Immunotherapy in triple-negative breast cancer: the role of immune checkpoint inhibitors

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ABSTRACT: Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer, associated with a poor prognosis in both early and advanced stages. Chemotherapy remains the standard treatment for these patients, despite its limited benefit. Due to the disease's aggressive features and lack of targeted therapies, several attempts have been made to disclose novel molecular targets. TNBC is now known to be an immunogenic breast cancer subtype. Therefore, immunotherapy has emerged as a promising treatment option for this disease. During the last few years, immune checkpoint inhibitors (anti-PD1/PD-L1 and anti-CTLA-4 monoclonal antibodies) have been investigated either as monotherapy or combined with conventional therapy in TNBC. Herein, we review the status of immunotherapy in TNBC, focusing on the value of immune checkpoint inhibitors.

Keywords: Triple-negative breast cancer (TNBC); Immune checkpoint inhibitors (ICIs); PD-L1; PD-1; CTLA-4.

Imunoterapia no cancro de mama triplo-negativo: o papel dos inibidores de *checkpoint* imunológico

RESUMO: O cancro de mama triplo-negativo (TNBC) é um subtipo agressivo de cancro de mama, associado a um mau prognóstico em estadios iniciais e avançados. A quimioterapia continua a ser o tratamento padrão preconizado para estes doentes, apesar de seu benefício limitado. Devido às características agressivas da doença e à falta de terapias dirigidas, várias tentativas foram feitas para investigar novos alvos moleculares. O TNBC é agora conhecido por ser um subtipo de cancro de mama imunogénico. Neste contexto, a imunoterapia surgiu como uma opção promissora de tratamento para esta doença. Durante os últimos anos, os inibidores de *checkpoint* imunológico (anti-PD1/PD-L1 e anti-CTLA-4 anticorpos monoclonais) foram investigados quer em monoterapia quer em combinação com a terapia convencional nesta neoplasia. Neste artigo apresenta-se uma revisão bibliográfica do papel da imunoterapia no contexto do TNBC, com enfoque no papel dos inibidores de *checkpoint* imunológico.

Palavras-chave: Cancro de mama triplo-negativo (TNBC); inibidores de checkpoint imunológico (ICIs); PD-L1; PD-1; CTLA-4.

Introduction

Breast cancer (BC) is the most frequent cancer among women in the world and it remains a leading cause of cancer-related death in women globally[1]. In Portugal, BC is the most frequent cancer diagnosed among women, with 7,041 new cases detected in 2020 (26.4% of total) and the first cause of cancer-related mortality (15.5% of total cancer cases in women)[1]. BC can be classified by tumour stage, histopathological type, grade, and the expression of certain genes and receptor proteins. According to receptor expression, BC can be categorized into four major subtypes: luminal A (*oestrogen* receptor (ER) positive, progesterone receptor (PR) positive, and human epidermal receptor 2 (HER2) negative); luminal B (ER-positive and/or PR-positive, HER2-positive); HER2 overexpressing (ER-negative, PR-negative, and HER2-positive); and triple negative[2]. Triple-negative breast cancer (TNBC) which is defined by the lack of ER and PR and HER2, accounts for

15% to 20% of all BC[3]. Compared to the other BC subtypes, TNBC presents a higher proliferative rate and frequent metastasis to the lung and brain[4]. Besides that, TNBC is more prevalent in black and young women (younger than 40 years), and typically displays aggressive behaviour, including earlier and higher recurrence (< 3 years) and distant metastasis[5]. Importantly, besides patients with germline BRCA-related TNBC which benefit from poly (ADP-ribose) polymerase (PARP) inhibitors, there are no currently available targeted therapies with curative means[6-7]. Chemotherapy is still the gold standard for most metastatic TNBC (mTNBC), however, responses are often short-lived, and patients have a median overall survival (OS) of 13 to 18 months[5].

Therefore, new emerging therapies for TNBC are urgently needed. Over the last decade, several investigations have demonstrated the major role of the immune system in TNBC establishment and progression. It is now known that TNBC presents not only higher expression levels of immune evasion molecules, such as programmed death ligand-1 (PD-L1), in tumour microenvironment, but also increased levels of tumour-infiltrating lymphocytes (TILs) which is a predictor of good response to immunotherapy and of better prognosis in early-stage disease[4-5,8-10]. Besides that, the presence of somatic nonsynonymous mutations (tumour mutation burden) that generate tumour neoantigens (MHC class I antigens) can lead to T cell activation giving rise to an immune response that can be enhanced by immune checkpoint inhibitors (ICIs). Therefore immunotherapy, especially ICIs, represents a promising treatment strategy for TNBC. ICIs act by blocking immunosuppressive receptors, such as programmed death 1 (PD-1), its ligand PD-L1, and cytotoxic T lymphocyte antigen-4 (CTLA-4), in order to improve TILs proliferation and cytotoxicity ability[11-14].

This review aims to discuss the role of the current and novel immune checkpoint inhibitors on TNBC treatment, either as monotherapy or in combination with standard or targeted therapy. Moreover, it will highlight the emerging immunotherapy biomarkers and point out the future directions for immunotherapy in TNBC.

ICIs mechanism of action

ICIs are normal control pathways that monitor the activity of immune cells. PD-1 is an inhibitory receptor expressed on activated T cells, B cells, macrophages, regulatory T cells (Tregs), and Natural Killers (NK) cells. Its ligand (PD-L1, CD274, or B7-H1) is expressed in T cells, B cells, Tregs, macrophages, dendritic cells (DCs), and on non-blood cells. Importantly, PD-L1 is highly expressed on the surface of tumour cells contributing to cancer immune escape[15]. Cancer cells lead to overstimulation of the PD-1/PD-L1 signalling pathway by induction of cytotoxic T-cell anergy, exhaustion, apoptosis, and decreased cytokine production, in order to bypass immune surveillance. Thus, the interaction of PD-1 with PD-L1 leads to increased tumour cell resistance to pro-apoptotic signals and immune escape of tumour cells, ultimately leading to poor cancer prognosis[16]. As observed in Figure 1, PD-1/PD-L1 checkpoint inhibitors suppress this pathway, by inhibiting the association between the ligand and its receptor, increasing immune cell proliferation and enhancing natural immune surveillance against cancer cells[17-19].

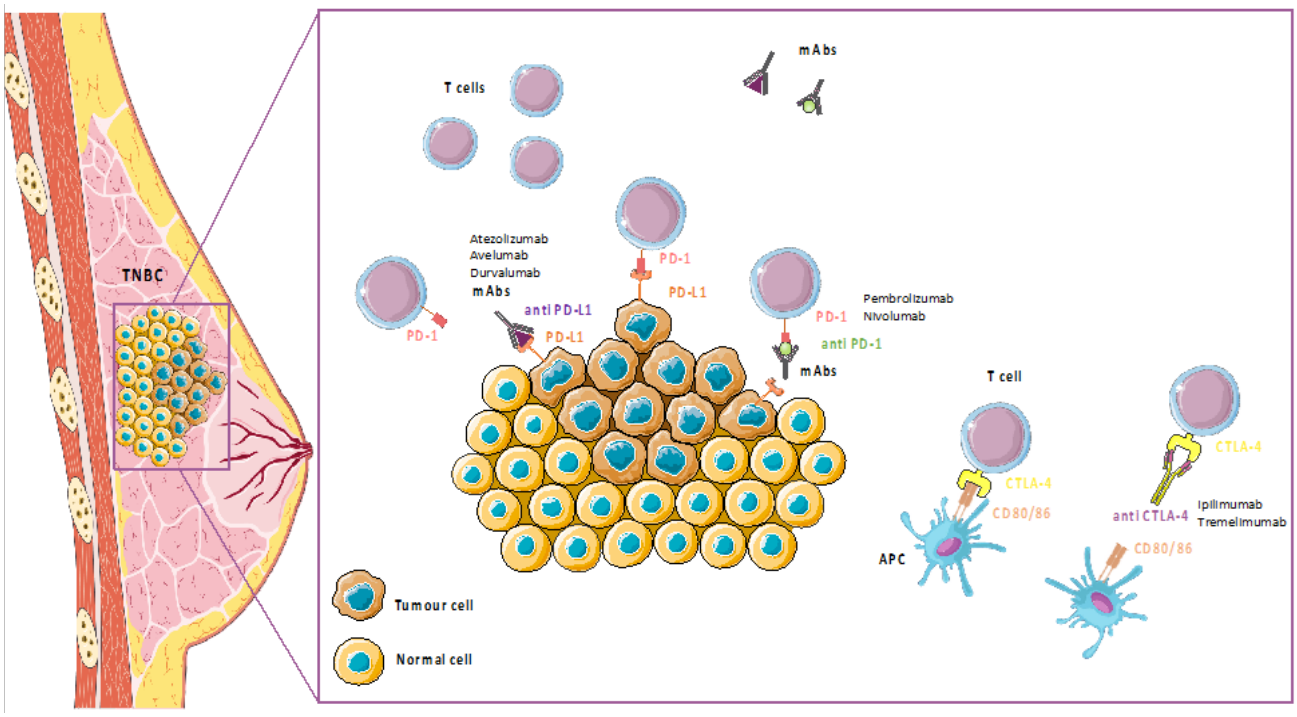

Figure 1. Immune check-point inhibitors mechanism of action in TNBC.

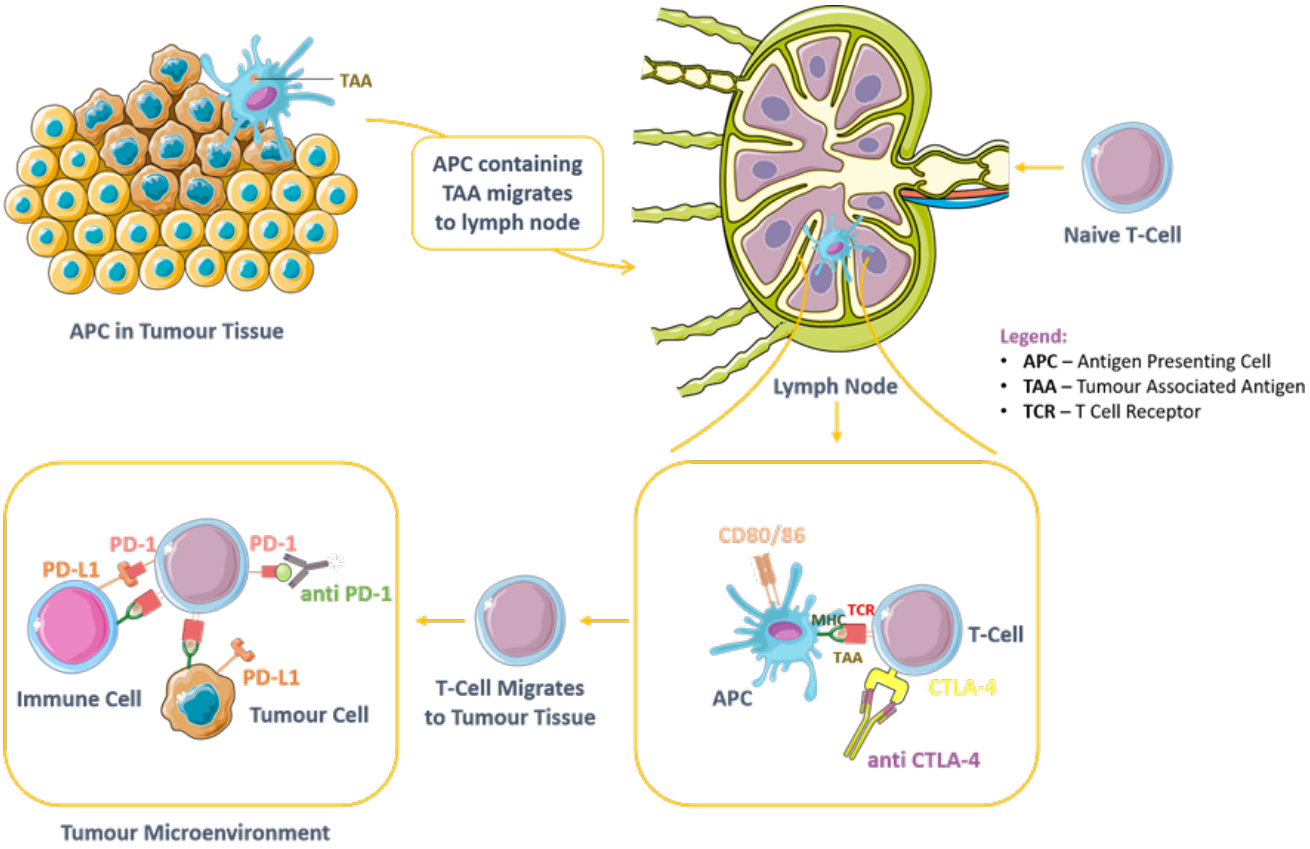

Figure 2. Role of PD-1/PD-L1 and CTLA-4 monoclonal antibodies on anti-tumour immune responses.

CTLA-4, or CD152, negatively regulates Tregs and activated T cell activity and proliferation[13]. The co-inhibitor CTLA-4 competes with the co-stimulator CD28, for B7 binding within the antigen-presenting cells (APC), whose function is to activate T cells. As CTLA-4 has a greater affinity to B7, it limits the CD28 connection. The inhibition of CTLA-4 leads to the activation of T cell-mediated antitumor immunity, increasing the number of activated $TCD8^+$ cells. By enhancing the proportion of $CD8^+/Foxp3^+$ Treg cells augments the host's immune response against neoplastic cells (Figures 1 and 2)[20-22].

As of April of 2021, 218 ongoing clinical trials investigating ICIs in TNBC (either as single agents or in combination with conventional and targeted therapy) have been listed on ClinicalTrials.gov. The selected ones are listed in supplementary Tables 1-3.

PD-L1 inhibitors

Atezolizumab

Atezolizumab is an engineered and humanized monoclonal antibody against PD-L1, which stimulates T cell activity against cancer cells[23].

A phase I clinical trial (NCT01375842) investigated the use of atezolizumab as the first and second line setting in TNBC. The first line setting presented significantly higher overall response rates (ORR) than the second line (24% *vs* 6%), with a median duration of response of 21 months. Interestingly high levels of PD-L1 in immune cells (> 10%) were independently associated with higher ORR and longer Overall Survival (OS)[24].

In 2019, the U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) granted accelerated approval for the use of atezolizumab (Tecentriq®) plus nab-paclitaxel (Abraxane®) as first-line treatment of PD-L1 positive ($PD\text{-}L1^+$), unresectable, locally advanced or metastatic TNBC, based on IMpassion130 clinical trial (NCT02425891)[25-26]. This phase III trial randomised 902 patients (1:1), with previously untreated metastatic TNBC, to receive either atezolizumab or placebo, plus paclitaxel protein-bound. Each group included 451 patients (median follow-up, 12.9 months). A clinically meaningful improvement with an OS of 7.0 months (25.4 months with atezolizumab *vs* 17.9 months with placebo), was achieved in $PD\text{-}L1^+$ mTNBC patients, reducing the risk of deaths by 33% in this subgroup[27]. In the intention-to-treat population, a median progression-free survival (PFS) was 7.2 months with atezolizumab plus nab-paclitaxel, as compared with 5.5 months with placebo plus nab-paclitaxel. Among patients with $PD\text{-}L1^+$ tumours (PD-L1 > 1%), the median PFS was greater favouring the atezolizumab group at 7.5 months versus 5.0 months. ORR in patients with confirmed responses

(PL-L1+) was 53.5% compared to 36.6% for the atezolizumab and placebo-containing arms, respectively[23,28-29]. The most common treatment related-adverse events (AEs) were similar in both groups. However, the incidence of grade ≥ 3 AEs was higher in the atezolizumab group. Alopecia was the most common event (56.4% in atezolizumab group and 57.5% in the placebo). The frequencies of nausea (46.0 % *vs* 38.1%), cough (24.8% *vs* 38.1%), neutropenia (20.8% *vs* 15.3%), pyrexia (18.8% *vs* 10.7%), and hypothyroidism (13.7% *vs* 3.4%) were at the least 5% greater in the atezolizumab group than in the placebo group[28].

Avelumab

Avelumab is another PD-L1 inhibitor in clinical development. The phase Ib JAVELIN trial (NCT01772004) enrolled 58 heavily pre-treated metastatic BC (mBC) patients (68.8% PD-L1+) who were treated with 10mg/kg every two weeks of avelumab (Bavencio®). An ORR of 3.0% was reported in mBC compared to 5.2% in mTNBC. As observed in previous reports, higher response rates were achieved in PD-L1+ (cut-off of 10%) versus PD-L1- patients (16.7% *vs* 1.6%) in the global population. Concerning TNBC the likelihood of response was 22.2% in PD-L1+ patients *vs* 2.6% in PD-L1- patients. This trial demonstrated an OS of 9.2 months and a PFS of 1.5 months[12-13,29].

Grade ≥ 3 treatment-related AEs occurred in 13.7% of patients, including two treatment-related deaths. The most common side effects were: fatigue (19%); infusion-related reaction (14.3%); nausea (13.1%); diarrhoea (8.9%); arthralgia (7.7%) and decreased appetite (7.1%)[30].

Durvalumab

In mTNBC, durvalumab is under evaluation combined with chemotherapy. The most promising results came from the GeparNuevo study, a phase II clinical trial (NCT02685059), which included 117 TNBC patients. The combination of durvalumab (Imfinzi®) with taxane-anthracycline-based neoadjuvant chemotherapy provided clinical benefit in early TNBC with an increase in pathologic complete response (pCR) from 44.2%, in the chemotherapy alone, to 53.4% with durvalumab[12-13,29]. The most common immune-related AE was thyroid dysfunction, reported in 47% of the patients. Like the other PD-L1 inhibitors, AEs such as alopecia (92.4%), peripheral sensory neuropathy (82.6%), nausea (58.7%), skin reaction (48.9%), constipation (31.5%), diarrhoea (28.3%), were also been seen in a large number of patients[31].

Several other clinical trials already ongoing in TNBC are listed in supplementary Table 1.

PD-1 inhibitors

Pembrolizumab

Pembrolizumab is a humanized monoclonal IgG4-k antibody with elevated affinity and selectivity against PD-1. TNBC has been evaluated as monotherapy or in combination with conventional or target therapy. The most relevant studies are described below, and the remaining ongoing studies are listed in supplementary Table 2.

The KEYNOTE-012 (NCT01848834) clinical trial, published in 2016, assessed the safety profile and clinical activity of this ICI in PD-L1+ pre-treated TNBC. An ORR of 18.5% (5/27) was observed and 25.9% of patients achieved stable disease ranging the median duration of response from 15.0 to 47.3 weeks[32].

A subsequent phase II clinical trial, KEYNOTE-086 (NCT02447003), enrolled 170 mTNBC patients who received prior systemic treatment. This study had as primary endpoints ORR (total and PD-L1+ population) and safety; and as secondary endpoints duration of response, disease control rate (percentage of patients with complete or partial response or stable disease for ≥ 24 weeks), PFS, and OS. An ORR of 5.3% in total and 5.7% in the PD-L1+ population was achieved. Median PFS was 2.0 months, with a 6-month rate of 14.9%. Median OS was 9.0 months being the 6-month rate 69.1%. Treatment-related AEs were present in 60.6% of the patients, with grade 3 or 4 toxicities reported in 12.9%. The most prevalent AEs were fatigue, nausea, hypothyroidism, anorexia, diarrhoea, and hyperthyroidism[33].

The open-label phase III trial, KEYNOTE-119 (NCT02555657) evaluated pembrolizumab monotherapy *vs* single-agent chemotherapy in pre-treated 622 mTNBC patients. Patients were stratified by PD-L1 status, previously received systemic therapy, and metastatic status at first diagnosis. The primary endpoints were OS and safety. Patients who had previously received at least one anthracycline or taxane therapy were randomly assigned to pembrolizumab or capecitabine/eribulin/gemcitabine/vinorelbine. Unfortunately, pembrolizumab did not show a significant increase in overall survival (OS) (median OS was 9.9 months for the pembrolizumab group *vs* 10.8 months for the chemotherapy group), although treatment effect augmented as PD-L1 enrichment increased. Grade 3 and 4 AEs were described, as being higher in the chemotherapy group compared to pembrolizumab. This study pointed out that monotherapy with pembrolizumab might have more efficacy in selected subpopulations of patients, with PD-L1-enriched tumours[34]. Given these results, occurred a shift of focus to pembrolizumab combined with systemic and target therapy in TNBC.

A phase II clinical trial (NCT02734290) investigated the combination of pembrolizumab with paclitaxel or capecitabine as early treatment in 22 patients with mTNBC. ORR was higher in capecitabine groups (43%) compared to the paclitaxel group (25%). This study pointed out that the combination of pembrolizumab with either capecitabine or paclitaxel is safe and has encouraging efficacy. However, both treatments were associated with iatrogenic declines in T-cell number[35].

In phase II, I-SPY2 multicentre trial (NCT01042379), the association between pembrolizumab and paclitaxel followed by doxorubicin and cyclophosphamide was investigated in 69 patients with HER2-negative BC. This study demonstrated that the combined therapy resulted in a higher pCR (60% *vs* 20%)[36].

Table 1. PD-L1 inhibitors clinical trials in TNBC

Trial ID	Agents	Target	Setting	Phase	Sample Size	Status	Estimated Study Completion
NCT03125902	Atezolizumab + Paclitaxel vs Placebo	PD-L1	Untreated locally advanced or metastatic TNBC	III	651	Active not recruiting	December 2021
NCT04148911	Atezolizumab + Nab-Paclitaxel	PD-L1	Unresectable locally advanced or metastatic PD-L1-positive TNBC	IIIb	180	Recruiting	October 2024
NCT02425891	Atezolizumab + Nab-Paclitaxel vs Placebo	PD-L1	Untreated mTNBC	III	900	Active not recruiting	July 2021
NCT03281954	Atezolizumab + Chemotherapy	PD-L1	TNBC	III	1520	Recruiting	June 2024
NCT03164993	Atezolizumab + Chemotherapy	PD-L1	mTNBC	II	75	Recruiting	December 2024
NCT04690855	Atezolizumab + TAlazoparib + Radiotherapy	PD-L1 PARP	gBRCA 1/2 negative patients with PD-L1+ mTNBC	II	23	Not yet recruiting	April 2023
NCT04584112	Atezolizumab + Tiragolumab + Chemotherapy	PD-L1 TIGIT	TNBC	Ib	80	Recruiting	Marh 2022
NCT03498716	Atezolizumab + Chemotherapy	PD-L1	Stage II/III TNBC	III	2300	Recruiting	August 2025
NCT03197935	Atezolizumab + Chemotherapy	PD-L1	Early stage TNBC	III	324	Active not recruiting	October 2022
NCT04177108	Atezolizumab + Ipatasertib + Paclitaxel	PD-L1 AKT	Locally advanced and mTNBC	III	242	Active not recruiting	October 2025
NCT03256344	Atezolizumab + Talimogene Laherparepvec	PD-L1	TNBC	Ib	36	Active not recruiting	August 2022
NCT04739670	Atezolizumab + Bevacizumab + Carboplatin + Gemcitabine	PD-L1 VEGF	mTNBC	II	31	Not yet recruiting	September 2025
NCT03371017	Atezolizumab + Chemotherapy	PD-L1	Early relapsing recurrent TNBC	III	572	Recruiting	March 2024
NCT04770272	Atezolizumab + Chemotherapy	PD-L1	TNBC	II	458	Recruiting	January 2026
NCT03206203	Atezolizumab + Carboplatin	PD-L1	Stage IV TNBC	II	106	Active not recruiting	November 2023
NCT03756298	Atezolizumab + Capecitabine	PD-L1	TNBC	II	284	Recruiting	January 2027
NCT02530489	Atezolizumab + Nab-Paclitaxel	PD-L1	TNBC (before surgery)	II	37	Active not recruiting	February 2023
NCT03483012	Atezolizumab + Stereotactic Radiation	PD-L1	mTNBC	II	45	Active not recruiting	September 2025
NCT04408118	Atezolizumab + Paclitaxel + Bevacizumab	PD-L1 VEGFA	mTNBC	II	100	Recruiting	April 2023
NCT03853707	Atezolizumab + Ipatasertib + Capecitabine	PD-L1 AKT	mTNBC	I/Ib	40	Recruiting	June 2022
NCT01898117	Atezolizumab + Carboplatin-cyclophosphamide vs Paclitaxel	PD-L1	Advanced TNBC	IIb	304	Recruiting	December 2030
NCT03464942	Atezolizumab + Stereotactic Radiation	PD-L1	Advanced TNBC	II	52	Recruiting	April 2022
NCT02883062	Atezolizumab + Carboplatin + Paclitaxel	PD-L1	Stage II/III TNBC	II	72	Active not recruiting	July 2021
NCT04249167	Atezolizumab + Nab-paclitaxel + Cryoablation	PD-L1	Locally advanced and mTNBC	I	5	Active not recruiting	December 2021
NCT02322814	Atezolizumab + Cobimetinib + Paclitaxel/ Nab-Paclitaxel	PD-L1	mTNBC	II	169	Active not recruiting	March 2021
NCT03800836	Atezolizumab + Ipatasertib + Paclitaxel/ Nab-Paclitaxel + AC	PD-L1 AKT	Locally advanced and mTNBC	Ib	140	Active not recruiting	October 2022
NCT03961698	Atezolizumab + IPI-549 + Nab-Paclitaxel	PD-L1 PI3K-γ	Locally advanced and mTNBC	II	90	Recruiting	August 2022
NCT02620280	Atezolizumab + Chemotherapy	PD-L1	Early high-risk and locally advanced TNBC	III	278	Active not recruiting	October 2022

Table 1. PD-L1 inhibitors clinical trials in TNBC (Continued)

Trial ID	Agents	Target	Setting	Phase	Sample Size	Status	Estimated Study Completion
NCT03915678	Atezolizumab + BDB001 + Radiotherapy	PD-L1 TLR7/8	TNBC	II	247	Not yet recruiting	March 2025
NCT03424005	Atezolizumab + Nab-Paclitaxel/ Tocilizumab/ Sacituzumab/ Govitecan/ Ipatasertib/ SGN-LIV1A/ Selicrelumab + Bevacizumab/ Gemcitabine + Carboplatin or Eribulin	PD-L1 IL-6 Topoisomerase AKT LIV-1 CD40 VEGF	Locally advanced TNBC	Ib/II	280	Recruiting	January 2023
NCT03289962	Atezolizumab + Autogene Cevumeran	PD-L1	Locally advanced or mTNBC	Ib	770	Recruiting	February 2024
NCT03829501	Atezolizumab + KY1044 (ICOS)	PD-L1	Advanced TNBC	I/II	412	Recruiting	May 2023
NCT03170960	Atezolizumab + Cabozantinib	PD-L1 RTK	Locally advanced or mTNBC	Ib	1732	Recruiting	December 2022
NCT03579472	M7824 + Eribulin Mesylate	PD-L1 + TGF-β	mTNBC	I	20	Recruiting	October 2021
NCT02926196	Avelumab	PD-L1	High-risk pre-treated TNBC	III	474	Active not recruiting	June 2023
NCT04360941	Avelumab + Palbociclib	PD-L1	mTNBC AR+	I	45	Recruiting	July 2024
NCT04188119	Avelumab + Aspirin	PD-L1	TNBC	II	42	Not yet recruiting	August 2021
NCT03971409	Avelumab + Binimetinib + Utomilumab, or Anti-OX40 Antibody PF-04518600	PD-L1 MEK CD137 CD134	TNBC	II	150	Recruiting	July 2021
NCT02554812	Avelumab + Utomilumab	PD-L1, CD137	Advanced TNBC	Ib/II	620	Recruiting	February 2024
NCT04551885	Avelumab + FT516	PD-L1, NK cells	Advanced TNBC	I	27	Recruiting	August 2037
NCT03167619	Durvalumab + Olaparib	PD-L1 PARP	mTNBC	II	50	Active not recruiting	August 2021
NCT03872505	Durvalumab + Non-Anthracycline Chemotherapy + Radiotherapy	PD-L1	Stage II/III TNBC	II	140	Not yet recruiting	July 2024
NCT03616886	Durvalumab + Paclitaxel + Carboplatin + Oleclumab	PD-L1 CD73	Untreated locally recurrent inoperable or mTNBC	I/II	171	Recruiting	October 2023
NCT03356860	Durvalumab + Neoadjuvant Chemotherapy	PD-L1	TNBC non-metastatic	Ib/II	57	Recruiting	April 2021
NCT03801369	Durvalumab + Olaparib	PD-L1 PARP	mTNBC	II	28	Recruiting	December 2026
NCT04176848	Durvalumab + CFI-400945	PD-L1, PLK4	Advanced TNBC	II	28	Recruiting	December 2022
NCT03742102	Durvalumab + Paclitaxel + Capivasertib or Durvalumab + Oleclumab/ Trastuzumab/ Datopotamab	PD-L1 AKT CD73 HER-2	mTNBC	Ib/II	200	Recruiting	February 2023
NCT02489448	Durvalumab + Chemotherapy	PD-L1	Stage I-III TNBC	I/II	71	Active not recruiting	December 2021
NCT03740893	Durvalumab	PD-L1	Neoadjuvant chemotherapy resistant residual TNBC	II	81	Recruiting	December 2025
NCT03983954	Durvalumab + Obinutuzumab + Naptumomab Estafenatox	PD-L1 CD20	Advanced or mTNBC	Ib/II	45	Recruiting	February 2022
NCT02484404	Durvalumab + Olaparib + Cediranib	PD-L1 PARP VEGF	Advanced TNBC	I/II	384	Recruiting	December 2022
NCT04504669	Durvalumab + AZD8701	PD-L1 FOXP3	Locally advanced TNBC	I	123	Recruiting	September 2023

Table 2. PD-1 inhibitors clinical trials in TNBC

Trial ID	Agents	Target	Setting	Phase	Sample Size	Status	Estimated Study Completion
NCT02768701	Pembrolizumab + Cyclophosphamide	PD-1	Pre-treated advanced/ mTNBC	II	40	Active not recruiting	March 2023
NCT02977468	Pembrolizumab	PD-1	Naïve TNBC (node negative)	I	15	Recruiting	December 2021
NCT03720431	Pembrolizumab + TTAC-001 (Tanibirumab)	PD-1 VEGFR-2	mTNBC	Ib	11	Active not recruiting	February 2022
NCT03121352	Pembrolizumab + Nab-paclitaxel + Carboplatin	PD-1	mTNBC	II	30	Active not recruiting	February 2022
NCT04095689	Docetaxel + Doxorubicin + Cyclophosphamide + Pembrolizumab + IL-12 gene therapy + L-NMMA	PD-1 IL-12 Nitric oxide synthase	Early-stage TNBC pre-treated with chemotherapy	II	43	Recruiting	August 2024
NCT04427293	Pembrolizumab + Lenvatinib	PD-1 VEGFR	Untreated early-stage TNBC	I	12	Recruiting	July 2026
NCT02730130	Pembrolizumab + Radiotherapy	PD-1	mTNBC	II	17	Active not recruiting	March 2022
NCT03639948	Pembrolizumab + Carboplatin + Docetaxel	PD-1	Stage I-III TNBC	II	100	Recruiting	November 2024
NCT04191135	Pembrolizumab + Olaparib or Carboplatin + Gemcitabine	PD-1 PARP	TNBC	II/III	932	Recruiting	January 2026
NCT04683679	Pembrolizumab + Olaparib + Radiotherapy	PD-1 PARP	mTNBC	II	56	Not yet recruiting	January 2025
NCT03012230	Pembrolizumab + Ruxolitinib	PD-1 JAK2	mTNBC stage IV	I	18	Recruiting	March 2020
NCT04373031	Pembrolizumab + IRX-2 + Chemotherapy	PD-1	TNBC	II	30	Recruiting	June 2025
NCT03225547	Pembrolizumab + Mifepristone	PD-1 Progesterone	Locally advanced or mTNBC	II	74	Recruiting	September 2022
NCT03310957	Pembrolizumab + SGN-LIV1A	PD-1 LIV-1	Untreated locally advanced or mTNBC	Ib/II	122	Recruiting	June 2023
NCT03567720	Pembrolizumab + Tavokinogene Telseplasmid + Nab-paclitaxel	PD-1 IL-12	Locally advanced or mTNBC	II	65	Recruiting	August 2024
NCT04468061	Pembrolizumab + Sacituzumab Govitecan	PD-1 Topoisomerasel	mTNBC	II	110	Recruiting	June 2026
NCT01676753	Pembrolizumab + Dinaciclib	PD-1 CDK	Unressectable mTNBC	Ib	32	Active not recruiting	December 2022
NCT03752723	Pembrolizumab + GX-I7 + Cyclophosphamide	PD-1 IL-7	Refractory or relapsed TNBC	Ib/II	83	Recruiting	December 2021
NCT03106415	Pembrolizumab + Binimetinib	PD-1 MEK	Locally advanced or mTNBC	I/II	38	Recruiting	July 2022
NCT02971761	Pembrolizumab + Enobosarm	PD-1 AR	mTNBC with AR+	II	29	Active not recruiting	November 2021
NCT03599453	Pembrolizumab + Celecoxib + Recombinant Interferon Alfa-2b + Rintatolimod	PD-1 INF TLR3	mTNBC	I	8	Active not recruiting	July 2022
NCT03004183	Pembrolizumab + Oncolytic virus + Radiotherapy	PD-1	mTNBC	II	57	Active not recruiting	November 2023
NCT03044730	Pembrolizumab + Capecitabine	PD-1	Locally advanced or mTNBC	II	30	Active not recruiting	May 2021
NCT02411656	Pembrolizumab	PD-1	mTNBC	II	35	Recruiting	December 2024
NCT02648477	Pembrolizumab + Doxorrubicin	PD-1	mTNBC	II	30	Active not recruiting	October 2021
NCT4230109	Pembrolizumab + Sacituzumab Govitecan	PD-1 Topoisomerasel	Localized TNBC	II	100	Recruiting	August 2024
NCT04443348	Pembrolizumab + Radiotherapy or Chemotherapy	PD-1	Locally advanced TNBC (nodule positive)	II	120	Recruiting	December 2023
NCT04265872	Pembrolizumab + Bortezomib + Cisplatin	PD1 Proteosome	mTNBC	I	20	Recruiting	September 2021
NCT03952325	Pembrolizumab / Nivolumab/ Atezolizumab + Tesetaxel	PD-1 PD-L1	mTNBC	II	320	Recruiting	August 2023
NCT04432857	Pembrolizumab + AN0025	PD-1 EP4	Locally advanced or mTNBC	Ib	84	Recruiting	March 2023
NCT03396445	Pembrolizumab + MK-5890-001 + Carboplatin/ Nab-paclitaxel	PD-1 CD27	Locally advanced or mTNBC	I	202	Recruiting	January 2024

Table 2. PD-1 inhibitors clinical trials in TNBC (Continued)

Trial ID	Agents	Target	Setting	Phase	Sample Size	Status	Estimated Study Completion
NCT02954874	Pembrolizumab + Radiotherapy	PD-1	TNBC	III	1155	Recruiting	May 2026
NCT04332653	Pembrolizumab +NT-17	PD-1 IL-7	Relapsed/Refractory mTNBC	Ib/IIa	168	Recruiting	April 2023
NCT03797326	Pembrolizumab + Levantinib	PD-1 Multi-kinase	TNBC	II	760	Recruiting	March 2024
NCT03213041	Pembrolizumab + Carboplatin	PD-1	mTNBC	II	100	Recruiting	July 2022
NCT02644369	Pembrolizumab	PD-1	Locally advanced TNBC	II	100	Active not recruiting	August 2021
NCT02957968	Pembrolizumab + Decitabine + Chemotherapy	PD-1 DNMTs	Locally advanced TNBC	II	32	Recruiting	February 2023
NCT03454451	Pembrolizumab + CPI-006	PD-1 CD73	Locally advanced TNBC	I/Ib	378	Recruiting	December 2023
NCT04331067	Neoadjuvant Chemotherapy + Nivolumab + Cabiralizumab	PD-1 CSF-1	Localized TNBC	I/II	50	Recruiting	February 2024
NCT03414684	Nivolumab + Carboplatin	PD-1	Untreated mTNBC	II	78	Active not recruiting	June 2025
NCT03487666	Nivolumab + Capecitabine	PD-1	TNBC	II	45	Recruiting	December 2022
NCT03098550	Nivolumab + Daratumumab	PD-1	Advanced TNBC	I/II	120	Active not recruiting	August 2021
NCT04159818	Nivolumab + Cisplatin + Low dose Doxorubicin	PD-1	mTNBC	II	52	Recruiting	December 2026
NCT03435640	Nivolumab + NKTR-262 + Bempegaldesleukin	PD-1 TLR IL-2	TNBC	I/II	64	Active not recruiting	December 2022
NCT02637531	Nivolumab + IPI-549	PD-1 PI3K-γ	Advanced TNBC	I/Ib	219	Active not recruiting	June 2021
NCT03829436	Nivolumab + TPST-1120	PD-1 PPARα	Advanced TNBC	I/Ib	138	Recruiting	June 2024
NCT03667716	Nivolumab + COM701	PD-1 PVRIG	Advanced TNBC	Ia/Ib	140	Recruiting	December 2021
NCT04243616	Cemiplimab + Chemotherapy	PD-1	Locally advanced TNBC	II	36	Recruiting	March 2023

Recently, the KEYNOTE-522 (NCT03036488), a phase III trial, assessed the combination of pembrolizumab plus paclitaxel and carboplatin *vs* placebo plus paclitaxel and carboplatin in a total of 1,174 untreated stage II or stage III TNBC patients (784 and 390 patients respectively). Both arms received four cycles of paclitaxel+carboplatin followed by 4 cycles of pembrolizumab *vs* placebo plus doxorubicin-cyclophosphamide or epirubicin-cyclophosphamide. In an adjuvant setting after curative surgery, patients received adjuvant pembrolizumab or placebo for nine cycles; until relapse or unacceptable toxicity. Primary endpoints were the pCR and event-free survival (EFS). The combination of chemotherapy with pembrolizumab significantly improved pCR compared to chemotherapy alone (64.8% *vs* 51.2% respectively). Concerning the PD-L1^{+} population, was described a pCR of 68.9% in the pembrolizumab arm *vs* 54.9% in the placebo. In the PD-L1 negative population, a pCR of 45.3% was achieved in the pembrolizumab arm *vs* 30.3% in the placebo arm. Additionally, the pembrolizumab arm also demonstrated a favourable trend in EFS. Grade 3 and 4 treatment-related AEs were found in 76.8% of the pembrolizumab arm and in 72.2% of the placebo arm[37].

The KEYNOTE-355 (NCT02819518), a multicentre, double--blind, randomized phase III study of pembrolizumab plus chemotherapy *vs* placebo plus chemotherapy enrolled 847 previously untreated, locally recurrent, inoperable or mTNBC patients. The main efficacy outcome was PFS. A median PFS of 9.7 months was achieved with pembrolizumab plus chemotherapy compared to 5.6 months observed in placebo plus chemotherapy. The most common AEs in patients were fatigue, nausea, diarrhoea, constipation, vomiting, alopecia, rash, cough, decreased appetite, and headache. Some laboratory abnormalities were also reported, namely, anaemia, leukopenia, neutropenia, lymphopenia, thrombocytopenia, elevated ALT and AST, hyperglycaemia, hypoalbuminemia, increased alkaline phosphatase, hypocalcaemia, hyponatremia, hypophosphatemia, and hypokalaemia. The PFS improvement led to accelerated FDA approval of pembrolizumab in combination with chemotherapy in the first line setting for locally recurrent unresectable or mTNBC whose tumours express PD-L1 (CPS ≥ 10), in November of 2020[38].

The phase II, KEYNOTE-162 trial (NCT02657889), investigated the combination of oral niraparib (PARP inhibitor) with pembrolizumab in 55 TNBC patients, 15 of which harbouring BRCA mutation. Overall, the ORR was 21%, being higher in BRCA mutation carriers (47%). Interestingly, positivity for PD-L1 was higher in patients harbouring mutated BRCA. The main adverse effects were haematological (thrombocytopenia and anaemia) and mainly due to the PARP inhibitor[39].

Nivolumab

The efficacy and safety of nivolumab in TNBC were assessed in several phase I and II clinical trials. A phase II study (NCT03316586) of nivolumab in combination with cabozantinib was performed on 18 patients with mTNBC. Despite, the absence of unexpected adverse reactions, this study only achieved with an ORR of 5.6% not reaching its primary endpoint (40). TONIC trial (NCT02499367) an adaptive phase II randomized noncomparative study evaluated nivolumab in 67 mTNBC patients with or without induction treatment with radiation, low-dose doxorubicin, metronomic cyclophosphamide, and cisplatin. The ORR 20% and included one (2%) complete response (CR) and 11 (22%) PR. Most responses were observed in the cisplatin (ORR: 23%) and doxorubicin (ORR: 35%) groups. The median duration of response was nine months. Doxorubicin and cisplatin induction contributed to an upregulation of genes involved in PD-1–PD-L1, T cell cytotoxicity, and inflammation pathways, like JAK-STAT and TNF-α. Therefore, leading to a more prone tumour microenvironment, increasing the immune response to PD-1 monoclonal antibodies[41].

Several other clinical trials are already ongoing and listed in supplementary Table 2.

CTLA-4 inhibitors

Currently, there is only a single anti-CTLA-4 antibody approved by the FDA, ipilimumab, for the treatment of melanoma, renal cell carcinoma, and non-small cell lung cancer[42-43]. CTLA-4 inhibitors are under investigation in several clinical trials for TNBC, mostly in combination with other immunomodulators or cytotoxic drugs[44].

The combination of nivolumab (anti-PD-1) with ipilimumab (anti-CTLA-4) is emerging as an attractive synergic therapy since the anti-CTLA-4 activates T cells at the lymph node whereas anti-PD-1 activates T cells at the tumour site (Figure 2)[45-46]. Several trials, with ipilimumab and nivolumab or durvalumab, in TNBC, are already ongoing and summarized in supplementary Table 3.

Table 3. CTLA-4 inhibitors clinical trials in TNBC

Trial ID	Agents	Target	Setting	Phase	Sample Size	Status	Estimated Study Completion
NCT03818685	Ipilimumab + Nivolumab + RT	CTL4-4 PD-1	Adjuvant to chemotherapy	II	114	Recruiting	December 2022
NCT03546686	Ipilimumab + Nivolumab + Pre-operative cryoablation	CTL4-4 PD-1	Residual and resectable TNBC after neoadjuvant chemotherapy	II	80	Recruiting	May 2023
NCT01928394	Ipilimumab + Nivolumab	CTL4-4 PD-1	Advanced or mTNBC	II	131	Active not recruiting	October 2022
NCT03789110	Ipilimumab + Nivolumab	CTL4-4 PD-1	Advanced or mTNBC	II	30	Recruiting	October 2022
NCT02983045	Ipilimumab + Nivolumab + NKTR-214	CTL4-4 PD-1 CD122	Advanced or mTNBC	I/II	557	Active not recruiting	December 2021
NCT04185311	Ipilimumab + Nivolumab + Talimogene laherparepvec	CTL4-4 PD-1	TNBC (before surgery)	I	6	Active not recruiting	July 2022
NCT03815890	Ipilimumab + Nivolumab	CTL4-4 PD-1	mTNBC	II	80	Recruiting	January 2025
NCT02536794	Tremelimumab + Durvalumab	CTL4-4 PD-L1	mTNBC	II	30	Recruiting	June 2023
NCT02527434	Tremelimumab + Durvalumab	CTL4-4 PD-L1	Advanced TNBC	II	64	Active not recruiting	December 2021
NCT03982173	Tremelimumab + Durvalumab	CTL4-4 PD-L1	mTNBC	II	88	Active not recruiting	April 2023
NCT03872791	KN046 + Nab-paclitaxel	CTLA-4/PD-L1	Locally advanced or mTNBC	Ib/II	90	Recruiting	September 2021

Regarding tremelimumab, clinical trials still are in the early stages (stage II) and also in combination with nab-paclitaxel or durvalumab. A single-arm phase II clinical trial, NCT02536794, has investigated the combination of durvalumab with tremelimumab in patients with positive oestrogen receptors and TNBC patients. Although the study did not reach the ORR result, it was demonstrated that 5/7 (71%) TNBC patients reached clinical benefit. In fact, three patients achieved PR and one patient with stable disease (SD) ≥ 6 months. Grade 4 AEs have not been reported[47].

A new drug was recently developed with an innovative design, being the world's first recombinant humanized PD-L1/CTLA-4 bispecific antibody named KN046 (Figure 3). A single domain of the antibody allows the blocking of the

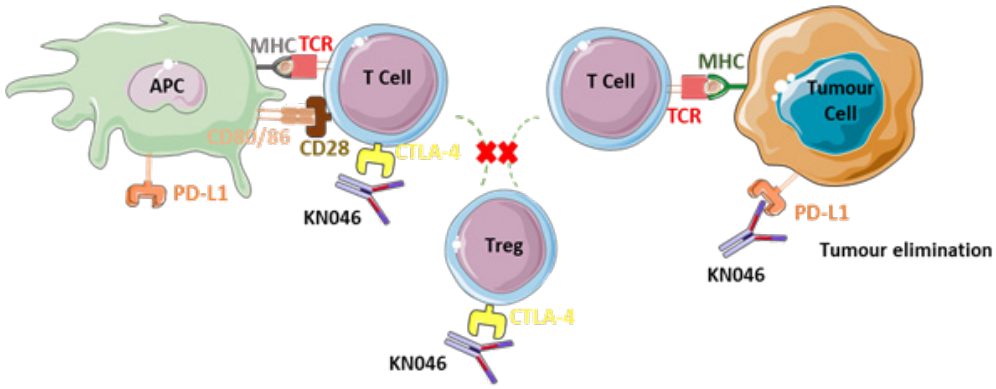

Figure 3. Mechanism of action of KN046 a recombinant humanized PD-L1/CTLA-4 bispecific antibody.

PD-L1 and CTLA-4 receptors, probably with more efficacy and less toxicity according to preclinical and clinical trial results. The phase I clinical trial (NCT03529526) enrolled 21 patients with advanced solid tumours. Interestingly, one TNBC patient treated with 1mg/kg KN046 had shown long-term stable disease for 14 weeks[48]. Currently, there is one ongoing clinical trial with this new molecule in TNBC (NCT03872791).

ICI antibody-vaccine combination therapy

Over the last decade, a multi-peptide vaccine for the treatment of metastatic solid tumours has emerged as a novel immunotherapeutic option, showing increased response rates[49]. Interestingly, the combination of ICIs with these anti-cancer vaccines might augment the immunological response induced by the multi-peptide vaccine. Regarding TNBC, several clinical trials investigating the efficacy of this combination are already ongoing and summarized in supplementary Table 4.

Predictive biomarkers

In order to accurately select patients that will benefit from ICIs therapy and overcome drug resistance, the identification of clinical biomarkers is urgently needed.

Currently, PD-L1 expression, TILs, transcriptional signatures of immune infiltration, TCR diversity, mismatch repair deficiency, and tumour mutational burden (TMB) were proposed as potential predictive biomarkers. PD-L1 expression either on tumour or immune cells has been evaluated as therapy

Table 4. Antibody-vaccine combination therapy in TNBC

Trial ID	Agents	Setting	Phase	Sample Size	Status	Estimated Study Completion
NCT02826434	Durvalumab + PVX-410	Stage II/III TNBC	Ib	22	Active not recruiting	February 2022
NCT03199040	Durvalumab + Neoantigen DNA vaccine	Stage II/III TNBC	I	24	Recruiting	December 2023
NCT03606967	Durvalumab or Tremelimumab + Nab-paclitaxel/ Carboplatin/ Gemcitabine + Neoantigen vaccine	mTNBC	II	70	Recruiting	December 2021
NCT03289962	Atezolizumab + Neoantigen vaccine	TNBC locally advanced or metastatic	Ia/Ib	770	Recruiting	February 2024
NCT04296942	M7824 (TGF β-trap/anti-PD-L1) + BN- Brachyury	mTNBC	Ib	65	Recruiting	January 2024
NCT03362060	Pembrolizumab + PVX-410	mTNBC + HLA-A2+	Ib	20	Recruiting	December 2024
NCT02432963	Pembrolizumab + p53MVA vaccine (modified vaccinia virus Ankara vaccine expressing p53)	mTNBC	I	19	Active not recruiting	December 2021
NCT03761914	Pembrolizumab + Galinpepimut-S (WT1 -targeting multivalent heteroclitic peptide	mTNBC	I/II	90	Recruiting	April 2024
NCT04024800	Pembrolizumab + AE37 Peptide vaccine	mTNBC	II	29	Recruiting	June 2024
NCT04634747	Pembrolizumab + PVX-410 + Chemotherapy	Naïve mTNBC, PD-L1 and HLA2+	II	53	Not yet recruiting	April 2025
NCT04301011	Pembrolizumab + TBio-6517 (oncolytic vaccine)	TNBC	I/IIa	84	Recruiting	December 2022

response biomarkers to anti-PD1 and anti-PD-L1 monoclonal antibodies. Stronger PD-L1 expression has been associated with better ORR, PFS, and OS in mTNBC treated with ICIs either in monotherapy or in combination with chemotherapy. However, a quantitative association between PD-L1 expression and response was not found yet. This may be related to the discrepancy between the methods and antibodies used for PD-L1 evaluation[24,27,30,50]. In fact, some patients whose tumours are PD-L1 negative could also benefit from ICIs[51]. Therefore, PD-L1 expression should be used to define the subgroup of patients more likely to benefit from ICIs rather than to exclude patients from therapy.

Increased levels of TILs have been associated with better responses to ICIs either on monotherapy or combined with chemotherapy[52]. The KEYNOTE-119 trial demonstrated that TILs > 5% were associated with better response and survival after monotherapy with pembrolizumab[53]. Precisely, the presence of stromal TILs was pointed out by several authors as a good prognostic factor in both adjuvant and neoadjuvant chemotherapy and was also associated with improved survival[8,54-57]. Interestingly, the percentage of TILs was found significantly decreased in mTNBC when compared to the corresponding primary tumour. This finding, together with the association between high PD1 expression and better prognosis in early-stage disease supports the usage of ICIs at this disease stage, where tumours are considered more immunogenic[58-60]. A recent study demonstrated that a specific subset of T cells ($CD8^+$, resident memory) was significantly correlated with augmented survival of patients with early-stage TNBC[61].

Mismatch repair deficiency is not common in breast cancer, being more prevalent in early-stage disease. Likewise, high TMB (> 10 mutations/Mb) in breast cancer is very low (3% in primary tumours and 8% in metastatic tumours), suggesting that TMB might not be a good predictive biomarker for these tumours[62-63]. Additional studies are required in TNBC in order to accurately identify solid immunotherapy response biomarkers.

Conclusion and future perspectives

TNBC is a heterogeneous disease that mostly affects young women and is associated with aggressive behaviour. Despite all the efforts, treatment of this disease remains a clinical challenge. Over the last few years, ICIs have emerged as a novel treatment option for this disease. On March 2019, the FDA approved atezolizumab in combination with nab-paclitaxel for PD-$L1^+$ mTNBC, the first licensed immunotherapy for breast cancer. Later, in November 2020, pembrolizumab in combination with chemotherapy was also approved by FDA. Interestingly, early phase studies indicated that treating patients with ICIs at the early disease stage and before exposing them to multiple lines of systemic therapy is associated with improved response. However, monotherapy with anti-PD-1/PD-L1 and anti-CTLA-4 antibodies only offers clinical benefit to a subgroup of patients. Another pitfall is that the results of most of the studies are not directly comparable. Besides enrolling patients with different disease stages and therapeutics there is also a lack of consistency not only in the evaluation method of PD-L1 expression but also in patient' stratification.

New investigations are necessary to understand drug resistance mechanisms to allow further advances in TNBC immunotherapy. The future of TNBC treatment might yield new combination strategies using immune checkpoint inhibitors, chemotherapy, radiotherapy, and targeted therapies. It is also crucial, for further research to identify new predictive biomarkers not only to monitor disease response but also to avoid treating patients that are unlikely to benefit from this type of therapy.

Authors contribution. All authors contributed equally to this manuscript.

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Conflict of interests
No conflicts to declare.

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