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Identification and use of biomarkers in treatment strategies for triple negative breast cancer subtypes

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Abstract

Triple negative breast cancer (TNBC) is a heterogeneous disease with distinct molecular subtypes that differentially respond to chemotherapy and targeted agents. The absence of high-frequency molecular alterations and a limited number of known biomarkers have limited the development of therapeutic strategies for the disease. Herein, we summarize the results of the first round of targeted therapy approaches in TNBC and discuss new preclinical strategies. Common themes emerge from the proposed strategies, such as the use of biomarkers to identify tumors with genomic instability, targeting adapted molecular states resulting from tumor suppressor loss, and targeting altered metabolic pathways.

Keywords

Triple negative breast cancer; EGFR; FGFR; TP53; WEE1; AR; VEGFR; INPP4B; genomic instability; subtypes; TNBCtype; PHGDH; LDHB; INPP4B; PTEN; PIK3CA; TP53; basal-like; therapy

Introduction

Triple negative breast cancer (TNBC) is defined by the absence of detectable estrogen receptor (ER) and progesterone receptor (PR) expression and the lack of human epidermal growth factor receptor 2 (HER2) gene amplification. This heterogeneous collection of tumors, representing ~15% to 20% of all breast cancers, is generally more aggressive, with higher rates of relapse and decreased overall survival in metastatic disease[1]. Despite overall poor outcomes, it is evident that a subset of patients respond to standard-of-care chemotherapy combinations and that those patients who achieve pathological complete response (pCR) after neoadjuvant chemotherapy have survival rates similar to other breast cancer subtypes [2,3]. However, TNBC patients with residual disease after chemotherapy have significantly worse survival and higher rates of relapse within the first three years after treatment [3].

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Current standard-of-care treatment regimes often include a combination of taxanes and genotoxic agents. Over the past decade there have been numerous attempts to use genomic data to identify targetable states in TNBC; however gross inter/intra-tumor variability has led to limited success. The heterogeneity of TNBC is further highlighted by the high prevalence of rare histopathological subtypes, such as metaplastic (90%), medullary (95%), adenoid cystic (90–100%) and apocrine (40–60%) carcinomas [4]. We provided further insight into the complexity of the disease by using gene expression (GE) analysis to identify distinct molecular TNBC subtypes displaying unique biologies and drug sensitivities[5].

One alteration that occurs in the majority of TNBC is mutation or loss of *TP53*. Of the 102 TNBC cases in The Cancer Genome Atlas (TCGA), 68% have *TP53* mutations in addition to homozygous deletion of the gene (3%) or *MDM2/4* amplifications (7%) (TCGA cbio portal) [6,7]. Confirming these data, Shah and colleagues sequenced 104 TNBC tumors and found that *TP53* mutations are the most frequent clonal event (53.8%), followed by *PIK3CA* mutations (10.7%) and after that a diverse catalogue of mutations in cytoskeletal, cell shape and motility proteins that occurred at much lower clonal frequencies[8]. The absence of a high-frequency, targetable oncogenic driver has hindered the development of successful therapeutic strategies.

Basal-like TNBC

Basal cells in the breast are defined as the cells in the basal position and adjacent to the basement membrane [9]. Interest in basal cells was stimulated after gene expression microarray profiling molecularly divided breast cancer into five intrinsic subtypes, with one of those subtypes displaying basal-like gene expression[10]. Tumors were categorized as basal-like due to the expression of genes found in normal basal/myoepithelial breast cells, including high-molecular weight basal cytokeratins (CK; CK5/6, CK14 and CK17)[11]. Basal-like breast cancers tend to occur in younger patients, frequently harbor *TP53* mutations, are high grade and are generally more aggressive[12]. While basal-like breast cancers largely consist of TNBC, these classifications are by no means synonymous. Evaluation of cancers classified on the basis of gene expression demonstrated that basal-like breast cancers are ER-, PR- and HER2-positive to varying degrees (15–54%)[13]. Further evidence supporting the incomplete overlap between TNBC and basal-like comes from Bastien *et al.*, who showed that 57% of TNBCs were basal-like and nearly 30% of with the HER2-enriched group were TNBC by PAM50 analysis[14]. Together, these results suggest that a majority of tumors with basal-like gene expression are TNBC, but not all TNBC are basal-like. Therefore, caution should be used when using the term basal-like to refer to TNBC at large.

Molecular subtyping of TNBC

Initial studies evaluating gene expression profiles from TNBC tumors concluded that TNBCs are synonymous with basal-like breast cancer, despite the observation of five distinct clusters in hierarchical analysis [15]. These results may have been confounded by the presence of false negative ER+ and HER2+ samples by IHC or report annotation, which have dramatic influence on gene normalization in the analysis. In order to decipher the inter-tumor molecular heterogeneity of TNBCs, we performed a large meta-analysis of 21

datasets and identified 587 TNBC expression profiles using bimodal filtering in order to remove PR+, ER+ and HER+ tumors from the analyses. When normalized in the absence of known receptor positive tumors, these TNBC profiles revealed at least six distinct molecular subtypes with differing biologies that include two basal-like (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem-like (MSL), and a luminal androgen receptor (LAR) subtype[5]. The BL1 subtype is heavily enriched in cell division pathway components, as well as DNA damage response (ATR/BRCA) pathways. The BL2 subtype displayed unique gene ontologies that involve growth factor signaling (EGF pathway, NGF pathway, MET pathway, Wnt/ β -catenin, and IGF1R pathway), glycolysis and gluconeogenesis and the expression of myoepithelial markers (TP63 and MME). The IM subtype is enriched for gene ontologies in immune cell processes and immune signal transduction pathways. It is still unclear whether this signature is reflective of the tumor cells or is a blend of tumor and immune infiltrate. However, others have identified an immune subtype of TNBCs that is associated with good prognosis {Rody: 2011gd, Desmedt:2008im, Teschendorff:2008ce, Kreike:2007ky}. Both the M and MSL subtypes are characterized by genes involved in motility, extracellular matrix, cell differentiation pathways, and genes associated with epithelial to mesenchymal transition (EMT). However, the MSL subtype differs in that it expresses low levels of proliferation genes and is enriched for mesenchymal stem cell-associated genes. The LAR subtype displayed luminal GE patterns and is heavily enriched for genes involved in steroid synthesis, and androgen/estrogen metabolism, including high levels of AR and its downstream targets and co-activators.

We also used GE signatures derived from the TNBC subtypes to identify representative TNBC cell lines that serve as models for each subtype. Five of the seven cell lines that harbor BRCA1/2 mutations were classified as BL1 and BL2 subtypes. The cell lines that were classified as MSL have dedifferentiated morphologies observed in breast carcinosarcoma as well as metaplastic and anaplastic carcinomas. The LAR cell lines have high levels of AR RNA and protein expression. Furthermore, the cell lines displayed differential sensitivity to cisplatin (BL1 cell lines and BRCA1/2 mutated lines most sensitive), the abl/src inhibitor dasatinib (M and MSL cell lines preferentially sensitive), the PI3K/mTOR inhibitor NVP-BEZ235 (M, MSL and LAR cell lines most sensitive) and the AR agonist bicalutamide (only LAR cell lines displaying sensitivity). These cell models provide the framework for future preclinical studies evaluating subtype-specific targeting of growth factor signaling and cell differentiation pathways.

Comparison of TNBC type to the intrinsic molecular subtypes

TNBCs clearly display distinct gene expression profiles that can be masked in the normalization process preceding genomic analyses. While TNBCs generally display basal-like gene expression patterns, this analysis is dependent on the population of samples during normalization. To determine the relationship between the PAM50 intrinsic subtypes[10] and the TNBC molecular subtypes (TNBCtype)[17], we extracted 374 TNBC samples from 14 datasets comprising 2441 gene expression profiles and performed both PAM50 and TNBC subtyping on each the cases (Fig. 1A). As anticipated when normalized with all samples including ER+, PR+ and HER2+ tumors, the majority of the TNBC samples are indeed

classified as basal-like (301, 81%) followed by HER2 (38, 10%), normal-like (17, 5%), luminal B (13, 3%) and luminal A (4, 1%). These subtype 'calls' are dependent on normalization, as intrinsic prediction of just the 374 TNBC samples normalized to one another provides a very different distribution: basal-like (194, 52%), luminal B (72, 19%) luminal A (44, 12%), normal-like (32, 9%), and HER2 (31, 8%). A similar scenario occurs when ER+ samples are normalized with TNBC samples prior to TNBC subtype prediction[17]. While these classifications provide hints at biology, they are highly dependent on normalization and by no means ready for clinical applications. However, new quantitative platforms such as RNA-seq will begin to address this bias and allow stable single sample predictions, provided the subtypes are reproducible across platforms. Using the intrinsic subtype tool that was generated from tumors representing all breast cancer, we examined the composition of each TNBC subtype. With the exception of MSL and LAR, all other TNBC subtypes are primarily composed of the basal-like intrinsic subtype [BL1 (99%), BL2 (95%), IM (84%) and M (97%)] (Fig. 1B). In contrast, MSL TNBCs are comprised of ~50% basal-like and the remainder normal-like (28%), luminal B (14%). Interestingly, the LAR subtype is classified as HER2 (74%) and Luminal B (14%) by PAM50 intrinsic subtyping (Fig. 1B). These differences can also be seen when TNBCs are separated into basal-like or non-basal groups by PAM50 intrinsic subtype, after which the vast majority of non-basal TNBC are classified as LAR and MSL tumors (Fig. 1C). Further evaluation of GE profiles identified a claudin-low subset of TNBC tumors characterized by the absence of luminal differentiation markers and tight junction proteins and enrichment for EMT markers, immune response genes and cancer stem cell-like features[18]. Our analysis showed that the vast majority of tumors classified as claudin-low are composed of M and MSL TNBC subtypes, consistent with the elevated levels of EMT-associated genes. (Figure 1D). Together, these data suggest that grouping TNBC into basal and non-basal subtypes is oversimplifying the molecular heterogeneity.

TNBC subtypes demonstrate clinical utility

In order to determine the potential clinical utility of classifying tumors by TNBC subtype, we generated an online tool (TNBCtype) that determines the molecular subtype from GE profiles, independent of platform[19]. Recently, Masuda *et al.* performed a retrospective analysis on 130 TNBC biopsies obtained prior to neoadjuvant anthracycline and taxane chemotherapy (insert masuda reference). While the overall pCR response was 28%, subtype-specific responses differed substantially, with the BL1 subtype achieving the highest pCR rate (52%) and the BL2, LAR and MSL subtypes having the poorest response (0% and 10% 23%, respectively). Furthermore, unlike PAM50 subtyping, TNBC subtype was shown to be an independent predictor of pCR status ($p = 0.022$) by a likelihood ratio test. These findings and additional retrospective and prospective validation have the potential to guide differential use of chemotherapy-based regimens based on molecular signature and the alignment of patients with select TNBC subtypes (e.g. LAR) to clinical trials investigating targeted therapies. There is a need for prospective validation of pCR rates amongst the TNBC subtypes and an assessment of whether subtyping is useful for predicting long-term patient outcome.

Targeting overexpressed growth factor receptors in TNBC

The generation of highly specific monoclonal antibodies and small molecule kinase inhibitors has greatly improved overall survival of HER2+ breast cancer. Several growth factor receptors and ligands are overexpressed in TNBC, however results from clinical trials aimed at targeting growth factor signaling pathways have been disappointing. The inter-tumor heterogeneity of TNBC amongst the patient cohorts enrolled may have contributed to the negative results of many of these trials.

EGFR

When Epidermal Growth Factor Receptor (EGFR) was found to be expressed at a higher level in 70–78% of basal-like TNBC compared to non-TNBC [19] [20], there was optimism for targeting this receptor based on the successes in EGFR mutant lung cancers. Two completed trials investigated the addition of the monoclonal anti-EGFR antibody cetuximab to a platinum crosslinking agent in metastatic TNBC. In TBCRC001, the response rates of patients treated with cetuximab alone or in combination with carboplatin were relatively low at 6% and 17%, respectively [21]. The BALI-1 trial demonstrated that the addition of cetuximab to cisplatin increased overall response rate of TNBC patients from 10% to 20% [22]. However, the combinations minimally increased progression free survival and overall survival. These disappointing results suggest that either cetuximab is not inhibiting its target, EGFR activity is ligand-independent, there is compensation by other growth factor pathways, or EGFR expression levels do not indicate tumor dependency on this pathway. Regardless, EGFR inhibition is unlikely to be beneficial in an unselected population of TNBC. While EGFR mutations and amplifications are rare in TNBC, there is evidence that cell lines with EGFR amplification have increased sensitivity to an anti-EGFR monoclonal antibody[23]. Future trials that prospectively identify patients with EGFR amplifications or activating mutations may yield more promising outcomes.

VEGFR

Similar to EGFR, the vascular endothelial growth factor receptor (VEGFR) has been explored as a target in breast cancer. There is indirect clinical evidence that angiogenesis plays an essential role in breast cancer development, invasion, and metastasis[24]. While there aren't any preclinical data indicating that targeting VEGFR would benefit TNBC, initial evidence came from a phase II trial in which the overall response to monotherapy with the VEGFR kinase inhibitor sunitinib was 11% in previously treated metastatic breast cancer, with a notably higher response in TNBC (15%)[25]. In the neoadjuvant setting, a monoclonal antibody against VEGFA, bevacizumab increased pCR of triple negative patients treated with epirubicin, cyclophosphamide and docetaxel from 27.9% to 39.3% [26]. Despite promising phase II results, the addition of sunitinib to capecitabine did not improve progression free survival of TNBC patients in a phase III study[27]. Similarly, there was no statistical improvement in progression free survival with the addition of bevacizumab to adjuvant chemotherapy in a phase III trial (BEATRICE)[28]. These trials demonstrate that the VEGFR inhibitors are ineffective in adjuvant treatment in unselected TNBC patients, but may have some efficacy in metastatic TNBC, which is likely enriched for chemo-resistant tumors.

FGFR

Targeting the fibroblast growth factor receptor (FGFR) has shown promise in a subset TNBC pre-clinical models, particularly when the receptor is amplified or mutated[29]. FGFR1 amplifications occur in approximately 10% of breast cancer[30] and 9% of TNBC[31]. Amplification of FGFR2 is more rare, and appears in approximately 1–2% of breast cancer and 2–4% of TNBC[29]. Mutations in FGFR family members are quite rare in TNBC (<1%) with only one TNBC tumor harboring a mutation in FGFR2 (S587C) (TGCA cbio portal) [6,7]. In preclinical investigations, cell lines with FGFR1 amplification or FGFR2 and FGFR4 mutations displayed constitutive activation and were sensitive to the FGFR ATP competitive inhibitor PD173074 [29]. In addition to genomic alterations, basal-like TNBC cell lines express an autocrine FGF2 signaling loop that may also be targetable by monoclonal antibodies[32]. Approximately 12% of TNBCs have altered FGFRs (FGFR1 n=9, 9%, FGFR2 n=2, 2%) suggesting that patients harboring those tumors may be candidates for FGFR-based targeted therapies.

Assessing and targeting genomic instability

Many tumors are characterized by some form of genomic instability easily identified by alterations in chromosome number and structure. To deal with the unremitting threat to DNA integrity, cells have evolved a complex DNA damage response (DDR) to maintain genomic integrity. The most deleterious lesion, double strand breaks are repaired by either homologous recombination (HR) or with non-homologous end joining (NHEJ). A subset of familial and sporadic breast, ovarian and pancreatic cancers are deficient in HR repair, resulting from mutations in the breast cancer susceptibility proteins BRCA1 and BRCA2.

It is now evident that commonly used chemotherapies and radiotherapy regimens are more effective in tumors with DDR defects. Platinum agents cause DNA inter- and intra-strand crosslinks, which are readily repaired by nucleotide excision repair and HR. Both isogenic cell line studies[33] and mouse models [34] demonstrated that BRCA1/2 mutations confer sensitivity to platinum agents. The observation that a majority of BRCA1 mutation carriers develop TNBC rather than other forms of breast cancer [35] led investigators to evaluate neoadjuvant cisplatin in unselected TNBC[36]. This study showed an overall pCR of 22% and associated both BRCA-mutated tumors and those with decreased BRCA1 expression with cisplatin sensitivity. Further evidence of the activity of platinum agents in TNBC comes from the GeparSixto trial that compared neoadjuvant paclitaxel, anthracycline, and bevacizumab +/- carboplatin, and found that the addition of carboplatin improved pCR from 38% to 59% [37].

Similar to platinum-derived agents, blocking poly (adenosine diphosphate [ADP]-ribose) (PARP) activity inhibits the growth of tumor cell lines lacking functional BRCA1 or BRCA2 [38]. Numerous clinical trials have investigated the efficacy of PARP inhibitors in the TNBC setting and produced mixed results with single agent PARP inhibitors not yielding objective responses in unselected patients[39]. However, when combined with chemotherapy, PARP inhibitors are well tolerated [40], displaying efficacy in BRCA1/2 mutant tumors and improving overall response from 32% to 52% when combined with gemcitabine and carboplatin [41]. A single arm phase II study of neoadjuvant gemcitabine,

carboplatin, and iniparib showed impressive responses, especially in BRCA 1/2 carriers[42]. However, subsequent phase III study evaluating gemcitabine/carboplatin ± iniparib did not meet the pre-specified criteria for significance for the co-primary endpoints of overall survival and progression free survival. The results should be interpreted cautiously as iniparib non-selectively modifies cysteine-containing proteins and its primary activity is likely not via PARP inhibition[43]. These findings have led many investigators to develop assays for identifying tumors with HR defects to better align patients with these agents[44–46].

Early attempts at identifying tumors with HR defects relied on a biological assay measuring the focal accumulation of the DNA recombinase protein RAD51 at sites of DNA damage by immunofluorescence after genotoxic stress [45]. Cancer cells with defects in HR such as those with BRCA1/2 loss of function are unable to induce RAD51 foci [47] and scoring of RAD51 foci in biopsies taken 24 h from the first cycle of neoadjuvant chemotherapy were strongly predictive for pCR[45]. These data suggest that a functional DDR assay would be able to detect HR-deficient tumors. One caveat however is that the DDR pathway is only fully “active” after DNA damage, and diagnostic tumor biopsies will likely not provide an accurate measurement of the status of DDR activity upon which to make a treatment decision.

Other approaches to assess deficiencies in DNA repair are represented by multiple studies that examined the association between HR defects and genomic patterns of loss of heterozygosity (LOH). One study demonstrated that the number of subchromosomal telomeric regions with allelic imbalance predicts cisplatin sensitivity *in vitro*, and is correlated with pCR to preoperative cisplatin in the TNBC setting [44]. Another study showed that the degree of LOH positively correlated with progression free survival in response to standard chemotherapy [48]. An assay has been developed that generates a homologous recombination deficiency (HRD) score measuring LOH of intermediate size frequently observed in tumors with BRCA1/2 deficiencies [46]. Successful validation and implementation of assays that measure global genomic instability could facilitate the selection of patients who would benefit from PARP inhibitors and platinum-based chemotherapy.

Targeting the adaptive cellular state caused by tumor suppressor loss

While it is evident from multiple genomic analyses that there is no unifying oncogenic driver in TNBC, there appears to be several tumor suppressors that are frequently lost or are present in a mutant form[31,49]. This loss of tumor suppressor function alters global gene expression patterns and signaling pathway activity in a manner that may provide clues to tumor-adaptive states that are amenable to targeted therapy.

PTEN and INPP4B

While PIK3CA mutations are strongly associated with ER+ luminal cancers, loss of the tumor suppressor phosphatases inositol polyphosphate 4-phosphatase type II (INPP4B) and phosphatase and tensin homolog (PTEN)[50] frequently occur in TNBC [51]. Despite the high frequency of activating PIK3CA mutations, reverse phase protein array data

demonstrated that pAKT, pS6 and p4EBP1, typical markers of PI3K pathway activation, were not elevated in luminal (ER+) breast cancer but rather were highly expressed in basal-like cancers and strongly correlated with PTEN and INPP4B loss[31]. PTEN is a well characterized negative regulator of the PI3K pathway through its dephosphorylation of the phosphoinositide capable of activating AKT (PI[3,4,5]P3 to PI[4,5]P2). INPP4B, however preferentially hydrolyzes PI(3,4)P2 and recent evidence demonstrates that INPP4B functions as a tumor suppressor capable of negatively regulating the PI3K/Akt signaling pathway[52]. These data and an initial report that AKT3 is frequently amplified or translocated in some TNBC[53], make AKT an attractive target in a large fraction of TNBC. Early preclinical evidence demonstrates that AKT inhibitors are highly selective, displaying greater efficacy in cell lines with endogenous PIK3CA mutations and PTEN loss or isogenic models of PTEN loss [54].

TP53

Because few tumors retain wild-type p53, investigators are exploring a number of approaches to target the p53 functionally null state. Since loss of p53 function disrupts cell cycle checkpoints, one option is to selectively target tumors cells that lack p53-dependent cell cycle checkpoints. While cells lacking functional p53 still can engage the S-phase checkpoint in response to DNA-damaging chemotherapy, researchers have demonstrated that the WEE1 kinase is needed to prevent premature entry of S-phase arrested cells into mitosis[58]. Therefore either chemotherapy combined with WEE1 inhibition or WEE1 and CHK1 inhibition may selectively force S-phase arrested cells lacking functional p53 into mitotic catastrophe and apoptosis. WEE1 inhibitors are currently being evaluated in phase I trials with and without platinum or gemcitabine in and have been shown to be well tolerated with strong target engagement[59].

Since the vast majority of tumors harbor missense mutations in p53, investigators are exploring a second approach that involves small-molecule targeting of p53 mutant proteins to restore p53 transcriptional activity. Mutant-specific inhibitors are currently under development[60]. The majority of missense mutations in p53 cluster into “hotspot” codons within the DNA-binding domain and there is evidence that these mutations can provide oncogenic potential beyond the simple loss of p53 function[61,62]. Targeting these gain-of-function mutations, restoring wild-type p53 function or targeting cell cycle checkpoint vulnerabilities have the potential to make the largest clinical impact, as *TP53* alteration is by far the most common event in TNBC.

Androgen receptor

While the androgen receptor (AR) is expressed in over 70% of breast cancers and is strongly associated with ER positivity, approximately 10–15% of TNBCs also express AR [63] [64]. Others have identified ER–, AR+ tumors displaying non-basal gene expression and termed these as a molecular apocrine subtype[65]. Using GE analysis, we identified that a similar percentage (12%) of TNBCs are highly enriched for AR and AR gene targets and display luminal gene expression. Furthermore, cell line models of the luminal AR subtype were in part dependent on androgen receptor (AR) signaling, as siRNA-mediated AR knockdown or pharmacological inhibition of AR by bicalutamide greatly decreased cell viability and tumor

growth[5]. In addition to AR-dependency, all LAR TNBC cell lines analyzed harbor an activating mutation in the kinase domain of PIK3CA (H1047R) and display greater sensitivity to PIK3CA inhibitors versus models of other subtypes[5]. Since AR protein is a good surrogate for the LAR subtype, it would serve as a robust biomarker for the selection of patients with TNBC for clinical trials exploring the efficacy of targeting AR and PI3K. Currently there is a clinical trial (NCT00468715/TBCRC011) in which bicalutamide as a single agent provided a clinical benefit rate of 19% in metastatic AR+, ER–/PR– breast cancers [66].

In contrast to other TNBC subtypes, the LAR subtype appears to be rather chemoresistant in both cell line models and retrospective analyses from clinical trials. The prospective GeparTrio phase-III trial analyzed core biopsies from primary breast cancer patients treated with neoadjuvant doxorubicin/cyclophosphamide/docetaxel (ACT) and showed a surprising disconnect between pCR and survival[67]. The trial results demonstrated that while AR+ patients as a whole have better disease free survival (DFS, AR+ 86% vs. AR–66%) and overall survival (OS, 95% vs. 76%), these patients displayed a decreased response to chemotherapy as measured by pCR (13% vs. 25%) compared to the study population at large. A similar finding was reported from a retrospective analysis of prospective TNBC biopsies obtained prior to neoadjuvant anthracycline and taxane chemotherapy in which molecular TNBC subtyping performed on these biopsies displayed differential pCR rates[68]. Compared to the study population, the LAR tumors had a significantly decreased response to neoadjuvant chemotherapy (pCR, 10% vs. 28%). Together, these studies provide strong rationale for prospectively identifying AR+ TNBC patients and aligning these patients to targeted therapies, while sparing toxicity associated with chemotherapy, as these patients are less likely to respond.

Summary

Recent genomic analysis have demonstrated the profound inter- and intra-tumoral heterogeneity of TNBCs, which may in part explain the disappointing results from early trials with targeted agents in unselected TNBC. However, a number of approaches have evolved that target adapted molecular states resulting from tumor suppressor loss, altered metabolic pathways or genomic instability. The success of future clinical trials depends on researchers deciphering the heterogeneity of TNBC and being willing to proceed with multi-institutional stratification of patients by subtype/pathway-specific genomic alterations to better align molecular state with proper therapy.

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Figure 1. Relationship between molecular TNBC subtypes and the intrinsic breast cancer subtypes

(A) Using a bimodal filter on ER, PR and HER2 expression, 374 TNBC samples were extracted from 2441 breast cancer gene expression microarray profiles originating from 14 datasets*. The TNBC samples were either normalized with all samples (left dendrogram branch) followed by PAM50 prediction for intrinsic breast subtypes or normalized alone (right dendrogram branch) followed by prediction with PAM50 (left) or TNBCtype (right). Doughnut pie charts display the relative distribution of the same 374 TNBC samples analyzed using the indicated subtype tools. (B) Pie charts represent analysis of the indicated TNBC subtypes using the PAM50 intrinsic subtype tool. Pie charts display the TNBCtype composition of either (C) basal-like or non basal-like TNBC or (D) low-claudin vs. high-claudin TNBC. Basal-like 1 (BL1), basal-like 2 (BL2), immunomodulatory (IM), mesenchymal (M), mesenchymal stem-like, and luminal AR (LAR). *(GSE1456, GSE1561, GSE2034, GSE2109, GSE2990, GSE2603, GSE5327, GSE5460, GSE5847, GSE7390, GSE11121, GSE12276, GSE18864, GSE20194)