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REVIEW

Triple-Negative Breast Cancer and the Need for New Therapeutic Targets

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Triple-negative breast cancers (TNBCs) are a diverse and heterogeneous group of tumors that by definition lack estrogen and progesterone receptors and amplification of the *HER2* gene. The majority of the tumors classified as TNBCs are highly malignant, and only a subgroup responds to conventional chemotherapy with a favorable prognosis. Results from decades of research have identified important molecular characteristics that can subdivide this group of breast cancers further. High-throughput molecular analyses including sequencing, pathway analyses, and integrated analyses of alterations at the genomic and transcriptomic levels have improved our understanding of the molecular alterations involved in tumor development and progression. How this knowledge should be used for rational selection of therapy is a challenging task and the subject of numerous ongoing research programs. This review summarizes the current knowledge on the clinical characteristics and molecular alterations of TNBCs. Currently used conventional therapeutic strategies and targeted therapy studies are discussed, with references to recently published results on the molecular characterization of TNBCs. *(Am J Pathol 2013, 183: 1064–1074; http://dx.doi.org/10.1016/j.ajpath.2013.05.033)*

Breast cancers show extensive heterogeneity despite their common tissue of origin. Decades of research in molecular pathology and molecular genetics have shown a number of distinct subtypes. Currently, only a limited number of clinical, pathologic, and molecular factors help clinicians make decisions on therapy selection and help evaluate prognosis at the time of diagnosis. Breast tumors are categorized into three main groups based on markers that reflect available treatment options: estrogen receptor (ER) or progesterone receptor positive, erbB2 amplified (HER2 positive) with and without ER/progesterone receptor, and triple-negative breast cancer (TNBC) defined by the absence of ER/progesterone receptor expression and HER2 amplification. Targeted therapy is available for patients with ER-positive and patients with HER2-positive tumors, leaving those with triple-negative (TN) disease as the only group of patients presently without an option for targeted therapy.

High-throughput molecular analyses have led to the discovery of distinct subtypes of breast cancer, which partly correspond to the histopathologic entities described above. The first gene expression–based classification was performed more than a decade ago,[1,2] and identified five different subgroups that are biologically diverse with different clinical outcomes: two luminal-cell–related groups (luminal A and luminal B), a myoepithelial-cell–related group (basal-like), a HER2-enriched group, and a normal-like group. A minimal gene set of 50 genes (Pam50) has been developed for classifying subtypes of breast cancer with high agreement in classification with the larger intrinsic gene sets originally used for subtyping.[3] This subgrouping has proven robust and has provided important insight into the biology of breast

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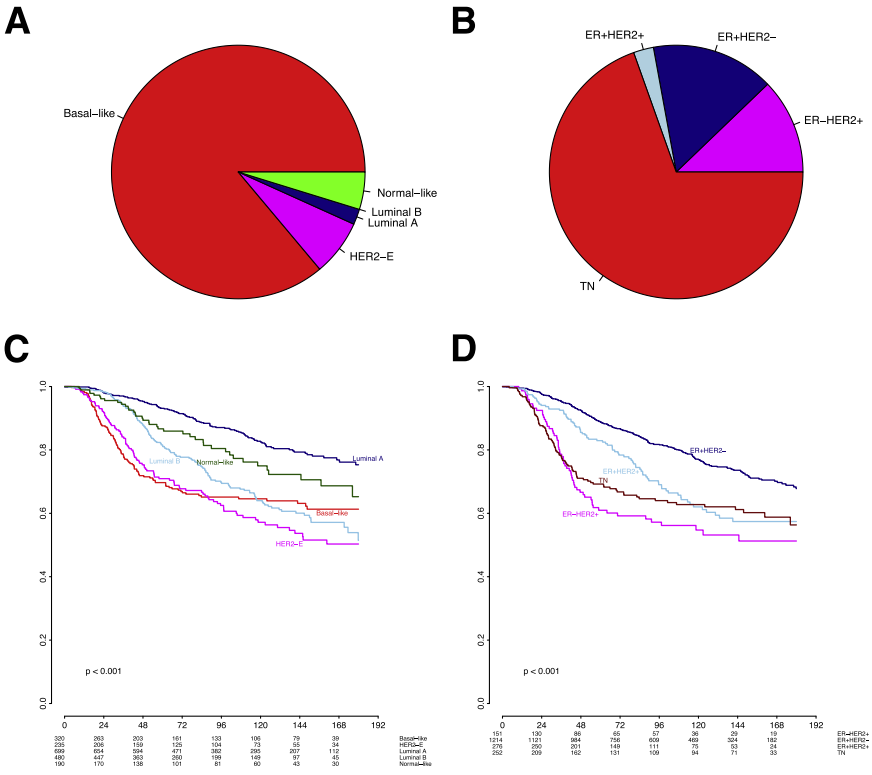

Figure 1 Distribution of subtypes and outcome in the METABRIC cohort.[9] **A:** Distribution of PAM50 subtypes in triple negative breast cancer (TNBC) (n = 252): 86.1% basal-like, 7.1% HER2-E), 4.8% normal-like, 2.0% luminal A (dark blue), and 0% luminal B. PAM50 subtypes were assigned from gene expression analysis as previously described.[9] **B:** Distribution of histopathologic groups in basal-like breast cancer (n = 321): 67.6% triple negative (TN), 15.3% ER+/HER2−, 11.8% ER−/HER2+, 2.5% ER+/HER2+. **C:** Breast cancer–specific survival by PAM50 subtypes in 1924 invasive breast carcinomas with 15 years of follow-up evaluation: luminal A, luminal B, basal-like, HER2-E, and normal-like. **D:** Breast cancer–specific survival by histopathologic grouping in 1893 invasive breast carcinomas: TN, ER+, and HER2+ including both progesterone receptor (PR)+ and PR−, ER+, and HER2− including both PR+ and PR−, and ER− and HER2+ including both PR+ and PR−. Cases that were ER−, PR+, and HER2− (n = 35) were excluded from this analysis. ER status was determined by immunohistochemical scoring, PR status was derived from gene expression arrays, and HER2 gene amplification status was based on scoring of array copy number analysis. PR, progesterone receptor.

cancer. However, methods for assigning a subtype to single samples have proven challenging and the methods are debated.[4–7] The basal-like and luminal carcinomas have different etiologies and for most purposes may be considered distinct diseases. Because the biology of these subtypes is substantially different, stratification is essential both for clinical and preclinical studies to identify common features of tumorigenesis that can be used for further therapy selection. The majority of basal-like tumors are TN, but not entirely, and further molecular profiling has shown extensive heterogeneity of both TNBC and basal-like tumors, which is of possible importance for treatment decisions. In this review the heterogeneity of TNBC/basal-like breast tumors is discussed, with a focus on the underlying molecular alterations of potential importance for developing targeting therapies.

Clinical Characteristics of TNBC

The fraction of TNBC is variable and has been reported to be in the range of 9% to 16%.[8–12] TNBC is known to be more frequent in younger patients, in *BRCA1* mutation carriers, and in specific ethnic groups (African American and Hispanic women).[12–15] Compared with Caucasians, African American women are twice as likely to develop TNBC, suggesting that germline genetic background is of major importance for the transcriptional program and tumor differentiation.[12,13,16] The majority of TNBC tumors, when classified by tumor morphology, are invasive ductal carcinomas (approximately 90%), with the rest classified as apocrine, lobular, adenoid cystic, and metaplastic.[11] Patients with TNBC often have unfavorable histopathologic features at diagnosis because they are of higher histologic grade, larger tumor size, and more often are lymph node positive.[17]

The basal-like subtype by gene expression most frequently is TN, but not always. Figure 1, A and B, shows the overlap between the basal and TN subtypes among cases from the large METABRIC cohort.[9] Of 252 TN tumors, 86.1% were basal-like, 7.1% were HER2-enriched, 4.8% were normal-like, and 2.0% were luminal A, illustrating the heterogeneity of TNBCs with respect to Pam50 subtypes (Figure 1A). Other investigators have reported a fraction of basal-like TN tumors of 72% to 79%.[18,19] Differences possibly could be owing to cohort selection or methodologic issues.[4,20] Conversely, basal-like tumors by gene expression (n = 321) are most frequently TNBCs (67.6%), but 15.3% are ER+/HER2−, 11.8% are ER−/HER2+, and 2.5% are ER+/HER2+ (Figure 1B). This lack of consistency between gene expression subtypes and standard markers has motivated several studies to refine classification by immunohistochemistry using additional markers. Expression of either cytokeratin 5/6 or epidermal growth factor receptor (EGFR) has been shown to identify basal-like tumors classified by gene expression.[8,13,21,22] Several investigators thus have used these as surrogate markers for a core basal subgroup (ER−, progesterone receptor−, HER−, cytokeratin 5/6+, and EGFR+).

Basal-like breast cancer has been shown to have an inferior prognosis compared with luminal subtypes in several studies.[2,4] In the recent METABRIC study this was confirmed, and Figure 1, C and D, show breast cancer–specific survival with 15 years of observation for the Pam50 subtypes and histopathologic groups, respectively. In this cohort, the

HER+/HER2-enriched group of patients had a poor prognosis, of whom none received HER2-targeted therapy. Basal-like tumors and TNBC have high breast cancer mortality the first few years after diagnosis, but then reach a plateau, suggesting that patients surviving the first high-risk phase are cured of the disease. This is in contrast to luminal disease, in which breast cancer mortality is constant over time (up to 15 years after diagnosis), as observed in several cohorts. With extended observation time after diagnosis the curves cross, leading to a higher risk of breast cancer death in luminal tumors.[8,23] Although most TNBCs are found in younger women, approximately 25% of the TN patients are ≥65 years, and because older patients are less likely to receive systemic treatment, this represents a subset of patients with aggressive disease and poor outcome.[24] It is known that African American women have a higher mortality rate from breast cancer, which potentially is explained by the higher incidence of TNBC.[25] However, no clear racial effect has been identified within TNBC.[26]

Prognosis is related closely to metastatic behavior and breast cancer subtypes are linked to different sites of metastases. Bone is the overall most common metastatic site, except in basal-like disease, which is dominated by brain, lung, and distant lymph node metastases.[27,28] TN nonbasal tumors showed a similar pattern to basal tumors, but with more liver metastases.

Molecular Characteristics of TNBC

Gene Expression Analysis of TNBCs by Microarrays

Gene expression analysis by microarrays was introduced more than a decade ago and has provided a basis for a comprehensive description of individual tumors and insight into the vast intertumor heterogeneity. Still, the classification derived from the intrinsic gene set[2] represents the basis for biological understanding of breast cancer with the luminal subtypes characterized by expression of estrogen receptor 1 and genes expressed by luminal epithelial cells, and the nonluminal subgroups (basal-like and HER2-enriched) characterized by expression of genes associated to basal/myoepithelial cells. The separation of luminal and nonluminal tumors has proven robust across most data sets.

Basal-like tumors were referred to as basal because of their expression of genes typically expressed in basal epithelial cells, such as cytokeratin 5, 6, or 17. These tumors are highly proliferative and frequently *TP53* mutated. Basal-like tumors are associated with *BRCA1* mutation status because the majority of *BRCA1* germline mutation carriers develop basal-like breast cancer.[14] This has led to the concept of BRCAness, tumors that share features of deregulated DNA repair by deficiency in homologous recombination with BRCA inactivated tumors from *BRCA* mutation carriers.[29] It is believed that the combined TP53, RB, and BRCA1 pathways loss of activity is responsible for the high level of genomic instability observed in basal-like tumors.[30] A fraction of TN tumors belong to the HER2-enriched gene expression subtype (Figure 1A) without HER2 gene amplification, but with an expression pattern associated with HER2 signaling with possible mutations in HER2 or downstream targets.[30] These patients do not receive any HER2-targeted treatment with the present accepted treatment recommendations based on the results in clinical trials, but the expression pattern suggests that they might benefit from treatment targeting relevant signaling pathways.

Further refinement of the intrinsic subgroups has identified the claudin-low group, which is characterized as TN with a low expression of claudin 3, 4, and 7 tight junction proteins and E-cadherin.[31] These were described to express mesenchymal expression features, with a low level of luminal genes and high expression of endothelial cell and lymphocyte markers. The tumors are enriched for epithelial-mesenchymal transition (EMT) markers and resemble mammary epithelial stem cells. In survival analyses the claudin-low group had an intermediate outcome compared with luminal and basal tumors, and an intermediate response to chemotherapy.[32]

The heterogeneity of TNBCs is widely acknowledged. In a study of 97 TN cases all tumors were found to be basal-like, but hierarchical clustering of gene expression revealed five subgroups.[33] A comprehensive characterization of 51 breast cancer cell lines showed that the transcriptional profile reflected primary breast tumors, except that more and higher levels of amplifications were seen.[34] Basal-like cell lines could be separated into two groups: basal A resembles basal-like, and basal B has a more stem cell—like expression profile. The most extensive study of gene expression in TNBCs to date was performed by Lehmann et al[35] and compiled a data set of 587 TNBC gene expression profiles from 21 published studies. Clustering analysis of the most differentially expressed genes identified seven subgroups: basal-like 1, basal-like 2, immunomodulatory, mesenchymal, mesenchymal stem—like, luminal androgen receptor, and an unstable cluster that was not classified. Basal-like 1 and basal-like 2 were highly proliferative and had a higher expression of cell-cycle and DNA damage response genes. The mesenchymal and mesenchymal stem—like groups were enriched for *EMT* genes, whereas the immune-modulatory subtype was characterized by immune cell signaling features. The luminal androgen receptor subtype was ER negative, but enriched in hormone-related pathways and found to be androgen receptor—expressing, suggesting a role for anti-androgenic treatment for these cases. However, in a recent neoadjuvant study, androgen-receptor expression in TNBC was associated with a favorable prognosis[36] and the biological relevance of androgen receptor in breast cancer and the potential role in therapy is debated.[37]

Copy Number Aberrations in TNBC Resulting from Genomic Instability

Copy number aberrations resulting from genomic instability is a key feature in the transformation of malignant cells,

leading to DNA translocations, deletions, and amplification. Array comparative genomic hybridization analysis of tumors provides genome-wide information of DNA copy number. The frequency of copy number alterations is found to differ between intrinsic subtypes.[38] Basal-like tumors show a high degree of genomic instability with recurrent copy number alterations being losses on 3q, 4p/q, 5q, 12q, and 14q, and gains on 1q, 3q, 6p, 7q, 8q, 10p, 17q, and 21q.[38–40] Some of the most frequently observed copy number alterations in TNBCs are affecting cancer-associated genes such as *RB1*, *PTEN*, *EGFR*, and *PARK2*.[41] Although extensive genomic instability is frequent in basal-like tumor genomes, a subset do not have such alterations, suggesting different mechanisms of carcinogenesis even within TNBC.[42]

The global patterns of chromosomal rearrangements can be used to group tumors as simple, complex sawtooth, and complex firestorm,[43] with patients whose tumors contain complex alterations experiencing an inferior outcome.[43] A more detailed characterization of such patterns quantified alterations of whole chromosomal arms and complex chromosomal rearrangements with two indices: whole-arm aberration index and complex arm aberration index.[44] Cases with a high complex arm aberration index score of complex genomic events, frequently basal-like, showed an increased risk for breast cancer–specific death, as compared with low complex arm aberration index tumors.

A challenge in analysis of chromosomal aberrations is the fact that a tumor is an admixture of tumor (aberrant) and normal (nonaberrant) cell fractions and that tumor ploidy usually is unknown. The allele-specific copy number analysis of tumors algorithm that deduces tumor ploidy showed that basal-like tumors have a significantly higher loss of heterozygosity and loss of genomic material during tumor development, followed by a duplication of the genome, resulting in near-triploid basal-like tumors.[45] This suggests that basal-like tumors tend to follow a progression path with an initial unstable hypodiploid state followed by whole-genome duplication. Because this pattern is different from the other subtypes it alludes to the importance of genomic instability in the pathogenesis of basal tumors. Sequencing single cells from two metastatic TNBCs showed that selected subclones were dominated by extensive loss of DNA, again suggesting the importance of a hypodiploid stage in basal-like tumor progression.[46] The translocation pattern of somatic rearrangements in 24 breast cancers (of which 6 tumors and 6 cell lines were TN) was studied using paired-end sequencing.[47] Most of these were intrachromosomal, as compared with interchromosomal translocations, most commonly tandem duplications, although diversity was observed within TN cancers. The ploidy distribution in basal-like tumors and the specific translocation pattern together may suggest a unique path of progression for TNBCs.[45,48,49]

Combined copy number aberrations and gene expression has been used in a study of 2000 cases for classification and categorization of breast cancer, with the major finding of 10 integrative cluster groups.[9] Most of the TNBCs were classified in integrative cluster 10, representing the core basal subgroup in this new classification. The highest rate of *TP53* mutations was found in integrative cluster 10 combined with intermediate levels of genomic instability, loss of 5q, and gains at 8q, 10p, and 12p.[9,50] Loss of 5q has been associated with the presence of a *TP53* mutation,[50] and a basal-specific gene expression pattern linked with cell-cycle checkpoint control, DNA damage repair, and apoptosis.[51] Approximately 25% of TNBCs were assigned to integrative cluster 4, consisting of both ER+ and ER− tumors. This subgroup has a good outcome, a low level of genomic instability, rearranged T-cell receptor, and extensive lymphocytic infiltration.[51] The pathway recognition algorithm using data integration on genomic models is a method that integrates molecular data from different genomic levels (copy number, methylation, miRNA, and mRNA expression) within a pathway-specific framework.[52] This approach has proven powerful because crucial pathways for tumor sustainability and evolution tend to be perturbed by a range of mechanisms and thus a pathway-based approach helps to decipher important regulators. In breast cancer this has led to identification of five clusters of tumors mostly separated by immune-related signaling pathways.[53] Basal-like tumors were split into two groups with differences in survival by their ratio of T-helper 1 and cytotoxic T-lymphocyte gene signature to the level of T-helper 2–associated humoral immunity signature, both with high FOXM1 signaling. Because perturbed immune signatures were the dominating factor in this pathway-driven stratification of breast tumors, it shows the importance of interaction between tumor and stromal cells and the complicated role of immune response in cancer.

Somatically Acquired Mutations in TNBC

Somatically acquired mutations in tumor DNA varies extensively from tumor to tumor.[54] The genes most frequently found mutated in three recent sequencing studies in TNBC are summarized in Table 1.[10,41,54] Somatic mutations of *TP53* are found in the majority of TN tumors (53.8% to 85.7%), and

Table 1 Top Mutated Genes in TNBC

	Shah et al[41] (n = 65)		Stephens et al[47] (n = 14)		TCGA10 (n = 86)	
Gene	n	%	n	%	n	%
TP53	35	53.8	12	85.7	68	79.1
PIK3CA	7	10.8	3	21.4	9	10.5
USH2A	6	9.2	1	7.1	9	10.5
TTN	6	9.2	8	57.1		
RB1	5	7.7	2	14.3	4	4.7
DST	2	3.1	5	35.7		
MLL3	2	3.1	1	7.1	3	3.5
MYO3A	6	9.2				
PTEN	5	7.7			1	1.2
SYNE1	4	6.2	2	14.3		

TCGA, The Cancer Genome Atlas Network.

when combined with inferred pathway analysis there is evidence for loss of TP53 function in nearly all basal-like tumors.[10] Interestingly, *TP53* mutations in basal-like tumors were more of the nonsense and frame-shift type, in contrast to mutations in luminal tumors that more frequently were missense. Shah et al[41] found that approximately 20% of cases had potential clinical druggable aberrations (ie, which may be inhibited by existing clinically used drugs) including *BRAF V600E*, *EGFR* amplifications, and *ERBB2/ERBB3* mutations. Integrative pathway analysis comparing basal-like and luminal tumors identified hyperactivated FOXM1 as a transcriptional driver of proliferation and found increased MYC and HIF1-a/ARNT as key regulators.[10] Integrative pathway analysis also confirmed that loss of *RB1* and *BRCA1* are basal-like features.

Sequencing studies[55,56] have shown that mutations are introduced continuously during tumor evolution, resulting in numerous subclones. Timing these events is possible by taking both mutational frequency and copy number alterations into account. *TP53* were found to frequently occur in most clones in the tumors, indicating an early event critical for tumorigenesis, and more rarely in smaller subclones, indicating a late event and hence probably less important in tumor development. Overall, basal TN had more subclonal populations than the nonbasal TNBCs. The subclones populate the tumor in a dynamic process in which the clone with the best selective advantage dominates. Treatment provides a selective pressure on the tumor and although chemotherapy may eradicate the bulk of the tumor, it could at the same time provide an opportunity for a subclone to proliferate and ultimately induce treatment resistance. Unless successfully treated, the genetic diversification in tumor cells does not stop, but is an ongoing process continued in the development of tumor metastases. This was addressed in a recent publication[57] on DNA and RNA sequencing of advanced (recurrent or metastatic) tumors from 14 patients with TN disease. Expression profiles associated with genes regulating cell-cycle control, G2-M checkpoint, and mitosis were seen in 8 of these tumors, and expression patterns were enriched for immune-related pathways in 5 tumors.[57] A single tumor was enriched for genes involved in androgen and ER metabolism. The most commonly mutated gene, *TP53*, was detected in 10 of the tumors. Alterations involving the RAS/RAF/MEK/ERK pathways or the PI3K/AKT/mTOR pathways were found in nine patients, which is of considerable interest from a therapeutic perspective. In addition, *FOXM1* genes were found overexpressed in 12 of the tumors, highlighting the importance of gene regulation of factors involved in the proliferation of cell-cycle and mitotic check point control.

Current and Future Therapy of TNBC

Therapy of TNBC is based on surgery, radiotherapy, and chemotherapy (Figure 2A), because currently there are no targeted treatment options are available. The most active cytotoxic agents are anthracyclines and taxanes. Large clinical studies and meta-analyses have shown survival benefit in both hormone receptor−positive and hormone receptor−negative tumors with anthracyclines.[58] The rate of pathologic complete response after neoadjuvant treatment in hormone receptor−negative tumors is higher compared with hormone receptor−positive tumors, and pathologic complete response has been shown to be predictive for outcome (recurrence-free survival).[59,60] A study combining data from seven neoadjuvant prospective clinical trials investigated the relationship between clinical subtypes, pathologic complete response, and RFS in 4193 of these patients.[59] A pathologic complete response, defined here as the absence of invasive breast cancer in the breast and lymph nodes, was achieved in 36% of the patients undergoing therapy with anthracyclines and taxanes. A summary of recent neoadjuvant clinical trials and data sets in patients with TNBC is shown in Figure 3.[59,61−74]

For therapy of TNBC, regimens other than standard anthracycline- and taxane-based may be of value (Figure 2A). Genetic instability associated with TNBC indicates that DNA interacting agents may be advantageous. Studies with cisplatin in combination with anthracyclines have shown activity in the adjuvant setting, but were not designed to detect or verify the superiority of platinum regimens in TNBC.[70] Other studies also have reported the activity of such combinations.[75] In a more recent study, the benefit of adjuvant cisplatin-containing therapy was similar to other commonly used regimens, but the outcome was not reported specifically for TNBC.[76] Recently, a neoadjuvant study of TNBC did not show an additional benefit of adding carboplatin to the standard treatment of anthracycline followed by taxane.[71] A few studies have reported the use of carboplatin with paclitaxel or docetaxel, without anthracyclines, in the neoadjuvant setting (Figure 3), with similar response rates to previous studies, but with a limited number of patients.[72−74] Although platinum-containing treatment regimens are active in breast cancer, there is no general evidence for superiority in TNBC.

Tumors associated with inactivated BRCA, as in *BRCA1/2* mutation carriers, may be candidates for platinum-containing therapy owing to the lack of BRCA protein function in homologous DNA recombination-mediated repair, making the cells dependent of the more error prone end-joining DNA repair mechanism (Figure 2, A and B). A high response rate has been reported, but the study was small (Figure 3), and the role of platinum in this subset of patients is debated.[77] Similarly, somatic inactivation of BRCA function has been described in basal-like breast cancers, and therefore could be a candidate for DNA-acting platinum-containing chemotherapy. However, recent neoadjuvant studies in patients with basal-like tumors have not confirmed this (Figure 3). Studies using specific signatures to predict the outcome of high-dose alkylating chemotherapy may indicate that some TNBCs could be responders to alkylating therapy.[78] Evidently, TNBC cannot be considered a single entity. Predictive markers of response to chemotherapeutic regimens may be patterns of DNA rearrangements, mutation patterns, or defective DNA repair mechanisms; hence, molecular analyses should be

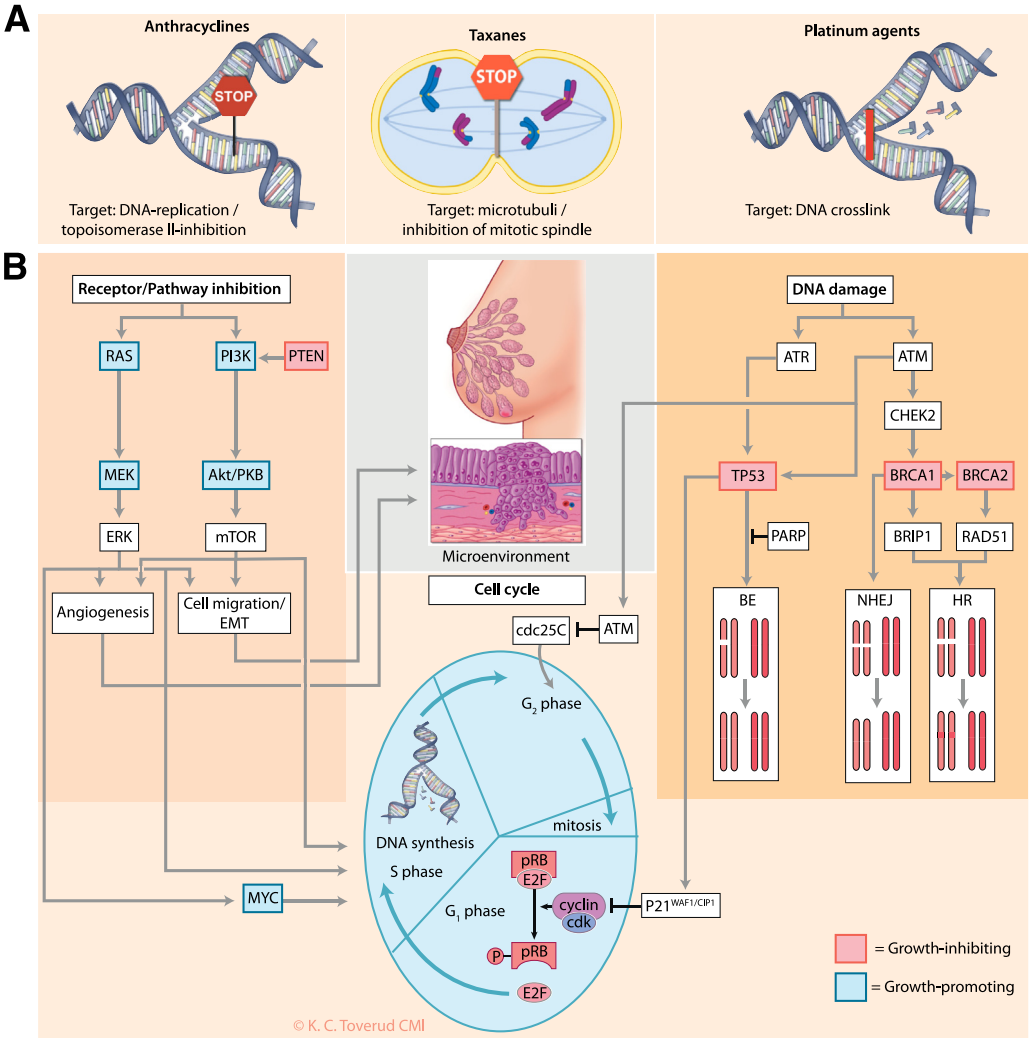

Figure 2 Currently used therapies and molecular targets for therapy in triple negative breast cancer (TNBC). **A:** Currently used therapy for TNBC includes anthracyclines, taxanes, and platinum-based agents (most commonly carboplatin and cisplatin). The mechanisms of action are indicated, and rational combinations of these therapies with new targeted treatment principles are investigated that may combat treatment resistance and increase therapeutic efficacy (eg, antiangiogenic therapy and platinum/PARPi). **B:** Molecular targeted therapies for TNBC have been studied involving multiple pathways, and druggable targets are exemplified in the figure. The different principles investigated are highly integrated, which may generate a rationale for combining different therapeutic approaches in the future. Of specific interest are future combinations involving chemotherapeutic agents (**A**) with targeted therapeutics because of the documented activity of the conventional treatment regimens. BE, base excision repair pathway; HR, homologous end-joining repair pathway; NHEJ, nonhomologous end-joining repair pathway. Printed with permission from Kari C. Toverud (copyright holder).

included in all preclinical and clinical trials for identification of relevant predictive factors in specific tumor types.

Molecular-Directed Therapy in TNBC

As reviewed above, a number of studies using high-throughput molecular technologies including deep sequencing have paved the way for a more rational selection of therapeutics in TNBC according to the molecular characteristics of the tumor itself (as shown in Figure 2B). A total of 121 studies on TNBC therapy were found in *http://www.clinicaltrials.gov* (last accessed at the time of the submission, April 5, 2013), using the search term "triple negative breast cancer," limiting the search to phase II and III intervention studies, or phase I studies only recruiting breast cancer patients, of which 77 were based on a targeted principle. The list is not exhaustive but provides an overview of agents currently in clinical trials in TNBC. The role of vascular endothelial growth factor inhibition (26 studies), poly (ADP-ribose) polymerase (PARP) inhibition (11 studies, of which 5 studies were on iniparib), erbB inhibition (14 studies), and phosphatidylinositol 3-kinase/AKT/mTOR targeted therapies (9 studies) in TNBC still are not settled, although some activity has been reported. In addition, a number of compounds are in early phase clinical trials, and are expected to be available for clinical trials in TNBC in the near future.

Molecular Targets for Therapy—PARP Inhibition

PARP inhibitors have been studied in breast cancer for some time with variable results. Initial studies on iniparib were encouraging,[79] but later studies were unable to confirm either

Treatment	Total No. patients in trial	Tumor	Percent pCR in TN (*N* pCR/*N* TN patients)	Percent pCR for specified treatment (fill indicates No. of TN patients in trial) 10 20 30 40 50 60 70 80	Reference
Trials with anthracyclin combination therapy					
A→T	3938	TN	36 (329/911)		59
EC→Doc	1948	TN	28 (95/340)*		61
EC→Doc+Bev			39 (127/323)*		
Doc → AC	1186	TN	47 (116/246)		62
Doc→ AC+Bev			52 (126/244)		
EC→Doc	59	TN	73 (8/11)		63
E Doc G	103	TN	28 (10/36)		64
DoxT→CMF	128	TN	54 (23/43)		65
DoxT→CMX			41 (17/42)		
DoxC→TX			40 (17/43)		
Doc → FEC	132	TN	48 (14/30)		66
G Doc → EV	74	TN	41 (9/22)		67
Trials with anthracyclin and cisplatin/carboplatin					
A→T			8 (2/25)		68
AC or FAC			22 (11/51)		
Cis			83 (10/12)		
Cis	28	Basal like	22 (6/28)†		69
DoxT → CisT or CisT → DoxT	88	TN	35 (10/29)		70
EC-Doc	94	TN	35 (16/46)		71
EC-Doc Carb			30 (14/48)		
Trials without anthracyclins					
Carb-Pac	55	TN	67 (8/12)‡		72
Doc-Carb	74	TN	55 (6/11)		73
Pac-Carb	107	TN	33 (8/24)		74

Figure 3 Efficacy of neoadjuvant chemotherapy in clinical trials and data sets in patients with TNBC. Treatments included anthracycline (A), doxorubicin + cyclophosphamide (AC), doxorubicin (Dox), taxane (T), epirubicin (E), cyclophosphamide (C), docetaxel (Doc), paclitaxel (Pac), bevacizumab (Bev), gemcitabine (G), methotrexate (M), 5-FU (F), capecitabine (X), vincristine (V), cisplatin (Cis), and carboplatin (Carb). For the percentage of pathologic complete response (pCR), black, >100 TN patients in the trial; hatched, >30 TN patients in the trial; and white, 11 to 30 TN patients in the trial. References 59, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, and 74. *pCR (ductal carcinoma *in situ* was not allowed). †All tumors were basal-like. ‡Seventeen Her2+ patients received trastuzumab.

the specific inhibition or the clinical relevance. The PARP inhibitor olaparib was shown to have significant activity when given as monotherapy in patients with advanced breast cancer and verified *BRCA* mutations.[80] This agent also has been used in another study with 8 BRCA mutated and 15 TNBC patients, but with disease stabilization as the best response.[81] Thus, monotherapy with these agents is not sufficient and further trials are needed for selection of the patient population and appropriate combination regimens for optimal disease control.[82] Therapy combining PARP inhibition and chemotherapy has been challenging owing to toxicity, but is currently in clinical trials (NCT01506609). New combinations involving specific inhibition of signaling pathways may be needed to optimize the effect of these agents, as described in a preclinical study in which phosphatidylinositol 3-kinase and PARP inhibition were combined.[83] A total of 20% of patients with basal-like tumors recently were found to have either germline or somatic BRCA1 or BRCA2 dysfunction, suggesting that one in five patients with basal-like tumors might benefit from PARP inhibition.[10]

Molecular Targeting of Signaling Pathways

The extensive studies on further TNBC subtyping may guide selection of therapeutics, as indicated in the previously discussed studies of gene expression patterns in TNBC.[35,84] In addition, sequencing studies of breast cancer are powerful tools for the detection of putative targeted therapeutic approaches for the evaluation and selection of agents[9,10,41,56] (Figure 2B).

Targeting the PI3K/mTOR/S6 Pathway and the receptor tyrosine kinase/RAS/MEK/MAPK Pathway

Activity of the PIK3CA/mTOR/S6 pathway as a driver in a subset of TNBC has been shown[35,84] with verified *PIK3CA* mutations in 10% to 21% of TNBCs (Table 1).[10,41,54] Mutations and loss of PTEN (1% to 8% mutated) and INPP4B (inositol polyphosphate-4-phosphatase, type II, 105 kDa) support the importance of this pathway in TNBC. Targeting is possible through inhibition of phosphatidylinositol 3-kinase by small molecular inhibitors, or combined inhibitors targeting other key pathway nodes. Optimal combinations need to be established to address the observed tumor heterogeneity and the redundancy of the cell signaling pathways. Recent results describing mutations in several other targets including tyrosine kinases and RAS may indicate that certain tumors may be candidates for inhibition of the RAS/RAF/MEK/ERK pathway for effective growth inhibition. However, direct targeting of upstream tyrosine kinases, in particular EGFR, which has been found amplified

and up-regulated in TN tumors, have been the focus of recent clinical studies with disappointing results.[85,86] Targeting downstream in the signaling pathway may be more effective, and studies are ongoing on the value of MEK inhibition in TNBC (Figure 2B). Inhibiting such a pathway, however, may trigger compensatory responses, as recently investigated.[87] A rational selection of targets therefore may be necessary to ensure optimal treatment efficacy.

Targeting the Rb/Proliferation Gene Pathways

Mutations and aberrations affecting gene expression in the Rb pathway are a highly relevant and significant feature of TNBC.[9,10] This is confirmed by RNA expression studies, with tumors overexpressing kinases related to cell-cycle and DNA replication.[35,84] High proliferative capacity is a hallmark of these TN tumors, with a dismal prognosis. A number of targets are druggable in this setting, exemplified by aurora kinase and polo-like kinase, in addition to more nonspecific inhibition by targeting heat shock protein 90. *MYC* is associated with basal-like breast cancer and was found amplified in approximately 30% of basal-like breast cancers in the Cancer Genome Atlas Network data set.[10] CDK inhibition is a possible therapeutic targeting strategy in MYC-activated basal-like breast cancers.[88] FOXM1 is a driver in basal-like breast cancers, which strengthens the argument that such growth-promoting pathways should be targeted for therapy.[10,57]

Targeting the Cytoskeleton, Cell Migration, and Vascular System

Mutations affecting the cytoskeleton,[41] and thus possibly influencing EMT, have been observed relatively frequently in TNBC.[89,90] Both basal-like and in particular claudin-low tumors have high expression levels of proteins involved in the EMT process.[32,85] The tyrosine kinase Src is involved in cell migration, and therefore may be one target for therapy involving the EMT and cell migration.[35] The efficacy of src inhibition in TNBC using dasatinib recently was investigated, but with limited activity.[91] However, monotherapy using such targeted agents is not expected to be highly active, and optimal therapeutic combinations in addition to markers for selecting responsive tumors should be sought for this therapeutic approach in TNBC. Subgroups of these tumors also show high expression levels of stem cell–associated markers that may confer resistance to conventional therapy. Targeting the EMT process thus may be important for a subset of TNBCs. The finding that genes coding for proteins that are important in extracellular matrix dynamics, receptors, and pathways associated with vascular endothelial growth factor signaling are affected in TNBC indicate that microenvironmental interactions may be of crucial importance for the development and progression of these tumors.[10,92] Such interactions also may constitute targets for therapy. Inhibition of angiogenesis has been suggested as a target in TNBC, and although initial positive results have been obtained,[61,62] the development of markers for selecting appropriate patients for antiangiogenic therapy are highly needed.

Conclusions

Heterogeneity of TNBC is widely acknowledged and is evident in both molecular and clinical studies. The large amount of information evolving from high-throughput molecular analyses including sequencing, represents an even more detailed basis for understanding the molecular biology of TNBC, but at the same time emphasizes the tumor heterogeneity and the continuous alterations associated with tumor evolution. How this translates to the molecular biology of metastatic disease only partially is understood and needs further study. New discoveries will generate opportunities for novel treatment strategies, as well as more rational selection of patients for existing treatment regimens. Future clinical studies should be designed for a better understanding of the relationship between the molecular biology and therapy response, in addition to traditional clinical end points.

References

1. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D: Molecular portraits of human breast tumours. Nature 2000, 406:747–752
2. Sorlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, van de Rijn M, Jeffrey SS, Thorsen T, Quist H, Matese JC, Brown PO, Botstein D, Lonning PE, Borresen-Dale AL: Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001, 98:10869–10874
3. Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, Quackenbush JF, Stijleman IJ, Palazzo J, Marron JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM, Bernard PS: Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009, 27:1160–1167
4. Weigelt B, Mackay A, A'Hern R, Natrajan R, Tan DS, Dowsett M, Ashworth A, Reis-Filho JS: Breast cancer molecular profiling with single sample predictors: a retrospective analysis. Lancet Oncol 2010, 11:339–349
5. Dunning MJ, Curtis C, Barbosa-Morais NL, Caldas C, Tavare S, Lynch AG: The importance of platform annotation in interpreting microarray data. Lancet Oncol 2010, 11:717
6. Sorlie T, Borgan E, Myhre S, Vollan HK, Russnes H, Zhao X, Nilsen G, Lingjaerde OC, Borresen-Dale AL, Rodland E: The importance of gene-centering microarray data. Lancet Oncol 2010, 11:719–720; author reply 20–21
7. Perou CM, Parker JS, Prat A, Ellis MJ, Bernard PS: Clinical implementation of the intrinsic subtypes of breast cancer. Lancet Oncol 2010, 11:718–719; author reply 20–21
8. Blows FM, Driver KE, Schmidt MK, Broeks A, van Leeuwen FE, Wesseling J, et al: Subtyping of breast cancer by immunohistochemistry to investigate a relationship between subtype and short and long term survival: a collaborative analysis of data for 10,159 cases from 12 studies. PLoS Med 2010, 7:e1000279
9. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, Speed D, Lynch AG, Samarajiwa S, Yuan Y, Graf S, Ha G,

Haffari G, Bashashati A, Russell R, McKinney S, Group M, Langerod A, Green A, Provenzano E, Wishart G, Pinder S, Watson P, Markowetz F, Murphy L, Ellis I, Purushotham A, Borresen-Dale AL, Brenton JD, Tavare S, Caldas C, Aparicio S: The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 2012, 486:346–352

10. Cancer Genome Atlas Network: Comprehensive molecular portraits of human breast tumours. Nature 2012, 490:61–70
11. Montagna E, Maisonneuve P, Rotmensz N, Cancello G, Iorfida M, Balduzzi A, Galimberti V, Veronesi P, Luini A, Pruneri G, Bottiglieri L, Mastropasqua MG, Goldhirsch A, Viale G, Colleoni M: Heterogeneity of triple-negative breast cancer: histologic subtyping to inform the outcome. Clin Breast Cancer 2013, 13:31–39
12. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V: Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer 2007, 109:1721–1728
13. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, Karaca G, Troester MA, Tse CK, Edmiston S, Deming SL, Geradts J, Cheang MC, Nielsen TO, Moorman PG, Earp HS, Millikan RC: Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA 2006, 295:2492–2502
14. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J, Perou CM, Lonning PE, Brown PO, Borresen-Dale AL, Botstein D: Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A 2003, 100:8418–8423
15. Foulkes WD, Smith IE, Reis-Filho JS: Triple-negative breast cancer. N Engl J Med 2010, 363:1938–1948
16. Lund MJ, Trivers KF, Porter PL, Coates RJ, Leyland-Jones B, Brawley OW, Flagg EW, O'Regan RM, Gabram SG, Eley JW: Race and triple negative threats to breast cancer survival: a population-based study in Atlanta. Breast Cancer Res Treatment 2009, 113:357–370
17. Dent R, Maureen T, Kathleen IP, Wedad MH, Harriet KK, Carol AS, Lavina AL, Ellen R, Ping S, Narod SA: Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007, 13: 4429–4434
18. Prat A, Adamo B, Cheang MC, Anders CK, Carey LA, Perou CM: Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. Oncologist 2013, 18:123–133
19. Prat A, Perou CM: Deconstructing the molecular portraits of breast cancer. Mol Oncol 2011, 5:5–23
20. Lusa L, McShane LM, Reid JF, De Cecco L, Ambrogi F, Biganzoli E, Gariboldi M, Pierotti MA: Challenges in projecting clustering results across gene expression-profiling datasets. J Natl Cancer Inst 2007, 99: 1715–1723
21. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, Hernandez-Boussard T, Livasy C, Cowan D, Dressler L, Akslen LA, Ragaz J, Gown AM, Gilks CB, van de Rijn M, Perou CM: Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004, 10:5367–5374
22. Broeks A, Schmidt MK, Sherman ME, Couch FJ, Hopper JL, Dite GS, et al: Low penetrance breast cancer susceptibility loci are associated with specific breast tumor subtypes: findings from the Breast Cancer Association Consortium. Hum Mol Genet 2011, 20:3289–3303
23. Langerod A, Zhao H, Borgan O, Nesland JM, Bukholm IR, Ikdahl T, Karesen R, Borresen-Dale AL, Jeffrey SS: TP53 mutation status and gene expression profiles are powerful prognostic markers of breast cancer. Breast Cancer Res 2007, 9:R30
24. Konigsberg R, Pfeiler G, Klement T, Hammerschmid N, Brunner A, Zeillinger R, Singer C, Dittrich C: Tumor characteristics and recurrence patterns in triple negative breast cancer: a comparison between younger (<65) and elderly (>/=65) patients. Eur J Cancer 2012, 48: 2962–2968
25. Siegel R, DeSantis C, Virgo K, Stein K, Mariotto A, Smith T, Cooper D, Gansler T, Lerro C, Fedewa S, Lin C, Leach C, Cannady RS, Cho H, Scoppa S, Hachey M, Kirch R, Jemal A, Ward E: Cancer treatment and survivorship statistics, 2012. CA Cancer J Clin 2012, 62:220–241
26. Pacheco JM, Gao F, Bumb C, Ellis MJ, Ma CX: Racial differences in outcomes of triple-negative breast cancer. Breast Cancer Res Treat 2013, 138:281–289
27. Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, Nielsen TO, Gelmon K: Metastatic behavior of breast cancer subtypes. J Clin Oncol 2010, 28:3271–3277
28. Sihto H, Lundin J, Lundin M, Lehtimaki T, Ristimaki A, Holli K, Sailas L, Kataja V, Turpeenniemi-Hujanen T, Isola J, Heikkila P, Joensuu H: Breast cancer biological subtypes and protein expression predict for the preferential distant metastasis sites: a nationwide cohort study. Breast Cancer Res 2011, 13:R87
29. Rehman FL, Lord CJ, Ashworth A: Synthetic lethal approaches to breast cancer therapy. Nat Rev Clin Oncol 2010, 7:718–724
30. Perou CM: Molecular stratification of triple-negative breast cancers. Oncologist 2010, 15(Suppl 5):39–48
31. Herschkowitz JI, Simin K, Weigman VJ, Mikaelian I, Usary J, Hu Z, Rasmussen KE, Jones LP, Assefnia S, Chandrasekharan S, Backlund MG, Yin Y, Khramtsov AI, Bastein R, Quackenbush J, Glazer RI, Brown PH, Green JE, Kopelovich L, Furth PA, Palazzo JP, Olopade OI, Bernard PS, Churchill GA, Van Dyke T, Perou CM: Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. Genome Biol 2007, 8:R76
32. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, He X, Perou CM: Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 2010, 12:R68
33. Kreike B, van Kouwenhove M, Horlings H, Weigelt B, Peterse H, Bartelink H, van de Vijver MJ: Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. Breast Cancer Res 2007, 9:R65
34. Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, Clark L, Bayani N, Coppe JP, Tong F, Speed T, Spellman PT, DeVries S, Lapuk A, Wang NJ, Kuo WL, Stilwell JL, Pinkel D, Albertson DG, Waldman FM, McCormick F, Dickson RB, Johnson MD, Lippman M, Ethier S, Gazdar A, Gray JW: A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. Cancer Cell 2006, 10:515–527
35. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, Pietenpol JA: Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011, 121:2750–2767
36. Loibl S, Muller BM, von Minckwitz G, Schwabe M, Roller M, Darb-Esfahani S, Ataseven B, du Bois A, Fissler-Eckhoff A, Gerber B, Kulmer U, Alles JU, Mehta K, Denkert C: Androgen receptor expression in primary breast cancer and its predictive and prognostic value in patients treated with neoadjuvant chemotherapy. Breast Cancer Res Treat 2011, 130:477–487
37. McNamara KM, Yoda T, Takagi K, Miki Y, Suzuki T, Sasano H: Androgen receptor in triple negative breast cancer. J Steroid Biochem Mol Biol 2013, 133:66–76
38. Bergamaschi A, Kim YH, Wang P, Sorlie T, Hernandez-Boussard T, Lonning PE, Tibshirani R, Borresen-Dale AL, Pollack JR: Distinct patterns of DNA copy number alteration are associated with different clinicopathological features and gene-expression subtypes of breast cancer. Genes Chromosomes Cancer 2006, 45:1033–1040
39. Chin K, DeVries S, Fridlyand J, Spellman PT, Roydasgupta R, Kuo WL, Lapuk A, Neve RM, Qian Z, Ryder T, Chen F, Feiler H, Tokuyasu T, Kingsley C, Dairkee S, Meng Z, Chew K, Pinkel D, Jain A, Ljung BM, Esserman L, Albertson DG, Waldman FM, Gray JW: Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. Cancer Cell 2006, 10:529–541
40. Chin SF, Wang Y, Thorne NP, Teschendorff AE, Pinder SE, Vias M, Naderi A, Roberts I, Barbosa-Morais NL, Garcia MJ, Iyer NG,

Kranjac T, Robertson JF, Aparicio S, Tavare S, Ellis I, Brenton JD, Caldas C: Using array-comparative genomic hybridization to define molecular portraits of primary breast cancers. Oncogene 2007, 26: 1959–1970
41. Shah SP, Roth A, Goya R, Oloumi A, Ha G, Zhao Y, et al: The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 2012, 486:395–399
42. Chin SF, Teschendorff AE, Marioni JC, Wang Y, Barbosa-Morais NL, Thorne NP, Costa JL, Pinder SE, van de Wiel MA, Green AR, Ellis IO, Porter PL, Tavare S, Brenton JD, Ylstra B, Caldas C: High-resolution aCGH and expression profiling identifies a novel genomic subtype of ER negative breast cancer. Genome Biol 2007, 8:R215
43. Hicks J, Krasnitz A, Lakshmi B, Navin NE, Riggs M, Leibu E, Esposito D, Alexander J, Troge J, Grubor V, Yoon S, Wigler M, Ye K, Borresen-Dale AL, Naume B, Schlicting E, Norton L, Hagerstrom T, Skoog L, Auer G, Maner S, Lundin P, Zetterberg A: Novel patterns of genome rearrangement and their association with survival in breast cancer. Genome Res 2006, 16:1465–1479
44. Russnes HG, Vollan HK, Lingjaerde OC, Krasnitz A, Lundin P, Naume B, Sorlie T, Borgen E, Rye IH, Langerod A, Chin SF, Teschendorff AE, Stephens PJ, Maner S, Schlichting E, Baumbusch LO, Karesen R, Stratton MP, Wigler M, Caldas C, Zetterberg A, Hicks J, Borresen-Dale AL: Genomic architecture characterizes tumor progression paths and fate in breast cancer patients. Science Transl Med 2010, 2:38ra47
45. Van Loo P, Nordgard SH, Lingjaerde OC, Russnes HG, Rye IH, Sun W, Weigman VJ, Marynen P, Zetterberg A, Naume B, Perou CM, Borresen-Dale AL, Kristensen VN: Allele-specific copy number analysis of tumors. Proc Natl Acad Sci U S A 2010, 107:16910–16915
46. Navin N, Kendall J, Troge J, Andrews P, Rodgers L, McIndoo J, Cook K, Stepansky A, Levy D, Esposito D, Muthuswamy L, Krasnitz A, McCombie WR, Hicks J, Wigler M: Tumour evolution inferred by single-cell sequencing. Nature 2011, 472:90–94
47. Stephens PJ, McBride DJ, Lin ML, Varela I, Pleasance ED, Simpson JT, Stebbings LA, Leroy C, Edkins S, Mudie LJ, Greenman CD, Jia M, Latimer C, Teague JW, Lau KW, Burton J, Quail MA, Swerdlow H, Churcher C, Natrajan R, Sieuwerts AM, Martens JW, Silver DP, Langerod A, Russnes HE, Foekens JA, Reis-Filho JS, van 't Veer L, Richardson AL, Borresen-Dale AL, Campbell PJ, Futreal PA, Stratton MR: Complex landscapes of somatic rearrangement in human breast cancer genomes. Nature 2009, 462:1005–1010
48. Dalgin GS, Alexe G, Scanfeld D, Tamayo P, Mesirov JP, Ganesan S, DeLisi C, Bhanot G: Portraits of breast cancer progression. BMC Bioinformatics 2007, 8:291
49. Navin N, Krasnitz A, Rodgers L, Cook K, Meth J, Kendall J, Riggs M, Eberling Y, Troge J, Grubor V, Levy D, Lundin P, Maner S, Zetterberg A, Hicks J, Wigler M: Inferring tumor progression from genomic heterogeneity. Genome Res 2010, 20:68–80
50. Jain AN, Chin K, Borresen-Dale AL, Erikstein BK, Eynstein Lonning P, Kaaresen R, Gray JW: Quantitative analysis of chromosomal CGH in human breast tumors associates copy number abnormalities with p53 status and patient survival. Proc Natl Acad Sci U S A 2001, 98:7952–7957
51. Dawson SJ, Rueda OM, Aparicio S, Caldas C: A new genome-driven integrated classification of breast cancer and its implications. EMBO J 2013, 32:617–628
52. Vaske CJ, Benz SC, Sanborn JZ, Earl D, Szeto C, Zhu J, Haussler D, Stuart JM: Inference of patient-specific pathway activities from multi-dimensional cancer genomics data using PARADIGM. Bioinformatics 2010, 26:i237–i245
53. Kristensen VN, Vaske CJ, Ursini-Siegel J, Van Loo P, Nordgard SH, Sachidanandam R, Sorlie T, Warnberg F, Haakensen VD, Helland A, Naume B, Perou CM, Haussler D, Troyanskaya OG, Borresen-Dale AL: Integrated molecular profiles of invasive breast tumors and ductal carcinoma in situ (DCIS) reveal differential vascular and interleukin signaling. Proc Natl Acad Sci U S A 2012, 109:2802–2807
54. Stephens PJ, Tarpey PS, Davies H, Van Loo P, Greenman C, Wedge DC, et al: The landscape of cancer genes and mutational processes in breast cancer. Nature 2012, 486:400–404
55. Nik-Zainal S, Van Loo P, Wedge DC, Alexandrov LB, Greenman CD, Lau KW, et al; Breast Cancer Working Group of the International Cancer Genome C: The life history of 21 breast cancers. Cell 2012, 149:994–1007
56. Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, et al; Breast Cancer Working Group of the International Cancer Genome C: Mutational processes molding the genomes of 21 breast cancers. Cell 2012, 149:979–993
57. Craig DW, O'Shaughnessy JA, Kiefer JA, Aldrich J, Sinari S, Moses TM, Wong S, Dinh J, Christoforides A, Blum JL, Aitelli CL, Osborne CR, Izatt T, Kurdoglu A, Baker A, Koeman J, Barbacioru C, Sakarya O, De La Vega FM, Siddiqui A, Hoang L, Billings PR, Salhia B, Tolcher AW, Trent JM, Mousses S, Von Hoff DD, Carpten JD: Genome and transcriptome sequencing in prospective refractory metastatic triple negative breast cancer uncovers therapeutic vulnerabilities. Mol Cancer Ther 2013, 12:104–116
58. Peto R, Davies C, Godwin J, Gray R, Pan HC, Clarke M, Cutter D, Darby S, McGale P, Taylor C, Wang YC, Bergh J, Di Leo A, Albain K, Swain S, Piccart M, Pritchard K: Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. Lancet 2012, 379:432–444
59. von Minckwitz G, Untch M, Blohmer JU, Costa SD, Eidtmann H, Fasching PA, Gerber B, Eiermann W, Hilfrich J, Huober J, Jackisch C, Kaufmann M, Konecny GE, Denkert C, Nekljudova V, Mehta K, Loibl S: Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. J Clin Oncol 2012, 30:1796–1804
60. Esserman LJ, Berry DA, Demichele A, Carey L, Davis SE, Buxton M, Hudis C, Gray JW, Perou C, Yau C, Livasy C, Krontiras H, Montgomery L, Tripathy D, Lehman C, Liu MC, Olopade OI, Rugo HS, Carpenter JT, Dressler L, Chhieng D, Singh B, Mies C, Rabban J, Chen YY, Giri D, van 't Veer L, Hylton N: Pathologic complete response predicts recurrence-free survival more effectively by cancer subset: results from the I-SPY 1 TRIAL–CALGB 150007/150012, ACRIN 6657. J Clin Oncol 2012, 30:3242–3249
61. von Minckwitz G, Eidtmann H, Rezai M, Fasching PA, Tesch H, Eggemann H, Schrader I, Kittel K, Hanusch C, Kreienberg R, Solbach C, Gerber B, Jackisch C, Kunz G, Blohmer JU, Huober J, Hauschild M, Fehm T, Muller BM, Denkert C, Loibl S, Nekljudova V, Untch M: Neoadjuvant chemotherapy and bevacizumab for HER2-negative breast cancer. N Engl J Med 2012, 366: 299–309
62. Bear HD, Tang G, Rastogi P, Geyer CE Jr., Robidoux A, Atkins JN, Baez-Diaz L, Brufsky AM, Mehta RS, Fehrenbacher L, Young JA, Senecal FM, Gaur R, Margolese RG, Adams PT, Gross HM, Costantino JP, Swain SM, Mamounas EP, Wolmark N: Bevacizumab added to neoadjuvant chemotherapy for breast cancer. N Engl J Med 2012, 366:310–320
63. Warm M, Kates R, Grosse-Onnebrink E, Stoff-Khalili M, Hoopmann M, Mallmann P, Thomas A, Harbeck N: Impact of tumor biology, particularly triple-negative status, on response to preoperative sequential, dose-dense epirubicin, cyclophosphamide followed by docetaxel in breast cancer. Anticancer Res 2010, 30: 4251–4259
64. Yardley DA, Peacock NW, Dickson NR, White MB, Vazquez ER, Foust JT, Grapski R, Hendricks LK, Scott WL, Hainsworth JD: A phase II trial of neoadjuvant gemcitabine, epirubicin, and docetaxel as primary treatment of patients with locally advanced or inflammatory breast cancer. Clin Breast Cancer 2010, 10:217–223
65. Zambetti M, Mansutti M, Gomez P, Lluch A, Dittrich C, Zamagni C, Ciruelos E, Pavesi L, Semiglazov V, De Benedictis E, Gaion F, Bari M, Morandi P, Valagussa P, Luca G: Pathological complete

response rates following different neoadjuvant chemotherapy regimens for operable breast cancer according to ER status, in two parallel, randomized phase II trials with an adaptive study design (ECTO II). Breast Cancer Res Treat 2012, 132:843–851
66. Iwata H, Sato N, Masuda N, Nakamura S, Yamamoto N, Kuroi K, Kurosumi M, Tsuda H, Akiyama F, Ohashi Y, Toi M: Docetaxel followed by fluorouracil/epirubicin/cyclophosphamide as neoadjuvant chemotherapy for patients with primary breast cancer. Jpn J Clin Oncol 2011, 41:867–875
67. Medioni J, Huchon C, Le Frere-Belda MA, Hofmann H, Bats AS, Eme D, Andrieu JM, Oudard S, Lecuru F, Levy E: Neoadjuvant dose-dense gemcitabine plus docetaxel and vinorelbine plus epirubicin for operable breast cancer: improved prognosis in triple-negative tumors. Drugs R D 2011, 11:147–157
68. Byrski T, Gronwald J, Huzarski T, Grzybowska E, Budryk M, Stawicka M, Mierzwa T, Szwiec M, Wisniowski R, Siolek M, Dent R, Lubinski J, Narod S: Pathologic complete response rates in young women with BRCA1-positive breast cancers after neoadjuvant chemotherapy. J Clin Oncol 2010, 28:375–379
69. Silver DP, Richardson AL, Eklund AC, Wang ZC, Szallasi Z, Li Q, Juul N, Leong CO, Calogrias D, Buraimoh A, Fatima A, Gelman RS, Ryan PD, Tung NM, De Nicolo A, Ganesan S, Miron A, Colin C, Sgroi DC, Ellisen LW, Winer EP, Garber JE: Efficacy of neoadjuvant Cisplatin in triple-negative breast cancer. J Clin Oncol 2010, 28:1145–1153
70. Yerushalmi R, Hayes MM, Gelmon KA, Chia S, Bajdik C, Norris B, Speers C, Hassell P, O'Reilly SE, Allan S, Shenkier TN: A phase II trial of a neoadjuvant platinum regimen for locally advanced breast cancer: pathologic response, long-term follow-up, and correlation with biomarkers. Clin Breast Cancer 2009, 9:166–172
71. Alba E, Chacon JI, Lluch A, Anton A, Estevez L, Cirauqui B, Carrasco E, Calvo L, Segui MA, Ribelles N, Alvarez R, Sanchez-Munoz A, Sanchez R, Garcia-Asenjo JA, Rodriguez-Martin C, Escudero MJ, Albanell J: A randomized phase II trial of platinum salts in basal-like breast cancer patients in the neoadjuvant setting. Results from the GEICAM/2006-03, multicenter study. Breast Cancer Res Treat 2012, 136:487–493
72. Sikov WM, Dizon DS, Strenger R, Legare RD, Theall KP, Graves TA, Gass JS, Kennedy TA, Fenton MA: Frequent pathologic complete responses in aggressive stages II to III breast cancers with every-4-week carboplatin and weekly paclitaxel with or without trastuzumab: a Brown University Oncology Group Study. J Clin Oncol 2009, 27:4693–4700
73. Chang HR, Glaspy J, Allison MA, Kass FC, Elashoff R, Chung DU, Gornbein J: Differential response of triple-negative breast cancer to a docetaxel and carboplatin-based neoadjuvant treatment. Cancer 2010, 116:4227–4237
74. Chen XS, Nie XQ, Chen CM, Wu JY, Wu J, Lu JS, Shao ZM, Shen ZZ, Shen KW: Weekly paclitaxel plus carboplatin is an effective nonanthracycline-containing regimen as neoadjuvant chemotherapy for breast cancer. Ann Oncol 2010, 21:961–967
75. Sirohi B, Arnedos M, Popat S, Ashley S, Nerurkar A, Walsh G, Johnston S, Smith IE: Platinum-based chemotherapy in triple-negative breast cancer. Ann Oncol 2008, 19:1847–1852
76. Sirohi B, A'Hern R, Coombes G, Bliss JM, Hickish T, Perren T, Crawford M, O'Brien M, Iveson T, Ebbs S, Skene A, Laing R, Smith IE: A randomised comparative trial of infusional ECisF versus conventional FEC as adjuvant chemotherapy in early breast cancer: the TRAFIC trial. Ann Oncol 2010, 21:1623–1629
77. Turner NC, Tutt AN: Platinum chemotherapy for BRCA1-related breast cancer: do we need more evidence? Breast Cancer Res 2012, 14:115
78. Vollebergh MA, Lips EH, Nederlof PM, Wessels LF, Schmidt MK, van Beers EH, Cornelissen S, Holtkamp M, Froklage FE, de Vries EG, Schrama JG, Wesseling J, van de Vijver MJ, van Tinteren H, de Bruin M, Hauptmann M, Rodenhuis S, Linn SC: An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. Ann Oncol 2011, 22:1561–1570
79. O'Shaughnessy J, Osborne C, Pippen JE, Yoffe M, Patt D, Rocha C, Koo IC, Sherman BM, Bradley C: Iniparib plus chemotherapy in metastatic triple-negative breast cancer. N Engl J Med 2011, 364:205–214
80. Tutt A, Robson M, Garber JE, Domchek SM, Audeh MW, Weitzel JN, Friedlander M, Arun B, Loman N, Schmutzler RK, Wardley A, Mitchell G, Earl H, Wickens M, Carmichael J: Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with BRCA1 or BRCA2 mutations and advanced breast cancer: a proof-of-concept trial. Lancet 2010, 376:235–244
81. Gelmon KA, Tischkowitz M, Mackay H, Swenerton K, Robidoux A, Tonkin K, Hirte H, Huntsman D, Clemons M, Gilks B, Yerushalmi R, Macpherson E, Carmichael J, Oza A: Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. Lancet Oncol 2011, 12:852–861
82. Kummar S, Ji J, Morgan R, Lenz HJ, Puhalla SL, Belani CP, Gandara DR, Allen D, Kiesel B, Beumer JH, Newman EM, Rubinstein L, Chen A, Zhang Y, Wang L, Kinders RJ, Parchment RE, Tomaszewski JE, Doroshow JH: A phase I study of veliparib in combination with metronomic cyclophosphamide in adults with refractory solid tumors and lymphomas. Clin Cancer Res 2012, 18:1726–1734
83. Ibrahim YH, Garcia-Garcia C, Serra V, He L, Torres-Lockhart K, Prat A, Anton P, Cozar P, Guzman M, Grueso J, Rodriguez O, Calvo MT, Aura C, Diez O, Rubio IT, Perez J, Rodon J, Cortes J, Ellisen LW, Scaltriti M, Baselga J: PI3K inhibition impairs BRCA1/2 expression and sensitizes BRCA-proficient triple-negative breast cancer to PARP inhibition. Cancer Discov 2012, 2:1036–1047
84. Speers C, Tsimelzon A, Sexton K, Herrick AM, Gutierrez C, Culhane A, Quackenbush J, Hilsenbeck S, Chang J, Brown P: Identification of novel kinase targets for the treatment of estrogen receptor-negative breast cancer. Clin Cancer Res 2009, 15:6327–6340
85. Dutta B, Pusztai L, Qi Y, Andre F, Lazar V, Bianchini G, Ueno N, Agarwal R, Wang B, Shiang CY, Hortobagyi GN, Mills GB, Symmans WF, Balazsi G: A network-based, integrative study to identify core biological pathways that drive breast cancer clinical subtypes. Br J Cancer 2012, 106:1107–1116
86. Carey LA, Rugo HS, Marcom PK, Mayer EL, Esteva FJ, Ma CX, Liu MC, Storniolo AM, Rimawi MF, Forero-Torres A, Wolff AC, Hobday TJ, Ivanova A, Chiu WK, Ferraro M, Burrows E, Bernard PS, Hoadley KA, Perou CM, Winer EP: TBCRC 001: randomized phase II study of cetuximab in combination with carboplatin in stage IV triple-negative breast cancer. J Clin Oncol 2012, 30:2615–2623
87. Duncan JS, Whittle MC, Nakamura K, Abell AN, Midland AA, Zawistowski JS, Johnson NL, Granger DA, Jordan NV, Darr DB, Usary J, Kuan PF, Smalley DM, Major B, He X, Hoadley KA, Zhou B, Sharpless NE, Perou CM, Kim WY, Gomez SM, Chen X, Jin J, Frye SV, Earp HS, Graves LM, Johnson GL: Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. Cell 2012, 149:307–321
88. Horiuchi D, Kusdra L, Huskey NE, Chandriani S, Lenburg ME, Gonzalez-Angulo AM, Creasman KJ, Bazarov AV, Smyth JW, Davis SE, Yaswen P, Mills GB, Esserman LJ, Goga A: MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. J Exp Med 2012, 209:679–696
89. Jeong H, Ryu YJ, An J, Lee Y, Kim A: Epithelial-mesenchymal transition in breast cancer correlates with high histological grade and triple-negative phenotype. Histopathology 2012, 60:E87–E95
90. Tomaskovic-Crook E, Thompson EW, Thiery JP: Epithelial to mesenchymal transition and breast cancer. Breast Cancer Res 2009, 11:213
91. Finn RS, Bengala C, Ibrahim N, Roche H, Sparano J, Strauss LC, Fairchild J, Sy O, Goldstein LJ: Dasatinib as a single agent in triple-negative breast cancer: results of an open-label phase 2 study. Clin Cancer Res 2011, 17:6905–6913
92. Bissell MJ, Hines WC: Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression Nat Med 2011, 17:320–329