research article

Understanding the biology of triple-negative breast cancer

C. Criscitiello1*, †, H. A. Azim, Jr1, †, P. C. Schouten2, S. C. Linn2, ‡ & C. Sotiriou1, ‡

1Breast Cancer Translational Research Laboratory J.C. Heuson, Université Libre de Bruxelles, Institut Jules Bordet, Brussels, Belgium; 2Divisions of Molecular Biology and Medical Oncology, Netherlands Cancer Institute–Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands

Greater understanding of the biology of triple-negative breast cancer (TNBC) is needed to discern the roughly 60% of node-negative patients who are already cured with locoregional therapy from the 40% who need adjuvant systemic therapy to be cured. Recent evidence suggests that patients with TNBC whose tumours have an activated immune response gene signature have a more favourable outcome than TNBC patients without this signature. For the group who needs additional systemic therapy, the challenge remains to choose the right systemic drug combination for the right TNBC sub-type. Significant heterogeneity exists within the TNBC class that is exemplified by differing chemotherapeutic sensitivity observed for some sub-types. This heterogeneity establishes the need for identifying differentiating molecular markers within the overall class of TNBC disease, which may help refine therapeutic management. In this review, we discuss some of these promising predictive molecular markers for tailoring therapy.

In addition, several gene expression profiling and functional studies employing genetic screens that help to establish TNBC sub-groups with varying sensitivities to a variety of targeted therapies currently under clinical investigation are conferred. It is anticipated that a greater understanding of the biology of TNBC and its complex heterogeneity will reveal novel targets or identify markers around which clinical trials in molecularly well-defined sub-groups can be designed.

Key words: gene expression, molecular markers, triple-negative breast cancer

introduction

Breast cancer is a heterogeneous disease encompassing a variety of entities, which are morphologically and clinically distinct. Immunohistochemical methods define the triple-negative breast cancer (TNBC) sub-type as staining negative for oestrogen receptor (ER) and progesterone receptor (PR), and not overexpressing human epidermal growth factor receptor 2 (HER2) [1]. Perou et al. [2] described the identification of five intrinsic sub-types of breast cancer determined by their different gene expression profiles. Despite their morphological and clinical heterogeneity, several of these intrinsic sub-types show a TNBC phenotype [3]. In general, patients with TNBC are at high risk of early relapse compared with other breast cancer sub-types [4]. However, chemotherapy treatment gives rise to a more favourable outcome in a subset of patients with TNBC compared with other breast cancer sub-types [5].

The lack of obvious targets is a major challenge in treating patients with TNBC. Thus, there is a need to elucidate targets specific to TNBC on which to base future therapies. In this review, we discuss the biology of TNBC at both the pathological and the molecular level. We also highlight the potential role of gene expression signatures in predicting outcome in this very challenging disease.

natural history of TNBC

TNBC represents around 15%–20% of newly diagnosed breast cancer cases [1]. It is associated with young age at diagnosis (<40 years), advanced disease, and African–American ethnicity [6–9]. Furthermore, TNBC is characterised by an early peak of recurrence between the first and third year after diagnosis followed by a sharp decrease in subsequent years with relapses seldom reported after 8 or 10 years [4]. Unlike other sub-types, TNBC outcome is less clearly related to stage [10]. Metastases tend to be more aggressive than other breast cancer sub-types and more likely to occur in the viscera, particularly in the lungs and brain [11], but less likely to spread to the bones [5].

heterogeneity of TNBC and overlap between TNBC and basal-like breast cancer

Although there is a substantial overlap between TNBC tumours and basal-like breast cancer, these breast cancer sub-types are not synonymous [3, 12]. To gain more insight into the heterogeneity of TNBC, researchers from the Netherlands...
Cancer Institute examined the histopathological features and gene expression profiles of 97 TNBCs [13, 14]. They found that all TNBCs were classified as basal-like tumours at the gene expression level; however, hierarchical cluster analysis revealed five distinct sub-groups of TNBC. Turner et al. [15] also found considerable heterogeneity of TNBC tumours resulting from gene expression profiling. Hence, this study suggests that although TNBC and basal-like tumours are frequently considered to be the same, there is significant heterogeneity within these largely overlapping sub-types. Very recently, Lehmann et al. [16] corroborated this concept by analysing gene expression profiles from 21 breast cancer datasets. They identified six different TNBC sub-types displaying unique gene expression patterns including two basal-like (BL1 and BL2), an immunomodulatory, a mesenchymal (M), a mesenchymal stem-like (MSL), and a luminal androgen receptor (LAR) subtype. BL1 and BL2 showed higher expression of cell cycle and DNA damage response genes whereas M and MSL were enriched with epithelial–mesenchymal transition genes. The LAR sub-type was characterised by higher expression of genes involved in androgen receptor signalling. Interestingly, the investigators identified breast cancer cell lines representative of each of these molecular sub-types. These showed different sensitivities to various targeted therapies currently under clinical investigation, providing an attractive platform for future drug development in TNBC.

From the histological perspective, the majority of TNBCs are unsurprisingly of the invasive duct carcinoma, not otherwise specified (IDC-NOS) histology [17–19]. However, a significant proportion (40%–100%) of other relatively rare histotypes (medullary and metaplastic carcinomas [20–22], adenoid cystic carcinoma, and apocrine carcinoma [23, 24]) also shows considerable overlap with BRCA1-mutated tumours [3]. BRCA1 is an important tumour suppressor gene that plays a vital role in DNA repair [25]. BRCA1 deficiency [as assessed by mutation, methylation, and BRCA1-like comparative genomic hybridisation (CGH) profile analysis] is observed in ~50% of patients with TNBC (Figure 1).

Apart from the basal-like sub-type, other relatively rare molecular entities have been claimed to exhibit the triple-negative phenotype. These include the recently described claudin-low molecular sub-type [17], which lacks expression of many of the claudin genes, such as cell–cell junction proteins. These tumours are highly immune cell infiltrated and show stem-cell and epithelial–mesenchymal transition characteristics [26, 27]. However, its exact nature at the molecular level as well as its clinical relevance requires further validation [28].

It should be also highlighted that molecular markers, such as androgen receptor, EGFR, C-KIT, and interferon/immunoglobulin-related genes, have been identified in TNBC by differential gene expression [29, 30]. In addition, other groups have also observed low expression of Bcl-2 and PTEN loss [31, 32]. However, the results in this area are too premature to draw solid conclusions at present.

Apart from gene expression and pathway deregulations associated with TNBC, copy number alterations have also been observed in a higher proportion than in the other breast cancer sub-types, suggesting gross genomic instability in this group [33–35]. Two specific copy number alterations were described; gene amplification and chromosomal deletion [34, 35]. In one study, amplification of the nuclear factor 1/B was observed and further validated by RT-PCR; however, the exact oncogenic function of this gene is not clear [35]. Hu et al. [34] showed deletion of chromosome 5q13-14 in TNBC, which harbours the RASA1 gene and regulates the RAS oncogene. Moreover, Andre et al. [33] found that TNBC is characterised by high frequency of copy number anomalies of PTEN, EGFR,

| Table 1. Incidence and prognosis of TNBC according to histology |

<table>
<thead>
<tr>
<th>Histology</th>
<th>Molecular hallmarks</th>
<th>Proportion TNBC (%)</th>
<th>Prognosis</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>IDC-NOS</td>
<td>HER1+and/or CK5/6+</td>
<td>12–17</td>
<td>Reference group:</td>
<td>[17–19]</td>
</tr>
<tr>
<td></td>
<td>Squamous epithelium differentiation; mesenchymal elements; EGFR+, CK5/6+, CK14+, p63+</td>
<td>90</td>
<td>Adverse in comparison with IDC-NOS</td>
<td>[20–22, 30, 46]</td>
</tr>
<tr>
<td>Metaplastic carcinoma</td>
<td>Squamous epithelium differentiation; mesenchymal elements; EGFR+, CK5/6+, CK14+, p63+</td>
<td>95</td>
<td>Favourable in comparison with IDC-NOS</td>
<td>[50]</td>
</tr>
<tr>
<td>Medullary carcinoma</td>
<td>Lymphoplasmacytic infiltrate; P53 mutation; BRCA1 mutation</td>
<td>90–100</td>
<td>Favourable in comparison with IDC-NOS</td>
<td>[24, 51]</td>
</tr>
<tr>
<td>Adenoid cystic carcinoma</td>
<td>Low grade; resembles tumours found in salivary glands; c-KIT+; fusion gene MYB-NFIB+; MYB overexpression</td>
<td>40–60</td>
<td>Favourable in comparison with IDC-NOS</td>
<td>[23, 52]</td>
</tr>
</tbody>
</table>

CK, cytokeratin; DFS, disease-free survival; EGFR, epidermal growth factor receptor; HER, human epidermal growth factor receptor; IDC-NOS, invasive duct carcinoma, not otherwise specified; OS, overall survival; TNBC, triple-negative breast cancer.
vascular endothelial growth factor A (VEGFA), the latter being gained in >30% of TNBCs.

**Prognostic and Predictive Values of Gene Expression Signatures in TNBC**

In recent years, gene expression profiles have been used to identify several prognostic and predictive signatures, including MammaPrint®, Oncotype Dx®, MapQuant Dx®, and Theros® [36]. These first-generation gene signatures are mainly informative in determining the risk of relapse in ER-positive/HER2-negative breast cancer. They show similar prognostic performance with proliferation genes being the main denominator [37]. As basal-like breast cancers are highly proliferative, the vast majority of these tumours are assigned as high risk [38].

Several investigators have tried to improve the prognostic performance of gene expression profiles by interrogating the role of breast cancer microenvironment. Teschendorff et al. [39] were the first to report a signature based on seven immune-related genes, prognostic for a subset of ER-negative breast cancer tumours (Table 2). Overexpression of these immune response genes was associated with a better prognosis in this high-risk population irrespective of other clinical prognostic parameters [38]. Building on these findings, Teschendorff et al. [40] used mixture discriminant analysis to develop a novel statistical tool capable of accurately identifying ER-negative breast cancer patients with a good prognosis [average predictive value = 94% (range 85%–100%), average hazard ratio = 0.15 (range 0.07–0.36), P < 0.000001]. The classifier was tested across a total of 469 ER-negative tumours, representing untreated and partially untreated patient cohorts.

Rody et al. [41] demonstrated that up-regulation of the lymphocyte-specific kinase metagene, which includes numerous T-cell-specific genes, was also predictive for a better outcome in ER-negative patients. Additionally, using a previously published 368-gene expression signature associated with medullary breast cancer, Sabatier et al. [42] found that immune-related genes are able to identify a sub-group of ER-negative patients associated with good prognosis. A meta-analysis performed by Desmedt et al. [38] further confirmed the prognostic power of immune response genes in ER-negative/HER2-negative tumours.

On the other hand, several gene-expression studies have shown that immune modules are predictive of pathological complete response to neoadjuvant chemotherapy in ER-negative/HER2-negative breast cancers; particularly anthracycline-based regimens [38, 43, 44]. Hence, these results suggest that immune-related gene expression signatures, unlike the first-generation signatures, have potential in refining prognosis and response to chemotherapy in ER-negative/HER2-negative patients, with concomitant implications for patients diagnosed with TNBC. Another aspect that deserves further attention is whether outcome in vaccination studies of patients with TNBC would be different between those having a tumour with an activated immune response gene signature versus those who have not. Stratification according to this feature in vaccination trials might shed more light on this issue.

**Future Directions**

A better understanding of the tumourigenesis and tumour progression of TNBC, and the causes of phenotypic
heterogeneity, may allow improvement in planning and designing novel, individualised treatments for this disease. An efficient way to move forward in this field is by testing potential hypotheses on tumour material from patients who have participated in randomised controlled trials. This would allow efficient progress in a field that is desperate to identify novel management strategies. A good example for this approach was recently demonstrated by Vollebergh et al. [45], who retrospectively tested a CGH classifier derived from BRCA1-mutated tumours as a predictive tool for patients who had received an adjuvant anthracycline-based regimen with or without one cycle of high-dose, platinum-based chemotherapy in the context of a prospective randomised trial [45]. In this study, it was found that patients with BRCA1-like breast cancers had a better disease-free and overall survivals when treated with the high-dose, platinum-based regimen compared with those treated with conventional chemotherapy. However, no difference was found in patients with non-BRCA1-like tumours.

Another method to find the Achilles heel of a tumour is to perform functional studies, such as genetic screens [46]. Using this approach, Sun et al. [46] identified the PTPN12 tyrosine phosphatase as a tumour suppressor in TNBC, which acts by inhibiting multiple oncogenic pathways including HER2 and EGFR. The tumourigenic and metastatic potential of PTPN12-deficient TNBC cells was found to be severely impaired upon restoration of PTPN12 function or combined inhibition of PTPN12-regulated tyrosine kinases. In the same study, around 60% of TNBCs were found to have negative expression at the protein level compared with only 9% in HER2-amplified tumours, suggesting that PTPN12 could represent a key driver of oncogenesis of TNBC. A second example of this approach was recently reported by Possemato et al. [47], who identified phosphoglycerate dehydrogenase (PHGDH) as a potential cancer target, specifically in ER-negative and TNBC. PHGDH catalyses the first step in the serine biosynthesis pathway. Breast cancer cells with increased expression of PHGDH have increased serine synthesis flux. Suppression of PHGDH overexpression in these cells results in a strong decrease in cell proliferation and a decrease in serine synthesis [47].

In our opinion, there is a continuous need for further molecular subdivision of the TNBC sub-type.

### Table 2. Summary of the prognostic value of immune gene signatures in TNBC

<table>
<thead>
<tr>
<th>Study and breast cancer population</th>
<th>Gene cluster name</th>
<th>Prognostic value</th>
<th>Overexpressed genes</th>
<th>Relevant breast cancer sub-types</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER-negative breast cancer [38]</td>
<td>IR+</td>
<td>Best prognosis</td>
<td>Immune response genes CIQA, IGLC2, LY9, TNFRSF17, SPP1, XCL2, HLA-F</td>
<td>Basal-like HER2-positive</td>
</tr>
<tr>
<td></td>
<td>CC+/IR+</td>
<td></td>
<td>Cell cycle and proliferation pathways and immune response genes</td>
<td>Basal-like Medullary</td>
</tr>
<tr>
<td></td>
<td>ECM+</td>
<td></td>
<td>Extracellular matrix genes</td>
<td>Basal-like BRCA1-mutated</td>
</tr>
<tr>
<td></td>
<td>SR+</td>
<td>Worst prognosis</td>
<td>Steroid response genes</td>
<td>Basal-like Normal breast-like ER-negative</td>
</tr>
<tr>
<td></td>
<td>CC+</td>
<td></td>
<td>Cell cycle and proliferation pathways</td>
<td>Non-TNBC (large majority HER2-positive)</td>
</tr>
<tr>
<td>General breast cancer [37]</td>
<td>Immune responses module</td>
<td>Predicts favourable prognosis of ER-</td>
<td>STAT1</td>
<td>ER-negative HER2-positive</td>
</tr>
<tr>
<td>ER-negative and HER2-positive breast cancer [39]</td>
<td>T-cell metagene</td>
<td>Predicts favourable prognosis of ER-</td>
<td>LCK metagene</td>
<td>ER-negative HER2-positive</td>
</tr>
<tr>
<td></td>
<td></td>
<td>negative tumours and HER2-</td>
<td>LCK and IgG metagenes</td>
<td>ER-negative</td>
</tr>
<tr>
<td>Medullary breast cancer [40]</td>
<td>SVM sub-group 1 (resembling MBC)</td>
<td>Less risk of relapse</td>
<td>Immune response, apoptosis, metastasis-inhibiting factors; low levels of metastasis-</td>
<td>Basal-like</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>promoting factors</td>
<td></td>
</tr>
<tr>
<td></td>
<td>SVM sub-group 2 (not resembling MBC)</td>
<td>Increased risk of relapse</td>
<td>Cell migration systems</td>
<td></td>
</tr>
<tr>
<td>ER-negative Neoadjuvant TOP [41]</td>
<td>Anthracycline-based A score</td>
<td>High negative predictive value</td>
<td>TOP2A amplification, tumour invasion, immune response</td>
<td>HER2-negative</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; HER, human epidermal growth factor receptor; LCK, lymphocyte-specific kinase; MBC, medullary breast cancer; SVM, support vector machine; TNBC, triple-negative breast cancer; TOP, trial of principle; IR, immune response; CC, cell cycle; ECM, extracellular matrix; SR, steroid response.
identification of TNBC-specific molecular markers will allow reinvention of existing drugs and the development of new targeted drugs that can be used against TNBC [48]. Hence, this will enable the clever design of randomised phase III clinical trials in well-defined molecular sub-groups, where fewer patients will be needed, to demonstrate superiority of the targeted approach over the standard treatment [48, 49].

acknowledgements
CC is supported by a TRANSBIG Fellowship. HAA Jr is supported by an ESMO Translational Research Fellowship. PCS is supported by a grant from A Sister’s Hope. SCL is supported by a grant from the Dutch Cancer Society (NKI 2006–3706). Editorial assistance was provided by ArticulateScience Ltd. funded by Sanofi. The authors retain full control of content.

disclosures
CS is co-inventor of the Gene Expression Grade Index and the Immune, Stroma, Topo II Signatures. SCL is a named inventor on a provisional patent application for the BRCA-like CGH classifier.

references