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

Katarzyna Uchimiak1, Anna M. Badowska-Kozakiewicz2, Aleksandra Sobiborowicz-Sadowska1 and Andrzej Deptała2

1Students’ Scientific Organization of Cancer Cell Biology, Department of Cancer Prevention, Medical University of Warsaw, Warsaw, Poland. 2Department of Cancer Prevention, Medical University of Warsaw, Warsaw, Poland.

**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-anchracycline 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

**Introduction**

Breast carcinoma (BC) is the most common malignancy among women worldwide1 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.5

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 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 response and enhances the efficacy of ICIs.
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. 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.

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. 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. 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. The disease control rate (DCR) was 7.6% in general, 9.5% in PD-L1-positive and 4.7% in PD-L1-negative populations. Previously untreated, PD-L1 positive cohort presented ORR of 21.4% and DCR of 23.8%.

However, phase 3 KEYNOTE-119 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.

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. 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. 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. An updating analysis of the study showed an increase in EFS in the pembrolizumab group that exceeded expectations based on pCR percentage. 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. The most common event was distant recurrence (7.7% in the pembrolizumab–ChT group and 13.1% in the placebo–ChT group). 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. 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. 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). Thus, the study provided further evidence for increased pembrolizumab efficacy in higher PD-L1 enrichment.
| AUTHOR, YEAR | TRIAL ID, PHASE | INCLUSION CRITERIA | MEDIAN AGE (YEARS) | STUDY ARMS, PATIENT COUNT (N) | RESULTS |
|-------------|----------------|-------------------|-------------------|-----------------------------|---------|
| Winer et al\textsuperscript{28} | KEYNOTE-119 Phase 3 | mTNBC, 1-2 ChT with anthracycline or taxane, PD after last ChT, ECOG ≤ 1 | 50 (43-59) | Pembro 200mg Q3W (n=312) | ORR 9.6% (95% CI: 6.6-13.4) DCR 12.2% (95% CI: 8.8-16.3) |
| | | | | | DCR 10.6% (95% CI: 7.4-14.6) |
| | | | | | 18.7% (95% CI: 14.5-23.5) |
| Cortes et al\textsuperscript{32} | KEYNOTE-355 Phase 3 | Untreated or locally recurrent mTNBC, ECOG ≤ 1 | 53 (44-63) | Pembro 200mg Q3W + ChT (Nab-PC or PC or gemcitabine + carboplatin) (n = 566) | PFS 7.5m |
| | | | | | PBO + ChT (n=281) | 5.6 m (HR 0.82, 95% CI: 0.69-0.97) |
| Schmid et al\textsuperscript{29,31} | KEYNOTE-522 Phase 3 | Untreated, locally advanced non-metastatic TNBC, ECOG ≤ 1 | 49 (22-80) | Pembro 200mg Q3W + PC + carboplatin (n=784) | pCR 64.8% (95% CI: 59.9-69.5) |
| | | | | | EFS at 36m 84.5% (95% CI: 81.7-86.9) |
| | | | | | 76.8% (95% CI: 72.2-80.7) |
| Adams et al\textsuperscript{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 18.0% (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\textsuperscript{35} | KEYNOTE-162 Phase 2 | Advanced or mTNBC, ≤ 2 lines of ChT, ECOG ≤ 1 | 54 (32-90) | Niraparib 200mg + Pembro 200g 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\textsuperscript{40} | NCT 02730130 Phase 2 | mTNBC, ECOG ≤ 2, > 2 sites of metastatic disease with ≥ 1 site requiring RT | 52 (37-73) | Pembro 200mg + 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<sup>30</sup> | IMpassion031 Phase 3 | Untreated, stage II-III TNBC | 51 (22-76) | Atezolizumab 840mg 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<sup>46</sup> | IMpassion130 Phase 3 | Untreated, locally advanced or mTNBC, ECOG ≤ 1 | 55 (46-64) | Atezolizumab 840mg Q2W + Nab-PC (n = 451) | OS 21 m (95% CI: 19-22.6) |
| | | | 56 (47-65) | PBO + Nab-PC (n = 451) | PFS 7.2 m (95% CI: 5.6-7.4) |
| Miles et al<sup>47</sup> | IMpassion131 Phase 3 | Locally advanced or mTNBC, no prior ChT or ≥ 12m since ChT | Mean 54.8 | Atezolizumab 840mg Q3W + PC (n = 431) | OS 19.2 m (95% CI: 16.8-22.5) |
| | | | Mean 52.7 | PBO + PC (n = 220) | PFS 5.7 m (95% CI: 5.4-7.2) |
| | | | | | ORR 47% (95% CI: 41-54) |
| Emens et al<sup>49</sup> | NCT01375842 Phase 1 | mTNBC, PD since last ChT, ECOG ≤ 1 | 53 (29-82) | Atezolizumab Q3W: 15mg/kg (n = 22), or 20mg/kg (n = 1), or 1200mg (n = 93) | OS First line (n = 21): 17.6 m (95% CI: 10.2-NR) |
| | | | | | 2-week W (n = 58) | 7.3 m (95% CI: 6.1-10.8) |
| | | | | | NW (n = 29) | 54% (95% CI: 49-58) |
| | | | | | | 5.6 m (95% CI: 4.7-6.5) |
| | | | | | | ORR RECiST: 10% (95% CI: 4.9-16.5) |
| Dirix et al<sup>50</sup> | 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: 1.1-1.4) |
| Loibl et al<sup>51</sup> | GeparNuevo Phase 2 | Untreated, non-metastatic, TNBC > 2 cm | 49.5 (25-74) | Durvalumab 1.5g Q4W + Nab-PC (n = 88) 2-week W (n = 59) NW (n = 29) | pCR Overall: 53.4% (95% CI: 42.5-61.4) |
| | | | 49.5 (23-76) | PBO + Nab-PC (n = 86) 2-week W (n = 58) NW (n = 28) | W: 61% NW: 37.9% |
| | | | | | 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: 0.21-1.47) |
| | | | | | DDFS<sup>32</sup> 3-year iDFS: 84.9% 3-year DDFS 91.4% 3-year OS<sup>32</sup> 95.1% |

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, ladiratzumab vedotin—an anti-LIV-1 antibody-drug conjugate with a protease-cleavable linker to monomethyl auristatin E, and sacituzumab govitecan—an 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, were found to increase PD-L1 expression on tumor cells providing more targets for PD-L1 inhibitors. 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. 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. 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. An ongoing phase 1b/2 trial (NCT03310957) studies the combination of ladiratzumab vedotin with pembrolizumab as a first-line treatment in patients with locally advanced or metastatic TNBC. 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.

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), 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. The primary completion date is estimated for April 2024.

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. 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. Overall, 33% of patients with durable responses presented them outside of the RT field, indicating certain efficacy of pembrolizumab in this combination. The treatment was presented as well tolerated.

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. 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. 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. At the time of writing, the published results regarding specifically patients with TNBC are limited to ORR which reached 83.3% in this subgroup. 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, potentiates T cell-mediated cytotoxicity, and suppresses cell invasion and mobility. 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. Atezolizumab with nab-paclitaxel was the first PD-L1 inhibitor-based regimen in TNBC approved by the European Medicines Agency (EMA) and FDA. The combination was granted an accelerated approval for locally advanced or metastatic, PD-L1-positive TNBC in March 2019, after promising results of IMpassion130 described below. The indication was then voluntarily withdrawn by the manufacturer in August 2021, due to unsatisfactory results of IMpassion131. 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 200mg iv Q3W + 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, 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 | 110 | PFS, AE rate |
| NCT03310957              | Phase 1b/2, Open-label | SGN-LIVIA + 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 | 1155 | iDFS |
| NCT04427293              | Phase 1, Open-label | Preoperative lenvatinib + pembrolizumab in early-stage TNBC | TNBC T1b-T2/N0-N1/M0, ECOG ≤ 2 | Lenvatinib 12mg + pembrolizumab 200mg 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 200mg iv Q3W + carboplatin and gemcitabine | 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 240mg 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, metastaticTNBC, 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; Q2/3W, every 2/3 weeks; TAM, tumor-associated macrophages; Til, Tumor-infiltrating lymphocytes; TNBC, triple-negative breast cancer.
IMpasion130, 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 IMpasion131 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 IMpasion031, 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 ≥ 6 months. Tumor shrinkage was noted in 45.7% of TNBC patients, in half of which reaching ≥ 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

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lymph nodes. 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. 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. 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. In 2 independent trials including patients with advanced melanoma, the HR in respect to median PFS was 0.42 and 0.40 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. 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. 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. 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. 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. 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 or sipuleucel-T for metastatic castration-resistant prostate cancer.

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 neoadtigen DNA vaccine (NCT03199040), and personalized synthetic neoadtigen 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 10mg/kg iv Q2W for 52 weeks | 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 | 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) and placebo + PC + doxorubicin/epirubicin + CP followed by placebo Q3W for 1 year | 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 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 | 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 | 50 | PFS |

(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 | 81 | PFS |
| NCT03971409 InCiTe Phase 2, Randomized, Open-label | Avelumab + binimetinib, sacituzumab govitacecan, 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 govitacecan + 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.

| STUDY DESIGN AND NAME | INCLUSION CRITERIA | PRIMARY OUTCOME | NO. OF PATIENTS | STUDY ARMS | AE rate | PRIMARY | PRIMARy | STUDY ARMS | AE rate | PRIMARY | PRIMARy | STUDY ARMS | AE rate |
|-----------------------|-------------------|-----------------|-----------------|------------|---------|---------|---------|------------|---------|---------|---------|------------|---------|
| NCT04551885 (FT516 with avelumab for solid tumors including TNBC) and completed, phase 1b QUILT-3.067 | Metastatic disease or unresectable locally advanced malignant, ECOG 0-1 | Resectable TNBC, ECOG = 1 | 88 | Ipilimumab + nivolumab + core biopsy/ cryoablation + breast surgery | 240 | ORR | 67% | DCR | 78% | CR | 22% | OS | 12 months |
| NCT03982173 (MATILDA) | Resectable TNBC, ECOG 0-1 | Metastatic disease or unresectable locally advanced malignant, ECOG 0-1 | 88 | Durvalumab and tremelimumab in metastatic solid tumors including TNBC | 75 | ORR | 67% | DCR | 78% | CR | 22% | OS | 12 months |

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. For instance, MHC-NK cell interaction results in the release of cytotoxic granules and proinflammatory cytokines, such as IFN-γ. IFN-γ acts as an activator of APCs, resulting in the induction of T-helper cell-mediated immune response.

PD-1 and CTLA-4 molecules act as negative regulators of NK cells' function, 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. NK cells' interaction with MHC and their ability to target cells with improper MHC function may comprise a potential gateway for achieving response in these patients. Meta-analysis by Nersesian et al 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).

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).

Safety Profile

Immunomodulation unbalances the immune system, therefore favoring the development of immune-related AEs (irAEs)—autoimmune side effects resulting from the treatment. Autoimmunity can be triggered by both suppression of immune response's negative regulation and cross-reactivity between the tumor neoantigens and healthy tissue antigens. IrAEs can affect any tissue, but most commonly involve the skin, gut, lungs, and endocrine glands. Although most irAEs respond to steroids, this treatment might compromise the antitumor effect. 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. No similar association was reported in respect to the PD-1 blockade.

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. In the KEYNOTE-162 study of pembrolizumab with niraparib, anemia (35%) and
| 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 unresctable or metastatic TNBC, lA2+, prior ChT in current setting, HLA A2+, ECOG ≤1 | PVX-410 vaccine + pembrolizumab Q3W | 20 | Immune response |
| NCT02432963              | Phase 1, Open-label | Vaccine therapy + pembrolizumab in solid tumors including TNBC with PD after prior therapy | Advanced (unresctable 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 1000mg 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 | 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. Tremelimumab and pembrolizumab + RT-based treatment studies described cases of lymphopenia. Overall, the irAEs in TNBC treatment, though frequent, are rather low-grade and controllable. Immunochemistry, as an alternative to ChT, seems to have an acceptable safety profile in TNBC, similar to other cancers. It was suggested that high BMI can be a potential risk factor for a worse tolerance of ICI in TNBC, despite its greater efficacy in these patients.

**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”). 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 (tumor-infiltrating lymphocytes [TILs]) can be identified as either stromal (sTILs) or intratumoral (iTILs). 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. TILs level is known to reflect the T\(\_\)1 immune response in BC and tends to be higher in more aggressive cancer types. 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). 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. Similarly, in KEYNOTE-173 (pembrolizumab + ChT), patients with pCR had higher median sTILs levels before and during treatment. The sTILs levels before the treatment for pCR\(\_\)ypT0 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 ypN0 and 65% (IQR 5–86) vs 25% (IQR 3–60) for pCR\(\_\)ypT0 ypN0. 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. 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. 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, which is currently being evaluated on its synergy with pembrolizumab. 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. 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.

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. The TIL level was reported to increase after ChT, 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. 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. The expression of 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. 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) and urothelial carcinoma. PD-L1 positivity is found in 20%-31% of
TNBC cases. 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.

The expression of PD-1 and PD-L1 on immune and tumor cells was as a predictive marker for immunotherapy-treated TNBC as 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]).

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. 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. Respective PD-L1 positivity (defined as ≥1% PD-L1 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%).

Thus, many cases that were PD-L1-negative based on SP142 were designated as positive with SP263 (29.6%) and 22C3 (29.0%). The difference in PD-L1 proportion yielded by SP142 and 22CC3 was also noted in the case of NSCLC and bladder cancer. 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.

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 exclusion 26.3% of SP142 PD-L1 ≥ 1% patients, 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. 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. In another aforementioned study—NCT01375842 (atezolizumab monotherapy), all responders were PD-L1-positive (at least 1% PD-L1 expression on tumor cells, SP142 assay). 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]). Moreover, the PD-L1 expression increased significantly after the exposure to atezolizumab in patients with mTNBC. In turn, in phase 1b JAVELIN solid tumor trial (avelumab monotherapy), PD-L1 expression on tumor cells did not affect the predicted response. 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).

For patients with CPS ≥ 1, ORR was 12% for pembrolizumab and 9% for ChT; for CPS ≥ 10, the ORR was 18% for pembrolizumab and 9% for ChT; and for CPS ≥ 20, it was 26% and 12%, respectively. 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. In KEYNOTE-162 (pembrolizumab + niraparib), the ORR was 32% in PD-L1-positive patients (CPS ≥ 1, 22C3 assay) and 8% in PD-L1-negative. Also, KEYNOTE-173 study (pembrolizumab + ChT) showed a positive association between higher PD-L1 expression (via 22C3 assay) and pCR rates. The median pre-treatment PD-L1 expressions for pCRypT0 ypN0 achieve rs vs non-achievers were 30% (IQR 5-69) vs 5% (IQR 2-38). In respect to pCRypT0 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. Importantly, in certain trials, ICI + ChT combination was significantly advantageous in early-stage TNBC regardless of PD-L1 status. These include Impassion031 and KEYNOTE-522.

**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). The neoantigens can be recognized by the immune system provoking an immune response. Its predictive value for immunotherapy was reported in the case of melanoma and NSCLC, but is of no significance for Hodgkin's lymphoma, which responds to ICI despite not having a high tumor mutational burden (TMB). 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. 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, 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 or lack of relationship 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. 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. 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, 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 incoherence between the criteria established in different studies. In the case of determining PD-L1 positivity, recent FDA approval for pembrolizumab-based treatment of CPS ≥ 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.

ORCID iD

Katarzyna Uchimiak https://orcid.org/0000-0002-3905-1708

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