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Immunotherapy for triple-negative breast cancer: A molecular insight into the microenvironment, treatment, and resistance

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ABSTRACT

Clinicians have very limited options to treat triple-negative breast cancer (TNBC) due to the lack of effective targeted drugs. Recently, the findings of the mechanism underlying tumor-intrinsic immune escape have fueled a wave of studies into immunotherapy in breast cancer (BC). Compared with other BC subtypes, TNBC shows a better response to immunotherapy due to the higher level of tumor mutation burden and lymphocyte infiltration. Thereinto, immune checkpoint inhibitors (ICIs) achieved the first success of immunotherapy for TNBC and are widely utilized with conventional treatments in the neoadjuvant/adjuvant and advanced stages. However, a large number of TNBC patients fail to demonstrate a good response to ICIs, and the acquired resistance to ICI-based therapies is clinically emerging, which is a major challenge for immunotherapy in TNBC. Here we review the latest advances in TNBC immune microenvironment, immunotherapy, and immunotherapeutic resistance and discuss the challenges and potential approaches to improve the clinical benefit of immunotherapy against TNBC.

Gene abbreviations: AKT, protein kinase B; APLNR, apelin receptor; AR, androgen receptor; AURKA, aurora kinase; BCL6, B cell lymphoma 6 protein; B2M, β2-microglobulin; C3, complement component 3; CALR, calreticulin; CCLs, C-X-C motif chemokines; CCR, C-C motif chemokine receptor; CXCR, C-X-C motif chemokine receptor; CX3CR1, C-X3-C motif chemokine receptor 1; CSFs, colony-stimulating factors; CTLA4, cytotoxic T-lymphocyte-associated protein 4; ELF5, E74-like factor 5; ERAP1, type 1 tumor necrosis factor receptor shedding aminopeptidase regulator; FBXW7, F-box/WD repeat-containing protein 7; GZMB, granzyme B; GZMK, granzyme K; HLA-A, human leukocyte antigen-A; HAVCR2, T-cell immunoglobulin and mucin-domain containing-3; HVEM, herpesvirus entry mediator; IDO1/2, indoleamine 2,3-dioxygenase-1/2; IFNγ, interferon-γ; IFNGR, IFNγ receptor; ILs, interleukins; IRF1, interferon regulatory factor 1; JAK, Janus kinase 1; LAG3, lymphocyte-activation gene 3; MEK, mitogen-activated protein kinase kinase; MEX3B, Mex-3 RNA binding family member B; MMP9, matrix metallopeptidase 9; PARP, poly-ADP ribose polymerase; PBAF, polybromo-associated BAF; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; PI3K, phosphoinositide 3-kinases; PTEN, phosphatase and tensin homolog; PRF1, perforin 1; STAT1, signal transducer and activator of transcription 1; TAP, transporter associated with antigen processing 1; TAPBP, TAP-binding protein; TGFs, transforming growth factors; TCF7, transcription factor 7; TIGIT, T cell immunoreceptor with Ig and ITIM domains; VEGFR, vascular endothelial growth factor receptor.

1. Introduction

Triple-negative breast cancer (TNBC) accounts for 10-30% of breast cancer (BC) cases and is likely to spread early and recur after treatment than other BC subtypes [1]. There are fewer TNBC-targeted treatments because there is no expression of estrogen receptor, progesterone receptor, and human epidermal growth factor receptor-2 (HER2) in these cancer cells, which is the main problem in TNBC treatment.

TNBC has a better response rate to chemotherapy compared with other BC subtypes. The management of TNBC mainly involves definitive surgery plus conventional chemotherapy (platinum, taxanes, and anthracycline), and a small number of patients with a better prognosis will receive breast-conserving surgery plus local radiotherapy (RT). While TNBC can be controlled by these options, the response is not long-lasting with very limited efficacy in metastatic and relapsed diseases [2]. Also, the overall survival (OS) rate of locally advanced or metastatic TNBC is not substantially improved by PARP inhibitor, though it demonstrates a clinical benefit on the progression-free survival (PFS) [3]. Furthermore, classic protein tyrosine kinase or phosphoinositide 3-kinase inhibitors have not yet shown convincing efficacy and safety against TNBC [4]. Novel therapies are still in urgent need.

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Recent advances in the immune landscape of the tumor microenvironment shed light on novel targeted opportunities for TNBC. The immunologic portrait of TNBC shows the tumor is featured with a unique microenvironment of higher levels of lymphocyte infiltration and PD-L1 expression than other BC subtypes [5]. Also, TNBC has a greater number of somatic mutations due to genomic instability, leading to the frequent presence of neoantigen [6]. These findings suggest that TNBC would be more likely to respond to immunotherapy. By blocking immune checkpoint proteins, inhibitors such as atezolizumab and pembrolizumab achieved the first success of immunotherapy in treating TNBC. The IMpassion130 trial provided strong confirmation that atezolizumab plus nab-paclitaxel (nab-PTX) improved both PFS (7.5 vs 5.0 months) and 3-year OS (36.0 vs 22.0 months) in the PD-L1$^+$ TNBC than nab-PTX monotherapy [7, 8]. The trial also confirmed their efficacy in the Asian subpopulation and is a significant milestone in tackling the heterogenicity of TNBC [9]. On the whole, we noticed a significant expansion of immunotherapy in the clinical trials for TNBC [4]. Undoubtedly, the emergence of immunotherapy will change the future treatment landscape for TNBC.

In this review, we summarize the latest advances in the immunologic portrait of the tumor microenvironment, immunotherapies, and immunotherapeutic resistance in TNBC and further discuss the challenges and potential strategies to improve the clinical benefit of immunotherapy against TNBC.

2. The immunologic portrait of TNBC

By describing the complexity of the tumor microenvironment in a pathway diagram, it can survey how the TNBC immune evasion is mediated by cancer cells, infiltrating immune cells, tumor stroma, and reciprocal communication within the microenvironment (Fig. 1). The continued genomic and clonal evolution of tumors in such microenvironment also generates cancer cells that can equilibrize the immune responses to metastasize and recur, which constitutes the aggressiveness of TNBC.

2.1. Cancer cell

TNBC is more associated with *TP53* (~80% vs ~33%) and *BRCA1/2* mutations (~30% vs ~5%) compared to other BC types, which often causes a deficiency in DNA damage repair [10, 19]. Also, the loss of mismatch repair (MMR) that allows DNA replication with mismatched bases is more frequently found in TNBC patients than in other types of BC (~4.7-6.9% vs ~2%) [20]. These mutations give TNBC the characteristics of genomic instability and elevated tumor mutation burden (TMB) as well as neoantigen levels. The mutant DNA and peptides make TNBC more immunogenic than other BC types and provide the prerequisites for the use of immunotherapy in TNBC. Nevertheless, TNBC also develops the immunosuppressive ability to avoid being killed by innate immune cells.

Analysis of TCGA-BRCA and METABRIC datasets suggests that *CTLA4, PD-L1/2, PD-1, LAG3, IDO1/2*, and *TIGIT*, are significantly upregulated in TNBC compared with other BC types [21]. The expression of these immune checkpoints is a crucial mechanism by which tumor cells escape the immunosurveillance via the “don’t eat me” signal. It is generally believed that IFNγ released by effector T cells activates the IFNGR of cancer cells and induces the expression of PD-L1 through the JAK/STAT1/IRF1 signal transcription machinery [22]. Furthermore, *MUC1-C* amplification, which is found in ~90% of TNBCs, also plays a critical role in immune checkpoint expression. The overexpressed MUC1-C in TNBC was found to recruit MYC and NF-κB to the PD-L1 promoter to accelerate PD-L1 transcription [11]. Moreover, MUC1-C can activate the IFNGR/JAK/STAT1/IRF1 pathway to induce the expression of IDO1 [23]. Similarly, an association of *TP53* mutation and MMR loss with PD-L1 upregulation in TNBC was also found by several studies though the potential mechanism remains unclear [21]. These findings suggest that the oncogenic mutation is closely associated with the immunodepleted state of the tumor.

CD73 functions as ecto-5′-nucleotidase and is also up-regulated on TNBC cells. It can convert extracellular adenosine monophosphate (AMP) into adenosine that induces immunosuppression through adenosine A2A receptor (A2AR) in T cells and NK cells. A high CD73 expression in TNBC leads to a reduced anti-tumor immunity and a poor prognosis in patients [24]. Thus, targeting the adenosinergic pathway is a promising strategy to improve the response of TNBC to immune checkpoint blockade. The combination of anti-CD73 (oleclumab) with PTX, carboplatin, and durvalumab is evaluating in the SYNERGY trials and demonstrated a favorable safety profile with a preliminary clinical benefit in the phase I stage [25].

So far, the cellular mechanism underlying immune evasion is not yet fully understood. A recent study identified 709 TNBC cell-based genes that regulate *in vivo* sensitivity to anti-tumor immune attack using a genome-wide RNAi screening platform [26]. However, how these genes are regulated and involved in TNBC immune escape is largely unknown. Further investigation may provide the key for novel mechanism discovery of TNBC immune escape.

2.2. Tumor microenvironment

TNBC comprises a unique immune microenvironment, distinct from other BC types due to higher TMB that attracts more tumor-infiltrating lymphocytes (TILs) (CD8$^+$ T cell, B cell, NK cell) to the tumor. Normally, these immune cells are thought to be able to coordinately attack and eliminate TNBC cells but are finally equilibrated by evolved cancer cells. Apart from cancer cell evasion, microenvironmental components, such as cancer-associated fibroblast (CAF), tumor-associated macrophage (TAM), and tumor-associated adipose (TAA), also play a critical role in the immunosuppression of TNBC. A large number of cytokines, such as CSFs, CCLs, ILs, and TGFs, and myeloid-derived suppressor cells (MDSCs) mediate this immune rejection. Furthermore, the CD4$^+$CD25$^+$ regulatory T cells (Tregs) are highly proliferative and active in such CCL12-enriched microenvironment caused by a subset of activated CAFs, which disrupts the function of cytotoxic T cells [13]. TAMs are likely to be polarized to the pro-inflammatory CD163$^+$ M2-like phenotype in the TNBC due to high levels of CSFs and TGF-β and then serve as a reservoir for cytokines, such as IL-10, that restrict the activity of infiltrating effector T cells [14, 15]. These TAMs can also express PD-L1 and B7-H4, thereby suppressing CD8$^+$ T cell stimulation in TNBC [16, 17]. Thus, targeting M2-like TAMs has been considered as a strategy to improve the efficacy of immunotherapy [27]. Similarly, a recent study indicated that TAA is the main source of the CCL2 with the ability to recruit monocytes and TAMs to the tumor [18]. Blocking CCL2 release enhanced the anti-tumor immunity in TNBC by decreasing the population of MDSCs and M2-like TAMs [18]. These studies suggest the role of cytokines in the potential of the tumor microenvironment to shape the phenotype of infiltrating immune cells. However, the pattern of these cytokines may vary considerably between individuals or even within the microenvironment. In particular, as TNBC is a heterogeneous disease, a cytokine profile is important to understand how the subtypes relate to their differential potential in altering the anti-tumor immunity. The correlation of this remains to be determined, though it is known that the phenotype of the TNBC microenvironment can be currently classified according to the residence status of innate immune cells and stromal cells.

On the other hand, the composition of intratumoral immune cells is heterogeneous. That is, with different local microenvironments, intratumoral immune cells exhibit phenotypic expansion and domination, suggesting that the immune cells shaping the heterogeneity of the tumor immune environment are not as simple as recruiting, differentiation, and activation. Recent single-cell studies provided further insights into this feature. The single-cell RNA-seq from Azizi, et al. [28] revealed that the lymphoid and myeloid cell lineages within BC are characterized by a

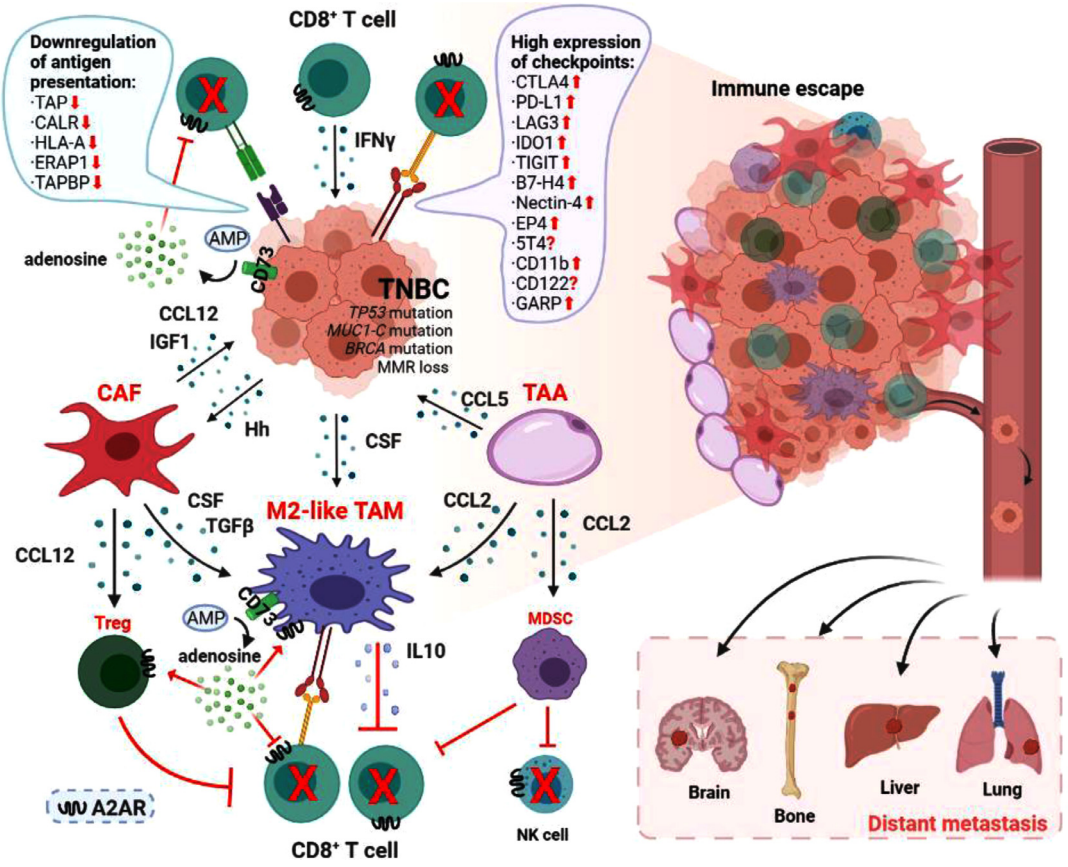

Fig. 1. The immunologic portrait of TNBC reveals the mechanism of immune escape. Although TNBC is more highly infiltrated with lymphocytes in the microenvironment, compared with other BC types, due to the higher mutation burden caused by an unstable genome (*BRCA* mutation and MMR loss), the cancer cells and microenvironmental components confer tumors a survival advantage to escape immunosurveillance and to grow or metastasize. TNBC is more associated with oncogenic *TP53* and *MUC1-C* mutations [10, 11]. These mutations may not only play a role in the uncontrolled growth and metastasis of tumors but also contribute to the high expression of immune checkpoints that mediate the 'don't eat me' signal and down-regulation of antigen presentation activity. Moreover, cancer cells can also express CD73 to catalyze the generation of adenosine that activates Treg cells and TAMs but inhibits the cytotoxic effect of $CD8^+$ T cells and NK cells and to induce an immunosuppressive microenvironment [12]. CAF is the most common stromal cell in the tumor microenvironment, while TAM is the tumor-infiltrating leukocytes that constitute approximately 50% of tumor cells. TNBC cells can secret Hh to activate CAFs which release CCL12 and IGF to increase the population of Tregs and to promote bone metastasis [13]. Both cancer cells and CAFs secret CSF to promote the polarization of TAM to an M2-like anti-inflammatory phenotype [14]. The M2-like TAMs with surface expression of PD-L1, B7-H4, and CD73 can also release IL-10 to limit the effect of $CD8^+$ T cells [15-17]. TAA is the most abundant stromal cell in breast tumors and plays a critical role in the obesity-related growth of TNBC by secreting CCL5. In addition, TAA can also release CCL5 to induce the differentiation of monocytes to immunosuppressive MDSCs that reduce the immune activity of $CD8^+$ T cells and NK cells [18]. **Abbreviations:** A2AR, adenosine A2A receptor; AMP, adenosine monophosphate; BRCA, breast cancer gene; CAF, cancer-associated fibroblast; CALR, calreticulin; CCL, chemokine (C-C motif) ligand; CSF, colony-stimulating factor; CTLA4, cytotoxic T-lymphocyte-associated protein 4; ERAP1, type 1 tumor necrosis factor receptor shedding aminopeptidase regulator; GARP, glycoprotein A repetitions predominant; Hh, hedgehog; HLA-A, human leukocyte antigen-A; IDO1, indoleamine 2,3-dioxygenase-1GARP; IFNγ, interferon-γ; IGF, insulin-like growth factor; IL, interleukin; LAG3, lymphocyte-activation gene 3; MDSC, myeloid-derived suppressor cells; MMR, mismatch repair; MUC1-C, mucin 1 C-terminal; NK cell, natural killer cell; PD-L1, programmed death-ligand 1; TAA, tumor-associated adipose; TAM, tumor-associated macrophage; TAP, transporter associated with antigen processing 1; TAPBP, TAP-binding protein; TGF, transforming growth factor; TIGIT, T cell immunoreceptor with Ig and ITIM domains; Treg, T regulatory cell.

significant phenotypic expansion with 14 specific myeloid cell clusters and 17 specific T cell clusters compared with normal breast tissue. Such diversity of cell states is caused by their response to various stimuli, such as cytokines, in the local microenvironment, such as inflammation and hypoxia. Taking T cells as an example, this heterogeneity is constituted by the specificity of T cell receptor expression. Similarly, Wagner, et al. [29] used single-cell mass cytometry to construct a map of the human BC ecosystem, which comprehensively uncovered the phenotypic diversity of tumor cells and homotypic and heterotypic tumor-immune cell relationships, proposing an ecosystem-based classification system for BC. Notably, the heterogeneity of intratumoral immune cell composition and phenotypic expansion may impact immunotherapeutic efficacy and prognostic features. Through single-cell transcriptomics, Bassez, et al. [30] found that intratumoral PD-L1$^+$CD8$^+$ T cells with the expression of PRF1, GZMB, CCL13, HAVCR2, and LAG3 and PD-L1$^+$CD4$^+$ T cells with the expression of IFNγ, BCL6, and CXCR5 clonally expanded in residue BC after pembrolizumab treatment. They also found that the percentage of PD-L1$^+$ dendritic cells and CCR2$^+$/MMP9$^+$ macrophages and the expression of major histocompatibility complex (MHC) class I/II of cancer cells in the pre-treatment biopsy were associated with such T cell expansion; while the percentage of TCF7$^+$/GZMK$^+$ pre-effector/memory T cells or CX3CR1$^+$/C3$^+$ macrophages was negatively correlated. These studies not only provide an extensive map of the intratumoral microenvironment related to the phenotypic status of immune cells but also propose molecular targets that may have a synergistic effect with immunotherapy. Predictions or hierarchical models based on these findings will help the precise patient stratification and therapeutic decision-making.

3. Role of immune checkpoint blockade in treating TNBC

3.1. Use with chemotherapy for advanced/metastatic TNBC

Immune checkpoint inhibitors (ICIs) achieved early success for TNBC. However, unlike advanced non-small cell lung cancer and melanoma where ICI monotherapy demonstrated a significant survival advantage over chemotherapy, the use of ICI in advanced TNBCs is accompanied by chemotherapy because their response to ICI monotherapy was modest and non-significant. One possible reason is that TNBC is simply a different disease with fewer PD-L1$^+$ tumor cells than other cancer types. The KEYNOTE-119 phase III study of pembrolizumab as monotherapy for PD-L1$^+$ metastatic TNBC reported an overall response rate (ORR) of 9.6% vs 10.6% in the single-agent chemotherapy group [31]. Pembrolizumab monotherapy also failed to improve OS and PFS, though the effect increased as PD-L1 increased. In contrast, the KEYNOTE-355 phase III trial of combinatorial pembrolizumab-chemotherapy (nab-PTX, PTX, or gemcitabine plus carboplatin) treatment reported a significant improvement in median PFS of patients with PD-L1$^+$ recurrent or metastatic TNBC, as compared to placebo-chemotherapy treatment (9.7 months vs 5.6 months) [32]. Consistent results were also obtained in the Asian subpopulation [33]. Along with the data of IMpassion130 which established the first immunotherapy approval for TNBC, these studies suggest that chemotherapy is critical in promoting the anti-tumor immune response and that the strategies with ICI monotherapy would be discarded for TNBC with more clinical trials of ICI being built in combination with chemotherapy, radiotherapy (RT), or other targeted drugs as shown in Table 1.

Chemotherapy can potentially remodel tumor immunity through various mechanisms, including enhancing tumor antigenicity, inhibiting Tregs and MDSCs, and increasing the activity of CD8$^+$ T cells and dendritic cells (DCs) [34]. Such mechanism was also demonstrated in TNBC where cytotoxic-induced reactive oxygen species (ROS) accumulation enhances the immune recognition by enriching CD47$^+$CD73$^+$PD-L1$^+$ tumor cells [12]. Likewise, the combinatorial therapy of cyclophosphamide and vinorelbine can also activate antigen-presenting cells (APC) and increases intratumoral Tcf1$^+$ stem-like CD8$^+$ T cells, which improves the effect of ICIs in TNBC [35]. However, notably, the immunogenicity-inducible effects of chemotherapy are mostly dose- and time-dependent. A higher dose and longer duration may cause the blunting of immune cells and thus abrogating ICI efficacy [36]. Moreover, different chemotherapies exert distinct effects on immunogenicity. The Impassion131 trial, which used PTX instead of nab-PTX in combination with atezolizumab, failed to obtain a clinical benefit in PD-L1$^+$ metastatic TNBC like the Impassion130 trial [37]. Also, patients treated with induction therapy of low-dose cisplatin or doxorubicin may obtain a better prognosis towards nivolumab than those with low-dose cyclophosphamide or RT [38], suggesting that chemotherapy may determine the response pf TNBC to immunotherapy and thus should be customized accordingly. Therefore, setting up an assessing framework of dosing and scheduling containing different cytotoxics on the immunomodulating endpoint, such as the up-regulation of immune-related genes and the expansion of PD-L1$^+$ tumor cells, CD8$^+$ T cells, and other TILs, as designed by the TONIC trial [39], may provide an answer to the failure of the IMpassion131 trial and will be critical in fine-tuning the combinatorial regimes to achieve the best synergism with immune checkpoint blockade.

3.2. Use with chemotherapy or RT as neoadjuvant/adjuvant therapy for early TNBC

Adding ICIs to the neoadjuvant settings also increases the pathological complete response (pCR) rate of early TNBC to chemotherapy. The evidence was first obtained from the GeparNuevo phase II study reporting that the addition of durvalumab to anthracycline-taxane-based neoadjuvant therapy in the window phase increased the pCR rate of patients with early TNBC from 41.4% to 61.0% [40]. A higher pCR rate was found in tumors with positive PD-L1 expression or higher stromal TILs in the durvalumab arm. The KEYNOTE-522 trial used a concomitant neoadjuvant therapy of pembrolizumab and PTX-carboplatin-based chemotherapy, followed by adjuvant pembrolizumab for early TNBC: they obtained similar improvement regardless of PD-L1 expression with a pCR rate of 64.8% as compared to 51.2% with placebo and chemotherapy [41]. The addition of pembrolizumab also effectively decreased the proportion of patients with the progressed disease after a median follow-up of 15.5 months (7.4% vs 11.8%), and consistent results were also obtained from the Asian subpopulation [41, 42]. In the IMpassion031 phase III study of atezolizumab in combination with neoadjuvant nab-PTX-anthracycline for early TNBC: the proportion of patients with pCR was increased from 41% to 58% compared with the placebo group [43]. Similar to the GeparNuevo study, the IMpassion031 study also observed a higher pCR rate in the PD-L1$^+$ tumors. These studies establish the role of ICI in the neoadjuvant settings for TNBC and yield crucial information on the potential of PD-L1 and stromal TILs as biomarkers to select patients responsive to ICI.

RT is an important treatment for local cancer control and often used in the neoadjuvant/adjuvant settings for TNBC. Similar to chemotherapy, RT can also elicit neoantigen-specific T cell priming by causing immunogenic cancer cell death or upregulating the expression of immunogenic mutant genes [44]. The addition of ICI to RT may generate a robust anti-tumor immune response and improve the efficacy of RT [45]. This is of great significance for early TNBC patients who are about to undergo breast-conserving surgery, where RT is commonly utilized. The survival benefit from the combination is under evaluation in several clinical trials (Table 1).

3.3. Use with other targeted drugs

Much has been reported about the use of other targeted therapies to improve the efficacy of ICI against advanced/metastatic TNBC. This is because the targets, such as MEK and AR, also play an important role in the regulation of tumor immunogenicity in TNBC. Blocking the genomic or transcriptomic activation of MEK and AR can increase TIL recruitment or retention and facilitate *de novo* anti-tumor immune responses [46, 47], which can directly improve the efficacy of ICI. Clinically, a modest clinical benefit rate of 25% was observed with the combinatorial treatment of enobosarm and pembrolizumab in heavily pre-treated AR$^+$ TNBC from a phase II study [48]. Furthermore, PARP inhibitor was found with the ability to up-regulate the expression of PD-L1 in BC cells by inactivating GSK3β [49]. Thus, the addition of ICI to the treatment can potentiate the efficacy of PARP inhibition. Results from a phase II study showed that the combination of niraparib and pembrolizumab achieved an ORR of 47% in *BRCA*-mutated advanced or metastatic TNBC compared with 21% in *BRCA* wild-type TNBC [50]. Additionally, it was reported that low-dose VEGFR2 blockade could promote immune cell priming and up-regulate PD-1 expression on immune cells, sensitizing TNBC to anti-PD-1 therapy [51]. The addition of apatinib to camrelizumab documented a higher ORR (43.3%) in advanced TNBC than either apatinib or camrelizumab in a phase II trial [52]. Other similar therapies are listed in Table 1. These combinations also demonstrated favorable safety profiles in the presence or absence of chemotherapy [53, 54]. While these studies suggest a role of other targeted drugs in the combinational modality of ICI for advanced TNBC, they are just getting started with small sample size, and further efficacy evaluation with safety monitoring is still in progress.

Preclinical models also demonstrated that dual ICI produces a better therapeutic effect than either treatment alone by expanding T-cell repertoires and enhancing their activity [10]. In particular, the combination of anti-PD1 and anti-CTLA4 with cisplatin significantly halted the growth of *BRCA1*-deficient TNBC and improved survival *in vivo* [19], which provides evidence of the complementary work of dual-ICI modality. Clinically, with more checkpoints and their inhibitors being novelly identified (**Table 1**), several studies are evaluating the efficacy of

Table 1
Immune checkpoint inhibitors and their representative combinational modalities for TNBC.

Drug	Target	Phase	Status	Regime Description	Primary Results	Identifier No.
Atezolizumab	PD-L1	**3**	**Complete**	**+ chemotherapy as neoadjuvant for early-stage TNBC (IMpassion031)**	**The pCR was documented with 58% in the atezolizumab + chemotherapy group versus 41% in the placebo + chemotherapy group; in the PD-L1$^+$ population, the pCR was 69% and 49%, respectively.**	**NCT03197935**
		3	Recruiting	+ anthracycline/taxane-based chemotherapy as adjuvant therapy for operable TNBC (IMpassion030)		NCT03498716
		3	Recruiting	+ chemotherapy for early recurrent inoperable locally advanced/metastatic TNBC (IMpassion132)		NCT03371017
		3	**Complete**	**+ nab-PTX for previously untreated metastatic TNBC (IMpassion130)**	**The median PFS was 7.2 months in the atezolizumab + nab-PTX group versus 5.5 months in the placebo + nab-PTX group; In PD-L1$^+$ tumors, the median PFS was 7.5 and 5.0 months, respectively. The median OS was 21.3 months in the atezolizumab + nab-PTX group versus 17.6 months in the placebo + nab-PTX group; In PD-L1$^+$ tumors, the median OS was 25.0 and 15.5 months, respectively.**	**NCT02425891**
		3	Recruiting	+ ipatasertib and PTX for locally advanced or metastatic TNBC		NCT04177108
		2	Recruiting	+ eganelisib and nab-PTX or bevacizumab for locally advanced or metastatic TNBC (MARIO-3)		NCT03961698
		2	Recruiting	+ bevacizumab and PTX for advanced or metastatic TNBC		NCT04408118
		2	**Complete**	**+ cobimetinib and PTX for locally advanced or metastatic TNBC (COLET)**	**The median PFS was 5.5 months in the cobimetinib + PTX group versus 3.8 months in the placebo + PTX group.**	**NCT02322814**
		1/2	Recruiting	+ KY1044 in advanced TNBC		NCT03829501
		1b	**Complete**	**+ rucaparib for previously treated advanced TNBC**	Undisclosed	**NCT03101280**
Avelumab	PD-L1	2	Recruiting	+ binimetinib, utomilumab, or PF-04518600 for stage IV or unresectable/recurrent TNBC (InCITe)		NCT03971409
		1	Recruiting	+ palbociclib in metastatic AR$^+$ TNBC (PAveMenT)		NCT04360941
Durvalumab	PD-L1	**2**	**Complete**	**+ anthracycline-/taxane-based chemotherapy as neoadjuvant therapy for early TNBC (GeparNuevo)**	**The pCR was documented with 53.4% in the durvalumab group versus 44.2% in the placebo group. Durvalumab effect was seen only in the window cohort and there was a trend for increased pCR rates in PD-L1$^+$ tumors.**	**NCT02685059**
		2	Not yet recruiting	+ RT as neoadjuvant therapy for stage II-III TNBC (PANDoRA) plus adjuvant RT		NCT03872505
		2	Recruiting	+ olaparib for platinum-treated metastatic TNBC (DORA)		NCT03801369
		2	Active, not recruiting	+ tremelimumab in metastatic solid tumors (MATILDA)		NCT03982173
TQB2450	PD-L1	3	Not yet recruiting	+ anlotinib for advanced TNBC		NCT04405505
FAZ053	PD-L1	1	Active, not recruiting	+ PDR001 for advanced TNBC		NCT02936102
KN046	PD-L1 CTLA-4	1b/2	Recruiting	+ nab-PTX for locally advanced unresectable or metastatic TNBC		NCT03872791

(*continued on next page*)

Table 1 (*continued*)

Drug	Target	Phase	Status	Regime Description	Primary Results	Identifier No.
Pembrolizumab	PD-1	**3**	**Complete**	**+ chemotherapy as neoadjuvant treatment, followed by pembrolizumab as adjuvant treatment for early TNBC (KEYNOTE-522)**	**The pCR was documented with 64.8% in the pembrolizumab + chemotherapy group versus 51.2% in the placebo + chemotherapy group; after a median follow-up of 15.5 months, 7.4% and 11.8% had disease progression, respectively.**	**NCT03036488**
		1	**Complete**	**+ RT as neoadjuvant therapy plus adjuvant RT**	**Neoadjuvant pembrolizumab + RT was safe/feasible and may increase pCR rate compared with chemotherapy alone. Baseline TIL count ≥ 10% in the initial biopsy was associated with pCR.**	**NCT03366844**
		3	**Complete**	**+ chemotherapy for previously untreated locally recurrent inoperable or metastatic TNBC (KEYNOTE-355)**	**In patients with PD-L1 baseline expression (combined positive score of 10 or more), the median PFS was 9.7 months in the pembrolizumab + chemotherapy group versus 5.6 months in the placebo + chemotherapy group.**	**NCT02819518**
		2	**Complete**	**+ standard therapy (PTX followed by DOX and cyclophosphamide) (I-SPY 2)**	**The pCR was documented with 60% in the pembrolizumab + standard therapy group versus 22% in the placebo + standard therapy group.**	**NCT01042379**
		2	**Complete**	**+ RT in metastatic TNBC**	**The combination of pembrolizumab + RT was safe with an ORR of 17.6%.**	**NCT02730130**
		2	**Complete**	**+ enobosarm in heavily pre-treated AR^+ metastatic TNBC without pre-selected PD-L1**	**The combination of enobosarm + pembrolizumab was safe with a clinical benefit rate of 25%.**	**NCT02971761**
		2	**Complete**	**+ niraparib for advanced or metastatic TNBC (TOPACIO)**	**The ORR was documented with 21% in the pembrolizumab + niraparib group; in *BRCA*-mutated tumors, the ORR was documented with 47%.**	**NCT02657889**
		1/2	Recruiting	+ binimetinib in locally advanced or metastatic TNBC		NCT03106415
		2	Recruiting	+ lenvatinib for previously treated solid tumors (LEAP-005)		NCT03797326
		2	Recruiting	+ bemcentinib for previously treated, locally advanced and unresectable, or metastatic TNBC		NCT03184558
		1/2	**Complete**	**+ epacadostat and INCAGN01876 for advanced or metastatic malignancies**	Undisclosed	**NCT03277352**
		1	Recruiting	+ ruxolitinib for metastatic stage IV TNBC		NCT03012230
Camrelizumab	PD-1	3	Recruiting	+ chemotherapy as neoadjuvant therapy for early or locally advanced TNBC		NCT04613674
		1/2	Recruiting	+ RT for early TNBC		NCT04481763
		2	Recruiting	+ nab-PTX and famitinib as a first-line treatment for unresectable locally advanced or metastatic immunomodulatory TNBC (FUTURE-C-PLUS)		NCT04129996
		2	**Complete**	**+ apatinib in advanced TNBC**	**The ORR was 43.3% in the continuous dosing cohort, while no objective response was observed in the intermittent dosing cohort.**	**NCT03394287**

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Table 1 (*continued*)

Drug	Target	Phase	Status	Regime Description	Primary Results	Identifier No.
Nivolumab	PD-1	2	Recruiting	+ ipilimumab as neoadjuvant therapy for early TNBC (BELLINI)		NCT03815890
		2	Recruiting	+ ipilimumab as neoadjuvant therapy for previously untreated and surgically-resectable solid tumor brain metastases		NCT04434560
		2	Recruiting	+ capecitabine as adjuvant therapy for TNBC with residual disease following neoadjuvant chemotherapy (OXEL)		NCT03487666
		2	Recruiting	+ RT and ipilimumab as adjuvant therapy for TNBC with residual disease (BreastImmune03)		NCT03818685
		2	**Complete**	**+ cabozantinib for metastatic TNBC**	**The ORR was 5.6% (missed primary endpoint). The study was closed to further accrual.**	**NCT03316586**
		1	Recruiting	+ TPST-1120 for advanced TNBC		NCT03829436
Toripalimab	PD-1	3	Recruiting	+ nab-PTX for metastatic or recurrent TNBC with or without systemic treatment (TORCHLIGHT)		NCT04085276
HLX10	PD-1	3	Recruiting	+ chemotherapy as neoadjuvant therapy for TNBC		NCT04301739
Budigalimab	PD-1	1	Recruiting	+ carboplatin and ABBV-927 for locally advanced or metastatic TNBC		NCT03893955
Spartalizumab	PD-1	1	Recruiting	+ novel immunotherapy combinations for advanced TNBC		NCT03742349
XmAb®20717	PD-1 CTLA-4	1	Recruiting	as monotherapy for advanced solid tumors		NCT03517488
XmAb®23104	PD-1 ICOS	1	Recruiting	+ ipilimumab for advanced solid tumors (DUET-3)		NCT03752398
Ipilimumab	CTLA-4	-	-	See 'Nivolumab'		-
Tremelimumab	CTLA-4	-	-	See 'Durvalumab'		-
XmAb®22841	CTLA-4 LAG-3	1	Recruiting	+ pembrolizumab for advanced solid tumors (DUET-4)		NCT03849469
LAG525	LAG-3	-	-	See 'Spartalizumab'		-
INCAGN02385	LAG-3	**1**	**Complete**	**as monotherapy for advanced malignancies**	Undisclosed	**NCT03538028**
ABBV-368	OX40	-	-	See 'Budigalimab'		-
INCAGN01949	OX40	**1/2**	**Complete**	**+ nivolumab and ipilimumab for advanced or metastatic malignancies**	Undisclosed	**NCT03241173**
Epacadostat	IDO-1	-	-	See 'Pembrolizumab'		-
INCAGN01876	GITR	-	-	See 'Pembrolizumab'		-
KY1044	ICOS	-	-	See 'Atezolizumab'		-
INCAGN02390	TIM-3	1	Active, not recruiting	as monotherapy for advanced malignancies		NCT03652077
SEA-TGT	TIGIT	1	Recruiting	+ pembrolizumab for advanced TNBC		NCT04254107
COM701	PVRIG	1a/1b	Recruiting	+ nivolumab for advanced solid tumors		NCT03667716
ABBV-927	CD40	-	-	See 'Budigalimab'		-
Utomilumab	CD137	-	-	See 'Avelumab'		-
NC318	Siglec-15	1/2	Recruiting	as monotherapy for advanced or metastatic solid tumors		NCT03665285
NKTR-262	TLR7/8	1/2	Active, not recruiting	+ bempegaldesleukin and nivolumab for locally advanced or metastatic solid tumors (REVEAL)		NCT03435640
BDB001	TLR7/8	2	Not yet recruiting	+ atezolizumab and RT for refractory TNBC		NCT03915678

Abbreviations: BRCA, breast cancer gene; CD137, tumor necrosis factor receptor superfamily member 9; CD40, cluster of differentiation 40; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; GITR, tumor necrosis factor receptor superfamily member 18; ICOS, inducible T cell costimulatory; IDO1, indoleamine 2,3-dioxygenase 1; LAG3, lymphocyte-activation gene 3; ORR, objective response rate; OS, overall survival; OX40, tumor necrosis factor receptor superfamily member 4; pCR, pathological complete response; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PFS, progression-free survival; PTX, paclitaxel; PVRIG, poliovirus receptor-related immunoglobulin domain-containing protein; RT, radiotherapy; TIGIT, T cell immunoreceptor with Ig and ITIM domains; TIL, tumor-infiltrating lymphocytes; TIM3, T-cell immunoglobulin and mucin-domain containing-3; TLR, Toll-like receptors.

dual ICI for advanced TNBC, yet no result is reported. Besides, the combination of nivolumab and ipilimumab is also evaluated as neoadjuvant therapy for early TNBC due to their excellent shrinkage effect on tumors.

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To date, reports regarding acquired resistance to ICI are rare in TNBC. However, judging from the utilization of ICI in melanoma and lung cancer, the resistance of TNBC to ICI is predictable, and a similar situation may be happening in TNBC. For instance, the ORR of ICI is only 5-30% in heavily pre-treated TNBC [55], which means treatment changes the immunologic portrait of a tumor and then exerts an impact on ICI. The mutational landscape of recurrent metastatic TNBC documented such molecular subtype shift from immunomodulatory to basal-like and mesenchymal-like phenotypes, with a suppressed immune activity within the tumor [56]. Thus, resistance to immunotherapy is a compensatory immune escape, currently mainly including down-regulation of targeted checkpoints or antigen presentation and up-regulation of alternative checkpoints (Fig. 2).

The expression of the immune checkpoint is the prerequisite for the efficacy of ICI. The release of IFNγ by T cell response to neoantigen activates the IFNGR/JAK/STAT1/IRF1 pathway to induce cellular PD-L1 and IDO1 expression. In TNBC, this pathway is further promoted by the amplification of *MUC-C* or loss of ELF5-FBXW7 and plays a critical role in the immune escape of TNBC [22, 23]. Data from genetic screening shows *IFNγ, JAK1*, and *STAT1* are highly expressed in tumors treated with ICI, and the disruption in IFNγ/JAK signaling by the loss-of-function mutations in APLNR [59, 65] and JAK [58], allelic loss of *IRF1* [60], and activation of PBAF complex [62] may increase tumor sensitivity to T cell-mediated killing but abrogate the effect of ICI by reducing PD-L1 expression. Such association has been confirmed in patients with melanoma refractory to PD-1 blockade therapy [58], yet there is no report from TNBC patients. Of note, Sceneay, et al. [57] noticed a decreased IFNγ signaling with age that limits the ICI efficacy in aged mice and patients (>65 years) with TNBC. Also, the IFN inducer demonstrates a coordinated effect with anti-PD-1 to induce a sustained immune response against TNBC *in vivo* [66]. These studies suggest a critical role of loss-of-function mutation of the IFN pathway in TNBC immunotherapeutic resistance. However, long-term IFNγ receptor activation may also cause tumor resistance to anti-CTLA4, which is induced by the epigenetic and transcriptomic alternations in IFNγ signaling that initiates a PD-L1-independent immune escape through up-regulating alternative immunosuppressive ligands such as HVEM and galectin-9 [61]. In this case, dual-ICI therapy is necessary.

Downregulation of proteins, such as TAP1/2, CALR, HLA-A, ERAP1, and TAPBP, in the MHC class I antigen-presenting pathway is a key mechanism for TNBC to avoid immune surveillance, especially for recurrent tumors [67]. Although the activation of the IFNγ signaling supports the surface expression of MHC class I, the acquired genetic alterations, such as the loss of *B2M* [58] and amplification of *MEX3B* [63], can still lead to the deficiency in MHC class I function, thereby loss of antigen-processing machinery and resistance to immunotherapy. Likewise, a recent study also identified an association of decreased antigen peptide-loading complex (PLC) components with elevated lncRNA LINK-A in anti-PD-1-resistant TNBC [64]. The authors found that LINK-A deactivates the E3 ubiquitin ligase TRIM71 via a G-protein-coupled signaling-mediated inhibition of phosphatidylinositol-(3,4,5)-trisphosphate and thus promotes K48-polyubiquitination-mediated PLC degradation. To date, the acquired functional mutations in the IFNγ signaling and antigen-presenting pathway that cause resistance to immunotherapy are rarely reported in TNBC, though some resistant phenotypes were noticed in many studies. As these mutations may determine the response to immunotherapy, understanding their alternations not only helps tailored immunotherapy but also helps predict disease outcomes more accurately.

5. Strategies to improve immunotherapy in TNBC

While the immunologic feature of TNBC provides a prerequisite for the use of ICI, the clinical response is still low and possibly due to the lack of patient selection. Analysis of the data from various anti-PD-L1/PD-1 trials suggests that patients with PD-L1$^+$ tumors are more responsive. Notably, in these studies, the methods they used to measure the expression of PD-L1 are different. For example, the Impassion studies only detected PD-L1 on tumor-infiltrating immune cells [7], while the KEYNOTE trials detected PD-L1 in tumor samples (including tumor cells, lymphocytes, and macrophages) [32]. The GeparNuevo study detected PD-L1 in both tumor cells and TILs, and they found that the durvalumab treatment effect remains only significant with PD-L1 detected in tumor cells [40]. Considering that PD-L1$^+$ tumors account for only 20% of TNBCs, the value of PD-L1 as a biomarker for selecting patients suitable for ICI is irreplaceable, despite the differences in its detection location. It is worth noting that some PD-L1$^-$ patients also respond to ICI, which indicates that a single PD-L1 is not enough to thoroughly identify the applicable patients.

The degree of tumor lymphocyte infiltration is closely associated with the prognosis of TNBC patients, as well as response to chemotherapy [68]. Since cytotoxic therapy can cause the immunogenic death of tumor cells, a high degree of tumor lymphocyte infiltration facilitates the host's immune system to recognize neoantigens and kill the tumor. Hence, TIL is a reliable biomarker to predict the response in the context of immuno-chemotherapy. Interestingly, the GeparNuevo study found that the higher level of baseline stromal TILs but not baseline intratumoral TILs predicted the efficacy of durvalumab [40]. This is consistent with a previous study showing that baseline stromal TILs but not the intratumoral TILs were associated with prognosis in TNBC [68]. Although the reasons are still unclear, these studies suggest that stromal TILs can be used with PD-L1 to select TNBC patients with a greater chance of achieving response to ICI.

Tumors with high TMB usually have a high neoantigen load and a microenvironment with high immune cell infiltration. However, analysis of the data from the Cancer Genome Atlas failed to support the role of TMB in predicting response to ICI in all solid cancer types [69]. In TNBC, the potential of TMB as a predictor for ICI response is not conclusive either. The GeparNuevo study demonstrated that TMB could independently predict pCR to neoadjuvant durvalumab + anthracycline taxane-based chemotherapy in early TNBC [70], whereas the IMpassion130 trial showed that TMB was not associated with the clinical benefit of atezolizumab + nab-PTX in advanced TNBC [71]. An important reason for this may be that not all mutations have the same influence on tumor immunogenicity. Compared with non-synonymous mutations that dominate the TMB measurement, indel mutations and splicing mutations generate more neoantigens [72], indicating that the mutation type is more decisive than the mutation number in determining tumor immunogenicity. Therefore, further exploration and utilization of the specific neoantigen-related TMB will be more helpful to predict the response to immunotherapy in solid tumors.

Recent studies stratify the immune subtype of TNBC using a multi-omics method and distinguish TNBC as 'hot' and 'cold' phenotypes. Collectively, a 'hot' tumor contains a lower clonal heterogeneity and high levels of TILs, macrophages, checkpoint molecules, and type 1 IFN signaling, and demonstrates sensitivity to ICI and a good prognosis. A 'cold' tumor is predominantly featured with stromal signatures and quiescent immune activity with a higher burden of mutations (*MYC* and *PTEN/PI3K*) and neoantigen (B7-H4), low level of TILs, and enrichment of immunosuppressive neutrophils or MDSCs, and associates with poor response to ICI [1, 73-75]. These studies not only draw a portrait to help select a subset of TNBCs with better therapeutic outcomes from ICI but also provide a model to identify potential targets that can converse 'cold' TNBC tumors to 'hot' ones. Based on the model, Wang, et al. [76] identified AURKA inhibitors that could promote T cell infiltrating through

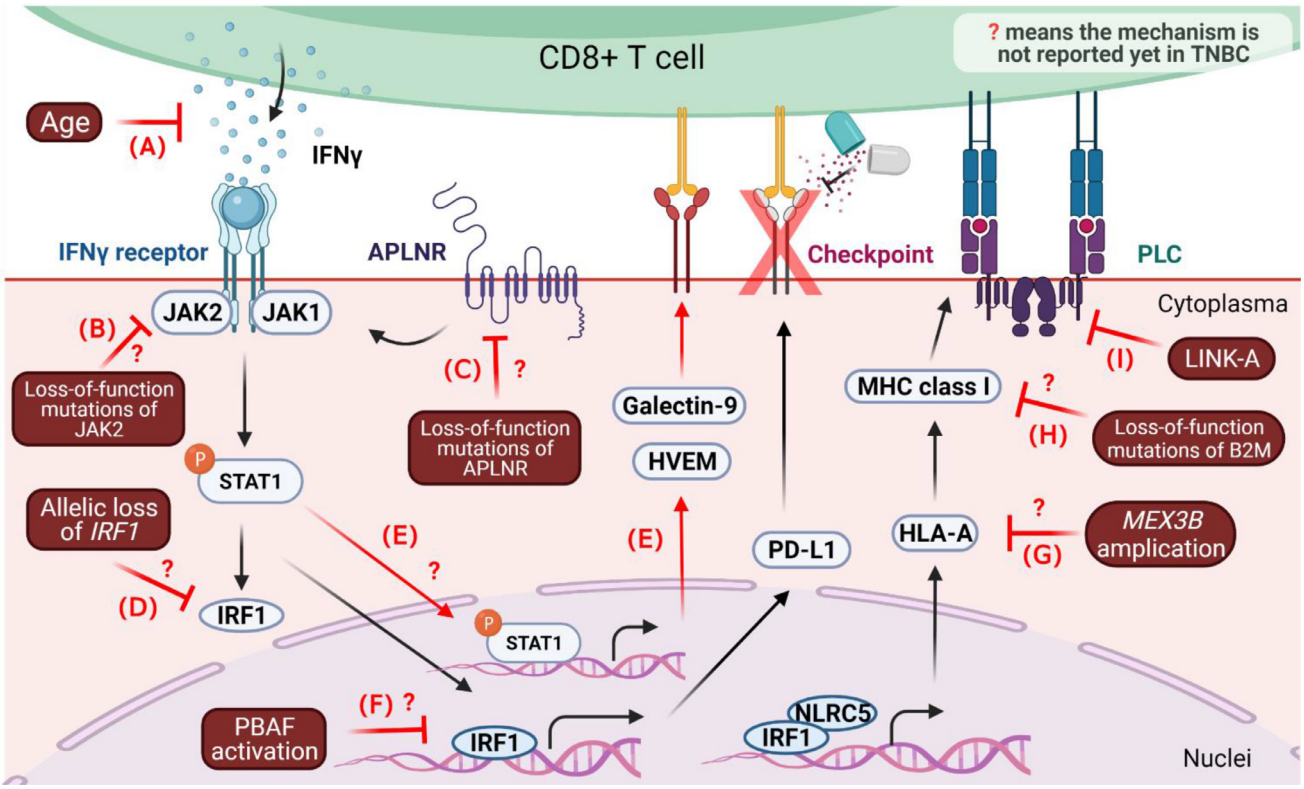

Fig. 2. Potential mechanisms that may cause therapeutic resistance to immune checkpoint blocking in TNBC. The IFNγ signaling is the central pathway to regulate cancer immunity. The activation of the IFNGR/JAK/STAT1/IRF1 by effector CD8+ T cells mediates the immune escape of TNBC by inducing PD-L1 expression. Also, it supports the expression of the MHC class I pathway for antigen presentation. The deficiency in this pathway may compromise the efficacy of ICI by down-regulating the PD-L1 and MHC class I pathways. In addition, prolonged IFNγ signaling may also induce an epigenetic and transcriptome alternation to up-regulating alternative checkpoints, leading to a PD-L1-independent resistance to ICI. **(A)** The IFNγ signaling was found diminished with age in TNBCs from mice and patients (>65 years vs <40 years), which is associated with a 'cold' immune microenvironment that limits the response to anti-CTLA4 and anti-PD-1 [57]. **(B)** Increased JAK in TNBC mediates the IFNγ signaling by activating STAT1. An F547 splice-site mutation in *JAK2* caused loss of JAK2 protein and functional response to IFNγ, which may be associated with acquired resistance to anti-PD-1 therapy [58]. **(C)** Increased apelin-APLNR signaling in obesity-related TNBC directly interacts with JAK1 to promote the IFN-γ response. Multiple loss-of-function mutations (T44S, C181S, P292L, G349E) in APLNR may cause a weak effector function of T cells and abrogate the effect of ICI [59]. **(D)** Allelic loss of IRF1 was found in approximately 32% of BC patients and may cause a lack of PD-L1 and MHC class I pathway expression [60]. **(E)** Prolonged IFNGR signaling may promote STAT1 transcriptional activation that triggers the expression of alternative immunosuppressive ligands for T cells [61]. **(F)** PBAF complex is an epigenetic regulator that controls the chromatin accessibility of the gene promoter for STAT1 and IRF1. Activation of the PBAF complex by *PBRM1* and *ARID2* that encode subunits of the PBAF complex may disrupt the binding of IRF1 and reduce the sensitivity to anti-PD-1/PD-L1 therapy [62]. **(G)** MEX3B is an RNA-binding protein and is often overexpressed in BC. It may reduce the expression of MHC class I by increasing the degradation of the HLA-A transcripts [63]. **(H)** Tumors treated with anti-PD-1 therapy may lose the expression of B2M, which disturbs the expression of MHC class I [58]. **(I)** TNBC with resistance to anti-PD-1 therapy shows an elevated LINK-A expression that down-regulates the expression of PLC components and decreases the antigen presentation activity [64]. **Abbreviations:** APLNR, apelin receptor; B2M, β2-microglobulin; HLA-A, major histocompatibility complex, class I, A; HVEM, herpesvirus entry mediator; IFNγ, interferon-gamma; IFNGR, IFNγ receptor; IRF1, interferon regulatory factor 1; JAK, Janus kinase; MEX3B, Mex-3 RNA binding family member B; MHC class I, major histocompatibility complex, class I; NLRC5, NLR family CARD domain containing 5; PBAF, polybromo-associated BAF; PD-L1, programmed death-ligand 1; PLC, peptide-loading complex. STAT1, signal transducer and activator of transcription 1.

reprograming the tumor immunological phenotype of TNBC [76], which proposes a novel strategy to improve the efficacy of ICI on 'cold' tumors.

On the other hand, other immune-based therapies have been developed over the past few years (Table 2), and many therapies demonstrate promising efficacy against heavily pre-treated TNBC. For example, antibody-drug conjugate sacituzumab govitecan documented a benefit rate of 45.4% in refractory metastatic TNBC and is approved by the FDA in 2019 [77]. Furthermore, the anti-tumor immunity activator that enhances host immune activity to recognize and kill the tumor also plays an increasingly important role in TNBC treatment. These targeted drugs inhibit immunosuppressive neoantigens, such as B7-H4 [78], Nectin-4 [79], EP4, CD11b, and GARP, and resume the anti-tumor immune response in immune-cold TNBC, which will be an important alternative to current immunotherapy in the future. Similarly, immunotherapeutic strategies by using the immune-activating cytokine analog [80], oncolytic virus [81], vaccine [82], and adoptive transfer of immune cells [83] are also under investigation to work in concert with ICI for advanced and metastatic TNBC. Preliminary data have shown the prominent effectiveness of these novel therapies in improving the response of heavily treated TNBC to ICI. Their role in TNBC immunotherapy is emerging.

6. Conclusion

Immunotherapy has brought new hope for effective targeted therapy of TNBC. Immunogenic chemotherapy combined with ICI has shown significant efficacy in the neoadjuvant/adjuvant treatment of TNBC and the late/metastatic stage. Additionally, their combination with other targeted drugs also provides more options for the treatment of heavily pre-treated refractory TNBC. Considering the correlation between TILs, MMR, TMB, or checkpoint expression and the efficacy of ICI, patient selection based on reliable biomarker systems and even immune microenvironment subtypes is necessary to improve the efficacy of ICI.

Table 2
Other immune-based strategies for TNBC.

Drug	Target	Phase	Status	Regime Description	Identifier No.
Antibody-drug conjugate					
Sacituzumab-Govitecan	TROP2	**3**	**Complete**	**monotherapy for metastatic TNBC refractory or relapsing after at least 2 prior chemotherapy (ASCENT)**	**NCT02574455**
		2	Recruiting	+ pembrolizumab for metastatic TNBC	NCT04468061
		1/2	Recruiting	+ atezolizumab for metastatic or inoperable locally advanced TNBC (Morpheus-TNBC)	NCT03424005
SKB264	TROP2	1/2	Recruiting	monotherapy for locally advanced unresectable/metastatic solid tumors who are refractory to available standard therapies (A264)	NCT04152499
SAR566658	CA6	**2**	**Complete**	**monotherapy for CA6+ metastatic TNBC**	**NCT02984683**
Enfortumab Vedotin	NECTIN4	2	Recruiting	monotherapy for previously treated locally advanced or metastatic malignant solid tumors (EV-202)	NCT04225117
Anti-EGFR-immunoliposomes loaded with DOX	EGFR	2	Active, not recruiting	monotherapy for EGFR+ advanced TNBC	NCT02833766
Ladiratuzumab Vedotin	LIV-1	1/2	Recruiting	+ pembrolizumab for first-Line treatment of unresectable locally-advanced or metastatic TNBC	NCT03310957
Anetumab Ravtansine	MSLN	1	Active, not recruiting	monotherapy for MSLN+ advanced or recurrent malignancies (ARCS-Multi)	NCT03102320
Camidanlumab Tesirine	CD25	1	Recruiting	monotherapy for advanced solid tumors	NCT03621982
Rovalpituzumab Tesirine	DLL3	1	Active, not recruiting	+ budigalimab and venetoclax for advanced solid tumors	NCT03000257
Anti-tumor immunity activator					
Oleclumab	CD73	1/2	Recruiting	+ PTX, carboplatin, and durvalumab for previously untreated locally recurrent inoperable or metastatic TNBC (SYNERGY)	NCT03616886
NZV930	CD73	1	Recruiting	+PDR001 and/or NIR178 for advanced cancers	NCT03549000
CPI-006	CD73	1	Recruiting	+ ciforadenant or pembrolizumab for advanced cancers	NCT03454451
LY3475070	CD73	1	Recruiting	+ pembrolizumab for advanced cancer	NCT04148937
NIR178	A2AR	2	Recruiting	+ PDR001 for multiple solid tumors	NCT03207867
AB928	A2AR A2BR	1	Active, not recruiting	+ DOX for advanced metastatic TNBC	NCT03719326
BT8009-100	Nectin-4	1/2	Recruiting	+ nivolumab for Nectin-4+ advanced solid tumors malignancies	NCT04561362
AN0025	EP4	1	Recruiting	+ pembrolizumab for advanced solid tumors	NCT04432857
Naptumomab Estafenatox	5T4	1	Recruiting	+ durvalumab for advanced or metastatic solid tumors	NCT03983954
GB1275	CD11b	1/2	Recruiting	+ pembrolizumab or chemotherapy for metastatic solid tumors	NCT04060342
NKTR-214	CD122	**1/2**	**Complete**	**+ nivolumab and ipilimumab for advanced solid tumors (PIVOT-02)**	**NCT02983045**
ABBV-151	GARP	1	Recruiting	+ budigalimab for locally advanced or metastatic solid tumors	NCT03821935
Chemokine					
Bempegaldesleukin	IL-2	1/2	Active, not recruiting	+ NKTR-262 and nivolumab for locally advanced or metastatic solid tumor malignancies (REVEAL)	NCT03435640
GX-I7	IL-7	1/2	Recruiting	+ pembrolizumab for refractory or relapsed TNBC (KEYNOTE-899)	NCT03752723
NT-I7	IL-7	1/2	Recruiting	+ pembrolizumab for advanced solid tumors (KEYNOTE A60)	NCT04332653
TAVO	IL-12	2	Recruiting	+ pembrolizumab and chemotherapy for inoperable locally advanced or metastatic TNBC	NCT03567720
SO-C101	IL-15	1	Recruiting	+ pembrolizumab for advanced/metastatic solid tumors	NCT04234113
PD-0360324	CSF1	2	Recruiting	+ other immunotherapies for advanced malignancies (JAVELIN Medley)	NCT02554812
IRX 2	IL-2, IL-1β, IL-6, IL-8, TNFα, CSF, and IFNγ	2	Recruiting	+ pembrolizumab and chemotherapy for TNBC	NCT04373031

(*continued on next page*)

Table 2 (*continued*)

Drug	Target	Phase	Status	Regime Description	Identifier No.
Celecoxib, recombinant IFNα-2b, and rintatolimod	-	1	Active, not recruiting	+ pembrolizumab for metastatic TNBC	
Vaccine					
Neoantigen DNA vaccine	DNA	1	Recruiting	+ durvalumab for stage II-III TNBC treated with standard therapy	NCT03199040
Individualized long peptide vaccine	Individualized neoantigens	2	Recruiting	+ nab-PTX, durvalumab, and tremelimumab for metastatic TNBC	NCT03606967
Dendritic cell vaccine	HER2 HER3	2	Not yet recruiting	+ pembrolizumab and celecoxib for brain metastasis of TNBC	NCT04348747
AE37 peptide vaccine	HER2	2	Recruiting	+ pembrolizumab to enhance tumor-specific immune response for TNBC	NCT04024800
PVX-410	XBP1 CD138 CS1	2	Recruiting	+ pembrolizumab and chemotherapy as frontline therapy for HLA-A2+ metastatic TNBC	NCT03362060
P10s-PADRE	CMP P10s	2	Recruiting	+ chemotherapy as neoadjuvant therapy for stage II and III TNBC	NCT02938442
Galinpepimut-S	WT1	1/2	Recruiting	+ pembrolizumab for advanced cancers	NCT03761914
BN-Brachyury poxvirus vaccine	Brachyury	1	Recruiting	+ entinostat, adotrastuzumab emtansine, and M7824 for advanced stage BC (BrEAsT)	NCT04296942
RO7198457	Individualized neoantigens	1	Recruiting	+ atezolizumab for locally advanced or metastatic tumors	NCT03289962
Oncolytic virus					
SBRT	-	2	Active, not recruiting	+ pembrolizumab for metastatic TNBC (STOMP)	NCT03004183
TBio-6517	-	2	Recruiting	+ pembrolizumab for solid tumors (RAPTOR)	NCT04301011
BT-001	-	1/2	Recruiting	+ pembrolizumab for metastatic or advanced solid tumors	NCT04725331
LTX-315	-	1	Complete	+ pembrolizumab or ipilimumab for transdermally accessible tumors	NCT01986426
ONCR-177	-	1	Recruiting	+ pembrolizumab for advanced and/or refractory cutaneous, subcutaneous or metastatic nodal solid tumors or with liver metastases of solid tumors	NCT04348916
Talimogene Laherparepvec	-	**1**	**Complete**	**+ PTX as neoadjuvant therapy for TNBC**	**NCT02779855**
		1	Recruiting	+ ipilimumab and nivolumab as neoadjuvant therapy for TNBC	NCT04185311
		1	Active, not recruiting	+ atezolizumab for TNBC	NCT03256344
NIS-expressing measles virus	-	1	Active, not recruiting	as monotherapy for metastatic BC	NCT01846091
Pelareorep	-	1	Recruiting	+ atezolizumab for early BC (AWARE-1)	NCT04102618
Adoptive transfer of immune cells					
FT516 NK cell	-	1	Recruiting	+ avelumab for advanced solid tumors	NCT04551885
Gene-edited autologous neoantigen-targeted TCR T cell	-	1	Recruiting	+ nivolumab and IL-2 for locally advanced or metastatic solid tumors	NCT03970382

Abbreviations: A2AR, adenosine A2A receptor; CA6, carbonic anhydrase 6; CD11b, integrin alpha M subunit; CD122, IL-2 receptor beta chain; CD138, syndecan-1; CD25, IL-2 receptor alpha chain; CD73, 5′-nucleotidase; CMP P10s, carbohydrate mimetic peptide P10s; CS1, CD2-like receptor-activating cytotoxic cell; CSF, colony-stimulating factor; DLL3, delta-like protein 3; DOX, doxorubicin; EGFR, epidermal growth factor receptor; GARP, glycoprotein A repetitions predominant; HER, human epidermal growth factor receptor; IFNγ, interferon-γ; MSLN, mesothelin; NECTIN4, nectin cell adhesion molecule 4; PTX, paclitaxel; TNFα, tumor necrosis factor-α; TROP2, trophoblast cell-surface antigen 2; WT1, Wilms tumor 1; XBP1, X-box binding protein 1.

Further, the use of *in vivo* and *in vitro* models to explore the mechanism of immune escape and to understand the functional mutations acquired during the formation of immunotherapeutic resistance will not only help better monitor the transformation of the tumor immune microenvironment before and after treatment and predict immunotherapeutic resistance but also help expand the combination of targeted drugs against refractory TNBC. Finally, more immunotherapeutic strategies, such as chemokine regulation, oncolytic viruses, vaccines, and adoptive cell therapy, will give a further boost to the immunotherapy for TNBC.

Declaration of competing interest

The authors declare that they have no conflict of interests.

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