Triple negative breast cancer: risk factors to potential targets

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Abstract

Triple negative breast cancer (TNBC) has recently been recognized as an important subgroup of breast cancer with a distinct outcome and therapeutic approach when compared to other subgroups of breast cancer. TNBC is primarily, but not exclusively, comprised of a molecularly distinct subtype of breast cancer, the basal-like subtype. We do not yet have an assay to identify basal-like breast cancer in clinical samples, so TNBC has become a commonly used proxy for this subtype. The molecular biology and pathophysiology of TNBC is incompletely understood but it is improving rapidly with the advent of sophisticated molecular biology platforms. Moreover, the established risk factors for breast cancer as a whole may not apply to this unique subgroup of patients. Finally, since TNBC is defined by the absence of a target, there are currently limitations to employing a tailored therapeutic approach, leaving conventional cytotoxic therapies as the mainstay. Active preclinical and clinical research programs focus on defining the clinical behavior, delineating the risk factors, and more completely understanding the molecular biology of TNBC in order to improve prevention, optimize conventional agents, and unveil novel therapeutic targets. This CCR focus article will review the current state of the art on TNBC.

Introduction

Triple-negative breast cancer (TNBC; ER, PR, and HER2-negative breast cancer) remains a major challenge to physicians and patients, and a source of great interest to laboratory investigators. Although TNBC accounts for a relatively small minority of breast cancer cases, it is responsible for a disproportionate number of breast cancer
deaths. Moreover, there have been fewer advances in the treatment of TNBC than has been seen with other subtypes. For these reasons, new research initiatives for TNBC are critical. The investigation of TNBC is one facet of an emerging effort that regards breast cancer as a collection of separate diseases rather than a single heterogeneous entity, an important step towards the individualization of therapy. Here we attempt to 1). Define TNBC and compare and contrast it with basal-like disease (a term frequently used interchangeably with triple negative disease); 2). Outline established and proposed risk factors; 3). Review the molecular, pathologic, and clinical features of triple negative disease; 4). Provide an overview of ongoing therapeutic trials and; 5). Suggest possible avenues for future research.

**Triple negative or basal-like breast cancer: which is it?**

TNBC has become a commonly used descriptor for malignancies that are estrogen receptor (ER), progesterone receptor (PR), and HER2 negative. The recent focus on this subgroup of tumors has arisen for two major reasons. First, unlike tumors that are ER and/or HER2 positive, triple negative tumors lack an established therapeutic target. As a result, conventional chemotherapy is the only effective systemic treatment for these patients and there is an urgent need for new treatment approaches. Second, recent developments in gene expression arrays have categorized breast cancer into distinct subgroups. One of these subgroups as defined by genetic clustering is the basal-like group of tumors (Figure 1). Among the features of this basal-like subgroup defined by gene expression pattern is low expression of hormone receptor- and HER2-related genes, so most of these are TNBCs. Because gene array profiling is not clinically available, and
immunohistochemical surrogate profiles for the basal-like profile have not been standardized or validated, clinicians do not have either direct or indirect access to the molecular subtype. For this reason, breast cancers in the clinical setting are more typically categorized by routine immunohistochemistry as TNBC as a proxy for the basal-like subtype. It is however crucial to note that while most basal-like cancers are TNBC, there is moderate discordance between TNBC and BLBC. In addition to variability in expression of known basal markers, there is also heterogeneity within TNBC for other potentially relevant features including p53 mutation, BRCA1 mutation or expression, expression of αB-crystallin and degree of expression of immune response genes (Figure 2). Because of this discordance and potential for misclassification, in this review we will refer to basal-like breast cancer (BLBC) when gene expression array or more sophisticated immunophenotypes were used for identification and TNBC when the analysis was limited to clinical assays.

**Basal-like breast cancer**

Gene expression arrays have reproducibly identified breast cancer molecular subtypes with clinical and prognostic implications. These studies confirmed the importance of hormone receptors and HER2 as central to the biologic variance among breast cancers, and have provided additional data regarding the biology of these subtypes. One of the subtypes is the basal-like. The term “basal-like” breast cancer comes from its expression pattern resemblance to that of the myoepithelial cell of the breast, however this definition does not necessarily imply that basal-like tumors arise from these cells. From a historical perspective, this subgroup had been identified long ago by studies examining phenotype...
and cytokeratin profiles. Using gene expression profiling or immunohistochemical surrogates, this subgroup of tumors are notable not only for low expression of hormone receptor- and HER2-related genes, but also for high expression of proliferative genes, including Ki-67, and for expression of a characteristic “basal-like” gene cluster, which includes high expression of basal cytokeratins (CK) such as CK5 and 17, caveolin-1, the epidermal growth factor receptor (EGFR), α-crystallin, and c-KIT (Figure 3). Other notable characteristics of this subtype include frequent p53 mutations, gene copy number aberrations and evidence of genomic instability, and Rb pathway inactivation (Figure 4).

There is an intriguing association of BLBC with germline BRCA1 mutations, one of the most important forms of hereditary breast cancer. At least three-quarters of BRCA1-related breast cancers are basal-like by microarray or by immunohistochemistry. Genomic instability appears characteristic of both BRCA1-related breast cancer and BLBC, which may reflect aberrant DNA repair pathways that are common to both subtypes of cancer. Indeed, they are, in general, remarkably similar whether the perspective is DNA, RNA or protein. For this reason, several investigators are exploring the role of the BRCA1 pathway in sporadic basal-like cancers. What has become clear is that the relationship is not simple. For example, a classical two-hit theory of carcinogenesis does not apply to BRCA1 and BLBC. Although BRCA1 is inactivated by one mechanism or another in at least some BLBC, somatic BRCA1 mutations are very rare in breast cancer. Methylation of the promoter of BRCA1 has been found (mainly in medullary and metaplastic breast cancers), but there are conflicting data, especially if the
studies are limited to BLBC that do not fall into these two categories. Moreover, decreased expression of BRCA1 in BLBC was seen in one study\textsuperscript{7} but not in another.\textsuperscript{29} In the latter study, one common factor seen in both BLBC and BRCA1-related breast cancer was a defect in the maintenance of normal chromosome X-inactivation. Thus a complex picture emerges: it seems likely that there is considerable heterogeneity within BLBC, as has been suggested by microarray studies\textsuperscript{30} (Figure 2). BRCA1-related breast cancers are also not homogeneous: mice conditionally mutant for \textit{Brca1} developed at least three different molecular and histological sub-types of breast cancer.\textsuperscript{31} This is mirrored in humans as not all breast cancers arising in BRCA1 mutation carriers are TNBC or BLBC. Interestingly, the proportion of ER-negative tumors in women with BRCA1 mutations decreases with age. Approximately 20\% of those younger than 45 years of age at diagnosis have ER-positive disease compared with \textasciitilde40\% for those 55-65 years of age.\textsuperscript{32}

The clinical outcomes for women with sporadic, BLBC compared with those with BRCA1-related cancers are broadly similar, and notable for early (within 5 years) relapse. In addition to the timing of relapse, the pattern of metastatic spread is also similar for BRCA1-related\textsuperscript{33} and BLBC.\textsuperscript{34} The characteristics of hereditary BRCA1-associated breast cancer found in sporadic cancers has been termed “BRCA-ness”, with potential clinical implications as described further below.\textsuperscript{22}

**Risk factors for basal-like breast cancer**

BRCA1 mutation confers an exceptionally high risk of developing BLBC, however there may be many other lower penetrance genes that raise this risk either alone or in concert
with other genes or with environmental exposures. Identifying these genes will require large scale genetic association studies such as genome wide association studies (GWAS), which are in development. Although not yet specifically designed to study by subtype, a recent GWAS study uncovered several powerful susceptibility loci for unselected breast cancer. These included five novel susceptibility loci that had never previously been reported in association studies. These included SNPs in: FGR2, chromosome 8q, CASP8, TNRC9, MAP3K1, and LSP1. Very weak associations confined to ER+ breast cancers were also identified with SNPs from 16q12 and 2q35. To date, the majority of these associations appear to be more significantly correlated with ER+ tumors. use of these powerful genetic platforms applied to more selected populations, will help to understand the biology of BLBC and hopefully provide insight to preventive approaches.

Several studies suggest that breast cancer subtypes vary by race and age; premenopausal women and African-American women are far more likely to develop basal-like, and far less likely to develop luminal A breast cancers than their postmenopausal and white counterparts. For example, in African-American women, there is a near-doubling of the percentage of BLBC (20-27%) when compared to Caucasian women (10-14%). While none of the older epidemiologic studies were designed to identify risk factors by molecular subtype, recent post hoc re-analyses also raise interesting questions about traditional risk factors and whether some risk factors are stronger for one subtype versus another or even have opposite effects in different subtypes. For example, in contradistinction to luminal breast cancer, higher parity and young age at first birth may be risk factors for BLBC, while lack of breast feeding and early age of menarche may be
stronger risk factors than for luminal breast cancers.\textsuperscript{41, 43, 44} Given our understanding of breast cancer as a heterogeneous disease, further research will need to focus upon examination of individual risk factors within subtypes.

**Clinical characteristic and outcomes of basal-like breast cancer**

There are several consistent clinical characteristics of this subtype. The basal-like group accounts for approximately 15% of all invasive breast cancers. Pathologically, most BLBCs possess high histologic and nuclear grade, poor tubule formation, high mitotic and proliferative indices, and can have a pushing border. Most are infiltrating ductal, although some unusual histologies such as metaplastic breast cancer share features of BLBC.\textsuperscript{45} Despite the relatively poor prognosis noted in historical datasets, this subtype presents at generally similar stage to other subtypes\textsuperscript{2}, however the highly proliferative nature of this tumor may explain the association with interval (becoming clinically detectable between mammograms) cancer.\textsuperscript{46}

Multiple datasets have consistently identified a poorer clinical outcome for women with BLBC \textsuperscript{47}, although modern chemotherapy may alter this history. Among triple negative tumors, the risk of recurrence is higher in the first 3-5 years after diagnosis than in women with ER+ tumors, with relatively few systemic recurrences after the first 5 years, suggesting that a substantial number of women are cured if they remain recurrence free for the first several years after a diagnosis.\textsuperscript{48, 49}
Since TNBC are a group of tumors without targeted therapeutics, clinicians are left to rely on relatively non-specific cytotoxic agents. Nevertheless, cytotoxic therapy can be quite effective in patients with TNBC. A retrospective evaluation of CALGB 9344 found that patients with either triple negative or HER2 positive breast cancer derived the greatest benefit from the addition of paclitaxel (T) to doxorubicin and cyclophosphamide (AC).\textsuperscript{50} Similarly, dose-dense therapy appears to have the greatest incremental benefit in women with ER negative tumors.\textsuperscript{51} Preoperative studies also suggest sensitivity to modern anthracycline/taxane-based regimens as measured by a high pathologic complete response (pCR) in BLBC or TNBC.\textsuperscript{52} While this appeared paradoxical given the poor prognosis associated with BLBC/TNBC, studies suggest that pCR carries a good outcome regardless of subtype. However, patients with BLBC or TNBC who do not achieve a pCR were more likely to relapse early than luminal or hormone receptor-positive breast cancers.\textsuperscript{52} In summary, women with primary basal-like tumors seem to have a high likelihood of response to chemotherapy, but if the disease is not chemosensitive, they have a worse outlook given the absence of known targetable molecules and the reliance on chemotherapy alone. Improved identification of those for whom modern adjuvant therapy is inadequate may rely upon approaches such as gene expression analysis to identify a panel predictive of pCR to T$\rightarrow$FAC (5-fluorouracil, doxorubicin, cyclophosphamide)\textsuperscript{52} or a panel that identifies regimen-specific signatures such as those developed for FEC (5-fluorouracil, epirubicin, cyclophosphamide) versus docetaxel followed by the combination of docetaxel with epirubicin.\textsuperscript{53}
Given the importance of chemotherapy in this disease, investigators have focused on optimizing drug selection of existing chemotherapeutic agents. From a biological standpoint, DNA damaging agents (such as platinating agents) are of a high priority based upon the BRCA1 pathway and DNA repair dysfunction in this subtype as described above, which may confer enhanced sensitivity to DNA-damaging agents. There are very few prospective studies that have specifically focused on patients with triple negative disease. Only small studies exist, but are supportive of this approach, including a neoadjuvant trial of single agent cisplatin in 28 women with triple negative disease demonstrating 22% pCR to the single agent, and another neoadjuvant trial of women with BRCA1 mutations and TNBC, in which 9 of 10 had pCR to single agent cisplatin. In two platinum-based regimens in the pretreated metastatic setting, response rates of 17% to carboplatin plus cetuximab and 30% to carboplatin plus irinotecan were seen. These results further support research into the utility of platinating agents in TNBC, however clearly there is a need for additional studies before cisplatin or any similar agent can be considered standard for front-line therapy. The additional benefit of carboplatin added to paclitaxel in triple negative disease will be directly studied in the neoadjuvant trial CALGB 40603. There is little question, however, that better therapies are needed. Given the lack of targeted therapy for TNBC, strategies that maximize the benefits associated with standard cytotoxic therapy could lead to further reductions in breast cancer recurrence and mortality in women with triple negative disease.

The promise of a targeted approach in this subtype of breast cancer is real, particularly with anti-angiogenic strategies. ECOG 2100, a phase III North American Breast Cancer
Intergroup trial, randomized over 700 women who had never received chemotherapy in the metastatic setting to paclitaxel with or without bevacizumab. There was a significant improvement in the primary endpoint of progression free survival with the addition of bevacizumab overall, including the subgroup of patients with largely triple negative disease. In this context, it is notable that BRCA1-related breast cancers are associated with the presence of glomeruloid microvascular proliferation, a marker of increased neo-angiogenesis in cancer. Another recently reported study of the multi-kinase vascular endothelial growth factor receptor (VEGFR) inhibitor, sunitinib, suggested a response rate of approximately 15% in the pretreated triple negative subset of patients. This approach will be tested directly in the neoadjuvant setting in CALGB 40603, which includes a second randomization to receive or not receive preoperative bevacizumab in addition to the assigned chemotherapy.

Based upon EGFR expression in gene profiling studies and EGFR dependence for growth and proliferation in BLBC cell lines, several groups have examined EGFR targeting in TNBC. Two studies completed to date shed light on this approach. TBCRC 001 was a randomized phase II trial evaluating the role of EGFR inhibition for triple negative metastatic breast cancer. In this study, eligible women received the anti-EGFR monoclonal antibody cetuximab combined with carboplatin, or cetuximab alone with a planned crossover to carboplatin at progression. Not surprisingly, cetuximab alone demonstrated a low response rate and was closed early by design, however response to the combination of cetuximab plus carboplatin was 17% with clinical benefit seen in 29% of a pretreated population. A similar study examining irinotecan plus carboplatin with
or without cetuximab suggested a modestly higher response rate (from 30% to 49%) with the combination in TNBCs on subset analysis.  

**The current plan of attack**

The optimal therapeutic approach for TNBC will likely include a mixture of targeting the host via an intimate understanding of pharmacogenetics and targeting the molecular biology of the tumor. Based on attempts to pair the molecular biology of TNBC with drug mechanism, multiple compounds are in testing for TNBC. These include taxanes, platinating agents, anti-angiogenic agents, EGFR inhibitors, Poly (ADP-ribose) polymerases (PARP) inhibitors, and Src-Abl inhibitors, among others. A number of trials have begun that are targeting this subgroup. As mentioned, CALGB 40603 is a neoadjuvant trial specifically designed for patients with triple negative disease and will address two very timely questions. It will attempt to discern the true value of adding either or both a platinating agent and an anti-angiogenic therapy to traditional chemotherapy in a 2 X 2 randomization schema. Another focus continues to be the role of EGFR in basal-like breast cancer. In TBCRC 001, in which women with TNBC received carboplatin plus cetuximab as described above, gene expression studies were performed on serial biopsies of the target lesions in 16 women. These studies suggest that EGFR targeting alone may be effective in some, but is insufficient in the majority of TNBC, in whom other agents may be needed for pathway inhibition. The VEGFR-2 small molecule tyrosine kinase inhibitor, sunitinib, is being studied in the advanced setting where patients are randomized to sunitinib versus standard of care. Other novel agents of interest include the multitargeted Src-Abl inhibitor dasatinib. A cell line-derived predictive model of
sensitivity to dasatinib applied to expression profiles from human tumors overlapped significantly with the TNBC tumors\textsuperscript{63}, leading to a recently completed but not yet reported Phase II trial in this subtype. PARP are molecules integrally involved in non-homologous DNA repair, which becomes the primary means of double strand DNA repair when the preferred homologous recombination mechanism is lost, as occurs when the BRCA1 pathway is defective. BRCA1 loss or inactivation thus sensitizes cells to PARP inhibitors.\textsuperscript{64} For the reasons described above, both hereditary and at least a subset of sporadic BLBC are thought to have dysfunctional BRCA1 pathways, resulting in several phase II studies of PARP inhibitors alone and in combination with DNA-damaging agents in both BRCA1 carriers and TNBC. In addition to the drug selection based on an intimate understanding of the molecular biology of TNBC (as described above), development of companion predictive markers will ultimately optimize the therapeutic success for this subgroup.\textsuperscript{65}

**SUMMARY**

While the risk factors for ER+ disease are well defined, those for triple negative disease are less well defined. A more comprehensive understanding of gene expression and genetic variability is central to understand both the etiology and pathogenesis of this disease. A validated risk model (similar to the modified Gail model) for triple negative disease would be clinically useful. Such a model might help facilitate the development of chemo-preventive agents or lifestyle modifications. With regard to our current approach to therapy, TNBC should be treated with conventional therapies in the curative setting at this time. Incorporation of platinating agents and other novel therapeutics, while
promising in the *in vitro* and advanced setting, should await further data from clinical trials. Retrospective correlative strategies focusing on the TNBC subgroup may help identify which subgroups will benefit most from standard drugs. Prospective trials are now underway, designed to study a given regimen by subgroup of disease (usually ER+ vs. HER2+ vs. TNBC). Simultaneously, novel agents are being evaluated in the advanced setting and added to more traditional regimens in the curative setting.

In the absence of agreed standards for staining and scoring of basal markers using immunohistochemistry, the coming generation of trials for patients with BLBC/TNBC should continue to classify the disease on the basis of ER/PR and HER status. At the present, these tests are commonly employed, represent relatively inexpensive biomarkers and will allow for rapid conversion of results into clinical application. We recognize, however, current and future gene array platforms have the power to yield a more thorough prediction of the molecular biology (which has the capacity to identify novel targets) and a greater sensitivity for distinguishing various subtypes of disease. At the current time, we believe that trials should focus on recruitment of TNBC but recruit a sufficiently large population to have statistical power to retrospectively analyze for a molecularly defined subgroup as well.

*On December 11, 2007, the Triple Negative Breast Cancer Foundation and Susan G. Komen for the Cure convened a meeting of clinicians, investigators, and advocates to review the state of current clinical and translational research on TNBC and make recommendations regarding opportunities for research in this breast cancer subtype. This is a report derived from that meeting. We would like to acknowledge and sincerely thank the Triple Negative Breast Cancer Foundation and the Susan G. Komen for the Cure for their support of this symposium.*
Members attending this symposium include: Anita Aggarwal, Sunil Badve, Jose Baselga, Lisa Carey, Jenny Chang, Ian Ellis, William Foulkes, Judy Garber,Montserrat Garcia-Closas, Lyndsay Harris, Gabriel Hortobagyi, Cliff Hudis, Ian Krop, Mary Jo Lund, Funmi Olopade, Charles Perou, Lajos Pusztai, Andrea Richardson, Hope Rugo, Bryan Schneider, Bill Sikov, Dan Silver, George Sledge, Sandra Swain, Andrew Tutt, Eric Winer, and Antonio Wolff. We also thank Lars Akslen and Jarle Arnes for contributing the photos for Figure 3 and Katherine Hoadley for composing Figure 4.

**Figure Legends:**

**Figure 1:** Immunohistochemical surrogate assay for intrinsic subtypes of breast cancer.

A) Hierarchical cluster dendrogram taken from Sorlie et al\textsuperscript{13} and color coded according to subtype. The gene expression data for the 4 genes/proteins used for IHC classification are shown. B) Tissue microarray results are shown for the representative IHC profiles used in Nielsen et al\textsuperscript{4} to identify the intrinsic subtypes.

**Figure 2:** Cartoon showing the relationship and overlap between breast cancer subgroups that share characteristics.

Triple negative (TN) breast cancers are shown in pink. It can be seen that breast cancers expressing basal markers, such as CK5 and EGFR, (shown in dark tan) overlap considerably with TN breast cancers, but the overlap is not complete, and not all TN tumors are positive for these markers. These “non-basal” TN tumors may be the result of technical failures in ER and HER2 testing (falsely “triple-negative”) or may be ultimately demonstrated to have basal features as additional markers of the basal phenotype are discovered and accepted. A subset of TN tumors have an expression profile indicating a strong immune response, presumably to breast cancer cell-surface antigens (shown in light tan), a feature that may have prognostic implications within the TN cancers. There are additional molecular features that characterize some but not all TN tumors including but not limited to p53 mutation (shown in light blue), BRCA1 germline mutation (shown in dark blue), and alpha B crystallin expression (shown in light green). Which of these various subsets of TN cancer will have clinical differences or treatment implications is yet to be determined.

**Figure 3:** Three "basal" breast cancer markers.

Examples of three basal markers that are characteristic of many triple negative breast cancers. Note the lymphocytic infiltration, indicative the presence of tumor-infiltrating lymphocytes in Figure 3c (Images courtesy of Drs. Lars Akslen and Jarle Arnes, The Gade Institute, Section for Pathology, Haukeland University Hospital, University of Bergen, Bergen, Norway). Of note, the absence of these 3, or any other specified markers, does not by definition make the tumor "non-basal".

A) EGFR: Strong membrane expression of EGFR in a poorly differentiated ductal carcinoma (Nottingham grade III) (x 400).

B) P-cadherin: Strong membrane and cytoplasmic expression of P-cadherin in an invasive ductal carcinoma (Nottingham grade III) (x 400).
C) CK5/6: Strong expression of Cytokeratin 5/6 in a poorly differentiated breast carcinoma with lymphocytic infiltration (Nottingham grade III) (x 400).

**Figure 4. Pathway analysis of the intrinsic subtypes.**
TP53, Retinoblastoma, and Receptor Tyrosine Kinase signaling pathways are shown for many of the major genes within each pathway. Each gene is color coded according to the average expression of that gene with each subtype.61 Pathway analyses are shown for (A) Luminal A, (B) Luminal B, (C) HER2-enriched, and (D) Basal-like tumors. An average value for the "proliferation signature" for each subtype is also shown within the RTK pathway box. TP53 mutation status is also shown for each subtype.2 These analyses highlight the Triple Negative phenotype, the high expression of EGFR and c-KIT in basal-like tumors, and show their high TP53 mutation rates.
Figure 1.

A

B

Figure 2.
Figure 3.
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