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Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease

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Abstract

Chemotherapy is the primary established systemic treatment for patients with triple-negative breast cancer (TNBC) in both the early and advanced-stages of the disease. The lack of targeted therapies and the poor prognosis of patients with TNBC have fostered a major effort to discover actionable molecular targets to treat patients with these tumours. Massively parallel sequencing and other ‘omics’ technologies have revealed an unexpected level of heterogeneity of TNBCs and have led to the identification of potentially actionable molecular features in some TNBCs, such as germline *BRCA1/2* mutations or ‘BRCAness’, the presence of the androgen receptor, and several rare genomic alterations. Whether these alterations are molecular ‘drivers’, however, has not been clearly established. A subgroup of TNBCs shows a high degree of tumour-infiltrating lymphocytes that also correlates with a lower risk of disease relapse and a higher likelihood of benefit from chemotherapy. Proof-of-principle studies with immune-checkpoint inhibitors in advanced-stage TNBC have yielded promising results, indicating the potential benefit of immunotherapy for patients with TNBC. In this Review, we discuss the most relevant molecular findings in TNBC from the past decade and the most promising therapeutic opportunities derived from these data.

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Author contributions

G.B., J.M.B., I.A.M. and L.G. researched data for the article. M.E.S. provided histological pictures. G.B., J.M.B., I.A.M., M.E.S. and L.G. contributed to discussing the article’s content. All authors wrote, reviewed and edited the manuscript before submission.

Competing interests statement

G.B. received honorarium from Roche. L.G. had an advisory role for Merck and Roche. J.M.B., I.A.M. and M.E.S. declare no competing interests.

FURTHER INFORMATION

ClinicalTrials.gov: <https://www.clinicaltrials.gov>

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The clinical and molecular heterogeneity of breast cancer is well known. The advancement and widespread application of ‘omics’ technologies (genomics, epigenomics, transcriptomics or proteomics, among others) has provided unprecedented insights and novel understanding of the molecular complexity of this disease^{1–5}. In spite of this complexity, clinical decisions still rely primarily on the assessment of three markers: the expression of the endocrine receptors for oestrogen and progesterone (ER and PgR, respectively) and the aberrant expression of HER2. The definition of triple-negative breast cancer (TNBC) applies to all tumours that lack the expression of ER, PgR and HER2, all of which are molecular targets of therapeutic agents. Nevertheless, chemotherapy is still the primary established treatment option for patients with early-stage and those with advanced-stage TNBC⁶.

Patients with TNBC typically have a relatively poorer outcome compared with those with other breast cancer subtypes owing to an inherently aggressive clinical behaviour and a lack of recognized molecular targets for therapy⁷. Herein, we summarize the current understanding of the molecular landscape of TNBC and describe the molecular and biological features that are emerging as possible actionable targets for the treatment of this disease.

Immunohistochemical definition of TNBC

The diagnosis of TNBC depends on the accurate assessment of ER and PgR protein expression levels by immunohistochemistry (IHC), and of HER2 by IHC and/or fluorescence *in situ* hybridization (FISH). The accuracy of this assessment is crucial to avoid the risk of a false diagnosis of ER-negative and/or HER2-negative disease in patients that would potentially benefit from endocrine therapy and/or HER2-targeted drugs. Many efforts have been made to optimize and standardize the methods for measuring the status of ER, PgR and HER2 (REFS 8,9). The assessment of these markers, however, is still subject to significant pre-analytical, analytical and post-analytical variability, as illustrated by the persistent discrepancy of the results from central and local laboratory assessments^{10,11}. Data from gene expression studies^{12,13} have confirmed that a conservative cut-off point of <1% of ER/PgR-positive tumour cells (assessed using IHC) should be adopted — as suggested by current guidelines⁸ — to reduce the number of breast tumours inappropriately defined as TNBC.

Key points

- The routine diagnosis of triple-negative breast cancer (TNBC) depends on the accurate assessment of the status of the oestrogen receptor (ER), progesterone receptor (PgR) and HER2
- Chemotherapy remains the standard therapeutic approach for TNBC at all stages, with platinum compounds having a relevant role, especially in patients harbouring *BRCA1/2* mutations or ‘BRCAness’
- ‘Omics’ technologies have provided unprecedented insights into the molecular complexity and heterogeneous clinical behaviour of TNBC but, to

date, none of the newly developed molecular classifications has demonstrated clinical utility

- Several potentially actionable molecular alterations, frequently affecting PI3K/mTOR or RAS/RAF/MEK, have been found in TNBC, but none have been confirmed as a ‘driver alteration’, nor have any TNBC subsets been shown to be ‘addicted’ to them
- Targeted agents currently under clinical investigation in TNBC include PARP inhibitors, PI3K inhibitors, MEK inhibitors, anti-androgen therapies, heat shock protein 90 inhibitors, histone deacetylase inhibitors, and their combinations
- TNBC is remarkably heterogeneous in terms of the tumour microenvironment; tumour lymphocyte infiltration is associated with good prognosis and a response to chemotherapy, which provides a strong rationale for testing immunotherapies in TNBC

The molecular landscape of TNBC

Beyond the need for an accurate diagnosis of TNBC, this type of tumour has a heterogeneous clinical behaviour, and patients with this disease frequently have a poor prognosis. Thus, a concerted effort has been undertaken to understand the molecular basis of this heterogeneity and to discover actionable molecular targets for TNBC. Many of these efforts have focused on assigning TNBC molecular characteristics into subgroups with specific courses of disease and homogeneous patterns of sensitivity to chemotherapy or new therapies.

Interpatient heterogeneity

TNBC is considered a single clinical entity and uniformly treated with chemotherapy, but molecular profiling with massively parallel sequencing and other ‘omics’ technologies has revealed an unexpectedly high level of heterogeneity as well as some common features^{1,4}. A balanced approach between the simple pragmatism of the current clinical therapeutic guidelines and the molecular complexity of TNBC would be the desirable approach to the management of patients with this disease. Thus, much research has focused on identifying practical TNBC subtypes bearing uniformly actionable molecular features.

Histological classification—The majority of TNBCs (95%) are classified histologically as invasive mammary carcinomas of no specific type (or, alternatively, invasive ductal carcinomas) and lack distinctive histological characteristics, but other different subtypes have been described¹⁴ (FIG. 1). The classically described medullary carcinoma, shown by gene-expression profiling to be a subgroup within TNBC¹⁵, is rare (0.4–1%) and characterized by high lymphoplasmacytic infiltration and good outcome compared to other subtypes¹⁶. The reproducibility of this histological definition, however, has not been confirmed and whether improved outcomes can be achieved by adapting adjuvant treatment selection for patients within this subgroup is unclear. Other subtypes with unique phenotypes

— including adenoid cystic carcinoma^{14,17}, adenosquamous carcinoma and fibromatosis-like spindle-cell metaplastic carcinomas — are rare (<1%) and less aggressive, and typically only capable of local recurrence, a feature that should be considered when planning adjuvant treatment¹⁷. Interestingly, adenoid cystic carcinoma constitutes a genomically-distinct subgroup characterized by a low frequency of copy-number aberrations and a characteristic chromosomal translocation t(6;9) (q22–23;p23–24), which leads to the *MYB–NFIB* fusion gene, present in ~90% of cases of this TNBC subtype¹⁷.

Molecular classification—Several attempts have been made to establish a molecular classification of TNBC based on the results of both transcriptomic and genomic studies. In this section, we do not aim to present all the existing classification systems for TNBC but rather, to discuss those that we consider potentially more relevant from the perspective of clinical benefit or more illustrative of the true degree of molecular heterogeneity, despite attempts to simplify classification. Pivotal studies of gene expression have identified different ‘intrinsic’ subtypes of breast cancer (basal-like, HER2-enriched, luminal A and B and normal-like; FIG. 1)^{18–20}, which largely correspond, but do not overlap, with groups defined by hormone receptors and HER2-status. Notably, the definition of intrinsic subtypes is consistent with a systematic study in which 3,527 specimens from 12 different types of cancer (including breast cancer) were classified into 11 major molecular subtypes mainly related to their ‘tissue-of-origin’ rather than ‘within-a-tissue’ features²¹. In this study, breast cancer represents one of the exceptions, confirming that basal-like breast cancer (BLBC) is a unique disease entity, despite having the same tissue of origin as luminal/ER-positive tumours^{18–20}. ‘Basal-like’ was the term chosen to define this subgroup because tumour cells express genes characteristic of normal basal/myoepithelial cells, such as *KRT5*, *KRT14* and *KRT17* (cytokeratins 5, 14 and 17, respectively), and *EGFR*²². More than 90% of BLBCs are TNBCs¹². Conversely, BLBC represents the most frequent subtype of TNBC (55–81%)^{23,24} (FIG. 1). Within TNBC, none of the intrinsic subtypes — including BLBC — differ significantly in terms of the rate of pathological complete response (pCR) or survival after neoadjuvant chemotherapy²⁴, and all derive similar benefit from platinum compounds^{25–27}. Basal-like tumours have distinct molecular features compared with other TNBC subtypes^{23,28}, but they also are markedly heterogeneous.

To better dissect TNBC-specific tumour heterogeneity, Lehmann and colleagues²⁹ defined six new TNBC subtypes (TNBCtype) on the basis of gene-expression profiles: two basal-like-related subgroups (basal-like 1 (BL1) and 2 (BL2)), two mesenchymal-related subgroups (mesenchymal (M) and mesenchymal stem-like (MSL)), one immunomodulatory subgroup (IM) and one luminal androgen receptor group (LAR)²⁹ (FIG. 1C). Tumours within the LAR subtype can be defined as TNBCs by IHC but histologically and genetically resemble luminal-like ER-positive breast cancer (FIG. 1). The LAR subtype of TNBC is characterized by expression of the androgen receptor (AR) in the presence of a luminal-like expression signature and thus, might be treated with agents that target AR, as is the case of prostate cancer²⁹. Interestingly, some of the TNBCtype subtypes are closely linked to histological types: IM tumours overlap with medullary breast cancer, M and MSL tumours with metaplastic breast cancer, and LAR tumours with apocrine tumours²⁹.

A direct comparison of 374 TNBC samples extracted from 14 datasets was performed to determine the relationship between the TNBCtype and intrinsic molecular (PAM50) subtypes³⁰. Most TNBCs were classified as basal-like (80.6%), followed by HER2-enriched (10.2%), normal-like (4.7%), luminal B (3.5%) and luminal A (1.1%)³⁰ using PAM50 subtyping. Comparison of the overlap between the PAM50 classification and the TNBCtype showed that, with the exception of MSL and LAR, the majority of the other TNBCtype subtypes were classified as basal-like tumours by PAM50 analysis (BL1(99%), BL2 (95%), IM (84%) and M (97%)). About 50% of MSL TNBCs were classified as basal-like, 28% as normal-like and 14% as luminal B tumours. Finally, tumours within the LAR subtype were mainly classified as HER2-enriched (74%) or luminal B (14%)³⁰.

The association between TNBCtype and response to neoadjuvant chemotherapy was evaluated in a retrospective analysis of 130 patients with TNBC treated with neoadjuvant taxane and anthracycline-based regimens³¹. Subtype-specific responses differed across groups, with patients with BL1 tumours achieving the highest pCR rate (52%) and patients with tumours classified as BL2, LAR and MSL having the lowest response rates (0%, 10% and 23%, respectively). Overall TNBC subtype was shown to be an independent predictor of pCR status ($P=0.022$) using a likelihood ratio test³¹. However, the sample size of this study³¹ was small and these findings require additional validation to determine whether TNBC subtypes are useful for predicting the risk of relapse and likelihood of benefit from chemotherapy.

In a different study⁵, 10 integrative clusters (IntClust) of breast cancer were defined on the basis of combined genomic and transcriptomic data. BLBCs were heterogeneously distributed among different groups (FIG. 1D), with IntClust 4 and 10 accounting for 80% of them⁵. Breast cancer heterogeneity has clinical relevance, as illustrated by the fact that tumours classified as IntClust 4 have extensive lymphocytic infiltration with a strong immune and inflammatory signature and few copy-number aberrations ('CNA-devoid' subgroup) and patients within this subgroup had a favourable outcome. Conversely, BLBCs within the IntClust 10 subtype have high genomic instability and frequently display major chromosomal aberrations, such as chromosome 5 loss, 8q gain, 10p gain or 12p gain⁵.

Novel druggable molecular alterations

On average, TNBCs carry 1.68 somatic mutations per Mb of coding regions (~60 somatic mutations in each tumour). The mutation burden is not uniform across TNBC, and some tumours have a high mutation burden (more than 4.68 somatic mutations per Mb)^{1,3}, and a frequent occurrence of multiple copy-number aberrations involving genes that lead to multiple pathway alterations^{1,4,5,32} (BOX 1).

Box 1

Potentially actionable pathways in TNBC

DNA repair pathway

- *BRCA1/2* mut/del

PI3K/mTOR pathway

- *PIK3CA* mut/amp, *AKT3* amp/mut, *PTEN* del/mut, *TSC1* del/mut, *INPP4B* del, *TSC1*

RAS/RAF/MEK pathway

- *FGFR1* amp, *EGFR* amp, *IGF1R* amp, *ERBB2* mut, *ERBB3* mut, *ERBB4* mut, *BRAF* amp/mut, *KRAS* amp/mut, *HRAS* mut, *DUSP4* del

Cell-cycle checkpoints

- *RB1* del, *CDK6* amp, *CCND1* amp, *CCND2* amp

JAK/STAT pathway

- *JAK2* amp

Other pathways

- Androgen receptor pathway
- Notch pathway
- JNK/AP-1 pathway
- HIF1- α /ARNT network

amp, gene amplification; del, gene deletion; mut, gene mutation; TNBC, triple-negative breast cancer.

Among the most frequent mutations in TNBC, only *TP53* mutations are found at a high frequency (60–70% of mutations), and are more common in basal-like (62–80%) than in non-basal TNBC (43%)^{1,4}. *PIK3CA* is the next most commonly mutated gene in TNBC (~10% globally)^{4,33}, although mutations in this gene are significantly more frequent in LAR TNBCs (46.2%) than in the other subtypes (average 4.5%)³³. All other mutations occur at a low (1–5%) to very low frequency (<1%) in TNBC, and some of them are actionable (for example, *ERBB2*)³⁴ or are a target of existing therapies (for example, *BRAF*^{V600E})⁴. The low frequency of TNBC driven by rare aberrations represents a challenge for ad hoc drug development, and requires the implementation of new trial designs and widespread adoption of genomic screening in clinical practice^{35,36}. Potentially actionable deletions (such as *PTEN* or *INPP4B*) and amplifications (such as *PIK3CA*, *KRAS*, *BRAF*, *EGFR*, *FGFR1*, *FGFR2*, *IGFR1*, *KIT* or *MET*) have been reported in TNBCs at variable frequencies (1–40%)^{1,4,5}, but their actionability has not been proven yet. Multiple aberrations can affect the same pathway at different levels (BOX 1), which can lead, for example, to a frequent PI3K/AKT pathway activation, meaning that considering targeting *PIK3CA* alterations alone could be a clinical oversight^{1,2,4,37}. Importantly, the results of these genomic studies have also provided evidence of the substantial clonality and intratumour heterogeneity of TNBC, which can have important clinical implications, such as for the development of resistance to therapies or the lack of responses to targeted therapies^{4,38–40}.

BRCA mutations and 'BRCAness'

The *BRCA1* and *BRCA2* genes encode proteins critical for maintaining DNA integrity and genomic stability^{41,42}. *BRCA1* and *BRCA2* are tumour-suppressor proteins essential for cell division, DNA replication error control, DNA repair and apoptosis. In breast cancer, the presence of germline mutations in *BRCA1* or *BRCA2* is characterized by a basal-like phenotype (in tumours with *BRCA1* mutations but not in those with *BRCA2* mutations), ER-negativity, *EGFR* overexpression, *MYC* amplification, *TP53* mutations, loss of RAD51-dependent focus formation, extreme genomic instability and sensitivity to DNA-crosslinking agents⁴³. The tight association between *BRCA1* mutations, a basal-like phenotype and TNBC will be explored in this section.

In addition to *BRCA1* and *BRCA2*, *ATM* and *TP53* are other critical genes in the DNA-damage response signalling pathway, which might have a role in breast tumorigenesis. *BRCA1* or *BRCA2* are required and critical for the repair of DNA double-strand breaks by homologous recombination⁴⁴, contribute to DNA repair and transcriptional regulation in response to DNA damage, and regulate other genes involved in DNA repair, the cell cycle and apoptosis⁴⁵. Increasing evidence supports a role for *BRCA1* in double-strand DNA break repair, in part through its interaction with RAD51 and proteins from the Fanconi anaemia group^{46,47}. Cell lines deficient in *BRCA1* or other components of the Fanconi anaemia/*BRCA1* pathway are more sensitive to X-ray-induced DNA damage and DNA-crosslinking agents, such as cisplatin^{46,48}.

BRCA1 mutations are rare (<5%) in sporadic tumours, but pathological high-grade breast cancers more frequently display loss of heterozygosity (LOH) and/or abnormal expression of *ATM*, *BRCA1* and *TP53* (REF. 49). The presence of germline mutations in *BRCA1/2* increases the lifetime risk of breast cancer to 60–70%⁵⁰ and occurs in ~10% of patients with TNBC^{1,4,51}. Promoter methylation⁵², somatic *BRCA1/2* mutation, and gene deletion have been described as alternative mechanisms to impair *BRCA1/2* function that likely contribute to 'BRCAness' genotypes^{1,4} — which are associated with a biological and clinical phenotype similar to that of tumours harbouring mutations in *BRCA1/2*, but without these alterations — but, whether these mechanisms confer the same functional deficiency as germline *BRCA1/2* mutations is currently unclear. In *BRCA1/2*-proficient TNBC, the overexpression of other genes, such as *ID4* (REF. 53) or *HORMAD1* (REF. 54), can be a potential driver of genomic instability and BRCAness.

Breast tumours arising in patients who carry *BRCA1* mutations have many molecular features of basal-like sporadic breast tumours, including a greater likelihood of being high-grade, ER/PgR-negative, HER2-negative, and a high frequency of *TP53* mutations⁵⁵. The existence of a tight association between *BRCA1* mutations, basal-like breast cancer and TNBC⁵⁶ has raised the question as to whether *BRCA1* loss of function through other mechanisms participates in the pathogenesis of sporadic BLBC and TNBC; such an association could be exploited therapeutically with rational clinical trials exploring the role of chemotherapy and biological agents targeting defective DNA-repair pathways.

Assays to evaluate BRCAness and HR deficiency—The role of *BRCA* mutations in homologous recombination-deficiency (HRD) has been well-established, but other diverse

molecular alterations can also drive HRD, such as mutations in *PALB2*, *BARD1*, *RAD51D*, *BRIP1* (REF. 57) and *RAD51C*⁵⁸, and HORMAD1 protein over-expression⁵⁴, which suppresses RAD51-dependent homologous repair. A number of specific, quantitative biochemical assays have been developed with the aim of establishing a clinical biomarker to define HRD in tumours, such as the HRD–loss of heterozygosity (HRD-LOH) assay⁵⁹, HRD–telomeric allelic imbalance (HRD-TAI) assay⁶⁰, and the HRD-large-scale state transition (HRD-LST) assay⁶¹. Although promising, the clinical utility of these assays remains to be proven.

Therapies for TNBC

Chemotherapy for TNBC

Cytotoxic chemotherapy remains the mainstay of treatment for TNBC, with data from many studies over the past two decades showing significant benefit of chemotherapy in the neoadjuvant, adjuvant and metastatic setting^{62,63}. Despite the lack of known targetable biomarkers, and an overall poor prognosis, patients with TNBC have a higher response to chemotherapy than patients with other breast cancer types, which is sometimes referred to as the TNBC paradox because of its high risk of recurrence without any treatment but also higher likelihood of benefit from treatment⁶⁴. Studies of neoadjuvant chemotherapy have consistently reported higher response rates in patients with TNBC than those with non-TNBC^{65,66}, and approximately 30–40% of patients with early-stage TNBC treated with standard neoadjuvant anthracycline and taxane-based chemotherapy regimens achieve a pCR after treatment⁶⁷. A preferred chemotherapy regimen in the neoadjuvant or adjuvant setting has not yet been determined, but dose-dense and high-dose regimens seem to be more effective in patients with TNBC^{68–70}. Taxanes and anthracyclines are effective treatments of TNBC and remain important agents in this setting^{71–73}.

Despite optimal systemic chemotherapy, fewer than 30% of women with metastatic breast cancer survive 5 years after diagnosis, and virtually all women with metastatic TNBC will ultimately die of their disease⁷⁴. Many combination chemotherapy regimens have been studied in an effort to improve the outcomes of these patients and, although combination therapies have resulted in improved response rates compared with single agents, this advance has been made at the expense of increased toxicity and with no benefits in patient survival⁷⁵. For patients with metastatic disease, the ESMO⁷⁶ and ASCO⁷⁷ guidelines recommend the use of sequential single-agent chemotherapy, except in the presence of visceral crisis or rapidly progressing disease^{76,77}. No specific chemotherapy agents are considered preferred agents in the metastatic setting; however, capecitabine might not be as effective for patients with TNBC as it is for patients with hormone receptor-positive breast cancer⁷⁸.

Clinical trials with platinum agents in TNBC—Platinum salts, including carboplatin and cisplatin, lead to DNA crosslink strand breaks that result in apoptosis specifically in cells unable to efficiently repair these lesions. Clinical and preclinical evidence of enhanced sensitivity to DNA-damaging agents in TNBC compared with other subtypes was established over a decade ago⁷⁹. The intrinsic genomic instability of a subset of TNBCs with

defects in DNA repair has generated a growing body of preclinical and clinical data examining the role of platinum-based chemotherapy for TNBC.

In a single arm, multicentre phase II study evaluating platinum monotherapy in 86 patients with metastatic TNBC (mTNBC)²⁶ overall response rates (ORR) of 32% and 19% for cisplatin and carboplatin, were reported, respectively. Patients who carried mutations in *BRCA1/2* ($n = 11$) had a higher ORR (54.5%) than those with wild type *BRCA1/2* ($n = 66$; ORR: 19.7%). Neither basal-like subtype, presence of *TP53* mutations, *PIK3CA* mutations, or p63 and/or p73 status were correlated with durable responses or longer progression-free, or overall survival²⁶. Higher HRD scores were detected in patients with mutations in *BRCA1/2* than in those with wild-type *BRCA1/2*, and were associated with platinum sensitivity. Subsequently, in the Triple-Negative Breast Cancer Trial (TNT)²⁷ investigators randomly allocated 376 patients with mTNBC to receive first-line treatment with either carboplatin or docetaxel. This trial provided no evidence that unselected patients with advanced-stage TNBC are more likely to respond to carboplatin than to docetaxel (ORR: 31.4% and 35.6%, respectively; $P=0.44$)²⁷. Patients with *BRCA1/2* mutations, however, had improved response rates to carboplatin than docetaxel (ORR: 68.0% and 33.3%, respectively; $P= 0.03$)²⁷. Interestingly, in this study the definition of a dichotomized HRD score did not enable selectivity for sensitivity to carboplatin over docetaxel. Furthermore, a similar performance was observed for treatment with carboplatin or docetaxel in patients with tumours defined as basal-like by PAM50-based Prosigna™ (Nanostring Technologies, Seattle, USA), but docetaxel outperformed carboplatin in patients with non-basal-like tumours²⁷. In summary, the overall clinical efficacy of platinum-based agents in patients with mTNBC has been modest, and molecular assays to identify those patients who will have a better response have not been convincingly better than determining the *BRCA1/2* mutation status alone.

Several clinical studies have examined the role of platinum-based agents in the neoadjuvant treatment of patients with TNBC. These studies utilize the pCR rate as an end point because the general assumption is that patients who achieve a pCR exhibit improved long-term outcomes⁶⁶, whereas a high residual disease burden at post-therapy surgery is correlated with a higher risk of disease recurrence and death^{80,81}. Approximately 30% of women with TNBC treated with anthracycline and taxane-based neoadjuvant chemotherapy will achieve a pCR after treatment^{63,67}. Consistently, patients with TNBC who achieved a pCR after neoadjuvant chemotherapy had improved disease-free survival rates than those patients who did not achieve a pCR (hazard ratio (HR) 0.24; 95% CI 0.18–0.33; $P<0.001$)^{63,67}. By contrast, Byrski *et al.*⁸² reported in a separate study that 50 out of 82 (61%) patients with stage I–III TNBC harbouring *BRCA1* mutations achieved a pCR after neoadjuvant therapy with cisplatin. In another small single-arm study in 28 women with stage II or III TNBC treated with four cycles of neoadjuvant single-agent cisplatin, the pCR rate was 22% (including the only two patients with *BRCA1* germline mutations), with four (14.2%) complete responses and 14 (50%) partial responses (overall clinical response rate 64%)⁸³. Finally, in a trial with 51 patients with TNBC treated with neoadjuvant cisplatin and the anti-VEGF antibody bevacizumab⁸⁴, the pCR was 15%. These results suggest that platinum-based agents are effective as single-agent regimens (and/or bevacizumab) in patients with

early-stage TNBC, and support the evaluation of addition of platinum-based agents to standard taxane–anthracycline-based neoadjuvant regimens.

Two large randomized trials further explored the effect of platinum-based agents on TNBC in the neo-adjuvant setting. The CALGB40603 trial⁸⁵ evaluated the effect of adding carboplatin and/or bevacizumab to paclitaxel followed by dose-dense doxorubicin and cyclophosphamide in 443 patients with stage II or III non-inflammatory TNBC. The addition of carboplatin increased the pCR rate in the breast from 46% to 60% ($P = 0.0018$), and in breast/axilla from 41% to 54% ($P = 0.0029$)⁸⁵. The GeparSixto trial⁸⁶ included 315 patients with stage II or III that were randomly assigned to receive a regimen containing anthracycline, taxane and bevacizumab, with or without carboplatin. The pCR rate in breast/axilla increased from 36.9% to 53.2% ($P = 0.005$) for those patients who received carboplatin⁸⁶. In both trials, however, a substantially increased toxicity was observed, and many patients (48%⁸⁶ and 20%⁸⁵) were unable to complete therapy as planned. The addition of cisplatin or carboplatin to neoadjuvant treatment of TNBC is clearly associated with significantly higher rates of pCR, which suggests that the absence of ER/PgR/HER2 could be a potential therapeutic target for these drugs in patients with early-stage disease.

In the PreCOG 0105 single-arm neoadjuvant trial⁵², 80 patients with TNBC were treated with gemcitabine, carboplatin and the poly(ADP-ribose) polymerase (PARP) inhibitor iniparib⁵². Patients with mutations in *BRCA1/2* had the highest response rate (75%, with residual cancer burden (RCB)⁸⁷ of 0 or 1). The HRD-LOH assay was used to identify patients without *BRCA1/2* mutation and a high HRD-LOH score (>10), who achieved a remarkable response rate (59%). By contrast, in the group of patients without mutations in *BRCA1/2* and also a low HRD-LOH score (<10), the favourable responses (RCB 0 or 1) were only 8%⁵². This result is concordant with the findings of the already mentioned TBCRC009 trial²⁶, but it is in contrast with the results of the TNT study, in which the HRD-LOH assay did not define higher sensitivity to carboplatin over docetaxel²⁷. One possible explanation is that the test might be more efficient at capturing high generic chemosensitivity than platinum-specific sensitivity and, therefore, the clinical utility of this assay remains to be defined.

Despite the added toxicity, a reasonable option is to consider the addition of a platinum-based agent in the neoadjuvant setting for patients with high-risk *BRCA*-mutated TNBC, and for patients with TNBC, for whom an increased clinical response to systemic treatment could improve locoregional control (for example, patients with inflammatory TNBC or with inoperable TNBC at the time of diagnosis). The clinical utility of the addition of platinum-based agents to standard neoadjuvant and adjuvant chemotherapy regimens, however, remains controversial mainly because of the added toxicities, but also because of the unclear correlation between an improved pCR rate and improved event-free survival (EFS) in the neoadjuvant setting. This lack of correlation was illustrated by the CtNEO meta-analysis across different molecular subtypes⁶³. This study was performed to evaluate whether the improvement of the pCR rate in a neoadjuvant trial can be a reliable predictor (surrogate end point) of an improvement in EFS. The GeparSixto⁸⁶ and CALGB40603 (REF. 85) phase II trials clearly demonstrated the benefit of adding a platinum-based agent to the systemic treatment of patients with TNBC in the neoadjuvant setting, but these trials were

underpowered to address improvements in disease-free survival and overall survival, and were non-informative on these crucial clinical end points. For the time being, the achievement of pCR has good prognostic value for a positive individual clinical outcome but not for predicting the survival benefit that would be derived from a new intervention⁶³.

Targeted therapies

Given the heterogeneity of TNBC, personalized treatment strategies targeting molecular tumour-specific alterations would be the most appropriate to effectively treat the 60–70% of patients with TNBC who do not fully respond to chemotherapy. A number of studies have focused on the identification of molecular targets that might be effectively targeted in TNBC as single agents, or that might circumvent intrinsic resistance to chemotherapy^{33,88–90}. As many as 90% of TNBCs that persist after chemotherapy contain alterations in pathways that can be targeted with agents currently under clinical investigation⁹¹, such as PARP inhibitors, PI3K inhibitors, MEK inhibitors, heat shock protein 90 (HSP 90) inhibitors and histone deacetylase (HDAC) inhibitors (FIG. 2).

PARP inhibitors—PARP is an abundant, constitutively expressed nuclear enzyme that catalyses the transfer of ADP-ribose from NAD⁺ to target proteins and facilitates DNA repair, cellular proliferation and signalling to other critical cell-cycle proteins and oncogenes through this mechanism⁹². At sites of DNA damage, PARP activates intracellular signalling pathways that modulate DNA repair and cell survival through poly(ADP)-ribosylation of several nuclear proteins involved in chromatin architecture and DNA metabolism. Immediate catalytic activation of PARP in response to DNA single-strand and double-strand breaks has been reported at levels up to 500-fold of basal levels^{93,94}.

PARP inhibition results in double-strand breaks in replicating cells⁹⁵. In cells with wild-type *BRCA1/2*, double-strand breaks are repaired via homologous recombination, but in *BRCA1/2*-deficient cells, homologous recombination is impaired and thus, DNA strand breaks rely on PARP1 functionality for repair^{95,96}. Therefore, inhibition of PARP1 by RNA interference or with chemical inhibitors leads to severe, highly selective toxicity in *BRCA1* and *BRCA2*-defective cells⁹⁷, so-called ‘synthetic lethality’ (REF. 98). Importantly, sensitivity to PARP inhibition depends on HRD, which can be caused by mechanisms other than inherited *BRCA1* or *BRCA2*-deficiency⁹⁵. PARP inhibitors have at least three roles in cancer treatment: sensitization to chemotherapy and radiotherapy, synthetic lethality in tumours from patients with hereditary mutations in *BRCA1/2* genes, and leveraging of putative ‘BRCA-like’ defects and defects in DNA repair, such as those identified in several TNBC subtypes^{95,99}.

Significant single-agent activity was reported with the PARP inhibitor olaparib in patients with *BRCA*-deficient metastatic breast cancer. Overall responses ranged from 22% (100 mg twice per day) to 41% (400 mg twice per day) with minimal toxicity¹⁰⁰. A phase II study evaluating olaparib as a single agent for patients with either recurrent high-grade serous or poorly differentiated ovarian carcinoma, or with TNBC¹⁰¹ did not report any responses in patients with TNBC harbouring sporadic mutations in *BRCA1/2* (BRCA-proficient), even though the target lesions were reduced in size by >30% in five out of 10 (50%) patients with

breast cancer who had *BRCA1* or *BRCA2* mutations, but were not confirmed objective responders¹⁰¹. Several phase III trials investigating the use of olaparib in the metastatic (NCT02000622) and neoadjuvant (NCT02032823) setting, for patients with mutations in *BRCA*, are ongoing. Importantly, there are several additional PARP inhibitors currently being tested in clinical trials, such as veliparib (phase III; NCT02163694), talazoparib (phase III; NCT01945775), niraparib (phase III; NCT01905592) and rucaparib (phase I/II; NCT01074970). Iniparib, a putative PARP inhibitor previously investigated in patients with breast cancer¹⁰², generated substantial excitement before failing phase III clinical trials (NCT00938652), however, subsequent laboratory investigations proved that this drug was not an effective inhibitor of PARP¹⁰².

Mature results from the adaptive neoadjuvant I-SPY2 biomarker trial¹⁰³ demonstrated that the addition of veliparib and carboplatin to standard chemotherapy regimens for patients with stage II or III TNBC improved pCR rates from 26% to 52%¹⁰³. However, whether the addition of veliparib to carboplatin contributed in any way to the increase in pCR rate is unclear because the results obtained with combination therapy were quite similar to those observed with the use of carboplatin only in the GeparSixto⁸⁶ and CALGB40603 (REF. 85) neoadjuvant trials.

Finally, a randomized phase II trial of cisplatin with or without rucaparib¹⁰⁴ was initiated for patients with TNBC and/or *BRCA* mutations who had a residual tumour burden in the breast or axilla after being treated with neoadjuvant chemotherapy consisting of an anthracycline plus a taxane¹⁰⁴. Preliminary data show that the disease-free survival at 1 year is similar (~76%) for both treatment groups, and rucaparib did not add substantial toxicity to the cisplatin regimen¹⁰⁴. Thus, although PARP inhibitors clearly demonstrate benefit when administered as single agents, their benefit when used in addition to platinum agents is unclear in HRD TNBC.

Anti-androgen therapy—The LAR subtype of TNBC shows sensitivity to AR antagonists *in vitro* and *in vivo*²⁹ (FIG. 3). A phase II trial of the androgen-blocking agent bicalutamide in patients with AR-positive mTNBC was reported¹⁰⁵. AR was evaluated using IHC in 424 patients with ER and PgR-negative breast cancer, 12% of whom showed >10% nuclear staining. Patients with AR-positive tumours received single-agent bicalutamide, and 19% of patients had a clinical benefit (defined as a complete or partial response, or stable disease) at 6 months of follow-up, and the median progression-free survival was short (12 weeks)¹⁰⁵. Nonetheless, the study provided evidence of the potential efficacy of hormone-directed therapy in AR-positive TNBC¹⁰⁵. Subsequently, the results of another phase II study with enzalutamide¹⁰⁶, a more-potent AR inhibitor, in advanced-stage AR-positive TNBC were reported. AR prevalence was higher than previously reported, with 55% of patients having 10% AR by IHC. In those 75 evaluable patients (AR 10%), two complete responses and five partial responses were observed¹⁰⁶. Interestingly, an androgen-driven gene signature denoted Dx⁺, which seemed to predict a superior clinical outcome, was defined by gene profiling¹⁰⁶. The proposed signature is of potential clinical relevance, but requires formal validation in independent series of patients.

LAR-subtype cell lines, which are also enriched with *PIK3CA* activating mutations, exhibit strong sensitivity to PI3K inhibitors, as well as to androgen blockers^{29,107}. The co-evolution of *PIK3CA* mutations with AR-dependency is similar to that observed for ER-positive breast cancers, which frequently contain *PIK3CA* mutations^{107,108}. Thus, the utility of targeting both PI3K and AR is being explored in a phase I/II study of the combination of an androgen blocker (enzalutamide) with a PI3K inhibitor (taselisib) for patients with AR-positive TNBC (NCT02457910). The additive efficacy and toxicity of PI3K inhibition in this patient population will be compared with the available data on enzalutamide monotherapy in TNBC¹⁰⁶.

PI3K inhibitors—Mutations affecting ‘hotspot’ regions of the *PIK3CA* gene, which encodes the PI3K catalytic subunit α , are the most frequent activating mutations in this gene in TNBC (10.2%)¹. Additionally, loss of *PTEN*, a negative regulator of the PI3K pathway, occurs in 9.6% of TNBCs⁴ and, in general, is mutually exclusive with *PIK3CA* hotspot mutations.

Preclinical studies of the combination of a DNA damaging agent with PI3K inhibitors have provided a rationale for using PI3K inhibitors in non-LAR tumours by demonstrating that, in addition to regulating cell growth, metabolism, and survival, PI3K also stabilizes double-strand breaks by interacting with the homologous recombination complex and creating a *BRCA1/2*-deficient-like state¹⁰⁹. PI3K blockade promotes HRD by downregulating *BRCA1/2*, creating a *BRCA*-mutant-like tumour state and thus, sensitizing *BRCA1/2*-proficient tumours to PARP inhibition. Consistent with this observation, the concomitant *PTEN* loss observed in tumours with mutant *BRCA1* can reverse HRD¹¹⁰. A phase I study of the pan-PI3K inhibitor buparlisib (BKM120, Novartis, Switzerland) in combination with olaparib for patients with solid tumours that include mTNBC is under way (NCT01623349).

MEK inhibitors—A large number of cell lines derived from TNBC and BLBC are sensitive to MEK inhibition *in vitro*; BLBC cell lines have broader sensitivity to MEK inhibitors than to PI3K inhibitors¹¹¹. Several TNBC cell lines that are sensitive to MEK inhibitors harbour mutations that affect the Ras/MAPK pathway, including activating mutations in *BRAF*, *HRAS*, or *KRAS*, which are extremely rare in patients with TNBC¹¹². Nonetheless, many TNBC cell lines show upregulation of the Ras/MAPK pathway without any oncogenic mutation identified in components from the Ras/MAPK pathway¹¹³. In those situations, aberrant activation of the Ras/MAPK pathway can be attributed to activation or overexpression of growth factor receptors (such as *EGFR*, *IGF1R*, *FGFR1* or *VEGFR*, among others), or to gene copy-number alterations (gains and amplifications) in key Ras/MAPK constituents such as *BRAF* and *KRAS*, which have been observed at moderate frequencies in BLBC (30 and 33%, respectively), and result in increased gene expression^{1,114,115}, although the contribution of the latter to pathway activation has not been determined experimentally. Another proposed mechanism of activation of the Ras/MAPK pathway in TNBC is the genetic and/or epigenetic loss of *DUSP4*, a negative regulator of ERK1/2 and JNK1/2, which is associated with BLBC Ras–ERK activation^{90,116}. In preclinical studies, loss or downregulation of *DUSP4* enhances chemotherapeutic resistance in TNBC, and contributes to the maintenance of the tumour-initiating cancer cell population,

which can be overcome with inhibitors of the Ras/MAPK pathway, and possibly the JNK/AP1 pathway^{28,51}.

MEK activation can support the stabilization of c-Myc, the product of an important oncogene amplified in ~30% of patients with TNBC or BLBC^{114,117}. Duncan *et al.*¹¹⁸ demonstrated that, although single-agent MEK inhibition can induce c-Myc degradation in TNBC, this effect simultaneously induces the expression and activation of receptor tyrosine kinases that can overcome MEK inhibition and cause resistance to therapy¹¹⁸. These data suggest that combinations of MEK inhibitors with small molecules or monoclonal antibodies targeting receptor-tyrosine kinases might be effective therapies, but the efficacy of such combinations has not been validated clinically¹¹⁸. The use of MEK inhibitors in combination with chemotherapy or other targeted agents to treat TNBC and BLBC is currently under clinical investigation; however, appropriate biomarkers that might enable optimal patient selection have not been defined. Little data on the efficacy of MEK inhibitors in TNBC have been published, but, importantly, in a phase Ib trial in patients with solid tumours ($n=31$) treated with gemcitabine and trametinib (an orally available potent inhibitor of MEK1/2), the only complete response to therapy occurred in a patient with mTNBC¹¹⁹.

Inhibitors of the cancer stem-cell population—Breast cancer stem cells, also known as tumour-initiating cells, represent a dynamic subpopulation of tumour cells that retain the properties of mammary stem cells and, most notably, the ability to repopulate a heterogeneous tumour (with both luminal and basal cytotokeratin compartments) from a single cell¹²⁰. Breast cancer stem cells have slower proliferation rates and higher degrees of resistance to chemotherapy compared with non-stem cancer cells¹²¹, and often demonstrate changes in phenotype similar to those associated with cells undergoing epithelial-to-mesenchymal transition^{122,123}. The stem cell population typically represents a small fraction of the overall tumour cell population and can be identified or enriched for in breast cancers through marker-based methods such as measurements of aldehyde dehydrogenase activity (ALDEFLUOR assay)^{120,124,125}, assessing the expression of integrin receptors, or the ability to exclude ABC transporter substrates^{126,127}. Initial findings suggested that this population of cells might represent the subset of tumour cells that, spared by chemotherapy, contribute to resistance and accelerated recurrence after traditional therapies¹²¹, and thus, would constitute an ideal target for novel therapeutic discovery in combination with chemotherapy. Among TNBC, BLBCs and some specific subtypes defined by independent groups^{128,129} are enriched for cancer stem cells. However, while the existence of phenotypic breast cancer stem cells is supported by a large body of preclinical and clinical evidence, the pathways that contribute to the maintenance of this cell population are not clearly defined. Data from independent studies have shown that the Ras/MAPK¹¹⁶, JAK/STAT¹³⁰, Wnt¹³¹, TGF- β ¹³², Hedgehog¹³³ and Notch¹³⁴ pathways can all contribute to the maintenance of breast cancer stem cells, depending on the particular model or cell line used. As such, no clearly defined ‘inhibitors’ of breast cancer stem cells have been established.

Other molecular targets—Several alterations in molecules and pathways are, or have been under investigation as therapeutic targets to treat metastatic TNBC. *EGFR* is amplified in about 2% of breast cancers¹, and is over-expressed more frequently in BLBC than in non-

BLBC. Given this finding, and the long-standing availability of clinically approved inhibitors of EGFR (such as erlotinib, gefitinib, lapatinib, cetuximab and panitumumab), several trials have tested the efficacy of these agents in patients with TNBC. Two phase II trials have reported non-statistically significant improvements in patient outcomes when cetuximab was combined with platinum agents^{135,136}. No patient selection criteria were included in these trials and, therefore, it remains possible that benefit could be derived if patients that harbour *EGFR* amplification were selected *a priori* for future trials.

Clinically approved HDAC inhibitors are also under investigation in combination with cisplatin for patients with mTNBC (NCT02393794), or as monotherapy (NCT02623751). Additional areas under investigation include targeting the hypoxia-inducible factor 1- α (HIF1- α) pathway¹³⁷, c-Met (NCT01738438)¹³⁸ and integrins (such as integrin β -5) involved in angiogenesis and other tumorigenic phenotypes¹³⁹. Finally, rare activating mutations in *ERBB2* are also found in TNBC, and a clinical trial with neratinib, an irreversible inhibitor of EGFR, is ongoing (NCT01953926)³⁴.

The immune system and TNBC

The key role of the immune system in cancer has been recognized for decades¹⁴⁰ but, only in the past few years, immunotherapy has been established as a major cancer therapy^{141–143}, with remarkable activity and curative potential in patients with a broad range of tumours^{144,145}. Our understanding of the complex interplay between cancer and the immune system has improved substantially by moving from the concept of ‘immune surveillance’ (REF. 146) to that of ‘immunoediting’ (REF. 147), a term that well describes the dual host-protecting and tumour-sculpting actions of the immune system, and has three phases: elimination, equilibrium and escape. The elimination phase in the immunoediting hypothesis corresponds with the immune surveillance function. In the equilibrium phase, although the immune system has failed in eliminating all clinically detected tumours, it can be actively and effectively engaged to keep the tumour under control, for instance reducing the risk of metastatic spread¹⁴⁸. During the equilibrium phase, the immune system can select cancer cells with a particular genotype and phenotype through evolutionary pressure favouring the development of immunological anergy, tolerance or indifference (escape phase)¹⁴⁷. An example of tumour immunoediting in TNBC is provided by the presence of *CASP8* mutations³⁷, which can abrogate the death induced by cytolytic CD8⁺ T cells, and has been described as a common mechanism of immune escape in many solid tumours¹⁴⁹.

The original idea that cancer arises as a failure of ‘immune surveillance’ has been the basis for immuno-therapy for many years, in which the main focus of this therapeutic modality was to attempt to induce more effective and specific tumour recognition through vaccines. The results from clinical trials have consistently been frustrating and the only clinical benefits reported have been anecdotal. The current understanding that unique mechanisms of immune regulation and escape are actively engaged during immunoediting to dampen host immunity is the basis for a different therapeutic strategy, consisting of targeting the immune checkpoints to elicit, reinvigorate and potentially expand the magnitude of pre-existing anticancer immune responses^{150,151}.

The higher genomic instability and mutational burden of TNBC results in a higher propensity to generate neoantigens, which can be recognized as ‘non-self’ by the adaptive immune system¹⁵². Accordingly, TNBCs have a higher amount of tumour-infiltrating lymphocytes (TILs)¹⁵³ and higher programmed cell death 1 ligand 1 (PD-L1) protein^{154,155} or mRNA^{156,157} expression compared with other breast cancer subtypes. The ligand PD-L1 and its receptor, programmed cell death protein 1 (PD-1), are involved in the regulation of immune tolerance. PD-L1 expression is significantly associated with the presence of TILs^{154–156}, which suggests that the most common mechanism of regulation of PD-L1 expression in TNBC is regulatory feedback (acquired resistance) to immune engagement. An extremely heterogeneous pattern of immune infiltration, however, has been described among TNBC subtypes¹⁵⁸ (FIG. 1E). A significant association has also been found between PD-L1 mRNA expression and the presence of PD-L1 copy-number alterations, with BLBC having the highest frequency of PD-L1 gains/amplifications (17%)¹⁵⁷. In addition, the loss of *PTEN* expression in TNBCs is associated with PD-L1 overexpression¹⁵⁶, confirming an association between increased PI3K signalling and the presence of PD-L1 (REF. 159). These findings suggest that, in addition to acquired resistance mechanisms, PD-L1 expression can also be regulated by molecular alterations and oncogenic pathways (intrinsic resistance), linking molecular and immune heterogeneity.

The immune system as a biomarker

Prognostic relevance of immune infiltration—High TIL infiltration has been associated with a lower risk of relapse in patients with breast cancer for over 50 years^{160–161}; now these findings can be explored in the context of molecular subtypes. Moreover, to understand the role and contribution of the immune system to the clinical behaviour of breast cancer, it is important to discriminate between ‘pure’ prognostic value (recurrence risk in the absence of any treatment) and predictive value, the latter indicating the effect on recurrence risk that is caused by benefit from systemic treatment. The association between immune system activation and risk of recurrence can be assessed in patients treated with adjuvant or neoadjuvant therapy, but in this patient population the risk is affected both by natural history and treatment benefit, and therefore it is not possible to discriminate between prognostic and predictive biomarkers.

The majority of TNBCs are treated with adjuvant or neoadjuvant chemotherapy and thus, few studies have investigated the prognostic value of assessing the immune system (TABLE 1). In patients with untreated ER-negative breast tumours, the high expression of B-cell and immunoglobulin-based metagenes is associated with a low risk of developing distant metastases^{162,163}, whereas in patients with untreated TNBC, elevated expression of the STAT1-related and T-cell-related metagenes is associated with a low risk of distant-metastasis^{164,165}. A similar trend has been described for a follicular helper T (T_{FH}) cell signature¹⁶⁶. Collectively, these studies^{162–166} suggest that effective engagement of the immune system, although insufficient to eliminate the tumour, can help to reduce the risk of tumour spreading, or maintain tumour dormancy¹⁴⁸. This observation supports the existence and relevance of the equilibrium phase described in the cancer immunoediting hypothesis¹⁵⁰, however, the mechanistic contribution of the immune system to improved patient outcomes has not been demonstrated yet.

Predictive value of the immune system—In TNBC, the association between the presence of TILs or expression of immune markers and the likelihood of achieving a pCR after neoadjuvant chemotherapy is consistent and strong (TABLES 1,2). A high level of expression of immune markers is associated with different immune cell types and functions and has been associated with benefit from chemotherapy in TNBCs^{25,157,165–171}. This association has also been confirmed by evaluation of the TIL density^{170,171}. Overall, these data suggest that the immune system collaborates with the action of chemotherapy in TNBCs, as suggested by data from preclinical studies^{172,173}. However, whether drug-specific immunomodulation properties¹⁷³, such as inducing immunogenic tumour cell death (postulated for anthracyclines) or engaging different immune effector mechanisms, are associated with different clinical outcomes is unknown and is currently an active area of investigation. Interestingly, assessments of the immune milieu modulation after neoadjuvant chemotherapy in patients with TNBC with residual disease has shown that the immune microenvironment can be turned from ‘cold’ (containing few TILs) to ‘hot’ (higher TIL presence) in some patients^{174,175}. Tumours that remain or become ‘cold’ after chemotherapy have a higher risk of relapse compared with tumours that remain or become ‘hot’ (REFS 176,177). These data also support the concept of chemotherapeutic agents having immunomodulatory activity, and thus, acting as an immunological adjuvant in the tumour microenvironment to stimulate vaccination-induced antitumour immunity¹⁷⁴. Whether the immune system has different prognostic and predictive roles in different molecular subtypes of TNBCs has not been defined yet.

Overall, considering that, in TNBCs, a ‘hot’ immune microenvironment is associated with a better prognosis and a higher likelihood of benefit from chemotherapy, it should not be surprising that many investigators have identified a strong association between high levels of immune markers or TILs and a low risk of relapse and/or death in patients with early-stage TNBC treated with systemic chemotherapy^{153,157,165,175,178–185} (TABLES 1,2). These results suggest that, in TNBC, the risk of recurrence in the early disease setting can be effectively defined by adopting the appropriate immune markers for risk stratification. These results also distinguish a subgroup of patients with TNBC characterized by a ‘cold’ immune microenvironment that has a high risk of relapse, despite treatment, and a low likelihood of benefit from cytotoxic chemotherapy¹⁶⁵. This subgroup is a high priority in the list of unmet needs in TNBCs¹⁶⁵. Evidence for the clinical utility of TIL evaluation, however, is still scarce, in part because TIL assessment lacks sufficient standardization; however, efforts to improve consistency and reproducibility are under way^{186,187}.

The new age of immunotherapy for TNBC

The findings that a population of TNBC is immunogenic and actively engaged by the immune system provides a strong rationale for testing immunotherapies. Two phase I trials with immune-checkpoint inhibitors in patients with advanced-stage TNBC have been reported^{188,189}. In one of them¹⁸⁸, patients with advanced-stage TNBC positive for PD-L1 expression were treated with the anti-PD-1 monoclonal antibody pembrolizumab with a response rate of 18.5% (five out of 27 patients). Seven additional patients had stable-disease¹⁸⁸. Of the screened patients, 1% PD-L1 expression was detected using IHC labelling of stromal or tumour cells in archival specimens from 58% of patients with the

22C3 antibody¹⁸⁸. In another trial with the anti-PD-L1 antibody MPDL3280A¹⁸⁹, PD-L1-positive disease (defined as PD-L1 expression in ≥ 5% TILs as determined by IHC with a proprietary Genentech/Roche antibody), the ORR in the 21 evaluable patients was similar (19%)¹⁸⁹. Despite patient selection criteria based on PD-L1 expression, patients with PD-L1-negative disease in three different solid malignancies (melanoma¹⁹¹, squamous-cell non-small-cell lung cancer⁹² and renal-cell carcinoma⁹³) have already derived substantial survival benefit from the PD-1-targeting antibody nivolumab, thus challenging the validity of PD-L1 as a selection marker. Furthermore, the two breast-cancer trials^{188,189} have brought into focus the remarkable problem of assay heterogeneity and the scoring criteria adopted, making standardization and harmonization of PD-L1-testing a major issue and an urgent goal. Different trials are ongoing to establish the role of immune-checkpoint inhibitors alone or in combination, and of other immunotherapies in TNBCs (TABLE 3). In light of the promising preliminary results obtained with immune-checkpoint inhibitors, their expected curative potential¹⁴⁵ and their beneficial safety profile, these agents are already being assessed for the treatment of patients with early-stage TNBC. Three trials in patients with stage I–III TNBC are currently ongoing to evaluate the potential activity of immune-checkpoint inhibitors in combination with chemotherapy in the neoadjuvant setting. In the phase III trial NeoTRIPaPDL1 (NCT02620280), patients with locally-advanced TNBC will be randomly assigned to receive nab-paclitaxel and carboplatin with or without a PD-L1-inhibitor (atezolizumab). Notably, the primary end point will be event-free survival. A phase II trial will evaluate atezolizumab in combination with nab-paclitaxel (NCT02530489). Finally, a phase I/II trial will test the safety and efficacy of durvalumab, another anti-PD-L1 antibody, in combination with weekly nab-paclitaxel followed by dose-dense chemotherapy containing cyclophosphamide and doxorubicin (NCT02489448).

Conclusions

Massively parallel sequencing technologies have helped to define the molecular landscape of TNBC to an unprecedented resolution. These results have demonstrated that TNBC is comprised of many different disease entities, in sharp contrast with the apparent homogeneity of their nomenclature. The vast heterogeneity of TNBC also extends to the tumour immune microenvironment, which displays a full array of different levels of lymphocyte and monocyte infiltration and activation of inhibitory checkpoints such as PD-1/PD-L1.

The molecular and immune features of TNBC have so far shaped treatment and patient selection. Patients with tumours harbouring *BRCA1/2* aberrations might benefit from treatment with platinum compounds and PARP inhibitors; patients with other TNBC types carrying defects in genes related to homologous recombination ('BRCAness') could have a similar pattern of drug sensitivity. The majority of actionable genomic alterations are rare in TNBC, but tend to affect more frequently a few molecular pathways (such as PI3K/mTOR or RAS/RAF/MEK). However, the identification of situations in which these are 'driver alterations' remains challenging. Several agents targeting these pathways are being investigated but, in light of the low frequency of the target population, the design of new modalities of drug development and novel approaches to genomic breast cancer screening needs to be undertaken.

The variegated nature of the immune microenvironment in TNBC considerably influences the risk of relapse and response to chemotherapy, and has provided the rationale for the application of immunotherapies. The importance of immune-checkpoint inhibitors has already been established in the treatment of many solid tumours, in which they have shown to be game changers. Immune-checkpoint inhibitors, which also have antitumour activity in metastatic TNBC, represent a very promising therapeutic option that is now ready for investigation in the setting of neoadjuvant and adjuvant therapy of early-stage TNBC. All in all, the field is prepared for major breakthroughs in the understanding and treatment of this, so far, therapeutically elusive group of breast cancers. One possible avenue is immuno-molecular therapy, which integrates immune and molecular features to devise novel combinatorial approaches based on targeting intracellular molecular alterations and modulating the immune response¹⁷⁶.

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Figure 1. The heterogeneous landscape of triple-negative breast cancer

Different approaches to dissect the complex molecular landscape of TNBCs are presented. **a** | Histological subtypes. Some rare but relevant subtypes are shown for illustrative purposes. **b** | Gene-expression-based subtypes of triple-negative breast cancer (TNBC) according to PAM50²³. **c** | Gene-expression-based subtypes defined by Lehmann *et al.*²⁹. **d** | Integrative clusters (IntClust) of genomic and transcriptomic data applied to basal-like breast cancer (BLBC) defined by gene-expression⁵. **e** | Heterogeneity of tumour-infiltrating lymphocytes. Tumours with low, intermediate and high lymphocyte infiltration are shown for illustrative purposes. BL1, basal-like 1; BL2, basal-like 2; IM, immunomodulatory; LAR, luminal androgen receptor; M, mesenchymal; MSL, mesenchymal stem-like; UNC, unclassified; UNS, unstable.

Figure 2. Active pharmacological interventional trials in TNBC

An overview of the clinical trials for triple-negative breast cancer (TNBC) active as of July 2015 is depicted (trials including immunotherapies were updated in November 2015). Targets of molecularly targeted therapies included angiogenesis (VEGF, VEGFR, integrins), homologous repair (poly(ADP-ribose) polymerase (PARP)), serine/threonine-protein kinase (Chk1/2), PI3K/mTOR, growth factor receptors (EGFR, MET), hormone receptors (androgen receptor (AR)), Ras/MAPK, and Notch/ γ -secretase, among others. Immune-checkpoint inhibitors included anti-programmed cell death 1 (PD-1), anti-programmed cell death 1 ligand 1 (PD-L1) and anti-cytotoxic T-lymphocyte protein 4 (CTLA 4) monoclonal antibodies (mAbs).

Figure 3. Histological characteristics of luminal androgen receptor (LAR) triple-negative breast cancer

a | On low magnification, infiltrative nests and cords of eosinophilic tumour cells surrounded by a desmoplastic tumour stroma devoid of tumour-infiltrating lymphocytes can be observed. **b** | Tumour cells contain large epithelioid nuclei with prominent nucleoli and abundant granular eosinophilic cytoplasm. As depicted, rates of mitosis are frequently low in LAR. **c** | Nuclear expression of androgen receptors, detectable by immunohistochemistry, is a hallmark of LAR.

Table 1

Gene-expression studies on the prognostic and predictive value of immune-related functions in TNBC

Study	TNBC definition	Immune-related signature	Association with outcome
<i>Prognostic value before adjuvant treatment</i>			
Desmedt <i>et al.</i> (2008) ¹⁶⁴	ER-negative/HER2-negative	95-gene STAT1-related immune metagene	Lower risk of distant metastasis in 'high expression' group
Callari <i>et al.</i> (2016) ¹⁶⁵	ER-negative/HER2-negative	6-consensus T-cell-related metagene	Lower risk of distant metastasis in 'high expression' group
Gu-Trantien <i>et al.</i> (2013) ¹⁶⁶	ER-negative/HER2-negative	8-gene follicular helper T (T _{FH})-cell signature	Marginal significant lower risk of distant metastasis in 'high expression' group
<i>Predictive value of benefit from neoadjuvant chemotherapy</i>			
Sikov <i>et al.</i> (2015) ²⁵	TNBC	IGG, T-cell, CD8 and B-cell signatures	Higher pCR rate in 'high expression' group
Sabatier <i>et al.</i> (2015) ¹⁵⁷	BLBC	PD-L1 expression (mRNA)	Higher pCR rate in 'high expression' group
Callari <i>et al.</i> (2016) ¹⁶⁵	ER-negative/HER2-negative	6-consensus T-cell-related metagene	Higher pCR rate in 'high expression' group
Gu-Trantien <i>et al.</i> (2013) ¹⁶⁶	ER-negative/HER2-negative	8-gene follicular helper T (T _{FH})-cell signature, T _{H1} -cell signature and CXCL13	Higher pCR rate in 'high expression' group
Desmedt <i>et al.</i> (2011) ¹⁶⁷	ER-negative/HER2-negative	95-gene STAT1-related immune metagene	Higher pCR rate in 'high expression' group
West <i>et al.</i> (2011) ¹⁶⁸	ER-negative and TNBC	8-gene lymphocyte expression signature	Higher pCR rate in 'high expression' group
Ignatiadis <i>et al.</i> (2012) ¹⁶⁹	ER-negative/HER2-negative	95-gene STAT1 related immune metagene and 7-gene IR module	Higher pCR rate in 'high expression' group
Denkert <i>et al.</i> (2015) ¹⁷⁰	TNBC	Several immune-related genes (<i>CXCL9</i> , <i>PDCD1</i> , <i>CCL5</i> , <i>CXCL13</i> , <i>CD279</i> , <i>CTLA4</i> , <i>IDO1</i>)	Higher pCR rate in 'high expression' group
<i>Mixed prognostic/predictive value in mixed setting (untreated and systemic CT)</i>			
Sabatier <i>et al.</i> (2015) ¹⁵⁷	Basal-like	PD-L1 expression (mRNA)	Lower risk of relapse and death in 'high expression' group
Teschendorff <i>et al.</i> (2007) ¹⁷⁸	ER-negative/HER2-negative	7-gene prognostic immune-related module	Lower risk of distant metastasis in 'high expression' group
Rody <i>et al.</i> (2009) ¹⁷⁹	ER-negative and ER-negative/HER2-negative	Lymphocyte-specific kinase metagene	Lower risk of relapse in 'high expression' group
Staaf <i>et al.</i> (2010) ¹⁸⁰	BLBC	HER2-derived prognostic predictor-enriched in immune genes	Lower risk of distant metastasis in 'high expression' group
Sabatier <i>et al.</i> (2011) ¹⁸¹	BLBC	28-kinase metagene associated with immune response	Superior disease-free survival in 'high expression' group
Rody <i>et al.</i> (2011) ¹⁸²	ER-negative/HER2-negative	High B-cell metagene/low IL-8-metagene subgroup	Lower risk of relapse in this group
Nagalla <i>et al.</i> (2013) ¹⁸³	BLBC	B-cell/plasma cell, monocyte/dendritic-cell and T-cell/NK metagenes	Lower risk of relapse in 'high expression' group

BLBC, basal-like breast cancer; CT, chemotherapy; ER, oestrogen receptor; IR, immune response; NK, natural killer cells; pCR, pathological complete response; TNBC, triple negative breast cancer.

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Table 2

Prognostic and predictive value of TILs in TNBC

Study	TNBC definition	Systemic treatment	TIL assessment ^{*,‡}	Association with outcome
<i>Predictive value of chemotherapy benefit</i>				
Denkert <i>et al.</i> (2015) ¹⁷⁰	TNBC	Neoadjuvant CT	sTILs as continuous and categorical marker to define LPBC versus non-LPBC	Higher pCR rate in LPBC group
Issa-Nummer <i>et al.</i> (2013) ¹⁷¹	ER-negative/HER2-negative	Neoadjuvant CT	TILs as categorical marker to define LPBC versus non-LPBC	No significant association
<i>Mixed prognostic/predictive value (baseline TIL assessment)</i>				
Loi <i>et al.</i> (2013) ¹⁵³	ER-negative/HER2-negative	Adjuvant CT	sTILs and iTILs as continuous and categorical marker to define LPBC versus non-LPBC	Better DFS and OS in LPBC group
Dieci <i>et al.</i> (2015) ¹⁷⁵	ER-negative/HER2-negative	Untreated and adjuvant CT	sTILs and iTILs as continuous marker to define LPBC versus non-LPBC	Lower risk of death in LPBC group
Loi <i>et al.</i> (2014) ¹⁸⁴	TNBC	Adjuvant CT	sTILs as continuous marker to define LPBC versus non-LPBC	Lower risk of distant relapse in LPBC group
Adams <i>et al.</i> (2014) ¹⁸⁵	TNBC	Adjuvant CT	sTILs as continuous and categorical marker to define LPBC versus non-LPBC	Lower risk of distant relapse and death in LPBC group
<i>Mixed prognostic/predictive value (post-treatment TIL assessment)</i>				
Loi <i>et al.</i> (2016) ¹⁷⁶	TNBC	Neoadjuvant CT	sTILs assessed in residual disease as continuous and categorical marker to define three groups with high, intermediate and low sTIL content	Lower risk of recurrence and death in high sTIL group in residual disease
Dieci <i>et al.</i> (2014) ¹⁷⁷	TNBC	Neoadjuvant CT (some also post-surgery CT)	sTILs and iTILs assessed in the residual disease as continuous and categorical marker to define LPBC versus non-LPBC	Lower risk of distant relapse and death in LPBC group

* In continuous evaluation, unit increases in the marker (sTILs or iTILs) — without any cut off points — are evaluated. In categorical evaluation, the marker is evaluated by applying cut-off points to define high and low groups; cut-off points can vary across different studies.

‡ The cut off for lymphocyte predominant breast cancer (LPBC) definition was 50% or >50% according to different studies.

CT, chemotherapy; DFS, disease free survival; ER, oestrogen receptor; iTIL, intratumoural TIL; OS, overall survival; pCR, pathological complete response; PgR, progesterone receptor; sTIL, stromal TIL; TIL, tumour-infiltrating lymphocyte; TNBC, triple-negative breast cancer.

Table 3

Clinical trials testing immunotherapies in patients with breast cancer

Disease setting	Phase	Clinical trial reference number	Breast cancer	Immunotherapies (alone or in combination)	Control arm treatment
<i>Trials including only patients with TNBC</i>					
Metastatic	I/II	NCT025113472	TNBC	Pembrolizumab [*] /eribulin mesylate	NA
	II	NCT02499367	TNBC	Nivolumab [*] /doxorubicin (low dose) or cyclophosphamide metronomic or radiation therapy or cisplatin	NA
		NCT02447003	TNBC	Pembrolizumab	NA
Adjuvant	III	NCT02555657	TNBC	Pembrolizumab	Single-agent CT (capecitabine, eribulin, gemcitabine or vinorelbine)
		NCT02425891	TNBC	Atezolizumab [‡] /nab-paclitaxel	Nab-paclitaxel
Neoadjuvant	II	NCT02539017	TNBC	Vaccine (DC-CIK)/EC followed by docetaxel	EC followed by docetaxel
	I/II	NCT02489448	TNBC	Durvalumab [‡] /nab-paclitaxel followed by ddAC	NA
	II	NCT02530489	TNBC	Atezolizumab/nab-paclitaxel	NA
III		NCT02620280	TNBC (LABC only)	Atezolizumab/nab-paclitaxel/carboplatin	Nab-paclitaxel/carboplatin
<i>Trials including patients with breast cancer</i>					
Metastatic	I	NCT02303366	All	Pembrolizumab/stereotactic ablative body radiosurgery	NA
	I/II	NCT00003432	All (CEA- positive only)	Vaccine (CEA RNA-pulsed DC)	NA
		NCT01421017	All (with skin metastasis)	Imiquimod (TLR7 agonist)/radiotherapy or cyclophosphamide	NA
II		NCT02536794	HER2- negative	Durvalumab/tremelimumab [§]	NA
		NCT02411656	HER2- negative	Pembrolizumab	NA
		NCT02563925	All (with brain metastasis)	Tremelimumab/brain radiotherapy or stereotactic	NA
		NCT00083278	All	Ipilimumab [§]	NA
		NCT01792050	HER2- negative	Indoximod (IDO inhibitor)/paclitaxel or docetaxel	Paclitaxel or docetaxel
		NCT02491697	All	Vaccine (DC-CIK)/capecitabine	Capecitabine

BC, breast cancer; CEA, carcinoembryonic antigen; CT, chemotherapy; DC, dendritic cells; DC-CIK, dendritic cells co-cultured with cytokine-induced killer cells; ddAC, dose-dense doxorubicin and cyclophosphamide; EC, epirubicin and cyclophosphamide; LABC, locally advanced breast cancer; TLR7, toll like receptor 7; NA, not applicable; TNBC, triple negative breast cancer.

^{*} Anti-programmed cell death protein 1 (PD-1) monoclonal antibodies (mAbs): nivolumab and pembrolizumab (anti-PD1).

[‡] Anti programmed cell death 1 ligand 1 (PD L1) mAbs: atezolizumab and durvalumab. [§] Anti cytotoxic T lymphocyte protein 4 (CTLA 4) mAbs: ipilimumab and tremelimumab.