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Insights into molecular classifications of triple-negative breast cancer: improving patient selection for treatment

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SUMMARY

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 comprehensive profiling of tumors for cellular composition and molecular features. Technological 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.

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 15–20% of all breast carcinomas (2). Compared to 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 TNBC patients, 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 OS (overall survival) with current treatment options is 13–18 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,

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transcriptomic, proteomic, epigenomic, and microenvironmental alterations have expanded our knowledge of TNBC. Here we review the most recent innovations in TNBC molecular taxonomy, the complex interaction between these classifications (Figure 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 (IHC)-defined triple-negative disease, basal-like tumors exhibit the greatest overlap with TNBC. Between 50–75% of TNBC have basal phenotype and approximately 80% of basal-like tumors are ER-negative/HER2-negative (Figure 2) (7,8). Characterization 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 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 (e.g., *CCNE, FANCA*) and low levels of estrogen signaling-related (e.g., *FOXA1, PGR*) genes were associated with pCR, while high expression of epithelial-mesenchymal transition (EMT) genes (e.g., *TWIST1, 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 dataset and in CALGB/9741, the two previously identified signatures (low luminal-A, 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) (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 to carboplatin in non-basal-like tumors (ORR 72.2% vs. 16.7%; p=0.002) (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 7–14% of all breast cancers (6). Approximately 70% of claudin-low tumors are TNBC, with high representation of metaplastic and medullary breast carcinomas. While 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*, *CD79a*). These mesenchymal features (including elevated expression of CD44, vimentin, N-cadherin) and low epithelial differentiation (low *CD24* gene expression) resemble a mammary stem cell-like phenotype ($CD44^{+}CD24^{-/low}$) that can be acquired by EMT (6). In retrospectives studies, claudin-low tumors were associated with lower (39%) pCR rates compared to 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 signaling may represent a potential therapeutic strategy to help prevent the development of chemo-refractory 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 *et al.* analyzed 21 public microarray datasets filtered for TNBC based on *ER*, *PR*, and *HER2* 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) (15). These subtypes are characterized by distinct patterns of molecular alterations, both 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*) (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, NFKB) 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, SPEDF, and XBP1*), with elevated mRNA and protein levels of 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%) (16). The 7-subtype classification independently predicted pCR, but not distant metastasis-free or overall survival (OS) in a retrospective analysis of patients with TNBC treated with neoadjuvant chemotherapy (17). Median OS was highest in 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 5 datasets 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 histological 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 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. Similarly to the 7-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 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 *et al.*: LAR, Mesenchymal (MES), Basal-like Immune Suppressed (BLIS), and Basal-like Immune Activated (BLIA) (21). Interestingly, the BLIS subtype exhibited the worst prognosis and the BLIA subgroup conferred the best outcome in terms of disease-free survival. 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 dataset, 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, since it does not reflect a clear molecular entity. What seems clear is that luminal (ER-positive or AR-positive) and non-luminal (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 androgen receptor (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 anti-androgens in this subgroup. *In vivo* studies have shown that tumors derived from LAR cell lines (e.g., MDA-MB-453, SUM185PE, 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) (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^+ TNBC. Enrichment in *PIK3CA* mutations has been described in triple-negative tumors that are AR^+ (36–40%) by IHC compared to AR^- (4–9%) (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^+ TNBC preclinical models, and a phase I trial is planned to explore enzalutamide plus alpelisib, an α-specific PI3K inhibitor, in patients with AR^+, $PTEN^{low}$ (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^+ MCF7 cell line (25). LAR cell lines exhibit lower transcriptomic levels of *CCNE1* and *CDK2* compared to basal-like TNBC and, thus, are dependent on CDK4/6 to phosphorylate RB1 and re-enter the cell cycle. *In vitro* PI3K inhibition decreases post-mitotic 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^{+-} 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 Trop-2, which is expressed in almost 90% of TNBC (32). In patients with heavily pre-treated metastatic TNBC, IMMU-132 achieved an ORR of 30%, and median 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 to 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 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) (36,37,39).

Most somatic mutations in TNBC occur in tumor suppressor genes (e.g., *TP53*, *RB1*, *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%) (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 twelve base-substitution signatures (including the five previously identified signatures), two indel signatures and six rearrangements signatures. Large 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 of 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) (15). Loss of PTEN and INPP4B, which also sensitizes cell lines to PI3K inhibition (42), are 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^+ *PIK3CA*-mutant breast cancer, though fewer studies have been conducted in TNBC. In the BELLE-4 trial, patients with locally advanced or metastatic $HER2^-$ 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. Addition of buparlisib to paclitaxel failed to demonstrate a significant improvement in progression-free survival (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 to placebo (5.5 versus 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 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 9.0 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 pre-selected 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% versus 22.4% in 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 10–30% 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*, *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, panitumumab) or tyrosine kinase inhibitors (e.g., lapatinib) (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) (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 pro-apoptotic BCL-2 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 targeted 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 co-targeting both pathways (65). In addition, amplifications at the *JAK2* locus (9p24) have been detected at a higher frequency in post-neoadjuvant TNBC samples compared to 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 to 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 objective response rate 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 (DSBs), 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, mytomicin 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 to docetaxel (68.0% vs. 33.3%, p=0.03) and PFS (6.8 vs. 4.4 months, interaction p=0.002) (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 to standard non-platinum 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 2–3 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 (57.0% *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 to standard chemotherapy, a significant increase in median PFS was observed with olaparib (7.0 months versus 4.2 months, HR 0.58; $p<0.001$) and with talazoparib (8.6 months versus 5.6 months, HR 0.54; $p<0.001$). Safety profiles were also comparable across trials and hematological 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 $BRCA1^{185delAG}$) (83); secondary *BRCA* reversion mutations that reinstate HR-proficiency through restoration of the open reading frame and *BRCA* re-expression (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* re-expression, 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).

¨*BRCA*ness¨ 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 non-responders (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) (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 dataset (97). This aggregated BRCA*ness* 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 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 re-sensitization 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 turns, 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%) (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 post-translational modification of histone proteins, are a recognized hallmark of cancer. Approximately 60–80% of basal-like and claudin-low breast cancers have aberrant DNA hypermethylation (102). Compared to 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 to breast cancers unmethylated for these genes (40% RFS at 70 and 30 months, respectively). A non-significant 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 non-luminal 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 advanced TNBC treated in a phase II study (105). No significant changes in gene expression in paired biopsies before and after two 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, 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) (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). While the absolute difference in median PFS in the PD-L1-positive population (2.5 months) was not strikingly different than that seen in the intent-to-treat (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 pre-treated 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 immunohistochemical 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 T-, B-, and E-axes (124). Furthermore, these axes strongly influenced the location of $CD8^+$ TIL (125), which may impact 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) (18), significantly differs across refined TNBCtypes, with the highest rates observed in BL1 (48%) and the lowest in M (0%) (126). Whether transcriptomic profiling could be incorporated to routine clinical practice to help select TNBC patients 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 impacts 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 is limited sequencing data in metastatic triple-negative tumors and 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 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 history of independent primary tumors (131). Given the differences in management of primary and recurrent tumors, sequencing of longitudinal samples could impact 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 10–12% of asynchronous recurrences, inducing a switch to TNBC in the metastasis (133), and has been associated with worse survival compared to cases with concordant hormone receptor-positive recurrence (134). To date, we do not fully understand the mechanisms that cause this conversion, and if 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 1–9% 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% versus 56.1%, respectively; HR 0.58; 95% CI: 0.39–0.87) and OS (78.8% versus 70.3%, respectively; HR 0.52; 95% CI: 0.30–0.90) (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 comprised 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 over 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 inter- 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 3-gene signature (elevated *TNFa*, 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 that 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

posthoc 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 of 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, 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 upcoming 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 datasets.

Furthermore, to address the need for advances in drug development and biomarker discovery in TNBC, the elaboration of prospective, large-scale, longitudinal multi-center 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 timeframe, 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.

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Significance

Triple-negative breast cancer is characterized by higher rates of relapse, greater metastatic potential and shorter overall survival compared to other major breast cancer subtypes. The identification of biomarkers that can help guide treatment decisions in triple-negative breast cancer 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.

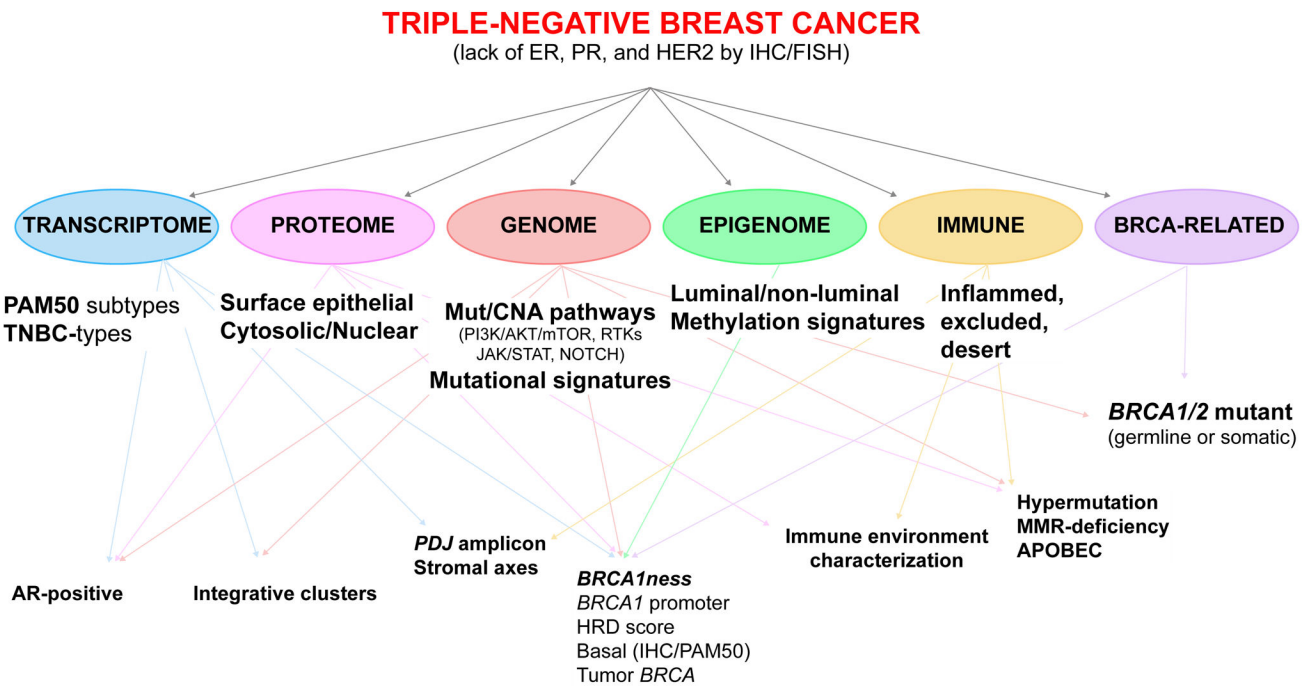

Figure 1.

Overview of the complex interactions between 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; CNA: copy number alterations; AR: androgen receptor; HRD: homologous recombination deficiency; IHC: immunohistochemistry; TIL: tumor-infiltrating lymphocytes.

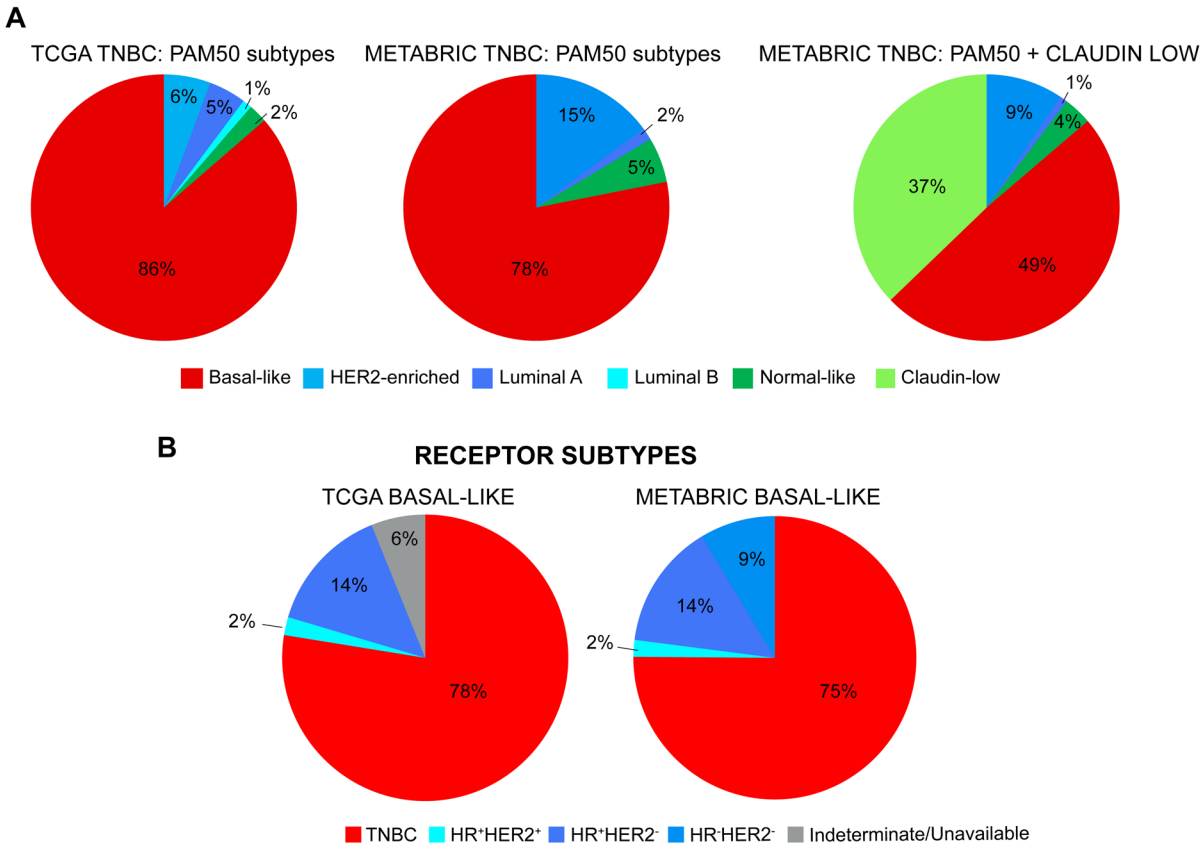

Figure 2.

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 TCGA and METABRIC datasets in triple-negative breast cancer (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 datasets 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 re-analysis of publicly available (22,36,37) using cBioPortal (150,151).

Table 1.

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

TCGA (Basal-like) (36)	**Genomic alteration (frequency, %)**
TP53 pathway	*TP53* mut (84); gain of *MDM2* (14)
PIK3CA/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) (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) (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); CCN*D3* 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)

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 somewhat platform and study dependent. In general, copy number gain ≥5 is considered an amplification, while copy number gain >2 but below 5 is considered a copy number gain. But, some studies define amplification when gene-specific vs. centromeric probe ratio is >2. Frequencies (%) of alterations are included when available.

Table 2.

Efficacy of Genomic-Based Targeted Therapies in Clinical Trials in Triple-Negative Breast Cancer.

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) (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) (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) (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) (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) (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

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Pathway	Drug	Mechanism	Patient population	Trial design (Total N patients)	Intervention	Exploratory biomarker	Efficacy	Clinicaltrials.gov Identifier
EGFR	Panitumumab	EGFR monoclonal antibody	Locally advanced/metastatic TNBC	Non-randomized phase II (n=71) (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	Non-randomized phase II (n=28) (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) (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) (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	Non-randomized phase II (n=21) (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) (67)	PF-03084014 + Docetaxel	NA	ORR: 16 (95% CI: 4.5-36.1)	NCT01876251

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

Table 3.

Ongoing clinical trials in *BRCA*-mutant or *BRCAness*-associated triple-negative breast cancer

Clinicaltrials.gov Identifier	Title	*BRCA* status eligibility criteria	Phase
Neoadjuvant			
NCT03109080	A Phase I of Olaparib With Radiation Therapy in Patients with Inflammatory, Loco-regionally 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 to 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 Multi-center Phase III Study to Assess the Efficacy and Safety of Olaparib Versus Placebo as Adjuvant Treatment in Patients With gBRCA1/2 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, Non-randomized, Multi-center 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, Loco-regionally Advanced or Metastatic TNBC or Patient 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

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 Anti-cancer 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 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, Multi-center 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

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¨.

Table 4.

Results of PD-1/PD-L1 Inhibition in Advanced Triple-Negative Breast Cancer

	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) (111)	**Pembrolizumab in Metastatic TNBC (KEYNOTE-086) (112,113)**	**Atezolizumab in TNBC Unselected for PD-L1 (108)**	**Avelumab in TNBC Unselected for PD-L1 (JAVELIN) (116)**	**Eribulin +/− Pembrolizumab in Metastatic TNBC (ENHANCE-1/ KEYNOTE-150) (115)**	**Atezolizumab + *nab*-Paclitaxel in TNBC Unselected for PD-L1 (114)**	**Atezolizumab + *nab*-Paclitaxel vs. Placebo + *nab*-Paclitaxel in Metastatic TNBC (Impassion130) (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: pre-treated, 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 (50.0)	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 ‡	58	107 (106 ‡)	32	902
Median prior lines of therapy in metastatic setting (range)	2 (0-9)	A: NA B: 0	7 (0-21)	NA *	S1: 0 S2: 1-2	5 (1-10)	0
EFFICACY							
ORR, %	18.5	A: 4.7 B: 22.6	Overall: 9.8 1st line: 26.3 2nd line: 3.6 3rd/+ line: 7.7	5.2	Overall: 26.4 S1: 29.2 S2: 22.0	Overall: 37.5 1st line: 46.1 2nd line: 22.2 3rd/+line: 40.0	ITT: 56.0 vs. 45.9
ORR in PD-L1+ cohort, %	18.5	A: 4.8 B: 22.6	12.7	22.2 **	30.6	36.3 **	58.9 vs. 42.6
CBR, %	25.9 †	A: 7.6 B: 25.0	NA	31.0 †	Overall: 36.8 S1: 40.0 S2: 31.7	81.3 †	NA

	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) (111)	**Pembrolizumab in Metastatic TNBC (KEYNOTE-086) (112,113)**	**Atezolizumab in TNBC Unselected for PD-L1 (108)**	**Avelumab in TNBC Unselected for PD-L1 (JAVELIN) (116)**	**Eribulin +/− Pembrolizumab in Metastatic TNBC (ENHANCE-1/ KEYNOTE-150) (115)**	**Atezolizumab + *nab*-Paclitaxel in TNBC Unselected for PD-L1 (114)**	**Atezolizumab + *nab*-Paclitaxel vs. Placebo + *nab*-Paclitaxel in Metastatic TNBC (Impassion130) (117)**
Median PFS, mo. (95% CI)	1.9 (1.7-5.5)	A: 2.0 (1.9-2.0) B: 2.1 (2.0-2.3)	NA	1.5 (1.4-1.7)	Overall: 4.2 (4.1-5.6) S1: 4.9 (4.1-6.1) S2: 4.1 (2.1-6.2)	NE	ITT: 7.2 vs. 5.5; HR 0.80 (0-69-0.92) PD-L1+: 7.5 vs. 5.0; HR 0.62 (0.49-0.78)
Median OS, mo. (95% CI)	11.2 (5.3-NR)	A: 8.9 (7.2-11.2) (CR, PR, or SD: NR; PD: 7.1 [6.3-8.8]) B: 19.2 (11.3-NE)	9.3 (7.0-12.6)	9.2 (4.3-NE)	Overall: 17.7 (13.7-NE) S1: 17.7 (13.3-NE) S2: 16.3 (12.4-19.2)	NE (8.0-NE)	ITT: 21.3 vs. 17.6; HR 0.84 (0.69-1.02) PD-L1+: 25.0 vs. 15.5; HR 0.62 (0.45-0.86)

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 ration.

Response rates per RECIST 1.1 criteria.

[†] DCR: defined as confirmed complete, partial response or stable disease as best response.

[‡] Number of patients considered Objective Response-Evaluable.

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

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