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Review

Controversial issues in the neoadjuvant treatment of triple-negative breast cancer

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Amanda Fitzpatrick and Andrew Tutt

***Abstract*:** Triple-negative breast cancer (TNBC), as a collective group of heterogenous tumours, displays the highest rate of distant recurrence and lowest survival from metastatic disease across breast cancer subtypes. However, a subset of TNBC display impressive primary tumour response to neoadjuvant chemotherapy, translating to reduction in future relapse and increased overall survival. Maximizing early treatment response is crucial to improving the outlook in this subtype. Numerous systemic therapy strategies are being assessed in the neoadjuvant setting and the current paradigm of generic chemotherapy components in regimens for high-risk breast cancers, regardless of biological subtype, is changing. Therapeutic approaches with evidence of benefit include platinum drugs, polyadenosine diphosphate ribose polymerase (PARP) inhibitors, immunotherapy and second adjuvant therapy for those not achieving pathological complete response. Importantly, molecular testing can identify subgroups within TNBC, such as deoxyribonucleic acid (DNA) homologous recombination repair deficiency, lymphocyte-predominant tumours, and TNBC type 4 molecular subtypes. Clinical trials that address the interaction between these biomarkers and treatment approaches are a priority, to identify subgroups benefiting from additional therapy.

Keywords: BRCA, breast cancer, immunotherapy, neoadjuvant chemotherapy, PARP inhibitor, triple negative

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Introduction

Triple-negative breast cancer (TNBC) is defined by the absence of oestrogen, progesterone and human epidermal growth factor receptor 2 (HER2).[1] Taken as a whole, this heterogeneous group of tumours display the highest distant metastasis rate and lowest overall survival (OS) of all breast cancer subtypes.[2] Despite surgery and adjuvant therapies, half of primary TNBC confined to breast and lymph nodes recur in distant sites by 5 years, and there is a strong predilection for metastasis to visceral organs and the central nervous system.[3] Systemic treatment of metastatic TNBC is currently limited to chemotherapy drugs, with successive regimens displaying diminishing effectiveness. Although the molecular landscape is largely known, no biologically targeted therapies have yet demonstrated applicability to this subtype.

Paradoxically, TNBC in the primary setting is the most chemotherapy responsive of all subtypes, revealed by the tumour response assessment possible when chemotherapy is given in the neoadjuvant rather than the adjuvant setting.[4] A large prospective study at MD Anderson between 1985 and 2004 was the first to comprehensively document the response to neoadjuvant chemotherapy (NACT) across breast cancer subtypes, and revealed that double the number of TNBC achieved pathological complete response (pCR; no remaining tumour in surgical specimen) than non-TNBC tumours (22 *versus* 11%).[5] pCR rate has increased further with more intensive regimens, and has become a major benchmark in assessing the most effective early breast cancer treatment regimens.

Why use neoadjuvant rather than adjuvant as systemic therapy in TNBC?

In contrast to hormone receptor (HR)-positive breast cancer, HR-negative tumours, including

Correspondence to:
Amanda Fitzpatrick
Breast Cancer Now Research Centre, The Institute of Cancer Research, 237 Fulham Road, London SW3 6JB, UK
amanda.fitzpatrick@icr.ac.uk

Andrew Tutt
Breast Cancer Now Research Centre, Institute of Cancer Research, London, UK Faculty of Life Sciences and Medicine, King's College London, London, UK

those free of lymph node involvement, display a high risk of distant recurrence. This can be reduced by chemotherapy as an adjuvant to surgery,[6] with equivalent outcomes whether given before (neoadjuvant) or after (adjuvant) surgery.[7] A key benefit of neoadjuvant therapy is the possibility of 'real-time' monitoring of treatment response, allowing the oncologist to assess chemosensitivity, or lack thereof, in each individual's tumour prior to surgical resection. Aside from a higher degree of breast-conserving surgery (BCS) or improved cosmesis with BCS,[8] this approach also allows for (a) addition of other systemic therapies to improve response during neoadjuvant therapy, (b) investigation of potential further 'adjuvant' therapy after surgery in clinical trials targeting those at highest risk, and (c) prognostication of future risk of relapse, with the potential to adopt close follow-up protocols. Furthermore, clinical trial design using primary tumour response to chemotherapy as the primary outcome expedites the assessment and approval of new agents, without the requirement to wait for several years of follow-up data.

pCR serves as a surrogate marker for improved distant relapse-free survival and OS in TNBC. Substantial evidence for this association comes from the CTNeoBC (Collaborative Trials in Neoadjuvant Breast Cancer) international working group, who performed a pooled analysis of 12 trials of anthracycline and taxane-based neoadjuvant regimens between 1990 and 2011.[9] The rate of achievement of pCR after chemotherapy was found at 34% in TNBC, 30% in HER2-positive (50% with addition of trastuzumab), 16% in high-grade HR positive, and 7.5% in low grade HR-positive tumours. All subgroups of breast cancer except for low grade, HR-positive tumours, revealed a significant association between achievement of pCR and event-free survival, with the largest magnitude of effect seen in the TNBC subgroup, where achievement of pCR was associated with 75% lower risk of recurrence. This analysis also demonstrated that the association with survival was stronger when complete tumour response was seen in both the breast and lymph nodes (ypT0 pN0 and ypT0/is ypN0) rather than the breast alone (ypT0/is), highlighting the importance of lymph node response to chemotherapy. The former is used as the definition of pCR throughout this review.

Failure to achieve pCR does not necessarily spell poor prognosis; however, there is clear evidence that lower volume of residual tumour following chemotherapy equates to better outcome. The quantity of residual disease in the surgical specimen, or residual cancer burden (RCB), following neoadjuvant therapy in breast cancer is internationally classified as RCB-0, I, II and III, considering size and cellularity of the tumour in the surgical specimen, where RCB-0 is equivalent to pCR, and RCB-III signifies no response or tumour progression.[10] The predictive value of RCB was investigated by Symmans and colleagues in a prospective clinical trial of neoadjuvant systemic chemotherapy conducted at the MD Anderson Cancer Centre. In the triple-negative cohort ($n=219$), 10-year relapse-free survival rates were 86%, 81%, 55%, and 23% for pCR/RCB-0, RCB-I, RCB-II and RCB-III, respectively.[11] Hence, with appropriate therapy, subgroups within TNBC achieving RCB-0/I with neoadjuvant therapy can achieve long-term prognoses similar to the non-TNBC setting.

There is therefore a strong rationale to aim for maximal response to the initial, presurgical systemic therapy in TNBC, with the knowledge that this can translate to improved overall outlook for this breast cancer subtype.

Taxane use in the neoadjuvant setting for TNBC

The incorporation of taxane chemotherapy into adjuvant regimens has become standard of care for 'high-risk' breast cancers, which generally includes all TNBC and HER2-positive tumours, and HR-positive/HER2-negative tumours which are high-grade and node positive. Numerous key adjuvant studies and a meta-analyses have displayed improved overall and relapse-free survival with postsurgical anthracycline–taxane *versus* anthracycline alone.[12,13] In the context of neoadjuvant therapy, the addition of taxanes also results in a higher pCR rate, for example, 26% for AC (doxorubicin and cyclophosphamide) followed by docetaxel, compared with 14% with AC alone in the B27 study.[14] The GeparTrio study, where participants received up to eight cycles of neoadjuvant TAC (docetaxel, doxorubicin, cyclophosphamide) displayed a pCR rate of 37% in the TNBC cohort.[15] The choice and schedule of the soluble taxane drug has not been evaluated specifically in the neoadjuvant setting; however, in the adjuvant setting, there is evidence that weekly paclitaxel may be more effective than docetaxel given 3 weekly.[16,17]

Albumin-bound paclitaxel (nab-paclitaxel) has the advantage of reduced rates of anaphylaxis and hypersensitivity. Its antitumour activity in the neoadjuvant setting has been investigated in two studies. The GeparSepto trial demonstrated that, in sequential combination with epirubicin and cyclophosphamide, pCR was significantly higher at 38% with nab-paclitaxel than 29% with soluble paclitaxel, both given weekly for 12 weeks.[18] Survival data recently published also showed a significant disease-free survival, but not OS benefit.[19] However, this result was not reproduced by the ETNA trial, which reported a small, but not statistically significant increase in pCR with nab-paclitaxel (22.5%) over paclitaxel (18.6%).[20] Both trials were similar in size, however study design differed in terms of nab-paclitaxel dose, which was 150 mg/m^2 in GeparSepto and 125 mg/m^2 in ETNA, and the administration schedule, with continuous weekly dosing in GeparSepto and in ETNA.

Currently, the albumin-bound formulation remains reserved for those who have experienced hypersensitivity to standard taxanes; however, the results of GeparSepto may lead some clinicians to choose this formulation for potential increased efficacy.

Is there a role for antiangiogenesis agents in TNBC neoadjuvant therapy?

Angiogenesis, a key step in tumourigenesis, is upregulated in TNBC compared with other subtypes, with higher tumour microvessel density[21] and increased levels of a key regulator, vascular endothelial growth factor (VEGF).[22] Bevacizumab, a monoclonal antibody directed against VEGF, was initially assessed in the metastatic TNBC setting, and displayed improved progression-free survival (PFS), but not OS across three phase II studies.[23]

Although no OS benefit, there was a clear rationale to assess antiangiogenesis at the primary tumour setting, since invasion and metastasis require blood vessels for tumour cell escape and intravasation to the circulation.[24,25]

GeparQuinto, a phase III neoadjuvant study, assessed the addition of bevacizumab to anthracycline–taxane chemotherapy, and found pCR improved from 33% to 43% with bevacizumab;[26] however, the 3.8-year survival data have since shown no difference in disease-free, nor overall, survival.[27]

A UK-led phase III study, ARTemis, evaluated the addition of four cycles of bevacizumab to neoadjuvant anthracycline–taxane chemotherapy for HER2-negative breast cancer. A total of 800 patients were recruited, of which 31% were also HR negative, therefore categorized as TNBC. The rate of pCR for both HR positive and negative combined was 22% with bevacizumab and 17% with chemotherapy alone.[28] Similar to GeparQuinto, 3.5-year survival data in ARTemis showed that the improved pCR was not associated with improved survival.[29] There was no effect of bevacizumab on survival in the adjuvant study BEATRICE.[30,31] Bevacizumab does not confer a reduction in distant recurrence and therefore is not recommended for routine use in neo/adjuvant TNBC. However, it may have a role in primary tumour downstaging, in the setting of large tumours, to achieve BCS.

Defining 'BRCA-ness' in TNBC

TNBC displays a high prevalence of chromosomal genome instability.[32] This is suggested as arising through frequent defects in deoxyribonucleic acid (DNA) repair pathways, in particular, those involving homologous recombination repair (HRR), a cellular mechanism for repairing DNA double-strand breaks (DSBs). HRR uses a homologous DNA sequence to guide repair at the DSB, thereby conserving the DNA sequence and integrity. In the setting of defective HRR, cells must rely instead on the error-prone pathway nonhomologous-end joining (NHEJ), which although effective in repairing the DSB, can result in DNA sequence aberrations and chromosome rearrangements.[33]

Defective HRR has been proposed as an important therapeutic vulnerability in TNBC. Cells can be pushed into replication fork crisis and thus cell death by use of DNA-damaging agents, such as platinum chemotherapy or polyadenosine diphosphate ribose polymerase (PARP) inhibitors, leading to DNA replication fork arrest and associated DSBs.[34]

At a molecular level, defective HRR in TNBC can in part be explained by somatic or germline mutations in HRR-pathway genes, such as *BRCA1* and *BRCA2*.[35] Germline *BRCA1/2*

mutation carriers (g*BRCA*m) have a markedly increased lifetime risk of developing breast and ovarian cancer.[36] There is a strong association between *BRCA1* and TNBC with 80–90% of *BRCA1*-mutated breast cancers being triple negative.[37] However not all TNBCs occur in the setting of germline *BRCA* mutation, as evidenced by a recent a large analysis of the germline DNA of 1824 patients with TNBC, which found 11% to have germline *BRCA* aberrations, and a further 4% having deleterious germline mutations in genes involved in other homologous recombination genes, such as *PALB2* and *RAD51*.[38] In the remaining 85%, no deleterious mutations were found in DNA repair genes; however, it has been suggested that a high proportion of TNBC exhibit functional evidence of defective homologous recombination, so called 'BRCA-ness'.[39]

There are currently many translational research groups endeavouring to deliver a clinically applicable test of defective HRR. This would also provide the opportunity to discover multiple potentially novel genetic and epigenetic drivers of HRR deficiency.[40]

Specific mechanisms such the epigenetic silencing of *BRCA*1 by promotor methylation, or reduced *BRCA* messenger ribonucleic acid (mRNA), may be easily assayed from tumour tissue specimens. A wide-angle approach to identifying HRR-deficient tumours is to identify genomic 'scars' of defective HRR: loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST).[40] These can be assessed using genome-wide single-nucleotide-polymorphism (SNP) arrays, or whole-genome sequencing, with the former being more cost effective and potentially clinically applicable. Myriad Genetics have developed a commercial test which measures all three genomic features using a custom whole-genome SNP profiling, and has been evaluated for its predictive value in response to DNA-damaging agents in both breast and ovarian cancer.[41] The resultant homologous recombination deficiency (HRD) score has now been used in various clinical trials to try defining potential TNBC subgroups beyond germline *BRCA* mutant who may benefit from additional therapies exploiting this vulnerability. While there has been some evidence from analysis of phase II trials that the Myriad HRD assay may predict of increase pCR with a platinum-containing regimen,[41] this assay did not distinguish between prediction of response to the carboplatin-containing regimen and the anthracycline and taxane control in the GeparSixto trial,[42] and is discussed below. Furthermore, the recently published TNT trial[43] did not show any evidence of predictive performance of a similar Myriad HRD assay for platinum selection over taxane in the metastatic setting. More recently a new 'HRDetect' assay[44] has been developed that requires whole-genome sequencing and the field eagerly awaits analysis of its performance as a specific platinum or PARP-inhibitor-response predictor.

Should platinums now be used in neoadjuvant management of TNBC?

Platinum drugs create DNA DSBs by creating adducts in DNA that arrest DNA replication. Striking responses are seen both preclinically and clinically to platinum chemotherapy in a BRCA-defective setting.[45,46] Platinum drugs appear more active in the TNBC subtype than other breast cancers. A Cochrane review of platinum-containing regimens concluded little to no effect on PFS or OS in unselected metastatic breast cancer, but evidence of a modest PFS improvement (hazard ratio 0.59; 95% confidence interval 0.49–0.70) in metastatic TNBC.[47] A direct comparison to docetaxel in the first-line metastatic setting was carried out in the TNT trial ($n=376$), which concluded that carboplatin is active in unselected metastatic TNBC, but offers no advantage over docetaxel.[43] However, the *bona fide* BRCA-defective subgroup, g*BRCA*m carriers, did display carboplatin sensitivity in the TNT trial, with doubling of response rates (68 *versus* 33%) over docetaxel, whereas 'BRCA-ness' subgroups, classified as tumours with *BRCA*1 methylation, low levels of *BRCA*1 mRNA, or high Myriad Genetics HRD score, displayed no increase in response with platinum over docetaxel.

Therefore, in the metastatic setting, platinum is an active chemotherapy agent and response appears enriched in germline *BRCA* mutation carriers, but not in epigenetically driven '*BRCA*-ness' TNBC.

Three phase II/III studies have assessed the addition of platinum to neoadjuvant therapy: GeparSixto ($n=595$ total, 315 TNBC), CALGB 40603 ($n=454$ all TNBC), and BrighTNess ($n=634$, all TNBC).

GeparSixto[48] investigated the addition of carboplatin to paclitaxel and liposomal doxorubicin given weekly for 18 weeks preoperatively in HR-negative breast cancer. In the TNBC subgroup ($n=315$), pCR was significantly higher at 57% *versus* 41% with the addition of carboplatin,[49] and there was a superior recurrence-free survival at 3 years with carboplatin than without (86 *versus* 76%), although no significant OS difference.[50]

CALGB 40603[51] employed a more standard chemotherapy backbone of weekly paclitaxel for 12 weeks followed by dose-dense doxorubicin and cyclophosphamide for four cycles. Analysis revealed that the pCR rate was significantly increased with carboplatin (54%) compared with the control arm (41%). The survival data at 3 years did not demonstrate a significant improvement with carboplatin; however, the study was not powered to detect event-free survival.

BrighTNess[52] again demonstrated the additive effect of carboplatin, in terms of achieving pCR. This study was primarily designed to determine the effectiveness a PARP inhibitor, veliparib, in combination with carboplatin, added to the standard backbone of paclitaxel followed by doxorubicin and cyclophosphamide as NACT for TNBC. pCR rate was 31% in the standard treatment group and rose significantly to 58% with addition of either carboplatin alone, or 53% with the added combination of carboplatin and veliparib. Survival data from this study have not yet been published.

Although clear that pCR rates are significantly higher with the addition of carboplatin to NACT, the survival data are currently equivocal and the BrighTNess data are eagerly awaited.

An explanation for the difference in survival results between GeparSixto and CALGB 40603 could be the use of cyclophosphamide in the chemotherapy backbone of the latter study. Cyclophosphamide is an alkylating drug, which, in a manner similar to carboplatin, exerts a cytotoxic effect by creating DSB-inducing DNA interstrand crosslinks. Therefore, in the absence of cyclophosphamide (GeparSixto), response to the DNA crosslinking effect of carboplatin may have been more apparent, whereas in CALGB 40603, the additional benefit of carboplatin may have been blunted.

The effect of cyclophosphamide is also supported by the GeparOcto trial, addressing the question of whether the high-dose intensity combination of epirubicin, taxane and cyclophosphamide (iddEPC) is equivalent to the carboplatin-containing treatment GeparSixto regimen of paclitaxel, liposomal doxorubicin and carboplatin (PMCb). This study suggests carboplatin and high-dose cyclophosphamide may be interchangeable, in combination with taxane and anthracycline, with similar pCR rates of 48.3% in the iddEPC arm and 48.0% in the PMCb arm.[53]

Given the current inconclusive survival benefit data, carboplatin is not yet universally considered standard of care in neoadjuvant therapy for TNBC. Many centres take an individualized approach per patient, adding carboplatin to the taxane phase of treatment in patients with higher stage of disease or sequentially, if tumour response is suboptimal following an initial anthracycline–cyclophosphamide phase of treatment. Adding carboplatin is not without toxicity, with increased haematological adverse events of neutropenia (including febrile neutropenia), anaemia and thrombocytopenia; therefore, there is a need to carefully define the patient subgroup that will benefit most with fewest complications. It seems most logical to consider adding platinum for patients with stage II or III cancers and few comorbidities, who have both higher recurrence risk and greater need for tumour response to improve cosmesis.

As with trials of platinum drugs in the metastatic setting, BRCA defectiveness has been investigated as a predictive biomarker for platinum-responsive subgroups in neoadjuvant trials. Contrary to the metastatic setting, germline *BRCA* mutation status was not a predictive biomarker for neoadjuvant carboplatin response in CALGB 40603. This was explored by a secondary analysis,[54] which discovered that in the 17% harbouring g*BRCA*m, the high pCR rate of 65% was not increased further by addition of carboplatin. Disease-free survival data showed that g*BRCA*m carriers had preferable prognoses regardless of chemotherapy regimen, whereas germline *BRCA* wild-type patients did experience additional improvement in 5-year survival rates with carboplatin. Recent reports of the g*BRCA*m subgroup of the GeparOcto study suggest, however, that mutation carriers with high-stage disease do gain greater benefit from the

high alkylating-agent-containing regimens and perhaps platinum regimens, specifically.[55]

In total, these data suggest that in the neoadjuvant setting, a g*BRCA*m subgroup with smaller tumours, perhaps receiving NACT while considering bilateral risk-reducing mastectomy, could perhaps be spared of the addition of carboplatin, since they already experience very high levels of chemotherapy response and excellent survival outlook with sequential anthracycline/cyclophosphamide and taxane regimens. Most patients with gBRCA1/2 mutations and particularly those with stage II or III tumours, and those who opt for breast conserving surgery who need to maximize tumour response for good cosmesis, should still be considered for platinum-based therapy. Some patients with stage I tumours or comorbidities should not be committed to receiving a platinum-based regimen based on *BRCA1/2* mutation status alone.

Further exploratory analysis of the GeparSixto trial assessed responses in HRR-deficient subgroups, using tumour *BRCA1/2* mutation status and the Myriad HRD score.[56] Of the 193 patient samples analysed, 136 were assessed to be HR deficient, while 129 had a high HRD score, 54 of which harboured either somatic or germline *BRCA* mutation, and an additional 7 tumours had *BRCA* mutation without high HRD score. HRR-deficient tumours had a higher pCR rate with chemotherapy overall (50% pCR rate in HRR deficient *versus* 24.6% pCR in nondeficient). Although HRR-deficient tumours experienced a higher pCR with added carboplatin (63.5% pCR rate) than without (33.9% pCR without carboplatin), the test for interaction was negative, therefore the HRD score did not act as a treatment selection biomarker to aid platinum selection in TNBC.

Assessment of alternative HR-deficiency assays, such as 'HRDetect', as therapy-specific predictive biomarkers in neoadjuvant trials is warranted to define TNBC subgroups who may benefit from additional therapy targeting defective HRR, and spare patients who may not benefit from the additional toxicity associated with platinum use.

The investigation of PARP inhibitors as neoadjuvant therapy for TNBC

In the setting of *BRCA* mutation, the inhibition and trapping on DNA of the PARP1 enzyme leads to cell death by synthetic lethality, as these cancers are more reliant on DNA repair pathways other than HRR.

Recently, olaparib became the first PARP inhibitor licensed by the US Food and Drug Administration (FDA) in the setting of g*BRCA*m breast cancer. This approval followed results of the phase III OlympiAD trial,[57] where olaparib was tested against clinicians' choice of chemotherapy in metastatic g*BRCA*m, HER2-negative breast cancer. Radiological response occurred in 60% of participants in the olaparib group compared with 29% with chemotherapy, and disease progression was delayed from 4.2 months to 7.0 months by olaparib. Subgroup analysis showed that HR-negative breast cancers were more responsive to PARP inhibition, with risk of progression decreased twofold compared with HR-positive subgroups (hazard ratio 0.43 *versus* 0.82).

In the neoadjuvant setting, PARP inhibitors are not yet used outside of clinical trials. Researchers in BrighTNess, as mentioned above in relation to carboplatin, assessed whether adding the combination of carboplatin and veliparib to Adriamycin® and cyclophosphamide (AC)–paclitaxel was superior to AC–paclitaxel alone or AC–paclitaxel plus carboplatin. Although the benefit of the addition of carboplatin was clear, there was no additional benefit from veliparib.[52]

There is an increasing understanding of the mode of action of PARP inhibitors. PARP1 enzymatic inhibition or PARP1 depletion leads to unrepaired single-strand breaks, which ultimately cause cell death in the setting of defective HRR, a phenomenon known as synthetic lethality. A more potent cytotoxic activity is *via* the 'trapping' of PARP onto DNA.[58] The various PARP inhibitors have differing PARP-trapping potency, with veliparib displaying very little, offering explanation for lack of effect in BrighTNess. Olaparib exhibits significant PARP-trapping activity and is currently being evaluated in the OlympiA adjuvant study[59] and in combination with platinum in the PARTNER neoadjuvant study [ClinicalTrials.gov identifier: NCT03150576].

Talazoparib, the most potent PARP inhibitor for both trapping and catalytic activity, has shown significant responses (78–88% tumour shrinkage) when used as monotherapy in the neoadjuvant setting in g*BRCA*m patients.[60] Results of a small phase II study comprising 20 patients with

confirmed g*BRCA*m who received talazoparib monotherapy for 6 months prior to surgery, followed by adjuvant chemotherapy, were presented at the American Society of Clinical Oncology (ASCO) conference 2018.[61] The majority of patients were triple negative ($n=17$), with the remainder being HR positive/HER2 negative. The rate of achieving RCB-0/I following neoadjuvant talazoparib monotherapy was 59%, and the larger-scale evaluation of this potent PARP inhibitor will be conducted.

There is a need to define whether benefit from PARP inhibitors is limited to the g*BRCA*m setting, or whether other the 'BRCA-ness' group may also benefit. A phase II study is currently underway in the advanced setting addressing talazoparib responses in germline *BRCA* wild-type TNBC with either high HRD score or germline or somatic mutation in other HRR-pathway genes [ClinicalTrials.gov identifier: NCT 02401347].[62]

Should gene expression subtypes of TNBC impact therapeutic strategies?

Classification of the molecular heterogeneity in TNBC has been sought using gene expression panels. PAM50 intrinsic subtypes segregate most TNBC as basal like (80%), however the remainder can fall into luminal-A, luminal-B and HER2-enriched subtypes.[63] To date, PAM50 basal-like subtype has not been found a validated predictive biomarker influencing NACT regimen selection; for example, for patient selection for addition of carboplatin, in contrast to the apparent interaction between basal-like and nonbasal-like status with the single-agent platinum *versus* taxane effect in the metastatic setting in the TNT trial.[43,64]

Lehmann and colleagues developed the TNBC type 4 classifier, using publicly available gene expression datasets, and classified 587 TNBC as BL-1 (basal-like 1), BL-2 (basal-like 2), M (mesenchymal tumour) and LAR (luminal androgen receptor).[65,66] The two basal-like tumour subgroups, BL-1 and BL-2, comprise 35% and 22% of TNBC, respectively, and are highly proliferative tumours with enriched expression of cell cycle and proliferation genes, with predominant DNA damage response profile in BL-1 and growth factor signalling in BL-2. BL-1, although highly proliferative, displays the best prognosis in terms of OS, most likely due to being enriched for BRCA-defective tumours known to have better prognosis and improved chemotherapy response.[67] M type, comprising 25% of tumours, are mesenchymal in nature with expression of genes involved in epithelial–mesenchymal-transition and growth factor pathways. Clinically, M type displays a pattern of early relapse and preferential metastasis to lungs. The LAR subtype, also identified in oestrogen-receptor-positive and HER2-positive breast cancers, comprised 16% of the TNBC analysed, and has a luminal pattern of gene expression and androgen-receptor signalling, and in keeping with other endocrine-regulated cancers, frequent metastasis to bone, lymph node and late relapses. TNBC type 4 has been investigated prospectively as a predictive biomarker tool in a nonrandomized trial of neoadjuvant docetaxel and carboplatin. BL-1 displayed the highest pCR rate (65.6%) followed by BL-2 (47.4%), M (36.4%) and LAR the lowest (21.4%).[66] Therefore, TNBC type 4 classification may have implications for approaches to neoadjuvant therapy. The lower pCR rates in the LAR subtype are in keeping with its luminal phenotype, and therapy targeting the androgen receptor may offer improvements. Various androgen-targeting agents are already available and used widely in prostate cancer, such as bicalutamide, enzalutamide and abiraterone. In addition to numerous metastatic studies of these agents in TNBC–LAR subtype, a neoadjuvant trial is currently underway at the MD Anderson Cancer Centre using enzalutamide plus paclitaxel [ClinicalTrials.gov identifier: NCT02689427].

Does immune infiltrate in TNBC help decision making?

The complex interplay between immune and tumour cells in the breast cancer microenvironment continues to be characterized. In TNBC, there is growing evidence that presence of tumour-infiltrating lymphocytes (TILs) is both predictive of response to chemotherapy and prognostic for better OS.[68–70]

TNBC in general has a relatively higher mutational load[71] and increased infiltration of TILs than other subtypes.[68] Some 70% TNBC has at least 20% TILs in the tumour itself or tumour stroma.[68] The definition of lymphocyte-predominant breast cancer (LPBC) defines a population which have more than 50–60% TIL abundance.[68] A prospective study involving both TNBC and HER2-positive breast cancer subjects from the GeparSixto trial, identified LPBC subtype in 28% of TNBC and in 20% of HER2-positive tumours.[72] Lymphocyte infiltrated

tumours displayed a significantly higher response to carboplatin than non-infiltrated tumours (pCR rate 75% LPBC *versus* 34% non-LPBC). The small pCR increase with 'standard' chemotherapy in LPBC (45% LPBC *versus* 34% non-LPBC) was not statistically significant, suggesting that the LPBC associated increase in chemotherapy response was attributable to carboplatin.

Combined with the discovery that *BRCA1* mutation associated breast cancers demonstrate increased lymphocyte infiltration,[73] and platinums may cause immunogenic cell death,[74] there is strong rationale underpinning the selection of platinum chemotherapy in the setting of *BRCA1*-mutated LPBCs.

Survival data pertaining to TIL content has been sought retrospectively by the BIG 02-98 trial group examining intratumoural TIL content in an adjuvant chemotherapy study. Using a threshold of 50% or greater TIL content, 10.6% of TNBC and 11.1% of HER2 positive were classified as LPBC, while only 2.9% of HR-positive/HER2-negative tumours were classified as such. Disease-free survival was demonstrated to be 92% at 5 years with an LPBC phenotype *versus* 62% for non-LPBC.[70]

Rather than using LPBC status as a dichotomous variable, it is now appreciated that the gradient of TIL-infiltrated breast cancer is important, with each 10% increase in TIL content equating to an increment in pCR rate and 14% reduction in recurrence or death.[75]

TIL content can be readily assessed from the tissue section on haematoxylin and eosin staining, does not involve expensive molecular testing platforms and can predict outcome following NACT. This information raises a new controversy: could assessment of TIL content be introduced as a standard reporting parameter for TNBC to aid in treatment selection?

Despite evidence for the predictive and prognostic role of TIL content, reporting has not been incorporated into routine clinical practice. This may be because TNBC tumours, regardless of high or low TIL content, would still be recommended neo/adjuvant chemotherapy. However, knowledge of TIL content may aid in the selection of patients who would benefit from additional chemotherapy, such as carboplatin. It should also be noted that there is considerable complexity within TIL populations, for example CD8+ cytotoxic T cells are antitumourigenic, whereas Foxp3+ regulatory T cells repress antitumour immune response;[76] therefore, this may need to be taken into account when incorporating immune assessment into clinical practice.

With the emerging use of immune-checkpoint inhibitors in breast cancer, increased TIL count may also be a predictive biomarker for response, as demonstrated in the Keynote-086 study of the programmed death-1 (PD-1) inhibitor pembrolizumab.[77] Furthermore, expression of programmed death-ligand 1 (PD-L1) on tumour-infiltrating immune cells was shown to predict response to atezolizumab in advanced TNBC in the IMpassion130 trial, and will be discussed further below.[78]

How much does the future of TNBC therapy involve immunotherapy?

PD-L1 is a transmembrane protein which can be expressed by a variety of cell types, including tumour cells and tumour-infiltrating T cells, and inhibits antitumour immune activity when bound to the cell-surface receptor PD-1 on CD8+ cytotoxic T cells. PD-1/PD-L1 immune-checkpoint inhibitors enhance the endogenous adaptive antitumour immune response and have brought major therapeutic advancement to a growing number of solid tumours.

Currently, major research efforts are underway to determine applicability of checkpoint inhibitors targeting the PD-1/PD-L1 immune checkpoint in breast cancer, appearing most promising in TNBC and HER2-enriched subtypes. As a monotherapy, only modest effects on survival were seen in heavily pretreated metastatic TNBC using the PD-1 inhibitor pembrolizumab;[79] however, numerous clinical trials combining checkpoint inhibitors with chemotherapy or PARP inhibitors earlier in metastatic disease management are ongoing.[80–82] A recently completed phase III study, IMpassion130, combined nab-paclitaxel chemotherapy with the anti-PD-L1 antibody atezolizumab, in the first-line therapy of advanced TNBC. Atezolizumab plus nab-paclitaxel led to PFS of 7.2 months, significantly longer than 5.5 months with nab-paclitaxel alone. The benefit was enhanced among patients with PD-L1-positive tumours, with median PFS of 7.5 months and 5.0 months, respectively. Interim OS analysis showed a numerically longer survival in both the intention-to-treat (ITT) and PD-L1 positive

subgroups at 21.3 *versus* 17.6 months (ITT) 25 *versus* 15.5 months (PD-L1 positive). These results have led to atezolizumab gaining FDA approval for first-line treatment of locally advanced or metastatic TNBC, combined with nab-paclitaxel. This is the first immune-checkpoint inhibitor therapy approval in breast cancer.

Durable responses in advanced disease with immunotherapy have spawned an attractive concept for neoadjuvant treatment, where release of immune checkpoints while macroscopic tumour is present and subjected to chemotherapy induced cytotoxicity may both improve tumour response and long-term eradication of minimal residual disease. A trial arm within the I-SPY 2 phase II platform trial evaluated the addition of pembrolizumab, a PD-1 inhibitor, to NACT in HER2-negative breast cancer with high-risk features on predefined molecular profiling. The initial results from this study involving 69 patients demonstrated an increase in pCR rate from 22.3% to 62.4% with the addition of pembrolizumab to standard AC–paclitaxel therapy.[83] Neoadjuvant pembrolizumab is also being investigated in the phase II neoadjuvant study KEYNOTE-173, and preliminary results were presented at the San Antonio Breast Cancer Symposium in 2018. This six-cohort study assesses the safety and efficacy of the combination of PD-1 inhibitor pembrolizumab (Keytruda) with platinum/taxane chemotherapy at varying doses. Pembrolizumab was administered in each cohort. The pCR rate across all cohorts was 60%, and the highest rates were reported in the cohorts administered nab-paclitaxel and carboplatin with pembrolizumab. Less encouraging results were seen with durvalumab, a PD-L1 antibody, which was investigated as a neoadjuvant therapy in the phase II placebo-controlled study GeparNuevo. This study included a window period where patients received durvalumab or placebo alone 2 weeks prior to commencement of durvalumab/placebo plus chemotherapy, which comprised nab-paclitaxel followed by epirubicin and cyclophosphamide.[84] There was a nonsignificant increase in pCR with the addition of durvalumab (53% *versus* 44%, $p = 0.287$). However, the subgroup that received durvalumab in the window period displayed significant increase in pCR (61% *versus* 41%, $p = 0.035$), raising the question of appropriate sequencing of immune-checkpoint inhibition when combined with chemotherapy.[85]

Preliminary data was also reported at ASCO 2019, suggesting higher NACT response associated with higher levels of tumour mutational burden but no interaction with durvalumab effect.[86]

Both pembrolizumab and atezolizumab are now being investigated in placebo-controlled phase III neoadjuvant studies, combined with taxane, platinum and anthracycline, in the KEYNOTE-522 trial [ClinicalTrials.gov identifier: NCT0303648][87] and IMpassion131 trial [ClinicalTrials.gov identifier: NCT03125902].[88] These large multicentre trials are expected to establish the role of neoadjuvant PD-1/PD-L1 inhibitor therapy in TNBC.

What is the role of second adjuvant therapy in patients with TNBC who don't achieve pCR?

A major advantage of NACT, and consequent ability to quantify chemotherapy-resistant residual disease burden, is the identification of patients who have a higher risk of relapse and may benefit from second adjuvant systemic therapy following surgery. The CREATE-X study was the first phase III trial conducted in this setting, randomizing patients with HER2-negative breast cancer and residual disease at surgery to six to eight cycles of adjuvant capecitabine.[89] The study met the primary outcome of improvement in disease-free survival, both for HR-positive and TNBC populations. The benefit was most prominent in the TNBC subgroup (30% of patients), where 5-year disease-free survival was 70% with capecitabine *versus* 56% without. Overall capecitabine was well tolerated, with hand–foot syndrome being the most common grade 3/4 toxicity (11% of patients), followed by neutropenia (6.3%) and diarrhoea (2.9%). Relative dose intensity was maintained in 80% of patients despite having recently completed 4–5 months of NACT. The results of this study are encouraging of the use of further systemic chemotherapy for residual disease, particularly in TNBC; however, as yet, this approach has not been widely adopted. A challenge to the generalization of the result of this single trial is that the study population was exclusively Japanese and Korean, therefore, the result should ideally be further verified in other populations. Another reason to seek further verification is that capecitabine did not add benefit when given concurrently with other chemotherapy in the adjuvant setting,[90] despite its clear activity in the metastatic setting.[91] The FinXX trial, where capecitabine was added to the anthracycline–taxane adjuvant chemotherapy, also showed no improvement in recurrence-free survival overall; however, both

the 5- and 10-year survival data demonstrated increased recurrence-free survival and OS in the TNBC subgroup. Therefore, there is a rationale to consider further evaluation of capecitabine in TNBC as opposed to other subtypes, particularly in the setting of residual disease. Currently, a US-based phase III trial (ECOG-ACRIN EA1131) is underway comparing second adjuvant platinum with second adjuvant capecitabine in basal-like TNBC with residual disease following NACT [ClinicalTrials.gov identifier: NCT02445391]. It is hoped this study clarifies the role of specific drugs for second adjuvant therapy, but will not address the role of either in comparison with placebo, meaning the use of second adjuvant chemotherapy after failure to achieve pCR will likely remain a controversial issue.

An attractive therapeutic option is the use of immune therapy in the setting of minimal residual disease, rather than clinical metastatic disease, in patients with TNBC and residual tumour in the breast resection specimen following NACT who are at high risk of such minimal residual disease. Researchers of a large phase III trial [ClinicalTrials.gov identifier: NCT02954874] currently underway aim to evaluate the effect of second adjuvant pembrolizumab in 1000 patients with TNBC who have completed definitive local treatment.[92] This trial has the potential to change the current adjuvant standard of care for TNBC patients with residual disease after NACT.

However, not only those with residual tumour experience future relapse, and the trial design of studies such as c-TRAK-TN [ClinicalTrials.gov identifier: NCT 03145961] incorporates the analysis of circulating tumour DNA (ctDNA) in plasma to identify those with minimal residual disease following NACT for TNBC, and tracks the effect of early intervention with therapies on clearance of tumour-specific ctDNA. On detection of tumour ctDNA in plasma, patients will be randomized to receive immunotherapy with pembrolizumab or placebo and continue with the monitoring of ctDNA and for the occurrence of clinical metastasis.

Conclusion

Altering the natural history of this generally poorer prognosis breast cancer subtype will rely upon reduction of distant metastatic recurrences, which appears linked to maximizing the response to therapy at the primary disease setting. pCR to chemotherapy before surgery is an important benchmark for the efficacy of new therapies linked to, but not always associated with, survival benefit, given the lack of survival benefit associated with primary tumour responses to antiangiogenesis inhibitors.[29,30]

pCR is demonstrated in response to platinum chemotherapy in unselected higher-stage TNBC and this is most prominent in two overlapping groups: g*BRCA*m carriers and tumours with high infiltrating lymphocytes.

PARP inhibitors are showing very promising results in g*BRCA*m if a potent PARP-trapping agent used; however, it is not yet clear which TNBC subgroups the PARP inhibitor benefit will extend to and if this will be defined by presence of mutation in HR-deficiency genes such as *BRCA1* and *BRCA2* or by HRD-mutational signature analysis.

Immuno-oncology approaches are attractive in TNBC with a rationale based in part on relatively higher mutational load and more frequent infiltration of lymphocytes than other breast cancer subtypes. The recent results of adding checkpoint inhibition to NACT in I-SPY2, if confirmed by definitive phase III studies, may change treatment paradigms. However, the long-lasting nature of some immune-related toxicities points to the need for identification of subgroups who could achieve maximal response with chemotherapy alone.

Lastly, further subdivision of molecular subtypes by gene expression and integrated mutation and gene expression profiles, such as PAM50 Intrinsic and TNBC type 4 subtypes, may better define the heterogeneity of TNBC and more accurately target the unique biological phenotypes associated with response to both standard-of-care chemotherapy and additional novel therapies.

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Conflict of interest statement

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ORCID iDs

Amanda Fitzpatrick https://orcid.org/0000-0001-9201-3797

Andrew Tutt https://orcid.org/0000-0001-8715-2901

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