How shall we treat early triple-negative breast cancer (TNBC): from the current standard to upcoming immuno-molecular strategies

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ABSTRACT
Triple-negative breast cancer (TNBC) is a long-lasting orphan disease in terms of little therapeutic progress during the past several decades and still the standard of care remains chemotherapy. Experimental discovery of molecular signatures including the ‘BRCAness’ highlighted the innate heterogeneity of TNBC, generating the diversity of TNB phenotypes. As it contributes to enhancing genomic instability, it has widened the therapeutic spectrum of TNBC. In particular, unusual sensitivity to DNA damaging agents was denoted in patients with BRCA deficiency, suggesting therapeutic benefit from platinum and poly(ADP-ribose) polymerase inhibitors. However, regardless of enriched chemosensitivity and immunogenicity, majority of patients with TNBC still suffer from dismal clinical outcomes including early relapse and metastatic spread. Therefore, efforts into more precise and personalised treatment are critical at this point. Accordingly, the advance of multiomics has revealed novel actionable targets including PI3K-Akt-mTOR and epidermal growth factor receptor signalling pathways, which might actively participate in modulating the chemosensitivity and immune system. Also, TNBC has long been considered a potential protagonist of immunotherapy in breast cancer, supported by abundant tumour-infiltrating lymphocytes and heterogeneous tumour microenvironment. Despite that, earlier studies showed somewhat unsatisfactory results of monotherapy with immune-checkpoint inhibitors, consistently durable responses in responders were noteworthy. Based on these results, further combinatorial trials either with other chemotherapy or targeted agents are underway. Incorporating immune-molecular interaction into combination as well as refining the standard chemotherapy might be key to unlock the future of TNBC. In this review, we share the current and upcoming treatment options of TNBC in the framework of scientific and clinical data, especially focusing on early stage of TNBC.

INTRODUCTION
Triple-negative breast cancer (TNBC) is immunohistochemically defined by the lack of oestrogen receptor (ER), progesterone receptor and human epidermal growth factor receptor 2 (HER2) expression.12 Although it is generally more chemosensitive than other types of breast cancer, it is also characterised to harbour the most aggressive behaviour with the front-loaded risk of relapse within the first 3–5 years after completion of adjuvant chemotherapy and its prevalence in younger women.3–6 Once metastasised, TNBC has a high predisposition to involve the critical visceral organs such as lung, liver and brain, eventually leading to a significantly shorter median overall survival than in other subtypes.7–8 Therefore, developing optimal therapeutic strategies for the treatment of early TNBC is crucial to alleviate the burden of TNBC. Accordingly, in the last decade, extensive efforts were undertaken to unravel newer therapeutic targets of TNBC based on its molecular landscape.9–15 But unfortunately, there has been little clinical success of targeted therapy, making most of patients with TNBC still mainly dependent on conventional chemotherapy. With the advances of multiomics,9–12 14–16 however, recent experimental investigations revealed a variety of potentially actionable targets including the immune signatures, which are actively engaged in and communicating with the tumour microenvironment.17 These give us a new insight on the molecular heterogeneity of TNBC and ultimately herald a new therapeutic horizon for TNBC beyond conventional chemotherapy.8 In parallel with these efforts, chemotherapeutic strategies have also been scientifically redefined. In this review, we describe recent scientific and therapeutic progress in TNBC, particularly focusing on the current standard and upcoming systemic treatment options in early stages of disease.

ELUCIDATING THE HETEROGENEITY OF TNBC
Attempts of molecular classification in TNBC
The first established molecular classification for TNBC was suggested by Lehmann et al with six subtypes based on their distinct gene expression profiles,9 which were the
basal-like (BL1 and BL2), mesenchymal (M) or mesenchymal stem-like (MSL), immunomodulatory (IM) or luminal androgen receptor (LAR)-enriched tumours. The BL subtypes represented BL-breast cancer (BLBC)-like phenotypes, with expression of genes involved in cell cycle and DNA damage repair (DDR) in the BL1 subtype and growth factor signalling pathways in the BL2 subtype. The two mesenchymal-related subtypes were closely associated with epithelial-mesenchymal transition and relative chemoresistance. Immune-related signatures were abundantly found in the IM subtype and the LAR subtype highly expressed the androgen receptors (ARs) with luminal-like gene expression signature. Interestingly, while phenotype of the LAR subtype resembled that of luminal-like ER-positive breast cancer, it was mainly categorised as HER2-enriched or luminal B subtype by the PAM50 algorithm. In a correlative analysis comparing PAM50 and Lehmann’s classifications, the majority of TNBC subtypes other than MSL and LAR were classified as BLBC. Recently, Lehmann et al revised their previous subclassification to a more concise system consisting of only four subtypes (TNBCtype-4): BL1, BL2, M and LAR. A noticeable remark from this study was that the IM phenotype was not an isolated molecular subtype, but can exist within all molecular subtypes to varying degrees. Burstein et al similarly classified TNBC into four subtypes: LAR, mesenchymal (MES), basal-like immunosuppressed (BLIS) and basal-like immune-activated (BLIA). These substantially overlapped with Lehmann’s classifications, but they uniquely incorporated immune signatures to further divide BL-related subtypes. As expected, BLIS and BLIA behaved clinically in disparate ways; BLIA showed a better prognosis, which was mainly attributable to its more favourable immunological milieu. Ten integrative clusters (IntClust) were identified by combined transcriptomics and genomics approach, and IntClust4 and 10 were suggested as two major subgroups comprising 80% of BLBCs. However, they clinically behaved in different manners due to their distinct molecular features; IntClust4 had a strong immune-related signature with a paucity of copy-number aberrations (CNA-devoid subgroups), whereas IntClust10 largely depended on the genomic instability of major chromosomal aberrations. Recently, a new classification which comprehensively incorporated the tumour microenvironment was proposed. With a hypothesis of possible intersection between immunological and metabolic signatures in tumour microenvironment, they suggested a subtype with enriched tumour-infiltrating lymphocytes (TILs) and programmed death ligand 1 (PD-L1) expression and activated glycolytic pathways. Despite their inherent immune-molecular divergence, an important commonality exists between the different molecular classifications of TNBC, which are represented by basal-likeness, abundant luminal/AR expression, mesenchymal potency and immune signatures. Regarding the clinical relevance of the molecular classifications, a few retrospective studies suggested the potential predictive role of certain molecular subtypes in patients treated with neoadjuvant chemotherapy. However, it still remains unclear whether the molecular classification itself could be a firm predictive biomarker for patients with TNBC.

**BRCAanness, a unique molecular trait of TNBC**

The term ‘BRCAanness’ describes a spectrum of phenotypes derived from the panoply of genotypes that share the biological features of BRCA-deficient tumours, typically with germline *BRCA1*/2 mutations. Although it is yet unclear whether non-canonical alterations such as promoter methylation, somatic BRCA mutations and copy-number variations result in exactly the same functional deficiency as germline *BRCA1*/2 mutations, these alterations were experimentally suggested to interact with BRCA-related molecules and induce the loss of function of BRCA proteins. BRCA1 signalling plays a critical role in prompting higher fidelity of DDR at the point of double-strand breaks (DSBs), mainly through the process of homologous recombination and also by activating other DNA repair pathways. It is referred to transcription-coupled repair, because BRCA1 interacts with other proteins of DNA repair including RAD51 and regulates the whole transcriptional machinery including cell cycle checkpoints, chromatin remodelling and apoptosis. While 10%–20% of patients carry germline *BRCA1*/2 mutations in TNBC that largely overlap with the phenotype of BLBC, most BLBCs do not carry *BRCA1*/2 mutations. In addition, aberrant expression of BRCA-related proteins was more frequently observed in BLBC except BRCA1. These heterogeneities underlie the complexity of BRCAness and suggest the existence of intricate networks between TNBC and BLBC and BRCAness. But, at the same time, it enriches the innate genomic instability of TNBC and contributes to therapeutic benefit by enhanced immunogenicity and chemosensitivity to DNA-damaging agents including platinum and poly(ADP-ribose) polymerase (PARP) inhibitors (PARPi).

**CURRENT TREATMENT OPTIONS IN EARLY TNBC**

The decision of treatment for early TNBC faces a great challenge due to the significantly higher risk of early relapse coupled with the lack of available treatment options beyond conventional chemotherapy. In this context, efforts should be made to optimise treatment efficacy in patients harbouring greater risk of relapse or high tumour burden, based on the tailoring of standard systemic treatment and further personalization strategy.

**Current standard of systemic treatment in early TNBC**

Despite the molecular heterogeneity, the standard of systemic treatment for TNBC follows the same general principle with other types of breast cancer. Thus, neoadjuvant or adjuvant chemotherapy remains a key component of systemic treatment in early TNBC, which is determined primarily by its clinical or pathologic stage.
Neoadjuvant and adjuvant chemotherapy

Neoadjuvant and adjuvant chemotherapy are the standard systemic treatment for early TNBC, and anthracycline and taxane-based chemotherapy regimens comprise the current standard of care.\textsuperscript{41 42} In previous pivotal neoadjuvant trials, patients with TNBC showed significantly higher response rates to anthracycline and taxane-based chemotherapy than those of other subtypes, achieving pathologic complete response (pCR) rates of approximately 40%.	extsuperscript{42-44} Moreover, as pCR showed a significant correlation with therapeutic benefits, it has been established a robust surrogate biomarker for long-term survival outcomes.\textsuperscript{7 44-47} Although the guideline for adjuvant chemotherapy is generally similar for each breast cancer subtype, adjuvant chemotherapy in TNBC is recommended for primary tumours larger than 0.5 cm due to their aggressive behaviour.\textsuperscript{48}

Regarding the paradox that TNBC carries both higher chemosensitivity and the risk of early relapse,\textsuperscript{49} efforts have been continued to develop more effective chemotherapeutic regimens for both responders and non-responders. Clinical trials of newer combinations and dose-determination studies that evaluated metronomic or dose-dense regimens suggested the feasibility of refining conventional chemotherapy.\textsuperscript{50-53} More recently, patients with an initially high tumour burden or residual disease after neoadjuvant chemotherapy were identified as compelling candidates for intensive systemic treatment, as they carry higher chance of relapse and metastatic spread. The CREATE-X trial demonstrated the potential survival benefit of adding capecitabine to the standard adjuvant chemotherapy regimen in early TNBC with a residual tumour burden after neoadjuvant treatment.\textsuperscript{54} A meta-analysis also provided a rationale for adding capecitabine to either neoadjuvant or adjuvant standard chemotherapy in patients with TNBC.\textsuperscript{55} Despite its substantial toxicity, additional capecitabine might be a reasonable option for patients with TNBC carrying a higher risk of relapse. Furthermore, in these patients, postneoadjuvant trials based on actionable molecular targets identified from residual tumour tissues should also be considered. Molecular profiling of residual TNBC might be helpful to identify genetic alterations involved in drug resistance in the neoadjuvant setting and to further guide adjuvant targeted therapy to eradicate the chance of clinically silent micrometastases at the time of surgery. The additive benefit of platinum-based agents, which have been consolidated in the neoadjuvant and adjuvant setting in the subset of patients with TNBC which had BRCAness, will be discussed more in the next section.

The role of platinum-based chemotherapy in early TNBC

In many preclinical studies, an unusual sensitivity to platinum-based agents was suggested in certain subset of TNBC, mainly due to genomic instability from DDR impairment.\textsuperscript{51 56} Subsequent clinical trials of metastatic TNBC showed a modest efficacy of platinum-based monotherapy,\textsuperscript{57-60} consistently suggesting a greater benefit in BRCA1/2 mutation carriers. In early TNBC, patients with stage II–III treated with neoadjuvant cisplatin alone showed a 22% pCR rate in a small retrospective study, in which only 7% of patients carried germline BRCA mutations. Further phase II studies exclusively for patients with BRCA1 mutation demonstrated markedly higher pCR rates from 61% to 90% after neoadjuvant cisplatin monotherapy, which validated the significance of BRCAness in predicting platinum sensitivity in TNBC.\textsuperscript{61-63} In another neoadjuvant study of carboplatin combined with eribulin, the homologous recombination deficiency (HRD) score was suggested as a potential predictive biomarker.\textsuperscript{64} As these earlier studies supported the rationale of adding platinum to the standard neoadjuvant chemotherapy in TNBC, two landmark phase II trials evaluated combination of platinum with anthracycline/taxane-based regimens, alone or with other targeted agents; In the CALGB40603 trial,\textsuperscript{65} the pCR rate was significantly improved from 41% to 54%, in patients who received neoadjuvant chemotherapy combining carboplatin and/or bevacizumab with paclitaxel followed by dose-dense doxorubicin and cyclophosphamide (ddAC). The GeparSixto trial\textsuperscript{66} similarly showed significantly enhanced pCR rates from 36.9% to 53.2% by adding carboplatin to combinations of ddAC and taxane-based chemotherapy with bevacizumab. In their recent secondary analysis, treatment benefit was consistently maintained even in patients without BRCA1/2 mutations.\textsuperscript{67} In another phase II PreECOG 0105 study, however, patients with BRCA1/2 mutations achieved the highest pCR rate (56%) after neoadjuvant combination chemotherapy with gemcitabine, carboplatin and the PARPi iniparib, while high score of HRD-loss of heterozygosity (LOH) showed a significant correlation with objective response rates (ORR) in patients without BRCA1/2 mutations. HRD-LOH score, as suggested in the study, has been often regarded a powerful estimation tool of DNA repair capacity, thus correlating with BRCAness. However, the predictive value of the HRD-LOH assay for platinum sensitivity still remains controversial when taking account of discordance from prior metastatic TNBC trials including the TBCR009 and the Triple Negative Breast Cancer Trial.\textsuperscript{57 60} In later phase II neoadjuvant trials, paclitaxel combined either with cisplatin or carboplatin yielded encouraging efficacy outcomes with improved pCR and survival outcomes, which also suggested a strong relationship between clinical benefit and genetic alterations of DDR pathway.\textsuperscript{68 69} These results altogether seemed to support the notion of adding platinum to conventional neoadjuvant chemotherapy in the subset of patients with early TNBC, which might be optimised in the context of BRCAness. However, we should give a particular concern about the absence of statistically valid survival benefits in previous studies, for pCR might not always translate into a long-term survival benefit. Because the CALGB40603 and GeparSixto trials were somewhat underpowered studies to draw robust conclusions about the long-term survival benefit beyond pCR improvement,
platinum-based combination chemotherapy has been hampered as a new standard neoadjuvant treatment. Safety concern with combination is another important issue that should not be overlooked. Therefore, platinum-based combination chemotherapy should be selectively applied in the optimal candidates carrying the BRCA1/2 deficiency and high risk of relapse with extensive tumour burden or in a younger age, who necessitate enriched locoregional control. Currently, several neoadjuvant trials of platinum-based combination chemotherapies are underway, including the PEARLY trial (NCT02441933), which is evaluating neoadjuvant taxane with or without carboplatin after doxorubicin/cyclophosphamide (AC) chemotherapy. In an adjuvant setting, a phase III trial of carboplatin monotherapy is about to begin for patients with residual TNBC after conventional neoadjuvant chemotherapy (NCT01752686), and trials adding cisplatin or carboplatin to taxanes with or without doxorubicin-based combination chemotherapy are underway. Completed and ongoing clinical trials in early TNBC are summarised in table 1.

UPCOMING TREATMENT OPTIONS BEYOND CONVENTIONAL CHEMOTHERAPY

The panoply of old and new targets

The advance of multiomics has introduced a vast range of actionable targets and accelerated the new horizon of targeted therapies, which could be particularly efficacious in TNBC subsets that are expected to be relatively chemoresistant (figure 1). However, widening the road of treatment spectrum does not always guarantee consequent therapeutic benefit. Therefore, we should recognise that molecular heterogeneity is a double-edged sword, which could be the hope or the hype for TNBC.

Targets that light the BRCA1ness of TNBC

PARPi

Because of their critical role in the process of DDR, PARPi have been thought to be a potential game changer for TNBC. PARPs, specifically PARP1 and 2, are enzymes that facilitate DDR at sites of single-strand breaks by activation of various intracellular signalling pathways through auto-poly(ADP)-ribosylation. A meaningful link between PARP inhibition and BRCA1ness is demonstrated by the concept of synthetic lethality. As BRCA1/2 deficiency leads to HRD, a dysfunction of the cell’s intrinsic DNA repair mechanism, repair of DNA damage solely depends on the action of PARP1 in BRCA-deficient breast cancer. Therefore, inhibiting PARP1 in patients with BRCA1/2 deficiency might induce accumulation of DSBs and eventually result in synthetic lethality, which could in turn enhance the sensitivity to PARPi. Based on this scientific evidence, earlier clinical data suggested that a subset of patients with TNBC having BRCA1/2 deficiency might benefit from PARPi treatment. In the pivotal phase III OlympiA trial of metastatic breast cancer, investigators compared the efficacy of monotherapy with olaparib with that of standard single chemotherapy at the discretion of physician in metastatic HER2-negative breast cancer with germline BRCA1/2 mutations. The subgroup of patients treated with olaparib had nearly double the response rate (59.5%) as well as a longer median progression-free survival (PFS; 7.0 months) and a better toxicity profile. Based on these dramatic treatment outcomes, PARPi have been recently approved for breast cancer with BRCA1/2 mutations, which mainly constitutes TNBC. Other PARPi than olaparib have been also vigorously investigated initially in metastatic breast cancer, which include veliparib, talazoparib and rucaparib. Once a phase I study of talazoparib showed favourable efficacy and safety profiles in advanced solid tumours with deleterious BRCA1/2 mutations including breast cancer (NCT01945775), feasibility trials of veliparib in combination with other alkylating agents are also undergoing in advanced or metastatic TNBC. And recently, a phase III trial EMBRACA comparing talazoparib and treatment by physician’s choice in metastatic TNBC revealed significant benefit of talazoparib with better PFS and ORRs.

In early TNBC, the phase III OlympiA trial (NCT02032823) is currently ongoing to evaluate neoadjuvant olaparib monotherapy for a year after standard neoadjuvant chemotherapy and local treatment in high-risk TNBC with germline BRCA1/2 mutations. PARTNER, another phase II/III trial of neoadjuvant olaparib in combination with carboplatin followed by the standard chemotherapy, is also under investigation in patients with breast cancer with TNBC or germline BRCA1/2 mutations (NCT03150576). The I-SPY 2 trial evaluated neoadjuvant veliparib and carboplatin (VC) in addition to the standard chemotherapy in patients with high-risk breast cancer and TNBC seemed to gain the most significant benefit from this combination therapy (pCR rates: 52% vs 24%). In a recent biomarker analysis of the I-SPY2, a genomics-based signature of BRCA1ness was suggested as a significant predictive biomarker of response to neoadjuvant VC. However, the true benefit from adding PARPi to platinum-based chemotherapy is still controversial, for platinum itself already showed its efficacy either as monotherapy or in combination. Another phase II neoadjuvant trial in high-risk, residual TNBC after standard neoadjuvant chemotherapy failed to show a significant therapeutic benefit from the combination of low-dose rucaparib and cisplatin compared with cisplatin alone, in terms of both toxicity and survival outcomes, although the applied dose of rucaparib has been considered therapeutically insufficient (NCT01074970). Therefore, the plausibility of combining these two chemosensitisers of germline BRCA1-mutant TNBC remains questionable at this point. Currently, the efficacy of neoadjuvant veliparib with radiation therapy is under exploration in a phase I study for node-positive, residual breast cancer after neoadjuvant chemotherapy (NCT01618357), and another phase I trial of neoadjuvant monotherapy with the novel PARPi niraparib is about to be initiated (NCT0329937) (table 2). Last, strategies for restoring BRCA1ness in order
Table 1  Key completed and ongoing clinical trials of platinum-based chemotherapy for early-stage TNBC, including both monotherapy and combination therapies

| Phase | NCT ID number | Defined breast cancer subtype | Setting | Stage | Experimental drugs | Control | Primary endpoint |
|-------|---------------|-------------------------------|---------|-------|---------------------|---------|-----------------|
| **Platinum monotherapy** |
| Completed |
| II | NCT00148694 | TNBC | Neoadjuvant | NA | Cisplatin (4C) | NA | pCR: 22% |
| II | NCT01630226 | LABC with BRCA1mt | Neoadjuvant | I–III | Cisplatin (4C) | NA | pCR: 61% (in TNBC) |
| Ongoing |
| III | Post-NAC study (NCT01752686) (not recruiting yet) | TNBC Residual after NAC | Adjuvant | NA | Carboplatin (6C) | Observation as per guideline | DFS |
| **Platinum-based combination** |
| Completed |
| II | NCT02934828 | Operable TNBC or HER2(+) | Neoadjuvant | I–III | Carboplatin+Eribulin (4C) | NA | pCR: 43.3%+gBRCAm 66.7% |
| II | SHPD001 (NCT02199418) | LABC, including TNBC | Neoadjuvant | I–II | Cisplatin (4C)+wPacli taxel (16 weeks) | NA | pCR: 64.7% in TNBC |
| II | PreECOG 0105 (NCT00813956) | TNBC or BRCA1/2mt | Neoadjuvant | I–IIA | Gemcitabine+Carbo platin+Iniparib | NA | pCR: 36% (all) vs 56% (gBRCAmt) |
| II-B | CALGB40603 (NCT00961705) | Hormone Receptor (-) and HER2(-) | Neoadjuvant | IIA–IIIA | Weekly Paclitaxel+Carboplatin+Bevacizumab + Followed by dose dense AC | Standard NAC | pCR: 62.4% vs 22.3% |
| II-B | GeparSixto (NCT01426880) | TNBC or HER2(+) | Neoadjuvant | II, III | Carboplatin+Bevaci zumab+standard NAC | Bevacizumab+standard NAC | pCR: 53.2% vs 36.9% |
| II-B | NCT00930930 | TNBC | Neoadjuvant | II–III | Weekly Cisplatin+wPacli taxel+Everolimus | wCisplatin+wPaclitaxel | pCR: 36% vs 49% |
| II-B | NCT01276769 (unknown) | Locally advanced TNBC | Neoadjuvant | NA | Paclitaxel+Carboplatin | Paclitaxel+Epirubicin | pCR: 38.6% vs 14.0% (0.014) 5-year RFS: 77.6% vs 56.2 (p=0.043) |

Continued
| Phase | NCT ID number | Defined breast cancer subtype | Setting | Stage | Experimental drugs | Control | Primary endpoint |
|-------|---------------|-------------------------------|---------|-------|--------------------|---------|------------------|
| Ongoing | II NCT02934828 | TNBC or HER2(+) | Neoadjuvant | II, III | Neoadjuvant: Carboplatin+Paclitaxel<br>Adjuvant: standard chemotherapy+Carboplatin | NA | DFS |
| | II NCT00148694 | TNBC | Neoadjuvant | II–III | Carboplatin+Docetaxel | Observation as per guideline | Predictors of pCR |
| | II NCT02315196 | TNBC | Neoadjuvant | II, III | Pegylated-liposomal doxorubicin+Carboplatin (4C)<br>Adjuvant paclitaxel after surgery | NA | pCR |
| | III SHPD002 (NCT02221999) | TNBC (and ER+)<br> | Neoadjuvant | NA | Weekly Paclitaxel+Cisplatin (4C) | NA | pCR |
| | III NCT03168880 | Large, operable TNBC | Neoadjuvant | NA | Weekly Carboplatin+Paclitaxel (8C)<br>Followed by AC/EC (4Q) | Paclitaxel | pCR: 62.4% vs 22.3% |
| | III PEARLY Trial (NCT02441933) | Operable TNBC | (Neo)adjuvant | II–III | AC → Taxane+Carboplatin (4C) | AC → Taxane | 5-year EFS |
| | III NCT02455141 | TNBC | Adjuvant | NA | EC → Taxane+Carboplatin (4C) | EC → Taxane | DFS |
| | III NCT0248967 | High-risk N(-) or N(+) TNBC | Adjuvant | IB–III | AC → Paclitaxel+Carboplatin | AC → Paclitaxel | pCR: 56% in gBRCAmt |
| | III NCT02879513 | LABC, pathologic PR after NAC | Adjuvant | NA | Paclitaxel+Cisplatin (2C) | CEF (4C) | |
| | II NCT03201861 (not recruiting yet) | High-risk HER2(-)<br>EBBC, including TNBC | Adjuvant | NA | Weekly Paclitaxel (12 weeks)+Cisplatin (3C)<br>Followed by EC (4C) | EC (4C) → Taxane | DFS |

AC, doxorubicin and cyclophosphamide; CEF, cyclophosphamide, epirubicin, and 5-fluorouracil; DFS, disease-free survival; EBC, early breast cancer; EC, epirubicin and cyclophosphamide; EFS, event-free survival; RFS, relapse-free survival; gBRCAmt, germline BRCA mutation; HER2, human epidermal growth factor receptor 2; LABC, locally advanced breast cancer; NA, not available; NAC, neoadjuvant chemotherapy; pCR, pathologic complete response; PR, progesterone receptor; TNBC, triple-negative breast cancer.
to prevent or counteract acquired resistance to PARPi are now actively underway.

Revisiting the PI3K-Akt-mTOR (PAM) pathway in TNBC

Data from the Cancer Genome Atlas (TCGA) revealed that the major genetic aberrations observed in TNBC occurred within the PI3K-Akt-mTOR (PAM) pathway and accumulating data from next-generation sequencing (NGS) studies further confirmed the PAM pathway as an appealing actionable target in TNBC. PIK3CA hotspot mutations and aberrations in phosphatase and tensin homolog (PTEN) are the two most frequent but mutually exclusive alterations, accounting for approximately 10% and 35%–50% of TNBC, respectively. As in other subtypes of breast cancer, targeting the PAM pathway has been heavily investigated in TNBC and expanded to other molecules that are in a circuit of cross-talk with the PAM signalling, including the epidermal growth factor receptor (EGFR) and mitogen-activated protein kinase (MAPK) pathways.

Inhibitors of the PAM pathway: from preclinical to clinical data

The PAM pathway comprehensively controls the cell cycle from survival to apoptosis and is therefore vitally involved in tumourigenesis and its progression. PAM signalling is primarily modulated by the key molecule PI3K, but is also regulated by active communication with other growth factor tyrosine kinase receptors, including EGFR and insulin-like growth factor 1 receptor (IGF1R). Activation of the PAM pathway often confers chemoresistance in breast cancer. In TNBC, PI3K was capable of enhancing the effects of BRCA1/2 mutations by interacting with the homologous recombination machinery, stabilising DNA DSBs. Thus, inhibition of the PI3K pathway with buparlisib, an oral pan-PI3K inhibitor, produced promising anti-tumour cytotoxicity in TNBC cell lines. Experimentally, it also enhanced sensitivity to PARPi in both BRCA1/2-deficient and BRCA1/2-sufficient TNBC cell lines by activation of extracellular signal-related kinase (ERK) and MAPK kinase (MEK1), which induced downregulation of BRCA1/2. Regardless of these encouraging preclinical studies, subsequent clinical trials showed somewhat disappointing results as in the recent BELL-4 trial, which failed to prove a benefit from combination treatment with buparlisib and paclitaxel in advanced HER2-negative breast cancer. Given that efficacy of cotargeting is strongly supported by recent preclinical data, combining another targeted therapy along with PI3K inhibitors might be a compelling overcoming strategy. Accordingly,
a phase I study with olaparib and an oral pan-PI3K inhibitor, buparlisib, is ongoing in recurrent TNBC and high-grade ovarian cancer (NCT01623349).

AKT, which is activated by PI3K, serves as another central node in the PAM signalling pathway. Once preclinical studies demonstrated the antitumour activity of the AKT inhibitors in TNBC,98–100 many clinical trials were initiated including the phase II PAKT trial evaluating the combination of AZD5363 and paclitaxel. The latest phase II trial, LOTUS,101 evaluated the combination of paclitaxel and ipatasertib, a highly selective ATP-competitive AKT inhibitor and showed its efficacy in patients with advanced TNBC, demonstrating significantly improved PFS compared with paclitaxel alone. This trial was the first study showing a significant PFS benefit of anti-PAM pathway targeted agents in IHC-defined TNBC population, although it was not maintained in the PTEN-low subset of patients, which was assumed as an appealing candidate for the drug. However, when PIK3CA/AKT1/PTEN alterations were refined based on NGS, the benefit in the subset was greater, suggesting that NGS-derived genomic biomarkers might better define the target population. Clever selection of targets might be another critical point of the LOTUS data, which newly highlighted AKT as a powerful druggable target of the PAM signalling pathway. Theoretically, AKT could be a preferable target to PI3K from the perspective of selectivity or it might act as a more direct functional regulator of PAM signalling, interacting with intertwining feedback loops across messenger molecules. There is a paucity of data regarding PAM inhibitors in early TNBC except for a previous phase II neoadjuvant trial combining mTOR inhibitors with conventional FEC (5-fluorouracil, epidoxorubicin and cyclophosphamide) chemotherapy, which unfortunately did not show clinical benefit.102 However, in a recent phase I study, temsirolimus or everolimus in addition to liposomal doxorubicin and bevacizumab showed notable efficacy in the mesenchymal subset of TNBC, predominantly in patients with an aberrant PI3K pathway (NCT00761644). 103

Table 2 Ongoing clinical trials of PARPi for patients with early-stage TNBC

| Phase | NCT ID number | Defined breast cancer subtype | Setting | Stage | Experimental drugs | Control | Primary endpoint |
|-------|---------------|--------------------------------|---------|-------|-------------------|---------|-----------------|
| PARPi monotherapy | | | | | | | |
| I NCT01618357 | Node (+) BC, Residual after NAC | Neoadjuvant | NA | Veliparib+radiation | Standard NAC | MTD |
| | NCT03239937 (not recruiting yet) | HER2(-), BRCA1/2mt | Neoadjuvant | NA | Niraparib | NA | Preliminary antitumour activity Measured by breast MRI |
| II NCT02282345 | Invasive BC and deleterious BRCAmt | Neoadjuvant | I–III | Talazoparib | Observation as per guideline | IDFS |
| III (NCT02032823) | gBRCA1/2mt High-risk HER2(-) BC including TNBC | Adjuvant (after NAC) | Olaparib up to maximum 1 year | Placebo | IDFS |
| PARPi-based combination | | | | | | | |
| II/III PARTNER (NCT03150576) | TNBC or gBRCAmt | Neoadjuvant | II, III | Olaparib+Carboplatin + Paclitaxel | Standard NAC | pCR |
| | NCT01042329*Neoadjuvant, personalised adaptive trial with novel agents | Locally advanced TNBC | | Followed by standard NAC by physician’s discretion | | |
| II I-SPY 2 (NCT01042379) | gBRCA1/2 mt or TNBC Residual disease after NAC | Neoadjuvant | II, III | Veliparib+Carboplatin | Standard NAC | pCR; 52% vs 24% (*preliminary result) |
| | | | | Followed by standard NAC (T → AC) | | |
| II NCT01074970 | gBRCA1/2 mt or TNBC Residual disease after NAC | Neoadjuvant | I–III | Cisplatin+Rucaparib (4C) → Rucaparib for 6 months | Cisplatin after neoadjuvant A or T | 2-year DFS |

AC, doxorubicin and cyclophosphamide; DFS, disease-free survival; gBRCAmt, germline BRCA mutation; HER2, human epidermal growth factor receptor 2; IDFS, invasive DFS; MTD, maximal tolerated dose; NA, not available; NAC, neoadjuvant chemotherapy; PARPi, poly(ADP-ribose) polymerase inhibitors; pCR, pathologic complete response; TNBC, triple-negative breast cancer.

**EGFR inhibitors and the PI3K pathway**

Although amplification of EGFR is rare among patients with breast cancer in general, the frequency of EGFR
overexpression is markedly higher in TNBC ranging from 13% to 76%, which is considered a poor prognostic factor for TNBC. Based on the dynamic molecular network between EGFR and PAM signalling, preclinical studies revealed a therapeutic synergism between anti-EGFR targeted treatment and DNA damaging agents such as platinum or PARPi, by increasing chemosensitivity in TNBC cell-lines with BRCA1 deficiency but intact PTEN. Unfortunately, subsequent clinical trials of EGFR tyrosine kinase inhibitors, alone or in combination, were disappointing and actually showed a more favourable response in non-TNBC subtypes. However, in two previous phase II trials of metastatic TNBC, adding cetuximab to cisplatin or carboplatin improved clinical outcomes compared with platinum-based monotherapy regardless of statistical insignificance. Cetuximab in combination with ixabepilone or irinotecan also showed a slightly higher response rate, but this did not translate to a survival benefit. Another recent phase II trial also failed to prove a significant benefit of adding panitumumab to the combination of gemcitabine and carboplatin in patients with metastatic TNBC, but the triple combination seemed feasible in the patient cohort. Given that these studies precluded biomarker-driven selection of target populations, further studies of EGFR inhibitors, particularly anti-EGFR mAbs, should be performed in a molecularly predefined subset of TNBC cases. In this context, several trials of metastatic TNBC are ongoing in selected cohorts harbouring alterations of the EGFR and/or PAM pathways, including the afatinib arm of the NCI-MATCH (NCT02465060) trial (online supplementary table 2).

In early TNBC, combination neoadjuvant trials of cetuximab and panitumumab with docetaxel after FEC showed modest efficacy. In these trials, the number of TILs could predict the response to anti-EGFR mAbs, suggesting an important association between the EGFR signalling pathway and T cell-mediated immunity. Currently, a phase II study of neoadjuvant panitumumab, carboplatin and paclitaxel (PaCT) is underway in patients with chemoresistance to standard treatment or inflammatory breast cancer (NCT02593175, NCT02876107). A phase II trial of neoadjuvant afatinib, alone and in combination with paclitaxel, is also recruiting patients (NCT02511847).

Significance of antagonising MEK activation

The mechanism of MAPK activation in TNBC and BLBC was initially investigated in vitro. Although these cell lines seldom harbour activating mutations of canonical oncoproteins such as Ras or c-Myc, they frequently harbour copy-number variations in these oncogenes or show overexpression of other growth factor receptors such as EGFR, IGF1R, vascular endothelial growth factor receptor (VEGFR) or fibroblast growth factor receptor 1. Dual-specificity phosphatase 4, a negative regulator of ERK1/2 and c-Jun N-terminal kinase 1/2, might also contribute to activation of MAPK signalling in TNBC, in that its loss or downregulation, either by genetic or epigenetic mechanisms, is associated with chemoresistance. Based on these molecular characteristics, MEK inhibitors were suggested as an attractive therapeutic option in TNBC, particularly in BLBC with intact PTEN, and PTEN itself was suggested as a potential negative predictor of response. However, in the presence of abundant signalling cross-talk, MEK inhibitors alone could not efficiently suppress activation of the MAPK pathway. Accordingly, combination treatment with MEK inhibitors and other targeted therapies or chemotherapeutic agents have been proposed to overcome the consequent chemoresistance including a phase II COLET trial evaluating upfront combination of cobimetinib and paclitaxel. The COLET showed a promising activity to control the resistance against taxane chemotherapy in the early data.

Another recent data reported a therapeutic synergy when combining the MEK inhibitor selumetinib with either buparlisib or the platelet-derived growth factor receptor inhibitor pazopanib, which was even effective for brain metastases of TNBC. Among numerous combination trials of MEK inhibitors currently ongoing, a phase Ib trial combining the MEK inhibitor trametinib with gemcitabine in advanced solid tumours showed a case of complete response in the patient with metastatic TNBC. On the other hand, phase I trials evaluating combination treatment of MEK inhibitors either with mTOR or PI3K inhibitors (NCT01476137, NCT00955757) revealed unacceptable fatal toxicities, prohibiting subsequent phase II studies. They showed the potential lethality of coblockade of master signalling pathways, including MAPK and PAM, which should be taken into consideration when designing future combinatorial trials with targeted agents.

Immunotherapy for TNBC

The immune microenvironment as a component of TNBC heterogeneity

The immune system plays a dual role in breast cancer, that is, the tumour initially induces innate immunity, but later suppresses adaptive immunity, ultimately resulting in disease progression. Due to its genomic instability and high mutational burden, tumour microenvironment of TNBC is considered to be ‘hot’ with abundant infiltrating immune cells, which are actively engaged in the process of ‘immunoediting’. TILs, mainly the CD8 +T cells, are the most famous immune-related player in breast cancer. In TNBC treated with neoadjuvant treatment, TILs was identified as a robust predictive biomarker of long-term survival and its significance in remnant disease was subsequently validated, revealing an active communication between immune system and cytotoxic agents. Although relatively unexplored, the significance of TILs in the adjuvant setting has also been suggested in recent years. Of note, the latest study showed the prognostic significance of TILs even in systemically untreated early TNBC, suggesting that the presence of TILs may refine the candidates for...
adjuvant chemotherapy or immunotherapy. Schmid and colleagues recently showed in metastatic TNBC that response rate and overall survival after atezolizumab treatment significantly correlated with the level of TILs. In addition, a lower level of TILs was significantly associated with aberrant activation of Ras-MAPK signalling, which can promote immune evasion in TNBC. Correlation between TILs and programmed cell death 1/ligand 1 (PD-1/PD-L1) expression was suggested in recent experiments, which assumed the existence of a feedback loop regulating PD-L1 expression as a means of immune homeostasis. Several retrospective studies of early TNBC demonstrated significantly worse survival outcomes in patients harbouring high PD-L1 expression and a low number of TILs or a high ratio of PD-L1/CD8 expression. By contrast, immunogenic factors potentially involved in the expression of neoantigens positively correlated with higher TILs and a more favourable prognosis. Taken together, TILs play a significant role in orchestrating the immune microenvironment and vigorously interact with cytotoxic signals including both chemotherapy and immunotherapy. Taken together, TILs could serve as a currently available predictive and prognostic immune biomarker, and at the same time, immune molecules could be another appealing therapeutic target of TNBC.

Efficacy outcomes of monotherapy with immune checkpoint inhibitors

Although TNBC has been assumed the potential protagonist of immunotherapy in breast cancer, no immunotherapy has yet been officially approved for breast cancer. However, earlier efficacy data on cytotoxic T-lymphocyte-associated protein 4 inhibitors and accumulating recent clinical evidences demonstrated a durable response in a small subset of patients with metastatic TNBC, raising the hope of success with immunotherapy.

In earlier phase I trial of atezolizumab in advanced solid cancers including heavily pretreated TNBC, the response was strikingly durable in responders although only 10% of patients responded; the survival rate of patients who achieved an ORRs was 100% at over 2 years. Phase I (KEYNOTE-012) and II (KEYNOTE-086) studies of pembrolizumab in metastatic TNBC showed modest ORRs of approximately 20% with several complete responses, while the median duration of the response was not reached at the time. Phase II trial was performed in two different cohorts (A and B) according to the disease setting and PD-L1 expression; cohort A enrolled patients with any level of PD-L1 expression who would receive pembrolizumab as salvage therapy, while cohort B only included patients with positive PD-L1 expression treated with first-line pembrolizumab. Although PD-L1 expression did not significantly affect the treatment outcomes in cohort A, patients in cohort B showed a higher response rate than cohort A. These studies with atezolizumab and pembrolizumab altogether suggest that the therapeutic benefit could be maximised when given as upfront treatment and/or possibly in patients with predefined PD-L1 expression. Also, in the phase I JAVELIN trial, patients with heavily pretreated metastatic TNBC treated with the PD-L1 antibody avelumab showed encouraging efficacy outcomes with a 31% disease control rate and PD-L1 expression were closely associated with response. Currently, a phase II trial of pembrolizumab monotherapy for BRCA-mutated breast cancer is underway (NCT03025035) (table 3).

Finding new combinatorial partners

Monotherapy with immune-checkpoint inhibitor revealed disappointing results in the metastatic trials with ORRs less than 10%. Hence, current efforts are concerted on developing combination strategies with immune checkpoint inhibitors, based on the remarkably durable responses in the subset of responders shown in previous studies (table 3).

Immune-based chemotherapy

Early data of nab-paclitaxel combined with atezolizumab showed a 40% ORR in metastatic TNBC, which hastened a phase III trial currently underway (NCT02425891). The combination of eribulin and pembrolizumab is being tested in an ongoing trial for heavily pretreated metastatic TNBC, and the interim analysis revealed a 42.1% ORR to first-line treatment and a 27.3% ORR to later-line treatment. These trials involving eribulin and paclitaxel, which are regarded modulators of immune priming, have once again suggested the importance of early application of immunotherapy in systemic treatment for metastatic TNBC. However, PD-L1 status failed to predict treatment response to either combination. Currently, KEYNOTE-355, a phase III trial evaluating the combination of pembrolizumab plus conventional chemotherapy compared with chemotherapy alone as the first-line treatment, is running in metastatic TNBC (NCT02819518). The combination of durvalumab and nab-paclitaxel followed by dose-dense conventional chemotherapy as well as the combination of avelumab and an antibody to another immune modulator, 41BB, is under investigation in advanced solid tumours, including TNBC (NCT02489448).

In early TNBC, preliminary results from the neoadjuvant I-SPY 2 trial demonstrated that pCR rates increased from 22.3% to 62.4% by adding neoadjuvant pembrolizumab to paclitaxel followed by anthracycline-based chemotherapy, which represents an approximately 40% improvement in pCR compared with standard chemotherapy alone. The KEYNOTE-173 trial also showed a remarkably increased pCR rate from 60% to 90% in high-risk patients by combining pembrolizumab with paclitaxel or conventional chemotherapy according to the physician’s discretion. In the adjuvant setting, the SWOG1418 phase III trial is evaluating adjuvant monotherapy with pembrolizumab after neoadjuvant chemotherapy followed by curative surgery. Another phase III trial for high-risk patients with early TNBC is investigating...
the addition of avelumab for a year after standard curative treatment including (neo)adjuvant chemotherapy (NCT02926196). A phase I study for the feasibility of adjuvant durvalumab with a peptide vaccine is underway for patients with stage II and III TNBC after completion of standard adjuvant therapy (NCT02826434).

Combining targeted agents with immune checkpoint inhibitors

In recent experimental study, PARPi modulated cancer-associated immunosuppression by upregulating PD-L1 in breast cancer cell lines, suggesting that blockade of PD-L1 could restore their sensitivity to PARPi. Followed xenograft study of combining PARPi to a PD-L1 inhibitor revealed significant synergistic effect compared with either agent alone.138 Accordingly, the feasibility of combination treatment with durvalumab with either a PARPi or a VEGFR inhibitor is under exploration in current phase II trial.139 As MAPK signalling pathway is highly activated and involved in regulating the level of TILs in TNBC,8 TNBC and BLBC cell lines showed broad sensitivity to MEK inhibition, and the generation of effector T cells was enriched by the treatment. These results suggested the MEK inhibitor as another weapon to harness immune surveillance, and more importantly, potential synergy with immune checkpoint inhibitors is suggested.139 On the basis of these experimental results, a phase Ib trial of MEK inhibitor in combination with a PD-L1 antibody is currently recruiting patients with advanced adenocarcinoma, including TNBC (NCT02900664).

Future challenges of TNBC treatment

Although a plethora of novel immune-molecular targets have been experimentally validated, the challenge lies in transforming these preclinical and clinical benefits into our daily practice and current standard treatment. To design next-generation targeted therapeutic

### Table 3

| Phase | NCT ID | Defined breast cancer subtype | Setting | Stage | Experimental drugs | Control | Primary endpoint |
|-------|--------|--------------------------------|---------|-------|-------------------|---------|-----------------|
| III   | SWOG1418 (NCT02954874) Residual TNBC (ypT>1 cm or ypN+) | Adjuvant after NAC | NA | Pembrolizumab for 1 year | Observation as per guideline | Invasive DFS (IDFS) |
| III   | NCT02926196 High-risk TNBC | Adjuvant or post-NAC | NA | Avelumab for 1 year | Observation as per guideline | Overall DFS, DFS in PD-L1(+) patients |
| II    | I-SPY 2 (NCT01042379) *Neoadjuvant, personalised adaptive trial with novel agents | LABC including TNBC | Neoadjuvant II, III | Pembrolizumab+Paclitaxel | Standard NAC | pCR: 62.4% vs 22.3% |
| II    | KEYNOTE-173 (NCT02022074) Locally advanced TNBC | Neoadjuvant II, III | (Arm A) | Pembrolizumab → Pembrolizumab+Nab-paclitaxel (Arm B) | NA | pCR (Arm A vs B): 60% vs 90% |
| II    | KEYNOTE-522 (NCT03036488) TNBC | Neoadjuvant NA | (Neoadjuvant) | Pembrolizumab+wPaclitaxel + Carboplatin (4C) → Pembrolizumab+AC (4C) (Adjuvant) | Placebo rather than Pembrolizumab | pCR, EFS |
| I/II  | NCT02489448 TNBC | Neoadjuvant I–III | Durvalumab+Nab-paclitaxel for 12 weeks | Followed by ddAC | NA | pCR |
| II    | Triple-negative first-line study (NCT02530489) | (Neo)adjuvant TNBC | NA | Neo: Atezolizumab+Nab-paclitaxel (4C) | NA | pCR |
| III   | NeoTRIPaPDL1 (NCT02620280) Locally advanced TNBC | Neoadjuvant TNBC | Atezolizumab+Nab-paclitaxel+Carboplatin | Nab-paclitaxel+Carboplatin | Nab-paclitaxel+Carboplatin | EFS |
| Ib    | NCT02826434 TNBC | Adjuvant II/III | Peptide vaccine PVX-410 (six infusions)+Durvalumab (2C) | After standard adjuvant chemotherapy | NA | DLT of PVX-410 in combination with Durvalumab |

(dd)AC, (dose-dense) doxorubicin and cyclophosphamide; DFS, disease-free survival; DLT, dose-limiting toxicity; EFS, event-free survival; IDFS, invasive DFS; LABC, locally advanced breast cancer; NA, not available; NAC, neoadjuvant chemotherapy; pCR, pathologic complete response; PD-L1, programmed death ligand 1; TNBC, triple-negative breast cancer.
strategies, we should return to the basics to perform personalised tumour genotyping based on valid actionable and druggable targets. In the era of modern immunotherapy, we need to figure out the optimal application of immunotherapy in TNBC including the disease setting and its combinatorial partners, based on more robust biomarkers. In addition, overcoming strategies for acquired and intrinsic resistance to immunotherapy and salvage treatment after failure of conventional immunotherapy should be defined. Selection of chemosensitive subset and enriching the efficacy of chemotherapy in these patients should not be also overlooked (figure 2).

CONCLUSION

TNBC is a unique disease entity with intrinsic molecular and immunological heterogeneity that therefore can manifest as a variety of clinical phenotypes. Extensive investigations to surmount this heterogeneity illustrated several comprehensive classification systems that incorporate immune-molecular signatures of TNBC. An important discovery was the identification of the BRCA-ness and its molecular synergism with PARPi as well as platinum-based agents. The BRCA-ness further unleashed inherent immunogenicity of TNBC by fostering dynamic tumour microenvironment, which conferred a rationale for immunotherapy in the subset. Witnessing a huge breakthrough in TNBC treatment, however, these promising scientific progresses have not yet been pertinently incorporated into our daily practice, and patients are still starved of available treatment options. To translate these therapeutic potentials into practical benefit, we must consistently and vigorously pursue the maximal opportunities of clinical trials for patients with TNBC. Customised clinical trials based on individualised genotyping seem also inevitable to set precise personalised medicine against its wide evolutionary mutational spectrum. In parallel, optimal tailoring of conventional chemotherapy in the landscape of immune-molecular heterogeneity should also be continued to establish the most effective regimens. Juggling with these efforts, the next chapter

Figure 2  Future aspects of therapeutic strategies in patients with TNBC based on its chemosensitivity and immune-molecular heterogeneity. Future challenge in TNBC is fundamentally to enrich the therapeutic efficacy to the optimal level both for chemosensitive and chemoresistant population. In this context, conventional chemotherapy and these four key entities constitute the main domain of upcoming treatment strategies. Targeting the BRCA-ness, revisiting our old but competent targets including PAM pathway and emerging immunotherapy can be the master molecular regulators of TNBC tumour microenvironment. Smart refining of conventional chemotherapy should be accompanied with these molecular targeting. Finally, combinatorial chains between these four independent domains would be the key of future therapeutics for TNBC. CTLA-4, cytotoxic T-lymphocyte-associated protein 4; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; PAM, PI3K-Akt-mTOR; PARPi, poly(ADP-ribose) polymerase inhibitors; PD-1, programmed cell death protein 1; PD-L1, programmed cell death ligand 1; TNBC, triple-negative breast cancer.
of TNBC treatment will be finally written on the novel combinatorial strategies, necessitating master refinement of target population and molecules.

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