Research progress on immunotherapy in triple-negative breast cancer (Review)

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Abstract. Triple-negative breast cancer (TNBC) is a highly heterogeneous and aggressive malignancy. Due to the absence of estrogen receptors and progesterone receptors and the lack of overexpression of human epidermal growth factor receptor 2, TNBC responds poorly to endocrine and targeted therapies. As a neoadjuvant therapy, chemotherapy is usually the only option for TNBC; however, chemotherapy may induce tumor resistance. The emergence of immunotherapy as an adjuvant therapy is expected to make up for the deficiency of chemotherapy. Most of the research on immunotherapies has been performed on advanced metastatic TNBC, which has provided significant clinical benefits. In the present review, possible immunotherapy targets and ongoing immunotherapy strategies were discussed. In addition, progress in research on immune checkpoint inhibitors in early TNBC was outlined.

Contents

1. Introduction

Triple-negative breast cancer (TNBC) has its name from the absence of estrogen receptors (ERs) and progesterone receptors, and its lack of overexpression of human epidermal growth factor receptor 2 (HER-2) on tumor cells. TNBC accounts for 15-20% of all breast cancer cases (1-3). TNBC is usually more aggressive, more likely to recur and has a poor long-term prognosis as compared with other types of breast cancer (4). Researchers are exploring and developing novel drugs to improve the prognosis of TNBC patients.

Immunotherapy has emerged as a therapy that uses the internal mechanisms of the host's immune system to fight cancer by enhancing the ability of the immune system to recognize and kill tumor cells. In the past, cancer treatment frequently relied on options that directly attacked tumor cells, including surgery, radiation therapy and chemotherapy (5). In recent years, the internal mechanism of the host's immune system has been harnessed to fight cancer cells and has achieved remarkable results. TNBC has higher numbers of tumor-infiltrating lymphocytes (TILs) and programmed cell death-ligand 1 (PD-L1) levels than other breast cancers, making immunotherapy promising for treating TNBC (6-9). The tumor mutation burden (TMB) is a measurement of the number of somatic/acquired mutations in the tumor genome, defined as the total number of nonsynonymous point mutations in each coding region of the tumor genome per million base pairs (mut/mbp) (10). Tumors with a TMB of ≥10 mut/mbp are considered tumor mutational burden-high TMB (TMB-H) (11). Tumors with TMB-H produce a relatively larger amount of neoantigens, making them more immunogenic (12). In June 2020, the US Food and Drug Administration (FDA) accelerated the approval of pembrolizumab (Keytruda) for the treatment of adults and children with unresectable or metastatic TMB-H solid tumors. The Targeted Agent and Profiling Utilization Registry study confirmed the anti-tumor activity of pembrolizumab in patients with metastatic TMB-H, supporting the FDA's decision (13). The frequency of TMB-H is lower in primary breast cancers than in metastatic breast cancers (3 vs. 11%) (14). TNBC carries a higher TMB than ER-positive or HER2-positive breast cancer (15-17). The frequency of TMB-H in the primary TNBC and brain metastasis TNBC cohort was 37.5 and 64.0%, respectively (18). TNBC with TMB-H may benefit from immunotherapy, but more research is required. At present, TMB-H is not an independent predictive marker for immunotherapy.

Several large-scale clinical studies applying immune checkpoint inhibitors (ICIs) to treat advanced TNBC have demonstrated anti-tumor effects (19-21). A study suggested

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that early application of immune checkpoint suppression enhanced the efficacy of the treatment. This enhancement in efficacy is because, compared with late metastatic TNBC, the immune microenvironment of an early tumor has a greater impact on tumor growth, which may enhance the anti-tumor action of immunotherapy (22). It is worth noting that the frequency of PD-L1 positivity in the metastatic TNBC cohort was lower than that in the primary TNBC cohort (18). The present article aimed to review the relevant research progress of immunotherapy in the neoadjuvant treatment of TNBC and provide ideas and directions for further related basic and clinical research.

2. Breast cancer immune microenvironment

The concept of the tumor microenvironment (TME) has an indispensable role in tumor occurrence, progression, metastasis, recurrence and drug resistance (23). TME is mainly composed of the extracellular matrix, fibroblasts, myofibroblasts, neuroendocrine cells, adipocytes, immune and inflammatory cells, and blood and lymphatic vascular networks (24). Immune cells of the TME mainly include T and B lymphocytes, natural killer (NK) cells and tumor-associated macrophages (TAMs).

TILs. TILs include innate lymphoid cells and T and B lymphoid cells (25). TILs may be used as prognostic markers in the neoadjuvant treatment of TNBC. Several studies on TILs in the neoadjuvant chemotherapy environment have demonstrated this marker's predictive and prognostic value (26-30). Compared with luminal breast cancer, high immune infiltration is more common in TNBC (31). In addition, the immunosuppressive markers programmed cell death-1 (PD-1) and PD-L1 were positively correlated with tumor-infiltrating lymphocytes (32). Higher levels of TILs are associated with an improved prognosis of TNBC, with every 10% increase in TILs being associated with a 15-25% reduction in the risk of recurrence and death (33-36). Iroquois homeobox protein 2 (IRX-2) is a natural cytokine consisting of multiple components, which may protect human T cells from tumor-induced apoptosis (37). A phase Ib study evaluated the safety and feasibility of pre-operative local IRX-2 treatment in early breast cancer and assessed intratumoral and peripheral immunomodulatory activity (38). The results suggested that IRX-2 was safe and effective in treating early breast cancer. The use of IRX-2 was associated with favorable changes in the TME, including PD-L1 upregulation and expansion of TILs. It was also indicated that modulation of TILs may be a potential strategy to improve TME immune regulation (38). There is an ongoing trial evaluating the clinical and immunological activity of pembrolizumab plus chemotherapy in combination with an IRX-2 induction regimen as a neoadjuvant therapy for TNBC (NCT04373031). Another clinical trial using IRX-2 with cyclophosphamide as a neoadjuvant treatment for TNBC is also underway (NCT02950259).

TAMs. Monocytes differentiate into two subtypes of TAM, namely M1 and M2. The M1 subtype mainly has an anti-tumor effect, while the M2 subtype is related to tumor progression and immunosuppression (39) (Figs. 1). Under normal physiological conditions, the polarization of M1 and M2 phenotypes is relatively stable. However, in a tumor state, the TME promotes the differentiation of monocytes to the M2 phenotype through different factors (40). Targeting the TME to regulate the number and function of TAMs is a promising immunotherapy strategy.

Macrophage-colony stimulating factor 1 (CSF-1) regulates the migration, proliferation, function and survival of macrophages and is an important regulator of macrophage homeostasis *in vivo* (41,42). When CSF-1 is artificially reduced by using CSF-1 blocking antibodies or CSF-1 receptor (CSF-1R) inhibitors, the number of macrophages is rapidly reduced (43,44). LY3022855, an antibody against CSF-1R, exhibited immunomodulatory effects in advanced TNBC (45). In a mouse tumor model of breast cancer, macrophage depletion with the CSF-1R inhibitor PLX3397 enhanced tumor infiltration by CD8 T cells and enhanced the efficacy of anti-PD-1 immunotherapy (46). Cabiralizumab is also an antibody that inhibits the CSF-1R and blocks the activation and survival of macrophages. A clinical trial examining whether the use of neoadjuvant therapy with the addition of Cabralizumab and Nivolumab in combination with chemotherapy reduces TAMs, increases TILs and improves clinical outcomes in patients with early-stage TNBC is currently underway (47).

The TME has a double-edged sword role in TNBC immune regulation and tumor progression, as it is both an immunosuppressive factor and an immune response factor (48). The TME is constantly changing in cancer progression. Ways to exert the immune activation response of the TME and reduce the tumor-promoting effect require to be discovered. Given the antitumor effects of TILs, it may be feasible to increase the number of TILs in patients. For instance, the use of adoptive cell therapy to enhance TILs in patients is a potentially effective immune strategy. Highly active TILs are isolated and infused into patients for therapy after large-scale expansion and activation *in vitro*. In addition, controlling the direction of differentiation of TAMs so that more TAMs differentiate towards the M1 subtype is also worthy of further exploration.

A study successfully distinguished four different spatial architectures of the TME [immune desert (ID), margin-restricted (MR), stroma-restricted and fully inflamed] in order to better understand the interactions between TNBC and the TME. This study showed that tumors with low CD8+ T cell expression (including MR and ID TME subtypes) had the worst prognosis (49). Identifying the different subtypes of TME allows us to stratify TNBC, predict outcomes and determine potential treatment goals for TNBC.

3. Immune checkpoint inhibition

Immune checkpoints comprise a large number of inhibitory pathways that closely regulate the immune system. These pathways are critical for maintaining self-tolerance and regulating the duration and amplitude of an immune response in peripheral tissues to minimize tissue damage (50). Under normal physiological conditions, immune checkpoints are essential to prevent autoimmunity and tissue damage from the immune system responding to infective pathogens. However, cancer cells use immune checkpoints to evade attacks from the immune system (51,52). One meta-analysis indicated that the

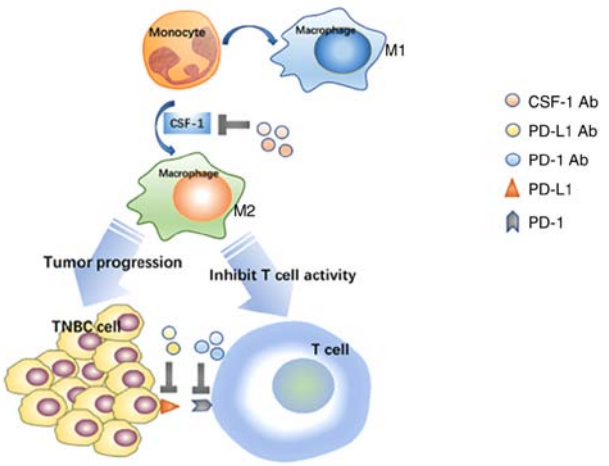

Figure 1. Schematic depicting the mechanism of tumor-associated macrophages impairing the ability of T cells to kill tumor cells. TNBC, triple-negative breast cancer; PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; Ab, antibody; CSF, colony-stimulating factor.

addition of PD-1/PD-L1 blockers to neoadjuvant chemotherapy significantly increased the pathologic complete response (pCR) rate of patients with TNBC, particularly those with a high risk of recurrence (53). The most extensively researched immune checkpoints for blocking are PD-1 and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4).

PD-1 and PD-L1. PD-1 is a co-inhibitory receptor expressed on immune cells, including T cells, B cells, dendritic cells, NK cells and TILs (54). PD-1 has two known ligands, PD-L1 and PD-L2 (55). Given that PD-L1 is more widely expressed than PD-L2 in normal and tumor cells, extensive research is being dedicated to exploring the role of PD-1/PD-L1 in the physiological and pathological immune responses and how to modify their interactions to treat cancer (56). The interactions between PD-1 and PD-L1 occur in the TME and PD-1 is highly expressed on activated T cells, while the PD-L1 is expressed on certain types of tumor cells and antigen-presenting cells (APCs). The interaction between PD-1 and PD-L1 inhibits the biological functions of T cells, including lymphocyte proliferation, cytokine secretion and CTL cytotoxicity, leading to tumor-specific T-cell failure and apoptosis, allowing tumor cells to evade T-cell immune surveillance (57).

It has been indicated that high PD-1+ immune cell infiltration was associated with significantly reduced patient survival (58). Of note, the PD-L1 expression rate in patients with TNBC was higher than that in other types of breast cancer (59). PD-L1 expression was observed in 20% of TNBC cases, meaning that targeting PD-1 or PD-L1 may benefit patients with TNBC (59). PD-1/PD-L1 inhibitors are able to specifically attenuate the inhibitory effects of PD-1/PD-L1 on activated antitumor T cells. The development of PD-1/PD-L1 inhibitor drugs has become a hot area of research on TNBC therapies.

PD-1 inhibitors. Pembrolizumab, a PD-1 inhibitor, has been mainly used to treat melanoma (60). The feasibility of using pembrolizumab for TNBC was first proposed in 2014 at the San Antonio Breast Cancer Symposium (61). The KEYNOTE-012 study confirmed the safety and anti-tumor activity of single-agent pembrolizumab use in advanced TNBC expressing PD-L1 (62). The subjects included in the KEYNOTE-086 phase II trial were divided into cohorts A and B. Cohort A included patients with advanced metastatic TNBC (mTNBC) who had previously received treatment, while cohort B included patients with mTNBC who had not received any treatment. All patients in cohort B had PD-L1 positive tumors, while 61.8% of patients in cohort A had PD-L1 positive tumors. The results demonstrated that pembrolizumab had significant efficacy as a first-line treatment for PD-1+ mTNBC. The safety was acceptable, as patients in both cohorts were able to tolerate pembrolizumab.

In the KEYNOTE-355 clinical trial, pembrolizumab was used in combination with chemotherapy as a first-line treatment for patients with locally recurrent inoperable or metastatic TNBC. The results indicated that the combination of pembrolizumab and chemotherapy significantly improved progression-free survival (PFS) in patients with tumors expressing higher PD-1 compared with the combination of placebo and chemotherapy. The median PFS (mPFS) of pembrolizumab combined with chemotherapy was determined to be 9.7 months, and it was 4.1 months longer than that in the group treated with placebo combined with chemotherapy, which was 5.6 months (19).

Of note, as tumor PD-L1 expression increased, the therapeutic effect of pembrolizumab also increased. Furthermore, the duration of response to pembrolizumab was also prolonged with increased tumor PD-L1 expression, suggesting that the clinical benefit of pembrolizumab may be related to PD-L1 expression. In addition, compared with chemotherapy, pembrolizumab had less high-grade toxicity (63). The conclusion that may be drawn from these results is that there is a greater benefit of using an immunosuppressant as a first-line treatment option for advanced TNBC as compared with chemotherapy alone.

However, the KEYNOTE-119 trial indicated that compared with chemotherapy, pembrolizumab monotherapy as a second-line or third-line treatment for metastatic TNBC did not significantly improve overall survival (OS). This difference may have resulted from the development of drug and immune resistance in patients who received a previous systemic therapy (64).

A systematic review indicated that the PD-1 inhibitor pembrolizumab was effective in patients with early as well as advanced TNBC independent of the PD-L1 status of the cancer. The addition of pembrolizumab to chemotherapy in early TNBC was more effective than chemotherapy alone (65). The KEYNOTE-173 study reported that using pembrolizumab combined with chemotherapy in the neoadjuvant treatment of early high-risk TNBC had controllable toxicity and good anti-tumor activity. However, limitations of this study included short follow-up times, a small sample size per group and the lack of a control group (66).

To further determine the benefits of pembrolizumab and chemotherapy in neoadjuvant treatment for TNBC, the I-SPY2 study was conducted as a validated randomized phase 3 neoadjuvant clinical trial. Among the 249 patients enrolled, 69 received pembrolizumab combined with paclitaxel and 180 received paclitaxel combined with doxorubicin (Adriamycin) and cyclophosphamide, the standard neoadjuvant chemotherapy

regimen. The results suggested that adding pembrolizumab increased the pCR rate from 20 to 60% in patients with TNBC, accounting for an improvement of 40% in comparison with the standard treatment (67). The KEYNOTE-522 trial expanded the number of study subjects. A total of 1,174 patients with TNBC received neoadjuvant therapy, of which 784 received pembrolizumab combined with chemotherapy and the other 390 received placebo and chemotherapy. The results indicated that in the neoadjuvant treatment of early TNBC, the pCR rate of patients who received pembrolizumab combined with chemotherapy was 64.8% and was significantly higher than the rate of 51.2% in those patients who received placebo combined with chemotherapy. Of note, this result was independent of PD-L1 expression (68,69). Based on these results, in July 2021, the FDA approved pembrolizumab combined with chemotherapy as a neoadjuvant treatment for early high-risk TNBC, with continued pembrolizumab monotherapy as adjuvant therapy after surgery.

The fourth interim analysis of the KEYNOTE-522 trial indicated that the median follow-up time was 39.1 months. The estimated 36-month event-free survival (EFS) was 84.5% (95% CI: 81.7-86.9) in the pembrolizumab + chemotherapy group and 76.8% (95% CI: 72.2-80.7) in the placebo + chemotherapy group. EFS was significantly improved in the pembrolizumab + chemotherapy group compared with the placebo + chemotherapy group. Treatment-related events or death occurred in 123 patients (15.7%) in the pembrolizumab + chemotherapy group and in 93 patients (23.8%) in the placebo + chemotherapy group (hazard ratio, 0.63; 95% CI: 0.48-0.82) (70).

PD-L1 inhibitors. Atezolizumab is a PD-L1 inhibitor that was proven to have the ability to delay disease progression in advanced metastatic TNBC (71). At present, clinical trials of neoadjuvant atezolizumab are ongoing. In the neoadjuvant therapy in TNBC with anti-PD-L1 (NeoTRIPaPDL1) study (NCT02620280), 280 patients with TNBC requiring neoadjuvant treatment were randomly divided into two groups; one received intravenous carboplatin and paclitaxel combined treatment on day one and day eight, and the other group received the same treatment with the addition of atezolizumab. The results indicated that the pCR rate was 43.5% when using atezolizumab, while that in the group without atezolizumab was comparable at 40.8% (odds ratio, 1.11) (72).

IMpassion130 is a phase 3 study with the endpoints of PFS and OS. The study was made up of patients with unresectable, locally advanced or metastatic TNBC. The results suggested that in the TNBC population with positive PD-L1 expression, atezolizumab combined with albumin-bound paclitaxel significantly improved PFS and OS compared with placebo plus albumin-bound paclitaxel. However, no such improvement was observed in a population of patients with TNBC with negative PD-L1 expression (20,73). In March 2019, atezolizumab received accelerated approval from the US FDA for use in combination with albumin-bound paclitaxel in patients with unresectable locally advanced or metastatic TNBC whose tumors express PD-L1. However, the IMpassion131 study indicated that the combination of atezolizumab and paclitaxel did not improve PFS or OS compared with paclitaxel alone (74). Based on this result, Roche voluntarily withdrew atezolizumab for the treatment of PD-L1-positive metastatic TNBC. The differences observed in Impassion130 and Impassion131 may be caused by the following factors: i) Paclitaxel, not albumin-bound paclitaxel, require to be pre-administered steroids, which may have reduced the efficacy of atezolizumab; ii) paclitaxel and albumin-bound paclitaxel had different effects on tumor-infiltrating macrophages and TILs, and albumin-bound paclitaxel may have had a stronger stimulating activity on T lymphocytes; iii) the study lacked reliable biomarkers to predict the benefits of atezolizumab (75).

The IMpassion031 study was a randomized, double-blinded, multi-center, phase 3 trial. A total of 333 patients with early-stage TNBC were randomly assigned to the atezolizumab plus chemotherapy group (n=165) or the placebo plus chemotherapy group (n=168). The results indicated that the median follow-up time of the atezolizumab plus chemotherapy group was 20.6 months [interquartile range (IQR), 8.7-24.9 months]. In comparison, the median follow-up time of the placebo plus chemotherapy group was 19.8 months (IQR, 8.1-24.5 months). In patients in the atezolizumab plus chemotherapy group, pCR was observed in 95 patients (58%; 95% CI: 50-65%). By contrast, in the placebo plus chemotherapy group, pCR was observed in 69 patients (41%; 95% CI: 34-49%) and the rate difference was 17% [95% CI: 6-27%; one-sided P=0.0044]. In the PD-L1-positive population, 53 of 77 (69%, 95% CI: 57-79%) patients in the atezolizumab plus chemotherapy group achieved pCR compared with 37 of 75 (49%, CI: 38-61%) patients in the placebo plus chemotherapy group [rate difference, 20%; 95% CI: 4-35%; one-sided P=0.021]. The addition of atezolizumab did not compromise the ability to receive chemotherapy. Commonly reported adverse events were similar between the groups and were mostly driven by chemotherapy. Regardless of the PD-L1 status, patients had achieved an improved pCR. The IMpassion031 study provided evidence for the benefits of ICIs in early TNBC (76).

Durvalumab is another PD-L1 inhibitor. GeparNuevo (NCT02685059) was a multi-center, prospective, randomized, double-blinded, placebo-controlled phase II clinical trial. The study consisted of 174 patients with early-stage TNBC that received durvalumab or placebo combined with chemotherapy. The results failed to prove a significant difference in pCR between the two cohorts. Of note, in an unplanned analysis, the cohort of patients who received additional durvalumab therapy prior to surgery achieved a higher pCR (61.0 vs. 41.4%) and it was indicated that immunotherapy enhanced the anti-tumor activity of cytotoxic drugs (77).

CTLA-4. CTLA-4 is a co-inhibitory receptor only expressed on T cells (78). As a homolog of CD28, CTLA-4 is able to bind with a higher affinity to CD80 and CD86 (79). The immune system requires CTLA-4's checkpoint function to prevent uncontrolled immune responses and autoimmune responses (50). Two signals are required for T-cell activation; the first is the presentation of an antigen by major histocompatibility complex I and II on APCs to be recognized by T-cell receptors (TCR) (80) and the second signal is the binding of CD28 receptors on T cells to the B7 ligand on the APC. However, the presence of CTLA-4 competes with CD28 for the B7 binding site, inhibiting the second activation signal, resulting in the failed activation of T cells (Fig. 2) (81,82).

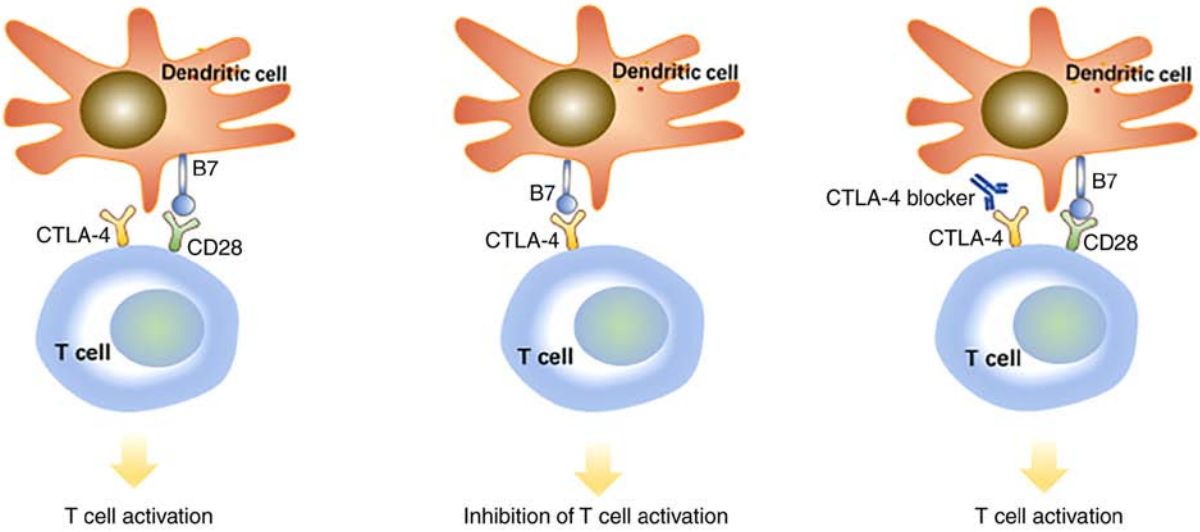

Figure 2. Schematic of CTLA-4 blocker allowing T-cell activation to proceed. CTLA-4, cytotoxic T-lymphocyte-associated protein 4.

Allison won the Nobel Prize for proposing to fight cancer by blocking CTLA-4 (83). The high expression of CTLA-4 in breast cancer cells is associated with poor prognosis (84,85). Among all types of breast cancer, the expression of CTLA-4 is highest in TNBC (86). Inhibition of CTLA-4 is able to prevent T-cell suppression and enhance the anti-tumor activity of T cells, which is a potential immune-related target for treating TNBC. The US FDA has approved the combination of the anti-CTLA-4 antibody ipilimumab with the anti-PD-1 antibody nivolumab for treating cancers, such as melanoma, lung cancer and colorectal cancer (87,88). There are currently no CTLA-4 inhibitors approved to treat breast cancer.

Compared with the rapid development of PD-1/PD-L1 inhibitors to treat TNBC, CTLA-4 inhibitor development has been slow due to the severe side effects caused by CTLA-4 inhibition. A randomized, double-blinded, phase 3 trial enrolled 906 patients undergoing complete resection of stage IIIB, IIIC or IV melanoma. Half of the patients received nivolumab and the other half received ipilimumab. Severe (grades 3-4) immune-related adverse events (irAEs) were reported in 14.4% of patients in the nivolumab group and 45.9% in the ipilimumab group; 9.7 and 42.6% of patients, respectively, discontinued treatment due to the adverse events. At >100 days after treatment, two deaths (0.4%) related to toxic effects were reported in the ipilimumab group (89). A clinical trial (NCT03546686) is underway to determine the effects of pre-operative treatments of cryoablation, ipilimumab and nivolumab on the 3-year EFS of females with TNBC after taxane-based neoadjuvant chemotherapy, but the safety of CTLA-4 inhibitors also requires to be studied.

4. Combination of immunotherapy and chemotherapy

For an immune response to effectively kill cancer cells, a series of step-by-step events must be initiated and an effective cycle (cancer immune cycle) must be formed (Fig. 3). First, the neoantigens produced and released by the tumor are captured and processed by APCs. The APCs then present the captured antigen to T cells, which triggers and activates the response of effector T cells to the cancer-specific antigens. The activated effector T cells then migrate and accumulate at the tumor and infiltrate the tumor bed. T cells recognize and bind cancer cells through TCRs and finally kill them. Killed cancer cells release additional tumor-associated antigens and increase the intensity of the immune response (90). There is evidence that several chemotherapeutics, including anthracyclines, cyclophosphamide and microtubule stabilizers, are able to promote the death of immunogenic tumor cells and release antigens recruiting antigen-presenting cells, which promotes the phagocytosis of dead cells and accelerates the maturation of dendritic cells (DCs) (91-93). The similarity in the immune response and cell death by chemotherapies indicates that the combination of chemotherapy and immune therapy may produce better anti-tumor effects.

A study selected two mice models that had spontaneous metastatic breast cancer and observed the effects on the outcomes of pre-operative neoadjuvant immunotherapy and post-operative adjuvant immunotherapy. The results indicated that neoadjuvant therapy had significantly higher efficacy for eradicating distant metastases after primary tumor resection than adjuvant immunotherapy. The sustained peripheral tumor-specific immune response was discovered to improve the outcomes in these mice (94,95). An *in vitro* study indicated that the combined use of carboplatin and PD-1 inhibitors as a pre-operative neoadjuvant therapy improved the prognosis by increasing the number of tumor-specific CD8+ T cells in the TME of the mice (96).

Another animal experiment indicated that as a neoadjuvant therapy, paclitaxel chemotherapy plus PD-L1 inhibitors significantly improved the OS of mice compared with paclitaxel chemotherapy plus anti-VEGF-A antibodies (97). A meta-analysis suggested that adding PD-1 or PD-L1 inhibitors to early TNBC neoadjuvant chemotherapy improved the prognosis of early TNBC and the status of PD-L1 did not affect the efficacy of ICIs (98).

In conclusion, existing studies suggest that combining chemotherapy and immunotherapy in treating TNBC is able to improve anti-tumor effects compared with chemotherapy

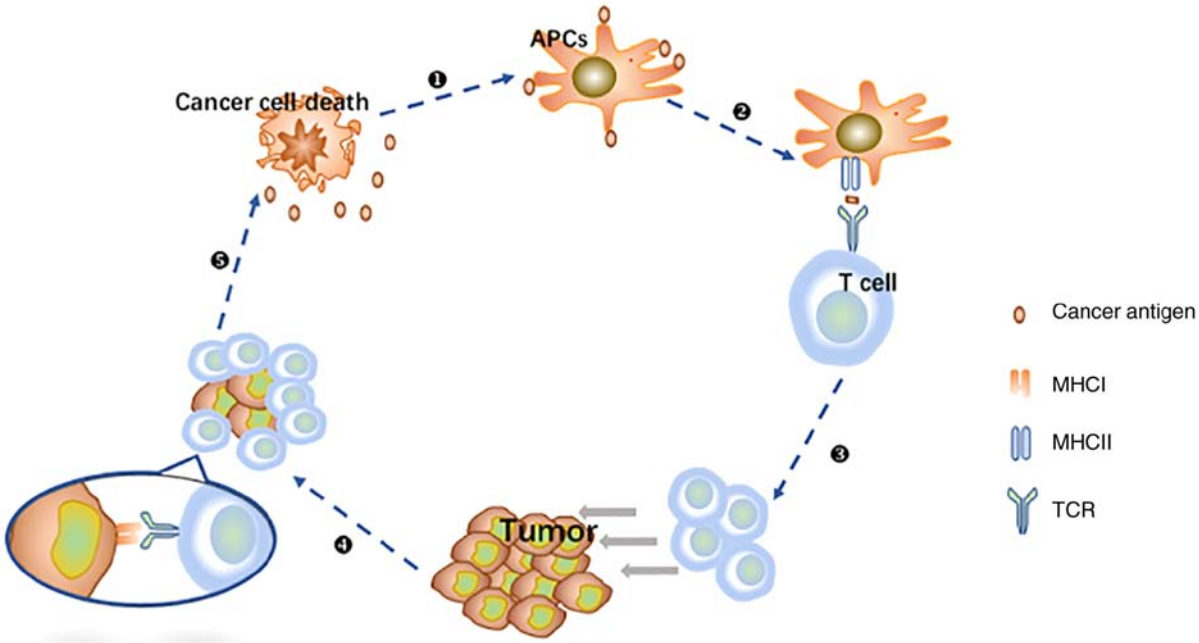

Figure 3. Schematic of the cancer immune cycle. ❶ Tumor cells die and release antigen, APCs recognize antigens; ❷ APCs present antigens to T cells and activate T cells; ❸ T cells gather towards the tumor and infiltrate the tumor bed; ❹ T cells recognize and kill tumors through T-cell receptors on the cell surface; ❺ tumor cells die and release antigen. APC, antigen-presenting cell.

alone. Furthermore, early combination therapy, i.e. adding immunotherapy to neoadjuvant chemotherapy, may have better and longer-lasting anti-tumor effects. However, additional large-scale clinical studies require to verify this. The published results of the ICI trials on the neoadjuvant treatment of TNBC are listed in Table I. Ongoing clinical trials are using ICIs in combination with chemotherapy for the neoadjuvant treatment of TNBC (Table II.

5. Other immunotherapies

Oncolytic viruses (OV) and intratumoral immunotherapy. OV immunotherapy is a cancer treatment that directly lyses tumor cells through selective viral replication in tumor cells, leading to the release of soluble antigens, danger signals and type I interferons (IFNs), thereby driving the collective anti-tumor immune reactions (99,100). In a tumor mouse model, a study observed that the immune stimulation of type I IFNs produced after OV treatment was necessary for the optimal induction of anti-tumor immunity and increased the number of tumor-specific regulatory T cells. It has been observed that in colon and ovarian cancer models, OV attracted effector T cells into the tumor and induced PD-L1 expression on cancer cells (101).

An effective T cell-mediated adaptive anti-tumor immune response requires two phases; the initial phase is marked by the production of anti-tumor T cells and the effect phase is marked by the destruction of cancer cells by T cells. Anti-PD-1/PDL-1 monoclonal antibodies have been indicated to work during the effect phase (102). Clinical studies using ICIs suggested that patients with existing anti-cancer immunity exhibit the strongest response (103). Therefore, a good strategy may be to simulate an anti-tumor immune response prior to applying ICIs. It has been indicated that intratumoral injections of immune agents, referred to as intratumor immunotherapy, may activate tumor-infiltrating T cells and produce an anti-tumor response (104). Intratumor immunotherapy avoids off-target toxicities and adverse effects that may accompany overall immune stimulation, since they are injected directly into the tumor. In addition, compared with systemic administration, direct intratumor immunotherapy achieved locally high concentrations of the drugs and improved the bioavailability of the immune-stimulating drugs. Therefore, those drugs are only required at low doses to induce local and systemic anti-tumor responses (102).

OVs and Newcastle disease viruses (NDVs) induced inflammatory responses when used in local intratumoral therapy for melanoma, resulting in lymphocytic infiltration and antitumor effects in tumors distant from the original injection site, without distant viral spread. Combination therapy of local NDV and systemic CTLA-4 inhibitors prevented tumor recurrence in hypoimmunogenic tumor models (105).

CF33-hNIS-ΔF14.5 is another oncolytic poxvirus. Researchers established a TNBC mouse model and injected the virus directly into the tumor. They observed that the virus increased the infiltration of CD8+ T cells into the tumor. In mice treated with a combination of virus and anti-PD-L1 antibodies, the immune regulation was significantly increased. Although CF33-hNIS-ΔF14.5 and anti-PD-L1 antibodies failed to exert a significant anti-tumor effect as a single drug, the combination of the two drugs resulted in significant anti-tumor effects. In addition, the mice did not grow new tumors after being injected with the same cancer cells after the combined treatment, indicating that immunity was developed to these cancer cells. This study demonstrated that CF33-hNIS-ΔF14.5 had a beneficial role in regulating immune cells in the TME and increased the response to PD-L1 ICIs (106).

Table I. Reported trials of neoadjuvant immune checkpoint inhibitors and chemotherapy for early TNBC.

Name and phase	Population	Agent	Pathologic complete response rate	Safety
GeparNuevo, II	Early TNBC	Durvalumab or placebo (with nab-paclitaxel) → EC	53.4 vs. 44.2% (P=0.29)	The most common immune-related adverse events were thyroid dysfunction any grade in 47%
NeoTRIPaPDL1, III	Early TNBC	Atezolizumab or placebo (with nab-paclitaxel and carboplatin)	43.5 vs. 40.8% (P=0.66)	-
Keynote-173, IB	High-risk, early-stage, non-metastatic TNBC	Pembrolizumab (with taxane +/- carboplatin) → AC	60.0%	No increased toxic effects
I-SPY2, II	HR-positive/ HER2-negative and TNBC	Paclitaxel ± pembrolizumab → AC	60% (with pembrolizumab) vs. 22% in TNBC	Adrenal insufficiency in 6 patients, at least 3 related to hypophysitis (5 late onset, after completion of AC; 1 during pembrolizumab)
Keynote-522, III	Stage II or stage III TNBC	Pembrolizumab or placebo (with paclitaxel and carboplatin) → AC or EC	64.8% (with pembrolizumab) vs. 51.2% (P=0.00055)	The incidence of treatment-related adverse events of grade 3 or higher was 78.0% in the pembrolizumab-chemotherapy group and 73.0% in the placebo-chemotherapy group, including death in 0.4% (3 patients) and 0.3% (1 patient), respectively
IMpassion031, III	Early TNBC	Atezolizumab or placebo (with nab-paclitaxel) → AC	58% (with pembrolizumab) vs. 41% (P=0.0044)	Treatment-related serious adverse events occurred in 37 (23%) and 26 (16%) patients, respectively

AC, doxorubicin/cyclophosphamide; EC, epirubicin/cyclophosphamide; TNBC, triple-negative breast cancer; HR, hormone receptor; HER2, human epidermal growth factor receptor 2.

The injection of the oncolytic MARABA virus (MG1) into TNBC tumors changed the local TME. In primary tumors, the combination of MG1 and ICIs significantly slowed the tumor growth in the TNBC model compared with the untreated or monotherapy groups (P<0.0001). After injecting MG1 directly into tumors in the fat pad, there was a reduction in lung tumor nodules, demonstrating that locally administered virus was able to reduce metastatic progression (103). The above results provided a strong theoretical basis for clinical research on combination therapies for TNBC. In addition, the combination of intratumor immunotherapy and chemotherapy in preclinical models achieved significant anti-tumor effects.

Intratumoral injections of LTX-315, an oncolytic virus, combined with doxorubicin, were administered to TNBC and a significant anti-tumor effect compared with the monotherapy was observed (P≤0.05). Imaging techniques and histological examination indicated that the combination induced strong local necrosis, followed by increased CD4+ and CD8+ immune cell infiltration into the tumor parenchymal tissue, and indicated that the TME had been remodeled after the combination treatment of LTX-315 and CAELYX® (107).

A clinical study using the oncolytic virus Talimogene Laherparepvec (T-VEC) combined with neoadjuvant chemotherapy in the treatment of TNBC (NCT02779855), and a clinical study of T-VEC combined with neoadjuvant immunotherapy in the treatment of TNBC (NCT04185311) are ongoing.

Vaccines. Cancer vaccines are designed to enhance the ability of the immune system to recognize and kill cancer cells and this is accomplished by injecting cancer-specific antigens into patients to trigger an immune response against the tumor (108). DNA and peptide vaccines are two well-studied types of anti-cancer immunotherapy (109). Synaptonemal complex protein 1 (SYCP1) and acrosin binding protein (ACRBP) are two well-known cancer/testis antigens that potently activate cellular and humoral immune responses against 4T1 murine

Table II. Clinical trials of adding immune checkpoint inhibitors to triple-negative breast cancer neoadjuvant chemotherapy.

Identifier no.	Phase	Target	Immune checkpoint inhibitor drug	Combinatorial chemotherapy agents
NCT04373031	II	PD-1	Pembrolizumab	IRX-2, Paclitaxel, Doxorubicin, Cyclophosphamide
NCT03639948	II	PD-1	Pembrolizumab	Carboplatin, Docetaxel
NCT03036488	III	PD-1	Pembrolizumab	Paclitaxel, Carboplatin, Doxorubicin or Epirubicin, Cyclophosphamide
NCT04722718	II	PD-1	Sintilimab	Apatinib, Nab-Paclitaxel, Carboplatin
NCT04877821	II	PD-1	Sintilimab	Anlotinib, Nab-Paclitaxel, Carboplatin, Epirubicin, Cyclophosphamid
NCT04809779	II	PD-1	Sintilimab	Epirubincin, Cyclophosphamide, Nab-Paclitaxel
NCT04213898	I/II	PD-1	Camrelizumab	Albumin-bound Paclitaxel, Epirubicin
NCT04676997	II	PD-1	Camrelizumab	Nab-Paclitaxel, Epirubicin, Cyclophosphamide
NCT05088057	II	PD-1	Camrelizumab	Doxorubicin, Cyclophosphamide, Docetaxel
NCT04676997	II	PD-1	Camrelizumab	Nab-Paclitaxel, Epirubicin, Cyclophosphamide
NCT04907344	II/III	PD-1	Camrelizumab	Nab-Paclitaxel, Carboplatin
NCT04613674	III	PD-1	Camrelizumab	Not stated
NCT04418154	II	PD-1	Toripalimab	Epirubicin hydrochloride, Cyclophosphamide, Nab-Paclitaxel
NCT02883062	II	PD-L1	Atezolizumab	Carboplatin, Paclitaxel
NCT04770272	II	PD-L1	Atezolizumab	Carboplatin, Paclitaxel, Epirubicin
NCT02530489	II	PD-L1	Atezolizumab	Nab-Paclitaxel
NCT03281954	III	PD-L1	Atezolizumab	Paclitaxel, Carboplatin, Doxorubicin or Epirubicin, Cyclophosphamide
NCT03498716	III	PD-L1	Atezolizumab	Doxorubicin, Epirubicin, Cyclophosphamide
NCT03197935	III	PD-L1	Atezolizumab	Nab-paclitaxel, Doxorubicin, Cyclophosphamide
NCT02489448	I/II	PD-L1	Durvalumab	Nab-Paclitaxel, Doxorubicin, Cyclophosphamide
NCT03356860	I/II	PD-L1	Durvalumab	Paclitaxel, Epirubicin, Cyclophosphamide

PD-1, programmed cell death 1; PD-L1, programmed cell death 1 ligand 1; IRX-2, iroquois homeobox protein 2.

mammary tumors. A study using combination immunotherapy of polyepitopic DNA and peptide cancer vaccines consisting of SYCP1 and ACRBP in a 4T1 breast cancer animal model indicated that combined immunization significantly inhibited the growth of murine triple-negative breast tumors (110).

In vivo studies revealed that nanoparticle (NPs)-based mRNA vaccines targeting the mannose receptors on DCs successfully expressed tumor antigens in lymph node DCs. NP vaccines induced a strong and antigen-specific cytotoxic T-lymphocyte response against TNBC 4T1 cells. The combination of vaccine immunotherapy and anti-CTLA-4 monoclonal antibody significantly enhanced the anti-tumor immune response compared with the monoclonal antibody-only group (111). Whether the vaccine is feasible in combination with other immunotherapies, or even chemotherapy, requires further study.

6. Genomics and immunotherapy

Although the antitumor effect of immunotherapy remains to be fully proven, not every TNBC patient benefits from it; however, precision immuno-oncology, the fusion of immunotherapy and precision oncology, appears to be a promising method for treating all patients with TNBC (112). Stratification of patients into a precise immuno-oncology framework requires information based on genomics and characteristics of the immune infiltrate. The cellular characteristics of the immune infiltrate indicate the tumor genotype and determine the immune phenotype and tumor escape mechanisms.

Researchers have characterized the intratumor immune profile and cancer antigenome of 20 types of solid cancer and created a cancer immune profile. Machine learning was used to identify the determinants of tumor immunogenicity and developed a quantitative scoring scheme called the immunophenotypic score. In two independent cohorts, the immunophenotypic score was a superior predictor of the anti-CTLA-4 and anti-PD-1 antibody response (113).

Tumor-infiltrating cells, particularly T-cell subsets, have a key role in cancer immunology and treatment. There are numerous subgroups of T cells with specific functions, some of which are usually associated with a favorable prognosis. However, other T cell types, such as regulatory T cells, have immunosuppressive effects (114). Therefore, cancer immunology research urgently requires a method to predict comprehensive T-cell subsets. Immune Cell Abundance Identifier (ImmuCellAI) is a method based on gene set characteristics and is used to accurately estimate the abundance of 24 immune cell types, including 18 T-cell subgroups, from gene expression data. ImmuCellAI results are able to predict the immunotherapy response with a high precision (area under the curve, 0.80-0.91). Therefore, ImmuCellAI has a powerful function in tumor immune infiltration and immunotherapy response predictions (115). Based on ImmuCellAI data,

patients were divided into groups based on the immune infiltration-related risk score (IRS), either the IRS-High group or IRS-Low group. In the IRS-Low group, 90% of patients were expected to respond to immunotherapy, but in the IRS-High group, the percentage was only 57%. When the survival analysis was performed in the immunotherapy response group and the non-response group, it was observed that the IRS level was able to predict the prognosis of the two groups. It is speculated that patients in the IRS-Low group may be more sensitive to immunotherapy (116).

Researchers developed and validated a compound clinicopathological immune-related genome nomogram by integrating large-scale clinically annotated TNBC gene expression profile data sets and dividing them into training or validation sets, which may be used to estimate the risk of recurrence or death in patients with TNBC. It was determined that a higher proportion of activated NK cells and naive B cells were respectively associated with a low risk of disease recurrence and death from all causes in patients with TNBC. However, activated mast cells represented an unfavorable prognostic indicator. The observed marginal trend is that several immunosuppressive proteins were higher expressed in low-risk subjects, and ICIs may be an effective treatment for this group (117).

TNBC was divided into three different subtypes through immune genome analysis: Immunity high, medium and low. The stability and reproducibility of these classifications were proved by machine learning methods in four independent data sets. Identifying TNBC subtypes associated with immune characteristics may help optimize the selection of patients with TNBC likely to respond to immunotherapy (118). The use of deep machine learning and artificial intelligence to manage integrated genomics data may accelerate the selective application of immunotherapy in clinical patients with TNBC. However, it would also require larger clinical samples to optimize the accuracy.

7. Immunotherapy and adverse events

Although monotherapy with PD-1 or PD-L1 agents is generally well tolerated, the risk of irAEs increases with combination regimens (112). One of the major risks of checkpoint inhibition is the induction of non-tumor inflammatory responses (119). These adverse reactions mainly manifest in the skin, gastrointestinal tract, liver, endocrine system and respiratory tract, such as rash, colitis, liver injury, hypophysitis and pneumonia (120,121). Adverse events involving these organs are usually not fatal (122). IrAEs that appear in the endocrine system frequently result in irreversible organ damage, which may require lifelong hormone supplements, thus seriously affecting the quality of life of patients (123). Joint inflammation occurs in ~10% of patients and this joint toxicity persists mainly as joint pain and decreased mobility long after discontinuing immunotherapy (124). Cardiac and neurotoxicity due to immunotherapy, although rare, develop rapidly and are fatal (122). Severe irAEs require discontinuation of immunotherapy. For less severe irAEs, high-dose corticosteroids are usually effective in relieving the symptoms. However, one underlying issue with high-dose corticosteroids is the reduced antitumor efficacy of immunotherapy while treating the irAEs (125). How to alleviate irAEs without compromising the therapeutic effect of the immunotherapies is an area of treatment that requires further research.

8. Conclusion

In short, TNBC is a highly heterogeneous tumor type with a poor prognosis. Pioneering work in the field of immunotherapy promises to improve the outcomes for patients with TNBC. The growing application and research using immunosuppressants have provided significant benefits as a cancer treatment. The early application of immunosuppressants in TNBC and the long-lasting anti-tumor effects are important points of development in immunotherapy research. One study indicated that the number of TILs and PD-L1 expression were significantly reduced in metastases and demonstrated that the benefit from the late application of immunosuppressants is not as obvious as that of early application (126). Furthermore, the changes in the TME caused by chemotherapy alone also affect the therapeutic effect of subsequent immune drugs, which may be effectively avoided by adding immune drugs to the first-line treatment. Given the safety and efficacy of cancer vaccines, more polyepitopic vaccines may be expected in the future.

One challenge is that only a minority of patients with TNBC respond well to ICIs (127). The heterogeneity of TNBC also makes the patient selection for immunotherapies difficult (128). Furthermore, combined immunotherapy and chemotherapy in conventional preoperative neoadjuvant therapy are bound to increase the adverse effects (129-131), as well as the physical and economic burden. These issues must be considered when prescribing the combination of immunotherapy and chemotherapy as neoadjuvant therapy. The development of immunotherapy based on precision medicine and artificial intelligence is expected to solve certain difficulties, such as selecting the most suitable patients for immunotherapy. Future clinical trials should also consider the choice of chemotherapy or immunotherapy alone or combined chemotherapy and immunotherapy according to the internal type of TNBC, the number of TILs and the expression of PD-L1.

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Availability of data and materials

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

All authors contributed to the study conception and design. Data collection and analysis were performed by XG, TJ and

RY. The first draft of the manuscript was written by XZ. Review and editing of the manuscript was done by SL. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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