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REVIEW

Progress and pitfalls in the use of immunotherapy for patients with triple negative breast cancer

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ABSTRACT

Introduction: Triple negative breast cancer (TNBC) is an area of high unmet medical need in terms of new effective treatment strategies. Although breast cancer is traditionally considered a 'cold' tumor type, TNBC is the most appropriate subtype for immunotherapeutic strategies; this is due to the high level of tumor infiltrating lymphocytes, PD-L1 expression, and tumor mutational burden compared to other breast cancer subtypes.

Areas covered: This review examines the available evidence on the use of immunotherapeutic strategies in early and advanced TNBC, discusses the pitfalls and limitations often encountered in clinical research, and summarizes data on novel promising immunomodulatory approaches that have been explored in early-phase trials.

Expert opinion: PD-1-blockade is approved for stage II/III TNBC and for first-line treatment of PD-L1-positive TNBC patients with metastatic disease and should be considered standard of care. However, question marks and difficulties remain; these include the identification of predictive biomarkers to select patients who benefit from the addition of PD1-blockade and the balance between efficacy and long-term toxicity for an individual patient. Numerous treatment combinations and new immunotherapeutic strategies beyond PD1 blockade are being evaluated, thus reflecting a promising evolution towards a more personalized approach, and extended clinical benefit in TNBC.

Abbreviations: Triple-negative breast cancer (TNBC); breast cancers (BCs); estrogen receptor (ER); progesterone receptor (PgR); human epidermal growth factor-2 (HER-2); basal-like 1 (BL1), basal-like 2 (BL2); mesenchymal (MES); mesenchymal stem-like (MSL); immunomodulatory (IM); luminal androgen receptor (LAR); basal-like immunosuppressed (BLIS); basal-like immune-activated (BLIA); tumor-infiltrating lymphocytes (TILs); tumor mutational burden (TMB); immune cells (ICs); immunohistochemistry (IHC); overall response rate (ORR); overall survival (OS); progression-free survival (PFS); intention-to-treat (ITT); hazard ratio (HR); confidence interval (CI); Food and Drug Administration (FDA); European Medicines Agency (EMA); immune checkpoint inhibitors (ICI); Combined Positive Score (CPS); disease control rate (DCR); neoadjuvant chemotherapy (NACT); pathological complete response (pCR); event-free survival (EFS); disease-free survival (DFS); residual cancer burden (RCB); San Antonio Breast Cancer Symposium (SABCS); antibody-drug conjugates (ADCs); PARP inhibitors (PARPi); clinical benefit rate (CBR); Histone deacetylase inhibitors (HDACi); Dendritic cell (DC); talimogene laherparepvec (TVEC); granulocyte-macrophage colony-stimulating factor (GM-CSF); mismatch repair deficiency (dMMR).

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1. Introduction

Triple-negative breast cancer (TNBC), accounting for 10–15% of breast cancers (BCs), is generally an aggressive and highly proliferative subtype affecting more frequently younger women [1,2]. TNBC still holds a poor prognosis, with around one-third of patients experiencing distant recurrences and eventually death. Most recurrence events peak at 3 years, and deaths predominantly occur in the first 5 years after diagnosis[1]. Due to these high rates of recurrence and mortality, with chemotherapy remaining the mainstay of systemic treatment for both early and advanced disease, TNBC naturally constitutes an area of high unmet medical need for treatment improvement and optimizing precision medicine [2,3].

TNBC has been immunohistochemically defined by the lack of estrogen receptor (ER), progesterone receptor (PgR) and

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Article Highlights

- Triple-negative breast cancer (TNBC) is an area of high unmet medical need in terms of development of new effective treatment strategies.
- Immunotherapy represents a promising approach in TNBC, due to its relatively higher level of tumor infiltrating lymphocytes, PD-L1 expression, and tumor mutational burden compared to other breast cancer subtypes.
- Pembrolizumab, in combination with chemotherapy, has been approved by the Food and Drug Administration (FDA) for patients with high-risk early TNBC as (neo)adjuvant treatment, and for first-line treatment of patients with advanced TNBC whose tumors express PD-L1 (Combined Positive Score [CPS] ≥10)
- Atezolizumab, in combination with chemotherapy, is no longer FDA-approved for the treatment of patients with advanced TNBC whose tumors express PD-L1 (Immune Cell score ≥ 1%), yet holding this indication according to the European Medicines Agency (EMA)
- Ongoing studies aim to extend the clinical benefit of immunotherapy, evaluating new treatment combinations able to overcome resistance to PD-(L)1 blockade
- Several new immunotherapeutic strategies beyond PD-(L)1 blockade are currently being tested, reflecting a promising evolution towards a more personalized approach in TNBC
- Several unmet needs remain to be fulfilled, including a deeper understanding of TNBC heterogeneity, the identification of reliable predictive biomarkers of response to immune checkpoint inhibitors, the definition of the most appropriate endpoints to evaluate their activity in clinical trials and investigation of less-explored potential long-term toxicities (e.g. on fertility, sexuality, and pregnancy)

This box summarizes key points contained in the article.

human epidermal growth factor-2 (HER-2) overexpression. Nevertheless, TNBCs harbor a heterogeneous nature and, in the last decade, molecular subtyping and genomic profiling have refined this classification, identifying various subtypes among TNBC, carrying prognostic differences and specific targets for precision treatment. In 2011, Lehmann and colleagues first identified, based on cluster analysis of gene expression profiles from 21 BC data sets (among whom 587 TNBC cases), six TNBC subtypes: basal-like 1 (BL1), basal-like 2 (BL2), mesenchymal (MES), mesenchymal stem-like (MSL), immunomodulatory (IM), and luminal androgen receptor (LAR)[4]. BL1 and BL2 subtypes had higher expression of cell cycle and DNA damage response genes, M, and MSL subtypes were enriched in epithelial–mesenchymal transition genes and growth factor pathways, the IM subtype was enriched for genes of the immune cell signaling (immune cell-surface antigens, cytokine signaling, complement cascade, chemokine receptors, and ligands, and antigen presentation), the LAR subtype was characterized by AR signaling[4]. Subsequently, Burstein and colleagues, using mRNA expression and DNA profiling from 198 TNBCs, identified four TNBC subtypes: LAR, MES, basal-like immunosuppressed (BLIS), and basal-like immune-activated (BLIA)[5]. Importantly, in this study, an immune signature seemed to retain an important clinical prognostic value for TNBCs, helping distinguish, among basal-like TNBCs, tumors that are highly infiltrated by immune cells, that harbor an intrinsic better prognosis and may benefit from immune-based therapeutic strategies[5].

BC has traditionally been considered as a 'cold' tumor from an immunological standpoint as compared to other tumor types [6,7]. Nevertheless, among all different BC subtypes, TNBC has been shown to be the preferred subtype for immunotherapeutic strategies, due to higher number of tumor infiltrating lymphocytes (TILs) [4,8–11], with concomitant higher PD-L1 expression [12,13], and higher tumor mutational burden (TMB) [14,15].

The present work aims to review the available evidence for the implementation of immunotherapeutic strategies into clinical practice for TNBCs in the early and advanced settings, discussing pitfalls and limitations of different studies and giving hints into new agents, which proved efficacy in early-phase trials and might translate into future development.

2. Immunotherapy in advanced TNBC

2.1. Atezolizumab

The first evidence of activity of the anti-PD-L1 antibody atezolizumab in patients with TNBC was observed in a multicenter, phase 1 trial investigating atezolizumab monotherapy[16]. Among 116 evaluable patients with metastatic TNBC, the median duration of response was 21 months[16]. Ninety-one (78%) patients had PD-L1 immune cells (ICs) expression ≥1% (using Ventana SP142 immunohistochemistry [IHC] assay) and these patients exhibited higher overall response rates (ORRs) and longer overall survival (OS) compared to those with less than 1% PD-L1-positive ICs (ORR 12% vs. 0%, and OS 10.1 vs. 6.0 months, respectively)[16].

Based on these results, the phase III trial (IMpassion130) was designed to test the efficacy of atezolizumab in combination with chemotherapy (nab-paclitaxel) as first-line treatment for patients with metastatic TNBC (Table 1)[17]. The two co-primary endpoints were progression-free survival (PFS) (both in the intention-to-treat [ITT] population and PD-L1-positive subgroup) and OS, tested hierarchically in the ITT population and, if significant, in the PD-L1-positive subgroup[17]. IMpassion130 demonstrated a modest but significant benefit in PFS in the ITT population (7.2 vs 5.5 months; HR 0.80; 95% CI 0.69 to 0.92; p = 0.002)[18] and in the PD-L1-positive subgroup (7.5 vs 5.3 months; hazard ratio [HR] 0.63; 95% confidence interval [CI] 0.50 to 0.79)[19]. The OS analysis did not reach statistical significance in the ITT population, while showing an improvement in the PD-L1-positive population (25.4 vs 17.9 months, HR 0.67; 95% CI 0.53 to 0.86), although this hypothesis was formally not allowed to be tested according to the statistical plan of the study[19]. Based on these data, in March 2019 the Food and Drug Administration (FDA) granted accelerated approval to atezolizumab plus nab-paclitaxel for patients with previously untreated PD-L1-positive TNBC, followed by the approval of the European Medicines Agency (EMA) in August 2019.

Due to accessibility and availability differences among countries in the use of nab-paclitaxel as first-line companion of atezolizumab, the IMpassion131 trial was designed to test whether weekly paclitaxel could be used as chemotherapy backbone, together with atezolizumab, in a population with similar inclusion criteria of IMpassion130 (Table 1)[21]. Differently from IMpassion130, the primary endpoint of the IMpassion131 trial was investigator-based PFS, assessed first in the PD-L1-positive population (using Ventana SP142 IHC

Table 1. Main studies with immune-checkpoint inhibitors in the advanced setting for patients with triple-negative breast cancer.

Immune-checkpoint inhibitor	Study name	Study design	Treatment	Setting	Population	Main results
Pembrolizumab	KEYNOTE-119[20]	Phase III	Pembrolizumab monotherapy vs. single agent chemotherapy (physician<apos;>s choice of capecitabine, eribulin, gemcitabine, or vinorelbine)	≥2nd line	Advanced TNBC (n = 622)	In PD-L1 CPS≥10 group: • mOS: 12.7 mo in pembrolizumab arm vs 11.6 in control arm (HR 0.78, 95%CI 0.57–1.06) In PD-L1 CPS≥1 group: • mOS: 10.7 mo in pembrolizumab arm vs 10.2 in control arm (HR 0.86, 95%CI 0.69–1.06) In all patients: • mOS: 9.9 mo in pembrolizumab arm vs 10.8 in control arm (HR 0.97, 95%CI 0.82–1.15)
	KEYNOTE-355[25,30]	Phase III	Pembrolizumab or placebo +chemotherapy (taxanes or carboplatin +gemcitabine)	1st line	Advanced TNBC (n = 847)	In PD-L1 CPS≥10 group: • mOS 23.0 mos in pembrolizumab arm vs. 16.1 in placebo arm (HR 0.73, 95%CI 0.55–0.95, p = 0.0093) • mPFS 9.7 mos vs 5.6 (HR 0.66, 95%CI 0.50–0.88) In PD-L1 CPS≥1 group: • mOS 17.6 mos vs. 16.0 (HR 0.86, 95%CI 0.72–1.04, p = 0.0563) • mPFS 7.6 mos vs. 5.6 (HR 0.75, 95%CI 0.62–0.91) In the ITT population: • mOS 17.2 mos vs. 15.5 (HR 0.89, 95%CI 0.76–1.05) • mPFS 7.5 mos vs. 5.6 (HR 0.82, 95%CI 0.70–0.98)
	KEYNOTE-158[31]*	Phase II	Pembrolizumab monotherapy	Heavily pretreated patients, with no alternative treatment options	MSI-H/dMMR advanced noncolorectal cancer (n = 233)	• Objective response rate 34.3% (95% CI, 28.3–40.8%). • mPFS 4.1 mos (95% CI, 2.4–4.9 months) • mOS 23.5 mos (95% CI, 13.5-not reached).
	TAPUR[32]	Phase II	Pembrolizumab monotherapy	Heavily pretreated patients, with no alternative treatment options	Advanced breast cancer with high TMB (9 to 37 mut/megabase) (n = 28)	• Objective response rate 21% (95%CI, 8–41) • mPFS: 10.6 weeks (95%CI, 7.7–21.1) • mOS: 30.6 weeks (95%CI, 18.3–103.3)

(*Continued*)

Table 1. (Continued).

Immune-checkpoint inhibitor	Study name	Study design	Treatment	Setting	Population	Main results
Atezolizumab	Impassion-130[18,19]	Phase III	Atezolizumab or placebo + nab-paclitaxel	1st line	Advanced TNBC (n = 902)	In the ITT population: • mPFS: 7.2 mos in atezolizumab arm vs 5.5 mos in control arm, HR 0.80; 95% CI 0.69–0.92, p = 0.002 • mOS: 21.0 mos in atezolizumab arm vs 18.7 in control arm, HR 0.87, 95% CI 0.75–1.02, p = 0.08 In PD-L1+ population: • mPFS: 7.5 mos in atezolizumab arm vs 5.3 in control arm, HR 0.63, 95% CI 0.50–0.79 • mOS: 25.4 mos in atezolizumab arm vs 17.9 in control arm, HR 0.67, 95%CI 0.53–0.86
	Impassion-131[21]	Phase III	Atezolizumab or placebo + paclitaxel	1st line	Advanced TNBC (n = 651)	In PD-L1+ population: • mPFS 6.0 mos with atezolizumab-paclitaxel vs 5.7 with placebo-paclitaxel (HR 0.82, 95% CI 0.60–1.12; p = 0.20). • ORR 63% vs 55%. • Median DOR 7.2 vs 5.5 mos. • mOS 22.1 vs. 28.3 mos (HR 1.11, 95% CI 0.76–1.64).
Durvalumab	SAFIRO2-IMMUNO [33]	Phase II	Durvalumab vs. maintenance chemotherapy	Maintenance therapy after six to eight cycles of chemotherapy without disease progression	HER2-negative metastatic breast cancer (n = 199, TNBC n = 82)	In the ITT population: • HR for PFS: 1.40, 95%CI 1.00–1.96; p = 0.047 • HR for OS: 0.84, 95 CI: 0.54–1.29; p = 0.423). In TNBC cohort (exploratory analysis): • HR for OS: 0.54, 95% CI 0.30–0.97, p = 0.0377). In TNBC PD-L1+ cohort (exploratory analysis): • HR for OS 0.37 (95% CI 0.12–1.13) In TNBC PD-L1- cohort (exploratory analysis): • HR for OS 0.49 (95% CI 0.18–1.34)
Nivolumab	TONIC[90]	Phase II	Nivolumab with (1) no induction or (2) irradiation or (3) cyclophosphamide or (4) cisplatin or (5) doxorubicin, all followed by nivolumab	Maximum of three prior lines of palliative systemic treatment	Advanced TNBC (n = 67)	Overall cohort: • ORR 20% Cisplatin cohort: • ORR 23% Doxorubicin cohort: • ORR 35%

(*Continued*)

Table 1. (Continued).

Immune-checkpoint inhibitor	Study name	Study design	Treatment	Setting	Population	Main results
						Overall cohort: • ORR 3.0% TNBC cohort: • ORR 5.2%
Avelumab	JAVELIN	Phase I	Avelumab monotherapy	Heavily pretreated patients, with no standard treatment options	Metastatic breast cancer (n = 168, TNBC n = 58)	• Overall cohort: • ORR 3.0% • TNBC cohort: • ORR 5.2%

*No patients with breast cancer were included in the present study.
Abbreviations: FDA: Food and Drug Administration; EMA: European Medicines Agency; TNBC: triple-negative breast cancer; CPS combined positive score; mOS: median overall survival; HR: hazard ratio; CI: confidence interval; mPFS: median progression-free survival; ITT: intention to treat; ORR: overall response rate; DOR: duration of response.

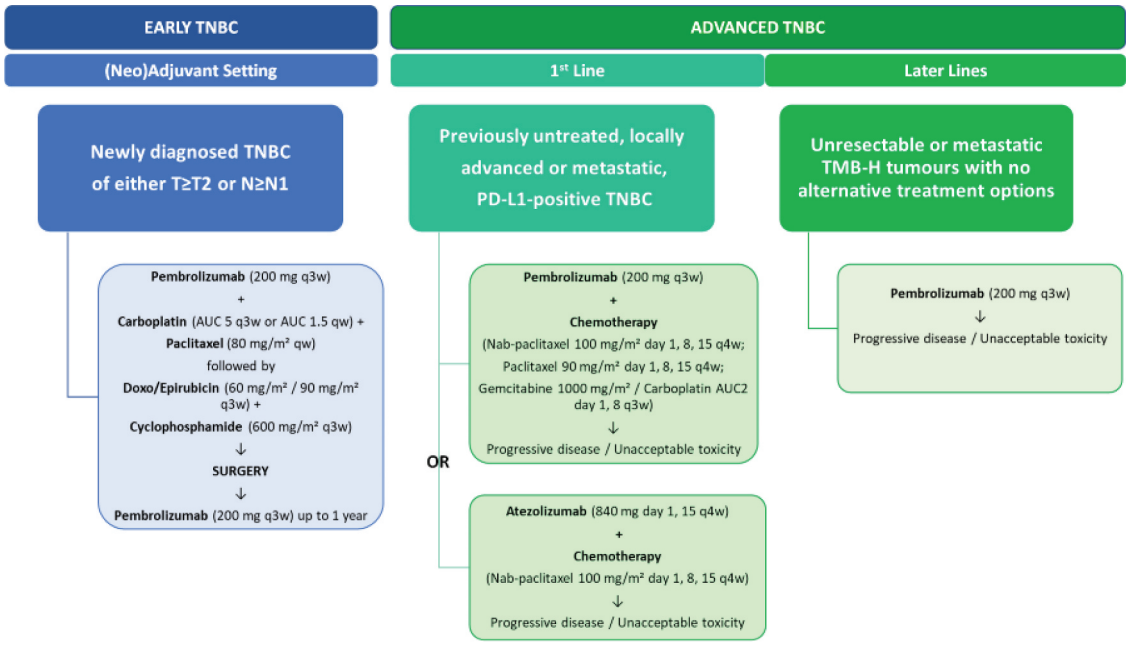

Figure 1. Treatment indication of the approved immune-checkpoint inhibitors (pembrolizumab and atezolizumab) in the treatment of patients with triple-negative breast cancer, in the early (blue boxes) and advanced (green boxes) settings.

Pembrolizumab, in combination with chemotherapy, has been approved by the Food and Drug Administration (FDA) for patients with high-risk early TNBC as (neo)adjuvant treatment, and for patients with advanced TNBC whose tumors express PD-L1 (combined positive score ≥10). Atezolizumab, in combination with chemotherapy, is no longer FDA-approved for the treatment of patients with advanced TNBC whose tumors express PD-L1 (immune cell score ≥ 1%), yet holding this indication according to the European Medicines Agency (EMA). Additionally, pembrolizumab is FDA-approved for the treatment of patients with unresectable or metastatic tumor mutational burden-high [TMB-H; ≥10 mutations/megabase (mut/Mb)] solid tumors, that have progressed following prior treatment and with no alternative treatment options.

assay), and, if significant, in the ITT population. OS was a secondary endpoint, to be tested only if the primary endpoint was positive; stratification factors included PD-L1 status and prior use of taxane-based chemotherapy[21]. Surprisingly, in the PD-L1-positive population (45% of patients), atezolizumab did not improve PFS as compared to placebo (median PFS 6.0 vs. 5.7 months, respectively)[21]. The reasons of such discrepant results are not completely clear, and, besides the different efficacy and immunomodulatory role of the chemotherapy backbone, several hypotheses have been raised [22]. Both trials enrolled a very similar study population in terms of age, performance status, disease setting, metastatic sites, PD-L1 expression, prior chemotherapy, and proportion of de novo metastatic breast cancer. However, it should be considered that TNBC is a remarkably heterogeneous disease, including several subtypes with different composition of the immune microenvironment (beyond PD-L1 expression), and with potentially discordant responses to immunotherapy, thus suggesting that an 'invisible difference' could exist between the two populations enrolled in IMpassion130 and IMpassion131, respectively[22]. Notably, the control arm of the IMpassion131 performed much better than what is expected in this patient population. Interestingly, in an abstract presented at ASCO Annual Meeting 2021, Emens and colleagues analyzed the tumor microenvironment of tumor specimens from patients enrolled in IMpassion130 (including PD-L1 status, CD8-based immune phenotypes and RNA-sequencing for molecular subtyping). This exploratory analysis showed that immune-inflamed tumors and basal-like immune activated tumors had the highest clinical benefit from the addition of immunotherapy to chemotherapy, while the LAR subtype did not seem to derive benefit from the addition of atezolizumab [23]. Moreover, the concomitant use of steroids associated with paclitaxel has been proposed as another possible explanation of the discordant results, because of their potential effect in dampening the immune response[22]. However, in other studies, it should be noted that the benefit from immune checkpoint inhibitors (ICI) has been observed despite the use of steroids [24,25]. Additionally, patient-level differences related to body mass index, body composition, and gut microbiome have not been fully investigated in neither IMpassion130 nor IMpassion131, but might account for dissimilarities in response to ICI[22].

In August 2021, Roche announced the decision to voluntary withdraw the FDA accelerated approval for atezolizumab in combination with nab-paclitaxel for the first-line treatment of PD-L1-positive metastatic TNBC. The withdrawal was not made due to changes in safety or efficacy observed in IMpassion 130, but due to the regular approval of pembrolizumab on 26 July (based on KEYNOTE-522), that changed the treatment landscape in the US, and that no longer allowed atezolizumab to fulfill the criteria for accelerated approval.

On the other hand, EMA reviewed the results of IMpassion131 and established that atezolizumab should be used only in combination with nab-paclitaxel.

2.2. Pembrolizumab

The safety profile and preliminary evidence of clinical activity of the anti-PD-1 antibody pembrolizumab as single agent was

Table 2. Main studies with immune-checkpoint inhibitors in the early setting for patients with triple-negative breast cancer.

Immune-checkpoint inhibitor	Study name	Study design	Treatment	Setting	Population	Main results
Pembrolizumab	I-SPY 2[35]	Phase II	Paclitaxel ± pembrolizumab followed by AC	Neoadjuvant setting	Stage II/III breast cancer (n = 250, TNBC n = 114)	• pCR: 44% vs 17% in HER2-negative population; • pCR: 60% vs 22% in TNBC cohort
	KEYNOTE-522 [36,37]	Phase III	Pembrolizumab or placebo + carboplatin and paclitaxel followed by AC/EC; Adjuvant pembrolizumab or placebo after surgery	Neoadjuvant and adjuvant settings	T1c N1-2 or T2-4 N0-2 TNBC (n = 602)	• pCR: 64.8% in pembrolizumab arm vs 51.2% in control arm; rate difference 13.6% (95% CI, 5.4–21.8; p < 0.001) • 36-months EFS: 84.5 vs. 76.8% (HR 0.63, 95%CI 0.48–0.82, p 0.00031).
Atezolizumab	NeoTRIPaPDL1 Michelangelo [42]	Phase III	Carboplatin and nab-paclitaxel ± Atezolizumab; Adjuvant AC/EC after surgery	Neoadjuvant setting	Early high-risk or locally advanced, TNBC (n = 280)	pCR: 43.5% in atezolizumab arm vs 40.8% in control arm, OR = 1.11 (95% CI 0.69–1.79)
	Impassion031 [40]	Phase III	Atezolizumab or placebo with sequential nab-paclitaxel followed by AC	Neoadjuvant and adjuvant settings	cT2-4 cN0-3 (stages II–III) TNBC (n = 333)	• ITT: pCR: 58% in atezolizumab arm vs. 41% in placebo arm (rate difference 17%, 95% CI 6–27; p = 0.0044). • PD-L1-positive cohort: pCR: 69% vs. 49% (rate difference 20%, 95% CI 4–35; one-sided p = 0.021).
Durvalumab	GeparNUEVO [45,46]	Phase II	Durvalumab or placebo + nab-paclitaxel followed by standard EC	Neoadjuvant setting	T1b-T4a-d TNBC (n = 117)	• pCR: 53.4% in durvalumab arm vs 44.2% in control arm, OR = 1.45 (95% CI 0.80–2.63), p = 0.224. • In the window-cohort (durvalumab/placebo alone given 2 weeks before nab-paclitaxel): pCR: 61.0% in durvalumab arm vs 41.4% in control arm, OR = 2.22 (95% CI 1.06–4.64), p = 0.035. • 3-year iDFS in pCR vs non pCR: 92.0% vs 71.9% (p = 0.002). • 3-year iDFS: 84.9% with durvalumab vs 76.9% with placebo (HR 0.54, 95%CI 0.27–1.09, p = 0.0559). • 3-year DDFS 91.4% vs 79.5% (HR 0.37, 95%CI 0.15–0.87, p = 0.0148). • 3-year OS 95.1% vs 83.1% (HR 0.26, 95%CI 0.09–0.79, p = 0.0076).
	Foldi et al [39].	Phase I/II	Durvalumab concurrent with weekly nab-paclitaxel and dose-dense AC	Neoadjuvant setting	Stages I–III TNBC (n = 55)	pCR rate of 44% (95% CI: 30–57%)

Abbreviations: FDA: Food and Drug Administration; AC: doxorubin and cyclophosphamide; TNBC: triple-negative breast cancer; pCR: pathological complete response; EC: epirubicin and cyclophosphamide; CI: confidence interval; ITT: intention to treat population; EFS: event-free survival; HR: hazard ratio; OR: odds ratio; iDFS: invasive disease-free survival; DDFS: distant disease-free survival; OS: overall survival.

first described in the phase 1b KEYNOTE-012 trial, in patients with advanced PD-L1-positive (expression in stroma or ≥1% of tumor cells by IHC) TNBC, gastric cancer, urothelial cancer, and head and neck cancer[26]. Among 27 evaluable patients with TNBC, the ORR was 18.5%, with a median duration of response not reached at the time of publication (range, 15.0 to ≥ 47.3 weeks)[26].

The phase II study KEYNOTE-086 evaluated pembrolizumab monotherapy in first (cohort B) or later (cohort A) lines of treatment for patients with metastatic TNBC. In cohort A, among 170, pretreated TNBC patients, 61.8% had PD-L1-positive tumors (defined as combined positive score [CPS] ≥1) and 43.5% had received ≥3 previous lines of therapy for metastatic disease; ORR was 5.3% and 5.7% in the overall and in the PD-L1-positive populations, respectively[27]. Median PFS was 2 months (95% CI, 1.9–2.0), and median OS was 9 months (95% CI, 7.6–11.2). Interestingly, at data cutoff, the duration of response was not reached: 75% and 62% of responders had a response duration of ≥6 and ≥12 months, respectively, highlighting the durable antitumor activity in a subset of patients with previously treated metastatic TNBC, a setting where standard chemotherapy usually accounts for very short duration of response (1–3 months)[27]. These data were confirmed and showed promising ORR in cohort B of KEYNOTE-086, where 84 previously untreated (86.9% of whom received prior (neo)adjuvant therapy) PD-L1-positive (CPS ≥1) patients received pembrolizumab for up to 2 years[28]. Among these patients, ORR was 21.4% (95% CI 13.9–31.4), and disease control rate (DCR) was 23.8% (95% CI 15.9–34.0), with a median duration of response of 10.4 months and a median time to response of 2.0 months.

The efficacy of pembrolizumab monotherapy, compared to standard chemotherapy, in second or third line of treatment for patients with metastatic TNBC, was evaluated in the phase III KEYNOTE-119 trial (Table 1)[29]. Patients were randomized to receive pembrolizumab or single-drug chemotherapy per investigator<apos;>s choice (capecitabine, eribulin, gemcitabine, or vinorelbine)[29]. Median OS in patients with a PD-L1 CPS ≥10 was 12.7 months for the pembrolizumab group and 11.6 months for the chemotherapy group (HR 0.78; log-rank p = 0.057); in patients with PD-L1 CPS ≥1, median OS was 10.7 months for the pembrolizumab group and 10.2 months for the chemotherapy group (HR 0.86; log-rank p = 0.073)[29]. Thus, pembrolizumab monotherapy did not significantly improve OS compared to chemotherapy, informing on the need for predictive biomarkers and combination approaches for future development, and supporting its investigation in earlier rather than lines of treatment.

KEYNOTE-355, a randomized phase III trial was designed to assess the efficacy of pembrolizumab plus chemotherapy as first-line treatment in patients with metastatic TNBC (Table 1)[30]. Patients were randomized to pembrolizumab or placebo plus chemotherapy (paclitaxel or nab-paclitaxel or gemcitabine plus carboplatin); stratification factors were PD-L1 expression at baseline (CPS ≥1 or <1, using Dako 22C3 IHC assay), type of on-study chemotherapy received, and previous treatment with the same class of chemotherapy in the (neo)adjuvant setting[30]. Dual primary efficacy endpoints were PFS and OS assessed in the PD-L1-positive CPS ≥10, CPS ≥1 and ITT populations. A hierarchical testing strategy was used, meaning that PFS would be assessed first in patients with CPS ≥10, and then, if significant, in patients with CPS ≥1, and ultimately in the ITT population[30]. At the second interim analysis, primary objective was met with a median PFS of 9.7 months with pembrolizumab-chemotherapy and 5.6 months with placebo-chemotherapy in the PD-L1-positive CPS ≥10 subgroup of patients, while no statistically significant difference was observed in the PD-L1-positive CPS ≥1 subgroup of patients and in the ITT population (not formally tested)[30].

Based on these PFS results, FDA granted accelerated approval to pembrolizumab in combination with chemotherapy for patients with locally recurrent unresectable or metastatic PD-L1-positive CPS ≥10 TNBC in November 2020. Final results of the KEYNOTE-355 trial were reported at ESMO 2021 after a median follow-up of 44.1 months. Pembrolizumab plus chemotherapy significantly improved OS compared to chemotherapy alone in TNBC patients with PD-L1-positive CPS ≥10 tumors; OS was 23 and 16.1 months in pembrolizumab/chemotherapy and placebo/chemotherapy group, respectively (HR 0.73; p = 0.0093), with no new safety signals identified[25]. The benefit was consistent across patient subgroups, irrespective of chemotherapy backbone. Of note, in KEYNOTE-355, 22% of patients in pembrolizumab arm had a disease-free interval between 6 and 12 months, while in IMpassion-130 prior chemotherapy was permitted only if treatment was completed ≥ 12 months before randomization.

An additional opportunity for patients with advanced TNBC to receive treatment with PD-1 blockade comes from the histology-agnostic FDA-approval of pembrolizumab based on the results of the KEYNOTE-158 trial (Table 1)[31]. In June 2020, FDA granted accelerated approval to pembrolizumab for the treatment of patients with unresectable or metastatic solid tumors with high TMB (defined as ≥10 mutations/megabase, as determined by an FDA-approved test), that have progressed following prior treatments and who have no satisfactory alternative treatment options. The approval was based on a retrospective analysis of 10 cohorts of patients of any solid tumor histology with high TMB from the non-randomized KEYNOTE-158 trial[31]. The ORR in patients with high TMB was 29% (95%CI, 21–39), and a half of patients had response durations of 24 months or more. Although the FDA approval is for any tumor type with high TMB, it should be considered that the dataset analyzed for this histology-agnostic approval did not include patients with breast cancer. Nevertheless, in the TAPUR study, pembrolizumab monotherapy in 28 patients with previously treated metastatic breast cancer with high TMB demonstrated antitumor activity (disease control and ORR in 37% [95% CI, 21–50] and 21% of patients [95% CI, 8–41], respectively) (Table 1), thus supporting and extending the results of the KEYNOTE-158 trial also to patients with metastatic breast cancer with high TMB[32].

Table 3. Ongoing studies with immune-checkpoint inhibitors (pembrolizumab, atezolizumab, durvalumab and avelumab) in the advanced setting for patients with triple-negative breast cancer. Data extracted from https://clinicaltrials.gov (date of search: November 4th, 2021).

NCT number	Study phase	Line of treatment	Experimental Treatment	Status
Pembrolizumab				
NCT02768701	Phase II	At least 1 prior line	Pembrolizumab + single-dose cyclophosphamide	Active, not recruiting
NCT03121352	Phase II	2 prior lines maximum	Pembrolizumab + carboplatin + nab-paclitaxel	Active, not recruiting
NCT02755272	Phase II	2 prior lines maximum	Pembrolizumab + carboplatin/gemcitabine	Recruiting
NCT02819518 MK-3475-355/ KEYNOTE-355	Phase III	1st line	Pembrolizumab + nab-paclitaxel or paclitaxel or carboplatin/gemcitabine	Active, not recruiting
NCT04024800 NSABP FB-14	Phase II	1 prior line maximum	Pembrolizumab + AE37 Peptide Vaccine	Active, not recruiting
NCT03720431	Phase I	Any line	Pembrolizumab + TTAC-0001	Active, not recruiting
NCT03362060	Phase I	At least 1 prior line	Pembrolizumab + PVX-410 Vaccine	Active, not recuiting
NCT04634747	Phase II	1st line	Pembrolizumab + PVX-410 + Chemotherapy	Not yet recruiting
NCT04986852	Phase II	1 or 2 prior lines	Pembrolizumab + Olinvacimab	Not yet recruiting
NCT02734290	Phase I/ II	1st or 2nd line	Pembrolizumab + paclitaxel or capecitabine	Active, not recruiting
NCT04683679	Phase II	2 prior lines maximum	Pembrolizumab + ablative radiotherapy ± olaparib	Recruiting
NCT03567720	Phase II	At least 1 prior line (chemotherapy-free cohort) or no prior lines (nab-paclitaxel cohort)	Pembrolizumab + TAVO (avokinogene telseplasmid) ± nab-paclitaxel	Recruiting
NCT04191135 MK-7339-009/ KEYLYNK-009	Phase II/III	1st line	Pembrolizumab + chemotherapy followed by pembrolizumab + chemotherapy or olaparib	Active, not recruiting
NCT02981303	Phase II	2nd line	Pembrolizumab + Imprime PGG	Completed
NCT03184558	Phase II	At least 1 prior line	Pembrolizumab + Bemcentinib	Completed
NCT03012230	Phase I	At least 1 prior line	Pembrolizumab + Ruxolitinib Phosphate	Recruiting
NCT04468061	Phase II	1st line	Pembrolizumab + Sacituzumab Govitecan	Recruiting
GS-US-592-6173	Phase III	1st line	Pembrolizumab + Sacituzumab Govitecan	Recruiting
NCT03752723 GX-I7-CA-006/ KEYNOTE-899	Phase I/ II	Any line	Pembrolizumab + GX-I7 ± cyclophosphamide	Recruiting
NCT02730130	Phase II	Any line	Pembrolizumab + Radiotherapy	Active, not recruiting
NCT03310957	Phase I/ II	1st line	Pembrolizumab + SGN-LIV1A (ladiratuzumab vedotin)	Recruiting
NCT03106415	Phase I/ II	3 prior lines maximum	Pembrolizumab + Binimetinib	Recruiting
NCT02971761	Phase II	Any line	Pembrolizumab + Enobosarm	Active, not recruiting
NCT03004183 STOMP	Phase II	Any line	Stereotactic body radiation therapy and Oncolytic Virus Therapy followed by Pembrolizumab	Active, not recruiting
NCT03599453	Phase I	Any line with no alternative treatment options	Pembrolizumab + Chemokine Modulation Therapy	Active, not recruiting
NCT01676753	Phase I	2 prior lines maximum	Pembrolizumab + Dinaciclib	Active, not recruiting
NCT03225547	Phase II	Any line	Pembrolizumab + Mifepristone	Active, not recruiting
NCT04348747	Phase II	Any line	Pembrolizumab + Dendritic Cell Vaccines against Her2/Her3, and Cytokine Modulation Regimen	Not yet recruiting

(*Continued*)

Table 3. (Continued).

NCT number	Study phase	Line of treatment	Experimental Treatment	Status
NCT02411656	Phase II	Any line	Pembrolizumab as maintenance therapy after clinical response or stable disease to prior chemotherapy	Recruiting
NCT05082259 ASTEROID	Phase I	Any line	Pembrolizumab + ASTX660	Not yet recruiting
NCT04301011 RAPTOR	Phase I/II	Any line with no alternative treatment options	Pembrolizumab + TBio-6517	Recruiting
NCT04879849	Phase I	Any line after completion of all standards of care treatment options, including prior checkpoint inhibitors	Pembrolizumab + TAK-676	Recruiting
NCT05070247	Phase I	Any line	TAK-500 ± Pembrolizumab	Not yet recruiting
NCT03775850	Phase I/II	Any line with no alternative treatment options	Pembrolizumab + EDP1503	Recruiting
NCT04432857	Phase I	3 prior lines maximum	Pembrolizumab + AN0025	Recruiting
NCT04265872	Phase I	3 prior lines maximum	Bortezomib followed by Pembrolizumab and Cisplatin	Recruiting
NCT03396445	Phase I	1st line	MK-5890 as Monotherapy and in Combination With Pembrolizumab	Recruiting
NCT03761914	Phase I/II	1 prior line maximum	Galinpepimut-S in Combination With Pembrolizumab	Recruiting
NCT05093387	Phase I	At least 1 prior line	SGT-53, Carboplatin, and Pembrolizumab	Not yet recruiting
NCT03797326	Phase II	1 or 2 prior lines	Pembrolizumab + Lenvatinib	Recruiting
NCT04332653 KEYNOTE A60	Phase I/II	At least 1 prior therapy	Pembrolizumab + NT-I7 (Efineptakin Alfa)	Recruiting
NCT05082610	Phase I	At least 1 prior therapy	HMBD-002 ± Pembrolizumab	Not yet recruiting
NCT03849469 DUET-4	Phase I	Any line with no alternative treatment options	XmAb22841 (monoclonal bispecific antibody) ± Pembrolizumab	Recruiting
NCT04234113	Phase I	Any line with no alternative treatment options	SO-C101 ± pembrolizumab	Recruiting
NCT03454451	Phase I	1–3 prior lines	CPI-006 ± Ciforadenant and Pembrolizumab	Recruiting
NCT04725331	Phase I/II	Any line with no alternative treatment options	BT-001 ± Pembrolizumab	Recruiting
NCT02648477	Phase II	Any line but no prior anthracyclines allowed	Pembrolizumab + doxorubicin	Active, not recruiting
NCT04429542	Phase I	Any line	BCA101 ± Pembrolizumab	Recruiting
NCT05094804	Phase I	At least 1 prior therapy including PARPi if eligible based on BRCA status	OR2805 (anti-CD163), ± Pembrolizumab or Nivolumab	Recruiting
NCT03621982	Phase I/II	Any line	ADCT-301 (camidanlumab tesirine) ± Pembrolizumab	Recruiting
NCT04348916	Phase I	Any line with no alternative treatment options	ONCR-177 ± Pembrolizumab	Recruiting
NCT05007106	Phase II	Any line	Pembrolizumab + Lenvatinib or Chemotherapy	Recruiting
NCT03213041	Phase II	1st line	Pembrolizumab and Carboplatin	Recruiting
NCT02432963	Phase I	At least 1 prior therapy	Vaccine Therapy and Pembrolizumab	Active, not recruiting
NCT04060342	Phase I/II	Any line	GB1275 + Pembrolizumab	Recruiting
NCT04148937	Phase I	Any line with no alternative treatment options	LY3475070 ± Pembrolizumab	Active, not recruiting
NCT02419495	Phase I	Any line	Selinexor + Chemotherapy or Pembrolizumab	Recruiting
NCT05069935	Phase I	Any line	FT538 + Chemotherapy + Pembrolizumab	Not yet recruiting
Atezolizumab				
NCT03164993 ALICE	Phase II	1 prior line maximum	Atezolizumab + pegylated liposomal doxorubicin + cyclophosphamide	Recruiting
NCT03206203	Phase II	1 prior line maximum	Atezolizumab + carboplatin	Active, not recruiting

(*Continued*)

Table 3. (Continued).

NCT number	Study phase	Line of treatment	Experimental Treatment	Status
NCT03464942 AZTEC	Phase II	1 prior line maximum	Atezolizumab + Stereotactic Ablative Body Radiotherapy	Recruiting
NCT03853707	Phase I/ II	0–2 prior lines	Atezolizumab + ipatasertib + capecitabine	Suspended (accrual goal met)
NCT03800836	Phase I	1st-3rd line according to the cohort	Ipatasertib + Atezolizumab and Paclitaxel or Nab-Paclitaxel	Active, not recruiting
NCT04408118 ATRACTIB	Phase II	1st line	Atezolizumab, Paclitaxel, and Bevacizumab	Recruiting
NCT03371017 Impassion132	Phase III	1st line	Atezolizumab + carboplatin + gemcitabine or capecitabine	Recruiting
NCT04148911 EL1SSAR	Phase III	1st line	Atezolizumab + Nab-Paclitaxel	Recruiting
NCT04690855 TARA	Phase II	1–2 prior lines	Atezoliaumab, Talazoparib, and Radiotherapy	Recruiting
NCT04584112	Phase I	1st line	Tiragolumab + Atezolizumab and Chemotherapy	Recruiting
NCT04177108	Phase III	1st line	Ipatasertib + Atezolizumab and Paclitaxel	Active, not recruiting
NCT04739670 BELLA	Phase II	1st line	Bevacizumab, Carboplatin, Gemcitabine and Atezolizumab	Not yet recruiting
NCT03483012	Phase II	Any line	Atezolizumab + Stereotactic Radiation for brain metastases	Active, not recruiting
NCT03101280	Phase I	At least 1 prior line	Atezolizumab + Rucaparib	Completed
NCT01898117 Triple-B	Phase II	1st line	Atezolizumab + carboplatin/cyclophosphamide or paclitaxel	Recruiting
NCT04249167	Phase I	Any line	Cryoablation, Atezolizumab/Nab-paclitaxel	Active, not recruiting
NCT03961698 MARIO-3	Phase II	1st line	IPI-549 (eganelisib) + Atezolizumab + Nab-paclitaxel	Recruiting
NCT04639245	Phase I/ II	Any line with no alternative treatment options	Atezolizumab + Genetically Engineered Cells (MAGE-A1-specific T Cell Receptor-transduced Autologous T-cells)	Recruiting
NCT03424005 Morpheus-TNBC	Phase I/ II	According to cohort of enrollment	Multiple Immunotherapy-Based Treatment Combinations including Atezolizumab, Ipatasertib, SGN-LIV1A, Bevacizumab, Selicrelumab, Tocilizumab, Sacituzumab Govitecan and Chemotherapy	Recruiting
NCT03289962	Phase I	At least 1 prior line	Autogene cevumeran (RO7198457) ± Atezolizuamb	Recruiting
NCT05001347	Phase II	2 prior lines or with no alternative treatment options	TJ004309 + Atezolizumab	Not yet recruiting
NCT03829501	Phase I/ II	Any line with no alternative treatment options	KY1044 + Atezolizumab	Recruiting
NCT03170960	Phase I/ II	NA	Atezolizumab + Cabozantinib	Recruiting
Durvalumab				
NCT03606967	Phase II	1st line	Durvalumab + nabpaclitaxel + neoantigen vaccine	Recruiting
NCT03616886 SYNERGY	Phase II	1st line	Durvalumab + paclitaxel + carboplatin + oleclumab	Active, not recruiting
NCT03167619 DORA	Phase II	2 prior lines maximum	Durvalumab + olaparib	Active, not recruiting
NCT03742102 BEGONIA	Phase I/ II	1st line	Durvalumab + paclitaxel + immune-modulating agents (selumetinib, danvatirsen, oleclumab and capivasertib)	Recruiting
NCT03199040	Phase I	Any line with no alternative treatment options	Neoantigen DNA Vaccine + Durvalumab	Active, not recruiting
NCT03801369	Phase II	0–2 prior lines	Olaparib and Durvalumab	Recruiting

(*Continued*)

Table 3. (Continued).

NCT number	Study phase	Line of treatment	Experimental Treatment	Status
NCT04176848	Phase II	Any line	CFI-400945 and Durvalumab	Recruiting
NCT03739931	Phase I	At least 1 prior line	mRNA-2752, Durvalumab	Recruiting
NCT03982173 MATILDA	Phase II	Any line with no alternative treatment options	Durvalumab + Tremelimumab	Active, not recruiting
NCT03983954	Phase I	Any line with al least 1 prior line	Naptumomab Estafenatox + Durvalumab	Recruiting
NCT04504669	Phase I	Any line	AZD8701 ± Durvalumab	Recruiting
NCT02484404	Phase I/II	Any line	Durvalumab ± Olaparib ± Cediranib	Recruiting
NCT04556773 DB-08	Phase I	At least 1 prior line or no prior lines according to different cohorts	Trastuzumab deruxtecan + Durvalumab + Paclitaxel	Recruiting
NCT04954599	Phase I/II	Any line	CP-506 (HAP) ± Carboplatin or ICI (including Durvalumab)	Not yet recruiting
Avelumab				
NCT03971409 InCITe	Phase II	2 prior lines maximum	Avelumab + Binimetinib, Sacituzumab Govitecan, or Liposomal Doxorubicin	Recruiting
NCT04360941 PAveMenT	Phase I	1–2 prior lines	Avelumab + Palbociclib	Recruiting
NCT02630368 METROmaJX	Phase I/II	Any line	Avelumab + JX-594 + Cyclophosphamide	Recruiting
NCT02554812 JAVELIN Medley	Phase II	Any line	Avelumab ± Utomilumab, PF-04518600, PD 0360324, CMP-001	Active, not recruiting
NCT05069935	Phase I	Any line	FT538 + Avelumab	Not yet recruiting
NCT04551885	Phase I	Any line with at least 1 prior line	FT516 + Avelumab	Recruiting
NCT04954599	Phase I/II	Any line	CP-506 (HAP) ± Carboplatin or ICI (including Avelumab)	Not yet recruiting
Nivolumab				
NCT02499367 TONIC	Phase II	3 prior lines maximum	Nivolumab + (1) no induction or (2) irradiation or (3) cyclophosphamide or (4) cisplatin or (5) doxorubicin, all followed by nivolumab	Active, not recruiting
NCT04159818 TONIC-2	Phase II	3 prior lines maximum	Nivolumab ± cisplatin or doxorubicin	Recruiting
NCT02393794	Phase I/II	Any line	Nivolumab + Romidepsin + cisplatin	Active, not recruiting
NCT03414684	Phase II	1st line	Nivolumab + carboplatin	Active, not recruiting
NCT03316586	Phase II	0–3 prior chemotherapeutic lines	Nivolumab + cabozantinib	Completed
NCT04142931	Phase I	At least 1 prior line	Sequentional immuno apheresis plasma volume escalation cohort study of reduction of soluble tumor necrosis factor receptors 1 and 2 ± Nivolumab	Recruiting
NCT03435640 REVEAL	Phase I/II	Any line	Nivolumab + NKTR-262 + bempegaldesleukin	Active, not recruiting
NCT03829436	Phase I	Any line with no alternative treatment options	TPST-1120 ± nivolumab	Recruiting
NCT04423029	Phase I	Any line with no alternative treatment options	Nivolumab + DF6002	Recruiting
NCT03667716	Phase I	At least 1 prior line	Nivolumab + COM701 (an Inhibitor of PVRIG)	Recruiting
NCT05094804	Phase I	At least 1 prior line	Nivolumab + OR2805, a Monoclonal Antibody Targeting CD163	Recruiting
NCT04561362	Phase I/II	Any line with no alternative treatment options	Nivolumab + BT8009-100	Recruiting
NCT02983045 PIVOT-2	Phase I/II	Any line	NKTR-214 + nivolumab ± ipilimumab	Active, not recruiting
NCT05069935	Phase I	At least 1 prior line	Nivolumab + FT538	Not yet recruiting
NCT04954599	Phase I/II	Any line with no alternative treatment options	Nivolumab + CP-506	Not yet recruiting

Figure 1 shows the treatment indications of the approved ICIs (pembrolizumab and atezolizumab) for patients with TNBC, in the early and advanced settings.

2.3. Durvalumab

The role of durvalumab for patients with advanced TNBC has been evaluated in the SAFIRO2-BREAST IMMUNO trial (Table 1) [33]. This phase II trial included 199 patients with HER2-negative BC whose disease had not progressed after 6–8 cycles of chemotherapy, who were randomized to receive either durvalumab or maintenance chemotherapy. Overall, durvalumab did not improve PFS (HR 1.40, 95% CI 1.00–1.96, p = 0.047) nor OS (HR 0.84, 95% CI 0.54–1.29; p = 0.423). However, in the exploratory subgroup analysis of patients with TNBC (n = 82), durvalumab significantly improved OS (HR 0.54, 95% CI 0.30–0.97, p = 0.0377). Moreover, the exploratory analysis of patients with TNBC and PD-L1-positive disease (n = 32) showed an HR of death of 0.37 (95% CI 0.12–1.13), compared to HR 0.49 (95% CI 0.18–1.34) for those with PD-L1-negative TNBC (n = 29)[33].

These findings should be interpreted with caution due to the small subgroups and exploratory nature of the analyses. Nevertheless, they provide a rationale to further evaluate single-agent durvalumab as maintenance therapy in patients with advanced TNBC.

Table 3 reports the ongoing studies with ICIs (pembrolizumab, atezolizumab, durvalumab, avelumab and nivolumab) for patients with advanced TNBC.

3. Immunotherapy in early TNBC

3.1. Pembrolizumab

PD-1 blockade has been recently introduced in the treatment of early TNBC. Following early evidence of safety and activity from KEYNOTE-173[34] and I-SPY2[35] trials, the incorporation of pembrolizumab into clinical practice was based on compelling data from the phase III KEYNOTE-522 trial[36], which led to the approval of this agent by the FDA in 2021 (Table 2). In this study, patients with stage II–III TNBC were randomized to receive neoadjuvant chemotherapy (NACT) with four cycles of paclitaxel and carboplatin plus pembrolizumab or placebo followed by an additional four cycles of doxorubicin–cyclophosphamide or epirubicin–cyclophosphamide plus pembrolizumab or placebo[36]. After surgery, patients received maintenance with pembrolizumab or placebo every 3 weeks for up to nine cycles. In the first interim analysis, consisting of the primary pathological complete response (pCR) analysis in 602 patients, pembrolizumab increased the pCR rate by 13.6% (51.2% vs 64.8%, p = 0.00055)[36]. At longer follow-up, after the randomization of 1174 patients, pembrolizumab improved event-free survival (EFS) (36 months-EFS 84.5% vs. 76.8%, HR 0.63, 95% CI 0.48–0.82) [37,38]. Albeit subgroup evaluation in the primary pCR analysis suggested that pembrolizumab benefit in terms of pCR was larger in patients with node-positive disease, similar EFS improvement was seen regardless of nodal status[37]. Moreover, while PD-L1 positivity was associated with higher pCR rates in both treatment arms, the EFS benefit from the addition of pembrolizumab was irrespective of PD-L1 status[38]. 36-month-EFS rates were higher in patients achieving pCR (94.4% and 92.5% in pembrolizumab and placebo arm, respectively), compared to patients who did not achieve pCR (67.4% and 56.8% in pembrolizumab and placebo arm, respectively)[38]. Hence, the magnitude of benefit from the addition of pembrolizumab was relatively higher in non-pCR patients (Δ = 10.6%) compared to pCR patients (Δ = 1.9%)[38].

3.2. Atezolizumab

The addition of atezolizumab to NACT has also been explored as a therapeutic option in early TNBC. The phase III IMpassion 031 randomized 333 patients with untreated stage II–III TNBC to receive NACT (nab-paclitaxel followed by dose-dense doxorubicin and cyclophosphamide) in combination with atezolizumab or placebo[40] (Table 2). After surgery, patients and physicians were unblinded and those randomized to the experimental arm received atezolizumab for 9 additional cycles. Atezolizumab led to an improvement in pCR rate from 41.1% to 57.6% (p = 0.0044). In the PD-L1-positive population, the increase in pCR (49.3% vs. 68.8%) did not cross the pre-specified significance boundary[40]. Although early analyses suggest encouraging trends in secondary time-to-event outcomes, this study was not formally powered for EFS, disease-free survival (DFS) or OS analyses[40]. Of note, in IMpassion031 carboplatin was not included in the chemotherapy regimen. Although current evidence tends to support the incorporation of carboplatin in the neoadjuvant treatment for TNBC[41], at the time IMpassion031 was published there was no clear global standard for the neoadjuvant treatment of early-stage TNBC[40]. Nevertheless, the results of IMpassion031 showed that atezolizumab in combination with a platinum-free regimen improved the rate of pCR and suggested that this combination could also provide benefit to patients who are unfit for platinum-containing chemotherapy[40].

The NeoTRIPaPDL1 study was designed to evaluate the role of neoadjuvant atezolizumab in combination with carboplatin and nab-paclitaxel followed by surgery and adjuvant anthracycline-based chemotherapy in patients with non-metastatic TNBC[42] (Table 2). No significant difference in pCR was observed between the atezolizumab and control arms (43.5% vs 40.8%)[42]. In this study, PD-L1 expression, evaluated as a continuous variable, demonstrated both prognostic value with higher pCR rate in the PD-L1-positive population in both treatment arms[43]. Data on EFS at 5 years, the other primary endpoint of NeoTRIPaPDL1 trial, are still immature.

Considering that not all patients will benefit from PD-1 blockade, Yam et al. evaluated the addition of neoadjuvant atezolizumab to nab-paclitaxel in patients with TNBC with suboptimal clinical response to four cycles of doxorubicin and cyclophosphamide[44]. In this single-arm, phase II trial, the pCR rate was 30% (10/33, 95% CI: 16–49%) and the pCR/residual cancer burden (RCB)-I rate was 42% (14/33, 95% CI: 25–61%), an interesting result considering the selection of an anthracycline-resistant population[44].

3.3. Durvalumab

Although not yet approved for clinical use, another ICI has also demonstrated promising activity and efficacy for the treatment of early TNBC. The phase II randomized GeparNuevo study evaluated the addition of durvalumab to NACT (nab-paclitaxel followed by dose-dense epirubicin/cyclophosphamide) in patients with non-metastatic TNBC of at least 2 cm (cT2 – cT4)[45] (Table 2). This trial was initially designed to have a 'window-phase' in which patients received a first injection of durvalumab 2 weeks before the start of chemotherapy. However, after the inclusion of 117 patients, the Independent Data Monitoring Committee considered that the mean time for starting NACT (47.7 days) was excessive, thus leading to the omission of the window-phase for patients enrolled thereafter, so that both durvalumab/placebo and chemotherapy could be started together on day 1[45]. The addition of durvalumab numerically increased pCR rates from 44.2% to 53.4%, although this difference was not statistically significant (p = 0.287)[45]. Nonetheless, for the subgroup of patients treated in the window-phase, there was a statistically significant increase in pCR rates favoring the durvalumab arm (OR 2.22, 95% CI 1.06–4.64, p = 0.035), which could suggest a potential 'priming' or blunting of T cells by upfront addition of chemotherapy[45]. Interestingly, despite the modest improvement in pCR, a subsequent report, with a median follow-up of 42 months, demonstrated a significant impact of durvalumab in invasive DFS at 3 years (HR 0.54, 95%CI 0.27–1.09, stratified log-rank p = 0.0559), distant DFS (HR 0.37, 95%CI 0.15–0.87, p = 0.0148), and also in OS (HR 0.26, 95%CI 0.09–0.79, p = 0.0076)[46]. Of note, as discussed for Impassion031, also in GeparNuevo carboplatin was not included in the chemotherapy regimen, although current evidence tends to support the incorporation of carboplatin in the neoadjuvant treatment for TNBC[41].

Despite the rapid evolution of immuno-oncology research, several important questions remain unanswered and are the object of ongoing investigation. Additional data about the role of atezolizumab for the treatment of early TNBC, as well as the best treatment schedule, will be provided by the GeparDouze (NSABP B-59/GBG96) trial (NCT03281954), which assesses neoadjuvant and adjuvant administration of atezolizumab/placebo in patients with high-risk TNBC, and also by ALEXANDRA-IMpassion030[47], which evaluates the addition of atezolizumab to adjuvant therapy for patients treated with upfront surgery. Trials assessing the efficacy and safety of other ICI, such as avelumab (A-BRAVE trial, NCT02926196), and nivolumab (BELLINI trial, NCT03815890), are also ongoing.

Table 4 reports the ongoing studies with ICI (pembrolizumab, atezolizumab, durvalumab, avelumab and nivolumab) for patients with early TNBC.

4. Immunotherapy combinations

4.1. Combinations with other immune-checkpoint inhibitors

Beyond PD-(L)1 inhibitors, several other immune-checkpoint inhibitors are under investigation for patients with TNBC, both as monotherapy and in combination with PD-(L)1 blockade (Figure 2).

The combination of anti-CTLA4 (e.g. ipilimumab, tremelimumab) and anti-PD-(L)1 is being tested aiming to improve antitumor activity in patients with pretreated advanced BC. In the phase I/II MOVIE trial, the combination of tremelimumab, durvalumab, and metronomic oral vinorelbine showed moderate activity, with two partial responses, of 1.6 and 3.8 months respectively, and two stable diseases longer than 24 weeks. The safety profile was consistent with previous anti-PD-L1/anti-CTLA4 combination regimens[48]. In the phase II study NIMBUS, recently presented at the San Antonio Breast Cancer Symposium (SABCS) 2021, the combination of nivolumab and ipilimumab in patients with hypermutated HER2-negative BCs met the primary study endpoint, with an ORR of 16.7% (5/30), with four of five responders showing durable responses longer than 1 year (NCT03789110). The combination of ipilimumab and nivolumab is also being tested in the preoperative setting in the ongoing BELLINI trial (NCT03815890).

LAG3 is an immune checkpoint that inhibits the activation of its host cell, thus promoting a more suppressive immune response. In the setting of metastatic TNBC, LAG525, an anti-LAG3 antibody, was tested in a phase II trial, in combination with PDR001, an anti-PD1 antibody and/or chemotherapy (carboplatin)[49]. The study had 3 treatment arms, and patients in first or second-line setting were randomized to receive LAG525 with or without PDR001, and/or chemotherapy. The LAG525 + PDR001 arm was early discontinued due to a high rate of progressive disease and no arms met proof of preliminary efficacy criteria, although an ORR of 32.4% was observed in the triplet arm[49].

ICOS, a member of the CD28 superfamily, binding to ligands expressed on B cells and phagocytes, triggers a downstream pathway regulating both T-cell proliferation and secretion of cytokines. KY1044 is a fully human anti-ICOS antibody, designed to stimulate anti-tumor CD8+ effector T cells (i.e. cytotoxic T-lymphocytes) and to deplete pro-tumor $ICOS^{high}$ regulatory T cells in the tumor microenvironment. KY1044 was tested, as single agent and in combination with atezolizumab, in a phase I/II open-label study in patients with advanced solid tumors[50]. The compound was well tolerated both as single agent and in combination with atezolizumab, and in the TNBC population one complete response and four partial responses were observed [50]. The phase 2 part of the study is ongoing[50].

VISTA is another immune-regulatory protein, expressed on both hematopoietic cells and tumor cells, that acts by repressing T-cell activation and cytokine production, and inducing an immunosuppressive environment[7]. HMBD-002 is a novel, anti-VISTA antibody, that showed a strong preclinical activity in blocking the inhibitory function of VISTA, without depleting VISTA-expressing cells[51]. A phase I study evaluating HMBD-002, both as a monotherapy and in combination with pembrolizumab, in patients with advanced solid tumor including TNBC is ongoing (NCT05082610).

Table 4. Ongoing studies with immune-checkpoint inhibitors (pembrolizumab, atezolizumab, durvalumab and avelumab) in the early setting for patients with triple-negative breast cancer. Data extracted from https://clinicaltrials.gov (date of search: November 4, 2021).

NCT number	Study phase	Setting	Experimental Treatment	Status
Pembrolizumab				
NCT03639948 NeoPACT	Phase II	Neoadjuvant	Pembrolizumab + Carboplatin + Docetaxel	Recruiting
NCT03289819 NIB	Phase II	Neoadjuvant	Pembrolizumab + Nab-Paclitaxel followed by Pembrolizumab + epirubicin and cyclophosphamide	Completed
NCT04373031	Phase II	Neoadjuvant	Pembrolizumab + IRX-2 + cyclophosphamide followed by pembrolizumab + pacliataxel followed by IRX-2, pembrolizumab + doxorubicine and cyclophosphamide	Recruiting
NCT04443348	Phase II	Neoadjuvant	Pembrolizumab + carboplatin + paclitaxel + cyclophosphamide and doxorubicine + preoperative radiation therapy	Recruiting
NCT04095689 INTEGRAL	Phase II	Neoadjuvant	Pembrolizumab + docetaxel + interleukin-12 gene therapy and L-NMMA	Recruiting
NCT02977468 Pembro-IORT	Phase I	Neoadjuvant	Pembrolizumab + Intraoperative radiation therapy (IORT)	Recruting
NCT04427293	Phase I	Neoadjuvant	Pembrolizumab + lenvatinib	Recruiting
NCT04373031	Phase II	Neoadjuvant	Pembrolizumab, IRX-2, and Chemotherapy	Recruiting
NCT04443348 P-RAD	Phase II	Neoadjuvant	Pembrolizumab + radiation therapy	Recruiting
NCT02957968	Phase II	Neoadjuvant	Pembrolizumab + Decitabine + Chemotherapy	Recruiting
NCT01042379 I-SPY2	Phase II	Neoadjuvant	Personalized Adaptive Novel Agents including Pembrolizumab	Recruiting
NCT03366844	Phase I/II	Neoadjuvant	Pembrolizumab + radiation therapy	Recruiting
NCT03036488 MK-3475-522/ KEYNOTE-522	Phase III	Neoadjuvant and adjuvant	Pembrolizumab + carboplatin + paclitaxel followed by anthracycline and cyclophosphamide followed by surgery and adjuvant pembrolizumab	Active, not recruiting
NCT02954874 SWOG S1418/NRG BR006	Phase III	Adjuvant	Pembrolizumab	Active, not recruiting
NCT03145961 c-TRAK-TN	Phase II	Adjuvant	Pembrolizumab	Active, not recruiting
Atezolizumab				
NCT02883062	Phase II	Neoadjuvant	Atezolizumab + carboplatin + paclitaxel	Active, not recruiting
NCT04770272 NeoMONO	Phase II	Neoadjuvant	Atezolizumab monotherapy followed by atezolizumab + chemotherapy	Recruiting
NCT02530489	Phase II	Neoadjuvant	Atezolizumab + nab-paclitaxel	Active, not recruiting
NCT04102618 AWARE-1	Phase I	Neoadjuvant	Pelareorep plus atezolizumab	Recruiting
NCT03281954 GeparDouze/NSABP B-59	Phase III	Neoadjuvant and adjuvant settings	Atezolizumab + doxorubicin and cyclophosphamide + paclitaxel + carboplatin followed by surgery and adjuvant atezolizumab	Active, not recruiting
NCT03498716 IMpassion030	Phase III	Neoadjuvant and adjuvant settings	Atezolizumab + paclitaxel followed by dose-dense doxorubicin/epirubicin + cyclophosphamide, surgery, and adjuvant atezolizumab	Recruiting
NCT04849364 PERSEVERE	Phase II	Post-neoadjuvant	Atezolizumab, Capecitabine, Talazoparib, Inavolisib according to plasma ctDNA and genomic markers	Recruiting
NCT03756298	Phase II	Adjuvant	Atezolizumab + capecitabine	Recruiting
NCT04434040 ASPRIA	Phase II	Adjuvant	Atezolizumab + Sacituzumab Govitecan	Recruiting
Durvalumab				
NCT03356860 B-IMMUNE	Phase I/II	Neoadjuvant	Durvalumab + paclitaxel + epirubicin and cyclophosphamide	Recruiting

(*Continued*)

Table 4. (Continued).

NCT number	Study phase	Setting	Experimental Treatment	Status
NCT02489448	Phase I/II	Neoadjuvant	Durvalumab + Nab-paclitaxel and Dose-dense AC	Active, not recruiting
NCT03594396	Phase I/II	Neoadjuvant	Olaparib + Durvalumab	Active, not recruiting
NCT01042379 I-SPY2	Phase II	Neoadjuvant	Personalized Adaptive Novel Agents including Durvalumab	Recruiting
NCT03740893 PHOENIX	Phase II	Neoadjuvant and adjuvant settings	Durvalumab, AZD6738, Olaparib	Recruiting
NCT02826434	Phase I	Adjuvant	PVX-410 Vaccine and Durvalumab	Active, not recruiting
Avelumab				
NCT04188119 IMpALA	Phase II	Neoadjuvant	Avelumab + aspirin	Not yet recruiting
NCT02926196 A-BRAVE	Phase III	Adjuvant	Avelumab	Active, not recruiting
Nivolumab				
NCT03815890 BELLINI	Phase II	Neoadjuvant	Nivolumab ± Ipilimumab	Recruiting
NCT04331067	Phase I/II	Neoadjuvant	Nivolumab + Cabiralizumab + chemotherapy	Recruiting
NCT04185311	Phase I	Neoadjuvant	Ipilimumab, Nivolumab, and Talimogene Laherparepvec	Active, not recruiting
NCT03546686	Phase II	Neoadjuvant and adjuvant settings	Neoadjuvant nivolumab + Ipilimumab followed by adjuvant Nivolumab	Recruiting
NCT03818685	Phase II	Adjuvant	Nivolumab + Ipilimumab + Radiotherapy Versus Radiotherapy + Capecitabine	Recruiting
NCT03487666 OXEL	Phase II	Adjuvant	Nivolumab ± capecitabine	Active, not recruiting

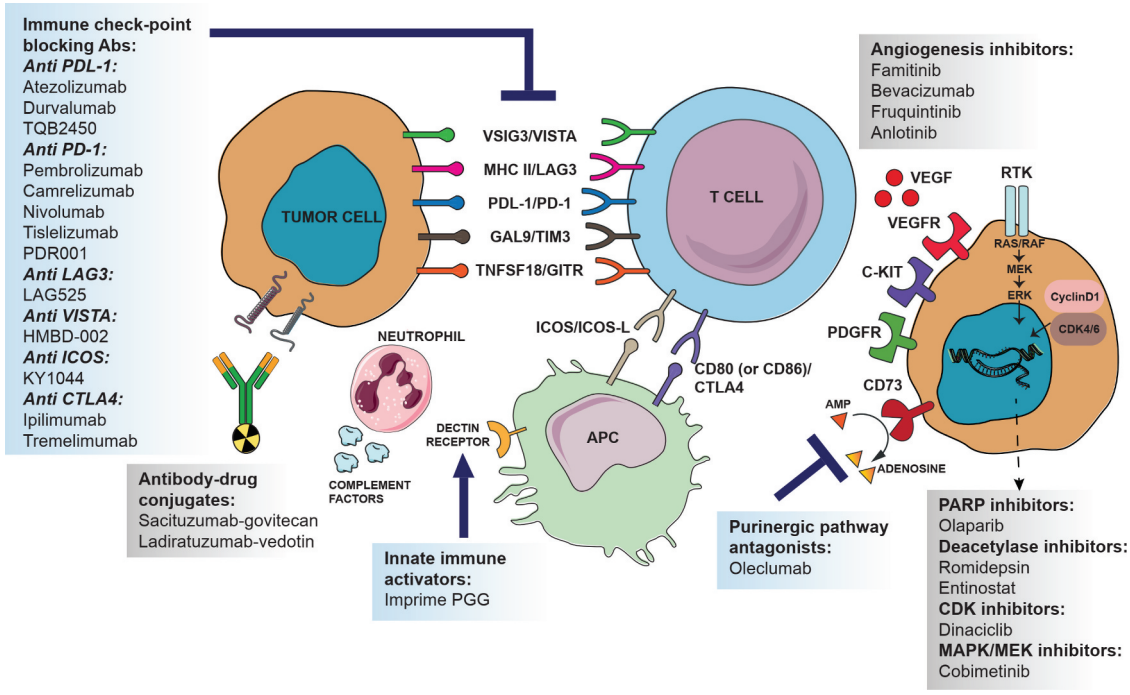

Figure 2. Immunotherapeutic strategies and their cellular/molecular targets in triple-negative breast cancer. The immunotherapeutic strategies are displayed in light blue boxes, and the main candidate combinatorial targeted agents are displayed in grey boxes.

Abbreviations: Abs: antibodies; APC: antigen-presenting cell; RTK: receptor tyrosine kinase.

4.2. Combinations with angiogenesis inhibitors

Antiangiogenics have immunomodulatory properties and are able to increase lymphocytic infiltration into the tumor, hereby enhancing antitumor immune responses[52].

Following the promising results of the umbrella phase Ib/II FUTURE trial in TNBC[53], a prospective, single-arm, phase II study (FUTURE-C-PLUS) was designed to assess efficacy and safety of the triple combination of the anti-PD-1 antibody camrelizumab plus chemotherapy (nab-paclitaxel) and the multityrosine kinase inhibitor famitinib (targeting VEGFR-2, PDGFR and c-kit) in metastatic TNBCs displaying IM subtype as determined using CD8 IHC with a cutoff of 10% or higher [54]. Objective responses were achieved in 39 (81.3%) of 48 patients in the ITT population and in 39 (84.8%) of 46 patients in the per-protocol population; the 9-month PFS rate was 60.2% (95%CI 43.2–77.3%). These interesting results led to the ongoing phase II randomized trial FUTURE-SUPER (NCT04395989).

Similarly, the anti-PD1 antibody nivolumab was tested in combination with the anti-VEGF antibody bevacizumab and paclitaxel chemotherapy in a phase II trial in patients with HER2-negative metastatic BC (18, 32% TNBC) as a first-line treatment[55]. The triple combination exhibited very promising efficacy results (ORR 75.4%, DCR 96.4%, 12-month PFS 75.8% and 12-month OS 87.1%), warranting further study in the HER2-negative population and specifically in TNBC patients who were poorly represented in this study.

Tislelizumab, a IgG4-variant monoclonal antibody against PD-1, is being tested in combination with the novel, highly selective, oral, tyrosine kinase inhibitor of VEGFR fruquintinib in phase Ib/II study in metastatic TNBC regardless of PD-L1 status, including both immunotherapy pretreated and naïve patients (NCT04579757). Study completion is estimated by August 2022. Moreover, TQB2450, a novel humanized monoclonal antibody targeting PD-L1, was tested in TNBC in a phase Ib trial in combination with anlotinib, an antiangiogenic small molecule, multi-target tyrosine kinase inhibitor, showing an acceptable safety profile and promising activity in heavily pretreated patients with advanced TNBC[56].

4.3. Combinations with antibody-drug conjugates (ADCs)

Sacituzumab govitecan, an ADC composed of a topoisomerase I inhibitor (SN-38) and an anti-Trop2 monoclonal antibody, linked together by a cleavable protein, is now approved by FDA and EMA for the treatment of patients with TNBC after two or more prior systemic therapies of whom at least one for metastatic disease, based on the results of the ASCENT trial [57]. In order to potentiate its antitumor activity, a randomized phase II trial is testing sacituzumab govitecan plus pembrolizumab in patients with PD-L1 negative (with 22C3 CPS < 10 or SP142 immune cells <1%) metastatic TNBC (NCT04468061). The rationale behind this study relies on the potential role of sacituzumab govitecan to act as immunomodulator, namely as promoter of antibody-dependent cellular toxicity, depletion of regulatory T cells, upregulation of MHC class I and PD-L1 expression, all features that were observed in murine models and that might overcome resistance to immunotherapeutic strategies in PD-L1-negative tumors[58]. Sacituzumab govitecan and pembrolizumab are also being tested in patients with

PD-L1 positive TNBC in a randomized, open-label, phase 3 study (Table 3).

Another ADC with similar immunomodulatory properties is ladiratuzumab-vedotin, an anti-LIV-1 ADC with a protease-cleavable linker to monomethyl auristatin E, that was tested in a phase Ib/II study in combination with pembrolizumab in first-line for patients with TNBC[59]. The combination appears tolerable, with promising initial signals of activity (ORR 54% among 26 patients followed for at least 3 months)[59].

4.4. Combinations with PARP inhibitors (PARPi)

The combination of PARPi and ICIs is another promising strategy, considering that neoantigens induced by PARPi-related DNA-damages could promote and enhance antitumoral immune response. In the MEDIOLA trial, the association of durvalumab and olaparib in patients with advanced solid tumors, including BRCA-mutated BCs, showed preliminary antitumor activity[60]. Particularly, in the subgroup of patients with TNBC (N = 17, 57%), median PFS and OS were 4.9 and 20.5 months, respectively[60].

A similar combination strategy is under investigation in the randomized, phase II/III KEYLYNK-009 trial, assessing the efficacy of pembrolizumab plus olaparib *versus* pembrolizumab plus chemotherapy as maintenance therapy in first line for patients with TNBC (NCT04191135).

In the phase II, single-arm, TOPACIO trial, the combination of niraparib and pembrolizumab in 47 evaluable patients with advanced TNBC showed promising antitumor activity (ORR 21%, DCR 49%), with numerically higher response rates in those with tumor BRCA mutations (N = 15, ORR 47%)[61]. The combination was safe, and warrants further investigation[61].

4.5. Combinations with innate-immune activators

Imprime-PGG is a novel, intravenously administered, Saccharomyces-derived beta-glucan, agonist of the dectin receptor that activates innate immune cells that could lead to reversion of immunosuppressive signals in the tumor microenvironment, activating antigen presenting cells and stimulating cytotoxic T cell activation[62]. According to its activity in preclinical models in enhancing ICI response, imprime-PGG was tested in the phase II IMPRIME-1 trial, in heavily pretreated metastatic TNBC patients in combination with pembolizumab. ORR was 15.9%, DCR was 54.5% and median OS was 16.4 months (compared to 9 months for those receiving pembrolizumab alone)[63]. Interestingly, imprime-PGG showed remarkable results in the subgroup of 12 patients who were originally hormone receptor-positive but then converted to TNBC, with 50% of patients having greater than 6-month DCR and a median OS of 17.1 months. Although the underlying biological mechanism for this impressive response is unclear, this particular subgroup of patients deserves further evaluation. Moreover, translational analyses showed that enhanced PFS and OS were associated with early innate immune and $CD8^+$ T cells activation in the peripheral blood, and with robust tumor infiltration by activated myeloid cells and $CD4^+$ and $CD8^+$ T cells ($Ki67^+$/granzyme B^+)[63].

4.6. Combinations with purinergic pathway antagonists

CD73 is an adenosine-generating enzyme, and the adenosine pathway has been shown to limit antitumor activity, favoring an immunosuppressive tumor microenvironment, with lower stromal TILs and worse prognosis in TNBC[64]. Oleclumab, an anti-CD73 monoclonal antibody, acts by blocking the generation of adenosine from a by-product of ATP, thus inhibiting the adenosine intracellular signaling pathway that mediates tumor cells growth and migration[65]. The preliminary results of the phase I/II SYNERGY trial showed that the combination of oleclumab, durvalumab and chemotherapy was safe, and active[66]. More mature data presented at SABCS 2021 failed to show a significant increase in clinical benefit rate (CBR) at 6 months with the addition of oleclumab to durvalumab and chemotherapy (CBR 47.1%), albeit some long-lasting responses were observed. Translational analyses on tissue and blood samples to investigate the heterogeneity of this disease are ongoing.

Oleclumab is under investigation also in the phase Ib/II BEGONIA study, in combination with durvalumab, with or without paclitaxel, as first-line treatment (NCT03742102).

4.7. Other combination strategies

ICIs are under investigation in early phase clinical trials in combination with several other molecules, targeting different pathways.

The MAPK pathway is frequently upregulated in chemotherapy-resistant TNBC[67], and the combination of a MAPK/MEK inhibitor (cobimetinib) with (nab)paclitaxel and atezolizumab has been tested in a phase II trial (COLET) enrolling patients with untreated advanced TNBC. The addition of cobimetinib did not demonstrate a significant increase in PFS or ORR[68].

Another class of agents under investigation in combination with ICIs are the cyclin-dependent kinase (CDK) inhibitors. Dinaciclib is an intravenous CDK-inhibitor, which demonstrated a synergistic antitumor effect with ICI, increasing immune cell activation and tumor infiltration in preclinical models of TNBC[69]. In a phase Ib, dose-escalation trial, dinaciclib was tested in combination with pembrolizumab in patients with advanced TNBC, and toxicities were generally manageable with dose reduction and dose delay. Dose expansion is ongoing[69]. The CDK4-6 inhibitor palbociclib is being tested in combination with avelumab in androgen receptor positive TNBC (NCT04360941).

Histone deacetylase inhibitors (HDACi) may also act synergistically with ICIs, due to their capacity of upregulating genes involved in antigen presentation and enhancing tumor cell recognition by activated ICs and response to PD-1/CTLA-4 blockade. The combination of romidepsin (an HDACi) plus cisplatin and nivolumab in 34 pre-treated metastatic TNBC showed encouraging signs of efficacy (ORR 44%, median PFS 4.4 months, median OS 10.3 months), warranting further evaluation in larger studies[70]. Controversial data come from another HDACi, entinostat, that, when evaluated in a phase II study for patients with advanced TNBC in combination with atezolizumab, did not improve PFS compared to placebo and

showed more treatment-related adverse events[71]. On the contrary, when tested in combination with nivolumab with or without ipilimumab in a phase I trial in advanced solid tumors, entinostat showed preliminary evidence of both clinical efficacy and immune modulation, supporting further investigation of the combination[72]. Results of the triple combination (entinostat, nivolumab, and ipilimumab) in patients with HER2-negative BC are awaited (NCT02453620).

5. Other immunotherapeutic agents

Moving beyond ICIs, other therapeutic strategies are being explored as ways to manipulate and educate the immune system for targeting and eliminating cancer cells. Dendritic cell (DC)-based antitumor vaccines use DC to act on the articulation between adaptive and innate immunity in order to acquire, process and present antigens to naïve T cells, polarizing them into effector or tolerogenic subsets[73]. In patients with HER2-negative BC, recently presented data demonstrated that the addition of DC vaccines to standard NACT improved the rate of pCR from 2.8% to 23.1% ($p < 0.05$) in PD-L1-negative population, although no significant differences were observed in 7-year EFS (HR = 1.7, 95%CI 0.42–6.8, P = 0.19) or in OS (HR = 2.5, 95%CI 0.56–11, p = 0.43)[74]. Although preliminary, these results suggest the possible activity of DC vaccines in a subgroup of patients with PD-L1-negative tumors, known to have lower response to cytotoxic NACT.

Another type of immune-based therapy, talimogene laherparepvec (TVEC) is a modified oncolytic herpes simplex 1 (HSV1) virus designed to preferentially lyse tumor cells to release tumor-associated antigens, as well as to produce granulocyte-macrophage colony-stimulating factor (GM-CSF) to activate DCs and stimulate T cells to infiltrate the tumor[75]. Already approved for the treatment of melanoma, TVEC has also been explored as a therapeutic strategy for early TNBC. In a recently presented single arm, phase II trial (n = 37), the addition to TVEC to anthracycline- and taxane-based NACT resulted in a pCR rate of 43% (95% CI 27–61%) and a rate of RCB 0/1 of 68% (95%CI 50–82%)[75].

The possibility to combine different approaches to increase the tumor recognition and response from the immune system is also being explored. SD-101 is a synthetic oligonucleotide that binds and activates toll-like receptor 9 in DCs, thus stimulating antigen presentation and cytotoxic T cells activity[76]. The combination of NACT with pembrolizumab and SD-101 was compared with anthracycline- and taxane-based NACT in patients with HER2-negative early BC in the adaptive I-SPY2 study [35,76]. The combination of pembrolizumab with SD-101 increased the pCR rates from 28% (95%CI 21–34%) to 44% (95%CI 28–60%) in the TNBC subpopulation[76]. Despite the proof of activity, the strategy did not meet the pre-specified threshold to be considered for further development within the I-SPY2 platform[76].

6. Expert opinion: challenges in immunotherapy for patients with TNBC

6.1. Predictive biomarkers

Currently available evidence clearly demonstrate that PD-1 blockade has an important role for the treatment of TNBC. It is also clear that these agents do not provide a similar level of benefit to all patients and that they are also associated with increased rates of adverse events. Therefore, integrating the information about the available biomarkers that predict response (or resistance) to ICI is of the utmost importance to select patients more likely to benefit from ICIs. The most extensively studied predictive biomarker for ICIs in TNBC is PD-L1 expression. In general, PD-L1 expression has shown predictive value only in the advanced setting. In both KEYNOTE-355 [24] and IMpassion130[77] trials, PD-L1 positivity predicted response to ICIs; on the contrary, in the early setting, PD-L1 expression was more a predictor of general response to chemotherapy and not associated with ICI benefit [36,40]. In line with these findings, pembrolizumab was approved by the FDA and EMA for patients with advanced TNBC with PD-L1 CPS ≥ 10, while no companion diagnostic was included in the FDA approval of this agent in the early setting.

A possible explanation for the difference in predictors may relate to the fact that anti-tumor immune response is different in the metastatic and early settings [13,78,79]. Indeed, in the early setting, the disease is confined to the breast, and patients have not been previously treated, thus having a more permissive tumor microenvironment, and a lower immune evasion. Patients with early breast cancer are usually immunocompetent, and the host can easily identify new antigens and create a new immune response [13,78,79]. On the contrary, in the metastatic setting, at the time of recurrence, cancer has already undergone an evolution toward immune evasion, with the activation of mechanisms of resistance (e.g. de-differentiation with lowering of immunogenic antigens, a more immunosuppressive microenvironment) [13,78,79]. Moreover, patients with metastatic breast cancer are often pre-treated, and, ultimately, immunosuppressed.

The controversies about the value of PD-L1 expression as predictive factor in TNBC are further complicated by the existence of different diagnostic assays (i.e. Ventana SP142 and, Dako 22C3), choice of cell subsets to be evaluated, scoring algorithms, inter-assay heterogeneity and site of evaluation (primary vs. metastatic)[80].

In the advanced setting, TMB and mismatch repair deficiency (dMMR) are two additional biomarkers that are currently FDA-approved in a 'tissue-agnostic' manner (i.e. for any cancer type – based on the results of biomarker testing). Although the prevalence of TMB > 10 mutations/Mb is low in BC (≈5%), high TMB has been shown to correlate with response to ICI in several cancer types[31]. As previously mentioned, in the TAPUR trial, pembrolizumab demonstrated a response rate of 21% in patients with heavily pretreated metastatic breast cancer with TMB ≥ 9 mutations/Mb.

Higher levels of TILs have also been associated with increased response to ICI[32]. In KEYNOTE-119, TILs ≥ 5%

were correlated with better clinical outcomes in patients treated with pembrolizumab, but not in those with chemotherapy[81].

Moreover, beyond the above-mentioned factors, several elements can influence the outcome of immunotherapy, including intrinsic and extrinsic tumor characteristics (e.g. molecular and intrinsic subtypes, tumor microenvironment, immune cell infiltration [82,83]) but also general features of each specific patient (e.g. immune status, circulating myeloid cells, body-mass index, microbiota)[84]. Additional studies are needed in BC to better define the predictive value of these and other biomarkers and their correlation with immunotherapy response.

In consideration of the several emerging biomarkers and of the limitations of each biomarker at some degree, it is likely that, in the next future, a 'composite biomarker,' containing the above-mentioned factors, could be more effective to provide a better patient selection[85]. Thus far, the search of predictive biomarkers still represents an open challenge in TNBC.

6.2. What are the best endpoints to evaluate ICI activity in clinical trials?

Due to their mechanism of action, ICIs may induce peculiar tumor responses, different from those observed in patients treated with cytotoxic chemotherapy agents. Indeed, ICIs act by 'releasing the brakes' of the immune system and by restoring an active infiltrate of T-cells in the tumor microenvironment, which ultimately translates into a specific immune response against cancer cells [86–88]. Thus, differently from chemotherapy that exerts its effect directly on tumor cells, ICIs, by acting on the immune system, tend to induce more durable responses that may persist also after treatment interruption in some cases [86–88]. Consistently, most trials investigating ICIs in different tumor types have demonstrated a significant survival benefit, despite occasional disappointing results in terms of response rate. An important lesson comes from the GeparNuevo trial [45,46]. At its first analysis of the primary endpoint (pCR), the addition of durvalumab to NACT did not significantly improve pCR rates in the ITT population, and durvalumab effect was seen only in the window cohort of patients receiving induction durvalumab prior to chemotherapy (Table 2)[45]. Interestingly, a longer follow-up revealed a significant improvement of 3-year invasive DFS, distant DFS and OS (Table 2), with no differences between the window and no window cohorts[46]. These findings raise several questions about the appropriate endpoints to evaluate response to ICIs in clinical trials. Whether pCR could represent a good surrogate for OS in patients receiving neoadjuvant treatment, has always been the topic of an intense academic discussion [89,90]. When considering ICIs, it could be hypothesized that pCR is not the optimal endpoint to assess their antitumor activity, thus suggesting that clinical trials powered according to survival endpoints (e.g. EFS) as primary endpoints could provide a better measure of immunotherapy efficacy over time. On the other hand, it should be considered that survival endpoints require longer follow-up in clinical trials, with higher number of events required to spot differences between treatment arms, and ultimately, a larger sample size, with additional costs and complexities in trial management.

6.3. Timing and duration of immunotherapy

The optimal duration of treatment with immunotherapy remains debated. Mature data from large, randomized trials show long-lasting responses induced by ICI, with persistent benefit also after treatment discontinuation, thus questioning whether immunotherapy should be continued until disease progression or unacceptable toxicity in the metastatic setting[91]. In the early setting, the most appropriate duration of immunotherapy is even more uncertain, and most trials are not designed to discern the relative contributions of the neoadjuvant and adjuvant treatment phases. Although in the pivotal KEYNOTE-522 trial pembrolizumab was started in the neoadjuvant phase and continued through the adjuvant phase[36], it is still not clear what the additional benefit is from the adjuvant part of the treatment. Theoretically, starting the ICI in the neoadjuvant phase could offer the possibility of more intense exposure to tumor antigens, thus increasing the odds of successful anti-tumor immune manipulation[92]. Nonetheless, considering that to date no biomarker demonstrated enough accuracy to discriminate those patients more likely to benefit from ICIs in the early setting, one interesting approach could be to use the response to NACT for patient selection. Although clinical data studying this approach are still preliminary[44], it seems interesting considering that it would have the potential to target the high-risk population of patients with residual disease while sparing those with pCR from the risk of immune-related adverse events. Potential caveats related with this approach emerge from KEYNOTE-522 data, in which patients achieving pCR had better long-term outcomes, but the relative benefit of pembrolizumab was demonstrated regardless of the pathologic response (although the magnitude of benefit associated with the addition of pembrolizumab was higher in patients not achieving pCR)[37]. Additionally, for patients treated with ICI-based neoadjuvant regimens, provocative data from GeparNuevo question the role of pCR in predicting long-term benefit from ICI[46]. Although the aforementioned completed and ongoing trials evaluating different possibilities of ICI timing will provide some evidence about the preferred approach, final answers to some of these questions will only be made available after the results of future studies designed to directly compare these different approaches. Interestingly, the ongoing SWOG S1418/NRG BR006 trial is investigating the role of adjuvant pembrolizumab only for patients with TNBC with ≥ 1 cm residual invasive disease or any positive lymph nodes following NACT (NCT02954874). Considering the risk of late toxicities, the quality of life implications and the costs of immunotherapy, studies investigating the optimal duration of therapy and/or discontinuation studies are warranted[91].

6.4. The choice of the chemotherapy backbone

Another important open question is whether the efficacy of ICI in the early setting could be influenced by the chemotherapy backbone[93]. The low efficacy of ICIs as monotherapy in TNBC [26,27], and the increased activity observed with regimens combining ICIs with chemotherapy [36,40], suggest that treatment backbone might play an important role. Moreover, preliminary clinical data suggest that the type of chemotherapy used may also influence the tumor microenvironment and the likelihood of response to ICI-based regimens[94]. In the phase II TONIC trial, patients with metastatic TNBC were randomly assigned to receive a 2-week low-dose induction with cyclophosphamide, cisplatin, doxorubicin, or irradiation, all followed by nivolumab [94]. In this trial, the highest ORR were achieved in the cisplatin (ORR = 23%) and doxorubicin (ORR = 35%) cohorts, in which an upregulation of immune-related genes involved in PD-1/PD-L1 and T-cell cytotoxicity pathways was observed. This is supported by the lack of increase in pCR in the Neo-TRIP trial in which no anthracyclines were used. Taken together, these data suggest that the NACT regimen used could influence the potential benefit of immune checkpoint blockade. However, if these effects are subtle, extremely large clinical studies are needed to yield final answers on this topic. Based on the available data, when incorporating pembrolizumab into NACT, carboplatin-based NACT used in KEYNOTE-522 is the preferred regimen[36], also considering the current available evidence of platinum-based chemotherapy in this setting [41,95].

6.5. How to combine or choose among the new emerging treatment options in the early setting?

An open issue of implementing the new drugs in clinical practice is related to overlapping indications of different drugs approved based on distinct studies. Post-neoadjuvant capecitabine is nowadays considered a standard option for patients with residual disease after NACT, according to international guidelines, based on the results of the CREATE-X study[96]. However, adjuvant capecitabine was not incorporated into the KEYNOTE-522 trial design. Moreover, important practice-changing data have been recently published for patients with high-risk, germline *BRCA*-mutated TNBC in the context of the Olympia study, showing a significant improvement in invasive and distant disease-free survival for high-risk patients receiving adjuvant olaparib for 1 year after the completion of local treatment and (neo)adjuvant chemotherapy [97]. Of note, neither the KEYNOTE-522 nor the CREATE-X trials used *BRCA* status as an inclusion criterion or a stratification factor for patient enrollment; therefore, these trials cannot inform on the relative efficacy of capecitabine and/or pembrolizumab as compared to olaparib in this context. Thus far, how to choose among these new emerging treatments in case of patients with overlapping indications remains an open question. Additionally, no safety data are available on the possible combination of these agents in the early setting. New treatment strategies of escalation (e.g. the combination of PARPi and ICIs with or without capecitabine for patients with a germline *BRCA* mutation and residual disease at surgery) or de-escalation (e.g. monotherapy with ICIs in patients with TNBC and high TILs) in selected patients may represent future options to be explored.

6.6. Fertility and immunotherapy: an unmet need

Considering the expanding indications of treatment with ICIs and their increasing use, it is of paramount importance to better understand the potential long-term toxicities for women receiving these treatments. Differently from chemotherapy, immunotherapy toxicities are associated with hyperactivation of the immune system, causing a variety of peculiar immune-related adverse events. While the most common adverse events are well known and described, with precise guidelines indicating their management in clinical practice[98], some areas remain to be further explored. Among others, the toxicities induced by ICI on fertility, pregnancy, and sexuality are poorly understood [99,100]. From the currently available evidence, these compounds could potentially cause libido and sexual impairment, as well as primary and secondary hypogonadism [101,102]. Moreover, based on preclinical data, conception, and pregnancy should be avoided during treatment with ICI, although, in some cases, a regular delivery seems to be possible[103]. Considerations on fertility, sexuality and pregnancy should represent a critical component of cancer care in patients receiving immunotherapeutic treatments, and an increasing research effort to fill the current knowledge gap should be made[104].

6.7. Other long-term toxicities

As ICI use is associated with immune-related adverse events, and it is of paramount importance to better understand the potential long-term toxicities for patients receiving these treatments, including endocrinopathies. Immune-mediated hypophysitis, in particular, is often unrecognized and can have a wide spectrum of clinical presentations, from mild forms to more severe ones, with isolated pituitary hormone deficiency or, more rarely, panhypopituitarism[105]. Severe cases can present with visual disturbances due to pituitary gland enlargement and optic chiasm compression, and/or lead to death for adrenal crisis[105]. Physicians should be aware of this potential toxicity to promptly recognize it and effectively treat this condition[105].

Several additional challenges exist in the use of immunotherapy for patients with TNBC, including the risk of other potential long-term immune-related toxicities, the high financial burden for public health-care systems associated with ICI use, and a deeper understanding of the underlying causes of different responses to immune checkpoint inhibitor beyond the PD-L1 expression, that are not extensively discussed in this review, but that deserve attention and further investigation.

7. Conclusions

PD-1 blockade is now approved for the treatment of TNBC, both in the early and advanced settings. However, issues remain, including the identification of predictive biomarkers

to select patients who are likely to benefit from the addition of ICIs, and the definition of the most appropriate endpoints to evaluate their activity in clinical trials. Additionally, a deeper understanding of the heterogeneity of TNBC is needed, both in terms of intrinsic and extrinsic tumor characteristics (e.g. molecular and intrinsic subtypes, tumor microenvironment, immune cell infiltration), and in terms of features of each specific patient (e.g. immune status, body-mass index, microbiota). Several new immunotherapeutic strategies are currently being evaluated, reflecting a promising evolution toward a more personalized approach and an extended clinical benefit in TNBC.

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