Insights into Molecular Classifications of Triple-Negative Breast Cancer: Improving Patient Selection for Treatment

Ana C. Garrido-Castro[1,2], Nancy U. Lin[1,2], and Kornelia Polyak[1,2]

ABSTRACT

Triple-negative breast cancer (TNBC) remains the most challenging breast cancer subtype to treat. To date, therapies directed to specific molecular targets have rarely achieved clinically meaningful improvements in outcomes of patients with TNBC, and chemotherapy remains the standard of care. Here, we seek to review the most recent efforts to classify TNBC based on the comprehensive profiling of tumors for cellular composition and molecular features. Technologic advances allow for tumor characterization at ever-increasing depth, generating data that, if integrated with clinical–pathologic features, may help improve risk stratification of patients, guide treatment decisions and surveillance, and help identify new targets for drug development.

Significance: TNBC is characterized by higher rates of relapse, greater metastatic potential, and shorter overall survival compared with other major breast cancer subtypes. The identification of biomarkers that can help guide treatment decisions in TNBC remains a clinically unmet need. Understanding the mechanisms that drive resistance is key to the design of novel therapeutic strategies to help prevent the development of metastatic disease and, ultimately, to improve survival in this patient population.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and the second most common cause of cancer mortality in women worldwide (1). Breast tumors that are immunohistochemically characterized by lack of estrogen receptor (ER), progesterone receptor (PR), and HER2 (also defined by lack of *HER2* amplification by FISH) are classified as triple-negative breast cancer (TNBC) and account for approximately 15% to 20% of all breast carcinomas (2). Compared with hormone receptor–positive or HER2-positive disease, TNBC has a highly aggressive clinical course, with earlier age of onset, greater metastatic potential, and poorer clinical outcomes as shown by the higher relapse and lower survival rates (2, 3). The molecular mechanisms that drive TNBC recurrence have not been fully elucidated. Consequently, to date, targeted therapies have not significantly improved survival in patients with TNBC, and chemotherapy remains the standard of care. Although many patients with early stages of TNBC are cured with chemotherapy, in those who develop metastatic disease, median overall survival (OS) with current treatment options is 13 to 18 months (4).

Major effort has been devoted over the past decade to classify TNBC into distinct clinical and molecular subtypes that could guide treatment decisions. Characterization of genomic, transcriptomic, proteomic, epigenomic, and microenvironmental alterations has expanded our knowledge of TNBC. Here, we review the most recent innovations in TNBC molecular taxonomy, the complex interaction between these classifications (Fig. 1), and their potential therapeutic implications.

TNBC AND INTRINSIC BREAST CANCER SUBTYPES

Early transcriptomic profiling of breast cancer using microarrays classified tumors into five intrinsic subtypes: luminal-A, luminal-B, HER2-enriched, basal-like, and a normal breast–like group (5, 6). Although all intrinsic subtypes can be found within immunohistochemically defined triple-negative disease, basal-like tumors exhibit the greatest overlap with TNBC. Between 50% and 75% of TNBC have basal phenotype, and approximately 80% of basal-like tumors are ER-negative/HER2-negative (Fig. 2; refs. 7, 8). Characterization

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts. [2]Department of Medicine, Harvard Medical School, Boston, Massachusetts.

Corresponding Author: Kornelia Polyak, Dana-Farber Cancer Institute, 450 Brookline Avenue, Boston, MA 02215. Phone: 617-632-2106; Fax: 617-582-8490; E-mail: kornelia_polyak@dfci.harvard.edu

doi: 10.1158/2159-8290.CD-18-1177

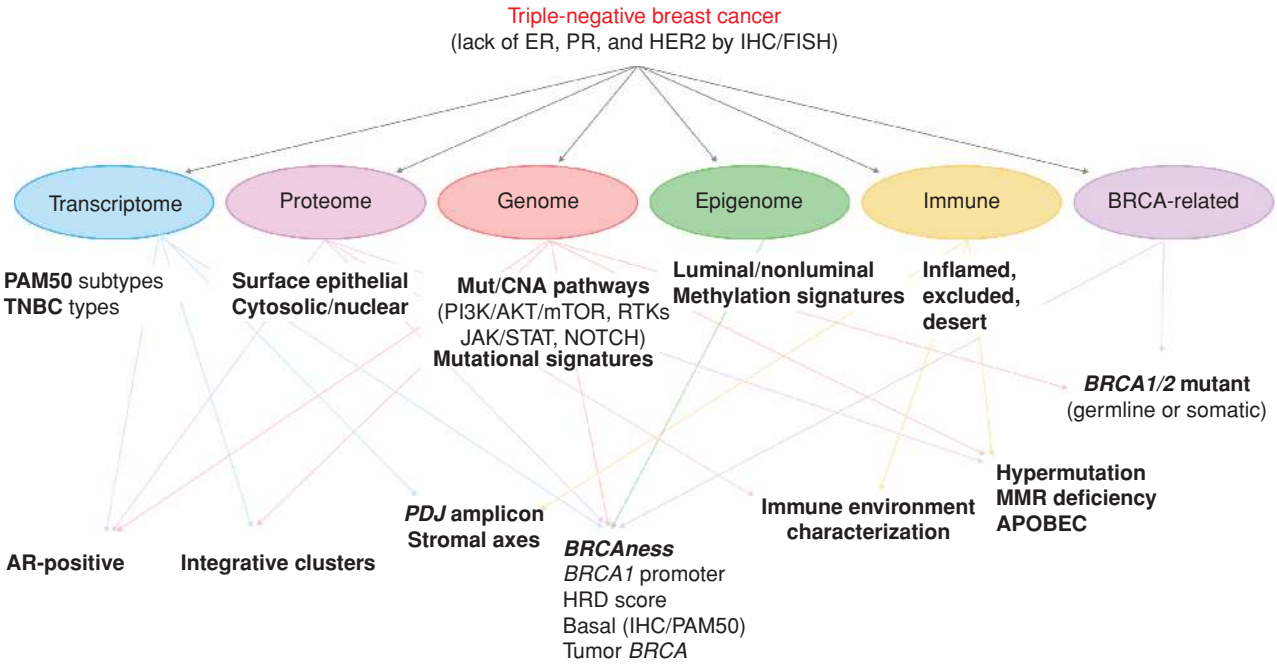

Figure 1. Overview of the complex interactions among molecular classifications of TNBC based on genomic, transcriptomic, proteomic, epigenomic, and immune characterization of the tumor and its microenvironment. ER, estrogen receptor; PR, progesterone receptor; Mut, mutant; RTK, receptor tyrosine kinase; MMR, mismatch repair; CNA, copy-number alteration; AR, androgen receptor; HRD, homologous recombination deficiency; IHC, immunohistochemistry.

of intrinsic subtypes using a 50-gene assay (established as the PAM50 subtype predictor) has provided independent predictive information of pathologic complete response (pCR) to neoadjuvant therapy across all subtypes (9), but when restricting analyses to TNBC, none of the PAM50 signatures at the time of diagnosis have significantly correlated with pCR (10). In basal-like TNBC, low expression of the luminal-A signature and high expression of the proliferation score were both significantly associated with pCR (10). High expression of cell cycle–related genes (e.g., *CCNE* and *FANCA*) and low levels of estrogen signaling–related genes (e.g., *FOXA1* and *PGR*) were associated with pCR, whereas high expression of epithelial–mesenchymal transition (EMT) genes (e.g., *TWIST1* and *ZEB1*) was significantly enriched in residual disease (10). Again, in the adjuvant setting, no significant gene-signature predictors of disease-free survival (DFS) have been found in TNBC (10). However, in basal-like TNBC in GEICAM/9906, and in basal-like tumors treated with adjuvant chemotherapy in the METABRIC data set and in CALGB/9741, the two previously identified signatures (low luminal-A and high proliferation score) predicted improved DFS and recurrence-free survival (RFS).

PAM50-defined subtypes have not yet been validated as predictors of benefit to individual chemotherapeutic agents in TNBC. An increase in pCR rates from 47% to 61% was noted with the addition of carboplatin to neoadjuvant therapy in patients with basal-like TNBC in CALGB/40603 (11), although this improvement did not differ from that observed in the overall population after incorporating the small number of non–basal-like tumors. In the metastatic setting, carboplatin and docetaxel achieved comparable objective response rates (ORR) in basal-like tumors in the TNT trial (32.5% vs. 31.0%, respectively; $P = 0.87$; ref. 12). Of note, though a significant interaction was observed between PAM50 subgroups and treatment arm, this was driven by the unexpected finding of greater efficacy of docetaxel compared with carboplatin in non–basal-like tumors (ORR, 72.2% vs. 16.7%; $P = 0.002$; ref. 12). Further studies prospectively evaluating taxanes and other agents in predefined subgroups are needed to confirm any differential activity in non–basal-like TNBC.

Additional gene-expression analyses later revealed the presence of another intrinsic subtype, claudin-low, present in 7% to 14% of all breast cancers (6). Approximately 70% of claudin-low tumors are TNBC, with high representation of metaplastic and medullary breast carcinomas. Although claudin-low and basal-like subtypes share low luminal and HER2 gene expression, claudin-low tumors do not highly express proliferation genes. They are uniquely characterized by low levels of cell adhesion proteins and elevated expression of immune-related genes (e.g., *CD4* and *CD79a*). These mesenchymal features (including elevated expression of CD44, vimentin, and N-cadherin) and low epithelial differentiation (low *CD24* gene expression) resemble a mammary stem cell–like phenotype ($CD44^{+}CD24^{-/lo}$) that can be acquired by the EMT (6). In retrospectives studies, claudin-low tumors were associated with lower (39%) pCR rates compared with basal-like subtype (73%), and worse prognosis than luminal-A tumors but similar survival as luminal-B, HER2-enriched, or basal-like tumors (6). Formation of cancer stem cells is induced by TGFβ in claudin-low cell lines (13), and in chemotherapy-resistant TNBC, TGFβ signaling and other stem cell markers are overexpressed (14). Thus, inhibition of TGFβ

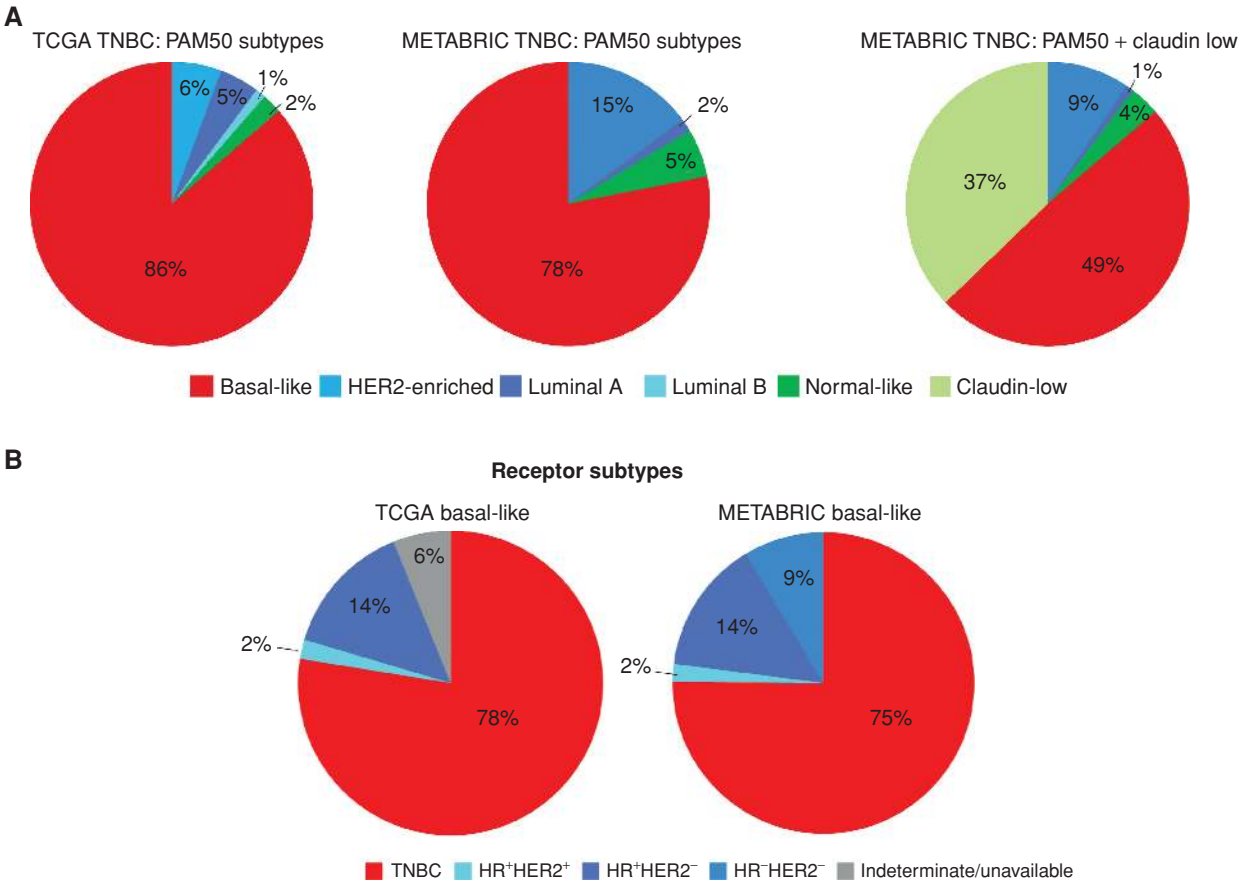

Figure 2. Distribution of intrinsic subtypes among TNBC and distribution of TNBC among basal-like breast cancer. **A,** Comparison of distribution of intrinsic subtypes defined by PAM50 and PAM50 + claudin-low in The Cancer Genome Atlas (TCGA) and METABRIC data sets in TNBC. TNBC was defined as clinical ER-, PR-, and HER2-negative testing per IHC. In TCGA, 88 TNBC samples had available PAM50 data. The distribution of intrinsic subtypes was basal-like (86%), HER2-enriched (6%), luminal A (5%), luminal B (1%), and normal-like (2%). In METABRIC, 320 TNBC samples had available intrinsic subtype data. When including claudin-low in the PAM50 predictor, the distribution of subtypes was basal-like (49%), claudin-low (37%), HER2-enriched (9%), normal-like (4%), luminal A (1%), and luminal B (0%). When excluding the 119 samples with claudin-low subtype, the distribution of subtypes was basal-like (78%), HER2-enriched (15%), normal-like (5%), luminal A (2%), and luminal B (0%). **B,** Comparison of distribution of breast cancer subtype according to receptor status defined by IHC in TCGA and METABRIC data sets in basal-like breast cancer. Of 98 basal-like breast cancers in TCGA, 78% were TNBC per IHC. Of 209 basal-like breast cancers (PAM50 + claudin-low classifier) in METABRIC, 75% were TNBC. Figures generated by reanalysis of publicly available studies (refs. 22, 36, 37) using cBioPortal (refs. 150, 151).

signaling may represent a potential therapeutic strategy to help prevent the development of chemorefractory disease, particularly in the claudin-low subtype.

MOLECULAR DEFINITION OF TNBC HETEROGENEITY

With evolving transcriptomic studies, the heterogeneity of TNBC has been further dissected. Lehmann and colleagues analyzed 21 public microarray data sets filtered for TNBC based on *ESR1, PGR*, and *ERBB2* expression and identified seven clusters within TNBC: basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal-stem-like (MSL), luminal androgen receptor (LAR), and an unstable cluster (UNS; ref. 15). These subtypes are characterized by distinct patterns of molecular alterations, in terms of RNA expression, somatic mutations, and copy-number variations, that tend to cluster in genes implicated in specific pathways. The BL1 subtype, enriched in genes involved in DNA-damage response and cell-cycle regulation [including the highest rate of *TP53* mutations (92%), high gain/amplifications of *MYC, CDK6*, or *CCNE1*, and deletions in *BRCA2, PTEN, MDM2*, and *RB1*; ref. 16), and the BL2 subtype, with high levels of growth factor signaling and metabolic pathway activity, share a highly proliferative phenotype that correlates with improved pCR with mitotic inhibitors, such as taxanes. Genes involved in antigen processing and presentation, immune cell and cytokine signaling (e.g., JAK/STAT, TNF, and NFκB) pathways are highly expressed in the IM subtype. Mesenchymal-like TNBC subtypes, M and MSL, display similar expression profiles related to cell motility, differentiation, and EMT, but are discernible by the unique enrichment in MSL of angiogenesis- and stem cell–associated genes, and low claudin expression. Finally, despite ER negativity, the LAR subtype displays a luminal pattern of gene expression (e.g., high levels of *FOXA1, GATA3, SPDEF*, and *XBP1*), with elevated

mRNA and protein levels of androgen receptor (AR), overlapping in 82% of cases with luminal-A– or luminal-B–intrinsic subtypes. Thus, not surprisingly, LAR tumors are enriched in mutations in *PIK3CA* (55%), *KMT2C* (19%), *CDH1* (13%, in conjunction with a higher prevalence of invasive lobular histology), *NF1* (13%), and *AKT1* (13%; ref. 16). The seven-subtype classification independently predicted pCR, but not distant metastasis-free or overall survival in a retrospective analysis of patients with TNBC treated with neoadjuvant chemotherapy (17). Median OS was highest in the LAR and BL1 subtypes, despite low pCR rate in the LAR group. Follow-up *in vitro* studies with representative cell lines of TNBC subtypes demonstrated differential drug sensitivity that, if validated, may have clinically relevant implications (15). Of note, all seven clusters were not detected in an independent analysis of five data sets of IHC-identified TNBC, as opposed to gene expression–defined TNBC (15). Even across other studies in which TNBC was identified using mRNA expression, reproducibility of the BL2 and UNS subtypes has not been consistent (16, 17).

In a follow-up study, by performing histologic assessment and laser microdissection prior to RNA isolation and gene-expression analysis, Lehmann and colleagues confirmed that the presence of stromal cells in tumor specimens—such as infiltrating lymphocytes and tumor-associated mesenchymal cells—influences the definition of the IM and MSL subtypes, respectively (18). This led to a revised classification, TNBCtype4, into four stable transcriptional subtypes (BL1, BL2, M, and LAR) that significantly differ not only in prognosis and response to chemotherapy, but also in initial presentation and patterns of recurrence, where regional nodal involvement is more common in LAR TNBC and metastatic recurrences have tropism to the lung in M subtypes and to the bone in LAR subtypes. Similar to the seven-subtype classification, response to neoadjuvant chemotherapy (platinum- and taxane-based regimen) is significantly associated with TNBCtype4 subtypes ($P = 0.027$), with the highest and lowest pCR rates reported in BL1 (65.6%) and LAR (21.4%), respectively (19). These findings highlight a major limitation of classifiers defined based on the profiling of bulk tumors that cannot distinguish between tumor and stromal cells and support the increasing use of single-cell techniques to improve the characterization of the tumor and its microenvironment. In fact, single-cell RNA sequencing has demonstrated the presence of multiple subtypes within most primary TNBC tumors, suggesting that the dominant signature identified through bulk sequencing may not accurately inform underlying biological processes, including interactions between malignant and normal stromal cell types (20). Differences in the prevalence of intratumoral heterogeneity between TNBC and ER-positive breast cancer could partly explain the challenges to date to apply commercially available gene-expression assays in routine clinical practice to provide prognostic and predictive information in TNBC.

Additional efforts to distinguish stable molecular TNBC phenotypes using gene-expression profiling include the classification into four subtypes by Burstein and colleagues: LAR, mesenchymal (MES), basal-like immune suppressed (BLIS), and basal-like immune activated (BLIA; ref. 21). Interestingly, the BLIS subtype exhibited the worst prognosis, and the BLIA subgroup conferred the best outcome in terms of DFS. In addition, specific DNA copy-number variations were identified in each subtype, such as focal gains on 11q13 (*CCND1*, FGF family) in the LAR subtype or BLIA-specific overexpression of *CTLA4*. In another analysis that integrated somatic copy-number variations and gene-expression profiles of primary breast tumors of any IHC subtype in the METABRIC data set, 10 integrative clusters were identified, where IntClust 10 exhibited the greatest overlap with PAM50 basal-like tumors and was characterized by 5 loss/8q gain/10p gain/12p gain (22). As exemplified by studies assessing the overlap between these different gene-expression classifications, a high correlation has been described between PAM50-defined basal-like, Lehmann BL1/BL2, and Baylor BLIA/BLIS subtypes (21–23), emphasizing the high stability of the basal subtype across TNBC. These studies also highlight the inherent problems associated with the TNBC definition, because it does not reflect a clear molecular entity. What seems clear is that luminal (ER-positive or AR-positive) and nonluminal (basal and mesenchymal) tumors have very different evolutionary paths, and this is in part likely driven by their normal cell-of-origin reflected in distinct epigenetic profiles. Thus, improved classifications based on epigenetic profiles and quantitative measures of intratumoral heterogeneity may lead to a better definition of clinically relevant TNBC subtypes.

ANDROGEN RECEPTOR–POSITIVE TNBC

As detailed above, a luminal phenotype, characterized by expression of the AR and luminal lineage-driving transcription factors, has been consistently identified across several studies in TNBC. In core-basal tumors, the prevalence of AR positivity defined by ≥1% of tumor cell nuclei IHC staining has been reported to be 32% (24). Interestingly, other studies have suggested that LAR tumors are characterized by a quiescent cell state (25), as opposed to rapidly proliferative basal tumors, raising the question of the optimal method of testing for AR positivity and possibly lack of a robust approach due to limited sample size. Altogether, this has prompted interest in exploring the role of antiandrogens in this subgroup. *In vivo* studies have shown that tumors derived from LAR cell lines (e.g., MDA-MB-453, SUM185PE, and CAL-148) are highly sensitive to the AR antagonist bicalutamide (15). In phase II single-arm trials conducted in patients with metastatic AR-positive, ER/PR-negative breast cancer, bicalutamide and enzalutamide demonstrated stable disease at 6 months of 19% and 28%, respectively, though no objective responses were observed (26, 27). Abiraterone acetate and prednisone achieved a similar 20% clinical benefit rate (CBR) at 6 months, and although the study failed to meet the prespecified >25% cutoff necessary to reject the null hypothesis, prolonged responses were observed (range, 6.4–23.4 months; ref. 28). An androgen-driven genomic signature, Dx, predicted improved OS with enzalutamide (29), and this led to the design of a phase III trial comparing enzalutamide, paclitaxel, and the combination in selected Dx-positive advanced TNBC (NCT02929576).

Similar to luminal tumors, strategies to enhance the effectiveness of hormone receptor blockade have been pursued in AR-positive TNBC. Enrichment in *PIK3CA* mutations has been described in triple-negative tumors that are AR-positive (36%–40%) by IHC compared with AR-negative (4%–9%; refs. 30, 31), the majority of which are located in the kinase domain

H1047 mutational hotspot and co-occur with amplification of the *PIK3CA* locus (30). Combination of PI3K/mTOR inhibition and AR antagonism has demonstrated synergistic activity in AR-positive TNBC preclinical models, and a phase I trial is planned to explore enzalutamide plus alpelisib, an α-specific PI3K inhibitor, in patients with AR-positive, PTEN^{lo} (IHC 0%) TNBC (NCT03207529). Additional studies have revealed that, in contrast to basal-like and mesenchymal subtypes, LAR TNBC cell lines are highly sensitive to CDK4/6 inhibitors, with comparable sensitivity to that observed in the ER-positive MCF7 cell line (25). LAR cell lines exhibit lower transcriptomic levels of *CCNE1* and *CDK2* compared with basal-like TNBC and, thus, are dependent on CDK4/6 to phosphorylate RB1 and reenter the cell cycle. *In vitro* PI3K inhibition decreases postmitotic CDK2 activity in *PIK3CA*-mutant TNBC, suggesting potential sensitization to CDK4/6 inhibitors, including in non-LAR TNBC (25); this has provided the rationale for the ongoing clinical trial testing palbociclib combined with either taselisib or pictilisib in *PIK3CA*-mutant ER-negative breast cancer (NCT02389842).

PROTEIN MARKERS IN TNBC FOR TARGETED ANTIBODY–DRUG CONJUGATES

Isolation of glycoproteins on the surface of epithelial cancer cells has triggered the development of antibody–drug conjugates (ADC) designed to improve delivery of elevated concentrations of cytotoxic drugs to cells expressing these molecules. Many of these targets are not necessarily cancer drivers or specific to breast cancer; instead, they require differential protein expression in malignant versus normal cells. Interestingly, several ADC have demonstrated encouraging activity in TNBC. Sacituzumab govitecan (IMMU-132) is an antibody–SN-38 conjugate targeting TROP2, which is expressed in almost 90% of TNBC (32). In patients with heavily pretreated metastatic TNBC, IMMU-132 achieved an ORR of 30%, and median progression-free survival (PFS) and OS were 6.0 and 16.6 months, respectively. LIV-1 is a transmembrane protein with metalloprotease activity expressed in 68% of metastatic TNBC samples. Ladiratuzumab vedotin (SGN-LIV1A), with monomethyl-auristatin-E (MMAE) as the payload, yielded a 25% ORR in a similar population of patients with TNBC, and median PFS was 11 months (33). Significant expression of glycoprotein-NMB (gpNMB), defined as staining ≥25% of tumor epithelial cells, is present in approximately 40% of TNBC, and in this subgroup, glembatumumab vedotin (CDX-011, an ADC that binds to gpNMB to deliver MMAE) achieved 40% ORR versus 0% with investigator's choice of therapy (34). However, when compared with capecitabine in preselected gpNMB-overexpressing metastatic TNBC in the METRIC phase II trial, glembatumumab vedotin failed to demonstrate improved PFS, ORR, or OS, leading to discontinuation of the development of this ADC (Celldex's METRIC Study Press release, April 16, 2018; https://globenewswire.com/news-release/2018/04/16/1471890/0/en/Celldex-s-METRIC-Study-in-Metastatic-Triple-negative-Breast-Cancer-Does-Not-Meet-Primary-Endpoint.html). SGN-LIV1A is currently being evaluated in phase II trials, and IMMU-132 has advanced to phase III development (ASCENT: NCT02574455). Given the high prevalence of many of these markers in TNBC, IHC confirmation may not be necessary prior to starting therapy, but other proteins overexpressed less frequently may require prescreening efforts to help identify patients who are more likely to benefit from ADC.

SOMATIC GENETIC ALTERATIONS IN TNBC

Cancers harbor numerous somatic genetic alterations, though only a small proportion of them confer clear fitness advantage, also known as "cancer drivers" (35). Large-scale exome and targeted sequencing studies in primary breast tumors have revealed the presence of many alterations in putative cancer-driver genes in TNBC (36–38). The average mutation rate in basal-like breast cancer is among the highest in breast tumors, 1.68 mutations per megabase (Mb); tumors that reach rates greater than three standard deviations above the mean (>4.68 mutations/Mb) are considered hypermutated (36). Different genomic classifications in breast cancer have been proposed by grouping next-generation sequencing (NGS)–detected alterations in known cancer-driver genes according to the intracellular pathways in which they are involved, such as PI3K/AKT and RAS/MAPK signaling, DNA-damage repair, and cell-cycle or transcriptional regulation (Table 1; refs. 36, 37, 39).

Most somatic mutations in TNBC occur in tumor suppressor genes (e.g., *TP53, RB1*, and *PTEN*), which have not been successfully targeted therapeutically to date. Although less prevalent, oncogenic alterations in the PI3K/AKT pathway have also been described in basal-like breast cancer (*PIK3CA* mutation, 7%; *AKT3* amplification, 28%; *PTEN* mutation or loss, 35%; ref. 36), potentially qualifying patients for clinical trials with matched therapies. Consistent with findings in untreated triple-negative tumors, targeted sequencing of residual disease post–neoadjuvant chemotherapy showed that >90% of patients had at least one altered pathway (39). However, only three alterations were found to be significantly prognostic for OS (*JAK2* amplification, *BRCA1* truncation or mutation: predicted poor OS; *PTEN* alteration: better OS). Drugs that inhibit these pathways have been explored in clinical trials in TNBC, mostly in combination with other therapies due to limited single-agent activity (Table 2).

Considering the underlying complexity of the genomic landscape of TNBC, analysis of single mutations in a putative driver or known oncogenic pathway is likely insufficient (40). Different processes, such as age, exposure to carcinogens, DNA replication errors, defects in DNA repair, and the family of APOBEC cytidine deaminases, imprint patterns of mutations known as mutational signatures on the cancer genome. Whole-genome sequencing of 21 breast tumors initially showed the presence of five different mutational signatures in breast cancer, including focal hypermutation and APOBEC (40). More recently, the expanded analysis of 560 breast cancers revealed somatic base substitutions, indels, rearrangements, and copy-number alterations in 93 candidate driver genes (41). Of the 10 most frequently mutated genes that accounted for 62% of drivers in the overall set, *TP53, MYC, PTEN, ERBB2*, and *RB1* appeared enriched in the ER-negative cohort. Application of mathematical algorithms discriminated 12 base-substitution signatures (including the five previously identified signatures), two indel signatures, and six rearrangement signatures. Large

Table 1. Classifications according to potentially targetable pathways based on exome or targeted sequencing

TCGA (basal-like; ref. 36)	Genomic alteration (frequency, %)
p53 pathway	*TP53* mut (84), gain of *MDM2* (14)
PI3K/PTEN pathway	*PTEN* mut/loss (35), *INPP4B* loss (30), *PIK3CA* mut (7)
RB1 pathway	*RB1* mut/loss (20), *CCNE1* amp (9), high expression of *CDKN2A*, low *RB1* expression
METABRIC (ER-negative; ref. 37)	**Mutated gene (frequency, %)**
AKT signaling	*PIK3CA* (24), *AKT1* (2), *PTEN* (4), *PIK3R1* (3), *FOXO3* (1)
Cell-cycle regulation	*RB1* (4), *CDKN2A* (1)
Chromatin function	*KMT2C* (9), *ARID1A* (3), *NCOR1* (2), *PBRM1* (3), *KDM6A* (2)
DNA damage and apoptosis	*TP53* (77), *BRCA1* (3), *BRCA2* (3)
MAPK signaling	*NF1* (4), *MAP3K1* (3), *MAP2K4* (1), *KRAS* (1)
Tissue organization	*CDH1* (3), *MLLT4* (3)
Transcription regulation	*TBX3* (2), *RUNX1* (2), *GATA3* (1), *ZFP36L1* (1), *MEN1* (1)
Ubiquitination	*USP9X* (3), *BAP1* (3)
Other	*ERBB2* (3), *SMAD4* (1), *AGTR2* (1)
Residual disease post–neoadjuvant chemotherapy (triple-negative; ref. 39)	**Genomic alteration (frequency, %)**
Cell cycle	*RB1* loss (11), *CDKN2A* loss (9), *CDKN2B* loss, *CDK4* amp, *CDK6* amp (6), *CCND1* amp (6), *CCND2* amp (6), CCND3 amp (6), *CCNE1* amp (6), *AURKA* amp
PI3K/mTOR pathway	*PTEN* mut/loss (16), *PIK3CA* mut/amp (12), *PIK3R1* mut/amp, *AKT1* amp, *AKT2* amp, *AKT3* amp (7), *RAPTOR* amp, *RICTOR* amp, *TSC1* truncations/mut
Growth factor receptor	*IGF1R* amp (6), *EGFR* amp (4), *MET* amp, *KIT* amp, *FGFR1* amp, *FGFR2* amp, *FGFR4* amp
RAS/MAPK pathway	*KRAS* amp/gain (7), *BRAF* amp/gain, *RAF1* amp/gain, *NF1* truncations (7)
DNA repair	*BRCA1* truncations/loss/mut (11), *BRCA2* truncations/loss/mut, *ATM* mut
JAK2/STAT3 pathway	*JAK2* amp (10)

NOTE: Mut, gene mutation; gain, gene copy-number gain (<5 but more than 2 copies); amp, gene amplification (≥5 copies and/or gene-specific and centromeric probe ratio >2). The definition of copy-number gain vs. amplification is partly platform and study dependent. In general, copy-number gain ≥5 is considered an amplification, whereas copy-number gain >2 but below 5 is considered a copy-number gain. However, some studies define amplification when gene-specific vs. centromeric probe ratio is >2. Frequencies (%) of alterations are included when available.

tandem duplications (>100 kb) were associated with rearrangement signature 1, mostly found in *TP53*-mutated, triple-negative tumors with high homologous recombination–deficiency (HRD) index but without *BRCA1/2* mutations or *BRCA1* promoter hypermethylation. In contrast, 91% of cases with *BRCA1* mutation or promoter hypermethylation fell into rearrangement signature 3, characterized predominantly by small tandem duplications (<10 kb). Additional research is required to fully understand the prognostic and therapeutic implications of these signatures.

TARGETING GENETICALLY ALTERED SIGNALING PATHWAYS IN TNBC

Tumors with genetic alterations that promote activation of the PI3K pathway, found at a higher frequency in TNBC cell lines classified as LAR and mesenchymal-like, demonstrate *in vitro* and *in vivo* sensitivity to BEZ235 (a dual PI3K and mTOR inhibitor; ref. 15). Loss of PTEN and INPP4B, which also sensitizes cell lines to PI3K inhibition (42), is more common in basal-like tumors (36). Oral pan-PI3K inhibitors, such as buparlisib (BKM120), or selective p110α–PI3K inhibitors, including alpelisib (BYL719) or taselisib (GDC-0032), have shown enhanced clinical activity in ER-positive *PIK3CA*-mutant breast cancer, though fewer studies have been conducted in TNBC. In the BELLE-4 trial, patients with locally advanced or metastatic HER2-negative breast cancer were randomized to buparlisib or placebo in combination with paclitaxel as first-line therapy (43). Stratification was performed according to PI3K pathway activation, defined as *PIK3CA* mutation (detected by Sanger sequencing in exons 1, 7, 9, or 20) and/or low PTEN expression (1+ in ≤10% tumor cells). Approximately 25% of all enrolled patients (99/416) had hormone receptor–negative disease (i.e., TNBC), and of these, 36 (36.4%) had tumors considered to be PI3K-pathway activated. The addition of buparlisib to paclitaxel failed to demonstrate a significant improvement in PFS in the overall population or in those with PI3K-activated tumors. In patients with TNBC, there was a trend toward shorter median PFS with buparlisib compared with placebo (5.5 vs. 9.3 months, respectively).

Ipatasertib, a highly selective AKT inhibitor, was evaluated in the phase II randomized trial LOTUS in combination with paclitaxel as first-line metastatic treatment for unselected TNBC (44). Ipatasertib improved PFS in the intent-to-treat (ITT) population, and a similar trend was also noted in patients with PTEN-low tumors (IHC 0 in ≥50% tumor cells). In a prespecified analysis in patients with *PIK3CA/AKT/PTEN*-altered tumors (presence of activating *PIK3CA/AKT1* mutations or *PTEN*-inactivating alterations using targeted NGS), median PFS with ipatasertib plus paclitaxel was

Table 2. Efficacy of genomic-based targeted therapies in clinical trials in TNBC

Pathway	Drug	Mechanism	Patient population	Trial design (total *N* patients)	Intervention	Exploratory biomarker	Efficacy	Clinicaltrials.gov identifier
PI3K/AKT/mTOR	Buparlisib	PI3K inhibitor	Locally advanced/metastatic HER2-negative	Randomized phase II (*n* = 416; ref. 43)	Buparlisib + paclitaxel vs. placebo + paclitaxel	Stratification by PI3K pathway activation	PFS (full population): 8.0 vs. 9.2 (HR, 1.18; 95% CI, 0.82–1.68) PFS (PI3K-activated): 9.1 vs. 9.2 (HR, 1.17; 95% CI, 0.63–2.17) PFS (TNBC): 5.5 vs. 9.3 (HR, 1.86; 95% CI, 0.91–3.79)	NCT01572727
	Ipatasertib	AKT inhibitor	Locally advanced/metastatic TNBC	Randomized phase II (*n* = 124; ref. 44)	Ipatasertib + paclitaxel vs. placebo + paclitaxel	Stratification by tumor PTEN status	PFS (intent-to-treat): 6.2 vs. 4.9 (HR, 0.60; 95% CI, 0.37–0.98; *P* = 0.037) PFS (PTEN-low): 6.2 vs. 3.7 (HR, 0.59; 95% CI, 0.26–1.32; *P* = 0.18) PFS (*PIK3CA/AKT1/PTEN*-altered): 9.0 vs. 4.9 (HR, 0.44; 95% CI, 0.20–0.99; *P* = 0.041)	NCT02162719
	MK2206	AKT inhibitor	Neoadjuvant stage II–III breast cancer (any subtype)	Randomized phase II (*n* = 149; ref. 45)	Paclitaxel ± MK2206 (followed by AC)	NA	pCR (all): 35.2 vs. 21.1 pCR (TNBC): 40.2 vs. 22.4	NCT01042379
	Temsirolimus, everolimus	mTORC1 inhibitor	Metastatic metaplastic TNBC	Phase I dose expansion (*n* = 52; ref. 46)	Liposomal doxorubicin + bevacizumab + (temsirolimus or everolimus)	Exploratory analysis by PI3K pathway activation	ORR (all): 21 (95% CI, 11–35) ORR (PI3K-activated): 31 (95% CI, 16–50)	NCT00761644
	Everolimus	mTORC1 inhibitor	Neoadjuvant stage II–III TNBC	Randomized phase II (*n* = 145; ref. 47)	Cisplatin + paclitaxel + everolimus vs. cisplatin + paclitaxel + placebo	Exploratory analysis of mutated genes, TNBC subtype, Ki67, AR, and TILs	pCR (all): 36 vs. 48 (*P* = 0.41)	NCT00930930

EGFR	Panitumumab	EGFR monoclonal antibody	Locally advanced/ metastatic TNBC	Nonrandomized phase II ($n = 71$; ref. 49)	Panitumumab + carboplatin + gemcitabine	*EGFR* amp, p53 loss, PTEN loss, *PIK3CA* mut	PFS (all): 4.4 (95% CI, 3.2–5.5) PFS (EGFR amp): 3.42 (95% CI, 1.51-NR)	NCT00894504
	Cetuximab	EGFR monoclonal antibody	Neoadjuvant stage II–IIIA TNBC	Nonrandomized phase II ($n = 28$; ref. 50)	Cetuximab + docetaxel	EGFR, Ki67, cytokeratins, CD8/FOXP3	pCR (intent-to-treat): 25 (95% CI, 9–41)	NCT00600249
	Lapatinib	EGFR/HER2 inhibitor	Locally advanced/ metastatic HER2-negative	Randomized phase III ($n = 580$; refs. 51, 149)	Lapatinib + paclitaxel vs. placebo + paclitaxel	EGFR	EFS (TNBC): 4.6 vs. 4.8 (HR: 1.25; 95% CI, 0.85–1.83) EFS (TNBC $EGFR^+$): 4.2 vs. 4.9 EFS (TNBC $EGFR^-$): 5.2 vs. 4.3	NCT00075270
RAS/ MAPK	Cobimetinib	MEK1/2 inhibitor	Locally advanced/ metastatic TNBC	Open-label safety run-in ($n = 16$), randomized phase II ($n = 90$; ref. 55)	Cobimetinib + paclitaxel vs. placebo + paclitaxel	TNBC subtype, genetic alterations, PD-L1 expression	PFS (intent-to-treat): 5.5 vs. 3.8 (HR, 0.73; 95% CI, 0.43–1.24; $P = 0.25$)	NCT02322814
JAK/ STAT	Ruxolitinib	JAK1/2 inhibitor	Metastatic TNBC or IBC of any subtype	Nonrandomized phase II ($n = 21$; ref. 66)	Ruxolitinib	*JAK2* amplification, pSTAT3	PFS (all): 1.2 (95% CI, 0.97–1.84)	NCT01562873
NOTCH	PF-03084014	Gamma-secretase inhibitor	Metastatic HER2-negative breast cancer	Phase I dose-finding/ dose expansion ($n = 29$; ref. 67)	PF-03084014 + docetaxel	NA	ORR: 16 (95% CI, 4.5–36.1)	NCT01876251

NOTE: Main efficacy analyses of biomarker-selected subgroups of interest are highlighted. HR, 95% CI, and *P* values are included when available.

Abbreviations: TNBC, triple-negative breast cancer; PFS, progression-free survival (months); pCR, pathologic complete response (%); ORR, objective response rate (%); IBC, inflammatory breast cancer; AR, androgen receptor; AC, adriamycin/cyclophosphamide; HR, hazard ratio; CI, confidence interval; *N*, number; NA, data not available; NR, not reached; amp, amplification; EFS, event-free survival (months); TIL, tumor-infiltrating lymphocyte.

9 months versus 4.9 months in the placebo plus paclitaxel group, suggesting that the pathway may drive oncogenesis in a subset of patients with TNBC and providing the rationale for the ongoing randomized phase III IPATunity130 trial assessing the combination in preselected patients with activation of the PI3K pathway (NCT03337724). In addition, results from I-SPY 2, an adaptive-design trial testing novel agents in the neoadjuvant setting, showed an improvement in pCR with the addition of an allosteric AKT inhibitor, MK-2206, to standard chemotherapy in TNBC (40.2% vs. 22.4% in the control group), with a predicted 75.9% probability of success in a phase III trial (45).

Considering the higher prevalence of PI3K pathway aberrations in mesenchymal TNBC, of which 10% to 30% are metaplastic, a phase I study was conducted in this histologic subgroup to evaluate the combination of mTOR inhibition (temsirolimus or everolimus) with liposomal doxorubicin and bevacizumab (46). Responses were limited to patients with NGS aberrations in *PIK3CA, AKT*, or *PTEN*. In the neoadjuvant setting, the addition of everolimus to cisplatin and paclitaxel did not increase pCR in molecularly unselected TNBC, and exploratory analyses showed that those who achieved pCR were not enriched for mutations in the PI3K/AKT/mTOR pathway (47).

Although alterations in genes encoding components of the RAS–MAPK pathway, such as *KRAS*, *HRAS*, *BRAF*, and *MEK1/2*, are not observed as frequently in treatment-naïve TNBC as in other cancer types, EGFR is highly expressed in TNBC and can lead to upregulation of RAS–MAPK signaling (48). Across phase II and III trials, EGFR overexpression has not selected patients with TNBC who are more likely to derive benefit from EGFR-targeting monoclonal antibodies (e.g., cetuximab and panitumumab) or tyrosine kinase inhibitors (e.g., lapatinib; refs. 49–52). Synergistic effects of combined RAF and MEK inhibition have been observed in MDA-MB-231 and MDA-MB-468 TNBC cell lines (53), likely due to the presence of an activating mutation in *KRAS* (codon 13; ref. 54) and amplification of *EGFR* (55), respectively, in these cells. In addition, MYC (an oncogenic transcription factor that regulates transcriptional activity of multiple genes involved in cell proliferation, metabolism, and survival) cooperates with RAS–MAPK to drive tumor progression in MCF10A triple-negative cell lines, and MEK inhibition potently inhibits tumor growth in *MYC*-overexpressed breast cancer (39). The presence of *MYC* amplification in 40% of basal-like tumors (36) suggests that MEK inhibition may be an attractive strategy in this selected population. Recently reported results from COLET, a randomized trial evaluating the MEK1/2 inhibitor cobimetinib with paclitaxel versus placebo and paclitaxel as first-line treatment for advanced TNBC, showed a modest but not statistically significant increase in PFS (56). Selumetinib (MEK1/2 inhibitor) is also being tested in combination with vistusetib (mTORC1/2 inhibitor) in treatment-refractory solid tumors (NCT02583542). Although no objective responses were observed in the phase I trial, stable disease for >16 weeks was confirmed across tumor types, including TNBC (57).

As previously described, elevated expression of MYC has been identified across breast cancer types, with a strong association observed in triple-negative and basal-like tumors (58). Downregulation of MYC alone is insufficient to induce synthetic lethality, and several combinatorial approaches have been investigated in preclinical models (59, 60). Activation of the MYC pathway sensitizes TNBC cell lines to CDK inhibition, possibly by promoting cellular apoptosis through upregulation of BIM, a proapoptotic BCL2 family member (58). CDK inhibitors, such as dinaciclib, downregulate MYC, and a synergistic effect has been observed in combination with PARP inhibitors in MYC-driven TNBC cell lines, regardless of *BRCA* status (59). Other strategies focus on epigenetic modulation of gene transcription, such as inhibition and/or degradation of BET bromodomain proteins. BET inhibitors/degraders also induce downstream suppression of MYC and an apoptotic effect that is significantly enhanced when combined with small-molecule BCL-XL inhibitors (61, 62). Altogether, these studies encourage further clinical research targeting MYC and exploring BET inhibitors in TNBC, and several clinical trials are ongoing in this area.

JAK-mediated activation of STAT transcription factors regulates transcriptional activity of target genes, including cell-cycle regulators (63), and the IL6/JAK2/STAT3 pathway plays an important role in the proliferation of $CD44^+CD24^-$ stem cell–like breast cancer cells, enriched in basal-like tumors (64). In TNBC cell lines, activation of JAK2/STAT5 has been implicated in PI3K/mTOR resistance and can be reversed by cotargeting both pathways (65). In addition, amplifications at the *JAK2* locus (9p24) have been detected at a higher frequency in post-neoadjuvant TNBC samples compared with basal-like untreated tumors in TCGA, suggesting possible clonal selection after acquired chemotherapy resistance (39, 66). Selective inhibition of JAK2 with NVP-BSK-805 (>20-fold selectivity of JAK2 over JAK1), administered with paclitaxel, significantly reduced pSTAT3 levels and tumor volume *in vitro* and *in vivo* compared with paclitaxel alone (66). In contrast, this effect was not observed with ruxolitinib (oral JAK1 and JAK2 inhibitor, with more limited activity against JAK2/STAT3) plus chemotherapy in *JAK2*-amplified TNBC cell lines. In a phase II trial in patients with metastatic TNBC, despite on-target inhibition and decreased pSTAT3 after two cycles of treatment, no responses were observed with single-agent ruxolitinib (67).

The NOTCH signaling pathway has been implicated in the differentiation and survival of stem cell–like tumor cells and resistance to cytotoxic chemotherapy (68). Neutralizing antibodies targeting NOTCH1 significantly inhibit tumor growth in $CD44^+CD24^-$ cells and enhance the activity of docetaxel (69). This synergistic effect with taxane-based therapy is also seen with PF-03084014, a reversible selective gamma-secretase inhibitor that blocks NOTCH signaling, in patient-derived TNBC xenograft models (70). NOTCH receptor mutations and focal amplifications are enriched in the triple-negative subtype, with most mutations either clustering in the heterodimerization domain or causing disruption of the PEST-negative regulatory domain (71). These aberrations show evidence of pathway activation in TNBC and exhibit sensitivity to PF-03084014. In cell lines expressing *NOTCH1* fusion alleles, gamma-secretase inhibition also downregulates expression of *MYC* and *CCND1*, two targets whose oncogenic role has been well established in murine

NOTCH-driven tumors (72). It is estimated that 13% of TNBC may be driven by these NOTCH-oncogenic alterations. In a phase Ib trial, 29 patients with molecularly unselected treatment-refractory HER2-negative breast cancer (TNBC: $n = 26$) were treated with PF-03084014 plus docetaxel. An ORR of 16% was confirmed among evaluable patients, and median PFS was 4.1 months in the expansion cohort (68).

As illustrated by the variable efficacy across clinical trials, the role that many of these genes play as potential oncogenic drivers in TNBC remains unclear. Many of these trials have not yielded clinically relevant improvements in outcomes. Although some of these studies show promising preliminary data for targeted therapies, many have yet to be explored either in larger, randomized studies or in populations enriched for molecular alterations. Also, up to 12% of TNBC carry low mutational burden and do not harbor mutations in known candidate driver or cytoskeletal genes (73), further highlighting the heterogeneity in the mutational landscape of TNBC and the need to improve our understanding of the functional implications of many of these alterations.

GERMLINE *BRCA*-ASSOCIATED TNBC

Cancers that lack functional BRCA1 or BRCA2 have a deficiency in homologous recombination (HR) repair of DNA double-strand breaks (DSB), leading to dependence on alternative mechanisms to repair these lesions, and genomic instability (74, 75). Drugs that generate DSBs, such as alkylating agents (e.g., platinum, mitomycin C) or PARP inhibitors, cause persistent DNA damage in HR-deficient cells and, consequently, induction of cell-cycle arrest and apoptosis (76, 77). Germline mutations in *BRCA1* or *BRCA2 (BRCA1/2)* are present in approximately 10% of patients with TNBC, and confer sensitivity to these drugs (78). In the previously mentioned TNT trial, despite failure to show a significant difference in activity between treatments in the overall population ($n = 376$), in the 43 patients with deleterious *BRCA1/2* germline mutations, carboplatin significantly improved ORR compared with docetaxel (68% vs. 33.3%, $P = 0.03$) and PFS (6.8 vs. 4.4 months, interaction $P = 0.002$; ref. 12). In the neoadjuvant setting, elevated pCR rates (61%–65%) have been observed with platinum agents in germline *BRCA*-associated TNBC, albeit *BRCA*-mutant patients in the GeparSixto trial obtained high pCR regardless of the addition of carboplatin (79, 80).

Recently, PARP inhibitors (e.g., olaparib and talazoparib) have been compared with standard nonplatinum chemotherapy in two phase III trials, OlympiAD and EMBRACA, respectively, in germline *BRCA*-associated metastatic HER2-negative breast cancer (81, 82). Eligibility criteria included receipt of no more than two to three previous lines of chemotherapy for metastatic disease, and receipt of an anthracycline and a taxane whether in the neoadjuvant, adjuvant, or metastatic setting. Neoadjuvant or adjuvant platinum was allowed if the time that had elapsed since the last dose was 12 months in OlympiAD and 6 months in EMBRACA. Both trials enrolled a similar patient population, with some differences including the distribution of germline mutations (57% *BRCA1* in OlympiAD; 54.5% *BRCA2* in EMBRACA) and, concordantly, a slightly greater proportion of patients with hormone receptor–positive disease in EMBRACA (55.9%) than OlympiAD (50.3%). Results of both studies were positive, with improvements in ORR, PFS, and quality of life, favoring the PARP inhibitor. Compared with standard chemotherapy, a significant increase in median PFS was observed with olaparib (7 months vs. 4.2 months, HR 0.58; $P < 0.001$) and with talazoparib (8.6 months vs. 5.6 months, HR 0.54; $P < 0.001$). Safety profiles were also comparable across trials, and hematologic toxicity was the most common cause of dose modifications with PARP inhibition. An adjuvant trial (OlympiA, NCT02032823) in patients with germline *BRCA*-associated breast cancer is currently accruing. Of note, the reported response rates in the metastatic phase III trials of olaparib and talazoparib (59.9% and 62.6%, respectively) were similar to those previously reported with carboplatin, and platinum agents were not allowed in the chemotherapy control arm. At the present time, the comparative efficacy and optimal sequencing (given potentially overlapping resistance mechanisms) of PARP inhibitors versus platinum agents is unknown. In addition, whether PARP inhibitors may have activity in patients with other germline DNA-repair defects (e.g., *PALB2*), or in patients with acquired somatic *BRCA1/2* deleterious mutations, is unknown but is being tested in an ongoing clinical trial (NCT03344965).

Multiple mechanisms underlie the development of primary and acquired resistance to both platinum agents and PARP inhibitors, many of which have also been well characterized in ovarian or prostate cancer. Molecular alterations leading to therapeutic resistance include, for example, small insertions/deletions that result in frameshift mutations and synthesis of truncated proteins (e.g., inherited founder mutation *BRCA1*^{185delAG}; ref. 83); secondary *BRCA* reversion mutations that reinstate HR proficiency through restoration of the open reading frame and *BRCA* reexpression (84); exon 11 deletion splice variants that produce truncated, hypomorphic proteins (85); or point mutations in *PARP1* that alter PARP trapping (86). In addition to genomic alterations, epigenetic changes such as loss of *BRCA1* promoter hypermethylation via *BRCA1* locus fusion rearrangements, with subsequent *BRCA1* reexpression, have also been described after acquired resistance to DNA-damaging drugs, including platinum or olaparib (87).

Several strategies to exploit potential synthetic lethality in HR-deficient tumors are being explored across solid tumors, including clinical trials combining PARP inhibitors with PI3K/AKT inhibitors (NCT02208375), immune-checkpoint inhibition (NCT02657889), and HSP90 inhibitors (NCT02898207). HSP90 is a chaperone that assists in intracellular protein homeostasis by mediating protein folding and stabilization. HSP90 inhibitors block adequate protein folding, leaving the "client" protein (e.g., BRCA1) in the cytoplasm to be degraded by the proteasome. *In vitro*, HSP90 inhibition results in loss of BRCA1 expression and function and impaired DSB repair, sensitizing tumors to DNA-damaging agents (88). Stabilizers of G-quadruplex DNAs such as CX-5641 bind to G4 DNA structures, interfering with progression of DNA replication complexes and inducing single-strand breaks that require HR for repair; thus, in BRCA-deficient tumors, failure to repair DNA damage leads to lethality, including in taxane-resistant BRCA1/2-deficient

TNBC patient-derived xenograft models (89). Given its promising *in vivo* activity, CX-5461 is currently being explored in a phase I trial, with an expansion phase for unresectable breast cancer in patients with known *BRCA1/2* or HRD germline aberrations (NCT02719977).

"*BRCAness*" IN SPORADIC TNBC

Somatic mutations and epigenetic alterations that inactivate *BRCA1/2* and other DNA-repair genes have been identified in sporadic cancers (90). Given that HR deficiency exposes specific therapeutic vulnerabilities, the detection of sporadic tumors with this so-called "*BRCAness*" phenotype could have clinical implications. Most *BRCA1*-related tumors are basal-like (91), and there is a marked resemblance in phenotype and biology between sporadic basal-like tumors and *BRCA*-associated cancers (90). Despite these similarities, targeting the HR pathway in sporadic basal-like cancer has revealed conflicting data in the metastatic and neoadjuvant settings. High HRD score or basal phenotype (by PAM50 or IHC) did not predict greater benefit from carboplatin in TNT (12). Similarly, gene-expression profiles were not associated with response to platinum in TBCRC-009, although a genomic instability signature based on HRD assays discriminated metastatic TNBC responders from nonresponders (92). HR deficiency (i.e., high HRD score or tumor *BRCA* mutation) predicted increased pCR to neoadjuvant platinum (93–95). In GeparSixto, the addition of carboplatin to paclitaxel/liposomal doxorubicin improved pCR in HR-deficient tumors (64.9% vs. 45.2%, $P = 0.025$), but not in HR-proficient tumors (40.7% vs. 20%, $P = 0.146$; ref. 94). Discrepancies across trials may be explained by significantly less methylated *BRCA1/2* in metastases than in primary tumors, leading to potential loss of HR deficiency (96). Treatment exposure to alkylating agents commonly used in early-stage TNBC could drive clonal selection of HR-proficient cells less likely to respond to platinum in the metastatic setting. However, another explanation for these observed differences could be the robustness of the genomic metrics used to calculate HRD scores. With advances in sequencing technologies, an algorithm using whole-genome sequencing, also known as the HRDetect model, identified six mutational signatures present in germline *BRCA1/2*-mutated tumors that were then found to also predict HR deficiency in sporadic tumors in the Sanger data set (97). This aggregated *BRCAness* score was independently associated with benefit from platinum-based chemotherapy after adjusting for germline *BRCA* status and treatment timing, although the relatively small sample size (33 patients with metastatic breast cancer treated with either carboplatin or cisplatin as a single agent or in combination regimens) and the retrospective nature of the study (clouding the ability to establish a causal relationship) are limitations to be considered (98). Direct comparisons of these different measures should be further evaluated in ongoing prospective trials in HR-deficient breast cancer.

Currently, we lack predictive biomarkers to guide the choice of chemotherapy in sporadic basal-like TNBC, which comprises the majority of TNBC. Beyond germline *BRCA* mutations and the recent approval of olaparib and talazoparib in these patients, much remains unknown about the *BRCAness* features that may confer sensitivity to PARP inhibitors and DNA-damaging agents. Trials assessing these drugs are ongoing both in unselected and biomarker-selected populations (Table 3). In addition, preclinical data have demonstrated upregulation of PD-L1 expression after exposure to PARP inhibition in triple-negative MDA-MB-231 cells, with subsequent resensitization to a PARP inhibitor when combined with a PD-L1 antibody (99). Furthermore, the accumulation of cytosolic damaged DNA induced by PARP inhibition activates the STING pathway, which in turn increases the expression of type-I IFN signaling and immune cell infiltration, regardless of *BRCA* mutational status (100). Altogether, this has provided the rationale to explore the combination of niraparib, a PARP inhibitor, and pembrolizumab, a PD-1 inhibitor, in the phase II clinical trial TOPACIO. Results from the TNBC cohort showed promising activity with an ORR of 28% in the 46 evaluable patients, and durable responses irrespective of tumor *BRCA* status, PD-L1 status, or prior platinum exposure, although the highest ORR was observed in patients with tumor *BRCA1* or *BRCA2* mutations (60%; ref. 101). A randomized phase II trial comparing olaparib in combination with the PD-L1 inhibitor atezolizumab versus olaparib alone in patients with *BRCA*-associated metastatic TNBC is currently ongoing (NCT02849496).

EPIGENETIC MARKERS AND THERAPIES IN TNBC

Epigenetic alterations, including changes in DNA methylation of gene promoter regions and posttranslational modification of histone proteins, are a recognized hallmark of cancer. Approximately 60% to 80% of basal-like and claudin-low breast cancers have aberrant DNA hypermethylation (102). Compared with luminal and HER2-positive cancers, TNBC exhibits extensive CpG methylation of the promoter regions of nine epigenetic biomarker genes (*CDH1, CEACAM6, CST6, GNA11, ESR1, MUC1, MYB, SCNN1A*, and *TFF3*). DNA hypermethylation–dependent silencing of these genes is associated with worse RFS across all molecular subtypes and stages, compared with breast cancers unmethylated for these genes (40% RFS at 70 and 30 months, respectively). A nonsignificant trend toward RFS disadvantage has also been described among basal-like and claudin-low tumors that have this 9-gene methylation signature (102). In addition, promoter hypomethylation of three breast cancer stem cell–related genes (*CD44, CD133*, and *MSH1*), which strongly correlates with positive IHC staining and thus gene activation, has been shown to predict triple-negative status (103). Differences in histone modifications are also associated with differences in the expression of breast cancer genes across subtypes, separating luminal tumors, enriched with H3K27me3-modified genes, from nonluminal tumors (TNBC/HER2-positive), enriched with H3K9ac-regulated genes (104).

Therapies targeting epigenetic modifications, such as inhibitors of DNA methyltransferases (DNMT; 5-azacitidine, decitabine) and histone deacetylases (HDAC; entinostat, vorinostat), have yielded disappointing results to date in TNBC. The combination of 5-azacitidine and entinostat did not achieve any responses among 13 women with

Table 3. Ongoing clinical trials in *BRCA*-mutant or *BRCAness*-associated TNBC

Clinicaltrials.gov identifier	Title	*BRCA* status eligibility criteria	Phase
Neoadjuvant			
NCT03109080	A Phase I of Olaparib with Radiation Therapy in Patients with Inflammatory, Locoregionally Advanced or Metastatic TNBC or Patient with Operated TNBC with Residual Disease	*BRCA* mutation not required.	I
NCT03329937	An Open-Label, Single-Arm Pilot Study Evaluating the Antitumor Activity and Safety of Niraparib as Neoadjuvant Treatment in Localized, HER2-Negative, *BRCA*-Mutant Breast Cancer Patients	Deleterious or suspected deleterious *BRCA1* or *BRCA2* mutation (germline or somatic).	I
NCT02978495	Neoadjuvant Carboplatin in Triple-Negative Breast Cancer—A Prospective Phase II Study (NACATRINE Trial)	*BRCA* mutation not required. Includes *BRCA*-mutant–specific cohorts.	II
NCT02789332	A Randomized Phase II Trial to Assess the Efficacy of Paclitaxel and Olaparib in Comparison with Paclitaxel/Carboplatin Followed by Epirubicin/Cyclophosphamide as Neoadjuvant Chemotherapy in Patients with HER2-Negative Early Breast Cancer and Homologous Recombination Deficiency	*BRCA* deleterious tumor or germline mutation and/or high HRD score.	II
NCT03150576	Randomized, Phase II/III, 3 Stage Trial to Evaluate the Safety and Efficacy of the Addition of Olaparib to Platinum-Based Neoadjuvant Chemotherapy in Breast Cancer Patients with TNBC and/or gBRCA	TNBC or germline *BRCA* mutation HER2-negative breast cancer.	II/III
Adjuvant			
NCT02032823	A Randomized, Double-Blind, Parallel Group, Placebo-Controlled Multicenter Phase III Study to Assess the Efficacy and Safety of Olaparib versus Placebo as Adjuvant Treatment in Patients with g*BRCA1/2* Mutations and High-Risk HER2-Negative Primary Breast Cancer Who Have Completed Definitive Local Treatment and Neoadjuvant or Adjuvant Chemotherapy	Suspected deleterious or deleterious *BRCA1* and/or *BRCA2* germline mutation.	III
Locally advanced, recurrent, or metastatic			
NCT02950064	Escalation Study of BTP-114 in Patients with Advanced Solid Tumors and *BRCA* or DNA-Repair Mutation	Deleterious germline or somatic BRCA mutation or DNA-repair mutation. Abnormal HRD tests are also allowed.	I
NCT00576654	A Phase I Dose-Escalation Study of Oral ABT-888 (NSC #737664) plus Intravenous Irinotecan (CPT-11, NSC#616348) Administered in Patients with Advanced Solid Tumors	*BRCA* mutation not required. Includes *BRCA*-mutant–specific cohort.	I
NCT02227082	Olaparib Dose Escalation in Combination with High Dose Radiotherapy to the Breast and Regional Lymph Nodes	*BRCA* mutation not required.	I
NCT02898207	A Phase 1 Study of PARP Inhibitor Olaparib and HSP90 Inhibitor AT13387 for Treatment of Advanced Solid Tumors with Expansion in Patients with Recurrent Epithelial Ovarian, Fallopian Tube, Peritoneal Cancer or Recurrent TNBC	*BRCA* mutation not required. Dose expansion excludes germline *BRCA1* or *BRCA2* mutations.	I
NCT03075462	An Open, Nonrandomized, Multicenter Phase I Study to Assess the Safety and Efficacy of Fluzoparib Given in Combination with Apatinib in Patients with Recurrent Ovarian Cancer or TNBC	*BRCA* mutation not required.	I
NCT03109080	A Phase I of Olaparib with Radiation Therapy in Patients with Inflammatory, Locoregionally Advanced or Metastatic TNBC or Patients with Operated TNBC with Residual Disease	*BRCA* mutation not required.	I
NCT03101280	A Phase IB Combination Study of Rucaparib (CO-338) and Atezolizumab (MPDL3280A) in Participants with Advanced Gynecologic Cancers and TNBC	Part 1: All comers; part 2: deleterious germline or somatic *BRCA* mutation, or wild-type tumor *BRCA* but high levels of LOH.	I

(*continued*)

Table 3. Ongoing clinical trials in *BRCA*-mutant or *BRCAness*-associated TNBC (*Continued*)

Clinicaltrials.gov identifier	Title	*BRCA* status eligibility criteria	Phase
NCT02393794	Phase I/II Study of Cisplatin plus Romidepsin and Nivolumab in Metastatic Triple-Negative Breast Cancer or BRCA Mutation-Associated Locally Recurrent or Metastatic Breast Cancer	TNBC or germline *BRCA* mutation breast cancer.	I/II
NCT02264678	A Modular Phase I, Open-Label, Multicenter Study to Assess the Safety, Tolerability, Pharmacokinetics and Preliminary Anti-tumor Activity of AZD6738 in Combination with Cytotoxic Chemotherapy and/or DNA Damage Repair/Novel Anticancer Agents in Patients with Advanced Solid Malignancies	Cohort HER2-negative breast cancer: with *BRCA* mutation (germline or somatic); cohort TNBC: without known *BRCA* mutation.	I/II
NCT02484404	Phase I/II Study of the Anti-Programmed Death Ligand-1 Antibody MEDI4736 in Combination with Olaparib and/or Cediranib for Advanced Solid Tumors and Advanced or Recurrent Ovarian, Triple-Negative Breast, Lung, Prostate and Colorectal Cancers	TNBC cohort requires germline *BRCA1* or *BRCA2* mutation.	I/II
NCT02401347	A Phase II Clinical Trial of the PARP Inhibitor Talazoparib in BRCA1 and BRCA2 Wild-Type Patients with (i) Advanced Triple-Negative Breast Cancer and Homologous Recombination Deficiency (HRD), and (ii) Advanced HER2-Negative Breast Cancer or Other Solid Tumors with Either a Mutation in Homologous Recombination (HR) Pathway Genes	No deleterious *BRCA* mutation. TNBC with high HRD score or HER2-negative breast cancer with germline or somatic mutation in the HR pathway.	II
NCT02203513	A Phase II Single-Arm Pilot Study of the Chk1/2 Inhibitor (LY2606368) in *BRCA1/2* Mutation-Associated Breast or Ovarian Cancer, TNBC, and High-Grade Serous Ovarian Cancer	TNBC or germline *BRCA* mutation breast cancer.	II
NCT03205761	A Phase II Clinical Trial to Analyze Olaparib Response in Patients with BRCA1 and/or 2 Promoter Methylation Diagnosed of Advanced Breast Cancer	Absence of deleterious or suspected deleterious germline *BRCA* mutations. Documented *BRCA1* and/or *BRCA2* promoter methylation.	II
NCT03330847	A Phase II, Open-Label, Randomized, Multicenter Study to Assess the Safety and Efficacy of Agents Targeting DNA Damage Repair in Combination with Olaparib Versus Olaparib Monotherapy in the Treatment of Metastatic TNBC Patients Stratified by Alterations in Homologous Recombinant Repair (HRR)-Related Genes (including *BRCA1/2*)	*BRCA* mutation not required. Stratification by mutation in *BRCA* and HRR genes.	II
NCT02595905	Phase II Randomized Placebo-Controlled Trial of Cisplatin with or without ABT-888 (Veliparib) in Metastatic TNBC and/or *BRCA* Mutation-Associated Breast Cancer, with or without Brain Metastases	TNBC or germline *BRCA* mutation breast cancer.	II
NCT01898117	Biomarker Discovery Randomized Phase IIb Trial with Carboplatin–Cyclophosphamide versus Paclitaxel with or without Atezolizumab as First-line Treatment in Advanced TNBC	*BRCA* mutation not required.	II
NCT03414684	A Randomized Phase II Trial of Carboplatin with or without Nivolumab in First- or Second-Line Metastatic TNBC	*BRCA* mutation not required. Stratification by germline *BRCA* mutation.	II
NCT02498613	A Phase 2 Study of Cediranib in Combination with Olaparib in Advanced Solid Tumors	*BRCA* mutation not required.	II

NOTE: The Clinicaltrials.gov database was searched for interventional-only clinical trials that are recruiting as of April 14, 2018. Only drug-based interventions were considered. Search terms included "triple-negative breast cancer," "HER2-negative breast cancer," "BRCA," and "PARP."

advanced TNBC treated in a phase II study (105). No significant changes in gene expression in paired biopsies before and after 2 months of treatment were observed, possibly due to absent ER promoter DNA methylation at baseline. Novel approaches in epigenetic modulation include BET bromodomain inhibitors that bind to acetylated lysine residues in histones, displacing bromodomain proteins from chromatin and inhibiting transcriptional activity (106). BET inhibitors achieve potent suppression of tumor growth in TNBC cell lines characterized by more basal-like and claudin-low/stem cell–like features (61). Several BET inhibitors are currently in early stages of clinical testing as single agents

or in combination with immunotherapy (NCT01587703, NCT02391480, and NCT02711137).

IMMUNE SUBTYPES OF TNBC

Increasing data suggest that the immune system is critical for disease outcome in TNBC. Analyses from neoadjuvant and adjuvant TNBC trials have shown that tumor-infiltrating lymphocytes (TIL), assessed by hematoxylin–eosin staining, are predictive of response to therapy and strongly associated with improved survival (107, 108). Stratification of TNBC based on quantitative TIL evaluation has distinguished immune "hot" (high-TIL) and "cold" (low-TIL) tumors, which also appear to correlate with response to immune-checkpoint inhibitors in the metastatic setting (109). Paired biopsies pre– and post–neoadjuvant therapy have shown that the immune microenvironment can be modulated by chemotherapy, converting tumors from "cold" to "hot," and these cases with highly infiltrated residual TNBC have improved survival (110). Phenotypic TIL characterization has also provided further insight into the populations of immune cells (e.g., $CD8^+$ T cells; elevated CD8/FOXP3 ratio) that may be responsible for this positive effect (111). Elevated expression in TNBC of immune markers of tumor evasion PD-1/PD-L1 has prompted clinical assessment of inhibitors of these checkpoints, with modest efficacy as monotherapy and encouraging results in combination with chemotherapy (Table 4; refs. 109, 112–118).

Recently, results from a large phase III trial (IMpassion130) that randomized patients in the first-line TNBC metastatic setting to receive nab-paclitaxel combined with either atezolizumab (PD-L1 inhibitor) or placebo were reported (118). Although the absolute difference in median PFS in the PD-L1–positive population (2.5 months) was not strikingly different from that seen in the ITT cohort (1.7 months), at a median follow-up of 12.9 months, a 9.5-month clinically meaningful improvement in median OS was noted in patients with PD-L1–positive tumors, in contrast to a 3.7-month difference in the ITT population (118). No PFS or OS differences were noted in the subset of patients with PD-L1–negative tumors (119). Several other randomized trials have completed accrual and are awaiting data maturity to report. Whether similar results may be achieved with chemotherapy plus immunotherapy in later lines is unknown at this time. Of note, increased ORR have been observed in patients with previously untreated metastatic TNBC with monotherapy PD-1/PD-L1 inhibitors, suggesting that these agents may be more active in less heavily pretreated metastatic disease (120).

Efforts to identify patients with tumors that are more or less likely to benefit from immunotherapy-based approaches are ongoing. As evidenced in the IMpassion130 trial, not all patients with PD-L1 tumors (defined by the presence of ≥1% IHC staining on immune cells) respond to PD-L1 inhibition and, contrarily, there are patients who, despite negative PD-L1 staining, appear to derive benefit from treatment. Beyond IHC classifications, genetic alterations of immune-regulatory genes have also segregated TNBC into subgroups with different prognostic and possibly therapeutic implications. *CD274* (encoding PD-L1) and *PDCD1LG2* (encoding PD-L2) genes localize to the 9p24 locus, adjacent to *JAK2*, constituting the *PDJ* amplicon. Overexpression of PD-L1 is observed in 88% of tumors with amplifications in the 9p24/*JAK2* locus, which are found at higher frequency in post-neoadjuvant residual TNBC (66). In TNBC, the *PDJ* amplicon identified a subset of patients at significantly greater risk of recurrence (121), and could be a potential biomarker for selection of high-risk patients who may benefit from PD-1/PD-L1 blockade. Activating mutations in the RAS/MAPK pathway, present in 15% of residual disease, correlated with reduced TIL; inhibition of MEK upregulated PD-L1 expression, synergizing with PD-1/PD-L1 antibodies in murine models (122). Furthermore, high tumor mutational burden has been associated with improved outcomes with PD-1 inhibition in other cancer types (123), and may represent an independent biomarker of response.

Transcriptomic analysis of tumor-associated stroma in TNBC has revealed the presence of four axes, each with differential expression of genes related to T-cell, B-cell, epithelial (E), and desmoplasia (D) markers. The E-axis inversely correlated with LAR Lehmann subtype, and the D-axis was positively associated with MSL while also determining the prognostic value of the T-, B-, and E-axes (124). Furthermore, these axes strongly influenced the location of $CD8^+$ TIL (125), which may affect antitumoral response to immune-checkpoint inhibitors. Similarly, when analyzing the tumor compartment, the presence of the immunomodulatory signature (associated with elevated lymphocytic infiltration and increased expression of immune-checkpoint regulators, e.g., PD-1/PD-L1; ref. 18), significantly differs across refined TNBC types, with the highest rates observed in BL1 (48%) and the lowest in M (0%; ref. 126). Whether transcriptomic profiling could be incorporated to routine clinical practice to help select patients with TNBC with a greater likelihood of responding to immune-checkpoint inhibitors, similar to the applicability of gene-expression assays (e.g., 21-gene recurrence score, oncotype) to predict chemotherapy benefit in ER-positive breast cancer (127), remains to be seen.

To date, one single marker has not been proven to effectively select patients who are more likely to respond to immunotherapies. Recently, the development of multiplexed imaging techniques has enabled analysis of the spatial distribution and interaction between tumor and immune cells, showing that in TNBC there is high intratumor topologic heterogeneity for the expression of PD-1 on cytotoxic $CD8^+$ and helper $CD4^+$ T cells (128). Tumors with immune cells that are spatially separated from tumor cells, also defined as compartmentalized (as opposed to mixed immune cells with tumor cells), predominantly express PD-1 on $CD4^+$ T cells and are independently associated with improved survival. Given the complexity of these interactions, integration of comprehensive omics analyses of samples with detailed clinical data annotation will be needed to better understand how the relationship between the tumor and its microenvironment affects response to treatment.

EVOLUTIONARY PATHS OF TNBC

Analyses of paired primary and metastatic TNBC samples are also needed to better understand the drivers of disease progression. Clonal frequencies vary significantly across TNBC at the time of diagnosis, suggesting their occurrence at different stages of tumorigenesis (73). There are limited sequencing data in metastatic triple-negative tumors, and

Table 4. Results of PD-1/PD-L1 inhibition in advanced TNBC

	Single-agent immunotherapy				Combination with chemotherapy		
	Anti–PD-1		Anti–PD-L1		Anti–PD-1	Anti–PD-L1	
	Pembrolizumab in PD-L1$^+$ TNBC (KEYNOTE-012; ref. 111)	Pembrolizumab in metastatic TNBC (KEYNOTE-086; refs. 112, 113)	Atezolizumab in TNBC unselected for PD-L1 (108)	Avelumab in TNBC unselected for PD-L1 (JAVELIN; ref. 116)	Eribulin ± pembrolizumab in metastatic TNBC (ENHANCE-1/KEYNOTE-150; ref. 115)	Atezolizumab + nab-paclitaxel in TNBC unselected for PD-L1 (114)	Atezolizumab + nab-paclitaxel vs. placebo + nab-paclitaxel in metastatic TNBC (IMpassion130; ref. 117)
Tumor characteristics							
Definition of PD-L1 positivity	≥1% TC or any staining in stroma	≥1% TC or any staining in stroma	≥5% IC	≥1% TC; ≥10% IC	≥1% TC or any staining in stroma	≥1% TC; ≥1% IC	≥1% IC
PD-L1 status inclusion criteria	Positive	All comers Cohort A: pretreated, any PD-L1 Cohort B: untreated, PD-L1$^+$	All comers	All comers	All comers Stratum 1: No prior therapy Stratum 2: 1–2 prior lines	All comers	All comers
Frequency of PD-L1 positivity among evaluable cases (%)	65/111 (58.6)	A: 105/169 (62.1) B: 128/207 (61.8)	71/108 (65.7)	TC: 33/48 (68.8) IC: 9/48 (18.8)	49/98 (50)	IC: 11/21 (52.4)	369/902 (40.9)
Patient characteristics							
Total number of patients enrolled	32	A: 170 B: 84	115	58	107 (S1: 66; S2: 41)	32	902
Total number of patients included in efficacy analysis	27	A: 170 B: 84	112[b]	58	107 (106[b])	32	902
Median prior lines of therapy in metastatic setting (range)	2 (0–9)	A: NA B: 0	7 (0–21)	NA[c]	S1: 0 S2: 1–2	5 (1–10)	0

Efficacy							
ORR, %	18.5	A: 4.7 B: 22.6	Overall: 9.8 First line: 26.3 Second line: 3.6 Third/+ line: 7.7	5.2	Overall: 26.4 S1: 29.2 S2: 22	Overall: 37.5 First line: 46.1 Second line: 22.2 Third/+ line: 40	ITT: 56 vs. 45.9
ORR in PD-L1^{+} cohort, %	18.5	A: 4.8 B: 22.6	12.7	22.2[d]	30.6	36.3[d]	58.9 vs. 42.6
CBR, %	25.9[a]	A: 7.6 B: 25	NA	31[a]	Overall: 36.8 S1: 40 S2: 31.7	81.3[a]	NA
Median PFS, mo.	1.9 (1.7–5.5)	A: 2.0 (1.9–2)	NA	1.5 (1.4–1.7)	Overall: 4.2 (4.1–5.6)	NE	ITT: 7.2 vs. 5.5; HR, 0.8 (0–69–0.92)
(95% CI)		B: 2.1 (2.0–2.3)			S1: 4.9 (4.1–6.1) S2: 4.1 (2.1–6.2)		PD-L1^{+}: 7.5 vs. 5; HR, 0.62 (0.49–0.78)
Median OS, mo.	11.2 (5.3–NR)	A: 8.9 (7.2–11.2)	9.3 (7.0–12.6)	9.2 (4.3-NE)	Overall: 17.7 (13.7–NE)	NE	ITT: 21.3 vs. 17.6; HR, 0.84 (0.69–1.02)
(95% CI)		[CR, PR, or SD: NR; PD: 7.1 (6.3–8.8)] B: 19.2 (11.3–NE)			S1: 17.7 (13.3–NE) S2: 16.3 (12.4–19.2)	(8–NE)	PD-L1^{+}: 25 vs. 15.5; HR, 0.62 (0.45–0.86)

NOTE: Response rates are per RECIST 1.1 criteria.

Abbreviations: TNBC, triple-negative breast cancer; TC, tumor cells; IC, immune cells; ORR, objective response rate; CBR, clinical benefit rate (defined as complete response, partial response, or stable disease for ≥24 weeks); mo., months; NR, not reached; NE, not estimable; NA, not available; PFS, progression-free survival; OS, overall survival; CI, confidence interval; ITT, intent-to-treat population; HR, hazard ratio.

[a]DCR is defined as confirmed complete, partial response, or stable disease as best response.

[b]Number of patients considered objective response–evaluable.

[c]In the overall population, the median number of prior lines of therapy in any setting was 4 (range, 1–10). In the TNBC cohort, 50% had received ≥2 prior lines of therapy for metastatic disease.

[d]According to PD-L1 positivity in IC.

much remains unknown about the differences in the molecular landscape of TNBC over its natural history. Multiclonal seeding from different cell populations in the primary tumor to the metastasis has been reported in two cases of basal-like TNBC, where, in addition, most putative driver mutations were shared, rather than acquired, between primary and metastatic lesions (129, 130). Also, most TNBC primary tumors and metastases are polyclonal, with overlapping clones, suggesting that polyclonal metastasis is common in TNBC. Phylogenetic analysis has the potential to distinguish local recurrences from second primary tumors and to help determine the origin of a metastatic lesion in a patient with a history of independent primary tumors (131). Given the differences in management of primary and recurrent tumors, sequencing of longitudinal samples could affect treatment decisions.

Receptor status, according to IHC, and also intrinsic subtype, can change at time of recurrence (132), but the clinical relevance of molecular phenotype switch remains unclear, and IHC subtypes largely drive current treatment decisions in breast cancer. Loss of ER and PR expression occurs in approximately 10% to 12% of asynchronous recurrences, inducing a switch to TNBC in the metastasis (133), and has been associated with worse survival compared with cases with concordant hormone receptor–positive recurrence (134). To date, we do not fully understand the mechanisms that cause this conversion, and whether there are special considerations that should be made when treating this patient population. Of note, there are also breast tumors that express low levels (1%–9%) of ER and PR, and it remains unclear whether these cases derive significant benefit from endocrine therapy (135). Retrospective studies have shown that almost half of tumors with 1% to 9% ER staining are basal-like (136), suggesting that we should consider these tumors similar to TNBC and apply treatment algorithms, including enrollment onto clinical trials, for TNBC in these patients.

The extent of residual disease post–neoadjuvant chemotherapy, quantified per residual cancer burden index, is a well-established risk factor for recurrence (137). Residual disease has been used as a marker to select patients for escalation of adjuvant therapy, particularly in TNBC, based on the significant absolute improvement observed in patients treated with versus without capecitabine in terms of 3-year DFS (69.8% vs. 56.1%, respectively; HR, 0.58; 95% CI, 0.39–0.87) and OS (78.8% vs. 70.3%, respectively; HR, 0.52; 95% CI, 0.30–0.90; ref. 138). However, not all patients with residual disease will recur. Distinguishing between the molecular mechanisms of chemoresistance and those that drive the development of metastatic disease remains a challenge. Intratumor genetic heterogeneity has been widely described in TNBC and may be associated with a decreased likelihood of achieving a pCR (139, 140). Bulk exome and single-cell sequencing in a small number of pre– and post–neoadjuvant therapy samples suggest the occurrence of adaptive clonal extinction or persistence and acquired transcriptional reprogramming as potential models of chemoresistance (140). Other single-cell resolution studies support the hypothesis that most mutation and copy-number events occur in early stages of tumor evolution, rather than develop gradually over time, implying punctuated evolution (141). Validation of these findings in larger sets of tumors with associated long-term outcome data is key to understand the impact of genomic and phenotypic evolution of triple-negative cancer cells.

CONCLUSIONS

In summary, TNBC is composed of a broad spectrum of biologically distinct subtypes with overlapping alterations. Despite advances in tumor characterization, separately, each classification has not yet translated to specific treatments or choices of treatments, with the exception of PARP inhibitors or platinum agents in germline *BRCA1/2* carriers, and potentially in the near future immune-checkpoint inhibition in tumors with PD-L1–positive immune cells. Comprehensive integrated analysis of data generated from different "omics" technologies may provide more insight into the etiology, evolution of TNBC, and, possibly, prevention and new treatment strategies. Nonetheless, as the volume of information exponentially increases, identifying alterations that are critical for tumor growth and survival continues to be a challenge. In addition, the utility of these profiles is largely limited by genetic and epigenetic heterogeneity within the tumor. There have been several large-scale efforts to find new targets, including shRNA/CRISPR screens (64, 142–144). Using loss-of-function RNAi-based screens across more than 500 cancer cell lines, biocomputational algorithms have been developed to help predict cancer dependencies (143), and novel potently selective inhibitors, as single agents or in combination, will be needed to effectively block these targets (61, 145, 146). Similarly to cell lines and organoids, patient-derived xenografts enable high-throughput drug screening, but with the potential advantages of analyzing tumor growth metrics and characterizing drug response in models that retain the histopathologic features and intertumor and intratumor genomic heterogeneity of the explanted tumor (147). Given the complexity of these techniques and sample size of individual cohorts, institutional collaborations should be forged to create biobanks that will provide a platform to help answer questions of interest in specific subsets of patients with TNBC.

Most trials to date have been performed in unselected TNBC, hoping to find a signal of efficacy in subgroup analyses. Prospective validation of biomarker-driven approaches has been widely considered a necessary step for approval of targeted therapies over the past years. Only recently were results published from the first trial in TNBC to prospectively stratify patients by the presence of a tumor gene signature (148). In this neoadjuvant study, patients were randomized to receive paclitaxel with or without LCL161, a small-molecule antagonist of inhibitor of apoptosis proteins. LCL161 induces tumor necrosis factor (TNF)–mediated apoptosis, and preclinical work identified a three-gene signature (elevated *TNFα*, elevated *RIPK1*, and reduced *STK39*) that was associated with sensitivity to LCL161. In patients with signature-positive tumors, the pCR rate was higher in the combination versus the control arm (38.2% vs. 17.2%, respectively), as opposed to lower pCR in those who were negative for the signature (5.6% vs. 16.4%, respectively), albeit with significant toxicity that led to treatment discontinuation in almost one fifth of patients treated with LCL161 and paclitaxel (148). Of the total of 312 patients who signed consent for molecular prescreening for this trial, 207 had a valid signature score

and were treated on study [of which 63 (30.4%) were found to be positive for the signature]. Enrollment was completed in approximately 25 months but required participation of 47 international sites across 11 countries. Inability to ship samples for testing (4.2%) and assay failure (7.1%) were among the reasons for exclusion of patients, highlighting the challenges of prospectively implementing molecular testing in clinical trials, including those evaluating biomarkers with a prevalence as high as the 30% rate observed in this trial.

Another limitation of conducting single oncogene–driven clinical trials is the fact that there are complex interactions and overlap between different genomic alterations (e.g., comparable prognosis between PI3K-activated, *TP53* wild-type TNBC, and ER-positive breast cancer) with consequences that are not clearly understood to date nor taken into consideration in study designs. As the field of genomics in TNBC evolves and new insights are gained, these factors may need to be incorporated into trial designs, particularly when *post hoc* stratification by various forms of analysis may be needed to interpret and demonstrate subgroup effects.

As NGS, immune-profiling, and other technologies become widely available, biomarker-selected basket trials across multiple cancer types are of particular importance to evaluate the efficacy of matched targeted therapies. Considering the multiple molecular hypotheses for treatment, dynamic biomarker-adjusted platforms, such as WSG-ADAPT or I-SPY, aim to improve the efficiency of early drug development by predicting the probability of success in phase III clinical trials (45, 149). However, given smaller and smaller potential subsets of interest, the success of biomarker-enriched designs will increasingly depend on more effective strategies to ensure that a larger pool of potentially eligible patients have the opportunity to be offered participation in such trials. Furthermore, given the evident heterogeneity in the molecular landscape of TNBC and current efforts to integrate omics data to better understand the underlying biological processes, the combinations of features in the tumor and its microenvironment that may be identified, and potentially targeted, seem endless. Conduction of randomized studies that require a large number of patients, aiming to test each individual hypothesis and demonstrate superiority of novel drugs to current standard-of-care regimens, is simply not feasible. As subsets of patients with rare, potentially actionable targets are identified, exploring multiple treatment options in these select populations is becoming more challenging, and this will likely translate into an increasing need to mindfully extrapolate results from subgroup analyses. Optimization of trial designs, including umbrella trials in TNBC (in which patients are assigned to an intervention based on genomic and/or immune profiling of the tumor at baseline), "pick-the-winner" strategies (with smaller sample sizes), and incorporation of comprehensive fresh biospecimen collection for correlative substudies, may provide proof-of-concept to help select therapies that are more likely to succeed in larger trials.

To overcome the challenges of limited, single-institution studies, multiple genomic data sharing initiatives such as Project GENIE (American Association for Cancer Research), Genomic Data Commons (National Cancer Institute), or cBioPortal have been developed as large repositories of sequencing data. Despite these efforts, one of the major limitations of these large-scale studies is the lack of detailed clinical annotation, making it difficult to answer specific questions such as the association between genomic features and prior exposure to therapy, changes in receptor subtype over time (i.e., due to absent ER, PR, and HER2 status at different time points) or clinical outcomes (e.g., response and survival). Another limitation is the heterogeneity in the utilization of exome/genome versus targeted panel sequencing across cancer centers, which limits the ability to perform in-depth analyses to genes that are common to all panels. In coming years, we anticipate standardization of clinical sequencing across institutions and implementation of machine-learning tools that will help extract clinical data from electronic medical records, facilitating a seamless integration of genomic and clinical information in both private and public data sets.

Furthermore, to address the need for advances in drug development and biomarker discovery in TNBC, the elaboration of prospective, large-scale, longitudinal multicenter cohort studies in TNBC that have the ability to capture a patient's clinical course and collect fresh–frozen tissue, blood, and other biospecimens over a longer time frame, over multiple treatments, regardless of trial participation, and across a larger number of patients has the potential to vastly improve our knowledge of the dynamic changes in tumor biology, and the markers of response or resistance to treatment. These platforms may also be utilized to effectively communicate, offer, and expand clinical trial participation to patients across collaborating institutions in order to help answer clinically relevant questions in a timely manner and, ultimately, improve outcomes in patients diagnosed with TNBC.

Disclosure of Potential Conflicts of Interest

K. Polyak is a consultant/advisory board member for Mitra Biotech. N.U. Lin reports receiving commercial research grants from Pfizer, Genentech, Novartis, Array Biopharma, and Seattle Genetics and is a consultant/advisory board member for Shionogi, Genentech, Novartis, Seattle Genetics, and Puma. No potential conflicts of interest were disclosed by the other author.

Acknowledgments

This work was supported in part by the NCI R35CA197623 (K. Polyak) and the Benderson Family Fund for Triple-Negative Breast Cancer Research.

Received October 5, 2018; revised December 19, 2018; accepted December 20, 2018; published first January 24, 2019.

REFERENCES

1. Bertucci F, Houlgatte R, Benziane A, Granjeaud S, Adelaide J, Tagett R, et al. Gene expression profiling of primary breast carcinomas using arrays of candidate genes. Hum Mol Genet 2000;9:2981–91.
2. Brown M, Tsodikov A, Bauer KR, Parise CA, Caggiano V. The role of human epidermal growth factor receptor 2 in the survival of women with estrogen and progesterone receptor-negative, invasive breast cancer: the California Cancer Registry, 1999–2004. Cancer 2008;112:737–47.
3. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007;13:4429–34.
4. Andre F, Zielinski CC. Optimal strategies for the treatment of metastatic triple-negative breast cancer with currently approved agents. Ann Oncol 2012;23(Suppl 6):vi46–51.

5. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature 2000; 406:747–52.
6. Prat A, Parker JS, Karginova O, Fan C, Livasy C, Herschkowitz JI, et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 2010;12: R68.
7. Perou CM. Molecular stratification of triple-negative breast cancers. Oncologist 2010;15(Suppl 5):39–48.
8. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Mol Oncol 2011;5:5–23.
9. Prat A, Parker JS, Fan C, Perou CM. PAM50 assay and the three-gene model for identifying the major and clinically relevant molecular subtypes of breast cancer. Breast Cancer Res Treat 2012;135:301–6.
10. Prat A, Lluch A, Albanell J, Barry WT, Fan C, Chacon JI, et al. Predicting response and survival in chemotherapy-treated triple-negative breast cancer. Br J Cancer 2014;111:1532–41.
11. Sikov WM, Barry WT, Hoadley KA, Pitcher BN, Singh B, Tolaney SM, et al. Abstract S4-05: Impact of intrinsic subtype by PAM50 and other gene signatures on pathologic complete response (pCR) rates in triple-negative breast cancer (TNBC) after neoadjuvant chemotherapy (NACT) +/− carboplatin (Cb) or bevacizumab (Bev): CALGB 40603/150709 (Alliance). Cancer Res 2015;75:S4–5.
12. Tutt A, Tovey H, Cheang MCU, Kernaghan S, Kilburn L, Gazinska P, et al. Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial. Nat Med 2018; 24:628–37.
13. Bruna A, Greenwood W, Le Quesne J, Teschendorff A, Miranda-Saavedra D, Rueda OM, et al. TGFbeta induces the formation of tumour-initiating cells in claudin-low breast cancer. Nat Commun 2012;3:1055.
14. Bhola NE, Balko JM, Dugger TC, Kuba MG, Sanchez V, Sanders M, et al. TGF-beta inhibition enhances chemotherapy action against triple-negative breast cancer. J Clin Invest 2013;123:1348–58.
15. Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750–67.
16. Bareche Y, Venet D, Ignatiadis M, Aftimos P, Piccart M, Rothe F, et al. Unravelling triple-negative breast cancer molecular heterogeneity using an integrative multiomic analysis. Ann Oncol 2018;29: 895–902.
17. Masuda H, Baggerly KA, Wang Y, Zhang Y, Gonzalez-Angulo AM, Meric-Bernstam F, et al. Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. Clin Cancer Res 2013;19:5533–40.
18. Lehmann BD, Jovanovic B, Chen X, Estrada MV, Johnson KN, Shyr Y, et al. Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. PLoS One 2016;11:e0157368.
19. Echavarria I, Lopez-Tarruella S, Picornell A, Garcia-Saenz JA, Jerez Y, Hoadley K, et al. Pathological response in a triple-negative breast cancer cohort treated with neoadjuvant carboplatin and docetaxel according to Lehmann's refined classification. Clin Cancer Res 2018; 24:1845–52.
20. Karaayvaz M, Cristea S, Gillespie SM, Patel AP, Mylvaganam R, Luo CC, et al. Unravelling subclonal heterogeneity and aggressive disease states in TNBC through single-cell RNA-seq. Nat Commun 2018;9:3588.
21. Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreras A, Fuqua SA, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin Cancer Res 2015;21:1688–98.
22. Curtis C, Shah SP, Chin SF, Turashvili G, Rueda OM, Dunning MJ, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 2012;486:346–52.
23. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol 2016;13:674–90.
24. Collins LC, Cole KS, Marotti JD, Hu R, Schnitt SJ, Tamimi RM. Androgen receptor expression in breast cancer in relation to molecular phenotype: results from the Nurses' Health Study. Mod Pathol 2011;24:924–31.
25. Asghar US, Barr AR, Cutts R, Beaney M, Babina I, Sampath D, et al. Single-cell dynamics determines response to CDK4/6 inhibition in triple-negative breast cancer. Clin Cancer Res 2017;23:5561–72.
26. Gucalp A, Tolaney S, Isakoff SJ, Ingle JN, Liu MC, Carey LA, et al. Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic Breast Cancer. Clin Cancer Res 2013;19:5505–12.
27. Traina TA, Miller K, Yardley DA, Eakle J, Schwartzberg LS, O'Shaughnessy J, et al. Enzalutamide for the treatment of androgen receptor-expressing triple-negative breast cancer. J Clin Oncol 2018; 36:884–90.
28. Bonnefoi H, Grellety T, Tredan O, Saghatchian M, Dalenc F, Mailliez A, et al. A phase II trial of abiraterone acetate plus prednisone in patients with triple-negative androgen receptor positive locally advanced or metastatic breast cancer (UCBG 12-1). Ann Oncol 2016; 27:812–8.
29. Traina TA, Yardley DA, Schwartzberg LS, O'Shaughnessy J, Cortes J, Awada A, et al. Overall survival (OS) in patients (Pts) with diagnostic positive (Dx+) breast cancer: subgroup analysis from a phase 2 study of enzalutamide (ENZA), an androgen receptor (AR) inhibitor, in AR+ triple-negative breast cancer (TNBC) treated with 0-1 prior lines of therapy. J Clin Oncol 2017;35:1089.
30. Lehmann BD, Bauer JA, Schafer JM, Pendleton CS, Tang L, Johnson KC, et al. PIK3CA mutations in androgen receptor-positive triple negative breast cancer confer sensitivity to the combination of PI3K and androgen receptor inhibitors. Breast Cancer Res 2014;16:406.
31. Millis SZ, Gatalica Z, Winkler J, Vranic S, Kimbrough J, Reddy S, et al. Predictive biomarker profiling of >6000 breast cancer patients shows heterogeneity in TNBC, with treatment implications. Clin Breast Cancer 2015;15:473–81e3.
32. Bardia A, Mayer IA, Diamond JR, Moroose RL, Isakoff SJ, Starodub AN, et al. Efficacy and safety of anti-Trop-2 antibody–drug conjugate sacituzumab govitecan (IMMU-132) in heavily pretreated patients with metastatic triple-negative breast cancer. J Clin Oncol 2017; 35:2141–8.
33. Modi S, Pusztai L, Forero A, Mita M, Miller K, Weise A, et al. Abstract PD3-14: phase 1 study of the antibody–drug conjugate SGN-LIV1A in patients with heavily pretreated triple-negative metastatic breast cancer. Cancer Res 2018;78:PD3-14-PD3.
34. Yardley DA, Weaver R, Melisko ME, Saleh MN, Arena FP, Forero A, et al. EMERGE: a randomized phase II study of the antibody–drug conjugate glembatumumab vedotin in advanced glycoprotein NMB-expressing breast cancer. J Clin Oncol 2015;33:1609–19.
35. Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science 2013;339:1546–58.
36. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature 2012;490:61–70.
37. Pereira B, Chin SF, Rueda OM, Vollan HK, Provenzano E, Bardwell HA, et al. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. Nat Commun 2016;7:11479.
38. Weisman PS, Ng CK, Brogi E, Eisenberg RE, Won HH, Piscuoglio S, et al. Genetic alterations of triple negative breast cancer by targeted next-generation sequencing and correlation with tumor morphology. Mod Pathol 2016;29:476–88.
39. Balko JM, Giltnane JM, Wang K, Schwarz LJ, Young CD, Cook RS, et al. Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets. Cancer Discov 2014;4:232–45.
40. Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, et al. Mutational processes molding the genomes of 21 breast cancers. Cell 2012;149:979–93.
41. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 2016;534:47–54.

42. Fedele CG, Ooms LM, Ho M, Vieusseux J, O'Toole SA, Millar EK, et al. Inositol polyphosphate 4-phosphatase II regulates PI3K/Akt signaling and is lost in human basal-like breast cancers. Proc Natl Acad Sci U S A 2010;107:22231–6.
43. Martin M, Chan A, Dirix L, O'Shaughnessy J, Hegg R, Manikhas A, et al. A randomized adaptive phase II/III study of buparlisib, a pan-class I PI3K inhibitor, combined with paclitaxel for the treatment of HER2- advanced breast cancer (BELLE-4). Ann Oncol 2017;28: 313–20.
44. Kim SB, Dent R, Im SA, Espie M, Blau S, Tan AR, et al. Ipatasertib plus paclitaxel versus placebo plus paclitaxel as first-line therapy for metastatic triple-negative breast cancer (LOTUS): a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet Oncol 2017;18:1360–72.
45. Tripathy D, Chien AJ, Hylton N, Buxton MB, Ewing CA, Wallace AM, et al. Adaptively randomized trial of neoadjuvant chemotherapy with or without the Akt inhibitor MK-2206: Graduation results from the I-SPY 2 trial. J Clin Oncol 2015;33:524.
46. Basho RK, Gilcrease M, Murthy RK, Helgason T, Karp DD, Meric-Bernstam F, et al. Targeting the PI3K/AKT/mTOR pathway for the treatment of mesenchymal triple-negative breast cancer: evidence from a phase 1 trial of mTOR inhibition in combination with liposomal doxorubicin and bevacizumab. JAMA Oncol 2017;3:509–15.
47. Jovanovic B, Mayer IA, Mayer EL, Abramson VG, Bardia A, Sanders ME, et al. A randomized phase II Neoadjuvant Study of Cisplatin, Paclitaxel With or Without Everolimus in Patients with Stage II/III Triple-Negative Breast Cancer (TNBC): Responses and long-term outcome correlated with increased frequency of DNA damage response gene mutations, TNBC subtype, AR Status, and Ki67. Clin Cancer Res 2017;23:4035–45.
48. Duncan JS, Whittle MC, Nakamura K, Abell AN, Midland AA, Zawistowski JS, et al. Dynamic reprogramming of the kinome in response to targeted MEK inhibition in triple-negative breast cancer. Cell 2012;149:307–21.
49. Yardley DA, Ward PJ, Daniel BR, Eakle JF, Lamar RE, Lane CM, et al. Panitumumab, gemcitabine, and carboplatin as treatment for women with metastatic triple-negative breast cancer: A Sarah Cannon Research Institute Phase II Trial. Clin Breast Cancer 2016; 16:349–55.
50. Nabholtz JM, Chalabi N, Radosevic-Robin N, Dauplat MM, Mouret-Reynier MA, Van Praagh I, et al. Multicentric neoadjuvant pilot Phase II study of cetuximab combined with docetaxel in operable triple negative breast cancer. Int J Cancer 2016;138:2274–80.
51. Finn RS, Press MF, Dering J, Arbushites M, Koehler M, Oliva C, et al. Estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 (HER2), and epidermal growth factor receptor expression and benefit from lapatinib in a randomized trial of paclitaxel with lapatinib or placebo as first-line treatment in HER2-negative or unknown metastatic breast cancer. J Clin Oncol 2009;27:3908–15.
52. Di Leo A, Gomez HL, Aziz Z, Zvirbule Z, Bines J, Arbushites MC, et al. Phase III, double-blind, randomized study comparing lapatinib plus paclitaxel with placebo plus paclitaxel as first-line treatment for metastatic breast cancer. J Clin Oncol 2008;26:5544–52.
53. Nagaria TS, Shi C, Leduc C, Hoskin V, Sikdar S, Sangrar W, et al. Combined targeting of Raf and Mek synergistically inhibits tumorigenesis in triple negative breast cancer model systems. Oncotarget 2017;8:80804–19.
54. Kozma SC, Bogaard ME, Buser K, Saurer SM, Bos JL, Groner B, et al. The human c-Kirsten ras gene is activated by a novel mutation in codon 13 in the breast carcinoma cell line MDA-MB231. Nucleic Acids Res 1987;15:5963–71.
55. Moasser MM, Basso A, Averbuch SD, Rosen N. The tyrosine kinase inhibitor ZD1839 ("Iressa") inhibits HER2-driven signaling and suppresses the growth of HER2-overexpressing tumor cells. Cancer Res 2001;61:7184–8.
56. Brufsky A, Miles D, Zvirbule Z, Eniu A, Lopez-Miranda E, Seo J, et al. Abstract P5-21-01: Cobimetinib combined with paclitaxel as first-line treatment for patients with advanced triple-negative breast cancer (COLET study): primary analysis of cohort I. Cancer Res 2018;78: P5-21-01.
57. Schmid P, Forster MD, Summers YJ, Good J, Sarker S-J, Lim L, et al. A study of vistusertib in combination with selumetinib in patients with advanced cancers: TORCMEK phase Ib results. J Clin Oncol 2017; 35:2548.
58. Horiuchi D, Kusdra L, Huskey NE, Chandriani S, Lenburg ME, Gonzalez-Angulo AM, et al. MYC pathway activation in triple-negative breast cancer is synthetic lethal with CDK inhibition. J Exp Med 2012;209:679–96.
59. Carey JPW, Karakas C, Bui T, Chen X, Vijayaraghavan S, Zhao Y, et al. Synthetic lethality of PARP inhibitors in combination with MYC blockade is independent of BRCA status in triple-negative breast cancer. Cancer Res 2018;78:742–57.
60. Gravina GL, Festuccia C, Popov VM, Di Rocco A, Colapietro A, Sanita P, et al. c-Myc sustains transformed phenotype and promotes radioresistance of embryonal rhabdomyosarcoma cell lines. Radiat Res 2016;185:411–22.
61. Shu S, Lin CY, He HH, Witwicki RM, Tabassum DP, Roberts JM, et al. Response and resistance to BET bromodomain inhibitors in triple-negative breast cancer. Nature 2016;529:413–7.
62. Bai L, Zhou B, Yang CY, Ji J, McEachern D, Przybranowski S, et al. Targeted degradation of BET proteins in triple-negative breast cancer. Cancer Res 2017;77:2476–87.
63. Desrivieres S, Kunz C, Barash I, Vafaizadeh V, Borghouts C, Groner B. The biological functions of the versatile transcription factors STAT3 and STAT5 and new strategies for their targeted inhibition. J Mammary Gland Biol Neoplasia 2006;11:75–87.
64. Marotta LL, Almendro V, Marusyk A, Shipitsin M, Schemme J, Walker SR, et al. The JAK2/STAT3 signaling pathway is required for growth of CD44(+)CD24(−) stem cell-like breast cancer cells in human tumors. J Clin Invest 2011;121:2723–35.
65. Britschgi A, Andraos R, Brinkhaus H, Klebba I, Romanet V, Muller U, et al. JAK2/STAT5 inhibition circumvents resistance to PI3K/mTOR blockade: a rationale for cotargeting these pathways in metastatic breast cancer. Cancer Cell 2012;22:796–811.
66. Balko JM, Schwarz LJ, Luo N, Estrada MV, Giltnane JM, Davila-Gonzalez D, et al. Triple-negative breast cancers with amplification of JAK2 at the 9p24 locus demonstrate JAK2-specific dependence. Sci Transl Med 2016;8:334ra53.
67. Stover DG, Gil Del Alcazar CR, Brock J, Guo H, Overmoyer B, Balko J, et al. Phase II study of ruxolitinib, a selective JAK1/2 inhibitor, in patients with metastatic triple-negative breast cancer. NPJ Breast Cancer 2018;4:10.
68. Locatelli MA, Aftimos P, Dees EC, LoRusso PM, Pegram MD, Awada A, et al. Phase I study of the gamma secretase inhibitor PF-03084014 in combination with docetaxel in patients with advanced triple-negative breast cancer. Oncotarget 2017;8:2320–8.
69. Qiu M, Peng Q, Jiang I, Carroll C, Han G, Rymer I, et al. Specific inhibition of Notch1 signaling enhances the antitumor efficacy of chemotherapy in triple negative breast cancer through reduction of cancer stem cells. Cancer Lett 2013;328:261–70.
70. Zhang CC, Yan Z, Zong Q, Fang DD, Painter C, Zhang Q, et al. Synergistic effect of the gamma-secretase inhibitor PF-03084014 and docetaxel in breast cancer models. Stem Cells Transl Med 2013;2:233–42.
71. Wang K, Zhang Q, Li D, Ching K, Zhang C, Zheng X, et al. PEST domain mutations in Notch receptors comprise an oncogenic driver segment in triple-negative breast cancer sensitive to a gamma-secretase inhibitor. Clin Cancer Res 2015;21:1487–96.
72. Robinson DR, Kalyana-Sundaram S, Wu YM, Shankar S, Cao X, Ateeq B, et al. Functionally recurrent rearrangements of the MAST kinase and Notch gene families in breast cancer. Nat Med 2011;17: 1646–51.
73. 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;486:395–9.
74. Moynahan ME, Chiu JW, Koller BH, Jasin M. Brca1 controls homology-directed DNA repair. Mol Cell 1999;4:511–8.

75. Tutt A, Bertwistle D, Valentine J, Gabriel A, Swift S, Ross G, et al. Mutation in Brca2 stimulates error-prone homology-directed repair of DNA double-strand breaks occurring between repeated sequences. EMBO J 2001;20:4704–16.
76. Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR, Bishop DK. The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. J Biol Chem 2000;275: 23899–903.
77. Farmer H, McCabe N, Lord CJ, Tutt AN, Johnson DA, Richardson TB, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature 2005;434:917–21.
78. Hartman AR, Kaldate RR, Sailer LM, Painter L, Grier CE, Endsley RR, et al. Prevalence of BRCA mutations in an unselected population of triple-negative breast cancer. Cancer 2012;118:2787–95.
79. Byrski T, Huzarski T, Dent R, Marczyk E, Jasiowka M, Gronwald J, et al. Pathologic complete response to neoadjuvant cisplatin in BRCA1-positive breast cancer patients. Breast Cancer Res Treat 2014; 147:401–5.
80. Hahnen E, Lederer B, Hauke J, Loibl S, Krober S, Schneeweiss A, et al. Germline mutation status, pathological complete response, and disease-free survival in triple-negative breast cancer: secondary analysis of the GeparSixto Randomized Clinical Trial. JAMA Oncol 2017;3:1378–85.
81. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med 2017;377:523–33.
82. Litton J, Rugo H, Ettl J, Hurvitz S, Gonçalves A, Lee K-H, et al. Abstract GS6-07: EMBRACA: a phase 3 trial comparing talazoparib, an oral PARP inhibitor, to physician's choice of therapy in patients with advanced breast cancer and a germline BRCA mutation. Cancer Res 2018;78:GS6-07-GS6.
83. Wang Y, Krais JJ, Bernhardy AJ, Nicolas E, Cai KQ, Harrell MI, et al. RING domain-deficient BRCA1 promotes PARP inhibitor and platinum resistance. J Clin Invest 2016;126:3145–57.
84. Banda K, Swisher EM, Wu D, Pritchard CC, Gadi VK. Somatic reversion of germline BRCA2 mutation confers resistance to Poly(ADP-ribose) polymerase inhibitor therapy. JCO Precision Oncol 2018:1–6.
85. Wang Y, Bernhardy AJ, Cruz C, Krais JJ, Nacson J, Nicolas E, et al. The BRCA1-Delta11q alternative splice isoform bypasses germline mutations and promotes therapeutic resistance to PARP inhibition and cisplatin. Cancer Res 2016;76:2778–90.
86. Pettitt SJ, Krastev DB, Brandsma I, Drean A, Song F, Aleksandrov R, et al. Genome-wide and high-density CRISPR-Cas9 screens identify point mutations in PARP1 causing PARP inhibitor resistance. Nat Commun 2018;9:1849.
87. Ter Brugge P, Kristel P, van der Burg E, Boon U, de Maaker M, Lips E, et al. Mechanisms of therapy resistance in patient-derived xenograft models of BRCA1-deficient breast cancer. J Natl Cancer Inst 2016;108.
88. Stecklein SR, Kumaraswamy E, Behbod F, Wang W, Chaguturu V, Harlan-Williams LM, et al. BRCA1 and HSP90 cooperate in homologous and non-homologous DNA double-strand-break repair and G2/M checkpoint activation. Proc Natl Acad Sci U S A 2012;109: 13650–5.
89. Xu H, Di Antonio M, McKinney S, Mathew V, Ho B, O'Neil NJ, et al. CX-5461 is a DNA G-quadruplex stabilizer with selective lethality in BRCA1/2 deficient tumours. Nat Commun 2017;8:14432.
90. Turner N, Tutt A, Ashworth A. Hallmarks of 'BRCAness' in sporadic cancers. Nat Rev Cancer 2004;4:814–9.
91. Foulkes WD, Stefansson IM, Chappuis PO, Begin LR, Goffin JR, Wong N, et al. Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst 2003;95: 1482–5.
92. Isakoff SJ, Mayer EL, He L, Traina TA, Carey LA, Krag KJ, et al. TBCRC009: a multicenter phase II clinical trial of platinum monotherapy with biomarker assessment in metastatic triple-negative breast cancer. J Clin Oncol 2015;33:1902–9.
93. Telli ML, Timms KM, Reid J, Hennessy B, Mills GB, Jensen KC, et al. Homologous recombination deficiency (HRD) score predicts response to platinum-containing neoadjuvant chemotherapy in patients with triple-negative breast cancer. Clin Cancer Res 2016;22:3764–73.
94. Minckwitz GV, Timms K, Untch M, Elkin EP, Fasching PA, Schneeweiss A, et al. Prediction of pathological complete response (pCR) by homologous recombination deficiency (HRD) after carboplatin-containing neoadjuvant chemotherapy in patients with TNBC: results from GeparSixto. J Clin Oncol 2015;33:1004.
95. von Minckwitz G, Schneeweiss A, Loibl S, Salat C, Denkert C, Rezai M, et al. Neoadjuvant carboplatin in patients with triple-negative and HER2-positive early breast cancer (GeparSixto; GBG 66): a randomised phase 2 trial. Lancet Oncol 2014;15:747–56.
96. Schrijver WA, Jiwa LS, van Diest PJ, Moelans CB. Promoter hypermethylation profiling of distant breast cancer metastases. Breast Cancer Res Treat 2015;151:41–55.
97. Davies H, Glodzik D, Morganella S, Yates LR, Staaf J, Zou X, et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. Nat Med 2017;23:517–25.
98. Zhao EY, Shen Y, Pleasance E, Kasaian K, Leelakumari S, Jones M, et al. Homologous recombination deficiency and platinum-based therapy outcomes in advanced breast cancer. Clin Cancer Res 2017; 23:7521–30.
99. Jiao S, Xia W, Yamaguchi H, Wei Y, Chen MK, Hsu JM, et al. PARP Inhibitor upregulates PD-L1 expression and enhances cancer-associated immunosuppression. Clin Cancer Res 2017;23:3711–20.
100. Nguyen M, Robillard L, Lin KK, Harding TC, Simmons AD. The PARP inhibitor rucaparib activates the STING pathway and enhances antitumor responses of immune checkpoint inhibitors in BRCA deficient syngeneic models [abstract]. In: Proceedings of the American Association for Cancer Research for Cancer Research Annual Meeting 2018; 2018 Apr 14–18; Chicago, IL. Philadelphia (PA): AACR; 2018. Abstract nr 1716.
101. Konstantinopoulos PA, Waggoner SE, Vidal GA, Mita MM, Fleming GF, Holloway RW, et al. TOPACIO/Keynote-162 (NCT02657889): a phase 1/2 study of niraparib + pembrolizumab in patients (pts) with advanced triple-negative breast cancer or recurrent ovarian cancer (ROC)—Results from ROC cohort. J Clin Oncol 2018;36:106.
102. Roll JD, Rivenbark AG, Sandhu R, Parker JS, Jones WD, Carey LA, et al. Dysregulation of the epigenome in triple-negative breast cancers: basal-like and claudin-low breast cancers express aberrant DNA hypermethylation. Exp Mol Pathol 2013;95:276–87.
103. Kagara N, Huynh KT, Kuo C, Okano H, Sim MS, Elashoff D, et al. Epigenetic regulation of cancer stem cell genes in triple-negative breast cancer. Am J Pathol 2012;181:257–67.
104. Karsli-Ceppioglu S, Dagdemir A, Judes G, Lebert A, Penault-Llorca F, Bignon YJ, et al. The epigenetic landscape of promoter genome-wide analysis in breast cancer. Sci Rep 2017;7:6597.
105. Connolly RM, Li H, Jankowitz RC, Zhang Z, Rudek MA, Jeter SC, et al. Combination epigenetic therapy in advanced breast cancer with 5-azacitidine and entinostat: a phase II National Cancer Institute/Stand Up to Cancer Study. Clin Cancer Res 2017;23:2691–701.
106. Belkina AC, Denis GV. BET domain co-regulators in obesity, inflammation and cancer. Nat Rev Cancer 2012;12:465–77.
107. Adams S, Gray RJ, Demaria S, Goldstein L, Perez EA, Shulman LN, et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. J Clin Oncol 2014;32:2959–66.
108. Denkert C, Loibl S, Noske A, Roller M, Muller BM, Komor M, et al. Tumor-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer. J Clin Oncol 2010;28:105–13.
109. Schmid P, Cruz C, Braiteh FS, Eder JP, Tolaney S, Kuter I, et al. Abstract 2986: Atezolizumab in metastatic TNBC (mTNBC): Long-term clinical outcomes and biomarker analyses. Cancer Res 2017;77:2986.
110. Dieci MV, Criscitiello C, Goubar A, Viale G, Conte P, Guarneri V, et al. Prognostic value of tumor-infiltrating lymphocytes on residual disease after primary chemotherapy for triple-negative breast cancer: a retrospective multicenter study. Ann Oncol 2014;25:611–8.

111. Miyashita M, Sasano H, Tamaki K, Hirakawa H, Takahashi Y, Nakagawa S, et al. Prognostic significance of tumor-infiltrating CD8+ and FOXP3+ lymphocytes in residual tumors and alterations in these parameters after neoadjuvant chemotherapy in triple-negative breast cancer: a retrospective multicenter study. Breast Cancer Res 2015;17:124.
112. Nanda R, Chow LQ, Dees EC, Berger R, Gupta S, Geva R, et al. Pembrolizumab in patients with advanced triple-negative breast cancer: phase Ib KEYNOTE-012 study. J Clin Oncol 2016;34:2460–7.
113. Adams S, Schmid P, Rugo HS, Winer E, Loirat D, Cescon DW, et al. Phase 2 study of pembrolizumab (pembro) monotherapy for previously treated metastatic triple-negative breast cancer (mTNBC): KEYNOTE-086 cohort A. J Clin Oncol 2017;35:1088.
114. Adams S, Loi S, Toppmeyer D, Cescon D, De Laurentiis M, Nanda R, et al. Abstract PD6-10: KEYNOTE-086 cohort B: Pembrolizumab monotherapy for PD-L1–positive, previously untreated, metastatic triple-negative breast cancer (mTNBC). Cancer Res 2018;78:PD6–10.
115. Adams S, Diamond JR, Hamilton EP, Pohlmann PR, Tolaney SM, Molinero L, et al. Phase Ib trial of atezolizumab in combination with nab-paclitaxel in patients with metastatic triple-negative breast cancer (mTNBC). J Clin Oncol 2016;34:1009.
116. Tolaney S, Kalinsky K, Kaklamani V, Savulsky C, Olivo M, Aktan G, et al. Abstract PD6-13: Phase 1b/2 study to evaluate eribulin mesylate in combination with pembrolizumab in patients with metastatic triple-negative breast cancer. Cancer Res 2018;78:PD6–13.
117. Dirix LY, Takacs I, Jerusalem G, Nikolinakos P, Arkenau HT, Forero-Torres A, et al. Avelumab, an anti-PD-L1 antibody, in patients with locally advanced or metastatic breast cancer: a phase 1b JAVELIN Solid Tumor study. Breast Cancer Res Treat 2018;167:671–86.
118. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med 2018;379:2108–21.
119. Emens L, Loi S, Rugo H, Schneeweiss A, Diéras V, Iwata H, et al. IMpassion130: efficacy in immune biomarker subgroups from the global, randomized, double-blind, placebo-controlled, phase III study of atezolizumab + nab-paclitaxel in patients with treatment-naïve, locally advanced or metastatic triple-negative breast cancer. San Antonio Breast Cancer Symposium 2018.
120. Kwa MJ, Adams S. Checkpoint inhibitors in triple-negative breast cancer (TNBC): where to go from here. Cancer 2018;124:2086–103.
121. Barrett MT, Anderson KS, Lenkiewicz E, Andreozzi M, Cunliffe HE, Klassen CL, et al. Genomic amplification of 9p24.1 targeting JAK2, PD-L1, and PD-L2 is enriched in high-risk triple negative breast cancer. Oncotarget 2015;6:26483–93.
122. Loi S, Dushyanthen S, Beavis PA, Salgado R, Denkert C, Savas P, et al. RAS/MAPK activation is associated with reduced tumor-infiltrating lymphocytes in triple-negative breast cancer: therapeutic cooperation between MEK and PD-1/PD-L1 immune checkpoint inhibitors. Clin Cancer Res 2016;22:1499–509.
123. Hellmann MD, Ciuleanu TE, Pluzanski A, Lee JS, Otterson GA, Audigier-Valette C, et al. Nivolumab plus ipilimumab in lung cancer with a high tumor mutational burden. N Engl J Med 2018;378:2093–104.
124. Saleh SMI, Bertos N, Gruosso T, Gigoux M, Souleimanova M, Zhao H, et al. Identification of interacting stromal axes in triple-negative breast cancer. Cancer Res 2017;77:4673–83.
125. Saleh S, Gruosso T, Gigoux M, Bertos N, Omeroglu A, Zuo D, et al. Abstract IA23: deconvolution of the triple-negative breast cancer microenvironment. Cancer Res 2016;76:IA23–IA.
126. Harano K, Wang Y, Lim B, Seitz RS, Morris SW, Bailey DB, et al. Rates of immune cell infiltration in patients with triple-negative breast cancer by molecular subtype. PLoS One 2018;13:e0204513.
127. Sparano JA, Gray RJ, Makower DF, Pritchard KI, Albain KS, Hayes DF, et al. Adjuvant chemotherapy guided by a 21-gene expression assay in breast cancer. N Engl J Med 2018;379:111–21.
128. Keren L, Bosse M, Marquez D, Angoshtari R, Jain S, Varma S, et al. A structured tumor-immune microenvironment in triple negative breast cancer revealed by multiplexed ion beam imaging. Cell 2018;174:1373–87e19.
129. Hoadley KA, Siegel MB, Kanchi KL, Miller CA, Ding L, Zhao W, et al. Tumor evolution in two patients with basal-like breast cancer: a retrospective genomics study of multiple metastases. PLoS Med 2016;13:e1002174.
130. Siegel MB, He X, Hoadley KA, Hoyle A, Pearce JB, Garrett AL, et al. Integrated RNA and DNA sequencing reveals early drivers of metastatic breast cancer. J Clin Invest 2018;128:1371–83.
131. Yates LR, Knappskog S, Wedge D, Farmery JHR, Gonzalez S, Martincorena I, et al. Genomic evolution of breast cancer metastasis and relapse. Cancer Cell 2017;32:169–84e7.
132. Cejalvo JM, Martinez de Duenas E, Galvan P, Garcia-Recio S, Burgues Gasion O, Pare L, et al. Intrinsic subtypes and gene expression profiles in primary and metastatic breast cancer. Cancer Res 2017;77:2213–21.
133. Hoefnagel LD, van de Vijver MJ, van Slooten HJ, Wesseling P, Wesseling J, Westenend PJ, et al. Receptor conversion in distant breast cancer metastases. Breast Cancer Res 2010;12:R75.
134. Dieci MV, Barbieri E, Piacentini F, Ficarra G, Bettelli S, Dominici M, et al. Discordance in receptor status between primary and recurrent breast cancer has a prognostic impact: a single-institution analysis. Ann Oncol 2013;24:101–8.
135. Chen T, Zhang N, Moran MS, Su P, Haffty BG, Yang Q. Borderline ER-positive primary breast cancer gains no significant survival benefit from endocrine therapy: a systematic review and meta-analysis. Clin Breast Cancer 2018;18:1–8.
136. Iwamoto T, Booser D, Valero V, Murray JL, Koenig K, Esteva FJ, et al. Estrogen receptor (ER) mRNA and ER-related gene expression in breast cancers that are 1% to 10% ER-positive by immunohistochemistry. J Clin Oncol 2012;30:729–34.
137. Symmans WF, Wei C, Gould R, Yu X, Zhang Y, Liu M, et al. Long-term prognostic risk after neoadjuvant chemotherapy associated with residual cancer burden and breast cancer subtype. J Clin Oncol 2017;35:1049–60.
138. Masuda N, Lee SJ, Ohtani S, Im YH, Lee ES, Yokota I, et al. Adjuvant capecitabine for breast cancer after preoperative chemotherapy. N Engl J Med 2017;376:2147–59.
139. Almendro V, Cheng YK, Randles A, Itzkovitz S, Marusyk A, Ametller E, et al. Inference of tumor evolution during chemotherapy by computational modeling and in situ analysis of genetic and phenotypic cellular diversity. Cell Rep 2014;6:514–27.
140. Kim C, Gao R, Sei E, Brandt R, Hartman J, Hatschek T, et al. Chemoresistance evolution in triple-negative breast cancer delineated by single-cell sequencing. Cell 2018;173:879–93e13.
141. Gao R, Davis A, McDonald TO, Sei E, Shi X, Wang Y, et al. Punctuated copy number evolution and clonal stasis in triple-negative breast cancer. Nat Genet 2016;48:1119–30.
142. Marcotte R, Sayad A, Brown KR, Sanchez-Garcia F, Reimand J, Haider M, et al. Functional genomic landscape of human breast cancer drivers, vulnerabilities, and resistance. Cell 2016;164:293–309.
143. Tsherniak A, Vazquez F, Montgomery PG, Weir BA, Kryukov G, Cowley GS, et al. Defining a cancer dependency map. Cell 2017;170:564–76e16.
144. Witwicki RM, Ekram MB, Qiu X, Janiszewska M, Shu S, Kwon M, et al. TRPS1 is a lineage-specific transcriptional dependency in breast cancer. Cell Rep 2018;25:1255–67e5.
145. Stratikopoulos EE, Dendy M, Szabolcs M, Khaykin AJ, Lefebvre C, Zhou MM, et al. Kinase and BET inhibitors together clamp inhibition of PI3K signaling and overcome resistance to therapy. Cancer Cell 2015;27:837–51.
146. Wang Y, Zhang T, Kwiatkowski N, Abraham BJ, Lee TI, Xie S, et al. CDK7-dependent transcriptional addiction in triple-negative breast cancer. Cell 2015;163:174–86.
147. Bruna A, Rueda OM, Greenwood W, Batra AS, Callari M, Batra RN, et al. A biobank of breast cancer explants with preserved intra-tumor heterogeneity to screen anticancer compounds. Cell 2016;167:260–74e22.

148. Bardia A, Parton M, Kummel S, Estevez LG, Huang CS, Cortes J, et al. Paclitaxel with inhibitor of apoptosis antagonist, LCL161, for localized triple-negative breast cancer, prospectively stratified by gene signature in a biomarker-driven neoadjuvant trial. J Clin Oncol 2018:JCO2017748392.
149. Hofmann D, Nitz U, Gluz O, Kates RE, Schinkoethe T, Staib P, et al. WSG ADAPT-adjuvant dynamic marker-adjusted personalized therapy trial optimizing risk assessment and therapy response prediction in early breast cancer: study protocol for a prospective, multi-center, controlled, non-blinded, randomized, investigator initiated phase II/III trial. Trials 2013;14:261.
150. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013;6:pl1.
151. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012;2:401–4.