Current State of Knowledge on the Immune Checkpoint Inhibitors in Triple-Negative Breast Cancer Treatment: Approaches, Efficacy, and Challenges

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ABSTRACT: Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype with limited treatment options. Recently, there has been a growing interest in immunotherapy with immune checkpoint inhibitors (ICIs) in TNBC, leading to extensive preclinical and clinical research. This review summarizes the current state of knowledge on ICIs efficacy and their predictive markers in TNBC and highlights the areas where the data are still limited. Currently, the only approved ICI-based regimen for TNBC is pembrolizumab with chemotherapy. Its advantage over chemotherapy alone was confirmed for non-metastatic TNBC regardless of programmed death-ligand 1 (PD-L1) expression (KEYNOTE-522) and for metastatic, PD-L1-positive TNBC (KEYNOTE-355). Pembrolizumab's efficacy was also evaluated in monotherapy, or in combination with niraparib and radiation therapy, showing potential efficacy and acceptable safety profile in phase 2 clinical trials. Atezolizumab + nab-paclitaxel increased the overall survival (OS) over placebo + nab-paclitaxel in early TNBC, regardless of PD-L1 status (IMpassion031). In IMpassion130 (untreated, advanced TNBC), the OS improvement was not statistically significant in the intention-to-treat population but clinically meaningful in the PD-L1 positive cohort. The durvalumab–anthracycline combination showed an increased response durability over placebo anthracycline in early TNBC (GeparNuevo). Several phase 1 clinical trials also showed a potential efficacy of atezolizumab and avelumab monotherapy in metastatic TNBC. ICIs appear to be applicable in both neoadjuvant and adjuvant settings, and are both pretreated and previously untreated patients. Further research is necessary to determine the most beneficial drug combinations and optimize patient selection. It is essential to identify the predictive markers for ICIs and factors affecting their expression.

KEYWORDS: Triple-negative breast cancer, immunotherapy, atezolizumab, pembrolizumab, immune checkpoint inhibitors

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Introduction

Breast carcinoma (BC) is the most common malignancy among women worldwide[1] and the leading cause of death among women.[2] BC can be classified into several clinically relevant subtypes based on the expression of estrogen receptor (ER), progesterone receptor (PrR), and overexpression of human epithelial growth factor receptor 2 (HER2).[3] The positivity of the expression of each identifies breast cancer clinical subtype and can predict the effectiveness of targeted therapeutic agents.[4]

A distinct breast cancer clinical subtype—triple-negative breast cancer (TNBC)—characterized by the lack of expression of ER, PrR, and no overexpression of HER2, represents approximately 12% to 20% of all BC diagnoses.[5-7] TNBC tends to occur more commonly in younger patients, with poor cellular differentiation and a higher stage at the diagnosis.[8,9] The rate of local recurrence in TNBC reaches more than 50%,[10] with a high rate of distant metastases.[11] Thus, TNBC is associated with the least favorable prognosis of all BC subtypes. From the biological point of view, TNBC is not a specific cancer type, but a heterogeneous subset of neoplasms brought together due to their immunohistochemical similarities.[10,12] Thus, due to the molecular differences, the search for new treatment modalities is significantly more complex. So far, there are no targeted molecular-based therapies for TNBC, and it is routinely managed with chemotherapy (ChT), including anthracyclines or taxane-based regimens.[13] The need to develop more effective treatment options for TNBC-affected patients results in extensive research in this field, bringing in many new therapeutic approaches evaluated in clinical trials, including immunotherapy with immune checkpoint inhibitors (ICI).

The immune checkpoints, programmed death-receptor 1 (PD-1) and cytotoxic T cell antigen 4 (CTLA-4), act as negative regulators of T cell immune function.[14] PD-1, expressed by T lymphocytes, interacts with programmed death-ligand 1 and 2 (PD-L1, PD-L2) on tumor cells, inhibiting the T cells' proliferation and production of interferon-γ (INF-γ) and tumor necrosis factor-α (TNF-α), and reducing their survival and cytotoxic abilities.[15] CTLA-4 inhibits the interaction between T cells and antigen-presenting cells (APCs), which weakens the immune response against the neoplastic cells.[16,17] It also binds to the T cells with higher affinity than CD28, a protein that provides co-stimulatory signals required for T cell activation and survival, but without providing co-stimulation. Inhibiting the checkpoints' function facilitates the immune

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response against the neoplastic cells, which is the desired outcome in the case of anticancer treatment.

Apart from monotherapy, ICIs have been tested in combinations with other anticancer treatment methods. As ICI therapy aims to facilitate the patient's immune response against the neoplastic cells, there are attempts to simultaneously support the immunogenic mechanisms in different areas. ChT acts as an immunomodulator by inducing cell death of the tumor cells, resulting in their specific antigens being released to the microenvironment, enhancing the immunologic response.[18] Moreover, certain drugs that target the tumors' mechanisms of avoiding the immune response, ie, cyclophosphamide, paclitaxel, cisplatin, and temozolomide can be used at low doses with an immunostimulatory effect.[19-23]

This review aims to summarize the reported data on ICI's efficacy in TNBC, possible drug combinations, results obtained in clinical trials, and emerging predictive markers of such therapy. It is to provide an overview of the current position and probable future research directions for ICI-based TNBC treatment.

Immunotherapy in TNBC—Clinical Trials

ICIs that are currently investigated for their efficacy in TNBC include PD-1 inhibitors (pembrolizumab, nivolumab), PD-L1 inhibitors (atezolizumab, avelumab, durvalumab), and CTLA-4 inhibitors (tremelimumab). Details of the clinical trials referred to below are shown in Table 1 (PD-1 inhibitors) and Table 2 (PD-L1 inhibitors). Due to the aforementioned synergy between ICIs and ChT, most of the described trials investigated the efficacy of different combinations of ICIs and ChT regimens.

PD-1 inhibitors

Pembrolizumab + ChT is currently the only ICI-based treatment combination approved by the Food and Drug Administration (FDA) for locally recurrent unresectable or metastatic, PD-L1-positive TNBC. On July 26, 2021, it was also granted accelerated approval for high-risk, early-stage TNBC as neoadjuvant treatment, continued as a single-agent adjuvant treatment.[24] Different pembrolizumab regimens were initially tested in open-label trials. Phase 2 KEYNOTE-086 evaluated pembrolizumab monotherapy as second- or later-line treatment in metastatic TNBC.[25,26] Pretreated patients reached the objective response rate (ORR) of 5.3% (95% CI: 2.7-9.9), and it was slightly higher in the PD-L1-positive subgroup—5.7% (95% CI: 2.4-12.2). Notably, the ORR was lower than in the case of single-agent ChT; however, it presented high durability and fewer adverse events than ChT.[26,27] The disease control rate (DCR) was 7.6% in general, 9.5% in PD-L1-positive and 4.7% in PD-L1-negative populations.[26] Previously untreated, PD-L1 positive cohort presented ORR of 21.4% and DCR of 23.8%.[25] However, phase 3 KEYNOTE-119[28] comparing pembrolizumab monotherapy to a single-agent ChT for pretreated (second- or third-line treatment) metastatic TNBC showed a median OS of 9.9 months (95% CI: 8.3-11.4) for the pembrolizumab group and 10.8 months (9.1-12.6) for the ChT group (HR 0.97 [95% CI: 0.82-1.15]), showing no advantage of pembrolizumab over ChT.[28]

The placebo-controlled, double-blind phase 3 KEYNOTE-522 trial assessed the efficacy of adding pembrolizumab to neoadjuvant ChT (paclitaxel + carboplatin) followed by adjuvant pembrolizumab vs ChT + placebo followed by adjuvant placebo for non-metastatic TNBC.[29] Results showed a significant increase in both primary endpoints—pathological complete response (pCR) and event-free survival (EFS) rates in the experimental arm. The pCR rate in the pembrolizumab-ChT group reached 64.8% (95% CI: 59.9-69.5) vs 51.2% (95% CI: 44.1-58.3) in placebo-ChT group.[29] The pCR in the PD-L1-positive population for pembrolizumab and placebo groups was 68.9% vs 54.9% respectively, while in the PD-L1-negative population, it was 45.3% vs 30.3%. This showed a benefit of adding ICI to neoadjuvant ChT regardless of PD-L1 expression, consistently with IMpassion031 trial.[30] An updating analysis of the study showed an increase in EFS in the pembrolizumab group that exceeded expectations based on pCR percentage.[31] At 36 months, the EFS was 84.5% (95% CI: 81.7-86.9) in the pembrolizumab–ChT group and 76.8% (95% CI: 72.2-80.7) in the placebo–ChT group.[31] The most common event was distant recurrence (7.7% in the pembrolizumab–ChT group and 13.1% in the placebo–ChT group).[31] A similar strategy—ICI (pembrolizumab) + ChT (nab-paclitaxel, paclitaxel, or gemcitabine + carboplatin) vs placebo + ChT for previously untreated metastatic TNBC was evaluated in KEYNOTE-355.[32] In the intention-to-treat population, the median progression-free survival (PFS) in the pembrolizumab-ChT group was 7.5 vs 5.6 months in the placebo–ChT group (HR 0.82, 95% CI: 0.69-0.97). In PD-L1-negative patients, median PFS was 6.3 months in the pembrolizumab–ChT group and 6.2 months in the placebo–ChT group (HR, 1.08, 95% CI: 0.77-1.53). Patients with PD-L1 positivity were further subdivided into groups with PD-L1 combined positive score (CPS) of ≥ 1 and ≥ 10. For the CPS ≥ 1 cohort, the median PFS in the pembrolizumab vs placebo group was 7.6 vs 5.6 months (HR 0.74, 95% CI: 0.61-0.90) and did not reach statistical significance. The respective pembrolizumab vs placebo PFS rates were 56.4% vs 46.6% at 6 months and 31.7% vs 19.4% at 12 months.[32] In the CPS ≥ 10 group, pembrolizumab significantly improved PFS duration, which reached 9.7 months in the pembrolizumab-ChT group and 5.6 months in the placebo–ChT group (HR for progression or death, 0.65, 95% CI: 0.49-0.86).[32] Thus, the study provided further evidence for increased pembrolizumab efficacy in higher PD-L1 enrichment.

Table 1. Summary of clinical trials assessing PD-1 inhibitors in TNBC management published to date.

AUTHOR, YEAR	TRIAL ID, PHASE	INCLUSION CRITERIA	MEDIAN AGE (YEARS)	STUDY ARMS, PATIENT COUNT (N)	RESULTS		
Winer et al[28]	KEYNOTE-119 Phase 3	mTNBC, 1-2 ChT with anthracycline or taxane, PD after last ChT, ECOG ⩽ 1	50 (43-59)	Pembro 200 mg Q3W (n = 312)	ORR 9.6% (95% CI: 6.6-13.4)		DCR 12.2% (95% CI: 8.8-16.3)
			53 (44-61)	Single-drug ChT (capecitabine, eribulin, gemcitabine, or vinorelbine) (n = 310)	10.6% (95% CI: 7.4-14.6)		18.7% (95% CI: 14.5-23.5)
Cortes et al[32]	KEYNOTE-355 Phase 3	Untreated or locally recurrent mTNBC, ECOG ⩽ 1	53 (44-63)	Pembro 200 mg Q3W + ChT (Nab-PC or PC or gemcitabine + carboplatin) (n = 566)	PFS 7.5 m		
			53 (43-63)	PBO + ChT (n = 281)	5.6 m (HR 0.82, 95% CI: 0.69-0.97)		
Schmid et al[29,31]	KEYNOTE-522 Phase 3	Untreated, locally advanced non-metastatic TNBC, ECOG ⩽ 1	49 (22-80)	Pembro 200 mg Q3W + PC + carboplatin (n = 784)	pCR 64.8% (95% CI: 59.9-69.5)		EFS at 36 m 84.5% (95% CI: 81.7-86.9)
			48 (24-79)	PBO + PC + carboplatin (n = 390)	51.2% (95% CI: 44.1-58.3)		76.8% (95% CI: 72.2-80.7)
Adams et al[25,26]	KEYNOTE-086 Phase 2	⩾ 1 ChT for mTNBC (with anthracycline and a taxane), PD	53.5 (28-85)	Cohort A: Pembro 200 mg Q3W (n = 170)	OS 9.0 m (95% CI: 7.6-11.2)	PFS 2.0 m (95% CI: 1.9-2.0)	ORR 5.3% (95% CI: 2.7-9.9)
		Untreated PD-L1-positive mTNBC	52.5 (26-91)	Cohort B: Pembro 200 mg Q3W (n = 84)	18.0 m (95% CI: 12.9-23.0)	2.1 m (95% CI: 2.0-2.2)	21.4% (95% CI: 13.9-31.4)
Vinayak et al[35]	KEYNOTE-162 Phase 2	Advanced or mTNBC, ⩽ 2 lines of ChT, ECOG ⩽ 1	54 (32-90)	Niraparib 200 mg + Pembro 200 g Q3W (n = 55)	DCR 49% (90% CI: 36-62)	PFS BRCAmut—8.3 m (95% CI: 2.1-NR) BRCAwt—2.1 m (95% CI: 1.4-2.5)	ORR 21% (90% CI: 12-33)
McArthur et al[40]	NCT 02730130 Phase 2	mTNBC, ECOG ⩽ 2, ⩾ 2 sites of metastatic disease with ⩾ 1 site requiring RT	52 (37-73)	Pembro 200 mg + RT 3000 cGy (n = 17)	PR rate wk 13 33%	SD rate wk 13 17%	PD rate wk 13 50%

Abbreviations: AC, doxorubicin + cyclophosphamide; BRCAmut, mutated BRCA; BRCAwt, wild-type BRCA; cGy, centigray; ChT, chemotherapy; CI, confidence interval; DCR, disease control rate; ECOG, Eastern Cooperative Oncology Group; EFS, event-free survival; HR, hazard ratio; m, months; mBC, metastatic breast cancer; (m)TNBC, (metastatic) triple-negative breast cancer; Nab-PC, nab-paclitaxel; NR, not reached; ORR, objective response rate; OS, overall survival; pCR, pathological complete response; PBO, placebo; PC, paclitaxel; PD, progressive disease; PD-1, programmed death receptor 1; PD-L1, programmed death-ligand 1; Pembro, pembrolizumab; PFS, progression-free survival; PR, partial response; Q2/3W, every 2/3 weeks; RT, radiation therapy; SD, stable disease; TNBC, Triple-negative breast cancer; wk, week; y, years.

Table 2. Summary of clinical trials assessing PD-L1 inhibitors in TNBC management published to date.

AUTHOR, YEAR	TRIAL ID, PHASE	INCLUSION CRITERIA	MEDIAN AGE (YEARS)	STUDY ARMS, PATIENT COUNT (N)	RESULTS		
Mittendorf et al[30]	IMpassion031 Phase 3	Untreated, stage II-III TNBC	51 (22-76)	Atezolizumab 840 mg Q2W + Nab-PC (n = 165)	pCR rate 58% (95% CI: 50-65)		
			51 (26-78)	PBO + Nab-PC (n = 168)	41% (95% CI: 34-49)		
Schmid et al[46]	IMpassion130 Phase 3	Untreated, locally advanced or mTNBC, ECOG ⩽ 1	55 (46-64)	Atezolizumab 840 mg Q2W + Nab-PC (n = 451)	OS 21 m (95% CI: 19-22.6)	PFS 7.2 m (95% CI: 5.6-7.4)	
			56 (47-65)	PBO + Nab-PC (n = 451)	18.7 m (95% CI: 16.9-20.3) Stratified HR 0.86 (95% CI: 0.72-1.02)	5.5 m (95% CI: 5.3-5.6) Stratified HR 0.80 (95% CI: 0.69-0.92)	
Miles et al[47]	IMpassion131 Phase 3	Locally advanced or mTNBC, no prior ChT or ⩾ 12 m since ChT	Mean 54.8	Atezolizumab 840 mg Q3W + PC (n = 431)	OS 19.2 m (95% CI: 16.8-22.5)	PFS 5.7 m (95% CI: 5.4-7.2)	ORR 54% (95% CI: 49-58)
			Mean 52.7	PBO + PC (n = 220)	22.8 m (95% CI: 17.1-28.3)	5.6 m (95% CI: 5.4-6.5)	47% (95% CI: 41-54)
Emens et al[49]	NCT01375842 Phase 1	mTNBC, PD since last ChT, ECOG ⩽ 1	53 (29-82)	Atezolizumab Q3W: 15 mg/kg (n = 22), or 20 mg/kg (n = 1), or 1200 mg (n = 93)	OS First line (n = 21): 17.6 m (95% CI: 10.2-NR) ⩾ Second line (n = 94): 7.3 m (95% CI: 6.1-10.8)	PFS RECIST: 1.4 m (95% CI: 1.3-1.6) irRC: 1.9 m (95% CI: 1.4-2.6)	ORR RECIST:10% (95% CI: 4.9-16.5) irRC: 13% (95% CI: 7.5-20.6)
Dirix et al[56]	JAVELIN Phase 1b	Locally advanced or mBC, ⩽ 3 prior lines of ChT, ECOG ⩽ 1	55 (31-81)	Avelumab 10 mg/kg Q2W (n = 58)	DCR 31%	ORR 5.2% (95% CI: 1.1-14.4)	
Loibl et al[51]	GeparNuevo Phase 2	Untreated, non-metastatic, TNBC ⩾ 2 cm	49.5 (25-74)	Durvalumab 1.5 g Q4W + Nab-PC (n = 88) 2-week W (n = 59) NW (n = 29)	pCR Overall: 53.4% (95% CI: 42.5-61.4) W: 61% NW: 37.9%	DFS[52] 3-year iDFS: 84.9% 3-year DDFS 91.4%	3-year OS[52] 95.1%
			49.5 (23-76)	PBO + Nab-PC (n = 86) 2-week W (n = 58) NW (n = 28)	Overall: 44.2% (95% CI: 33.5-55.3) W: 41.4% (OR 2.22, 95% CI: 1.06-4.64) NW: 50% (OR 0.61, 95% CI: 0.21-4.17)	3-year iDFS: 76.9% (HR 0.54, 95% CI: 0.27-1.09) 3-year DDFS: 79.5% (HR 0.37, 95% CI: 0.15-0.87)	83.1% (HR 0.26, 95% CI: 0.09-0.79)

Abbreviations: CI, confidence interval; DCR, disease control rate; DDFS, distant disease-free survival; DFS, disease-free survival; ECOG, Eastern Cooperative Oncology Group; HR, hazard ratio; iDFS, invasive disease-free survival; irRC, immune-related response criteria; m, months; mBC, metastatic breast cancer; mTNBC, metastatic triple-negative breast cancer; Nab-PC, nab-paclitaxel; ND, no data; NR, not reached; NW, non-window; OR, odds ratio; ORR, objective response rate; OS, overall survival; PBO, placebo; PC, paclitaxel; pCR, pathological complete response; PD, progressive disease; PD-L1, programmed death-ligand 1; PFS, progression-free survival; Q2W, every 2 weeks; RECIST, Response Evaluation Criteria In Solid Tumors; TNBC, triple-negative breast cancer; W, window; y, years.

In addition to combining ICIs with ChT, pembrolizumab is also being evaluated on its synergy with other therapeutics. Examples include niraparib—a poly (adenosine diphosphate ribose) polymerase (PARP) inhibitor, ladiratuzumab vedotin—an anti-LIV-1 antibody-drug conjugate with a protease-cleavable linker to monomethyl auristatin E, and sacituzumab govitecan—antibody-drug conjugate composed of an anti-trophoblast cell surface antigen 2 IgG1 kappa antibody and SN-38, the active metabolite of irinotecan, and a topoisomerase I inhibitor.

PARP inhibitors, apart from inhibiting the detection and repair of DNA damage,[33] were found to increase PD-L1 expression on tumor cells providing more targets for PD-L1 inhibitors.[34] The efficacy of the combination of pembrolizumab and niraparib for metastatic or locally advanced TNBC was studied in phase 2 KEYNOTE-162. Enrolled patients had a median history of 1 prior treatment in the metastatic setting. The ORR and DCR were 21% and 49%, respectively.[35] In the efficacy-evaluable population, 11% achieved a complete response (CR), 11% had partial response (PR), 28% experienced stable disease (SD), and 51% had disease progression. OS could not be determined at the time of publishing.[35] A numerically higher response rate was achieved in groups with confirmed tBRCA mutation vs tBRCA wild-type (ORR = 47% vs 11%, DCR = 80% vs 33%, median PFS = 8.3 vs 2.1 months) and PD-L1-positive vs PD-L1-negative disease (ORR = 32% vs 8%). As long as the ORR difference between BRCA types was similar to the one in the case of PARP inhibitors monotherapy, PFS was nearly 3 months longer.[35]

An ongoing phase 1b/2 trial (NCT03310957) studies the combination of ladiratuzumab vedotin with pembrolizumab as a first-line treatment in patients with locally advanced or metastatic TNBC.[36] At the time of writing, after a follow-up of ⩾ 3 months, ORR was 54% (95% CI, 33.4, 73.4), showing an encouraging clinical activity of this regimen and a manageable safety profile.[36]

Sacituzumab govitecan is an FDA-approved drug in pretreated metastatic TNBC. Due to promising results of the trials comparing it to ChT's efficacy (significant increase in PFS and OS in the sacituzumab govitecan cohort[37]), it is now being explored in different combinations. An ongoing phase 2 trial NCT04468061 aims to compare the efficacy of sacituzumab govitecan with pembrolizumab to that of sacituzumab govitecan monotherapy in metastatic, PD-L1-negative TNBC, with PFS being the primary endpoint.[38] The primary completion date is estimated for April 2024.[39]

A single-arm, phase 2 clinical trial no. NCT02730130 aimed to determine the safety and efficacy of pembrolizumab with radiation therapy (RT) for mTNBC treatment.[40] By the 13th week of the study, 29% of the patients had died of disease-related complications. Out of the participants evaluable at week 13, 50% had disease progression, 33% had a PR, and 17% had SD which was durable for 30 weeks.[40] Overall, 33% of patients with durable responses presented them outside of the RT field,[40] indicating certain efficacy of pembrolizumab in this combination. The treatment was presented as well tolerated.[40]

Another PD-1 inhibitor, nivolumab, was found to inhibit the growth of tumors derived from injecting TNBC cell line into mice model which develops a significant population of human B and T lymphocytes.[41] A phase 2 TONIC trial investigated the efficacy of nivolumab in metastatic TNBC administered after different induction protocols, such as hypofractionated irradiation, low-dose cyclophosphamide, cisplatin, or doxorubicin. Overall, the ORR was 20%, with most responses presented in the cisplatin (ORR 23%) and doxorubicin (ORR 35%) cohorts.[42] The study provided a solid rationale for considering induction treatment before introducing ICIs; however, the specific regimens and timelines are to be explored in further trials.

The combination of nivolumab, paclitaxel, and bevacizumab (anti-vascular endothelial growth factor antibody) as a first-line treatment in patients with HER2-negative metastatic breast cancer is a subject of a single-arm, phase 2, NEWBEAT trial.[43] At the time of writing, the published results regarding specifically patients with TNBC are limited to ORR which reached 83.3% in this subgroup.[43] As the trial is still ongoing, more data can be expected in the future.

To date, research on nivolumab's efficacy in TNBC is not as advanced as in the case of pembrolizumab. Nonetheless, many noteworthy combinations including nivolumab are currently being evaluated for TNBC and we are likely to find out more about its most promising regimes in the following years. The ongoing trials on pembrolizumab and nivolumab in different combinations for TNBC are summarized in Table 3.

PD-L1 inhibitors

Atezolizumab blocks the PD-L1 antigen specifically without altering its expression,[44] potentiates T cell-mediated cytotoxicity, and suppresses cell invasion and mobility.[45] Moreover, atezolizumab is known to inhibit signaling pathways, such as NF-kB, PI3 K/Akt/mTOR, MAPK, and CD40, which mediate tumor growth, cell migration, invasion, epithelial-mesenchymal transition, and development of metastases.[44]

Atezolizumab with nab-paclitaxel was the first PD-L1 inhibitor-based regimen in TNBC approved by the European Medicines Agency (EMA)[26] and FDA.[27] The combination was granted an accelerated approval for locally advanced or metastatic, PD-L1-positive TNBC in March 2019, after promising results of IMpassion130[46] described below. The indication was then voluntarily withdrawn by the manufacturer in August 2021, due to unsatisfactory results of IMpassion131.[47] Nonetheless, the data obtained from trials regarding this and similar atezolizumab-based regimens remain valuable for potential future research.

Table 3. Summary of the ongoing clinical trials assessing PD-1 inhibitors in TNBC management.

TRIAL IDENTIFIER AND NAME	STUDY DESIGN	BRIEF SUMMARY	INCLUSION CRITERIA	STUDY ARMS	NO. OF PATIENTS	PRIMARY OUTCOME MEASURE
NCT02755272	Phase 2, Randomized, Open-label	Pembrolizumab with ChT in metastatic TNBC	mTNBC, ⩾2 prior ChT in metastatic setting, ECOG ⩽2	Pembrolizumab 200 mg iv Q3W + carboplatin + gemcitabine Carboplatin + gemcitabine	87	ORR, AE rate
NCT03106415	Phase 1/2, Open-label	Pembrolizumab and binimetinib in unresectable TNBC	Locally advanced unresectable or metastatic TNBC, ⩽3 prior ChT regimens in metastatic setting, ECOG ⩽1	Binimetinib po bid + pembrolizumab iv Q2W	23	ORR, MTD
NCT04468061	Phase 2, Randomized, Open-label	Sacituzumab govitecan ± pembrolizumab in metastatic TNBC	PD-L1-negative mTNBC, no prior ChT, ECOG ⩽1	Sacituzumab govitecan + pembrolizumab Q3W Sacituzumab govitecan Q3W	110	PFS, AE rate
NCT03310957	Phase 1b/2, Open-label	SGN-LIV1A + pembrolizumab for locally advanced or metastatic TNBC	Locally advanced or metastatic TNBC, no prior ChT, ECOG ⩽1	Ladiratuzumab vedotin iv + pembrolizumab iv Q3W	211	ORR, AE rate, Laboratory abnormalities, Dose-limiting toxicity rate
NCT03012230	Phase 1, Open-label	Pembrolizumab and ruxolitinib phosphate for metastatic stage IV TNBC	mTNBC, ⩾1 prior ChT in metastatic setting, ECOG ⩽1	Pembrolizumab iv Q3W + ruxolitinib phosphate po bid	18	MTD, AE rate
NCT04265872	Early phase 1 Open-label	Bortezomib followed by pembrolizumab and cisplatin in mTNBC	mTNBC previously treated with standard anthracycline, cyclophosphamide, and taxane ChT, ⩽3 prior ChT regimens in metastatic setting, ECOG ⩽1	Bortezomib until PD, followed by pembrolizumab and cisplatin	20	ORR
NCT02954874	Phase 3, Randomized, Open-label	Adjuvant pembrolizumab for TNBC after neoadjuvant ChT	Non-metastatic TNBC after neoadjuvant ChT followed by surgery	Observation as per guidelines Pembrolizumab iv Q3W	1155	iDFS
NCT04427293	Phase 1, Open-label	Preoperative lenvatinib + pembrolizumab in early-stage TNBC	TNBC T1b-T2/N0-N1/M0, ECOG ⩽2	Lenvatinib 12 mg + pembrolizumab 200 mg iv Q3W	12	Clinical response
NCT04191135 KEYLYNK-009	Phase 2/3, Randomized, Open-label	Olaparib + pembrolizumab vs ChT + pembrolizumab after induction with first-line ChT + pembrolizumab in TNBC	Locally recurrent inoperable or metastatic TNBC, ECOG ⩽1	Pembrolizumab 200 mg iv Q3W + carboplatin and gemcitabine Pembrolizumab 200 mg iv Q3W + olaparib 300 mg po bid	1225	PFS, OS
NCT04331067	Phase 1b/2, Randomized, Open-label	Cabiralizumab + nivolumab and neoadjuvant chemotherapy in localized TNBC	TNBC T2, any N, M0 or any T N +, no prior therapy for TNBC, ECOG ⩽1	Neoadjuvant PC and carboplatin + nivolumab 240 mg iv Q2W Neoadjuvant PC and carboplatin + nivolumab 240 mg iv Q2W + cabiralizumab 4 mh/kg iv Q2W	50	%TIL change, %TAM change, AE rate

Abbreviations: AE, adverse events; bid, twice a day; ChT, chemotherapy; ECOG, Eastern Cooperative Oncology Group; iDFS, invasive disease-free survival; iv, intravenous; MTD, maximum tolerated dose; mTNBC, metastatic triple-negative breast cancer; Nab-PC, nab-paclitaxel; ORR, objective response rate; OS, overall survival; PC, paclitaxel; pCR, pathological complete response; PD, progressive disease; PD-1, programmed death-receptor 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; po, oral; Q2/3W, every 2/3 weeks; TAM, tumor-associated macrophages; TIL, Tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancer.

IMpassion130, a multicenter, randomized, placebo-controlled, double-blind phase 3 study assessed the efficacy of atezolizumab + nab-paclitaxel vs placebo + nab-paclitaxel in patients with previously untreated, locally advanced or metastatic TNBC.[46] Generally, taking into account PD-L1-positive and PD-L1-negative cases, the study found no advantage of atezolizumab over placebo in combination with nab-paclitaxel in the intention-to-treat population: median OS in the atezolizumab group reached 21 vs 18.7 months in the placebo group (HR 0.86, 95% CI: 0.72-1.02). However, the updating analysis of IMpasion130 study provided evidence for atezolizumab's efficacy in patients with PD-L1 immune cell-positive tumors, as in those patients, atezolizumab group median OS reached 25 vs 18 months in the placebo group (stratified HR 0.71, 0.54-0.94) showing a clinically meaningful, nearly 30% reduction in the risk of death in the atezolizumab group.[46]

Preliminary results of IMpassion131 study of previously untreated metastatic TNBC showed that atezolizumab with conventional paclitaxel had no survival advantage over placebo + paclitaxel treatment.[47] The respective OS durations were 19.2 m (95% CI: 16.8-22.5) for atezolizumab vs 22.8 m (95% CI: 17.1-28.3) for placebo group. Similarly, no benefit of adding atezolizumab was found in terms of PFS—5.7 m (95% CI 5.4-7.2) and 5.6 m (95% CI 5.4-6.5), respectively, for the atezolizumab and placebo groups. Mature survival results are to be expected; however, so far, atezolizumab + paclitaxel appears not to be an effective regimen in TNBC and is not recommended by EMA.[48]

A particularly noteworthy trial was IMpassion031, assessing neoadjuvant atezolizumab and nab-paclitaxel for early TNBC.[30] The study showed a statistically significant difference between atezolizumab vs placebo with respective pCR rates of 58% (95% CI: 50-65) and 41% (95% CI: 34-49). Interestingly, the study found no statistically significant difference in pCR rates between PD-L1-positive and PD-L1-negative populations.[30] The pCR rates for atezolizumab vs placebo were 69% vs 49% for PD-L1-positive and 48% vs 34% for PD-L1-negative patients, suggesting the potential effectiveness of this combination in early TNBC regardless of PD-L1 status.

In an open-label, multicenter phase 1 study no. NCT01375842, atezolizumab monotherapy administered intravenously every 3 weeks for patients with metastatic TNBC was found to be generally well tolerated and of effectiveness similar to ChT.[49] The ORR reached 24% in patients receiving atezolizumab as the first-line treatment and 6% for second- or later-line treatment groups. OS was 17.6 months for first-line patients and 7.3 months for second- or later-line patients. The duration of response ranged between 3 and 38 months with a median of 21 months.[49] As a part of the same study, a 48-year-old woman with a 31-year history of PD-L1-positive TNBC received atezolizumab monotherapy and showed a remarkable CR.[50] Previously, the patient had been treated surgically with adjuvant RT, followed by surgical resection of regional recurrences with adjuvant ChT. She met the PR criteria and immune-related response criteria (irRC) after 4 cycles of atezolizumab,[50] and after re-treatment due to disease progression, she had a PR and a CR 2 months later.[50]

Another emerging combination of ChT and PD-L1 inhibitors in early TNBC is durvalumab with anthracycline in the neoadjuvant approach. It was assessed in a multicenter, prospective, randomized, double-blind, placebo-controlled phase 2 trial GeparNuevo.[51] Out of the patients treated with durvalumab, 53.4% achieved a pCR compared with 44.2% treated with placebo, although the difference did not reach statistical significance. However, the difference in pCR in the window cohort (single-agent durvalumab vs placebo 2 weeks prior to neoadjuvant chemotherapy) and the no-window cohort was statistically significant (window: 61.0% vs 41.4%, OR 2.22; non-window: 37.9% vs 50.0%; OR 0.61), suggesting the window treatment regimen to be more promising.[51] Notably, recently presented follow-up results showed a significant increase in response durability in durvalumab-treated patients.[52] A 3-year invasive disease-free survival (iDFS) in pCR achievers vs non-achievers was 92.0% vs 71.9% showing significantly longer response durability despite a small pCR increase in the durvalumab cohort. A 3-year iDFS was 84.9% with durvalumab vs 76.9% with placebo, 3-year distant DFS was 91.4% vs 79.5%, and 3-year OS was 95.1% vs 83.1%[52] (HR values presented in Table 3.). The results were consistent regardless of window vs non-window approach.[52] This would further confirm an emerging claim that achieving pCR does not necessarily drive long-term survival in ICI-treated TNBC and may not be as meaningful as in the case of ChT-based treatment.[53] It could be justified by the different mechanisms of ChT's and ICIs' action, as the latter does not aim at tumor reduction via cytotoxicity. Thus, patients with residual disease after ICI are still likely to benefit from the therapy in the long run.[53]

Avelumab, apart from acting as a PD-L1 inhibitor, was also found to facilitate the antibody-dependent cellular cytotoxicity of natural killer (NK) cells against tumor cells.[54] In a study on TNBC cancer cell lines in vitro, avelumab's effect on enhancing antibody-dependent cell-mediated cytotoxicity was stronger against tumor cells with higher PD-L1 expression.[54,55] The efficacy of avelumab in monotherapy of locally advanced or metastatic breast cancer was studied in a phase 1 JAVELIN Solid Tumor trial.[56] The ORR was 3% overall, and 5.2% in the TNBC subset all responses being durable. Out of the patients with a CR, PR, or SD, 29.8% had no progression of the disease for $\geq$ 6 months. Tumor shrinkage was noted in 45.7% of TNBC patients, in half of which reaching $\geq$ 30%. The overall DCR was 31% in the TNBC subset.[56] A case report was published describing a 48-year-old woman with locally advanced TNBC involved in an aforementioned study of avelumab monotherapy, who had also received adjuvant RT on tumor bed and regional

lymph nodes.[57] At the moment of writing, 16 months after the initial diagnosis, the patient remained alive and disease-free. Therefore, the combination of ICIs with RT could also present a potential therapeutic regimen worth further research.

All atezolizumab, avelumab, and durvalumab are currently being evaluated in different combinations in phase 1 to 3 trials as shown in Table 4. There are now attempts to combine PD-L1 inhibitors with PARP inhibitors, sacituzumab govitecan, cytotoxic agents, and others, so further advances in PD-L1-inhibitor-based regimens for TNBC are warranted.

CTLA-4 inhibitors

In contrast to a list of trials evaluating the efficacy of PD-1 and PD-L1 inhibitors in TNBC, the data on CTLA-4 inhibitors' efficacy are more limited. The combination of tremelimumab and RT was examined in a phase 1 study, which enrolled 5 patients with metastatic hormone-receptor-positive BC and 1 patient with mTNBC.[58] It was shown that tremelimumab in combination with RT was generally well tolerated, with manageable adverse events. The overall DCR was 33%, however with no objective response, and the mTNBC patient did not achieve SD. Median PFS was 1.5 months and median OS was 50.8 months since the diagnosis and 27 months since initiating tremelimumab + RT treatment.[58] Currently, tremelimumab monotherapy in advanced solid tumors including TNBC is a subject of an ongoing, phase 2 NCT02527434 trial. After disease progression, patients will have the option of being sequenced to durvalumab monotherapy or durvalumab + tremelimumab combination therapy, for up to 12 months or until disease progression.

Combining different ICIs

The existence of synergy between PD-1 or PD-L1 and CTLA-4 inhibitors' efficacy has been well studied in a setting of metastatic melanoma in a number of clinical trials. In patients with unresectable and metastatic melanoma, combined ICIs turned out significantly more effective, however with an increased risk of adverse events.[59-61] In 2 independent trials including patients with advanced melanoma, the HR in respect to median PFS was 0.42[59] and 0.4[60] when comparing the efficacy of nivolumab + ipilimumab vs ipilimumab only. Pre-clinical studies and case reports referred to below showed the potential benefit of this approach in TNBC.

In BRCA1-deficient mice with TNBC, the combination of cisplatin with a simultaneous PD-1 and CTLA-4 blockade inhibited the tumor growth and significantly increased subjects' OS.[62] In the same study, a single checkpoint blockade or double checkpoint blockade without cisplatin gave unsatisfactory results, providing a rationale for the clinical studies of the dual immune blockade in combination with classic ChT agents.

Moreover, a case has been reported of a 50-year-old woman with wild-type BRCA1 (BRCA1wt) and stage IV TNBC with bilateral pulmonary metastases.[63] The patient received treatment of concurrent nivolumab and ipilimumab with regional hyperthermia, followed by 1 low dose of cyclophosphamide and IL-2 with taurolidine. Taurolidine had been suggested to reduce IL-2-caused vascular leak syndrome while maintaining its therapeutic effect in patients with stage IV melanoma.[64] The patient was brought to a durable, complete remission of pulmonary metastases, though the disease progressed in mediastinal and axillary lymph nodes. The patient, initially with a very poor prognosis, remained alive for another 27 months after initiating the treatment.[63] The combination of nivolumab and ipilimumab for TNBC treatment is being evaluated in a few ongoing trials summarized in Table 5.

The combination of a PD-L1 and CTLA-4 inhibitor (durvalumab + tremelimumab) in TNBC was assessed in an open-label, pilot study, which enrolled 18 patients, 7 of whom had TNBC.[65] Among TNBC patients, the ORR reached 43% and median PFS was not reached, whereas none of the hormone receptor-positive BC patients had an objective response, and the median PFS in this group was 2.2 months. The most common adverse events were hepatitis, electrolyte abnormalities, and rash, while there were no grade 4 or 5 adverse events observed. The regimen is now a subject of phase 2 MATILDA trial (Table 5) for solid tumors including TNBC, so more data on this approach can be expected.

ICIs with cancer vaccines

There are several ongoing clinical trials assessing the efficacy and tolerability of ICIs with cancer vaccines in TNBC treatment. The rationale behind combining cancer vaccines with ICIs focuses on enhancing the vaccine-elicited tumor-directed immune response via immune checkpoint blockade. The need for combination therapy derives from overall modest results of cancer vaccine monotherapy even in FDA-approved indications, such as talimogene laherparepvec in advanced unresectable melanoma[66] or sipuleucel-T for metastatic castration-resistant prostate cancer.[67]

Several trials evaluating ICI with cancer vaccines in advanced TNBC focus on pembrolizumab with either investigational multi-peptide vaccine PVX-410 (NCT03362060), specific vaccine targeting p53 (NCT02432963) or Galinpepimut-S—a Wilms Tumor-1-targeting vaccine (NCT03761914). Other combinations include durvalumab with PVX-410 (NCT02826434), durvalumab with neoantigen DNA vaccine (NCT03199040), and personalized synthetic neoantigen vaccine with nab-paclitaxel + durvalumab and tremelimumab or ChT (NCT03606967). The ongoing trials evaluating ICI-vaccine combinations are summarized in Table 6.

ICIs with NK cells

NK cells are a part of an innate non-specific immune system and have their role in malignancy-targeted response. They

Table 4. Summary of the ongoing clinical trials assessing PD-L1 inhibitors in TNBC management.

TRIAL IDENTIFIER AND NAME	STUDY DESIGN	BRIEF SUMMARY	INCLUSION CRITERIA	STUDY ARMS	NO. OF PATIENTS	PRIMARY OUTCOME
NCT02926196 A-Brave	Phase 3, Randomized, Open-label	Avelumab as adjuvant or post-neoadjuvant treatment for high-risk TNBC	Locally advanced, non-metastatic TNBC, adequate tumor excision, ⩾3 courses of anthracycline and taxane, ECOG⩽1	Avelumab 10 mg/kg iv Q2W for 52 weeks Observation as per guidelines	474	DFS
NCT03371017 IMpassion132	Phase 3, Randomized, Double-blind, Placebo-controlled	Atezolizumab with ChT in inoperable recurrent TNBC	Unresectable or metastatic TNBC, PD within 12 months after treatment with curative intent, no prior ChT in the current setting, ECOG⩽1	Atezolizumab 1200 mg iv on first and third day Q3W + carboplatin + gemcitabine + capecitabine Placebo + carboplatin + gemcitabine + capecitabine	572	OS
NCT02620280 NeoTRIPaPDL1	Phase 3, Randomized, Open-label	Neoadjuvant atezolizumab + ChT in early high-risk and locally advanced TNBC	Early high-risk and locally advanced, non-metastatic TNBC, no prior treatment, ECOG⩽1	Atezolizumab 1200 mg iv Q3W + carboplatin + nab-PC followed by surgery and adjuvant ChT (AC, EC or FEC) Carboplatin + nab-PC followed by surgery and adjuvant ChT (AC, EC, or FEC)	278	EFS
NCT03281954	Phase 3, Randomized, Double-blind	Neoadjuvant atezolizumab + ChT followed by adjuvant atezolizumab in TNBC	TNBC, T2/T3 if N0 or T1c/T2/T3 if nodal involvement, no prior treatment, ECOG⩽1	Atezolizumab 1200 mg iv Q3W + PC + carboplatin followed by atezolizumab + AC/EC followed by surgery and adjuvant atezolizumab Q3W for 1 year Placebo + PC + carboplatin followed by placebo + AC/EC followed by surgery and adjuvant placebo Q3W for 1 year	1520	pCR rate, EFS
NCT03498716 IMpassion030	Phase 3, Randomized, Open-label	Adjuvant atezolizumab + ChT followed by atezolizumab maintenance in stage II-III TNBC	Non-metastatic, adequately excised, stage II-III TNBC	Atezolizumab 840 mg iv Q2W + PC + doxorubicin/epirubicin + CP followed by atezolizumab 1200 mg Q3W for 1 year PC + doxorubicin/epirubicin + CP	2300	iDFS
NCT03167619, DORA	Phase 2, Randomized, Open-label	Olaparib + durvalumab in platinum-treated TNBC	Unresectable locally advanced or metastatic TNBC, previous ChT with platinum, ⩽2 prior ChT regimens, ECOG⩽2	Olaparib 300 mg po bid + durvalumab Q4W Olaparib 300 mg po bid	50	PFS

(Continued)

Table 4. (Continued)

TRIAL IDENTIFIER AND NAME	STUDY DESIGN	BRIEF SUMMARY	INCLUSION CRITERIA	STUDY ARMS	NO. OF PATIENTS	PRIMARY OUTCOME
NCT03801369	Phase 2, Open-label	Olaparib + durvalumab in mTNBC	mTNBC, ⩽2 prior ChT in metastatic setting, ECOG ⩽1	Olaparib po + durvalumab Q4W	28	ORR
NCT02849496	Phase 2, Randomized, Open-label	Olaparib + atezolizumab in unresectable TNBC	Locally advanced unresectable or metastatic TNBC, BRCA 1/2 mutation, ECOG ⩽2	Olaparib po + atezolizumab Q3W Olaparib po	81	PFS
NCT03971409 InCITe	Phase 2, Randomized, Open-label	Avelumab + binimetinib, sacituzumab govitecan, or liposomal doxorubicin in unresectable TNBC	Stage IV or unresectable locoregional recurrence of TNBC, ECOG ⩽1	Binimetinib po bid + avelumab iv Q2W Anti-OX40 iv + avelumab iv Q2W Utomilumab iv Q4W + avelumab iv Q2W Binimetinib po bid + avelumab iv Q2W + liposomal doxorubicin Sacituzumab govitecan + avelumab iv Q2W Avelumab iv Q2W + liposomal doxorubicin	150	Best ORR
NCT04360941 PAveMenT: Part B	Phase 1b Open-label	Palbociclib and avelumab in metastatic AR + triple-negative breast cancer	Recurrent inoperable locally advanced or metastatic AR + TNBC, 1-2 prior lines of ChT for advanced disease	Palbociclib + avelumab	27	MTD ORR

Abbreviations: AC, adriamycin + cyclophosphamide; AE, adverse events; AR, androgen receptor; bid, twice a day; ChT, chemotherapy; CP, cyclophosphamide; DFS, disease-free survival; EC, epirubicin + cyclophosphamide; ECOG, Eastern Cooperative Oncology Group; EFS, event-free survival; FEC, fluorouracil + epirubicin, + cyclophosphamide; iDFS, invasive disease-free survival; iv, intravenous; m, months; MTD, maximum tolerated dose; (m)TNBC, (metastatic) triple-negative breast cancer; Nab-PC, nab-paclitaxel; ORR, objective response rate; OS, overall survival; PC, paclitaxel; pCR, pathological complete response; PD, progressive disease; PD-L1, programmed death-ligand 1; PFS, progression-free survival; po, oral; Q2/3/4W, every 2/3/4 weeks; TNBC, triple-negative breast cancer; y, year.

Table 5. Summary of the ongoing clinical trials assessing the combinations of different ICIs in cancer management, including TNBC.

TRIAL IDENTIFIER AND NAME	STUDY DESIGN	BRIEF SUMMARY	INCLUSION CRITERIA	STUDY ARMS	NO. OF PATIENTS	PRIMARY OUTCOME
NCT04185311	Phase 1, Open-label	Ipilimumab, nivolumab, and talimogene laherparepvec before surgery in localized TNBC or ER +, HER2—BC	TNBC or ER +, HER2—BC, ECOG ⩽ 1	Talimogene laherparepvec + nivolumab iv Q2W + ipilimumab Q6W	6	AE rate
NCT03818685 BreastImmune03	Phase 2, Randomized, Open-label	Post-operative treatment associating RT + nivolumab + ipilimumab vs RT + capecitabine for TNBC with RD	Non-metastatic TNBC with RD after surgery, ECOG ⩽ 1	Nivolumab 360 mg iv Q3W + ipilimumab 1 mg/kg iv Q6W Capecitabine 1000 mg/m² bid	114	DFS
NCT03546686	Phase 2, Open-label	Peri-operative ipilimumab + nivolumab and cryoablation in TNBC	Resectable TNBC, ECOG ⩽ 1	Ipilimumab + nivolumab + core biopsy/cryoablation + breast surgery + post-surgery nivolumab	80	EFS
NCT03982173 MATILDA	Phase 2, Open-label	Durvalumab and tremelimumab in metastatic solid tumors including TNBC	Metastatic disease or unresectable locally advanced malignancy, ECOG ⩽ 1	Tremelimumab 75 mg + durvalumab 1500 mg	88	ORR

Abbreviations: AE, adverse events; ChT, chemotherapy; DFS, disease-free survival; ECOG, Eastern Cooperative Oncology Group; EFS, event-free survival; ER, estrogen receptor; HER2, human epithelial growth factor receptor 2; ICI, immune checkpoint inhibitors; iv, intravenous; (m)TNBC, (metastatic) triple-negative breast cancer; Nab-PC, nab-paclitaxel; ORR, objective response rate; PC, paclitaxel; Q2/3/6W, every 2/3/6 weeks; RD, residual disease; RT, radiation therapy; TNBC, triple-negative breast cancer.

interact with major histocompatibility complex (MHC) on altered cells by multiple activating and inhibitory receptors, promoting cytotoxicity through a number of pathways.[68] For instance, MHC-NK cell interaction results in the release of cytotoxic granules and proinflammatory cytokines, such as IFN-γ.[68] IFN-γ acts as an activator of APCs, resulting in the induction of T-helper cell-mediated immune response.[69]

PD-1 and CTLA-4 molecules act as negative regulators of NK cells' function,[70,71] which justifies the evaluation of synergy between NK cells and ICI in cancer treatment. Moreover, ICI-resistant TNBC often presents downregulation of major MHC class I elements.[72] NK cells' interaction with MHC and their ability to target cells with improper MHC function[72] may comprise a potential gateway for achieving response in these patients. Meta-analysis by Nersesian et al[73] showed an association between increased NK cell infiltration and more favorable prognosis in solid tumors including BC (8 studies on BC, n = 1631 patients, including 278 patients with TNBC). Overall, the BC studies showed a decreased risk of death in patients with documented increased NK cell tumor infiltration (HR = 0.27, 95% CI: 0.09-0.68, $P = .027$).[73]

At the moment of writing, ICI-NK cell regimens for TNBC treatment are a subject of 2 trials—an ongoing phase 1 NCT04551885 (FT516 with avelumab for solid tumors including TNBC) and completed, phase 1b QUILT-3.067 (NCT03387085), assessing avelumab with high-affinity NK (haNK) cell therapy, IL-15 cytokine administration, cancer vaccines, and metronomic chemoradiation for metastatic TNBC. Interim results of the latter appear particularly encouraging with the ORR of 67%, DCR of 78%, CR of 22%, and a PFS ranging from 2 to over 12 months (n = 9 patients).[74]

Safety Profile

Immunomodulation unbalances the immune system, therefore favoring the development of immune-related AEs (irAEs)—autoimmune side effects resulting from the treatment.[75-77] Autoimmunity can be triggered by both suppression of immune response's negative regulation and cross-reactivity between the tumor neoantigens and healthy tissue antigens.[78] IrAEs can affect any tissue, but most commonly involve the skin, gut, lungs, and endocrine glands.[77] Although most irAEs respond to steroids, this treatment might compromise the antitumor effect.[77] The data regarding irAEs after ICI derive mainly from clinical trials involving patients with melanoma. In this malignancy, the AEs after the CTLA-4 blockade were found to depend on the cumulative dose.[79,80] No similar association was reported in respect to the PD-1 blockade.[81]

Listed TNBC clinical trials most frequently reported fatigue (7%-44%), nausea (11%-55%), pyrexia (4%-19%), and diarrhea (1.8%-31%) or constipation (pembrolizumab + niraparib—24%-25%) followed by rash, hypothyroidism, hyperthyroidism (less frequently), pneumonitis, hyperglycemia, and lichen planus.[25,26,35,46,49-51,56-58,63,65,82,83] In the KEYNOTE-162 study of pembrolizumab with niraparib, anemia (35%) and

Table 6. Summary of the ongoing clinical trials assessing ICI-vaccine combination in TNBC management.

TRIAL IDENTIFIER AND NAME	STUDY DESIGN	BRIEF SUMMARY	INCLUSION CRITERIA	STUDY ARMS	NO. OF PATIENTS	PRIMARY OUTCOME
NCT03362060	Phase 1b, Open-label	PVX-410 vaccine + pembrolizumab in HLA-A2 + patients with mTNBC	Locally advanced unresectable or metastatic TNBC, ⩾1 prior ChT in current setting, HLA A2 +, ECOG ⩽1	PVX-410 vaccine + pembolizumab Q3W	20	Immune response
NCT02432963	Phase 1, Open-label	Vaccine therapy + pembrolizumab in solid tumors including TNBC with PD after prior therapy	Advanced (unresectable) solid tumors including TNBC, ECOG ⩽2	Pembrolizumab iv + ankara vaccine expressing p53 sc	19 total	Tolerability
NCT03761914 TNBC arm	Phase 1/2, Open-label	Galinpepimut-S + pembrolizumab in patients with selected advanced cancers including TNBC	Advanced or metastatic TNBC, ⩽1 prior lines of therapy for metastatic disease, ECOG ⩽1	Galinpepimut-S monotherapy followed by galinpepimut-S + pembrolizumab	15 in TNBC arm	TRAEs, ORR, CR
NCT04024800 NSABP FB-14	Phase 2, Open-label	AE37 peptide vaccine + pembrolizumab in advanced TNBC	Invasive TNBC with ⩽1 prior line of therapy for metastatic disease ECOG ⩽1	AE37 vaccine starting at 1000 mg Q3W + pembrolizumab 200 mg iv	29	Recommended dose, ORR
NCT02826434	Phase 1, Open-label	Adjuvant PVX-410 vaccine + durvalumab in stage II/III TNBC	Stage II/III TNBC, completed all planned therapy, HLA A2 +, ECOG ⩽1	PVX-410 vaccine Q2W + durvalumab iv on fourth and sixth after vaccine + hiltonol im	22	Dose-limiting toxicity rate
NCT03199040	Phase 1, Randomized, Open-label	Neoantigen DNA vaccine + durvalumab in TNBC following standard-of-care therapy	Stage II-III TNBC, standard-of-care therapy, ECOG ⩽1	Neoantigen DNA vaccine Q3W + durvalumab iv Q4W Neoantigen DNA vaccine Q3W	18	AE rate
NCT03606967	Phase 2, Randomized, Open-label	Individualized vaccine + nab-PC, durvalumab, tremelimumab, and ChT in mTNBC	mTNBC, PD-L1 negative, no prior ChT in metastatic setting	Gemcitabine + carboplatin followed by Nab-PC Neoantigen vaccine + tremelimumab + durvalumab iv + Nab-PC Tremelimumab + durvalumab iv + Nab-PC	70	PFS

Abbreviations: AE, adverse events; ChT, chemotherapy; CR, complete response; DNA, deoxyribonucleic acid; ECOG, Eastern Cooperative Oncology Group; HLA, human leukocyte antigen; ICI, immune checkpoint inhibitors; im, intramuscular; iv, intravenous; (m)TNBC, (metastatic) triple-negative breast cancer; Nab-PC, nab-paclitaxel; ORR, objective response rate; PC, paclitaxel; PD, progressive disease; PD-L1, programmed death-ligand 1; PFS, progression-free survival; Q2/3/4W, every 2/3/4 weeks; sc, subcutaneous; TNBC, triple-negative breast cancer; TRAEs, treatment-related adverse events.

thrombocytopenia (25%) were also reported.[35] Tremelimumab and pembrolizumab + RT-based treatment studies described cases of lymphopenia.[58,82] Overall, the irAEs in TNBC treatment, though frequent, are rather low-grade and controllable. Immunotherapy, as an alternative to ChT, seems to have an acceptable safety profile in TNBC, similar to other cancers.[77,84] It was suggested that high BMI can be a potential risk factor for a worse tolerance of ICI in TNBC,[85] despite its greater efficacy in these patients.[86]

Predictive Markers

Overall, TNBC is associated with poor prognosis and high mortality rate. However, this heterogeneous group of neoplasms includes subtypes that respond relatively well to ChT (a so-called "triple-negative paradox").[87] Research shows that depending on several factors, the immunotherapy's efficacy can also vary in different cases of TNBC. Several predictive markers of the tumor's response to the treatment have been proposed so far. They are highly probable to comprise potential criteria for the choice of treatment methods with the most accurate prediction for a particular patient.

Tumor-infiltrating lymphocytes

The mononuclear immune cells that infiltrate tumor tissue[88,89] (tumor-infiltrating lymphocyte [TILs]) can be identified as either stromal (sTILs) or intratumoral (iTILs).[90] Depending on the study, these can be considered as separate TIL groups or taken together as a whole due to the continuity of the infiltration.[90] TILs level is known to reflect the T_H1 immune response in BC[91] and tends to be higher in more aggressive cancer types.[88] It was confirmed to be both a prognostic and a predictive marker for both ChT and immunotherapy-treated patients with TNBC in a number of studies referred to below.

In the KEYNOTE-086 trial (pembrolizumab monotherapy), patients with TILs levels higher or equal to median vs lower than median had ORR of 6% vs 2% in previously treated patients (cohort A) and 39% vs 9% in previously untreated (cohort B).[92] Responders vs non-responders had the mean TILs level of 10% vs 5% in cohort A and 50% vs 15% in cohort B. The relationship between higher TILs level and higher ORR was statistically significant in combined cohorts.[92] Similarly, in KEYNOTE-173 (pembrolizumab + ChT), patients with pCR had higher median sTILs levels before and during treatment.[83] The sTILs levels before the treatment for $pCR_{ypT0/Tis\ ypN0}$ were 42% (IQR 10-74) among achievers vs 10% (IQR 5-25) in non-achievers and for $pCR_{ypT0\ ypN0}$ 40% (IQR 10-75) for achievers vs 10% (IQR 5-38) for non-achievers. The respective data on median on-treatment sTILs levels were 65% (IQR, 5-89) vs 25% (IQR, 2-48) in case of $pCR_{ypT0/Tis\ ypN0\%}$ and 65% (IQR, 5-86) vs 25% (IQR, 3-60) for $pCR_{ypT0\ ypN0}$.[83] The GeparNuevo study (durvalumab + nab-paclitaxel) showed that sTILs levels at the baselines were a statistically significant predictor of pCR in the durvalumab arm, placebo arm, and complete cohort, thus, were not a specific predictor of response to durvalumab.[51] However, change in iTILs during treatment significantly predicted achieving pCR in the durvalumab arm. Similar conclusions for ER-negative/HER2-negative tumors were drawn from the BIG 02-98 study comparing doxorubicin-based treatment with the addition of docetaxel.[93] An increase in 10% in TILs level was associated with 17% decreased risk of relapse in the case of iTILs and 15% for sTILs. The risk of death was reduced by 27% and 17% for iTILs and sTILs levels, respectively. In GeparSixto, a study investigating the addition of carboplatin to anthracycline with a taxane; patients with increased sTILs levels had pCR of 59.9% vs 33.8% in patients with low sTILs levels. Thus, it was concluded that sTILs level might be a predictive marker for a response to carboplatin in TNBC,[94] which is currently being evaluated on its synergy with pembrolizumab.[83,95] In FinHER, ECOG 2197, and ECOG 1199 trials, TILs were confirmed to be a significant prognostic factor for patients with ChT-treated TNBC. In FinHER, 10% of TILs increase led to a 13% decrease in the risk of distant recurrence.[96] The ECOG-sponsored studies showed a 10% increase in sTILs level to decrease the risk of recurrence or death by 14%, distant recurrence by 18%, and death by 19% in a median follow-up of 10.6 years.[97]

At the moment of publishing, the correlation between TILs level and both response to different treatment methods and prognosis is clearly documented for TNBC. Similar results were obtained in the case of non-luminal HER2-positive tumors, however not for luminal BC.[93,98] The TIL level was reported to increase after ChT,[88,99] which can comprise a promising approach for patients with low TILs and provides further justification for the pursuit of finding optimal combinations of ChT and ICI in TNBC treatment. It was further confirmed by the previously mentioned TONIC trial, aimed to evaluate the effects of induction treatment on the tumor microenvironment, which showed a statistically significant increase in the T cell infiltration after induction with cisplatin and doxorubicin.[42] Even though the KEYNOTE-86 trial showed a less favorable response in the previously treated cohort, it did not consider patients' TIL levels, which may have affected the final conclusions.[26] The expression of 4 particular genes—HLF, CXCL13, SULTE1, and GBP1—was found to be associated with the increase in TILs after anthracycline-containing neoadjuvant ChT in TNBC in the training set, but not confirmed in the validation set.[100] Thus, the mechanisms affecting TILs expression and the response to treatment remain unclear and are to be determined in further studies.

PD-1 and PD-L1 expression

The level of PD-L1 expression is a well-established predictive marker of response to immunotherapy in certain malignancies, such as non-small cell lung carcinoma (NSCLC)[101] or urothelial carcinoma.[102] PD-L1 positivity is found in 20%-31% of

TNBC cases.[103,104] However, the methods of assessing PD-L1 expression, establishing PD-L1 cut-off values, and type of studied cells (tumor cells, TILs, or both) have greatly varied between FDA-approved studies, resulting in heterogeneity in concluded PD-L1 predictiveness.[105]

The expression of PD-1 and PD-L1 on immune and tumor cells as a predictive marker for immunotherapy-treated TNBC was assessed in several aforementioned clinical trials. However, the immunohistochemistry (IHC) assays used to determine PD-L1 status tend to differ between studies. In IMpassion130 (atezolizumab + nab-paclitaxel) PD-L1 positivity (defined as ⩾ 1% PD-L1 expression on immune cells evaluated via SP142 IHC assay) was associated with a mean increase in median OS of 7 months (HR 0.71 [95% CI: 0.54-0.94])[46] Median PFS was 7.5 months (95% CI: 6.7-9.2) in the PD-L1 immune cell-positive population and 5.6 months (95% CI: 5.5-7.3) in the PD-L1 immune cell-negative group.[46] Interestingly, a post hoc analysis of 614 patients (68.1% of the IMpassion130 intention-to-treat population) showed a lack of equivalence in PD-L1 positivity prevalence determined by SP142, SP263, and 22C3 IHC assays.[106] Respective PD-L1 positivity (⩾ 1% expression) rates were 46.4% (95% CI: 42.5%-50.4%), 74.9% (95% CI: 71.5%-78.3%), and 73.1% (95% CI: 69.6%-76.6%).[106] Thus, many cases that were PD-L1-negative based on SP142 were designated as positive with SP263 (29.6%) and 22C3 (29.0%).[106] The difference in PD-L1 proportion yielded by SP142 and 22CC3 was also noted in the case of NSCLC[107] and bladder cancer.[108] In IMpassion130, SP142 seemed to be the most accurate assay in terms of determining a potential OS benefit from the therapy; however, the PFS benefit appeared consistent across different IHC assay-defined groups.[106] SP263 PD-L1 ⩾ 4% subgroup could then comprise a potential additional population that would benefit in terms of PFS. Importantly, SP263 PD-L1 ⩾ 4% population excluded 26.3% of SP142 PD-L1 ⩾ 1% patients,[106] suggesting that the optimal patient selection requires considering different IHC assays rather than one specific method with a fixed cut-off.

In GeparNuevo (durvalumab + nab-paclitaxel), the PD-L1 positivity (defined as ⩾ 1% of PD-L1 expression on both tumor cells and TILs, SP263 assay) was a significant predictor of 54.3% of pCR in the PD-L1-positive and 30% of pCR in the PD-L1-negative group.[51] Similarly, in previously treated patients enrolled in KEYNOTE-086 study, the PD-L1 status (defined as the ratio of PD-L1-positive cells—tumor cells, lymphocytes, and macrophages- ⩾ 1% of the total number of tumor cells, 22C3 assay) was significantly correlated with DCR—it reached 9.5% in the PD-L1-positive population and 4.7% in PD-L1-negative population.[26] In another aforementioned study—NCT01375842 (atezolizumab monotherapy), all responders were PD-L1-positive (at least 1% PD-L1 expression on tumor cells, SP142 assay).[49] The PD-L1-positive group had a greater DCR than the PD-L1-negative group (15% vs 5%) and longer median OS (10.1 months [95% CI: 7.0-13.8] vs 6.0 months [95% CI: 2.6-12.6]).[49] Moreover, the PD-L1 expression increased significantly after the exposure to atezolizumab in patients with mTNBC.[49] In turn, in phase 1b JAVELIN solid tumor trial (avelumab monotherapy), PD-L1 expression on tumor cells did not affect the predicted response.[56] However, with respect to the PD-L1 expression on the tumor-associated immune cells, the ORR was 22.2% for PD-L1-positive patients (10% expression cut-off, Dako PD-L1 IHC 73-10 pharmDx assay) vs 2.6% for PD-L1-negative patients. In KEYNOTE-119 (pembrolizumab vs ChT), the ORR in the pembrolizumab group was positively correlated with PD-L1 CPS, defined as the percentage of PD-L1 positively staining cells of the total number of viable tumor cells (22C3 assay).[28] For patients with CPS ⩾ 1, ORR was 12% for pembrolizumab and 9% for ChT; for CPS ⩾ 10 group, the ORR was 18% for pembrolizumab and 9% for ChT; and for CPS ⩾ 20, it was 26% and 12%, respectively.[28] Therefore, despite the disappointing overall results of the study, it still showed high response rates to pembrolizumab in patients with greater PD-L1 expression, even in the case of pretreated, metastatic TNBC.[28] In KEYNOTE-162 (pembrolizumab + niraparib), the ORR was 32% in PD-L1-positive patients (CPS ⩾ 1, 22C3 assay) and 8% in PD-L1-negative.[35] Also, KEYNOTE-173 study (pembrolizumab + ChT) showed a positive association between higher PD-L1 expression (via 22C3 assay) and pCR rates.[83] The median pre-treatment PD-L1 expressions for $pCR_{ypT0/Tis\ ypN0}$ achievers vs non-achievers were 30% (IQR 5-69) vs 5% (IQR 2-38). In respect to $pCR_{ypT0\ ypN0}$, the values were 30% (IQR 5-66) for achievers vs 10% (IQR 4-42) for non-achievers. As no control arm was included in the trial, PD-L1's predictive and prognostic value could not be evaluated.[83] Importantly, in certain trials, ICI + ChT combination was significantly advantageous in early-stage TNBC regardless of PD-L1 status. These include Impassion031[30] and KEYNOTE-522.[29]

Tumor mutational burden

The accumulation of somatic mutations within a tumor cell can lead to the creation of neoantigens that are associated with either malignant transformations (driver mutation) or raised genetic instability (passenger mutation).[109] The neoantigens can be recognized by the immune system provoking an immune response.[110,111] Its predictive value for immunotherapy was reported in the case of melanoma[78,112] and NSCLC,[113] but is of no significance for Hodgkin's lymphoma, which responds to ICI despite not having a high tumor mutational burden (TMB).[114] As for metastatic BC, the responders to durvalumab and tremelimumab were found to have a greater number of non-synonymous somatic mutations and higher numbers of predicted neoantigens compared with non-responders.[65] BCs, in general, are associated with relatively low TMB; however, this potential marker is more abundant in

the case of TNBC,[65,109] indicating its greater immunogenicity. Within the TNBC group, relatively high TMB was found in the luminal androgen receptor subtype and low in the case of mesenchymal stem-like subtype.[115] As mentioned, TNBC is a highly heterogeneous set of tumors. The differences in TMB in this group indicate that further evaluation of specific TNBC subtypes could lead to a more precise tumor profiling and better-tailored treatment selection.

Moreover, there seems to be an association between TMB and the level of TILs. In one of the studies on TNBC, for patients with high TMB, the 5-year OS was 100% in highly infiltrated, 76% for moderately infiltrated, and 60% for immune-cold tumors.[116] In the case of TMB-low cancers, the difference between tumors of different levels of infiltration was absent with a 5-year OS of 81%-86%.[116] The difference in OS was statistically significant in the case of highly and moderately infiltrated tumors, but not in low-infiltrated. In immune-cold patients, the OS was reversely correlated with TMB levels suggesting a less favorable prognosis for TMB-Hi cases with low immune infiltration.[116] The actual impact of TMB on immune activities[117] and the correlation between TMB and TIL/PD-L1 levels[118-120] and its predictive value in ICI-treated TNBC require further evaluation.

Mismatch-repair deficiency and microsatellite instability

Deficiencies in DNA mismatch-repair (MMR) leading to microsatellite instability (MSI) are known to cause the development of certain cancers, such as colorectal cancer (CRC) and endometrial cancer.[121] In a study regarding the impact of MSI on OS, the combined HR estimate was 0.65, which indicated a better prognosis for patients with ChT-treated MSI. However, it did not provide satisfying evidence for the predictive value of MSI in respect to ChT for CRC.[121] A study of MSI as a predictive marker of pembrolizumab-treated CRC and non-CRC showed a greater clinical benefit in the MMR-deficient cohort.[122] An analysis of MMR deficiency among BCs suggested a low frequency of this phenomenon in BCs in general and particularly low in non-TNBC.[123] It also showed that not all MMR deficiencies may lead to MSI. However, the small sample did not give satisfactory evidence for MSI being either a prognostic or predictive marker in TNBC.

Gene signatures

Research regarding predictive markers for immune manipulations used in cancer treatment resulted in identifying several pathways more frequently occurring in patients presenting a better response. These pathways include Th-1 signaling and CXCR3/CCR5 ligands and effector immune functions and are referred to as Immunologic Constant of Rejection (ICR).[124] Other immune-regulatory genes include, eg, CD274/PD-L1, PDCD1/PD1, CTLA4, FOXP3, and IDO1. Their expression was found to be strongly correlated with ICR.[124] When divided into 4 clusters based on the immune gene expression level (ICR1 for tumors with the lowest expression—ICR4 for the highest), the prognosis of the tumors representing different groups differed to a certain extent. For instance, basal-like tumors classified as ICR4 had a significantly higher OS than subgroups ICR1 to 3. Overall, the ICR4 tumors had a greater frequency of amplifications and deletions, with a potential immunomodulatory impact. The analysis of TMB also showed a significantly greater number of non-silent mutations with increasing immune-related genes' level.[124]

Another 3-gene signature, consisting of the B cell/plasma cell (B/P), T cell/natural killer cell (T/NK), and monocyte/dendritic cell (M/D) immune metagenes, was reported to be associated with a more favorable response to ChT in BCs in general.[125] Its prognostic value was particularly significant in the case of highly proliferating tumors, with more favorable distant metastasis-free survival in most basal-like tumors.[125]

A 1-unit increase in the expression of HLF, CXCL13, SULT1E1, and GBP1 was reported to be significantly associated with better distant relapse-free survival in patients with residual disease after ChT (HR: 0.17, 95% CI: 0.06-0.43) and regardless of the response to ChT (HR: 0.29, 95% CI: 0.13-0.67).[100] No association was found between the expression of the 4-gene signature and the probability to achieve pCR.[100]

In the TONIC trial (induction treatment + nivolumab) of metastatic TNBC, the inflammation-related gene signatures were significantly higher in responders than in non-responders.[42] They were found to be upregulated after induction treatment with cisplatin and doxorubicin, which was even more pronounced after nivolumab treatment.[42] No similar trend was observed after irradiation-based induction,[42] suggesting ChT as a preferred induction method of inflammatory-gene signature upregulation.

BRCA1/2 mutation

The proportion of driver mutations and several variants of frequent alleles were reported to be higher in the case of BRCAwt rather than hereditary BRCA mutation (BRCAmut).[126] However, the sole number of mutations is higher in hereditary tumors.[126] Therefore, hereditary BRCA mutation may result in a lesser number of driver mutations being sufficient for the development of cancer. In KEYNOTE-162 study (pembrolizumab + niraparib), the BRCA status was analyzed giving a numerically higher ORR in tBRCAmut group—47% (90% CI: 24-70) vs tBRCAwt group—11% (90% CI: 3-26). The DCR was 80% (90% CI: 56-94) and 33% (90% CI: 19-51) for respective populations.[35] It was also suggested that the presence of BRCA1/2 mutation is associated with a greater expression of PD-1 and PD-L1, thus leading to a better potential response to ICI. In KEYNOTE-162, the PD-L1 positivity was higher in tBRCAmut patients (80%) compared with tBRCAwt patients (56%).[35] However, at this point, the research on the

association between BRCA1 and BRCA2 type and PD-1/PD-L1 expression has given conflicting results, suggesting either a correlation[127] or lack of relationship[128] between these variables in TNBC.

Body mass index

Interestingly, despite the increased frequency of adverse effects among obese patients, the tumor response to ICIs in TNBC was found to be higher in this group.[86] Higher BMI was also reported to be a positive predictive factor in patients with NSCLC treated with ICI as a second- or later-line of treatment.[129] It may comprise a potential predictive factor for ICI-treated TNBC, though at the moment of writing the data on its significance is limited.

Conclusions

Completed and ongoing clinical trials show that ICIs in TNBC treatment are of promising efficacy and acceptable safety profile. While certain ICIs are already a subject of randomized trials both in monotherapy and in various combinations, some regimens remain described only in case reports or preclinical studies, so future advances in ICI-based therapies in TNBC are warranted. ICIs appear to be applicable in both neoadjuvant and adjuvant approaches, and in both pretreated and previously untreated patients, which raises hope for developing well-tailored, targeted treatment for TNBC in the future. Currently, more attention seems to be drawn toward combination therapy, especially the synergistic effect of ICIs and ChT. Pembrolizumab + ChT is currently the only FDA-approved ICI-based treatment regimen for TNBC. However, given the impressive long-term response to durvalumab + nab-paclitaxel vs nab-paclitaxel only,[52] this combination is likely to follow. Overall, nab-paclitaxel appears to be the most promising co-agent for ICIs, along with carboplatin, known for its efficacy in TNBC and recently reported effectiveness in combination with pembrolizumab.

Further research is particularly necessary for determining the most beneficial drug combinations and optimizing patient selection. An issue of essence is identifying the predictive markers for ICIs and factors affecting their expression. Currently, progress appears to be limited by the inconsistency of reported data and incoherencies between the criteria established in different studies. In the case of determining PD-L1 positivity, recent FDA approval for pembrolizumab-based treatment of CPS $\geqslant$ 10 TNBC is likely to draw the researchers toward the CPS-based approach. The post hoc analysis of IMpassion130 also indicates the importance of the IHC assay choice and its impact on determining PD-L1 positivity. Optimal criteria establishing TIL status are still to be determined.

Thorough evaluation of different TNBC subtypes regarding their molecular and histological profile could also lead to a better understanding of this heterogeneous group and possibly contribute to more accurate treatment tailoring. The attempts to use monoclonal antibodies in TNBC treatment are not limited to ICIs, so establishing the predictive markers for newly emerging therapies together with better profiling of tumors within the TNBC group may greatly facilitate research advances in this field.

Author Contributions

KU and AS-S wrote the manuscript in consultation with AMB-K and AD.

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REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69:7-34.
2. Callahan R, Hurvitz S. Human epidermal growth factor receptor-2-positive breast cancer: current management of early, advanced, and recurrent disease. *Curr Opin Obstet Gynecol*. 2011;23:37-43.
3. Carey LA, Perou CM, Livasy CA, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. *JAMA*. 2006;295:2492-2502.
4. Bonotto M, Gerratana L, Poletto E, et al. Measures of outcome in metastatic breast cancer: insights from a real-world scenario. *Oncologist*. 2014;19:608-615.
5. Chen W, Zheng R, Baade PD, et al. Cancer statistics in China, 2015. *CA Cancer J Clin*. 2016;66:115-132.
6. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin*. 2015;65:87-108.
7. Anders CK, Carey LA. Biology, metastatic patterns, and treatment of patients with triple-negative breast cancer. *Clin Breast Cancer*. 2009;9:S73-S81.
8. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. *Cancer*. 2007;109:1721-1728.
9. Dent R, Trudeau M, Pritchard KI, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res*. 2007;13:4429-4434.
10. Januškevičienė I, Petrikaitė V. Heterogeneity of breast cancer: the importance of interaction between different tumor cell populations. *Life Sci*. 2019;239:117009.
11. Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci USA*. 2003;100:8418-8423.
12. Wahba HA, El-Hadaad HA. Current approaches in treatment of triple-negative breast cancer. *Cancer Biol Med*. 2015;12:106-116.
13. Cardoso F, Kyriakides S, Ohno S, et al. Early breast cancer: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2019;30:1194-1220.
14. Buchbinder EI, Desai A. CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition. *Am J Clin Oncol*. 2016;39:98-106.
15. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008;26:677-704.
16. Fallarino F, Fields PE, Gajewski TF. B7-1 engagement of cytotoxic T lymphocyte antigen 4 inhibits T cell activation in the absence of CD28. *J Exp Med*. 1998;188:205-210.
17. Masteller EL, Chuang E, Mullen AC, Reiner SL, Thompson CB. Structural analysis of CTLA-4 function in vivo. *J Immunol*. 2000;164:5319-5327.
18. Emens LA, Middleton G. The interplay of immunotherapy and chemotherapy: harnessing potential synergies. *Cancer Immunol Res*. 2015;3:436-443.
19. Emens LA, Machiels JP, Reilly RT, Jaffee EM. Chemotherapy: friend or foe to cancer vaccines? *Curr Opin Mol Ther*. 2001;3:77-84.
20. Ghiringhelli F, Menard C, Puig PE, et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. *Cancer Immunol Immunother*. 2007;56:641-648.
21. Nizar S, Copier J, Meyer B, et al. T-regulatory cell modulation: the future of cancer immunotherapy? *Br J Cancer*. 2009;100:1697-1703.
22. Pfannenstiel LW, Lam SS, Emens LA, Jaffee EM, Armstrong TD. Paclitaxel enhances early dendritic cell maturation and function through TLR4 signaling in mice. *Cell Immunol*. 2010;263:79-87.
23. Chen G, Emens LA. Chemoimmunotherapy: reengineering tumor immunity. *Cancer Immunol Immunother*. 2013;62:203-216.

24. U.S. Food and Drug Administration. FDA approves pembrolizumab for high-risk early-stage triple-negative breast cancer, https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-pembrolizumab-high-risk-early-stage-triple-negative-breast-cancer?utm_medium=email&utm_source=govdelivery. Published 2021. Accessed November 12, 2021.
25. Adams S, Loi S, Toppmeyer D, et al. Pembrolizumab monotherapy for previously untreated, PD-L1-positive, metastatic triple-negative breast cancer: cohort B of the phase II KEYNOTE-086 study. *Ann Oncol*. 2019;30:405-411.
26. Adams S, Schmid P, Rugo HS, et al. Pembrolizumab monotherapy for previously treated metastatic triple-negative breast cancer: cohort A of the phase II KEYNOTE-086 study. *Ann Oncol*. 2019;30:397-404.
27. Senkus E, Cardoso F, Pagani O. Time for more optimism in metastatic breast cancer? *Cancer Treat Rev*. 2014;40:220-228.
28. Winer EP, Lipatov O, Im SA, et al. Pembrolizumab versus investigator-choice chemotherapy for metastatic triple-negative breast cancer (KEYNOTE-119): a randomised, open-label, phase 3 trial. *Lancet Oncol*. 2021;22:499-511.
29. Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for early triple-negative breast cancer. *N Engl J Med*. 2020;382:810-821.
30. Mittendorf EA, Zhang H, Barrios CH, et al. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. *Lancet*. 2020;396:1090-1100.
31. Schmid P, Cortes J, Dent R, et al. Event-free survival with pembrolizumab in early triple-negative breast cancer. *N Engl J Med*. 2022;386:556-567.
32. Cortes J, Cescon DW, Rugo HS, et al. Pembrolizumab plus chemotherapy versus placebo plus chemotherapy for previously untreated locally recurrent inoperable or metastatic triple-negative breast cancer (KEYNOTE-355): a randomised, placebo-controlled, double-blind, phase 3 clinical trial. *Lancet*. 2020;396:1817-1828.
33. Papadimitriou M, Mountzios G, Papadimitriou CA. The role of PARP inhibition in triple-negative breast cancer: unraveling the wide spectrum of synthetic lethality. *Cancer Treat Rev*. 2018;67:34-44.
34. Sato H, Niimi A, Yasuhara T, et al. DNA double-strand break repair pathway regulates PD-L1 expression in cancer cells. *Nat Commun*. 2017;8:1751.
35. Vinayak S, Tolaney SM, Schwartzberg L, et al. Open-label clinical trial of niraparib combined with pembrolizumab for treatment of advanced or metastatic triple-negative breast cancer. *JAMA Oncol*. 2019;5:1132-1140.
36. Han H, Diab S, Alemany C, et al. Abstract PD1-06: open label phase 1b/2 study of ladiratuzumab vedotin in combination with pembrolizumab for first-line treatment of patients with unresectable locally-advanced or metastatic triple-negative breast cancer. *Cancer Res*. 2020;80:PD1-06.
37. Bardia A, Hurvitz SA, Tolaney SM, et al. Sacituzumab govitecan in metastatic triple-negative breast cancer. *N Engl J Med*. 2021;384:1529-1541.
38. Garrido-Castro AC, Keenan TE, Li T, et al. Saci-IO TNBC: randomized phase II trial of sacituzumab govitecan (SG) +/- pembrolizumab in PD-L1–metastatic triple-negative breast cancer (mTNBC). *J Clin Oncol*. 2021;39:TPS1106.
39. Sacituzumab Govitecan +/- Pembrolizumab in Metastatic TNBC, https://clinicaltrials.gov/ct2/show/study/NCT04468061. Published 2020. Updated January 11, 2022. Accessed March 11, 2022.
40. McArthur H, Barker C, Gucalp A, et al. A single-arm, phase II study assessing the efficacy of pembrolizumab (pembro) plus radiotherapy (RT) in metastatic triple negative breast cancer (mTNBC). *J Clin Oncol*. 2018;36:14.
41. Capasso A, Lang J, Pitts TM, et al. Characterization of immune responses to anti-PD-1 mono and combination immunotherapy in hematopoietic humanized mice implanted with tumor xenografts. *J Immunother Cancer*. 2019;7:37.
42. Voorwerk L, Slagter M, Horlings HM, et al. Immune induction strategies in metastatic triple-negative breast cancer to enhance the sensitivity to PD-1 blockade: the TONIC trial. *Nat Med*. 2019;25:920-928.
43. Ozaki Y, Mukohara T, Tsurutani J, et al. Abstract PD1-03: a multicenter phase II study evaluating the efficacy of nivolumab plus paclitaxel plus bevacizumab triple-combination therapy as a first-line treatment in patients with HER2-negative metastatic breast cancer: WJOG9917B NEWBEAT trial. *Cancer Res*. 2020;80:PD1-03.
44. Saleh R, Taha RZ, Sasidharan Nair V, Alajez NM, Elkord E. PD-L1 blockade by atezolizumab downregulates signaling pathways associated with tumor growth, metastasis, and hypoxia in human triple negative breast cancer. *Cancers (Basel)*. 2019;11:1050.
45. Mohan N, Hosain S, Zhao J, et al. Atezolizumab potentiates Tcell-mediated cytotoxicity and coordinates with FAK to suppress cell invasion and motility in PD-L1(+) triple negative breast cancer cells. *Oncoimmunology*. 2019;8:e1624128.
46. Schmid P, Rugo HS, Adams S, et al. Atezolizumab plus nab-paclitaxel as first-line treatment for unresectable, locally advanced or metastatic triple-negative breast cancer (IMpassion130): updated efficacy results from a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol*. 2020;21:44-59.
47. Miles DW, Gligorov J, Andre F, et al. LBA15 Primary results from IMpassion131, a double-blind placebo-controlled randomised phase III trial of first-line paclitaxel (PAC) ± atezolizumab (atezo) for unresectable locally advanced/metastatic triple-negative breast cancer (mTNBC). *Ann Oncol*. 2020;31:S1147-S1148.
48. European Medicines Agency. EMA reminds physicians to use Tecentriq with nab-paclitaxel for treating breast cancer, https://www.ema.europa.eu/en/news/ema-reminds-physicians-use-tecentriq-nab-paclitaxel-treating-breast-cancer. Published 2020. Updated May 21, 2021. Accessed November 28, 2021.
49. Emens LA, Cruz C, Eder JP, et al. Long-term clinical outcomes and biomarker analyses of atezolizumab therapy for patients with metastatic triple-negative breast cancer: a phase 1 study. *JAMA Oncol*. 2019;5:74-82.
50. Molinero L, Li Y, Chang CW, et al. Tumor immune microenvironment and genomic evolution in a patient with metastatic triple negative breast cancer and a complete response to atezolizumab. *J Immunother Cancer*. 2019;7:274.
51. Loibl S, Untch M, Burchardi N, et al. A randomised phase II study investigating durvalumab in addition to an anthracycline taxane—based neoadjuvant therapy in early triple negative breast cancer—clinical results and biomarker analysis of GeparNuevo study. *Ann Oncol*. 2019;30:1279-1288.
52. Loibl S, Schneeweiss A, Huober JB, et al. Durvalumab improves long-term outcome in TNBC: results from the phase II randomized GeparNUEVO study investigating neodjuvant durvalumab in addition to an anthracycline/taxane based neoadjuvant chemotherapy in early triple-negative breast cancer (TNBC). *J Clin Oncol*. 2021;39:506-506.
53. Brown LC, Loi S. Immune checkpoint inhibition in the treatment of early stage triple negative breast cancer: 2021 update. *Breast*. 2022;62:S29-S33.
54. Boyerinas B, Jochems C, Fantini M, et al. Antibody-dependent cellular cytotoxicity activity of a novel anti-PD-L1 antibody avelumab (MSB0010718C) on human tumor cells. *Cancer Immunol Res*. 2015;3:1148-1157.
55. Julia EP, Amante A, Pampena MB, Mordoh J, Levy EM. Avelumab, an IgG1 anti-PD-L1 immune checkpoint inhibitor, triggers NK Cell-mediated cytotoxicity and cytokine production against triple negative breast cancer cells. *Front Immunol*. 2018;9:2140.
56. Dirix LY, Takacs I, Jerusalem G, et al. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1b JAVELIN Solid Tumor study. *Breast Cancer Res Treat*. 2018;167:671-686.
57. La Rocca E, Dispinzieri M, Lozza L, et al. Radiotherapy with the anti-programmed cell death ligand-1 immune checkpoint blocker avelumab: acute toxicities in triple-negative breast cancer. *Med Oncol*. 2018;36:4.
58. Jiang DM, Fyles A, Nguyen LT, et al. Phase I study of local radiation and tremelimumab in patients with inoperable locally recurrent or metastatic breast cancer. *Oncotarget*. 2019;10:2947-2958.
59. Hodi FS, Chiarion-Sileni V, Gonzalez R, et al. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. *Lancet Oncol*. 2018;19:1480-1492.
60. Postow MA, Chesney J, Pavlick AC, et al. Nivolumab and ipilimumab versus ipilimumab in untreated melanoma. *N Engl J Med*. 2015;372:2006-2017.
61. Wolchok JD, Kluger H, Callahan MK, et al. Nivolumab plus ipilimumab in advanced melanoma. *N Engl J Med*. 2013;369:122-133.
62. Nolan E, Savas P, Policheni AN, et al. Combined immune checkpoint blockade as a therapeutic strategy for BRCA1-mutated breast cancer. *Sci Transl Med*. 2017;9:eaal4922.
63. Kleef R, Moss R, Szasz AM, Bohdjalian A, Bojar H, Bakacs T. Complete clinical remission of stage IV triple-negative breast cancer lung metastasis administering low-dose immune checkpoint blockade in combination with hyperthermia and interleukin-2. *Integr Cancer Ther*. 2018;17:1297-1303.
64. O'Brien GC, Cahill RA, Bouchier-Hayes DJ, Redmond HP. Co-immunotherapy with interleukin-2 and taurolidine for progressive metastatic melanoma. *Ir J Med Sci*. 2006;175:10-14.
65. Santa-Maria CA, Kato T, Park JH, et al. A pilot study of durvalumab and tremelimumab and immunogenomic dynamics in metastatic breast cancer. *Oncotarget*. 2018;9:18985-18996.
66. Andtbacka RH, Ross M, Puzanov I, et al. Patterns of clinical response with talimogene laherparepvec (T-VEC) in patients with melanoma treated in the OPTiM phase III clinical trial. *Ann Surg Oncol*. 2016;23:4169-4177.
67. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010;363:411-422.
68. Zingoni A, Fionda C, Borrelli C, Cippitelli M, Santoni A, Soriani A. Natural killer cell response to chemotherapy-stressed cancer cells: role in tumor immunosurveillance. *Front Immunol*. 2017;8:1194.
69. Guermonprez P, Valladeau J, Zitvogel L, Thery C, Amigorena S. Antigen presentation and T cell stimulation by dendritic cells. *Annu Rev Immunol*. 2002;20:621-667.
70. Stojanovic A, Fiegler N, Brunner-Weinzierl M, Cerwenka A. CTLA-4 is expressed by activated mouse NK cells and inhibits NK Cell IFN-gamma production in response to mature dendritic cells. *J Immunol*. 2014;192:4184-4191.
71. Alvarez IB, Pasquinelli V, Jurado JO, et al. Role played by the programmed death-1-programmed death ligand pathway during innate immunity against Mycobacterium tuberculosis. *J Infect Dis*. 2010;202:524-532.

72. Abdel-Latif M, Youness RA. Why natural killer cells in triple negative breast cancer? *World J Clin Oncol*. 2020;11:464-476.
73. Nersesian S, Schwartz SL, Grantham SR, et al. NK cell infiltration is associated with improved overall survival in solid cancers: a systematic review and meta-analysis. *Transl Oncol*. 2021;14:100930.
74. Nangia C, Soon-Shiong P, Rabizadeh S, et al. 358P—complete responses in patients with second-line or greater metastatic triple negative breast cancer (TNBC) following first-in-human immunotherapy combining NK and T cell activation with off-the-shelf high-affinity CD16 NK cell line (haNK). *Ann Oncol*. 2019;30:v130.
75. Devaud C, John LB, Westwood JA, Darcy PK, Kershaw MH. Immune modulation of the tumor microenvironment for enhancing cancer immunotherapy. *Oncoimmunology*. 2013;2:e25961.
76. Kuehn HS, Ouyang W, Lo B, et al. Immune dysregulation in human subjects with heterozygous germline mutations in CTLA4. *Science*. 2014;345:1623-1627.
77. Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer*. 2016;54:139-148.
78. Snyder A, Makarov V, Merghoub T, et al. Genetic basis for clinical response to CTLA-4 blockade in melanoma. *N Engl J Med*. 2014;371:2189-2199.
79. Wolchok JD, Neyns B, Linette G, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. *Lancet Oncol*. 2010;11:155-164.
80. Weber JS, Kahler KC, Hauschild A. Management of immune-related adverse events and kinetics of response with ipilimumab. *J Clin Oncol*. 2012;30:2691-2697.
81. Topalian SL, Sznol M, McDermott DF, et al. Survival, durable tumor remission, and long-term safety in patients with advanced melanoma receiving nivolumab. *J Clin Oncol*. 2014;32:1020-1030.
82. Bonvalot S, Gronchi A, Le Pechoux C, et al. STRASS (EORTC 62092): a phase III randomized study of preoperative radiotherapy plus surgery versus surgery alone for patients with retroperitoneal sarcoma. *J Clin Oncol*. 2019;37:11001.
83. Schmid P, Salgado R, Park YH, et al. Pembrolizumab plus chemotherapy as neoadjuvant treatment of high-risk, early-stage triple-negative breast cancer: results from the phase 1b open-label, multicohort KEYNOTE-173 study. *Ann Oncol*. 2020;31:569-581.
84. Abou Alaiwi S, Xie W, Nassar AH, et al. Safety and efficacy of restarting immune checkpoint inhibitors after clinically significant immune-related adverse events in metastatic renal cell carcinoma. *J Immunother Cancer*. 2020;8:e000144.
85. Hu JB, Ravichandran S, Rushing C, et al. Higher BMI, but not sarcopenia, is associated with pembrolizumab-related toxicity in patients with advanced melanoma. *Anticancer Res*. 2020;40:5245-5254.
86. Naik A, Monjazeb AM, Decock J. The obesity paradox in cancer, tumor immunology, and immunotherapy: potential therapeutic implications in triple negative breast cancer. *Front Immunol*. 2019;10:1940.
87. Carey LA, Dees EC, Sawyer L, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res*. 2007;13:2329-2334.
88. Criscitiello C, Esposito A, Trapani D, Curigliano G. Prognostic and predictive value of tumor infiltrating lymphocytes in early breast cancer. *Cancer Treat Rev*. 2016;50:205-207.
89. Underwood JC. Lymphoreticular infiltration in human tumours: prognostic and biological implications: a review. *Br J Cancer*. 1974;30:538-548.
90. Salgado R, Denkert C, Demaria S, et al. The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014. *Ann Oncol*. 2015;26:259-271.
91. Loi S. Tumor-infiltrating lymphocytes, breast cancer subtypes and therapeutic efficacy. *Oncoimmunology*. 2013;2:e24720.
92. Loi SA, dams S, Schmid P, et al. Relationship between tumor infiltrating lymphocyte (TIL) levels and response to pembrolizumab (pembro) in metastatic triple-negative breast cancer (mTNBC): results from KEYNOTE-086. *Ann Oncol*. 2017;28:V608.
93. Loi S, Sirtaine N, Piette F, et al. Prognostic and predictive value of tumor-infiltrating lymphocytes in a phase III randomized adjuvant breast cancer trial in node-positive breast cancer comparing the addition of docetaxel to doxorubicin with doxorubicin-based chemotherapy: BIG 02-98. *J Clin Oncol*. 2013;31:860-867.
94. Denkert C, von Minckwitz G, Brase JC, et al. Tumor-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers. *J Clin Oncol*. 2015;33:983-991.
95. Schmid P, Dent R, O'Shaughnessy J. Pembrolizumab for early triple-negative breast cancer. Reply. *N Engl J Med*. 2020;382:e108.
96. Loi S, Michiels S, Salgado R, et al. Tumor infiltrating lymphocytes are prognostic in triple negative breast cancer and predictive for trastuzumab benefit in early breast cancer: results from the FinHER trial. *Ann Oncol*. 2014;25:1544-1550.
97. Adams S, Gray RJ, Demaria S, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. *J Clin Oncol*. 2014;32:2959-2966.
98. Ingold Heppner B, Loibl S, Denkert C. Tumor-infiltrating lymphocytes: a promising biomarker in breast cancer. *Breast Care (Basel)*. 2016;11:96-100.
99. Miyashita M, Sasano H, Tamaki K, et al. Prognostic significance of tumor-infiltrating CD8+ and FOXP3+ lymphocytes in residual tumors and alterations in these parameters after neoadjuvant chemotherapy in triple-negative breast cancer: a retrospective multicenter study. *Breast Cancer Res*. 2015;17:124.
100. Criscitiello C, Bayar MA, Curigliano G, et al. A gene signature to predict high tumor-infiltrating lymphocytes after neoadjuvant chemotherapy and outcome in patients with triple-negative breast cancer. *Ann Oncol*. 2018;29:162-169.
101. Matthew Hellmann HJW. Management of advanced non-small cell lung cancer lacking a driver mutation: immunotherapy. https://www.uptodate.com/contents/management-of-advanced-non-small-cell-lung-cancer-lacking-a-driver-mutation-immunotherapy. Published 2020. Updated March 17, 2020.
102. Ding X, Chen Q, Yang Z, et al. Clinicopathological and prognostic value of PD-L1 in urothelial carcinoma: a meta-analysis. *Cancer Manag Res*. 2019;11:4171-4184.
103. Dill EA, Gru AA, Atkins KA, et al. PD-L1 expression and intratumoral heterogeneity across breast cancer subtypes and stages: an assessment of 245 primary and 40 metastatic tumors. *Am J Surg Pathol*. 2017;41:334-342.
104. Mittendorf EA, Philips AV, Meric-Bernstam F, et al. PD-L1 expression in triple-negative breast cancer. *Cancer Immunol Res*. 2014;2:361-370.
105. Davis AA, Patel VG. The role of PD-L1 expression as a predictive biomarker: an analysis of all US Food and Drug Administration (FDA) approvals of immune checkpoint inhibitors. *J Immunother Cancer*. 2019;7:278.
106. Rugo HS, Loi S, Adams S, et al. PD-L1 immunohistochemistry assay comparison in atezolizumab plus nab-paclitaxel-treated advanced triple-negative breast cancer. *J Natl Cancer Inst*. 2021;113:1733-1743.
107. Rimm DL, Han G, Taube JM, et al. A prospective, multi-institutional, pathologist-based assessment of 4 immunohistochemistry assays for PD-L1 expression in non-small cell lung cancer. *JAMA Oncol*. 2017;3:1051-1058.
108. Zavalishina L, Tsimafeyeu I, Povilaitite P, et al. RUSSCO-RSP comparative study of immunohistochemistry diagnostic assays for PD-L1 expression in urothelial bladder cancer. *Virchows Arch*. 2018;473:719-724.
109. Sugie T. Immunotherapy for metastatic breast cancer. *Chin Clin Oncol*. 2018;7:28.
110. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. *Science*. 2015;348:69-74.
111. Budczies J, Bockmayr M, Denkert C, et al. Classical pathology and mutational load of breast cancer—integration of two worlds. *J Pathol Clin Res*. 2015;1:225-238.
112. Hugo W, Zaretsky JM, Sun L, et al. Genomic and transcriptomic features of response to anti-PD-1 therapy in metastatic melanoma. *Cell*. 2016;165:35-44.
113. Rizvi NA, Hellmann MD, Snyder A, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. *Science*. 2015;348:124-128.
114. Ansell SM, Lesokhin AM, Borrello I, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. *N Engl J Med*. 2015;372:311-319.
115. Bareche Y, Venet D, Ignatiadis M, et al. Unravelling triple-negative breast cancer molecular heterogeneity using an integrative multiomic analysis. *Ann Oncol*. 2018;29:895-902.
116. Thomas A, Routh ED, Pullikuth A, et al. Tumor mutational burden is a determinant of immune-mediated survival in breast cancer. *Oncoimmunology*. 2018;7:e1490854.
117. Liu Z, Li M, Jiang Z, Wang X. A comprehensive immunologic portrait of triple-negative breast cancer. *Transl Oncol*. 2018;11:311-329.
118. Xiao Y, Ma D, Zhao S, et al. Multi-omics profiling reveals distinct microenvironment characterization and suggests immune escape mechanisms of triple-negative breast cancer. *Clin Cancer Res*. 2019;25:5002-5014.
119. Rooney MS, Shukla SA, Wu CJ, Getz G, Hacohen N. Molecular and genetic properties of tumors associated with local immune cytolytic activity. *Cell*. 2015;160:48-61.
120. Safonov A, Jiang T, Bianchini G, et al. Immune gene expression is associated with genomic aberrations in breast cancer. *Cancer Res*. 2017;77:3317-3324.

121. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. *J Clin Oncol*. 2005;23:609-618.
122. Le DT, Uram JN, Wang H, et al. PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372:2509-2520.
123. Wen YH, Brogi E, Zeng Z, et al. DNA mismatch repair deficiency in breast carcinoma: a pilot study of triple-negative and non-triple-negative tumors. *Am J Surg Pathol*. 2012;36:1700-1708.
124. Hendrickx W, Simeone I, Anjum S, et al. Identification of genetic determinants of breast cancer immune phenotypes by integrative genome-scale analysis. *Oncoimmunology*. 2017;6:e1253654.
125. Alistar A, Chou JW, Nagalla S, Black MA, D'Agostino R Jr, Miller LD. Dual roles for immune metagenes in breast cancer prognosis and therapy prediction. *Genome Med*. 2014;6:80.
126. Ferreira EN, Brianese RC, de Almeida RVB, et al. Influence of BRCA1 germline mutations in the somatic mutational burden of triple-negative breast cancer. *Transl Oncol*. 2019;12:1453-1460.
127. Audeh MW, Dadmanesh F, Yearley J. Abstract P4-04-01: PDL-1 expression in primary breast cancers with germline mutations in BRCA 1 and 2. *Cancer Res*. 2016;76:P4-04-01.
128. Sobral-Leite M, Van de Vijver K, Michaut M, et al. Assessment of PD-L1 expression across breast cancer molecular subtypes, in relation to mutation rate, BRCA1-like status, tumor-infiltrating immune cells and survival. *Oncoimmunology*. 2018;7:e1509820.
129. Ichihara E, Harada D, Inoue K, et al. The impact of body mass index on the efficacy of anti-PD-1/PD-L1 antibodies in patients with non-small cell lung cancer. *Lung Cancer*. 2020;139:140-145.