Therapeutic pattern and progress of neoadjuvant treatment for triple-negative breast cancer (Review)

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Abstract. Triple-negative breast cancer (TNBC) is a heterogeneous disease, accounting for about 15.0-20.0% of all breast cancer cases. TNBC is associated with early recurrence and metastasis, strong invasiveness and a poor prognosis. Chemotherapy is currently the mainstay of treatment for TNBC, and achievement of a pathological complete response is closely associated with a long-term good prognosis. Improving the long-term prognosis in patients with TNBC is a challenge in breast cancer treatment, and more clinical evidence is needed to guide the choice of treatment strategies. The current study reviews the conventional treatment modality for TNBC and the selection of neoadjuvant chemotherapy (NACT) regimens available. The research progress on optimizing NACT regimens is also reviewed, and the uniqueness of the treatment of this breast cancer subtype is emphasized, in order to provide reference for the clinical practice and research with regard to TNBC treatment.

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1. Introduction

Breast cancer is the most common cancer among women globally, accounting for 25.0% of all cancer cases, with triple-negative breast cancer (TNBC) accounting for 15.0-20.0% of all breast cancer cases (1). TNBC refers to a subtype of breast cancer that lacks expression of oestrogen receptor (ER), progesterone receptor and human epidermal growth factor receptor 2 (HER2). TNBC is common in premenopausal women and women who are carriers of breast cancer susceptibility gene (BRCA) mutations (2,3). As a special subtype of breast cancer, TNBC is associated with early recurrence and metastasis, strong invasiveness and a poor prognosis (4,5).

Despite the poor prognosis and high aggressiveness of the disease, certain patients with TNBC seem to be particularly sensitive to chemotherapy compared with those with ER-positive breast cancer. In total, 30.0-40.0% of patients with TNBC achieve a pathological complete response (pCR) after neoadjuvant chemotherapy (NACT) (4). Compared with patients with residual disease, patients who reach a pCR after NACT show a good prognosis, and the risk of recurrence is reduced by >70% (6,7). In addition, in the field of TNBC research in recent years, the application of immune checkpoint inhibitors and poly(ADP-ribose) polymerase (PARP) inhibitors has also been actively explored. Several studies and clinical trials have been performed to explore the addition of targeted therapy to chemotherapy for the neoadjuvant treatment of TNBC, but in unselected patients with TNBC, and this treatment strategy has only resulted in limited clinical survival advantages. This may be explained by the molecular heterogeneity of breast cancer subtypes (8). Genomic and transcriptome analysis showed that TNBC includes various subtypes, which are characterized by specific genomic drivers and potential therapeutic targets. Therefore, there are still unmet clinical needs for developing targeted therapies and optimizing treatment strategies for TNBC neoadjuvant therapy.

The present review focuses on the different subtypes of TNBC and the latest research progress of neoadjuvant treatment strategies for TNBC, including platinum drugs, PARP inhibitors, immune checkpoint inhibitors and some emerging targeted therapies, as well as potential biomarkers for predicting the response or resistance of these drugs, which makes this review different to previous reviews.

2. Molecular characteristics and clinicopathological characteristics of TNBC

TNBC is a heterogeneous disease, and there are differences in the sensitivity and prognosis of treatment. The PAM50 microarray set of 50 genes is used to identify breast cancer intrinsic subtypes. A set of 374 TNBC samples taken from 14 microarray datasets was analyzed to characterize TNBC subtypes using PAM50 (9). Lehmann *et al* (10) divided TNBC into six subtypes according to different gene expression: Basal-like (BL1 and BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like (MSL) and luminal androgen receptor type (LAR). Basal-like subtypes account for 70.0-80.0%, and are characterized by a high chromosomal recombination rate, poor gene stability and vulnerability to BRCA1/2 mutations. The BL1 type cell cycle and DNA damage-related genes were highly expressed, and were sensitive to chemotherapy, especially DNA-damaging drugs. The BL2 type is usually overexpressed by growth factor receptors and may be less sensitive to chemotherapy. The M and MSL subtypes are rich in epithelial-mesenchymal transition (EMT) mechanism-related pathways, often with PIK3CA mutations, and tyrosine kinase inhibitors and PIK3/mTOR inhibitors may be effective. The LAR subtype expresses the androgen receptor (AR), is sensitive to AR antagonists and has a relatively good prognosis. The IM subtype expresses genes related to immune cell processing and is considered to be the most valuable subtype in immunotherapy (10). Burstein *et al* (11) used a non-negative matrix factorization method to derive a panel consisting of 80 core genes that divided TNBC into four subtypes, luminal-AR (LAR), mesenchymal (MES), basal-like immune-suppressed (BLIS) and basal-like immune-activated (BLIA). Liu *et al* (12) performed mRNA and long non-coding RNA expression analysis in 165 TNBC tumour samples at Fudan University Shanghai Cancer Centre. The tumour samples were categorized into four subtypes (IM, LAR, MES and BLIS subtypes), consistent with the number of classification by Burstein *et al* (11) (IM is not included).

Patients with TNBC mainly have the following clinicopathological characteristics: Relatively young age of onset; large primary mass; high TNM stage (13); high histological grade, generally grade III; poorly differentiated, with high proliferation; most of the pathological types are invasive ductal carcinoma; and easily transferred to the liver, lungs and central nervous system (14); insensitive to hormones and targeted therapies (except for BRCA mutations), and resistant to chemotherapy; extremely aggressive, with a high recurrence rate; long-term prognosis is worse than other subtypes of breast cancer, and the median survival time of patients with recurrence and metastasis is ~9.6 months; the 5-year survival rate is only 14.0% and TNBC accounts for ~25.0% of breast cancer deaths (4,5).

3. Conventional mode of treatment for TNBC

The traditional treatment for TNBC includes surgery, radiotherapy and chemotherapy, and new treatment methods have been explored to improve the survival rate and prognosis of patients, such as targeted therapy and immunotherapy. Tumor resection and mastectomy are traditional surgical procedures performed on patients with TNBC, and adjuvant radiotherapy and chemotherapy are usually required after surgery (15). The neoadjuvant therapy given before surgery can help reduce the tumor burden and achieve the goal of breast preservation (16). Simply performing chemotherapy earlier does not improve the survival of patients, but prognostic information can be obtained, and the prognosis of patients with a pCR is improved. Exploring TNBC optimized neoadjuvant chemotherapy to increase the pCR rate is an effective way to improve the prognosis of patients. At the same time, follow-up intensive adjuvant treatment for patients who have not reached pCR can further reduce the risk of recurrence and improve the prognosis (17).

NACT is the standard and preferred treatment for stage II-III TNBC (18-20). Achieving a pCR after NACT is an important prognostic factor with a good long-term prognosis; it is characterized by no residual invasive tumor cells in the pathological examination of the primary breast and axillary lymph node surgical specimens, which is associated with a reduced risk of recurrence and death (7,21). Studies (22,23) have confirmed that compared with Luminal and HER2 overexpression breast cancer types, TNBC is more sensitive to NACT. The prognosis of those who achieve a pCR after NACT is significantly improved, and their 5- and 10-year relapse-free survival (RFS) rates can reach 89.0 and 86.0%, while those with obvious residual lesions have a poor prognosis, and their 5- and 10-year RFS rates are 62.0 and 55.0% (24,25). In addition to achieving the purpose of reducing TNM stage and breast preservation, NACT also has the advantages of evaluating drug sensitivity and obtaining prognostic related information.

Taxanes and anthracyclines form the current standard of care for TNBC in the neoadjuvant setting. Paclitaxel and docetaxel are familiar examples of taxanes used in the first line of therapy (26). The pCR rate in patients with TNBC receiving anthracycline combined with taxane neoadjuvant chemotherapy is 28.0-35.0%, which is better than that for the anthracycline-only regimen (pCR rate, ~20.0%) or taxane single-agent chemotherapy (pCR rate, ~12.0%) (6,25). Regarding the role of nab-paclitaxel in TNBC, the available evidence is not conclusive, with the phase III GeparSepto trial suggesting a pCR benefit for nab-paclitaxel over paclitaxel in the TNBC subgroup (27), and the subsequent phase III ETNA trial conversely failing to formally establish the superiority of this agent (28).

4. Research progress in neoadjuvant therapy for TNBC

Although TNBC has high sensitivity to anthracyclines and taxanes, numerous patients are prone to drug resistance after treatment (23). In the further exploration of more effective chemotherapy regimens, it was found that platinum-containing chemotherapy regimens have significant efficacy in

neoadjuvant chemotherapy for TNBC, and have good safety and tolerability (29).

Platinum-based chemotherapy. After platinum drugs enter tumor cells, they can break DNA double-strands and cause cell death, which has a prominent effect on tumors with DNA repair barriers. BRCA genes play an important role in maintaining the double-stranded structure of DNA. BRCA mutations (deletion or inactivation) cause damage to the DNA repair mechanism. As 15.0-25.0% of TNBC cases have BRCA1/2 mutations, researchers at home and abroad have begun to explore the role of platinum drugs in neoadjuvant chemotherapy for TNBC (30-32). Platinum drugs can lead to 70.0-90.0% of patients with BRCA1 mutant TNBC achieving a pCR. For TNBC without BRCA1, the pCR rate of carboplatin combined with taxanes is still very high (56.0%) (33,34).

In the field of TNBC neoadjuvant therapy research, a series of phase II clinical studies has shown that platinum-based chemotherapy may bring survival benefits. The most important randomized controlled trials (RCT) are the GEICAM/2006-03 (35), GeparS6xto (36) and CALGB40603 trials (37) (Table I). The GeparSixto 66 study (36,38) is a phase II RCT of neoadjuvant chemotherapy with a carboplatin-containing regimen. Carboplatin was added to the combination of paclitaxel, doxorubicin liposomes and bevacizumab. The results showed that in the TNBC subgroup, the pCR rate increased from 36.9 to 53.2%, and the disease-free survival (DFS) rate, with a median follow-up time of 35 months, also increased significantly (76.1 vs. 85.8%). The CALGB 40603 study (37) is a phase II RCT for TNBC. Carboplatin was added to a paclitaxel regimen that was with or without bevacizumab. After the sequential doxorubicin and cyclophosphamide (AC) regimen, the pCR rate was increased from 41.0 to 54.0% There was no survival benefit after 39 months of follow-up. In the aforementioned two studies, bevacizumab was added to the chemotherapy. Although the four-drug combination or sequential treatment increased the pCR rate, hematological adverse reactions also increased significantly, and the survival benefit was uncertain. The GEICAM/2006-03 study (35) randomly assigned 94 patients to receive epirubicin combined with cyclophosphamide (EC) and sequential docetaxel, or EC and sequential docetaxel combined with carboplatin. Unlike the aforementioned two studies, there was no difference in the reported pCR rates (35.0 vs. 30.0%; P=0.606). In this study, only patients with basal-like TNBC (defined as hormone receptor-negative/HER2-negative and cytokeratin 5/6- or EGFR-positive) were included, and the lower dose of docetaxel (75 mg/m^2) and the higher dose of docetaxel (100 mg/m^2) in the platinum-free chemotherapy group were included. Unlike the GeparSixto and CALGB40603 trials, the patients participating in the GEICAM/2006-03 trial were treated with cyclophosphamide before receiving platinum-based chemotherapy. Moreover, basal-like TNBC seems to be more difficult to treat and is usually resistant to standard chemotherapy. On the other hand, it cannot be ruled out that previous treatment with the DNA disrupting agent cyclophosphamide may reduce the possibility of adding platinum drugs to standard NACT. These two factors can partly explain why the addition of platinum in this trial had no effect on the pCR rate (39).

The BrighTNess (40) and GeparOcto (41) studies, which are phase III trials, also studied the role of platinum in the neoadjuvant treatment of TNBC (Table I). The BrightTNess trial (40) randomly assigned 634 patients with TNBC to receive paclitaxel combined with carboplatin and veliparib (VC) sequential AC, paclitaxel combined with carboplatin sequential AC, or paclitaxel sequential AC. The BRCA status was the stratification factor, and the primary end point was pCR. The pCR rate of patients treated with paclitaxel combined with VC was significantly higher than that of patients treated with paclitaxel alone (53.0 vs. 31.0%; P<0.0001). Compared with paclitaxel alone, patients receiving paclitaxel and carboplatin also had a higher pCR rate (58.0 vs. 31.0%) (40). The GeparOcto 84 study (41) was a phase III RCT designed based on the GeparSixto 66 study. The GeparOcto 84 study compared the three-drug combination of paclitaxel, doxorubicin liposomes and carboplatin with epirubicin, paclitaxel and cyclophosphamide. The effectiveness and safety of the sequential regimen of neoadjuvant chemotherapy showed that the pCR rates in the TNBC subgroup (403 cases) were similar, 51.7 and 48.5% (P=0.584), and the adverse reactions in the two groups were severe.

The meta-analysis published in 2018 (39) included 9 RCTs with stage II to III platinum-containing regimens of neoadjuvant chemotherapy, including the aforementioned phase II and III clinical studies, with a total of 2,109 TNBC patients. 7 of the RCTs added carboplatin to anthracycline and taxane chemotherapy. The results showed that platinum-containing regimens could increase the pCR rate of TNBC neoadjuvant chemotherapy (52.1 vs. 37.0%, P<0.001), but that there was no obvious survival benefit. Moreover, complications such as grade 3 to 4 neutropenia (53.1 vs. 23.2%; P=0.002), grade 3 to 4 anemia (10.8 vs. 0.4%; P<0.001) and grade 3 to 4 thrombocytopenia (11.0 vs. 1.0%; P<0.001) occur. For unselected TNBC patients, adding carboplatin to anthracycline and taxane neoadjuvant chemotherapy can increase the pCR rate, but the adverse reactions also increase significantly, and survival does not improve, so the clinical application is limited.

Potential predictive biomarkers for platinum-based chemotherapy in TNBC. Due to the different chemotherapy regimens used by NACT and the differences in patient characteristics, for some patients who are not sensitive to chemotherapy drugs, neoadjuvant chemotherapy may delay their treatment. Thus, exploring and evaluating the efficacy of predictors for NACT and how to use associated indexes to predict the efficacy of neoadjuvant chemotherapy has become a very important issue. It is important to be able to identify the patients who benefit the most from NACT and implement the chemotherapy regimen with the highest probability of obtaining a pCR.

The GeparSixto (36) and BrighTNess (40) trials reported the pCR rate of platinum added based on BRCA status. There were 50 (17.2%) patients with BRCA mutation in the GeparSixto study and 46 (14.5%) in the BrightTNess trial. A previous meta-analysis of the pCR rates of these 96 patients with BRCA mutation and another 513 patients with the BRCA wild-type (wt) showed that among the patients with the BRCA mutation, 54 out of 96 patients (56.3%) achieved a pCR [29/50 patients (58.0%) in the platinum-containing chemotherapy group and 25/46 patients (54.3%) in the platinum-free

Table I. Neoadjuvant randomized trials exploring the addition of carboplatin in TNBC.

Clinical trial	Phase	TNBC cases, n	TNBC treatment arms	TNBC pCR	TNBC survival rates
GeparSixto	II	158 vs. 157	P + Dox + Bev + Cp vs. P + Dox + Bev (P 80 mg/m^2 qw x 18 + liposomal Dox 20 mg/m^2 qw x 18 + Bev 15 mg/kg q3w x 6 ± Cp AUC 2 qw x 18)	53.2 vs. 36.9%; P=0.005	35-month DFS: 76.1 vs. 85.8%; P=0.035
CALGB 40603	II	225 vs. 218	P + Cp ± Bev; ddAC vs. P ± Bev; ddAC (P 80 mg/m^2 qw x 12 ± Cp AUC 6 3w x 4; Dox 60 mg/m^2 + CTX 600 mg/m^2 q2w x 4, Bev 10 mg/kg q2w x 9)	54 vs. 41%; P=0.0029	39-month EFS: HR, 0.84; P=0.36; OS: HR, 1.15; P=0.53
GEICAM/2006-03	II	48 vs. 46	EC: T + Cp vs. T (EPI 90 mg/m^2 + CTX 600 mg/m^2 q3w x 4; T 100 mg/m^2 q3w x 4) vs. (EPI 90 mg/m^2 + CTX 600 mg/m^2 q3w x 4; T 75 mg/m^2 q3w x 4)	30 vs. 35%; P=0.606	NA
BrighTness	III	316 vs. 160 vs. 158	A: Veliparib (50 mg orally bid) + Cp AUC 6 q3w x 4+ P 80 mg/m^2 qwx12; AC (Dox 60 mg/m^{2+}CTX 600 mg/m^2 q2w or q3w x 4); B: Placebo + Cp AUC 6 q3w x 4 + P80 mg/m^2 qwx12; AC (Dox 60 mg/m^2 + CTX 600 mg/m^2 q2w or q3w x 4); C: Placebo + P 80 mg/m^2 qwx12; AC(Dox 60 mg/m^2 + CTX 600 mg/m^2 q2w or q3w x 4)	A: 53%; B: 58%; C: 31%; P<0.0001 (B vs. C); P<0.0001 (A vs. C)	NA
GeparOcto	III	203 vs. 200	PDoxCp vs. ddEPC (P 80 mg/m^2 + liposomal Dox 20 mg/m^2 + Cp AUC 1.5 qw x 18) vs. (EPI 150 mg/m^2 q2w x 3; P 225 mg/m^2 q2w x 3; CTX 2,000 mg/m^2 q2w x 3)	51.7 vs. 48.5%; P=0.584	NA

P<0.05 shows the success of the trials as indicated in the studies. TNBC, triple-negative breast cancer; pCR, pathological complete response; P, paclitaxel; M, non-pegylated liposomal doxorubicin; Cp, carboplatin; T, docetaxel; Dox, doxorubicin; Bev, bevacizumab; CTX, cyclophosphamide; EPI, epirubicin; AC, doxorubicin and cyclophosphamide; EC, epirubicin and cyclophosphamide; dd, dose dense; qw, every week; EFS, event-free survival; OS, overall survival; NA, No answer.

chemotherapy group; OR, 1.17; 95% CI, 0.51-2.67; P=0.711]. Among the patients with the BRCA-wt, 230 out of 513 patients (44.8%) achieved a pCR [146/256 (57.0%) in the platinum-containing chemotherapy group and 84/257 (32.7%) in the platinum-free chemotherapy group; OR, 2.72; 95% CI, 1.71-4.32; P<0.001] (42). Therefore, whether platinum drugs are added or not, the pCR rate after NACT is generally higher in patients with BRCA mutation. Moreover, in patients with BRCA-mutated breast cancer, paclitaxel combined with carboplatin, sequential anthracyclines and cyclophosphamide seem to be non-contributory to the increase in pCR rate. By contrast, the benefit is significant in patients without BRCA mutations, among which the pCR rate is lower compared with BRCA-mutated patients.

Furthermore, the homologous recombination deficiency (HRD) status has been studied as a predictor of platinum drug response. The pooled analysis of five phase II studies (43) included 166 patients with TNBC who received platinum-based

NACT, and estimated the pCR rates based on HRD status. The HRD status is defined as high if the score is ≥42 points and/or in the presence of BRCA1/2 tumor mutations. Patients with HRD tumors are more likely to achieve a pCR than patients with non-HRD tumors (44.0 vs. 8.0%; P<0.01). This pooled analysis showed that HRD status can be used to identify patients with TNBC who are highly likely to obtain a pCR through platinum-based NACT. An exploratory analysis in the GeparSixto trial (44) evaluated the HRD status of 193 of the 315 (61.3%) TNBC participants using formalin-fixed and paraffin-embedded tumor samples. The HR defects were defined as HRD scores ≥42 and/or if there was a tumor with BRCA mutation. Of the 193 patients with TNBC, 136 (70.5%) had detectable homologous recombination defects, of which 82 (60.3%) showed high HRD scores but no BRCA mutations. A high HRD score independently predicted the pCR rate (OR, 2.60; 95% CI, 1.26-5.37; P=0.008). In fact, the addition of carboplatin to paclitaxel and non-pegylated liposomal Adriamycin significantly increased the pCR rate in HRD tumors (33.9 vs. 63.5%; P=0.001), but not in non-HRD tumors (20.0 vs. 29.6%; P=0.540; test for interaction, P=0.327). In addition, in patients with high HRD scores but no BRCA mutations, the pCR rate of carboplatin was also higher than that of patients without carboplatin (63.2 vs. 31.7%; OR, 3.69; 95% CI, 1.46-9.37; P=0.005). Although this study did not conclude a survival analysis, the addition of carboplatin showed disease-free survival time (DFS) improvement in both HRD tumors (HR, 0.44; 95% CI; 0.17-1.17; P=0.086) and non-HRD tumors (HR, 0.49; 95% CI; 0.23-1.04; P=0.059), but the difference was not statistically significant (40).

The potential predictive role of HRD status on treatment selection was demonstrated in the study by Jiang *et al* (45), which showed that patients with high HRD scores may benefit substantially from DNA-damaging therapies, such as platinum-based chemotherapy. The vast majority of tumors with high HRD consist of the BLIS transcriptional subtype, representing a subgroup of TNBC with a poor prognosis. Basal-like and immune-suppressed patients generally had higher HRD scores than those with other subtypes, independent of BRCA1/2 germline mutations. The BLIS subtypes were further divided into high-HRD BLIS and low-HRD BLIS subgroups, and the patients with low-HRD BLIS tumors had a worse prognosis than those with high-HRD scores (5-year RFS rate, 73.0 vs. 95.0%; P=0.002). High-HRD TNBC metastases were highly sensitive to platinum-based chemotherapy (45). By contrast, in the metastatic setting, the TNT trial did not show a difference in objective response rate (ORR), progression-free survival time (PFS) and overall survival time (OS) between carboplatin and docetaxel according to HRD status. The trial was designed to compare the activity of docetaxel with carboplatin, not the addition of carboplatin to standard chemotherapy (46).

5. Targeted TNBC therapies newly approved by the Food and Drug Administration (FDA)

The innovation of genetic testing technology and the development of new targeted drugs have brought new hope to patients with TNBC. Currently, the screening of eligible populations for TNBC targeted therapy based on biomarkers has become a research hotspot.

Poly(ADP-ribose) polymerase (PARP)-inhibitors. PARP is involved in cell DNA single-strand damage repair, repairing DNA damage through base excision. For tumors with DNA repair disorders (such as BRCA mutations), PARP inhibitors inhibit tumor growth through 'synthetic lethality' effects (47).

The application of PARP inhibitors in neoadjuvant therapy is gradually developing. A series of studies have explored the efficacy of PARP inhibitors in neoadjuvant treatments of TNBC, such as monotherapy or combination chemotherapy (Table II). The GeparOLA Phase II study (48), reported at the 2019 ASCO meeting, compared the efficacy of olaparib or carboplatin for neoadjuvant treatment of BRCA-mutated and/or homologous recombination deficiency (HRD) high-score HER2-negative early breast cancer. In this study, 102 patients (72.6% for TNBC and 60.4% for BRCA mutations) were randomly combined with olaparib or carboplatin on the basis of paclitaxel sequential epirubicin and cyclophosphamide (P-EC) neoadjuvant chemotherapy. The results showed that in the total population, the pCR rates of the paclitaxel plus olaparil group and the paclitaxel plus carboplatin group were 55.1 and 48.6%, respectively; in the TNBC subgroup, the pCR rates of the paclitaxel plus olaparil group and the paclitaxel plus carboplatin group were 56.0 and 59.3%, respectively, were not significantly different. The study found that patients <40 years old, who were HR-positive, with a high HRD score and no BRCA mutation were more likely to benefit from the combination treatment with olaparib. A study (48) suggested that in patients with BRCA mutations or high HRD HER2-negative breast cancer, PARP inhibitors have the same effect as platinum. To the best of our knowledge, there has been no comparison between PARP inhibitors and platinum single drugs.

The I-SPY2 trial (49) added veliparib and carboplatin (VC) to the standard neoadjuvant chemotherapy (paclitaxel sequential AC) regimen, and the pCR rate of the TNBC subgroup increased from 26.0 to 51.0%, among which BRCA1 mutation had a good response to V-C (P=0.023) (50). However, due to the limitations of the trial design, it was impossible to speculate whether the benefits came from veliparib, carboplatin or the synergy of the two. The BrighTNess Phase III clinical study (40) identified the source of benefit in the I-SPY-2 trial. The study included a total of 634 TNBC patients, 15.0% of which had BRCA mutations, who were randomly divided into three groups: Neoadjuvant chemotherapy (P-AC), chemotherapy plus carboplatin, and chemotherapy plus veliparib and carboplatin. The results showed that the pCR rates of the three groups were 31.0, 58.0 and 53.0%, respectively, while the pCR rates of the BRCA mutants were 41.0, 50.0 and 57.0%, respectively. The pCR of the paclitaxel + carboplatin + veliparib group was significantly higher than that of the paclitaxel single-agent group (53.0 vs. 31.0%; P<0.0001). However, there was no significant difference compared with the paclitaxel combined with carboplatin group (58.0%; P=0.36), indicating that the increase in pCR was due to the carboplatin rather than the veliparib. This suggests that carboplatin can increase the pCR rate of TNBC neoadjuvant chemotherapy, and that further combination with PARP inhibitors has no synergistic effect. Lastly, there appears to be no obvious additional benefits in patients with BRCA mutations.

Table II. Neoadjuvant clinical trials with PARP-inhibitors in TNBC.

Clinical trial	Phase	PARP-inhibitors	TNBCs, n	TNBC treatment arms	Primary endpoint	pCR	P-value
PrECOG 0105	II (single arm)	Iniparib	80	Cp + Gmz + iniparib	pCR	36%	-
GeparOLA	II	Olaparib	50 vs. 27	P + olaparib; EC; P + Cp; EC	pCR	56 vs. 59.3%	NA
I-SPY2	II	Veliparib	72 vs. 44	P + V + Cp; AC; P; AC	pCR	51 vs. 26%	NA
Brightness	III	Veliparib	316 vs. 160 vs. 158	A: Veliparib + Cp + P; AC; B: Placebo + Cp + P; AC; C: Placebo + P; AC	pCR	53 vs. 58 vs. 31%	0.36 (A vs. B) <0.0001 (A vs. C)

P<0.05 shows the success of the trials as indicated in the studies. TNBC, triple-negative breast cancer; pCR, pathological complete response; CT, chemotherapy; Cp, carboplatin; Gmz, gemcitabine; P, paclitaxel; NA, no answer; EC, epirubicin and cyclophosphamide; V, veliparib; AC, doxorubicin and cyclophosphamide.

In summary, PARP inhibitor neoadjuvant treatment of BRCA-mutated TNBC has a significant effect, and there is no additional benefit in combining it with platinum-containing chemotherapy. However, most of the existing data are the results of phase II studies, and further research and exploration are needed in terms of survival benefits, safety and target population selection.

Potential predictive biomarkers for use of PARP-inhibitors in TNBC. It is also important to explore specific biomarkers to enable screening for the population that will benefit from PARP inhibitors. This would enable PARP inhibitors to bring survival benefits to more patients with TNBC.

The PrECOG 0105 (51), I-SPY2 (49), and BrightTness trials (40) explored BRCA mutation status and HRD score as predictors of response to PARP inhibitors. In the PrECOG 0105 trial, 19 patients (24.0%) had germline BRCA1/2 mutations. The pCR rates of TNBC in BRCA1/2-wt patients, BRCA1/2 mutation carriers and BRCA1/2 mutation carriers were 33.0, 47.0, and 56.0%, respectively. The HRD phenotype was evaluated using HRD-loss of heteroygosity (LOH) in the preconditioning core breast core biopsy. Regardless of the BRCA1/2 mutation status, the presence of HRD was associated with a higher response rate. In fact, the average HRD-LOH score of responders was higher than that of non-responders (P=0.02), and this factor was still significant when BRCA1/2 germline mutation carriers were excluded (P=0.021). This study showed for the first time that the HRD-LOH in TNBC may be able to distinguish responders from non-responders after administration of platinum-based and PARP inhibitor-based treatments. HRD-LOH allows the identification of TNBC BRCA1/2-wt sporadic patients, and an elevated HRD-LOH score can indicate good pathological remission (49).

In the I-SPY2 (49) and BrightTNess (40) trials, a series of exploratory analyses evaluated potential predictors of response to PARP inhibitors. In the I-SPY2 trial, only a few patients had BRCA mutations: 12 (17.0%) patients in the veliparib/carboplatin group and 2 (5.0%) patients in the control group. In the veliparib/carboplatin group, BRCA mutation carriers were more likely to achieve a pCR compared with wild-type patients (75.0 vs. 29.0%; OR, 7.25; P=0.006), but the mutation rate was too low to compare with the control group response (49). In the BrightTNess trial, ~15.0% of patients had BRCA mutations. Although a benefit of adding veliparib plus carboplatin or carboplatin alone was observed compared with the standard group (P-AC), no difference in pCR rate was observed in the mutant BRCA population between the treatment groups (40).

In the GeparOLA trial (48), which included patients with high TNBC and luminal HRD scores, the BRCA mutation subgroup had a higher pCR rate. The pCR rate of patients with BRCA mutation in the carboplatin group was higher than that of BRCA-wt patients (51.7 vs. 37.5%), while the pCR rate in the olaparib group was comparable between the BRCA mutation and BRCA-wt subgroups (59.0 vs. 57.1%) (48).

Immune checkpoint inhibitors. TNBC has a high tumor mutation burden (TMB), programmed cell death 1 ligand 1 (PD-L1) expression, and a number of tumor-infiltrating lymphocytes (TILs). These characteristics suggest that TNBC may be sensitive to immunotherapy (52,53).

Several studies have explored the application of immune checkpoint inhibitors in neoadjuvant therapy (Table III). KEYNOTE-173 is a multi-cohort phase Ib study for patients with locally advanced TNBC. The trial investigated different doses and schedules of platinum and taxanes combined with pembrolizumab, followed by AC with pembrolizumab. The overall pCR rate was 60%, indicating that pembrolizumab combined with chemotherapy had good antitumor activity and controllable toxicity (54). In this trial, both TILs and PD-L1 combined positive score were significantly associated with higher pCR and ORR and with each other (55). The I-SPY2 trial randomized 69 HER2-negative patients to receive paclitaxel ± pembrolizumab sequential AC weekly. In 29 patients with TNBC, pembrolizumab increased the original and estimated pCR rates by >50 and 40.0%, respectively. The original and estimated pCR rates in the pembrolizumab group were 71.0 and 62.0%, respectively, while those of the control group were 19.0 and 22.0%, respectively (56).

The GeparNew Phase II trial studied the use of nab-paclitaxel followed by EC combined with durvalumab/placebo in

Table III. Neoadjuvant clinical trials with immune checkpoint inhibitors in TNBC.

Clinical trial	Phase	Immunotherapy drug	TNBC treatment arms	Primary endpoint	pCR	P-value
Keynote-173	Ib (6 cohorts)	Pembrolizumab	A: Pembro; Pembro + Nab-pac; Pembro + AC; B: Pembro; Pembro + Cp + Nab-pac; Pembro + AC; C-D-E-F: Pembro; Pembro + different doses and schedules of Cp and taxanes; Pembro + AC	Safety and phase II dose; key efficacy endpoint: pCR	A: 60%; B: 90%; overall: 60%	-
I-SPY2	II	Pembrolizumab	P + Pembro; AC; P; AC	pCR	71.4 vs. 62.4 vs. 19.2 vs. 22.3%	NA
GeparNew	II	Durvalumab	Durva; Durva + Nab-pac; Durva + AC	pCR	53.4 vs. 44.2%	0.224
KEYNOTE-522	III	Pembrolizumab	Pembro + P + Cp; Pembro + AC; P + Cp; AC	pCR and DFS	64.8 vs. 51.2%	0.00055
NeoTRIP	III	Atezolizumab	Atezo + Nab-pac + Cp	DFS	43.5%	NA
IMPassion 031	III	Atezolizumab	Atezo + Nab-pac; AC	pCR	57.6%	0.0044

P<0.05 shows the success of the trials as indicated in the studies. DFS, disease-free survival; pCR, pathological complete response; TNBC, triple-negative breast cancer; Pembro, Pembrolizumab; Nab-pac, nab-paclitaxel; AC, doxorubicin and cyclophosphamide; Cp, carboplatin; P, paclitaxel; Durva, Durvalumab; Atezo, Atezolizumab; NA, no answer.

patients with early TNBC (57). The patients were randomized to receive durvalumab/placebo monotherapy (window period) 2 weeks before the start of chemotherapy, and then received durvalumab/placebo plus albumin-bound paclitaxel followed by durvalumab/placebo plus EC treatment. Compared with patients who received chemotherapy alone, patients who received durvalumab achieved a higher pCR rate, although this was not statistically significant (53.4 vs. 44.2%; OR, 1.45; 95% CI, 0.80-2.63; P=0.224). Subgroup analysis showed that patients with the highest expression of TILs had the best results and durvalumab benefited them the most. The study indicated that durvalumab seems to increase the pCR rate, especially in patients with a high rate of lymphocyte infiltration in tumors (57).

KEYNOTE-522 is the first prospective randomized placebo-controlled phase III trial to show the benefits of adding pembrolizumab to TNBC in the early stages of neoadjuvant therapy. A total of 1,174 patients were enrolled and randomly received carboplatin + paclitaxel ± pembrolizumab at a ratio of 2:1, followed by AC/EC ± pembrolizumab. The pCR rate and event-free survival (EFS) were the common primary endpoints. In the first interim analysis, the addition of pembrolizumab had a statistically significant (and clinically significant) pCR rate increase of 13.6% (64.8 vs. 51.2%; P=0.00055). The benefit of pembrolizumab had no link to PD-L1 status. The pCR rate of the PD-L1-positive group increased by 14.2% (68.9 vs. 54.9%) and that of the PD-L1-negative group increased by 18.3% (45.3 vs. 30.3%). The latest research data showed that with a median follow-up of 39 months, the pembrolizumab group reduced the risk of EFS events by 37.0% (HR, 0.63; P=0.00031) compared with the chemotherapy-placebo regimen (58). However, the phase III NeoTRIP clinical trial concluded that the addition of atezolizumab (1.200 mg) intravenous injection infusion every 3 weeks to nab-paclitaxel (125 mg/m^2) and carboplatin (AUC 2) given intravenous injection on day 1 and day 8 every 3 weeks for a total of 8 cycles. Similar to the KEYNOTE-522 trial, it did not significantly increase the pCR in the patient population (43.5 vs. 40.8%) (59). Nevertheless, the main goal of NeoTRIP research is DFS (not yet reached), which is not the same as evaluating the pCR in the GeparNew and KEYNOTE-522 trials. In addition, the NACT regimen is different between clinical trials, excluding the neoadjuvant anthracyclines and cyclophosphamide in the NeoTRIP trial, both of which are chemotherapeutics with considerable immunogenicity.

The results of the Impassion 031 trial were announced at ESMO 2020 (60). Impassion031 was a phase III, double-blind, randomized, multi-centre, placebo-controlled study, which enrolled TNBC patients with cT2-4N0-3 disease (primary tumor size >2 cm) and no previous systemic treatment. A total of 333 patients were randomized to receive NACT combined with atezolizumab or placebo at a ratio of 1:1. The immunization regimen was 840 mg atezolizumab every 2 weeks, while the chemotherapy regimen was 125 mg/m^2 albumin paclitaxel every week for 12 weeks and sequential doxorubicin (60 mg/m^2) + cyclophosphamide (600 mg/m^2) every 2 weeks for 8 weeks. This was followed by surgical treatment. After surgery, the immunotherapy group was administered 11 doses of atezolizumab every 3 weeks. The pCR rate in the atezolizumab plus chemotherapy group was 57.6%, while that in the placebo-chemotherapy group was 41.0%, and the results were statistically significant (P=0.0044). In the PD-L1-positive population, the pCR rate increased by 19.5% (68.8 vs. 49.3%), and in the PD-L1 negative population, the pCR rate increased by 14% (48.0 vs. 34.0%).

Potential predictive biomarkers for use of immune checkpoint inhibitors in TNBC. Immune checkpoint inhibitors have entered clinical practice as a first-line or second-line treatment for a variety of cancer types. However, it is still a challenge to select the patients who will benefit the most. The aforementioned clinical trials demonstrate that immune checkpoint inhibitors have activity in neoadjuvant settings of TNBC. However, only a minority of patients will experience any real benefit from these therapies.

The following sections outline how biomarker assessments across these trials suggest particular patient subgroups that may be more likely to respond to immune checkpoint inhibitors. These biomarkers either investigate features of multiple cell types in the tumor immune microenvironment (PD-L1, TILs and bulk tumor gene expression profiling) or are specific features of the tumor cells (TMB, DNA damage repair mutations and somatic mutations).

Routine clinical detection of PD-L1 expression is currently performed using five different FDA-approved companion diagnostic immunohistochemical tests (61). However, the use of different antibody clones (pembrolizumab 22C3, nivolumab 28-8, durvalumab SP263, atezolizumab SP142 and avelumab 73-10), biomarker staining platforms, scoring systems and cut-off values for PD-L1 positivity makes it makes it difficult to consolidate the predictive value of PD-L1 expression across tumor types and across studies. In addition, some tests only define PD-L1 positivity based on tumor cell surface expression, while other tests quantify the cytoplasm and cell surface PD-L1 expression of the tumor and immune cells. The prospective, multicentre Blueprint study compared the performance of all five PD-L1 antibody clones in non-small cell lung cancer specimens (62). The study reported good agreement between three antibodies (22C3, 28-8 and SP263), while 73-10 showed higher sensitivity and SP142 performed poorly with lower sensitivity. In TNBC, few studies have compared the performance of FDA-approved detection methods and confirmed the previous findings in TNBC (63). The positivity rate of PD-L1 detected by SP142 was reported to be significantly lower than that of SP263 and 22C3. These findings emphasized the need for more specific and reproducible predictive biomarkers for immune checkpoint suppression. Notably, PD-L1 did not predict a response to immune checkpoint inhibitors in either of the phase III KEYNOTE-522 or NeoTRIP trials (58,59). In the I-SPY trial, gene expression microarrays were performed to identify cellular expression signatures that would predict the response to pembrolizumab. It was found that in the TNBC subset, a dendritic cell and Th1 gene signature showed a significant interaction with the pCR to pembrolizumab compared with the control arm (64). In a subsequent retrospective analysis using the dendritic cell gene signatures (CCL13, CD209 and HSD11B1) to classify patients as immune-positive or -negative, 67.0% of patients were found to be positive and 33.0% were found to be negative in the trial population (65). It was found that the pCR rate with pembrolizumab was 87.0% in patients classified as immune-positive, but only 29.0% in the immune-negative group (65). Likewise, in the GeparNew trial, investigators also examined RNA gene expression and identified that 44 genes were significantly associated with pCR in the durvalumab arm. There were 3 genes (HLA-A, HLA-B and PSIP1) that were significantly upregulated and 2 genes (HEY2 and THBS4) that were significantly downregulated and associated with a treatment interaction, suggesting they may be candidate genes to evaluate the benefit of durvalumab in future studies (66).

TILs are an additional microenvironment biomarker. Several studies have shown that the presence of TILs is the most constant prognostic factor in TNBC (67), which implicates the involvement of the immune system in the pathophysiology and treatment of these tumors (68). It has been demonstrated that greater lymphocytic infiltration in the initial biopsy predicts a higher pCR rate after neoadjuvant therapy, thus providing a better prognosis in early TNBC regardless of the systemic therapy used and conferring an improvement of ~10.0% in terms of DFS and OS rate from each 10.0% increase in TILs (69-71). In patients with TNBC receiving ICI monotherapy or combination chemotherapy, an increase in the number of TILs is associated with a better overall survival time (57,72). The relative importance of intratumoral TILs (iTILs) and interstitial TILs (sTILs) has not been clearly defined and may vary by tumor type. In breast cancer, both iTILs and sTILs are related to clinical outcome and chemotherapy response (73-75). In addition, in metastatic TNBC, sTILs are associated with treatment response to pembrolizumab, atezolizumab and nivolumab (72,76). Therefore, the International Immuno-oncology Biomarker Working Group has issued guidelines for evaluating iTILs and sTILs in a variety of solid tumor types (77). However, robust scoring of sTILs is hindered by differences in relative iTIL and sTIL distribution, inaccurate delineation of tumor boundaries, small areas of intratumoral stroma, and the presence of necrosis and extracellular mucin (78).

In addition, among 27 solid tumor types, TMB is associated with a higher ORR of anti-programmed cell death protein 1 or anti-PD-L1 monotherapy (79). Notably, the response rate observed in breast cancer is lower than expected based on TMB, which suggests that TMB may not be a good predictive biomarker in these tumors. We hypothesize that combining predictive biomarkers (such as PD-L1 expression, and iTIL and sTIL density) with TMB, T-cell receptor (TCR) diversity and immune gene characteristics will be more likely to produce better performance than using these biomarkers alone. Therefore, further studies are necessary.

6. Emerging targeted therapies in TNBC

In addition to PARP inhibitors and immune checkpoint inhibitors, new drugs that are undergoing phase II and phase III trials for TNBC neoadjuvant therapy mainly involve tyrosine kinase inhibitors with anti-angiogenic activity, such as apatinib, and proliferation inhibitors, such as the androgen receptor inhibitor enzalutamide and the luteinizing hormone-releasing hormone analogue goserelin (80). Epigenetic changes and JAK/STAT pathways have also been explored by using hypomethylation drugs (decitabine) and JAK1/2 inhibitors (ruxolitinib) as therapeutic targets, and are expected to be included in the future clinical treatment of TNBC (81).

7. Conclusions

The conventional treatment for early TNBC is surgery first and adjuvant chemotherapy (with or without radiotherapy) after the operation. There is still a large percentage of patients (30-40%) who experience recurrence and metastasis, and for whom the prognosis is poor. According to the molecular characteristics of TNBC, an optimized and targeted neoadjuvant treatment plan should be selected to increase the pCR rate. Additionally, follow-up adjuvant treatment should be strengthened for non-pCR patients (with a poor prognosis) to reduce the risk of recurrence, which is expected to improve the prognosis of the overall TNBC population. Therefore, the treatment model of chemotherapy after surgery is worthy of further exploration. The development of precision therapy based on individual molecular characteristics is the future direction of development. Related clinical trials on TNBC-targeted therapy and immunotherapy are in progress. The combination of anti-angiogenic drugs and immunotherapy, the combination of immunotherapy and chemotherapy, and the combination of targeted therapy and chemotherapy are also under ongoing investigation. As the results of these clinical trials continue to be updated, the optimization strategy for TNBC treatment will become clearer.

In short, choosing different treatment options according to different subtypes of TNBC will bring more benefits to affected patients; however, further research is needed. It is believed that soon, the classification treatment strategy of TNBC will enter the clinical practice guidelines, thereby bringing more clinical benefits to patients with the disease.

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YX was a major contributor in writing the manuscript. WG conceived the review and revised the manuscript. Both authors read and approved the final manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

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