Triple-negative breast cancers: an updated review on treatment options

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ABSTRACT

Morphologic features of tumour cells have long been validated for the clinical classification of breast cancers and are regularly used as a “gold standard” to ascertain prognostic outcome in patients. Identification of molecular markers such as expression of the receptors for estrogen (ER) and progesterone (PR) and the human epidermal growth factor receptor 2 (HER2) has played an important role in determining targets for the development of efficacious drugs for treatment and has also offered additional predictive value for the therapeutic assessment of patients with breast cancer. More recent technical advancements in identifying several cancer-related genes have provided further opportunities to identify specific subtypes of breast cancer. Among the subtypes, tumours with triple-negative cells are identified using specific staining procedures for basal markers such as cytokeratin 5 and 6 and the absence of ER, PR, and HER2 expression. Patients with triple-negative breast cancers therefore have the disadvantage of not benefiting from currently available receptor-targeted systemic therapy. Optimal conditions for the therapeutic assessment of women with triple-negative breast tumours and for the management of their disease have yet to be validated in prospective investigations. The present review discusses the differences between triple-negative breast tumours and basal-like breast tumours and also the role of mutations in the BRCA genes. Attention is also paid to treatment options available to patients with triple-negative breast tumours.

KEY WORDS

Triple-negative breast tumours, epidermal growth factor receptor, chemotherapy

1. INTRODUCTION

Tumours in the breast have long been classified according to their morphologic features, histologic type, and grade (severity). Identification of molecular markers such as expression of the estrogen (ER) and progesterone (PR) and the human epidermal growth factor receptor 2 (HER2) has offered additional predictive value for the therapeutic assessment of women diagnosed with breast cancer. More recently, gene expression analysis using DNA microarray technology has identified additional breast tumour subtypes that were not apparent using traditional histopathologic methods. Based on gene expression profiles, breast cancer can be classified into 5 main groups:

- Luminal A
- Luminal B
- Basal-like
- HER2
- Normal breast-like

Most breast cancers originate from the inner (“luminal”) cells that line the mammary ducts. Luminal A and luminal B tumours are similar in that both are typically ER+ or PR+, or both. However, they are dissimilar in that the A type is usually HER2− and the B type is more likely to be HER2+ and lymph node-positive.

Women with luminal A tumours are often diagnosed at a younger age. They tend to have the best prognosis, with relatively high rates of overall survival and relatively low rates of recurrence. Those with luminal B tumours tend to have a higher tumour grade and a poorer prognosis (Table 1).

Basal-like tumours originate in the outer (“basal”) cells that line the mammary ducts. Their incidence has been estimated to be between 13% and 25% [12–14]. These tumours are diagnosed more frequently among younger women and are associated with hereditary BRCA1-related breast cancers. They are often aggressive [15–17] and are associated with a prognosis poorer than those for the luminal A, luminal B, and normal breast-like types [11,16]. Metastatically, they seem to disseminate to the axillary nodes and, less frequently, to bone [18]. In a population-based study, basal-like breast cancer was suggested to be more prevalent...
TABLE 1  Microarray-based breast tumour classification

A—Subtype (hormone and HER2 receptor status)

<table>
<thead>
<tr>
<th>Subtype</th>
<th>ER+ or PR+ or both, HER2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>(ER+ or PR+ or both, HER2+)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>(ER+, PR+, HER2+)</td>
</tr>
<tr>
<td>HER2+</td>
<td>(ER+, PR+, HER2+)</td>
</tr>
<tr>
<td>Basal-like</td>
<td>(ER+, PR+, HER2+)</td>
</tr>
<tr>
<td>Triple-negative tumours</td>
<td>(ER+, PR+, HER2+)</td>
</tr>
<tr>
<td>Normal breast-like tumours</td>
<td>(tumours that do not fall into any of the foregoing categories)</td>
</tr>
</tbody>
</table>

B—“Triple-negative tumours” encompasses:

- Basal-like breast tumours
- Normal breast-like tumours
- BRCA1-deficient breast tumours

ER = estrogen receptor; PR = progesterone receptor; HER2 = human epidermal growth factor receptor 2; BRCA1 = protein encoded by the breast cancer 1 gene.

among premenopausal African American women (39% vs. 16% in non–African American women and 14% in postmenopausal African American women) 11.

The mechanism or mechanisms by which basal-like breast cancer metastasizes to the brain is not currently clear. However, the incidence of brain metastasis among patients with HER2+ or cytokeratin 14 tumours has been documented 18,19. Data developed by sequencing primary breast tumours, brain metastases, matched normal tissue, and a mouse xenograft developed from the primary tumour identified 50 point mutations and small insertions or deletions that were present in the tumour genomes but not in the matched normal tissue 20. The increased incidence of brain metastases in patients with HER2+ metastatic breast cancer has been documented, and a targeted therapeutic—namely, lapatinib (Tykerb: GlaxoSmithKline, Mississauga, ON)—has shown promise in the treatment of progressive HER2+ brain metastases refractory to trastuzumab (Herceptin: Genentech, San Francisco, CA, U.S.A.) 19,21.

The HER2 tumours are named for their status as HER2+. They tend to be ER−, PR−, and lymph node−positive, with poorer grades. They may contain p53 mutations. The HER2+ tumours have relatively poor prognoses and are prone to early and frequent relapse and to distant metastasis 11,16,22.

The normal breast−like tumours are those that do not fall into any of the other categories. They account for 6%−10% of all breast cancers 11,16. These tumours are usually small and typically have a good prognosis 10,11. They are more common in postmenopausal than in premenopausal women 10. From a clinical and therapeutic point of view, tumours in the breast can be divided into three main groups:

- Hormone receptor−positive breast tumours, which are treated with a number of hormone−targeted therapies such as tamoxifen, aromatase inhibitors, fulvestrant (Faslodex: AstraZeneca, London, U.K.), and ovarian suppression [oophorectomy or analogs of luteinizing−hormone releasing−hormone and gonadotropin−releasing hormone such as goserelin (Zoladex: AstraZeneca) or leuprolide (Lupron: Abbott Laboratories, Abbott Park, IL, U.S.A.)] with or without chemotherapy
  - HER2−positive breast tumours, which are managed with HER2−directed therapies such as trastuzumab (and lapatinib in some cases) with or without chemotherapy
  - Basal and triple-negative breast tumours, which are resistant to the existing ER, PR, and HER2−targeted therapies because of loss of target receptors; hence, chemotherapy appears to be the only available treatment modality

2. TRIPLE-NEGATIVE AND BASAL-LIKE TUMOUR CELLS

Triple-negative breast cancer is a recent term derived from tumours that are characterized by the absence of ER, PR, and HER2. Triple-negative disease is diagnosed more frequently in younger and premenopausal women 11,23−26 and is highly prevalent in African American women 27,28. It remains unclear whether this racial association is related to germ-line genetic factors, exposure to environmental factors, or a combination of both.

Triple-negative and basal-like tumour cells proliferate at a higher rate, exhibit central necrosis with a pushing border, and are identified mostly using simple immunohistochemical staining for the expression of cytokeratins 5 and 6 (CK5/6), ER, PR, HER2, and vimentin 8,17,29. In about 60% of cases, these tumour cells express the receptor for epidermal growth factor (EGFR) 17,30−32 and may also contain mutations in the p53 gene 10,11,33.

If expression profiling analysis using the intrinsic gene list is considered the “gold standard” for identification, triple-negative tumours and a great preponderance of tumours overall do not express ER, PR, and HER2 17,34. However, among basal-like tumours, 5%–45% were reported to be ER+, and 14% were shown to be HER2+ 9. Furthermore, during investigations of the expression of basal CK5 and EGFR in separate cohorts of triple-negative tumours, only 56%−84% expressed those markers 25,26. In addition, patients with triple-negative tumours that express a basal-like tumour phenotype have a significantly shorter disease-free survival than do patients with triple-negative tumours lacking the expression of basal-like tumour markers 25,26. When triple-negative and basal-like tumours were analyzed by gene expression profiling, 71% of triple-negative tumours showed a basal-like phenotype, and 77% of basal-like tumours showed a triple-negative phenotype 35.
Although the terminology relating to triple-negative and basal-like tumours tends to be used interchangeably, it is not entirely interchangeable or synonymous. Caution should therefore be exercised not to equate these tumour types.

From a pathologist’s point of view, triple-negative tumours and basal-like tumours are predominantly of high histologic grade 23,25,26. Approximately 10% of triple-negative tumours have been shown to be of grade 1 23. In random cohort studies of 148 Nigerian patients, 66.9% were premenopausal women with a mean age of 43.8 years when diagnosed with triple-negative tumours. Overall, 87 of the patients (59%) were thought to have a basal-like tumour 36. Among triple-negative tumours, most recurrences are observed during the 1st and 3rd years after therapy. Most deaths take place in the first 5 years, even after a strict therapeutic regimen 23,26. Additional data also suggest that, compared with control patients having non-basal-like or non-triple-negative tumours, patients with triple-negative tumours and basal-like tumours experience significantly shorter survival after the first metastatic event 18,23,37. Although triple-negative tumours and basal-like tumours share some characteristics, a careful analysis of microarray-based expression profiles suggests that triple-negative tumours also encompass phenotypes of normal breast-like tumours, and in most studies, normal breast-like tumours have been shown to cluster together with basal-like and HER2 tumours in the ER-negative arm 6,8,16,22.

3. TRIPLE-NEGATIVE AND BRCA-MUTANT TUMOUR CELLS

Hereditary breast cancers account for only 5%–10% of all breast cancer cases. However, individuals carrying mutations in the BRCA gene (BRCA1 or BRCA2) have a 40%–80% chance of developing breast cancer. Thus, identification of BRCA mutations has been used as one of the strongest breast cancer predictors 38–41. By contrast, the incidence of sporadic mutations in BRCA1 and BRCA2 are in the ranges 4%–29% and 0.6%–16% respectively 39. In nonfamilial cancer cohorts in the United States, mutation frequencies are 0.6% for BRCA1 and 0.9% for BRCA2 42. However, U.S. Ashkenazi families have much higher frequencies of BRCA1 and BRCA2 mutations, at 28.6% and 15.6% respectively 42–44.

The contribution of BRCA mutations to the development of breast cancer within any specific population depends on prevalence and penetrance power. Mutations in the BRCA1 and BRCA2 genes occur with different frequencies in individuals of different ethnicities living in different geographic regions in the world. For example, 64% of high-risk families in Poland are reported to carry BRCA1 mutations (1 in 9 are recurring mutations), but rarely any BRCA2 mutations 45. In Sweden, 34% of high-risk families carry BRCA1 mutations, but only 2% carry BRCA2 mutations 46. By contrast, 32% of high-risk Sardinian families carry mutations in BRCA2, and 11%, mutations in BRCA1 47. Interestingly, DNA microarray and immunohistochemical analyses revealed that 80%–90% of breast cancers in women with germ-line mutations in BRCA1 are triple-negative (ER−, PR−, HER2−) 26,48–52. The extent to which the BRCA1 pathway contributes to the behavior of triple-negative cancers is an area of active research.

Among triple-negative tumours, the incidence of BRCA1 or BRCA2 mutations was reported to be approximately 12.5%. In the United States, approximately 3.3% of woman with triple-negative tumours show BRCA1 mutations; however, the incidence of triple-negative breast tumours in nonfamilial BRCA mutations is not currently clear. In some triple-negative tumours of high histologic grade, BRCA1 protein levels have been shown to be significantly lower, suggesting that the BRCA1 pathway may be dysfunctional in these tumour cells 50,52. Several lines of evidence suggest a link between basal-like breast cancer and BRCA1 deficiency 51. The functions of BRCA1 are diverse, and one is the repair of double-stranded DNA breaks by the potentially error-free mechanism of homologous recombination 50. Lack of BRCA1 could result in DNA repair by more error-prone mechanisms such as nonhomologous end-joining and single-strand annealing, resulting in genomic instability and therefore cancer predisposition 53. Most BRCA1-associated tumours are triple-negative, and the patients in which they arise have a poor outcome 54. Clustering analyses of microarray expression profiling data have shown that familial BRCA1 mutant tumours tend to fall into a basal-like category, suggesting similar carcinogenic pathways or causes in these cancer subtypes 55.

4. TREATMENT OPTIONS FOR TRIPLE-NEGATIVE TUMOURS

Patients with triple-negative breast cancer do not benefit from hormonal or trastuzumab-based therapies because of the loss of target receptors such as ER, PR, and HER2. Hence, surgery and chemotherapy, individually or in combination, appear to be the only available modalities. However, some studies have identified certain receptors as targets for new therapeutic drugs—for example, cell-surface receptors such as EGFR and c-Kit, both of which have been shown to play a major role in the progression of triple-negative tumours. Nielsen et al. 17 observed the expression of c-Kit in 31% of basal-like tumour cells and also in 11% of non-basal-like tumour cells. In addition, some available reports suggest that because BRCA1-deficient breast tumours share features and behavior associated with the basal-like tumours, the therapies that effectively target basal-like tumours should also
be highly effective in the treatment of BRCA1-deficient breast cancer and triple-negative tumours.

Mutations in the BRCA1 gene have been demonstrated to lead to error-prone DNA repair, resulting in genomic instability and thus predisposition to carcinogenesis. This phenomenon could be relevant to the control of proliferation in BRCA1-related, triple-negative, and basal-like tumour cells. Data from several in vitro studies have indicated that breast tumour cells with BRCA1 mutations are extremely sensitive to drugs that induce cross links (mitomycin-C and platinum) and single- and double-strand breaks (etoposide and bleomycin) in DNA. Furthermore, single-strand breaks in DNA are repaired by the base-excision repair pathway in which poly(ADP-ribose) polymerase 1 (PARP1) enzyme is one of the essential components. Cells null for BRCA1 were shown to be incapable of repairing DNA strand breaks, and inhibition of PARP1 resulted in enhanced apoptotic processes. Thus, BRCA1-null cells were reported to be more sensitive to chemotherapies. By contrast, these cells were shown to be resistant to mitotic-spindle poisons such as taxanes and vinorelbine. Previous observations provided strong circumstantial evidence that the BRCA1 and PARP1 pathways could be dysfunctional in a significant subgroup of triple-negative and basal-like breast tumours and, therefore, that those pathways could be targeted for therapy. Because ionizing radiation also induces DNA strand breaks, additional local or regional radiotherapy may possibly be of particular benefit for patients with advanced triple-negative cancers.

Approximately 66% of breast cancer patients with triple-negative tumour cells and basal-like tumour cells have been reported to express EGFR. Inhibition of EGFR might be a useful therapeutic strategy. Clinical trials evaluating the efficacy of humanized anti-EGFR monoclonal antibodies and EGFR tyrosine kinase inhibitors in patients with triple-negative breast cancer are currently under way.

Numerous phase I and II clinical trials for the treatment or management of patients with triple-negative breast tumours are under way. The strategies used in those trials are broadly divided into these five groups:

- **Agents that target EGFR** Overexpression of EGFR in triple-negative and basal-like tumour cells is well established. At least a couple of phase II clinical trials are using cetuximab (antibody treatment), which targets the expression of EGFR or its signalling pathways. One of the EGFR-inhibitor phase II clinical trials, by U.S. Oncology Research, is evaluating weekly gefitinib (Iressa: AstraZeneca) plus carboplatin, with or without cetuximab, in treating patients with metastatic breast cancer.

- **Agents that inhibit PARP1** Phase I clinical trials using PARP1 inhibitors have been successfully completed, and phase II studies have begun. The data from these studies have already indicated that PARP1 inhibitors could protect against the nephrotoxicity of cisplatin and the cardiotoxicity of doxorubicin, thus potentially helping to provide additional chemotherapeutic efficacy. The PARP inhibitors effectively disarm the ability of cancer cells to repair themselves and cause the death of those cells. Importantly, PARP inhibition, which kills cancer cells, spares identical normal cells that lack cancer-related alterations such as those from mutated BRCA1 and BRCA2. In combination with chemotherapy, PARP inhibitor (BSI-201) shows promise in the treatment of triple-negative tumours not harbouring BRCA mutations.

- **Agents that inhibit c-Kit** The efficacy of imatinib, a drug that inhibits c-Kit tyrosine kinase, is being evaluated in patients with triple-negative and basal-like tumours. However, the immunohistochemical detection of c-Kit overexpression does not guarantee a positive response to imatinib in patients with metastatic disease because most commercially available antibodies for c-Kit recognize total c-Kit and do not distinguish the activated or phosphorylated version, which is the actual target of imatinib.

- **Agents that inhibit second messengers** (Ras, MEK/ERK, mammalian target of rapamycin, heat shock proteins, and anti-vascular endothelial growth factor, among others) Many components of the proliferation pathway are overexpressed or mutated in cancer cells and therefore represent potential targets.

Triple-negative cancers are reported to respond to neoadjuvant chemotherapy, but overall, survival in patients with such tumours is still poor, and management of these patients may require more aggressive intervention. The data obtained from various investigations using triple-negative tumour cells suggest that patients with these breast cancers are more likely to benefit from chemotherapeutic agents in development that use a strategy of inhibiting DNA repair. Researchers are pursuing new treatments and drug combinations. A full list of clinical
TREATMENT OPTIONS FOR TRIPLE-NEGATIVE BREAST CANCERS

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6. CONFLICT OF INTEREST DISCLOSURES

The author has no financial conflicts of interest to disclose.

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