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What is triple-negative breast cancer?

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ABSTRACT

Triple-negative (ER-negative, PR-negative, HER2/neu not overexpressed) breast cancer has distinct clinical and pathologic features, and is a clinical problem because of its relatively poor prognosis, aggressive behaviour and lack of targeted therapies, leaving chemotherapy as the mainstay of treatment. Most triple-negative tumours fall into the basal-like molecular subtype of breast cancer, but the terms are not completely synonymous. Among the intriguing characteristics of triple-negative breast cancer is its association with cancers arising in BRCA1 mutation carriers, in young women and in African–American women. The reasons for these associations are unclear but may ultimately provide avenues for prevention and targeted therapy. This review discusses the definitions and characteristics of as well as current and evolving therapies for triple-negative and basal-like breast cancer.

1. Introduction

In 2007, 1.3 million women worldwide were diagnosed and 465,000 died from breast cancer making this the most common cancer in women and the leading cause of death.[1] Though impressive, these statistics treat breast cancer as a homogeneous entity, which we increasingly recognise as inaccurate. Gene expression studies have identified several major subtypes of breast cancer[2]: the luminal subtypes, which typically express hormone receptor-related genes, and two hormone receptor-negative subtypes – the human epidermal growth factor receptor 2 (HER2) positive/oestrogen receptor (ER) negative subtype and the basal-like subtype. The subtypes vary in prognosis, with worse outcomes traditionally seen among the two hormone receptor-negative subgroups compared with the luminal subgroups[3–5]; however, improvements in chemotherapy, endocrine therapy and HER2-targeted therapy may change the prognostic landscape of breast cancer.

A subtype of particular interest is the basal-like breast cancer BBC. In population-based studies, this subtype comprises approximately 15–20% of breast cancers.[6–8] In research studies, BBC has been reproducibly identified using gene expression methods[4,5] and immunohistochemistry,[9–11] however, a validated method to identify BBC and other intrinsic subtypes of breast cancer for clinical use does not exist. In arrays, BBCs are characterised by low expression of ER-related genes and HER2-related genes; for this reason in clinical specimens they are usually ER-negative, progesterone receptor (PR) PR-negative and lack HER2 overexpression. This is called the 'triple-negative' phenotype.

Since triple-negative breast cancer is resistant to our current HER2-targeted therapies such as trastuzumab, and hormonal therapies such as tamoxifen and aromatase inhibitors, chemotherapy is the mainstay of treatment. This lack of targeted therapies has intensified the interest in this group of patients. This review will focus on the definition and features of triple-negative breast cancer, current treatment strategies and future directions for treatment.

2. Nomenclature

As mentioned above, most triple-negative breast cancers cluster with the BBC,[10] however, these are not synonyms. 'Triple negative' is a term based upon clinical assays for ER, PR

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and HER2, while 'basal-like' is a molecular phenotype. The 'basal-cell phenotype' was first described by Wetzels et al. by using immunohistochemical markers to identify cytokeratins in breast tumours that normally were found only in the cell layer lying closest to the basement membrane of the mammary gland epithelium.[12] These markers are also expressed in BRCA1-associated cancers, as discussed below. The original basal-like intrinsic subtype was defined using cDNA microarrays[2,3]; however, it has been reproducibly identified using other gene expression platforms,[5,13–15] mean expression-based and similar approaches to subtype categorisation,[16–18] and multiplex immunohistochemical profiling involving either multiple markers[9,11] or a simpler surrogate (ER-negative, PR- negative, HER2-negative, plus either cytokeratin 5 positive or EGFR-positive),[10] which is 76% sensitive and 100% specific.[10] The triple-negative proxy is the least accurate; although most triple-negative tumours have basal-like expression profiles and most BBCs are triple negative, both categories have up to 30% discordance.[19,20] The interest in developing other diagnostic tools to identify subtypes is because although RNA-based microarrays are the gold standard to identify breast cancer subgroups, these assays are not routinely used in clinical environments for both technical reasons and the need for frozen tumours.

3. Molecular features of triple-negative breast cancer

'Unsupervised' (heedless of clinical characteristics or outcome) gene expression array profiling studies on breast tumours have allowed breast cancers to be clustered according to their intrinsic gene expression patterns, revealing at least five intrinsic subtypes[2–5]: luminal A and B, HER2+/ER-negative, normal breast-like, basal-like and potentially a 'claudin-low' subtype.[21] These breast cancer subtypes are highly reproducible,[3–5] persist before and after therapy, are concordant between the primary tumour and the metastasis[22] and are found in the preneoplastic lesion ductal carcinoma *in situ*.[23–26]

Gene expression of the novel 'basal cluster' includes HER1 (epidermal growth factor receptor, EGFR), high molecular weight cytokeratins 5, 14 and 17, vimentin, p-cadherin, fascin, caveolins 1 and 2 and alpha-B-crystallin.[3,10,11,16,27–32] The hormone receptor cluster of genes is underexpressed, and the proliferation cluster is highly expressed, befitting the largely grade 3 nature of these tumours.[6] Myoepithelial markers SMA, p63 and CD 10 are generally expressed[27] and have been suggested as a means of identifying BBC. Some of these characteristic markers are potentially targetable. As mentioned, HER1/EGFR is expressed in approximately 60% of triple-negative tumours.[10,33] c-Kit expression is higher in basal-like tumours; in one study, 31% of tumours expressing basal cytokeratins had c-kit staining compared to 11% in basal cytokeratin-negative tumours ($p < 0.001$).[10] Several molecules integrally important in response to DNA damage are aberrantly expressed in BBC, which may have implications for chemosensitivity. For example, high p53 IHC expression or TP53 gene mutations are common in BBC[3,11,34,35]; in one study 82% of BBC had p53 mutations compared with only 13% in the luminal A subtype ($p < 0.001$).[3] In addition, the strong association of BBC with BRCA1 mutation carriers, described further below, raises the question of whether this pathway, which is integrally involved in repair of DNA damage, may be dysfunctional in both sporadic and BRCA1-associated BBC.[36] These observations, as well as the high grade nature and the high proportion of gene copy number aberrations speak to underlying genomic instability in BBC.[37] In BBC, mRNA levels of p16, cyclin E and E2F3 are elevated compared to other tumour types, while the levels of Rb and cyclin D1 are lower; this suggests that Rb inactivation is integrally linked to BBC.[38] The Rb pathway is a key component of the response to cellular stress. Deranged p16/Rb signalling and abnormal stress response are also characteristics of BBC.[38] Table 1 summarises these molecular features.

Table 1 – Summary of relevant molecular features in triple-negative tumours.

Increased	Decreased or not found
c-kit	ER
p53 protein /TP53 gene mutations	PR
cyclin E	HER2
p16	Cyclin D1
EGFR	Rb
Basal cytokeratins 5, 14, 17	
αB crystallin	

Updated examination of the gene expression portraits of large numbers of breast cancers suggests that there may be other smaller subtypes. Among these is the 'claudin-low' subtype,[21] which is typically triple-negative so it warrants a discussion here. The relationship of breast cancer subtypes and the controversial area of mammary stem cells is of great interest because of the implications for treatment.[39,40] There are several lines of evidence in support of mammary stem cell involvement in breast cancer pathogenesis. Al-Hajj and colleagues isolated CD 44+/CD24$^{-/low}$ tumourigenic breast cancer cells that were capable of generating phenotypic heterogeneity[41]; another suggested that the loss of BRCA1 contributes to the development of such tumourigenic cells.[42] While some studies have suggested shared characteristics cancer cells with myoepithelial/basal cell phenotype with breast progenitor cells,[43,44] this does not necessarily imply derivation. More recent studies suggest that there exists a subpopulation of CD44+/CD24$^{-/low}$ cells that share some characteristics with the basal-like subtype, including triple-negative status, but are biologically distinct and more phenotypically consistent with 'stemness'.[21,40,45]

4. Clinical features and risk factors

Triple-negative tumours typically have a higher histologic grade, elevated mitotic count, scant stromal content, central necrosis, pushing margins of invasion, a stromal lymphocytic response and multiple apoptotic cells[27,46]; histologically they are largely ductal,[6] but several unusual histologies are also overrepresented, including metaplastic,[27,47,48] atypical or typical medullary,[27,49] or adenoid cystic carcinomas.[50] A case series evaluating 65 metaplastic breast cancers by

immunohistochemistry revealed 91% to be basal-like (ER-negative, HER2-negative, CK 5+ and EGFR+)[47]; another case series of 24 tumours showed 96% to be triple-negative.[48] The majority of medullary carcinomas also show a basal phenotype.[49,51]

In the population-based Carolina Breast Cancer Study (CBCS), basal-like breast cancer (defined by triple-negative status plus EGFR or cytokeratin 5 positivity) were virtually all of ductal or mixed histology (90%) and of high grade (84%). They did not significantly differ from other breast cancers in stage at diagnosis or lymph node positivity.[6] In a cohort study of 1601 breast cancer patients (180 triple-negative), the mean age at diagnosis was younger for the triple-negative group (53 years versus 58 years); the triple-negative breast cancers were also more likely to be grade III and have a greater mean tumour size. In that study, they were more likely to have axillary node involvement at diagnosis,[52] although that was not seen in the CBCS.[6]

A significant interaction with age and race has been seen in multiple datasets.[6–8,53,54] In the CBCS, 16% of non-African–American women had BBC compared with 26% of African–American women, and 24% of premenopausal women compared with 15% of postmenopausal women.[6] The group of breast cancer patients who are most likely to have BBC are premenopausal African–American women, in whom this subtype comprises 27–47% of tumours[6,7,53,54]; the group at lowest risk are postmenopausal non-African–American women, in whom only approximately 14% are BBC.[6] Given the poor prognosis of BBC confirmed in these and other datasets,[3–7,53,54] this has raised the question of whether the higher proportion of BBC might contribute to the worse outcomes suffered by African-American women with breast cancer. Table 2 summarises the clinical characteristics of triple-negative tumours.

Reanalysis of epidemiologic risk factors for breast cancer stratified by subtype has contributed some intriguing but preliminary findings. In the CBCS, for example, several traditional risk factors had a different magnitude or direction of effect between basal-like and luminal breast cancers. In contrast to the relationship with luminal cancers, an increased risk of BBC was seen with parity and younger age at first term birth. Not breastfeeding or taking medications to suppress lactation were risk factors for basal-like, but not for luminal A breast cancer. An elevated waist-hip ratio was a risk factor for basal-like breast cancer in both pre- and postmenopausal women, whereas it was a lesser risk factor for luminal A cancers and then only in postmenopausal women.[53] Another population-based study, the Polish Breast Cancer Study, similarly suggested a differential effect by subtype, with increasing age at menarche associated with reduced risk of basal-like but not luminal cancers, and increasing body mass index among premenopausal women was associated with reduced risk of luminal but not basal-like cancers.[55] The potential for differential risk factor indices by subtype illustrates the need for more specific performance and analysis of epidemiologic studies from both a traditional risk factor and a molecular epidemiology standpoint.[8,56,57]

Table 2 – Summary of clinical features of triple-negative tumours.

Patient characteristics	Young age at diagnosis African–American Primary tumour type in BRCA1 mutation carriers
Tumour characteristics	Ductal or mixed histology High grade
Treatment/prognosis	Poorer prognosis Sensitive to primary chemotherapy No known targeted therapy (yet) Elevated risk of early relapse

5. BRCA 1 and basal breast cancer

BRCA1-associated breast cancers are mostly basal-like and triple negative[4,58] and express basal markers such as cytokeratins 5, 14, 17, and EGFR.[59] Efforts to link sporadic (occurring in women without germline BRCA1 mutations) BBC with dysfunction of the BRCA1 pathway are ongoing.[36,60] While BRCA1 methylation and localisation appear similar across subtypes,[60,61] in one study BRCA1 mRNA was lower in BBC than in matched controls, and ID4, a downregulator of BRCA1, was expressed nine times more in BBC.[36] In addition to triple-negative phenotype, sporadic and BRCA1-associated breast cancers share other characteristics such as evidence of genomic instability and similar X-chromosome inactivation patterns,[61] lending credence to the concept that even if BRCA1 itself is unaffected, BRCA1 pathway function is compromised in both sporadic and inherited forms of BBC. Since the BRCA1 pathway is in part responsible for DNA repair, this putative association may have therapeutic implications, as described further below.

6. Prognosis

BBC has molecular characteristics predictive of a poor prognosis. In one study comparing intrinsic subtypes with prognostic profiles in 53 patients with BBC, all had high Recurrence Scores, all had poor 70-gene profiles, 50 had activated wound response signatures and 42 had poor two-gene ratio signatures.[62] The initial studies examining outcome by intrinsic subtype uniformly found a poor prognosis in BBC.[3,5] In population-based studies, the triple-negative phenotype demonstrated reduced breast cancer-specific survival compared with luminal phenotypes as predicted by the early translational studies.[6]

As described further below and counter to a commonly held misconception, triple-negative breast cancer is sensitive to chemotherapy. In two neoadjuvant studies, one anthracycline-based, the other anthracycline and taxane-based, the pathologic complete response rate (pCR) was significantly higher in triple negative (25–45%) than in luminal breast cancers (6–7%).[63,64] Despite this higher pCR rate, patients with triple-negative tumours had worse four-year distant disease-free and overall survival. Interestingly, those with pCR did well; the worse outcome was due to a higher relapse among those with residual disease. The good outcome of triple-negative breast cancers that achieve pCR with poor outcome of the group driven by excessively high relapse risk among those

with residual disease has been confirmed in a larger neoadjuvant cohort treated with various regimens.[65]

There is also a difference in timing of relapse; all the relapses in the neoadjuvant study after 40 months occurred in the luminal phenotypes, all of whom received five years of adjuvant tamoxifen.[63] Stronger evidence comes from larger, although also heterogeneously treated, datasets. In one single institution cohort study involving over 1600 patients, triple-negative breast cancer had an increased likelihood of distant recurrence (HR 2.6, 95% confidence interval (CI) 2–3.5) and death (HR 3.2, 95% CI 2.3–4.5) within five years of diagnosis, but not after five years; the peak of distance recurrence peaked at approximately three years.[52] Another Canadian study demonstrated a difference in overall survival between triple-negative and non-triple-negative cancers that were most obvious at three years and decreased to no difference at 10 years.[66]

7. Therapy

Since there is no role for hormonal or HER2-targeted agents, the primary adjuvant therapy for triple-negative breast cancer is chemotherapy. As previously noted, these tumours respond to anthracycline- and anthracycline/taxane-based regimens; however, they have a high risk of relapse. Several promising avenues of improving our treatment armamentarium are in investigation (Table 3). The association of BBC with BRCA1 mutation carriers raises other chemotherapeutic possibilities. Tumours with BRCA1 dysfunction have deficient double-stranded DNA break repair[67] which leads to an increased sensitivity to chemotherapeutic agents that cause DNA damage, such as platinum agents.[68] For this there is little clinical evidence; however, several small studies suggest activity of platinum-based regimens in triple-negative breast cancer.[69–71] The association with BRCA1 pathway dysfunction also raises the possibility of efficacy of a novel targeted therapy, poly(ADP-ribose) polymerase (PARP) inhibitors. BRCA1's role in homologous recombination repair of DNA damage means that in the setting of BRCA1 dysfunction, the primary mechanism for the repair of double strand DNA breaks becomes the PARP-dependent non-homologous recombination pathway. For this reason, cell death from PARP inhibition is augmented in BRCA1 or BRCA2-deficient cells,[72] and PARP inhibitors synergise with DNA damaging chemotherapy.[73]

Antiangiogenic therapy targeting vascular endothelial growth factor (VEGF) clearly has a role in breast cancer therapy, and may be a targeted therapy with efficacy in the basal-like subtype. In a randomised phase III trial comparing the anti-VEGF monoclonal antibody bevacizumab plus paclitaxel to paclitaxel alone in metastatic breast cancer (ECOG 2100), the combination doubled progression-free survival.[74] Subset analysis revealed that the hormone receptor-negative subset, which is largely triple negative given the HER2-negative nature of the study population, had the same benefit (hazard ratio of progression approximately 0.5) as the remainder of the study population. Sunitinib, an oral tyrosine kinase inhibitor that has antiangiogenic activity, has shown evidence that it may have activity in breast cancer, and is currently being studied in triple-negative metastatic breast cancer.[75]

EGFR is present in approximately 60% of triple-negative tumours.[10,33] *In vitro*, BBC cell lines are more sensitive to EGFR inhibitors than luminal cell lines, and demonstrate synergy to the combination of carboplatin and cetuximab.[76] Single agent cetuximab is well tolerated in triple-negative metastatic breast cancer, but has low single agent response in the pretreated setting, whereas the combination of cetuximab plus carboplatin after progression on single agent cetuximab was modestly active (18% response rate) in a pretreated population.[70] The combination of cetuximab plus carboplatin from initiation of therapy similarly produced 17% response and 31% clinical benefit in this population.[77] In an *a priori*-defined subset of a randomised phase II study of weekly irinotecan/carboplatin with or without cetuximab, the addition of cetuximab increased the objective response rate associated with irinotecan/carboplatin in metastatic triple-negative breast cancer from 30% to 49%.[69] However, a small retrospective analysis of the dual EGFR/HER2 inhibitor added to paclitaxel in the first-line setting did not appear to prolong disease-free survival in the triple-negative subset (Di Leo A, personal communication). Among the challenges we face in targeted therapy for tumours with redundant pathways is the possibility, or likelihood, of alternate pathways for cell signalling and the potential that combination biologic therapy may be necessary in many cases.[70]

Several targeted approaches to the therapy of basal-like breast cancer are of interest, but these currently lack any clinical evidence. Histone deacetylase (HDAC) plays a key role in gene expression through chromatin remodelling, and the role of HDAC inhibitors in breast cancer is the subject of ongoing study, especially in combination with agents that target DNA repair.[78] Another focus of study is the oral small molecule tyrosine kinase inhibitor dasatinib. Dasatinib is a multitargeted kinase inhibitor that inhibits src and abl, and is approved for imatinib-resistant chronic myelogenous leukaemia. A cell-line derived response predictor for dasatinib suggests that of all breast cancers, BBCs are the most likely to respond.[79,80] A phase II study limited to stage IV triple-negative breast cancer is testing this hypothesis. The intracellular signalling pathway, Akt/PI3K/mammalian target of rapamycin (mTOR), has been strongly linked to drug resistance.[81]

Table 3 – Therapeutic strategies in investigation for triple-negative breast cancer.

Targeting aberrant DNA repair	Platinum agents PARP inhibitors (AZD2281; BSI-201) Trabectedin (DNA transcription inhibitor)
Antiangiogenesis	Bevacizumab Sunitinib
EGFR targeting	Cetuximab Erlotinib
Epigenetic modifications	Trichostatin A Butyrate Vorinostat
Src inhibitor	Dasatinib
PI3K/Akt pathway	mTOR inhibitors (Everolimus)

Targeting this pathway may lead to novel therapies in overcoming drug resistance in triple-negative disease.[82]

8. Conclusion

Triple-negative breast cancers mostly comprise the basal-like molecular subtype of breast cancer, which has distinctive clinical and pathological features. While triple-negative breast cancers do not necessarily present at later stages, in most datasets they have worse survival than the more common luminal subtype of breast cancer and have no known targeted agents, making chemotherapy the primary adjuvant and metastatic modality of treatment. They have unique risk factors such as an association with BRCA1 mutation carriers. Current research is focused on improving our understanding of the risk factors for this subtype and on developing improved treatment options.

Conflict of interest statement

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