembrolizumab and abemaciclib in women with advanced luminal BC previously treated with 1-2 lines of chemotherapy, without prior PD-(L)1 ICB nor CDK4-6 inhibitor achieved an ORR of 29% and a CBR of 46% [95]. Similar results were obtained with nivolumab administered with letrozole and abemaciclib (ORR 29%); the response rate increased slightly when the CDK4/6 inhibitor and nivolumab were combined with fulvestrant (ORR 54%) but the trial was stopped due to toxicity [96].

Other agents used in immunotherapy combination strategies are histone deacetylase inhibitors [97]. A phase I trial is testing pembrolizumab and histone deacetylase inhibitors in combination with tamoxifene.

ICB in HRR-deficient BC

Results from the trials employing ICB in HRR-deficient BC are summarized in Tables 1 and 3. HRR-deficient BC represents a subgroup of tumors that might derive benefit from specific anti-cancer treatments (eg, ATR, CHK1 or Wee inhibitors) that are under investigation in a variety of clinical trials. The role of the immune response has been evaluated in this setting [28,98]. One of the mechanisms proposed for turning a non-inflamed ("cold") tumor into a highly-inflamed ("hot") tumor is the creation of replication stress by targeting PARP and or ATR. This might lead to the activation of type I interferon response via cGAS STING. Other proposed mechanisms are: 1) an increase in the TMB, which was associated with a major benefit from ICB although the type of neoantigens generated has a greater impact on the likelihood of response to these treatments [99] and 2) the induction of immunogenic cell death, that could be achieved through the use of a variety of chemotherapeutic drugs [100]. Jiao *et al.*, demonstrated the synergism for concurrently targeting PARP and the PD-L1 pathways at the pre-clinical level [28,101]. This combination strategy has been tested in the TOPACIO [102] and MEDIOLA [26], but showed ORRs similar to PARPi monotherapy in the same setting.

Conclusions

ICBs are currently under investigation across all BC subtypes with best results achieved in patients with early-stage TNBC. Further progress with the use of ICB in BC will require a greater understanding of which patients are the ideal candidates to receive these novel treatments given the available data that demonstrates benefit only in a subset of patients.

While results to date have been globally very modest, the field of immunotherapy is rapidly evolving, and innovative strategies may help augment the benefit of immunotherapy in BC in the future. Given that as a possibility, additional questions that might need to be addressed, include: [1] might there be long-lasting toxicities and what impact might these have on fertility and/or on the quality of life? [2] Is hyperprogression frequent in BC and, if so, how should this be handled? [103] [3] Would the evaluation of responses and toxicities at imaging [104–108] represent a new clinical need in this new era of ICB? [4] What is the impact of financial costs? [109–111] [5] Might radiomics offer an innovative predictive biomarker [112,113] of benefit from ICB? [6] Can TIL assessment [114,115] aid in patient stratification in terms of prognosis and benefit from treatments?

Conflicts of interest

BP, OEC, CT, EM, PDS, THS, MS, DF, KWG and CS have no COI to declare. AM reports grants and personal fees from Roche, grants and personal fees from Novartis, personal fees from Macrogenics, grants and personal fees from Pfizer, grants and personal fees from Lilly, grants and personal fees from EISAI, outside the submitted work. AM is member of advisory board of Roche, Lilly, Macrogenics and MSD. MS is consultant/ member of advisory board and/or received speakers' bureau from Amgen, Sanofi, MSD, EISAI, Astra Zeneca, Merck, Bayer.

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