Triple negative and basal-like breast cancer: one or many diseases?
Implications for surgical pathologists

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Take home messages
- The majority of basal-like breast cancers have a triple negative phenotype and the vast majority of triple negative cancers display a basal-like transcriptome, however the two are not synonymous.
- Basal-like and triple negative breast cancers comprise a heterogeneous group of diseases, which are characterised by a constellation of morphological features.
- Not all basal-like and triple negative breast cancers have a poor outcome.
- The precursors of invasive breast carcinomas of basal-like and triple negative phenotype include ductal carcinoma in situ harbouring a similar phenotype and, possibly, a subgroup of microglandular adenosis.
- A subgroup of basal-like and triple negative breast cancers displays an exquisite sensitivity to anthracycline-based neoadjuvant chemotherapy.
- Defects in the p53, pRB and p16 pathways are found in a significant proportion of basal-like and triple negative cancers.
- BRCA1 pathway dysfunction is found in a substantial proportion of basal-like and triple negative breast cancers and can be exploited therapeutically (e.g. inhibitors of the PARP enzyme and cross-linking agents)

Introduction
Breast cancer is a heterogeneous disease, encompassing a plethora of entities which not only have distinct morphological features but also clinical behaviour. In recent years, it has become apparent that this diversity may be underpinned by distinct patterns of genetic, epigenetic and transcriptomic aberrations 1-4. In fact, the marriage of pathology and genetics has led to the establishment of clear examples of genotypic-phenotypic correlations. For instance, secretory carcinomas of the breast consistently harbour a t(12;15)(p13;q25) translocation, leading to the formation of a fusion transcript ETV6-NTRK31,5-8. Lobular carcinomas have been shown to be characterised by inactivation of the CDH1 gene, which encodes E-cadherin, a transmembrane adhesion molecule that mediates homophilic-homotypic adhesions. A recent conditional mouse model has provided strong circumstantial
evidence to suggest that CDH1 gene inactivation is not only involved in the characteristic discohesiveness of lobular lesions, but may be also involved in the peculiar metastatic pattern of invasive lobular carcinomas.\(^9\)

Although morphology is often associated with the pattern of molecular aberrations in breast cancers, it is also clear that tumours of the same histological type display remarkably different clinical behaviour. This is most evident in invasive ductal carcinomas of no special type (IDC-NST), where even tumours of the same histological grade may have distinct outcome and remarkably different responses to systemic therapy.\(^2,3\) With the boom of high throughput technologies and the apocalyptic promise of microarray analysis, several groups endeavoured in devising a new taxonomy solely based on the molecular features of breast cancers. The gene expression microarray-based class discovery studies pioneered by the Stanford group have led to the identification of at least five subgroups of breast cancer: luminal A, luminal B, normal breast-like, HER2 and basal-like breast cancer.\(^10-14\) This taxonomy was devised based on the analysis of IDC-NSTs and a limited number of lobular carcinomas and has proven to be of prognostic significance. Although based on the analysis of a rather limited number of samples and with varying definitions in each study, this classification has captured the attention of oncologists, pathologists and scientists alike, with some authorities in the field claiming that the gold standard for the classification of breast cancers is microarray-based gene expression profiling.

It should be noted, however, that this taxonomy identified subgroups of breast cancer that were to some extent already known. In fact, the most robust distinction observed by microarray analysis is between the transcriptome of oestrogen receptor (ER)-positive and ER-negative breast cancers. Luminal tumours are described as those that show expression patterns reminiscent of normal luminal epithelial cells of the mammary gland, including consistent expression of low molecular weight cytokeratins 8/18, ER and genes associated with an active ER pathway.\(^11,12,15,16\) At least two subgroups of luminal tumours have been identified: luminal A, which are usually of low histological grade, have an excellent prognosis and show high levels of expression of ER-activated genes; and luminal B, which are more often of higher histological grade, have higher proliferation rates and a significantly worse prognosis than luminal A tumours.\(^11,12,14-16\) Normal breast-like cancers are rather poorly characterised; one of the defining features of these tumours is that they consistently cluster together with samples of fibroadenomas and normal breast. The clinical significance of normal breast-like tumours is yet to be determined and some have suggested that this subgroup may be a mere artefact of expression profiling (i.e. disproportionately high content of stromal cells). HER2 tumours are usually ER-negative and characterised by
overexpression of the human epidermal growth factor receptor type 2 (HER2) and genes associated with HER2 pathway and/or HER2 amplicon on 17q12. HER2 cancers have a very aggressive clinical behaviour, however they are amenable to novel tailored therapies using either humanised monoclonal antibodies against HER2 or HER2 tyrosine kinase inhibitors. Although the vast majority (>80%) of HER2 cancers as defined by microarrays harbour HER2 gene amplification or HER2 3+ immunohistochemical expression, not all tumours that are HER2 amplified fall into the HER2 cluster by expression arrays analysis. There is also evidence to suggest that some HER2 amplified, ER-positive cancers fall within the luminal B subtype rather than the HER2-microarray subtype. Basal-like cancers, another group of ER-negative cancers, are so named because the neoplastic cells of this tumour type consistently express genes usually found in normal basal/myoepithelial cells of the breast, including cytokeratins 5 and 17. It should be emphasised that basal-like breast cancers, unlike ‘basal’/myoepithelial cells of normal breast, almost uniformly express cytokeratins 8 and/or 18, calling into question the initial histogenetic implications of this microarray-based taxonomy of breast cancers.

What is a basal-like breast cancer?
The characteristics of basal-like breast cancer have been extensively reviewed in the last 18 months. It should be noted that there is still no internationally accepted definition for basal-like breast cancers. Some groups have employed microarray-based expression profiling to define basal-like breast cancers, whereas others have used immunohistochemical surrogates. In fact, there are several surrogate markers for basal-like breast cancers already described, the most used of which are i) the panel based on the mRNA expression profiling of basal-like breast cancers defined by Nielsen et al. and Cheang et al., ii) lack of ER, progesterone receptor (PR) and HER2 (triple negative phenotype), or iii) expression of high-molecular weight cytokeratins.

Despite using distinct definitions for basal-like breast cancers, we and others have demonstrated that basal-like tumours have distinctive clinical presentations, histological features, response to chemotherapy, and outcome. In brief, basal-like tumours comprise a heterogeneous group of cancers that account for up to 15% of all breast cancers. These tumours affect younger patients, are more prevalent in African-American women and more often present as interval cancers. Histologically, as a group, the vast majority of basal-like breast cancers are IDC-NSTs of high histological grade and characterised by high mitotic indices, the presence of central necrotic zones, pushing borders, conspicuous lymphocytic infiltrate and typical/atypical medullary features. However, the vast majority of medullary and atypical medullary, metaplastic cancer...
secretory, myoepithelial and adenoid cystic carcinomas of the breast also display a basal-like phenotype. More recently, a subgroup of lobular carcinomas has been shown to express high molecular weight cytokeratins, however it remains to be determined whether these cases truly display a basal-like transcriptome. At the immunohistochemical level, the majority of basal-like breast cancers lack or display low levels of ER and PR, lack HER2 gene amplification and express genes usually found in ‘basal’ myoepithelial cells of the normal breast including high molecular weight cytokeratins (5/6, 14 and 17), P-cadherin, caveolins 1 and 2, nestin, αB crystallin and epidermal growth factor receptor (EGFR) and, in a minority of cases, harbour EGFR gene amplification or aneusomy. p53 immunohistochemical expression or TP53 gene mutations in up to 85% of cases and alterations of the pRB and p16 G1/S cell cycle checkpoint are remarkably prevalent in these cancers. In fact, a recent study demonstrated that approximately 30% of basal-like breast cancers concurrently display lack of pRB expression, p16 overexpression and p53 immunoreactivity, whereas this profile was rarely seen in tumours of other molecular subgroups. Basal-like display remarkably high proliferation indices as defined by mitotic counting or by MIB1 (Ki67) labelling index.

Basal-like cancers, as defined by microarrays or by immunohistochemical surrogates, have been shown to have a more aggressive clinical behaviour. In fact, some studies have demonstrated that expression of basal keratins is a prognostic factor independent of tumour size, grade and lymph node status. However, when compared to either ER-negative non-basal-like cancers or to grade-matched non-basal-like cancers, carcinomas with a basal-like phenotype do not seem to be associated with a poorer outcome. In addition, the pattern of metastatic spread of tumours with a basal-like phenotype seems to be different from that of non-basal-like cancers: they are reported to less frequently disseminate to axillary nodes and bones and to favour a haematogenous spread, with a peculiar proclivity to develop metastatic deposits in the brain and lungs.

**What is a triple negative breast cancer?**

Triple negative cancers are defined as tumours that lack ER, PR and HER2 expression, accounting for 10-17% of all breast carcinomas, depending on the thresholds used to define ER and PR positivity and the methods for HER2 assessment. It should be noted that different studies have employed different methods and thresholds to define lack of expression of these markers. Furthermore, future studies are likely to produce slightly different results given the change in the definition of HER2 positivity according to the new ASCO/ CAP guidelines. Despite the issues with the definitions of triple negative cancers,
the interest in these tumours stems from the lack of tailored therapies for this group of breast cancer patients and the overlap with the profiles of basal-like cancers.

The main characteristics of triple negative cancers that have emerged from the literature illustrate the similarities between basal-like and triple negative tumours, including the fact that they are more frequently affect younger patients (<50 years) \(^{32,61,63,64}\), are more prevalent in African-American women \(^{64-66}\), often present as interval cancers and are significantly more aggressive than tumours pertaining to other molecular subgroups \(^{32,34,61,63-65}\). This aggressiveness is best translated by the fact that the peak risk of recurrence is between the 1st and 3rd years and the majority of deaths occur in the first 5 years following therapy \(^{61,63}\). On the other hand, the differences in outcome between triple negative cancers and tumour with other phenotypes are reduced at 10 years of follow up. Interestingly, patients with basal-like \(^{42}\) or triple negative cancers \(^{61,65}\) have a significantly shorter survival following the first metastatic event when compared to those with non-basal-like/ non-triple negative controls.

From a pathologist point of view, the differences between triple negative and non-triple negative breast cancers are not surprising, given that the majority of triple negative cancers are of histological grade III \(^{61,62}\) and basal-like phenotype. It should be noted, however, that the group of cancers defined by triple negativity is more heterogeneous than that defined by basal-like phenotype \(^{26,69,70}\). Apart from the more heterogeneous transcriptome, triple negative cancers also show more varied histological features. In fact, up to 10% of triple negative tumours were shown to be of grade I in one study \(^{61}\). Furthermore, apart from medullary, metaplastic, secretory, myoepithelial and adenoid cystic carcinomas \(^{1,4,39,40,52}\), which are preferentially triple negative tumours, several other histological special types of breast cancer may display a triple negative phenotype, including apocrine carcinomas, pleomorphic lobular carcinomas and duct-lobular cancers \(^{13,52,70}\).

There are conflicting results on the prevalence of lymph node metastasis at diagnosis in patients with triple negative cancers: whilst in one study, there was a higher prevalence of lymph node metastasis in triple negative cancers when compared to controls \(^{61}\), others found no difference \(^{32,62}\) or an inverse association between triple negative phenotype and lymph node metastasis \(^{63}\). Interestingly, it has been described that unlike non-triple negative cancers, no correlation between tumour size and presence of lymph node metastasis was observed in the triple negative group \(^{61}\). A similar dissociation between tumour size and prevalence of lymph node metastasis at diagnosis was identified by Foulkes et al. \(^{71}\) in tumours arising in \(BRCA1\) germline mutation carriers.
Are basal-like and triple negative cancers synonymous?

Given that there is no internationally accepted definition for basal-like breast cancers, it is not surprising that there has been a great deal of confusion as to whether triple negative and basal-like breast cancers are synonymous. Although several groups have used these terms interchangeably, it should be noted that not all basal-like cancers lack ER, PR and HER2 and not all triple negative cancers display a basal-like phenotype. The vast majority of triple negative cancers are of basal-like phenotype. Likewise, the vast majority of tumours expressing 'basal' markers are triple negative. It should be noted, however, that there is a significant number of triple negative cancers that do not express basal markers and a small, but still significant, subgroup of basal-like cancers that express either hormone receptors or HER2. Bertucci et al. have addressed this issue directly and confirmed that not all triple negative tumours when analysed by gene expression profiling were classified as basal-like cancers (i.e. only 71% were of basal-like phenotype) and not all basal-like breast carcinomas classified by expression arrays displayed a triple negative phenotype (i.e. 77% were of triple negative phenotype). Taken all together, these results are in accord with the concept that the triple negative phenotype is not an ideal surrogate marker for basal-like breast cancers and call for caution in the interpretation of ongoing therapeutic trials whose selection of patients was made on the basis of lack of ER, PR and HER2 expression. Furthermore, there are several lines of evidence to suggest that the group of triple negative cancers is substantially more heterogeneous than the group encompassed by basal-like breast cancers.

Precursors of basal-like and triple negative cancers: getting beyond the high grade ductal carcinoma in situ (DCIS)

Despite the multiple definitions of basal-like breast cancer, a group of high grade DCIS lacking ER, PR and HER2 and expressing 'basal' markers has been identified. However, it should be noted that its prevalence is lower than that of invasive triple negative and basal-like breast cancer and that triple negative and basal-like cancers often lack an overt in situ component.

It has more recently been established a link between microglandular adenosis and triple negative and basal-like cancers. In fact, the vast majority of invasive cancers developing in the context of microglandular adenosis have been shown to be of triple negative phenotype. Interestingly, invasive carcinomas developing in the context of microglandular adenosis not uncommonly display metaplastic elements or are of adenoid cystic morphology. Shin et al. have recently demonstrated that microglandular adenosis may be a non-obligate precursor of triple negative and basal-like breast cancers.
Microglandular adenosis was shown to harbour the expected pattern of genetic aberrations of basal-like and triple negative cancers. Furthermore, similar patterns of genetic aberrations were found in matched microglandular adenosis, atypical microglandular adenosis and invasive carcinomas and a stepwise progression in the number of gross chromosomal changes from microglandular adenosis to invasive carcinoma was observed. Our group has recently identified examples of basal-like and triple negative cancers arising in the context of microglandular adenosis, including one case in a BRCA1 germline mutation carrier (Geyer F, Jones RL and Reis-Filho JS, unpublished observations).

**Basal-like and tumours arising in BRCA1 germ-line mutation carriers**

There is increasingly more coherent evidence to suggest a link between BRCA1 pathway and basal-like breast cancers. In fact, the vast majority of tumours arising in BRCA1 germline mutation carriers, in particular those diagnosed before 50 years of age, have morphological features similar to those described in basal-like cancers and display a basal-like phenotype as defined by immunohistochemistry or expression arrays.

The immunohistochemical similarities between BRCA1 tumours and basal-like breast carcinomas are deeper than those illustrated by the expression of high molecular weight cytokeratins and other proteins expressed by myoepithelial cells. In fact, both basal-like breast cancers and tumours arising in BRCA1 germline mutation carriers show a peculiar pattern of cell cycle protein expression, however they express significantly lower levels of p27 and higher levels of Skp2, cyclin E and caspase 3 when compared to sporadic breast carcinomas and BRCA2 mutation tumours.

Although, even at the genetic level, sporadic basal-like cancers and tumours arising in BRCA1 mutation carriers show similar molecular genetic profiles, they differ by the lack of BRCA1 somatic mutations in sporadic basal-like cancers. Despite the lack of BRCA1 mutations, it has been recently demonstrated that BRCA1 pathway may be dysfunctional in sporadic basal-like tumours. BRCA1 protein expression levels have been shown to be significantly lower in tumours of high histological grade, lacking ER and PR expression and of basal-like phenotype. We and others have hypothesised that this downregulation would be mediated by epigenetic mechanisms, such as gene promoter methylation and/or transcriptional silencing of BRCA1. In fact, BRCA1 gene promoter is methylated in >60% of medullary and metaplastic breast cancers of basal-like phenotype. However, despite the significantly lower levels of BRCA1 mRNA expression in sporadic basal-like cancers than grade matched controls, both sporadic invasive ductal carcinomas with and without basal-
like phenotype showed a similarly low prevalence of BRCA1 gene promoter methylation $^{35,107}$. We therefore investigated alternative epigenetic mechanisms of BRCA1 pathway inactivation and found that sporadic invasive ductal carcinomas with basal-like phenotype expressed ID4, a negative regulator of BRCA1 $^{108,109}$, at significantly higher levels than grade-matched controls $^{35}$. This mechanism may account for the low levels of BRCA1 expression in sporadic basal-like carcinomas of ductal morphology. Interestingly, a recent study has suggested that BRCA1 plays a critical role in the differentiation of ER-negative stem/progenitor cells to ER-positive luminal cells $^{110}$, however it remains to be determined whether BRCA1 inactivation in luminal epithelial cells cannot lead to de-differentiation or acquisition of a stem-like phenotype.

Based on the fact that the majority of basal-like breast cancers show a dysfunctional BRCA1 pathway $^{35,90,91}$ and harbour TP53 gene mutations $^{12,16,76}$, we have engineered the conditional mouse $^{BLG-Cre;Brca1^{F22-24/F22-24};p53^{+/−}}$, where the $Brca1$ gene is inactivated in β-lactoglobulin-expressing cells (i.e. luminal epithelial cells of the mouse mammary gland) and all cells of the animal have only one wild-type allele of p53 $^{111}$. Consistent with our findings in human tumours, pathological analysis of the tumours arising in the above mice revealed that 78% lacked hormone receptors and HER2 and expressed basal markers (cytokeratins 14 and/or EGFR) and 88% showed homologous metaplastic elements. This mouse model provides another line of evidence for the link between basal-like phenotype and BRCA1 pathway dysfunction and may prove useful for testing novel therapies for basal-like cancers $^{111}$. Interestingly, another conditional mouse model $^{K14cre;Brca1^{F/F};p53^{F/F}}$, where $Brca1$ and $Trp53$ were inactivated in basal cells of the mouse mammary gland, has been shown lead to the development of tumours whose morphological and phenotypic characteristics are remarkably similar to those observed in our study $^{111}$. Taken together, these findings provide circumstantial evidence to suggest that despite the cell of origin, $Brca1$ inactivation may lead to basal-like breast cancers. This is not surprising, given that BRCA1 has been shown to play a pivotal role in the regulation of ER expression and that RNA interference-mediated silencing of BRCA1 in breast cancer cell lines leads to a marked reduction in expression of endogenous ER protein levels $^{112}$.

**Basal-like and triple negative breast cancers are heterogeneous**

Recent studies have clearly demonstrated that, contrary to the initial idea that basal-like breast cancers would constitute a homogeneous group of cancers with aggressive clinical behaviour, basal-like and triple negative cancers are heterogeneous in their histological features, immunohistochemical profiles, outcome and response to therapy.
As discussed above, although the majority of basal-like breast cancers are high-grade tumours characterised by a constellation of morphological features, there are low-grade lesions that display a triple negative and basal-like phenotype. It is rather arguable to classify under the same term high grade IDC-NST of triple negative phenotype with secretory and adenoid cystic carcinomas, given that their genetic features and clinical behaviour are remarkably different. However, these lesions consistently display a triple negative immunophenotype and harbour a basal-like transcriptome.

Detailed analysis of the immunophenotype of basal-like breast cancers demonstrates that, again, these tumours are heterogeneous. Although defects of p53, pRB and p16 are significantly more frequently found in triple negative and basal-like breast cancers, these proteins are concurrently expressed in an abnormal pattern in 30% and 50% of basal-like and non-basal-like triple negative cancers, respectively. BRCA1 pathway appears to be dysfunctional in a significant proportion of triple negative and basal-like breast cancers, however the proportion of cases that lack competent homologous recombination DNA repair due to BRCA1 pathway abnormalities remain to be determined.

Contrary to the widely held belief that basal-like and triple negative breast tumours would be chemotherapy resistant, several studies have demonstrated that a subgroup of these cancers display a remarkable sensitivity to conventional chemotherapy regimens. In fact, 17% to 58% of patients with triple negative breast cancers have been shown to evolve to pathological complete response after anthracycline- or anthracycline+taxane-based neoadjuvant chemotherapy and 17% of triple negative cancers evolved to pathological complete response after neoadjuvant platinum-based chemotherapy. However, when followed up, despite the higher prevalence of pathological complete response, patients with basal-like and triple negative cancers have been shown to have a worse outcome than those with non-triple negative or non-basal-like tumours. This apparent paradox has been recently resolved. There are several lines of evidence to suggest that patients with triple negative or basal-like cancers that evolve to pathological complete response after neoadjuvant chemotherapy have an excellent prognosis, whereas those who fail to achieve pathological complete response have a dismal outcome. These results provide another level of evidence of the heterogeneity of triple negative and basal-like breast cancers and suggest that a subgroup of these cancers is sensitive to genotoxic agents. It should be noted, however, that markers for the identification of patients with triple negative and basal-like cancers that benefit most from chemotherapy remain to be defined. Furthermore, several groups have recently identified a subgroup of good prognosis ER-
negative cancers, encompassing a subgroup of triple negative and basal-like tumours, that is characterised by the expression of an immune response module. This transcriptomic profile may prove helpful for the identification of patients with triple negative and basal-like cancers that have a better outcome and, once more, illustrates the heterogeneity of this group of tumours.

Finally, a subgroup of triple negative and basal-like breast carcinomas have been shown to have a dysfunctional BRCA1 pathway and this subgroup may be amenable to specific therapeutic strategies. Given that tumours that have a dysfunctional BRCA1 pathway lack competent homologous recombination DNA repair, our group and others have hypothesised that these cancers would be exquisitely sensitive to cross-linking agents and inhibitors of the PARP enzyme. Reassuringly, in vitro studies and animal models have demonstrated that tumours with BRCA1 or BRCA2 loss of function are indeed sensitive to these agents. Consistent with this hypothesis, results of PARP inhibitor phase I clinical trials that included patients with BRCA deficient tumours have been encouraging and sustained responses in patients with BRCA1/2 deficient breast or ovarian metastatic cancers have been observed. Given these exciting results, several clinical trials testing cross-linking agents (e.g. carboplatin and cisplatin) and PARP inhibitors in patients with BRCA1 germline mutations and sporadic basal-like breast cancers are currently ongoing (for a list of clinical trials, please see ). If positive, these studies may render the identification of tumours lacking competent homologous recombination compulsory in our diagnostic practice.

Conclusions
Basal-like breast cancer is a heterogeneous group of tumours that is more prevalent in young, African-American patients. The majority of these cancers are of triple negative phenotype and have a poor outcome. Despite their sensitivity to chemotherapy agents, basal-like and triple negative cancers have a relatively poor outcome. Currently, triple negative cancers are routinely identified in clinical practice, whereas there is no internationally accepted definition for basal-like cancers, there is still no clear clinical indication for the routine identification of these tumours, and microarray-based gene expression profiling is far from becoming the 'gold standard' for breast cancer classification in clinical practice.

Given that basal-like breast cancers are still heterogeneous, regardless of the definition employed, it is possible that in the next few years markers that identify subgroups of basal-like or triple negative cancers that respond to specific agents, rather than the identification of
basal-like cancers per se, will become part of our diagnostic armamentarium. Perhaps then, the contribution of microarray-based gene expression profiling will come full circle and deliver on the promise of individualised therapies for every cancer patient. With the advent of massively parallel sequencing \(^{130}\), which allows for the genome-wide quantitative and qualitative genomic and transcriptomic characterisation of cancers, and the imminent death of microarrays \(^{131,132}\), it is likely that the taxonomy of breast cancers will be revisited again \(^{1,24}\). This time, it is possible that more homogeneous molecular subgroups, their biological drivers and therapeutic targets will be identified. Until then, we, pathologists, have to strive for providing optimal assessment of the histological features, and ER, PR and HER2 status of breast cancers.

References


