

A Four-gene Decision Tree Signature Classification of Triple-negative Breast Cancer: Implications for Targeted Therapeutics



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Abstract

The molecular complexity of triple-negative breast cancers (TNBCs) provides a challenge for patient management. We set out to characterize this heterogeneous disease by combining transcriptomics and genomics data, with the aim of revealing convergent pathway dependencies with the potential for treatment intervention. A Bayesian algorithm was used to integrate molecular profiles in two TNBC cohorts, followed by validation using five independent cohorts ($n = 1,168$), including three clinical trials. A four-gene decision tree signature was identified, which robustly classified TNBCs into six subtypes. All four genes in the signature (*EXO1*, *TP53BP2*, *FOXM1*, and *RSU1*) are associated with either genomic instability, malignant growth, or treatment response. One of the six subtypes, MC6, encompassed the largest proportion of tumors (~50%)

in early diagnosed TNBCs. In TNBC patients with metastatic disease, the MC6 proportion was reduced to 25%, and was independently associated with a higher response rate to platinum-based chemotherapy. In TNBC cell line data, platinum sensitivity was recapitulated, and a sensitivity to the inhibition of the phosphatase PPM1D was revealed. Molecularly, MC6-TNBCs displayed high levels of telomeric allelic imbalances, enrichment of CD4⁺ and CD8⁺ immune signatures, and reduced expression of genes negatively regulating the MAPK signaling pathway. These observations suggest that our integrative classification approach may identify TNBC patients with discernible and theoretically pharmacologically tractable features that merit further studies in prospective trials.

Introduction

Triple-negative breast cancers (TNBCs), defined by the lack of estrogen receptor (ER), progesterone receptor (PgR), and human epidermal growth factor receptor 2 (HER2) expression, display remarkable molecular complexity and heterogeneous clinical behavior (1). The overall prognosis of women with TNBC after metastatic relapse is significantly poorer when compared with that of women with other breast cancer subtypes (2). Chemotherapy remains the only systemic therapeutic approach for patients with TNBC. Although subpopulations can be identified that are more

responsive to chemotherapy, such as those with *BRCA1* mutations, its effectiveness remains limited in an unselected TNBC population. Many agents are presently in clinical development, including PARP inhibitors and platinum salts (3–5), MEK inhibitors (6), and immunological agents (7). For some, predictive biomarkers have been recognized, while for others appropriate molecular features enabling optimal patient selection are still lacking (8, 9).

Several studies have sought to take advantage of molecular profiling to classify TNBCs and subsequently provide clinically relevant information (10–13). One of the most recent classifications, TNBCtype-4, is based on gene expression and classifies TNBC into four subtypes. Among those, basal-like 1 (BL1) TNBCs were shown to achieve significantly higher pathological complete response (pCR) rates (49%) compared with all other subtypes (31%) when treated with neoadjuvant chemotherapy. However, classification approaches making use of multi-omics data are warranted to identify patients that may benefit from treatment beyond the standard-of-care (12).

TNBCs are characterized by extensive genomic instability (14, 15). Measures capturing overall levels of genomic instability, like a chromosomal instability gene signature (CIN70), are informative for outcome prediction in patients with ER⁻ breast cancer (16). Similarly, genomic signatures, including Scars of Chromosomal Instability measures (SCINS; ref. 17), Number of Telomeric Allelic Imbalance (NtAI; ref. 18), the Homologous Recombination Deficiency (HRD) score (19), and HRDetect (20), are indicative of BRCAness and predictive of response to chemotherapy, particularly in the neoadjuvant setting (21). In metastatic TNBC trials [TNT (22) and TBCRC009 (23)], these methods were not

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able to identify patients specifically responding to platinum-based chemotherapy, suggesting some refinement of such approaches is required.

Here, we developed a four-gene decision tree signature based on integrated transcriptomics and genomics data that robustly classifies TNBC into six subtypes across 1,168 TNBCs. In TNBC patients with metastatic disease, our classification identified a subgroup of tumors sensitive to platinum-based chemotherapy. Molecular characteristics of this subgroup included increased number of allelic imbalanced aberrations in their telomeres, decreased inactivation of MAPK signaling, and enrichment of CD4⁺ and CD8⁺ immune signatures.

Materials and Methods

Clinical sample data

TNBCs from the previously described Guy's TNBC (E-MTAB-5270 and E-MTAB-2626; ref. 24) were selected on the basis of IHC status of HER2, ER, and PgR. For 88 TNBCs, patient matched Affymetrix GeneChip Human Exon 1.0ST gene expression and Affymetrix SNP 6.0 copy number data were available.

METABRIC TNBC is a subset of the METABRIC study (EGAD00010000164; ref. 10). Triple-negative status was based on IHC-assessed ER and HER2 status. Patient matched Illumina Human HT-12 v3 gene expression and Affymetrix SNP 6.0 copy number data were available for 112 TNBCs and processed, as reported previously (24).

A triple-negative subset of TCGA BRCA (25) was used as the TCGA TNBC cohort. For 95 TNBCs, defined by ER and HER2 status, gene expression was available on the Agilent 244K Custom Gene Expression array.

The TNBC616 cohort is a compilation of 24 different breast cancer cohorts (Supplementary Table S1). A total of 3,495 breast cancers were obtained, of which 616 were defined as triple negative based on HER2, ER, and PgR status. Details are provided in the online Supplementary Information.

Clinical trial data

The PrECOG 0105 cohort comprised 80 patients enrolled in a single-arm, phase II, early-stage study assessing the efficacy of neoadjuvant treatment using gemcitabine, carboplatin and iniparib (NCT00813956; refs. 26, 5). Response to treatment was stratified using the residual cancer burden (RCB) index (27). Patients with RCB > 1 were considered treatment-unresponsive; those with an RCB of 0 were deemed to have pCR, while those with an RCB of 1 had a partial response. Affymetrix HuGene 1.0st microarray gene expression was available and processed using the affy Bioconductor package (28) following RMA normalization (29).

Sanofi phase II (NCT00540358; ref. 3) and Sanofi phase III (NCT00938652; ref. 4) are both two-arm clinical trials in TNBC patients with metastatic disease testing the efficacy of gemcitabine and carboplatin, with or without iniparib. A total of 123 and 519 patients in phase II and phase III, respectively, were randomly assigned to either arm. From the 74 (phase II) and 319 (phase III) patients for which gene expression data from the primary lesions were obtained, objective response was available for 61 and 224 patients. Gene expression was available on the Affymetrix Human Genome U133 Plus 2.0 microarray and was processed similarly to the PrECOG 0105 data.

Affymetrix SNP 6.0 copy number data

Raw Affymetrix SNP 6.0 data were normalized, allele-specific signal intensity measures were generated, and log R ratios and B-allele frequencies were obtained using PennCNV-Affy (30, 31). Allele specific copy number data were obtained using the ASCAT algorithm (32).

Cancer cell line data

Transcriptomics and drug response data for TNBC cell lines ($n = 16$) were acquired from the Genomics of Drug Sensitivity in Cancer (GDSC; ref. 33). The area under the dose-response curve (AUC) values represent the relative sensitivity (low AUC) and resistance (high AUC) for each drug within each cell line. Associations between drug response and MC6 were assessed using Mann-Whitney U tests.

Statistical methods

Detailed information on the integrative analysis and statistical methods are described in the online Supplementary Information. In brief, genomics and transcriptomics data were integrated independently in Guy's TNBC and METABRIC TNBC using the COpy Number and EXpression In Cancer (CONEXIC) algorithm (34). The identified four-gene signature required for TNBC subtyping was normalized before being applied to additional cohorts.

Results

Integrative analysis of transcriptomics and genomics data identifies a four-gene decision tree signature

To determine whether combined transcriptomics and genomics data could lead to a robust TNBC classification, we applied the previously published CONEXIC algorithm (34) to two independent TNBC cohorts; Guy's TNBC ($n = 88$; ref. 24) and TNBCs from the METABRIC study (METABRIC TNBC; $n = 112$; ref. 10; Fig. 1A; Supplementary Table S2). CONEXIC constructs gene sets, defined as groups of genes, which share similar expression patterns across all samples. As a next step, CONEXIC builds decision trees for each of these gene sets, using gene expression levels of copy number-driven genes (modulators, copy number ≥ 5). Within Guy's TNBC and METABRIC TNBC, CONEXIC identified 38 and 36 gene sets with decision trees, respectively (Supplementary Tables S3–S6). We next sought to identify concurrent gene sets between the 38 Guy's and 36 METABRIC gene sets by comparing all 1,368 possible gene set combinations for overlapping genes (Fig. 1B) and identified 92 sets with statistically significant concurrent genes (Fig. 1C, gray squares), including 3 gene sets that shared a common modulator in their respective decision trees, namely *EXO1* [Fig. 1C (black squares) and D]. In *Guy's-set 33* (Supplementary Table S7) and *METABRIC-set 15* (Supplementary Table S8), genes involved in the inactivation of the MAPK pathway were significantly enriched. As no overlapping GO terms were identified between *Guy's-set 27* (Supplementary Fig. S9) and *METABRIC-set 15*, we focused on *Guy's-set 33* and *METABRIC-set 15* (Supplementary Fig. S1).

A four-gene decision tree signature consistently classifies TNBCs into six subtypes in test cohorts

We applied both decision tree signatures to a total of 1,168 TNBCs, including (i) 95 TNBCs of TCGA BRCA (25); (ii) TNBC616 (see Supplementary Table S1 and online

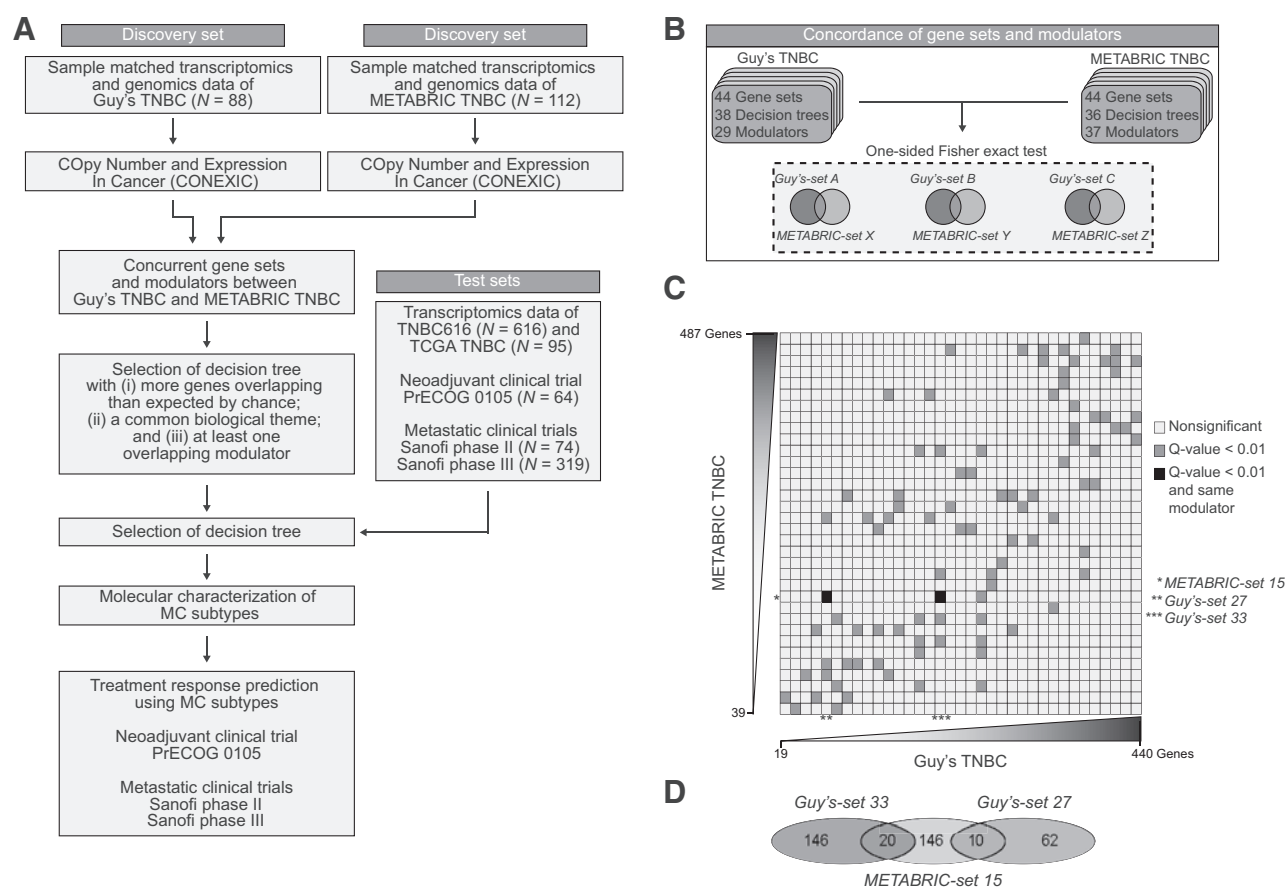


Figure 1. Identification of concurrent cancer gene sets with CONEXIC. **A**, Research design and workflow. After selecting a decision tree, a molecular characterization of the subtypes was performed in two discovery and five validation sets. Treatment response to DNA-damaging agents was assessed in three clinical trials. **B**, The number of gene sets, decision trees, and modulators identified by the CONEXIC algorithm in Guy's TNBC and METABRIC TNBC. Overlap in gene set composition between the two cohorts was assessed using a one-sided Fisher exact test. **C**, Level plot of concurrent gene sets between Guy's TNBC and METABRIC TNBC. Dark gray boxes indicate gene sets with significantly overlapping genes. Black boxes, in addition to overlapping modulators. Gene sets are ordered by size, as indicated by the scales. **D**, Venn diagram depicting the number of common genes between *Guy's-set 27*, *Guy's-set 33*, and *METABRIC-set 15*.

Supplementary Information); (iii) PrECOG 0105 ($n = 64$; ref. 5), and both a phase II and a phase III trial in TNBC patients with metastatic disease; (iv) Sanofi phase II ($n = 74$; ref. 3); and (v) Sanofi phase III ($n = 319$; ref. 4; Fig. 1A). Transcriptomics data in each of the trials were derived from treatment-naïve primary breast cancer tissue.

The classification defined by the decision tree of *Guy's-set 33*, which is based on the expression levels of five genes (*ST8SIA1*, *EXO1*, *NEK2*, *C8orf46*, and *MMS22L*), nominated TNBCs in some cohorts, such as TCGA BRCA and Sanofi III, mostly as one class (Supplementary Fig. S2) and could not be tested in TNBC616 as expression levels for *MMS22L* and *C8orf46* were not available for assessment. Furthermore, investigations based on the *Guy's-set 33* decision tree were therefore not performed. In contrast, applying the *METABRIC-set 15* decision tree signature based on the expression levels of four genes (*TP53BP2*, *EXO1*, *FOXM1*, and *RSU1*), established six subtypes, named MC1 to MC6, including one large subtype and five of varying sizes (Fig. 2). The most frequent subtype (MC6) comprised 44.6%, 47.7%, 46.6%, and 56.8% of all patients in METABRIC TNBC, Guy's TNBC, TNBC616, and

TCGA TNBC, respectively. In the PrECOG 0105 trial, the proportion of the MC6 subtype increased to 64%, whereas in TNBC patients with metastatic disease (Sanofi phase II and III), its proportion reduced to approximately 24%. In contrast, the frequency of MC1-TNBCs increased from approximately 7.2% in the nontrial cohorts to approximately 22.4% in the metastatic setting. No difference in overall survival or risk of developing metastasis was observed when the four genes were tested separately in a univariate or multivariate Cox survival analysis, neither do the different MC subtypes demonstrate any association with increased or decreased overall and progression-free survival or risk of developing metastasis (Supplementary Tables S10–S13).

Molecular characterization of MC subtypes

MC subtypes are nonsynonymous with other breast cancer classifications. Currently, TNBCs are classified by TNBCtype-4 (12), the PAM50 subtypes (35–37) and the IntClust breast cancer classification (10). We sought to evaluate whether the four-gene decision tree classification converges to these ones (Fig. 3A; Supplementary Tables S14 and S15). MC6-TNBCs consisted exclusively of PAM50

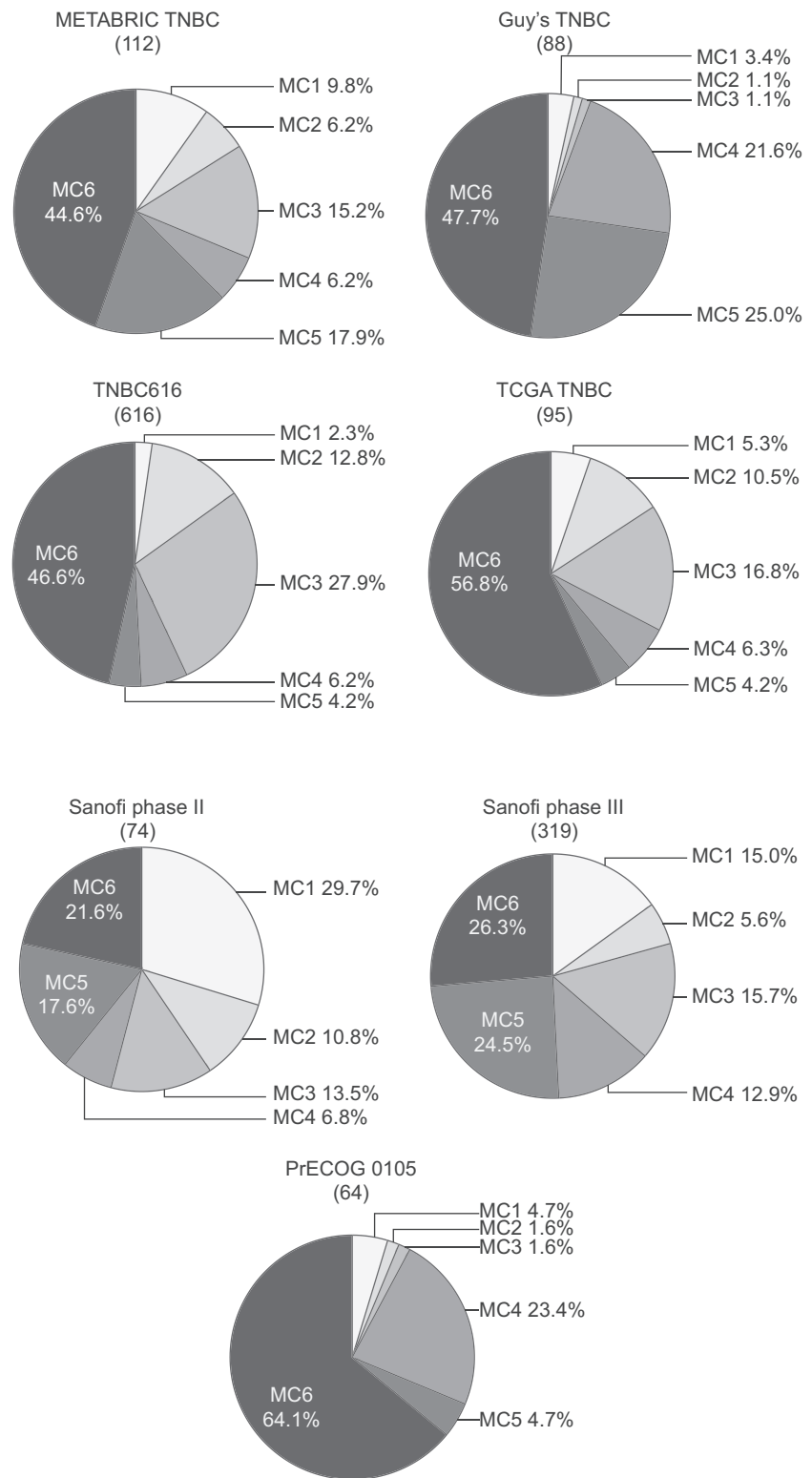


Figure 2. TNBC cohorts classified using the *METABRIC-set 15* four-gene decision tree signature. Pie charts illustrating the proportion of the MC subtypes in four primary invasive TNBC cohorts, namely METABRIC TNBC, Guy's TNBC, TNBC616, and TCGA TNBC, as well as three clinical TNBC studies, including Sanofi phase II, Sanofi phase III, and PrECOG 0105. The total number of tumors in each cohort is listed in brackets. The percentage for each subgroup is shown next to the respective pies.

basal-like cases (100%, $P = 2e-08$, Fisher exact test), although only 57% of the basal-like TNBCs were classified as MC6. MC1-TNBCs were enriched for Luminal A (36%, $P = 3e-04$, Fisher exact test) and Normal-like (36%, $P = 3e-03$, Fisher exact test),

and MC2-TNBCs for HER2-enriched (57%, $P = 2e-03$, Fisher exact test). TNBCs of the remaining three MC subtypes fell across all PAM50 subtypes. Except for three TNBCs, MC6-TNBCs belonged to the IntClust 10 group (78%, $P = 1e-02$, Fisher exact

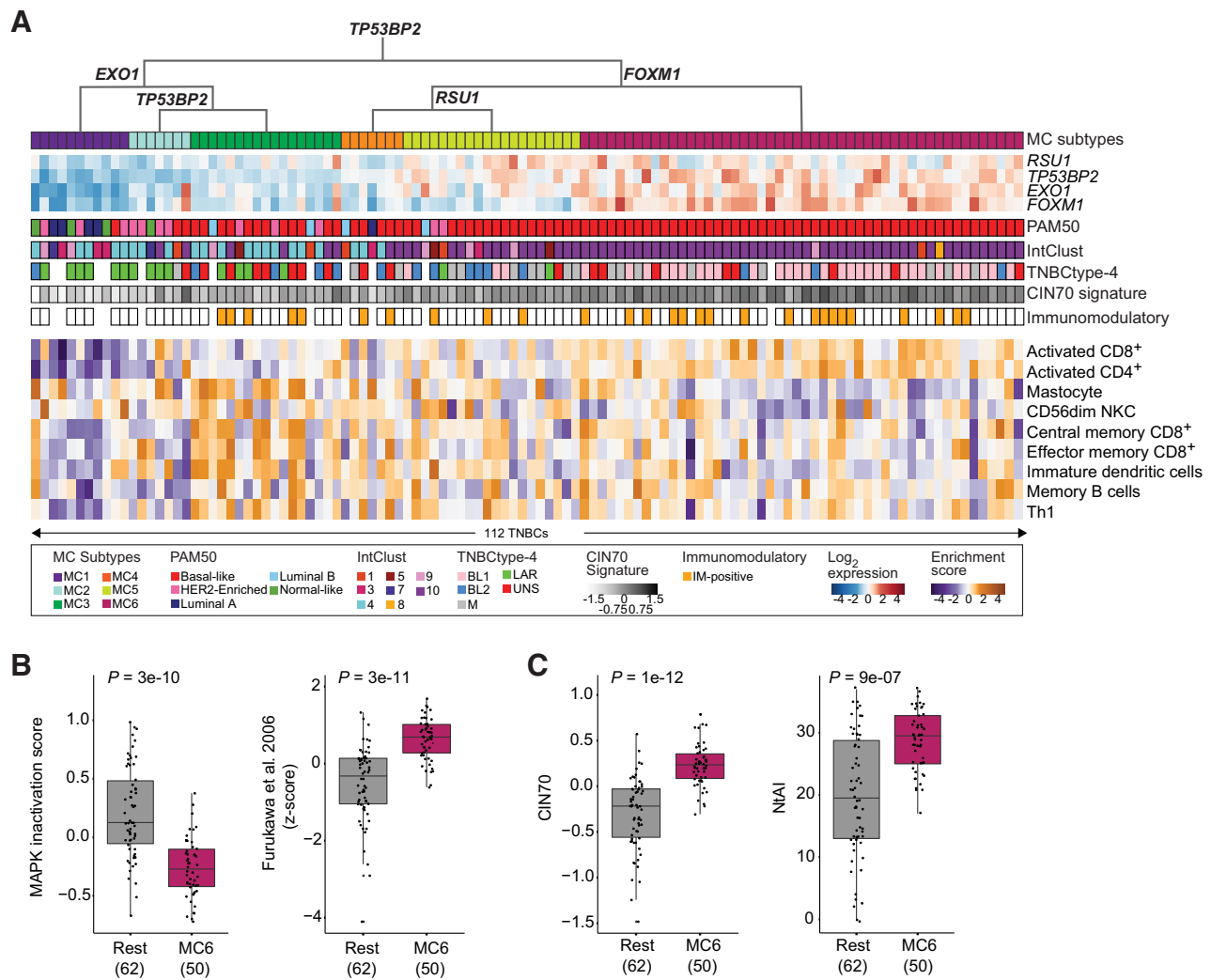


Figure 3. Molecular characterization of the MC subtypes in METABRIC TNBC. **A**, The four-gene decision tree underlying the MC subtypes is shown, followed by a heatmap representing the expression levels of the four modulatory genes. The colored bars represent sample-specific characteristics, including PAM50, IntClust, TNBCtype-4, CIN70 signature, and the immunomodulatory status. The heatmap at the bottom illustrates the enrichment of immune gene signatures. **B**, (left) MAPK inactivation scores, calculated by summarizing the expression levels of *DUSP4*, *DUSP5*, *DUSP6*, *DUSP10*, and *SPRED2*, in MC6-TNBCs (magenta) and the remaining TNBCs (gray). Significance was assessed using Wilcoxon rank-sum tests. (right) Z-scores for the *DUSP6* gene set were obtained using ssGSEA. Significance was assessed using a Wilcoxon rank-sum test. **C**, Boxplots displaying the CIN70 signature (left) and NtAI (right) in MC6-TNBCs (magenta) and the remaining TNBCs (gray). Significance was assessed using Wilcoxon rank-sum tests.

test), whereas TNBCs of other MC subtypes were classified mostly as IntClust 4, 5, 3, and 1, although no significant enrichments were observed. MC6-TNBCs were found to be enriched for the BL1 subtype (61%, $P = 1e-06$, Fisher exact test), whereas MC1-TNBCs and MC2-TNBCs were primarily of the LAR subtype (86% and 67%, $P = 1e-04$ and $1e-02$, respectively, Fisher exact test). Thus, the MC subtypes diverged from established breast cancer classifications

MC subtypes differ in their immune gene enrichment. On the basis of Lehmann’s immunomodulatory (IM) classification (12), 37% of the MC6-TNBCs were IM-positive, MC1- and MC2-TNBCs were exclusively IM-negative, and MC3-, MC4- and MC5-TNBCs were mostly IM-negative (74%; Fig. 3A). Next, we performed a gene set

enrichment analysis (38) on gene expression data from METABRIC TNBC to further deconvolute immune cell enrichment across the MC subtypes. Using 28 different immune gene sets (39), activated CD4⁺ and CD8⁺ immune signatures were found enriched in MC6-TNBCs ($Q = 3e-08$, and $1e-04$, respectively, Mann-Whitney *U* test), and low in MC1-TNBCs ($Q = 2e-05$ and $1e-05$, Mann-Whitney *U* test; Fig. 3A; Supplementary Table S16). MC6-TNBC had lower mastocytes and CD56^{dim} natural killer cell activation in comparison with other MC subtypes ($Q = 5e-03$, and $3e-02$, respectively, Mann-Whitney *U* test). In MC3-TNBCs, increased central memory CD8⁺, effector memory CD8⁺, and immature dendritic cells expression levels were observed ($Q = 4e-02$, $4e-02$, and $4e-02$, respectively, Mann-Whitney *U* test). MC2-TNBCs had reduced expression of the

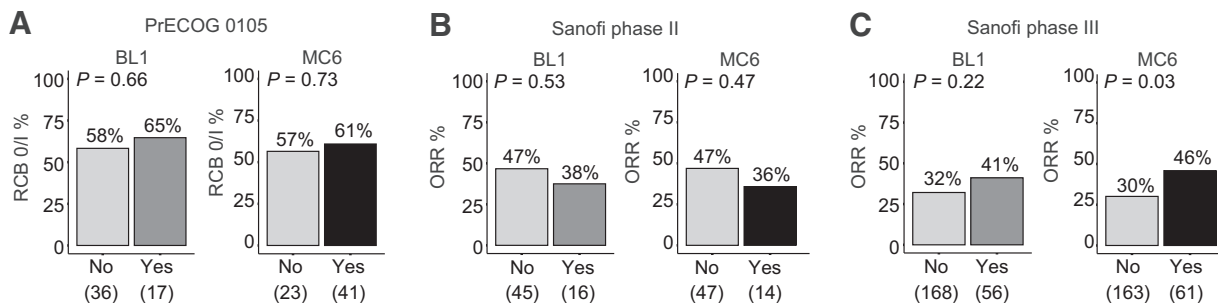


Figure 4.

Response rates for the MC6 and BL1 subtypes in clinical trials. **A**, Response to neoadjuvant platinum-based chemotherapy, as assessed by the RCB index, in BL1-TNBCs and MC6-TNBCs obtained from the PrECOG 0105 clinical trial. ORR in Sanofi phase II (**B**) and Sanofi phase III (**C**). Response rates are dichotomized by being BL1 or not (left), and MC6 or not (right). Subtypes were assessed as predictors of treatment response using a multivariate logistic regression, by including age and race for Sanofi II, and age, race, and grade for Sanofi III into the models.

memory B-cell signature ($Q = 4e-02$, Mann-Whitney U test), whereas in MC1-TNBCs, Th1 expression was reduced ($Q = 4e-02$, Mann-Whitney U test). This clearly demonstrates a variety of immune cell features across the MC subtypes.

Pathway deregulation in MC subtypes. *DUSP4*, *DUSP5*, *DUSP6*, *DUSP10*, and *SPRED2* were amongst the *METABRIC-set 15 gene set* (Fig. 1D; Supplementary Table S8) and are involved in the negative regulation of the MAPK signaling pathway. We investigated their expression levels across the MC subtypes and found that MC6-TNBCs had the lowest expression of these genes in comparison with the other subtypes ($P < 0.05$, Mann-Whitney U test; Fig. 3B; Supplementary Fig. S3A). Genomic regions encompassing *DUSP4*, *DUSP5*, and *DUSP6* were deleted (Supplementary Table S17), potentially resulting in decreased expression levels. To further corroborate the lack of negative regulation of MAPK inactivation, a set of genes previously reported under control of this inhibitory mechanism (40) was interrogated in the MC subtypes and found increased in MC6-TNBCs compared with TNBCs of other MC subtypes ($P < 0.05$, Mann-Whitney U test; Fig. 3B; Supplementary Fig. S3B). These results demonstrate the highly selective features of MC6-TNBCs.

In MC5-TNBCs, genes highly expressed were enriched for ErbB signaling ($Q = 6e-03$, Hypergeometric test), particularly through the EGFR pathway ($P = 1e-02$, Fisher exact test). MC4-TNBCs were enriched for PI3K/Akt signaling ($Q = 5e-03$, Hypergeometric test) and DNA replication ($P = 2e-02$, Fisher exact test). Although not enriched for Lehmann's IM classification, many genes upregulated in MC3-TNBCs are involved in innate and adaptive immune response ($P = 7e-04$ and $1e-03$, respectively, Fisher exact test), and chemokine signaling ($Q = 3e-03$, Hypergeometric test). MC2-TNBCs were enriched for metabolic processes ($Q = 2e-28$, Hypergeometric test), and MC1-TNBCs for steroid hormone-mediated signaling ($P = 3e-02$, Fisher exact test), particularly estrogen and androgen receptor signaling ($P = 7e-04$ and $2e-02$, respectively, Fisher exact test). The unique pathway activities underlying the MC subtypes further demonstrate the molecular complexity of TNBC and further strengthen our TNBC classification.

Increased levels of telomeric allelic imbalanced aberrations in MC6-TNBCs. Next, we investigated the levels of genomic instability. MC6-TNBCs showed significantly higher levels of chromo-

somal instability ($P < 0.05$, Mann-Whitney U test; Fig. 3C; Supplementary Fig. S3C) as defined by the CIN70 gene expression signature (16, 41). MC1-TNBCs had the lowest levels of chromosomal instability. To further decipher this genomic instability, we investigated diverse genomic instability measurements based on copy number data (17, 18). MC6-TNBCs displayed a high burden of allelic imbalanced aberrations in their telomeres ($P = 9e-07$, Mann-Whitney U test; Fig. 3C). MC5-TNBCs exhibited a medium burden. In contrast, the remaining MC subtypes did not harbor specific types of copy number aberrations.

MC6 identifies TNBCs responsive to platinum-based chemotherapy in Sanofi phase III clinical trial. Given the selective features of MC6-TNBCs, we next assessed whether this subtype carried any predictive value in treatment response to DNA-damaging agents. Using three clinical trials (Fig. 1A), TNBCs from each cohort were dichotomized as either being MC6-TNBCs or non-MC6 (referred to as remaining TNBCs). We included the TNBCType-4 classification as a comparator, as BL1-TNBCs were previously shown to be responsive to neoadjuvant chemotherapy (12). In the neoadjuvant PrECOG 0105 trial, the RCB 0/I pathological response rate in BL1-TNBCs (11/17, 65%) was similar to that of MC6-TNBCs (25/41, 61%; Fig. 4A). However, of the 64 patients enrolled in the PrECOG 0105 trial, the MC6 subtype identified 39% of the responders with an accuracy of 55%, in contrast to the BL1 subtype, identifying 17%, with an accuracy of 41%. In the metastatic TNBC Sanofi phase II trial, neither the BL1 nor the MC6 subtype was predictive of the overall response rate (ORR; Fig. 4B; Supplementary Table S18). However, in the metastatic TNBC Sanofi phase III trial, the MC6 subtype was a significant predictor of treatment response in a multivariate model [OR = 2.41, confidence interval (CI) = 1.01 to 5.81, $P < 0.05$], with an ORR in MC6-TNBCs of 46% (28/61), compared with an ORR of 30% (49/163) in the remaining TNBCs (Fig. 4C). The ORR in BL1-TNBCs was 41% (23/56), as compared with 32% (54/168) in the remaining TNBCs. In contrast to BL1, the MC6 subtype did reach significance in the univariate and in the multivariate model (OR = 1.78, CI = 0.76 to 4.19, $P = 0.18$; Supplementary Table S19).

MC6 cell lines are sensitive to cisplatin. To test the hypothesis that MC6-TNBCs are responsive to platinum-based chemotherapeutics, we exploited drug sensitivity profiles of 16 TNBC tumor cell

lines generated as part of the GDSC project (33). We found that the median AUC of cisplatin in the MC6 TNBC cell lines ($n = 4$) was 0.89, compared with 0.92 in cell lines from other MC subtypes ($n = 11$; $P = 5.6e-02$, Mann-Whitney U test), suggesting an enhanced sensitivity to platinum salts in the MC6 subtype. With the aim of identifying novel alternative therapeutic sensitivities, we also assessed the associations between >200 GDSC drug response profiles and the MC6 subtype (Supplementary Table S20). We found that among the GDSC drug sensitivity profiles, MC6-TNBC cell lines displayed the greatest sensitivity to CCT007093 (42), a small-molecule inhibitor of the DNA damage-activated phosphatase, PPM1D ($P = 7.7e-03$, Mann-Whitney U test, Supplementary Fig. S4; ref. 43).

Discussion

With the purpose of classifying molecular heterogeneous TNBCs into clinically relevant subtypes, our multi-omics integrative approach differs from previously published methods (10–13). Our classification was constructed using data exclusively from TNBCs, strengthening the exploration of their molecular complexity in more detail. Robustness of our approach was increased by applying the CONEXIC algorithm (34) to two independent TNBC cohorts to then identify concurrent gene sets and modulatory genes. *Guy's-set 33* and *METABRIC-set 15* shared similarities in both their gene set and modulatory genes. The latter represented well-known cancer-associated genes potentially contributing to tumor progression, and thereby substantiating our analytical approach to identify candidate drivers with effects on gene expression patterns. However, upon validation of both decision trees, only *METABRIC-set 15* was taken forward due to the consistently reproducible MC subtypes. Of note, the molecular features of primary tumors of patients that will be developing metastasis seem to be distinct, as was reflected by the change in proportion of the MC1 and MC6 subtype between nontrial and metastatic TNBC cohorts. The MC6 subtype identified patients with metastatic TNBC who showed improved response to platinum-based chemotherapy in the Sanofi phase III, but not in the neoadjuvant PrECOG 0105 trial (5). In early-stage TNBCs, which are more likely to respond to platinum-based chemotherapy, the MC6 subtype is not able to differentiate between those that do, and do not respond to this treatment. However, in the metastatic setting, where patients are less responsive to platinum-based chemotherapy, MC6 appears to be more discriminative as a predictor. In contrast, genomic signatures lack predictive value in the metastatic setting (22, 23).

The four-gene decision tree signature consists of *TP53BP2*, *RSU1*, *FOXM1*, and *EXO1*. The expression of *TP53BP2*, a known regulator of apoptosis and cell growth, has been reported to be copy number dependent, and is associated with poor response to chemotherapy in TNBC (14). *RSU1*, Ras suppressor-1, is involved in the RAS signal transduction pathway and was proposed as a biomarker for metastasis in breast cancers (44). The proto-oncogenic transcription factor *FOXM1* has frequently been shown to mediate cell proliferation, survival, migration, progression, and tumorigenesis in TNBC (45); can modulate cisplatin sensitivity by regulating the expression of *EXO1* in ovarian cancer (46); and is part of a recently identified KRAS-associated signature in colorectal cancer (47). All four genes provide biological rationales for being good candidates for classification approaches.

To further substantiate the finding that MC6-TNBCs were more sensitive to platinum-based chemotherapeutics, cisplatin drug response profiles were assessed across 16 TNBC cell lines categorized by MC subtypes. In line with findings from Sanofi phase III, MC6 cell lines appeared overall more sensitive to cisplatin than those of other subtypes. In addition, MC6-TNBC cell lines were found to exhibit enhanced sensitivity to CCT007093, a chemical inhibitor of PPM1D (42). In parallel, MC6-TNBC cell lines displayed lower expression of *DUSP10* and *SPRED2* as a part of the MAPK inactivation signature; both are negative regulators of p38. Loss of *DUSP4*, *DUSP5*, and *DUSP6* in TNBC has been previously reported by others (48–50). Thus, we hypothesize that the sensitivity to CCT007093 may be a result of p38-dependent cell death, thereby pointing to a potential PPM1D dependency for this group of TNBCs. Given that PPM1D modulates the activity of a series of substrates including p38, ATM, CHK1, and CHK2 (43), this association further demonstrates the connectivity between genomic instability and TNBC.

The proposed decision tree is an ideal classification approach to be performed with relative ease using other platforms, including qPCR and NanoString, due to the small number of genes required. However, there are some limitations, as in its current form, the four-gene decision tree signature relies on the distribution of each of the four genes and careful standardization experiments would need to be implemented. Furthermore, validation of our classification across independent clinical trials are warranted to investigate whether association with treatment effect to platinum-based chemotherapy is specific or rather reflects a combinatorial effect with gemcitabine in Sanofi phase III.

The management of TNBC, especially in the metastatic setting, can be complex. With single-agent chemotherapy still considered the standard-of-care, targeted therapeutic strategies are required, which will rely on appropriate biomarkers for optimal patient selection. The unique molecular features of the MC subtypes reflect the intrinsic heterogeneity of TNBC and revealed targetable pathways, such as the p38 MAPK signaling pathway in MC6-TNBC. The same subset also showed elevated levels of genomic instability in telomeric regions, a type of genomic instability, which may be the result of escaping from telomere crisis. *EXO1*, a modulatory gene, may further contribute to this process (51, 52). Further characterizations of the MC subtypes are warranted to establish their association with genomic signatures from whole-genome sequence data (53, 54).

In conclusion, we sought to decipher the complex nature of TNBC, with the goal of informing patient selection for current and future treatment strategies. We showed that a four-gene decision tree signature based on copy number-dependent genes classifies TNBCs into six subtypes. Given the current lack of selection criteria for TNBC patients with metastatic disease, this classification warrants further testing in randomized metastatic TNBC trials, such as TNT (22).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

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