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CLINICAL TRIAL

Exploratory biomarker analysis from a phase II clinical trial of eribulin plus gemcitabine versus paclitaxel plus gemcitabine for HER2-negative metastatic breast cancer patients (KCSG BR13-11)

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Abstract

Purpose We conducted an exploratory biomarker study from a phase II clinical trial of eribulin plus gemcitabine (EG) versus paclitaxel plus gemcitabine (PG) in HER2-negative metastatic breast cancer (BC) patients.

Methods We performed targeted deep sequencing with a customized cancer gene panel and RNA expression assay. Tumor mutation burden (TMB) and mutation signatures were determined based on genetic alteration in targeted regions. Gene set variation analysis was performed with PanCancer Immune Profiling and PanCancer Pathway Panels. Statistical analyses were conducted to identify the associations between genetic alterations and clinical outcomes.

Results Of 119 patients, 40 had available biomarker data. Among the 40 patients, 4 supported their post-treatment tissues. In targeted deep sequencing, FAT3 (48%) was the most frequently mutated gene, followed by PKHD1, TP53, GATA3, PARP4, and PIK3CA. In terms of gene expression, low expression of epithelial-mesenchymal transition (EMT) pathway genes was associated with prolonged progression-free survival (PFS) in the EG group, while high expression of the EMT pathway was associated with good prognosis in the PG group. Median TMB was 6.5 (range 2.44–46.34) and there was no relationship between TMB and patient prognosis. Analysis of mutation signatures showed that signatures 3, 20, and 26 were frequently observed in our cohort. Further survival analysis according to mutation signature showed that mutation signature 3, as a homologous recombinant deficiency-related signature, was highly associated with disease progression (hazard ratio (log2 scale) 8.21, 95% confidence interval 2.93–13.48, $p = 0.002$). Kaplan–Meier plot also showed that BCs with signature 3 had short PFS compared to those without these signatures (median PFS (months) for signature 3 (low vs. high): 17.2 vs. 8.1, $p = 0.0026$).

Conclusions Mutation signature 3, found in about 30% of MBCs regardless of hormone receptor status, was associated with short PFS for patients with cytotoxic chemotherapy.

Trial registry ClinicalTrials.gov number: NCT02263495.

Keywords Metastatic breast cancer · Eribulin · Paclitaxel · Next-generation sequencing

Introduction

Exploratory biomarker analysis has been performed in many clinical trials to predict response to drugs [1, 2]. Most recently, development of next-generation sequencing (NGS) techniques allows prompt multigene assays; therefore, exploratory translational research has been actively performed.

In breast cancer (BC), parallel translational research has revealed that PIK3CA and ESR1 mutations are associated with drug resistance and patient prognosis [1, 2]. Patients

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with hormone receptor-positive and human epidermal growth factor receptor 2 (HER2)-positive BC harboring PIK3CA alteration had shorter progression-free survival compared to BC patients without PIK3CA mutation under everolimus and trastuzumab combination treatment [1]. ESR1 and PIK3CA mutations were frequently observed in patients with short progression-free survival (PFS) [2]. In addition, ERBB2 and ERBB3 mutations are potential biomarkers of neratinib, a pan-HER kinase inhibitor [3]. Germline BRCA1/2 mutations also had a predictive role as platinum chemotherapy and PARP1 inhibitors [4–6].

In general, triple-negative breast cancer (TNBC) and hormone receptor-positive BC that must be treated with cytotoxic chemotherapy has a relatively aggressive phenotype and worse prognosis rather hormone receptor-positive BC treated with an anti-hormonal agent [7, 8]. However, regarding cytotoxic chemotherapy, parallel translational research has been rarely performed. Based on the BR9601 trial, HER family genes (Epidermal growth factor receptor; EGFR, HER2, and HER3), Ki67, and topoisomerase IIa were evaluated to identify associations with anthracycline treatment response [9]. In this study, BC without HER family gene expression had a higher response rate to epirubicin plus cyclophosphamide, methotrexate, and fluorouracil (CMF) chemotherapy compared with CMF. Besides, other microarray-based parallel translational research has been performed to identify chemotherapy biomarkers [10, 11].

In terms of genetic mutation, BC harboring germline BRCA mutation increased response rate to platinum-based chemotherapy compared with anthracycline-based chemotherapy [12]. Recently, cancer with germline BRCA1/2 mutation had double response rate of carboplatin compared with docetaxel [6]. Therefore, biomarkers predicting treatment response can aid when choosing effective chemotherapeutic agents and prolong survival in BC patients treated with a cytotoxic agent.

We previously conducted a phase II clinical trial of eribulin plus gemcitabine (EG) versus paclitaxel plus gemcitabine (PG) as first-line chemotherapy in HER2-negative metastatic breast cancer (MBC) patients [13]. The result of this clinical trial showed that EG chemotherapy has similar efficacy and less neurotoxicity compared to PG chemotherapy. In this biomarker analysis, we aimed to identify biomarkers that predict drug response and prognosis using targeted deep sequencing and gene expression assays.

Patients and methods

Patients

Patients with histologically confirmed HER2-negative MBC and with no prior history of chemotherapy for metastatic disease were eligible for the study. Patients were eligible for the study if at least 12 months had passed since the completion of prior chemotherapy, even if they had received an anthracycline- or taxane-containing regimen as neoadjuvant or adjuvant therapy. This parallel biomarker study was conducted using tumor samples at diagnosis and after disease progression. All patients consented to provide tumor samples (NCT02263495).

Study design

This study was a multicenter, randomized phase II, open-label study of the Korean Cancer Study Group (KCSG) (KCSG-BR13-11). Patients were randomly assigned, in a 1:1 ratio, to either the EG or PG chemotherapy group.

EG chemotherapy comprised of intravenous (IV) administration of 1.0 mg/m^2 eribulin and administration of 1000 mg/m^2 gemcitabine as a 30-min IV infusion on days 1 and 8 q 3 weeks. PG chemotherapy comprised of IV administration of 175 mg/m^2 paclitaxel on day 1, with 1250 mg/m^2 gemcitabine administered as a 30-min IV infusion on days 1 and 8 q 3 weeks.

The study was conducted in full accordance with the guidelines for Good Clinical Practice and the Declaration of Helsinki and was approved by the Institutional Ethics Committees of each hospital and the KCSG Institutional Review Board. The ClinicalTrials.gov identifier number was NCT02263495. Written informed consent was obtained from each participant.

Biomarker analysis

We evaluated genetic alterations and gene expression. Tumor DNA was extracted from formalin-fixed, paraffin-embedded (FFPE), or fresh frozen tissue.

To perform targeted deep sequencing, we used CancerScan™, a 375-cancer gene panel (Table S1). After enriched exome libraries were multiplexed, they were sequenced on a HiSeq 2500 sequencing platform (Illumina). A paired-end DNA sequencing library was prepared through gDNA shearing, end-repair, A-tailing, paired-end adapter ligation, and amplification. After hybridization of the library with bait sequences for 27 h, the captured library was purified and amplified with an index barcode tag, and the library quality and quantity were assessed. Sequencing of the exome library was performed using the 100-bp paired-end mode of the TruSeq Rapid PE ClusterKit and TruSeq Rapid SBS Kit (Illumina). This targeted deep sequencing panel included entire exons and some upstream areas.

Sequence reads were mapped to the human genome (hg19) using Burrows-Wheeler Aligner (BWA, v0.7.5a), and duplicates were removed by Picard (v1.93) [http://broadinstitute.github.io/picard/] and SAMtools (v0.1.19).

The Genome Analysis Toolkit (GATK, v2.4-7) was used to recalibrate base quality and optimize local realignment. Variant calling was performed only in the targeted region. To detect single-nucleotide variants (SNVs), we integrated the results of the variant callers LoFreq (v0.6.1) and Mutect (v.1.1.4) to increase sensitivity. Indels and copy number variations (CNVs) were called using Pindel (v0.2.4w) and GATK (v3.1.1), respectively. Variants were annotated using ANNOVAR [14], with gene and chromosome information, exonic function (synonymous, nonsynonymous, stop gain, nonframeshift, or frameshift indel), amino acid changes, and allele frequencies extracted from public databases such as the 1000 Genomes Project (2015 August version), dbSNP (version 137, 138), and ClinVar (2017 January version). To remove common germline polymorphisms, variants were filtered using public and in-house databases. Variants were chosen if they were located in an exonic region, and synonymous variants were filtered out. SNVs with a frequency of 1% with at least five supporting reads were chosen for further analyses. Indels having at least 8 supporting reads were reported. Read alignments were manually investigated using the Integrative Genomic Viewer (IGV) [http://www.broadinstitute.org/igv/].

For gene expression analysis, we performed an nCounter expression assay (NanoString Technologies, Seattle, WA) using a 730-gene PanCancer Immune Profiling Panel and a 730-gene PanCancer Pathway Panel. These two gene panels shared 130 genes (Table S2). An nCounter CodeSet (NanoString Technologies) containing a biotinylated capture probe for 170 genes and five housekeeping genes and reporter probes attached to color-barcode tags, according to the nCounter CodeSet Design, was hybridized in solution to 200 ng of total RNA for 18 h at 65 °C according to the manufacturer's instructions. Hybridized samples were loaded into the nCounter Prep Station for post-hybridization processing. On the deck of the Prep Station, hybridized samples were purified and immobilized in a sample cartridge for data collection, followed by quantification of target mRNA in each sample using the nCounter Digital Analyzer. Quantified expression data were analyzed using NanoString nSolver analysis software. After performing image quality control using a predefined cutoff value, outlier samples were excluded using a normalization factor based on the sum of positive control counts greater than threefold. The counts of the probes were then normalized to the geometric mean of the five housekeeping genes. Accordingly, expression level represents normalized mRNA transcript counts per 200 ng of total RNA extracted from tumor tissue. Gene set analyses were performed with PanCancer Immune Profiling Panel and PanCancer Pathway Panel using GSVA [15], which estimates gene set enrichment for each sample based on gene expression data.

Tumor mutation burden and mutation signature analyses

Tumor mutation burden (TMB) was defined as the number of somatic variants per megabase of genome. We assessed TMB based on variants detected by targeted deep sequencing, CancerScan™. Among variants in coding regions, nonsynonymous SNVs and frameshift indels were counted. Germline variants with population frequency > 0.001 in the Exome Aggregation Consortium (ExAC) [16] database and Korean population were also excluded. Variants listed in the Catalogue of Somatic Mutations in Cancer (COSMIC) [17] were filtered. To calculate the TMB per megabase, the total number of mutations counted was divided by the size of the coding region of the targeted region.

We obtained a set of 30 mutation signatures that represent distinct characteristics of human cancer types based on base substitution at the mutation site [18]. Mutation signature was inferred from all base substitutions detected by targeted deep sequencing. We calculated 30 mutation signatures for each sample with R package deconstructSigs [19], which identifies the weighted combination of predefined signatures to best explain the mutational profiles used.

Statistical analysis

We evaluated the impact of genetic alterations on treatment efficacy in each treatment group. Progression-free survival (PFS) and overall survival (OS) according to genetic information in each treatment group were estimated using the Kaplan–Meier method. A Cox proportional hazards model was used to test the interactions between treatment and biomarkers and to compute hazard ratios (HRs) and the associated 95% confidence interval (CI) for the biomarker-defined subpopulations. We also evaluated correlation between genetic alteration and overall response rate (ORR) using Fisher's exact test.

Results

Population with biomarker analysis

Clinical characteristics of patients included in the biomarker analysis group are described in Fig. 1. From 52 patients, we obtained 57 tumor tissues. Tumor DNA was extracted from 53 FFPE tissues from 43 patients, and RNA was extracted from 50 FFPE tumor tissues from 46 patients. Finally, 38 baseline and 6 progression tissues from 40 patients were analyzed using nCounter expression assays, and 26 baseline and 5 progression tissues from 28 patients were analyzed using targeted ultra-deep sequencing. Twenty-two patients

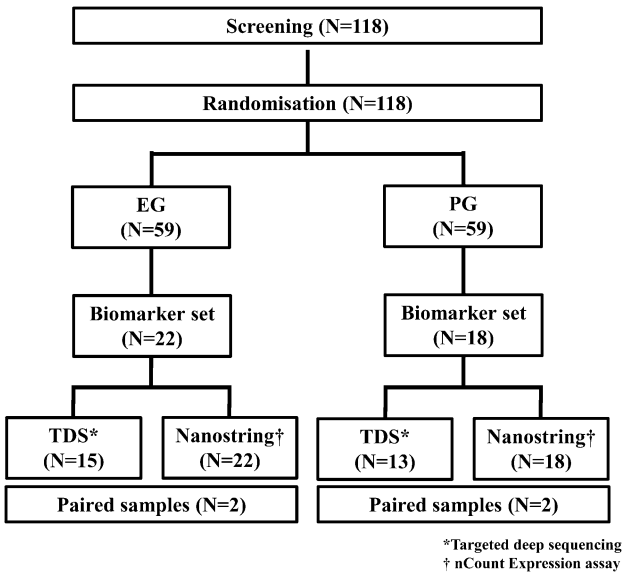

Fig. 1 Consort diagram

were enrolled in the EG group, and 18 patients were in the PG group.

Table 1 presents clinical characteristics of the biomarker subgroup according to treatment group. HR-positive BCs were found in 37 (92.5%) cases and TNBCs in 3 (7.5%). Recurred stage IV BCs after curative resection were found in 28 (70.0%) cases and de novo metastatic BC in 12 (30.0%).

Genetic alterations and gene expression assay

The genetic alterations of MBCs are described in Fig. 2. The most commonly mutated gene was FAT3 (48%), followed by PKHD1 (42%), TP53 (39%), GATA3 (29%), PARP4 (29%), and PIK3CA (29%). In terms of type of alteration, single-nucleotide substitutions were frequent, while frame shift deletions were commonly detected in TP53, and only frame shift insertions were seen in GATA3. Further survival analysis showed that genetic alterations were not associated with patient survival. FAT3 mutation seemed to be related to poor prognosis, but not statistically significant ($p = 0.079$) (Fig. S1).s

In terms of gene expression, DEG analysis and GSVA were performed to determine a relationship between gene expression and patient prognosis. We chose a 24-months PFS (the median follow-up duration) as a measure of disease progression. According to this criterion, 8 MBCs had not recurred (3 in the EG group and 5 in the PG group).

In DEG analysis using cancer panels, high expression of SHC3, ITGB3, IL1B, COL6A6, and FGF10 was associated with long duration of treatment response, while expression of CCNE1, LEFTY2, BAMBI, and RNF43 were related to disease progression ($p < 0.05$, respectively) (Fig. 3a). In the immune panels, DEG analysis showed that BCL6, CSF1, and IL6ST expression predicted no disease progression in contrast to CD24 and C8G expression, which was associated with disease progression ($p < 0.05$, respectively) (Fig. 3b). Among all gene panels, genes related to angiogenesis were most intensively associated with disease prognosis. To identify the impact of expression of angiogenesis associated genes on patient prognosis, further DEG analyses were performed according to treatment group. In the EG group, tumors with disease progression expressed low levels of CD34, TNFSF12, VEGFA, FOXO4, and HDAC5, and high levels of NODAL, LAMA5, and S100A7 ($p < 0.05$, respectively) (Figs. S2 and S3). In the PG group, low expression levels of 13 genes including IL18 and IL1B were associated with disease progression (Figs. S2 and S3).

In GSVA using cancer panels, the expression levels of gene sets associated with tumor invasion, PI3K, and ECM remodeling were associated with lack of disease progression (Fig. S4). Among immune panels, hypoxia, angiogenesis, and leukocyte function were associated with tumors with better prognosis (Fig. S4). According to treatment group, low expression of epithelial-mesenchymal transition (EMT) pathway genes and high expression of Notch pathways were associated with prolonged PFS in the EG group (Fig. 3c). In contrast, high expression of the EMT pathway was associated with good prognosis in the PG group (Fig. 3d).

Tumor mutation burden

We calculated TMB in MBCs (Fig. 4a). Median TMB was 6.5 (2.44–46.34), and mean TMB was 10.88. Further survival analyses were performed and showed neither a PFS nor OS difference according to TMB (median PFS (months) (low vs. high): 12.7 vs. 15.4, $p = 0.2174$, and median OS: 32 vs. not reached, $p = 0.2079$, respectively) (Fig. 4b, c).

Mutation signature

Mutation signatures in MBCs are described in Fig. 5a. Among 30 mutation signatures, the age-associated signature (signature 1) was expressed in the largest number of cancer tissues. In terms of BC-specific signatures, we frequently observed signatures 3, 20, and 26 in our cohort.

Further survival analysis was performed according to mutation signature. Mutation signature 3, a homologous recombinant deficiency-related signature, was highly associated with disease progression (HR, log2 scale: 8.21, 95% CI 2.93–13.48, $p = 0.002$) (Fig. 5b). In addition, mutation signature 20, related to defective mismatch repair, was also associated with disease progression (HR 4.83, 95% CI − 0.53–10.19, $p = 0.078$) (Fig. 5c). Kaplan–Meier plots showed that BCs with signature 3 or signature 20 had short PFSs compared to those without these signatures (median

Table 1 Clinical characteristics of patients with biomarker analysis

Characteristics	Clinical trial group (n =118)	Biomarker analysis group (n=40)	P-value	EG in biomarker group (n=22)	PG in biomarker group (n=18)	P-value
Median age (range)	50 (24, 65)	50 (37, 65)	0.851	50 (38, 64)	50 (37, 65)	0.907
Performance status			0.150			0.999
0	51 (43.2)	13 (32.5)		7 (31.9)	6 (33.3)	
1	61 (51.7)	27 (67.5)		15 (68.1)	12 (66.7)	
2	6 (5.1)	0 (0.0)		0 (0.0)	0 (0.0)	
Menopausal status			0.762			0.734
Premenopause	52 (44.1)	20 (50.0)		11 (50.0)	9 (50.0)	
Postmenopause	56 (47.5)	16 (40.0)		8 (36.4)	8 (44.4)	
Unknown	10 (8.4)	4 (10.0)		3 (13.6)	1 (5.6)	
Histology			0.999			0.093
Invasive ductal carcinoma	97 (82.2)	33 (82.5)		19 (86.4)	14 (77.8)	
Others	21 (17.8)	7 (17.5)		3 (13.6)	4 (22.2)	
Hormone receptor			0.036			0.999
ER[a] or PgR[b] positive	91 (77.1)	37 (92.5)		20 (90.1)	17 (94.4)	
ER and PgR Negative	27 (22.9)	3 (7.5)		2 (9.9)	1 (5.6)	
Disease status			0.999			0.165
De novo stage IV	34 (28.8)	12 (30.0)		9 (40.1)	3 (16.7)	
Recurred	84 (71.2)	28 (70.0)		13 (59.9)	15 (83.3)	
Number of metastatic site			0.890			0.014
1	25 (21.2)	9 (22.5)		3 (13.6)	6 (33.3)	
2	44 (37.3)	13 (32.5)		10 (45.4)	3 (16.7)	
≥3	49 (41.5)	18 (45.0)		9 (41.0)	9 (50.0)	
Metastatic sites						
Lung	52 (44.1)	18 (45.0)	0.999	12 (54.5)	6 (33.3)	0.216
Liver	55 (46.6)	18 (45.0)	0.999	9 (40.9)	9 (50.0)	0.750
Stage IV Lymph nodes	69 (58.5)	27 (67.5)	0.353	14 (63.6)	13 (72.2)	0.737
Bone	68 (57.6)	23 (57.5)	0.999	16 (72.7)	7 (38.9)	0.053
Others	30 (25.4)	14 (35.0)	0.307	7 (31.8)	7 (38.9)	0.744
Visceral metastasis			0.421			0.744
Yes	86 (72.9)	26 (65.0)		15 (68.2)	11 (61.1)	
No	32 (27.1)	14 (35.0)		7 (31.8)	7 (38.9)	
Tissue status			NA[c]			0.748
Surgical specimen	NA	16 (40.0)		8 (36.4)	8 (44.4)	
Biopsy specimen	NA	24 (60.0)		14 (63.6)	10 (55.6)	
Tissue status		All (n =44)		EG (n =24)	PG (n =20)	
Initial tissue	NA	38 (86.4)		21 (87.5)	17 (85.0)	
After disease progression	NA	6 (13.6)		3 (12.5)	3 (15.0)	
Breast	NA	30 (68.2)		18 (75.0)	12 (60.0)	
Lymph node	NA	7 (15.9)		2 (8.3)	5 (25.0)	
Liver	NA	4 (9.1)		2 (8.3)	2 (10.0)	
Lung	NA	3 (6.8)		2 (8.3)	1 (5.0)	

[a] Estrogen receptor; [b] Progesterone receptor; [c] Not applicable

PFS [months] for signature 3 [low vs. high]: 17.2 vs. 8.1, $p = 0.0026$, and median PFS for signature 20: 15.4 vs. 8.9, $p = 0.0261$, respectively) (Fig. 5d, e).

Gene expression after disease progression

We biopsied tissues after disease progression in four patients, two who had received eribulin plus gemcitabine (EG) and two who had received PG. Compared to pre-treatment tissues, all resistant tumors had decreased hedgehog (HH), Wnt, cytokine, and transcription factor pathway signals and upregulated antigen processing pathway (Fig. S5). In terms of treatment arms, JAK/STAT, RAS pathway, and EMT signals decreased after disease progression of EG treatment but increased after PG treatment.

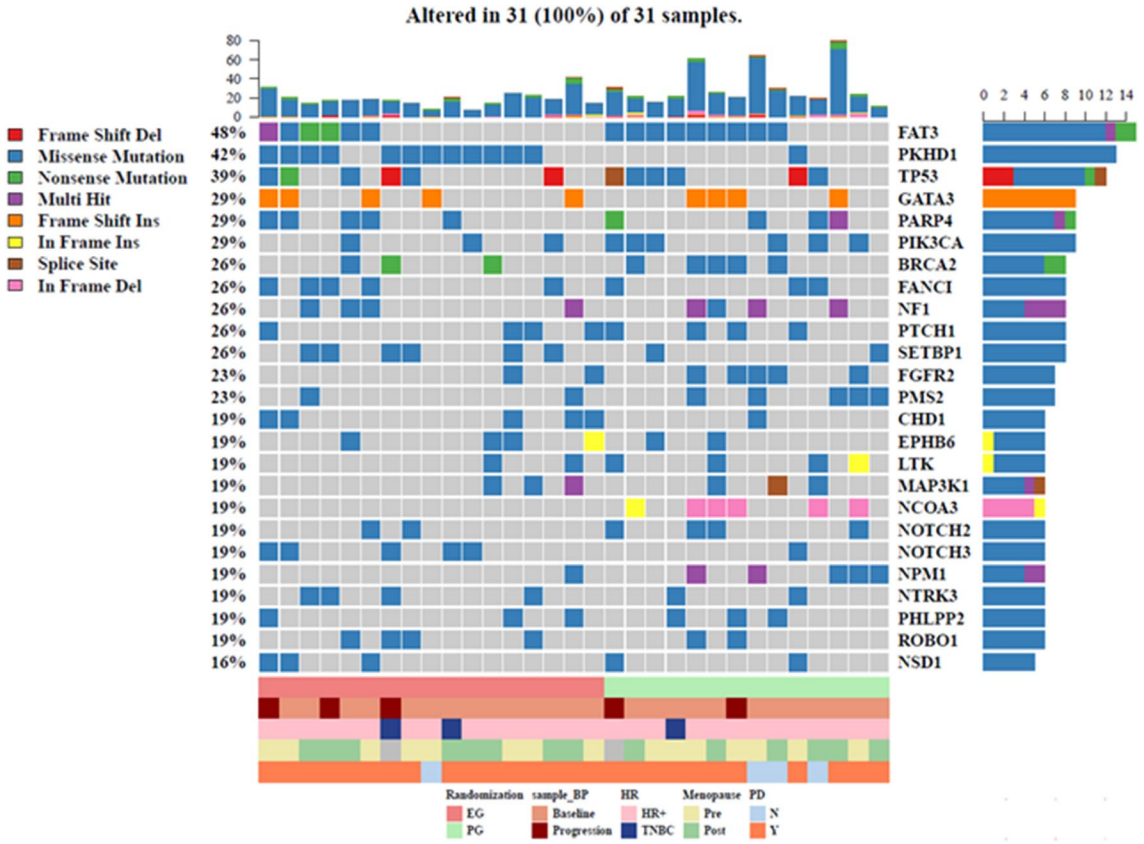

Fig. 2 Genetic landscape of metastatic breast cancers

Discussion

This translational research for discovering biomarkers for chemotherapy in MBC patients suggested that mutation signature associated with DNA repair could be a potential prognostic factor to identify disease progression and shorten PFS. EMT pathway activation influenced disease progression for PG treatment and in the opposite direction for EG treatment, respectively. Tumor mutation burden did not affect patient prognosis.

Mutation signatures, specific mutational processes of each cancer, have been discovered among all cancer types, and these mutation signatures indicate the mechanism of genetic alterations in cancers [18, 20]. To date, 30 mutation signatures have been discovered among cancer types [21].

Mutation signatures are mutational processes regardless of cancer type; however, signatures 2, 3, 8, 17, 18, 20, 26, and 30 are frequently observed in BCs. Among these, signature 3 is associated with failure in DNA homologous recombination, as seen in BRCA1 and BRCA2 mutations [18]. A recent genome study suggested that signature 3 is strongly associated with homologous recombination defects, abrogation of BRCA1, triple negativity, and basal intrinsic subtypes of BC [21].

In our study, about 90% of BCs were hormone receptor-positive and mutation signature 3 was observed in 27% of BCs, which was associated with short PFS. Especially, EG chemotherapy provided less benefit to MBCs with signature 3 (Fig. S6). Therefore, regardless of subtype, mutation signature 3 in BC is a compelling prognostic genetic marker of cytotoxic chemotherapy.

Hormone receptor-positive BCs with signature 3 were rarely observed in previous studies [21]. Another study presented fewer than 10% of hormone receptor-positive BCs with a basal-like intrinsic subtype [22]. However, these studies were conducted using early-stage BCs, not metastatic cases. During metastasis processes, tumors undergo clonal evolution and experience changes in characteristics [23]. Therefore, signature 3 could be an evolutional mechanism sending a strong survival signal with chemotherapy resistance in hormone receptor-positive BCs.

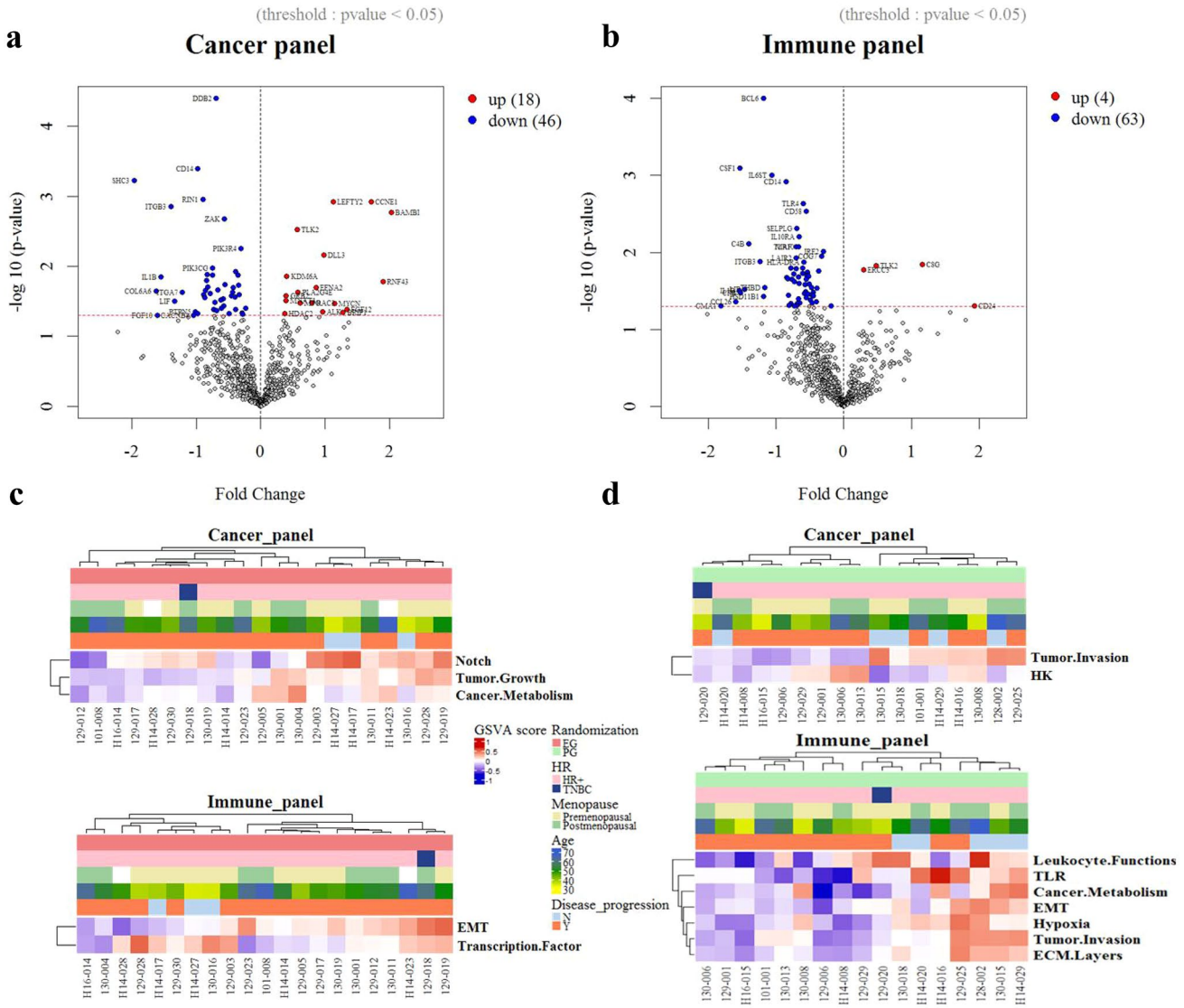

Fig. 3 **a** Differentially expressed gene (DEG) analysis for nanostring cancer panel, **b** DEG analysis for nanostring Immune panel, **c** gene set variation analysis (GSVA) for nanostring cancer and immune panel in patients with eribulin plus gemcitabine chemotherapy, and **d** GSVA in patients with paclitaxel plus gemcitabine chemotherapy

Eribulin, a microtubule inhibitor, is a cytotoxic agent with mechanisms that suppress the EMT of cancer cells [24]. In our study, EMT pathway downregulation was observed in patients with good response to EG chemotherapy, while relatively high expression of the EMT pathway was found in responders to PG. Although we genetically analyzed only two paired samples, EMT suppression was observed in the EG group after disease progression. Therefore, we suggest that EMT is not associated with drug resistance.

Past microarray analyses have been conducted to characterize predictive and prognostic biomarkers of cytotoxic chemotherapy [9, 11]. In the era of NGS, many investigators conducted translational research using whole exome sequencing, targeted deep sequencing, and RNAseq. Most parallel translational research has been conducted in clinical trials with endocrine therapy and/or targeted therapy [1, 2]. Currently, only platinum among cytotoxic chemotherapeutic agents for BC patients has been associated with a predictive genetic biomarker, germline BRCA1/2 mutations [6].

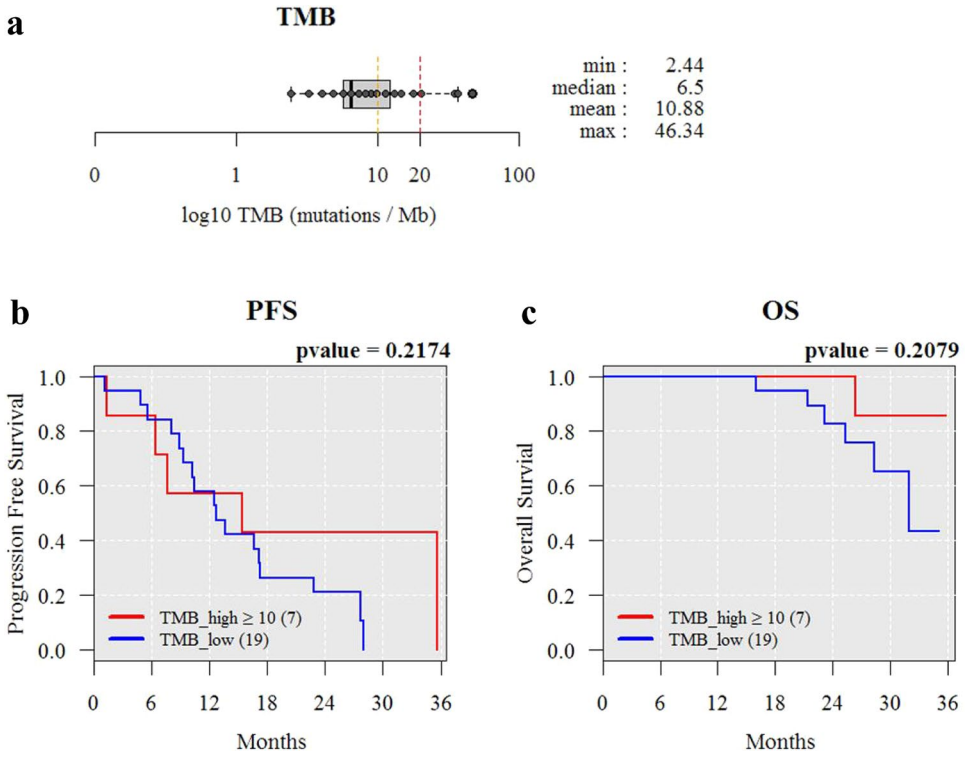

Fig. 4 **a** Tumor mutation burden (TMB). **b** Progression-free survival (PFS) according to TMB. **c** Overall survival (OS) according to TMB

Our translational research was conducted in a prospective clinical trial of cytotoxic chemotherapy [13]. We acquired 44 tumor tissues from 40 (33.6%) of 119 patients enrolled in this clinical trial. In those with TNBC, only 3 of 27 participated in this translational research. Although biomarker analysis was conducted in a limited number of patients, this study was a comprehensive genetic analysis to determine the genetic mechanism of chemotherapy-sensitive and -resistant MBCs.

In conclusion, mutation signature 3, found in about 30% of MBCs regardless of hormone receptor status, was associated with short progression-free survival for patients with cytotoxic chemotherapy. Considering the rarity of genetic studies for cytotoxic chemotherapy, although most MBC patients are eventually treated with cytotoxic agents, comprehensive genetic analysis would help many MBC patients to expect their response to cytotoxic chemotherapy.

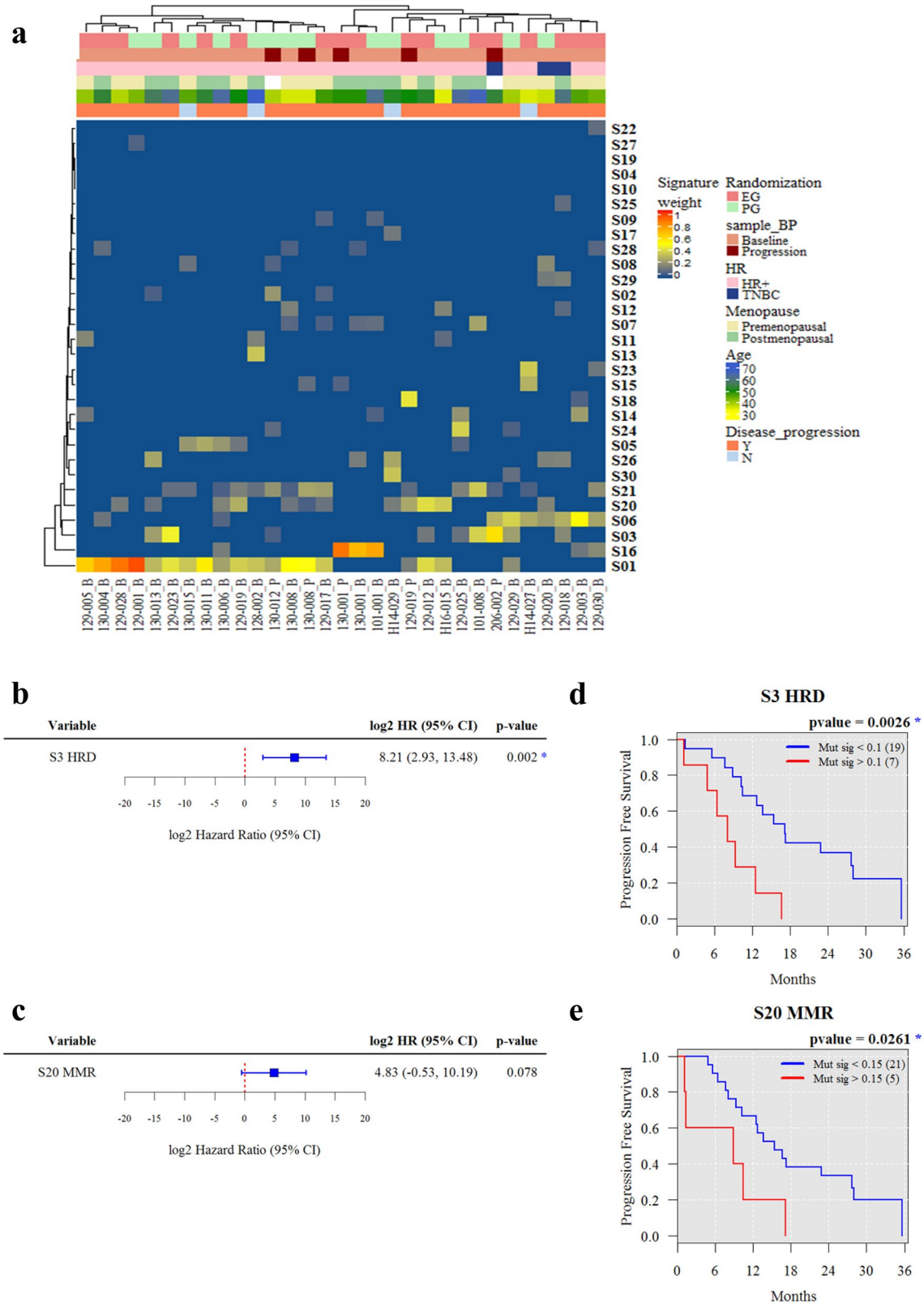

Fig. 5 **a** Mutation Signature of metastatic breast cancers. **b** Cox-regression analysis for PFS according to the mutation signature 3. **c** Cox-regression analysis for PFS according to mutation signature 20. **d** Kaplan–Meier (KM) plot for PFS according to mutation signature 3. **e** KM plot for PFS according to mutation signature 20

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards our institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

References

1. Andre F, Hurvitz S, Fasolo A, Tseng LM, Jerusalem G, Wilks S, O'Regan R, Isaacs C, Toi M, Burris H, He W, Robinson D, Riester M, Taran T, Chen D, Slamon D (2016) Molecular alterations and everolimus efficacy in human epidermal growth factor receptor 2-overexpressing metastatic breast cancers: combined exploratory biomarker analysis From BOLERO-1 and BOLERO-3. J Clin Oncol 34:2115–2124. https://doi.org/10.1200/JCO.2015.63.9161
2. Cristofanilli M, DeMichele A, Giorgetti C, Turner NC, Slamon DJ, Im SA, Masuda N, Verma S, Loi S, Colleoni M, Theall KP, Huang X, Liu Y, Bartlett CH (2018) Predictors of prolonged benefit from palbociclib plus fulvestrant in women with endocrine-resistant hormone receptor-positive/human epidermal growth factor receptor 2-negative metastatic breast cancer in PALOMA-3. Eur J Cancer 104:21–31. https://doi.org/10.1016/j.ejca.2018.08.011
3. Hyman DM, Piha-Paul SA, Won H, Rodon J, Saura C, Shapiro GI, Juric D, Quinn DI, Moreno V, Doger B, Mayer IA, Boni V, Calvo E, Loi S, Lockhart AC, Erinjeri JP, Scaltriti M, Ulaner GA, Patel J, Tang J, Beer H, Selcuklu SD, Hanrahan AJ, Bouvier N, Melcer M, Murali R, Schram AM, Smyth LM, Jhaveri K, Li BT, Drilon A, Harding JJ, Iyer G, Taylor BS, Berger MF, Cutler RE Jr, Xu F, Butturini A, Eli LD, Mann G, Farrell C, Lalani AS, Bryce RP, Arteaga CL, Meric-Bernstam F, Baselga J, Solit DB (2018) HER kinase inhibition in patients with HER2- and HER3-mutant cancers. Nature 554:189–194. https://doi.org/10.1038/nature25475
4. Tutt A, Tovey H, Cheang MCU, Kernaghan S, Kilburn L, Gazinska P, Owen J, Abraham J, Barrett S, Barrett-Lee P, Brown R, Chan S, Dowsett M, Flanagan JM, Fox L, Grigoriadis A, Gutin A, Harper-Wynne C, Hatton MQ, Hoadley KA, Parikh J, Parker P, Perou CM, Roylance R, Shah V, Shaw A, Smith IE, Timms KM, Wardley AM, Wilson G, Gillett C, Lanchbury JS, Ashworth A, Rahman N, Harries M, Ellis P, Pinder SE, Bliss JM (2018) Carboplatin in BRCA1/2-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial. Nat Med 24:628–637. https://doi.org/10.1038/s41591-018-0009-7
5. Litton JK, Rugo HS, Ettl J, Hurvitz SA, Goncalves A, Lee KH, Fehrenbacher L, Yerushalmi R, Mina LA, Martin M, Roche H, Im YH, Quek RGW, Markova D, Tudor IC, Hannah AL, Eiermann W, Blum JL (2018) Talazoparib in Patients with Advanced Breast Cancer and a Germline BRCA Mutation. N Engl J Med 379:753–763. https://doi.org/10.1056/NEJMoa1802905
6. Robson M, Im SA, Senkus E, Xu B, Domchek SM, Masuda N, Delaloge S, Li W, Tung N, Armstrong A, Wu W, Goessl C, Runswick S, Conte P (2017) Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. N Engl J Med 377:523–533. https://doi.org/10.1056/NEJMoa1706450
7. Pichon MF, Broet P, Magdelenat H, Delarue JC, Spyratos F, Basuyau JP, Saez S, Rallet A, Courriere P, Millon R, Asselain B (1996) Prognostic value of steroid receptors after long-term follow-up of 2257 operable breast cancers. Br J Cancer 73:1545–1551
8. Kim HK, Lee SH, Kim YJ, Park SE, Lee HS, Lim SW, Cho JH, Kim JY, Ahn JS, Im YH, Yu JH, Park YH (2019) Does guideline non-adherence result in worse clinical outcomes for hormone receptor-positive and HER2-negative metastatic breast cancer in premenopausal women?: result of an institution database from South Korea. BMC Cancer 19:84. https://doi.org/10.1186/s12885-018-5258-9
9. Bartlett JM, Munro A, Cameron DA, Thomas J, Prescott R, Twelves CJ (2008) Type 1 receptor tyrosine kinase profiles identify patients with enhanced benefit from anthracyclines in the BR9601 adjuvant breast cancer chemotherapy trial. J Clin Oncol 26:5027–5035. https://doi.org/10.1200/JCO.2007.14.6597
10. Okuma HS, Koizumi F, Hirakawa A, Nakatochi M, Komori O, Hashimoto J, Kodaira M, Yunokawa M, Yamamoto H, Yonemori K, Shimizu C, Fujiwara Y, Tamura K (2016) Clinical and microarray analysis of breast cancers of all subtypes from two prospective preoperative chemotherapy studies. Br J Cancer 115:411–419. https://doi.org/10.1038/bjc.2016.184
11. Kim JY, Jung HH, Ahn S, Bae S, Lee SK, Kim SW, Lee JE, Nam SJ, Ahn JS, Im YH, Park YH (2016) The relationship between nuclear factor (NF)-kappaB family gene expression and prognosis in triple-negative breast cancer (TNBC) patients receiving adjuvant doxorubicin treatment. Sci Rep 6:31804. https://doi.org/10.1038/srep31804
12. Vollebergh MA, Lips EH, Nederlof PM, Wessels LF, Schmidt MK, van Beers EH, Cornelissen S, Holtkamp M, Froklage FE, de Vries EG, Schrama JG, Wesseling J, van de Vijver MJ, van Tinteren H, de Bruin M, Hauptmann M, Rodenhuis S, Linn SC (2011) An aCGH classifier derived from BRCA1-mutated breast cancer and benefit of high-dose platinum-based chemotherapy in HER2-negative breast cancer patients. Ann Oncol 22:1561–1570. https://doi.org/10.1093/annonc/mdq624
13. Park YH, Im SA, Kim SB, Sohn JH, Lee KS, Chae YS, Lee KH, Kim JH, Im YH, Kim JY, Kim TY, Lee KH, Ahn JH, Kim GM, Park IH, Lee SJ, Han HS, Kim SH, Jung KH, Korean Cancer Study G (2017) Phase II, multicentre, randomised trial of eribulin plus gemcitabine versus paclitaxel plus gemcitabine as first-line chemotherapy in patients with HER2-negative metastatic breast cancer. Eur J Cancer 86:385–393. https://doi.org/10.1016/j.ejca.2017.10.002
14. Wang K, Li M, Hakonarson H (2010) ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 38:e164. https://doi.org/10.1093/nar/gkq603
15. Hanzelmann S, Castelo R, Guinney J (2013) GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinform 14:7. https://doi.org/10.1186/1471-2105-14-7
16. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, O'Donnell-Luria AH, Ware JS, Hill AJ, Cummings BB, Tukiainen T, Birnbaum DP, Kosmicki JA, Duncan LE, Estrada K, Zhao F, Zou J, Pierce-Hoffman E, Berghout J, Cooper DN, Deflaux N, DePristo M, Do R, Flannick J, Fromer M, Gauthier L, Goldstein J, Gupta N, Howrigan D, Kiezun A, Kurki MI, Moonshine AL, Natarajan P, Orozco L, Peloso GM, Poplin R, Rivas MA, Ruano-Rubio V, Rose SA, Ruderfer DM, Shakir K, Stenson PD, Stevens C, Thomas BP, Tiao G, Tusie-Luna MT, Weisburd B, Won HH, Yu D, Altshuler DM, Ardissino D, Boehnke M, Danesh J, Donnelly S, Elosua R, Florez JC, Gabriel SB, Getz G, Glatt SJ, Hultman CM, Kathiresan S, Laakso M, McCarroll S, McCarthy

MI, McGovern D, McPherson R, Neale BM, Palotie A, Purcell SM, Saleheen D, Scharf JM, Sklar P, Sullivan PF, Tuomilehto J, Tsuang MT, Watkins HC, Wilson JG, Daly MJ, MacArthur DG, Exome Aggregation C (2016) Analysis of protein-coding genetic variation in 60,706 humans. Nature 536:285–291. https://doi.org/10.1038/nature19057

17. Bamford S, Dawson E, Forbes S, Clements J, Pettett R, Dogan A, Flanagan A, Teague J, Futreal PA, Stratton MR, Wooster R (2004) The COSMIC (Catalogue of Somatic Mutations in Cancer) database and website. Br J Cancer 91:355–358. https://doi.org/10.1038/sj.bjc.6601894
18. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, Bignell GR, Bolli N, Borg A, Borresen-Dale AL, Boyault S, Burkhardt B, Butler AP, Caldas C, Davies HR, Desmedt C, Eils R, Eyfjord JE, Foekens JA, Greaves M, Hosoda F, Hutter B, Ilicic T, Imbeaud S, Imielinski M, Jager N, Jones DT, Jones D, Knappskog S, Kool M, Lakhani SR, Lopez-Otin C, Martin S, Munshi NC, Nakamura H, Northcott PA, Pajic M, Papaemmanuil E, Paradiso A, Pearson JV, Puente XS, Raine K, Ramakrishna M, Richardson AL, Richter J, Rosenstiel P, Schlesner M, Schumacher TN, Span PN, Teague JW, Totoki Y, Tutt AN, Valdes-Mas R, Buuren MM, Veer L, Vincent-Salomon A, Waddell N, Yates LR, Australian Pancreatic Cancer Genome I, Consortium IBC, Consortium IM-S, PedBrain I, Zucman-Rossi J, Futreal PA, McDermott U, Lichter P, Meyerson M, Grimmond SM, Siebert R, Campo E, Shibata T, Pfister SM, Campbell PJ, Stratton MR (2013) Signatures of mutational processes in human cancer. Nature 500:415–421. https://doi.org/10.1038/nature12477
19. Rosenthal R, McGranahan N, Herrero J, Taylor BS, Swanton C (2016) DeconstructSigs: delineating mutational processes in single tumors distinguishes DNA repair deficiencies and patterns of carcinoma evolution. Genome Biol 17:31. https://doi.org/10.1186/s13059-016-0893-4
20. Nik-Zainal S, Alexandrov LB, Wedge DC, Van Loo P, Greenman CD, Raine K, Jones D, Hinton J, Marshall J, Stebbings LA, Menzies A, Martin S, Leung K, Chen L, Leroy C, Ramakrishna M, Rance R, Lau KW, Mudie LJ, Varela I, McBride DJ, Bignell GR, Cooke SL, Shlien A, Gamble J, Whitmore I, Maddison M, Tarpey PS, Davies HR, Papaemmanuil E, Stephens PJ, McLaren S, Butler AP, Teague JW, Jonsson G, Garber JE, Silver D, Miron P, Fatima A, Boyault S, Langerod A, Tutt A, Martens JW, Aparicio SA, Borg A, Salomon AV, Thomas G, Borresen-Dale AL, Richardson AL, Neuberger MS, Futreal PA, Campbell PJ, Stratton MR, Breast Cancer Working Group of the International Cancer Genome C (2012) Mutational processes molding the genomes of 21 breast cancers. Cell 149:979–993. https://doi.org/10.1016/j.cell.2012.04.024
21. Nik-Zainal S, Davies H, Staaf J, Ramakrishna M, Glodzik D, Zou X, Martincorena I, Alexandrov LB, Martin S, Wedge DC, Van Loo P, Ju YS, Smid M, Brinkman AB, Morganella S, Aure MR, Lingjaerde OC, Langerod A, Ringner M, Ahn SM, Boyault S, Brock JE, Broeks A, Butler A, Desmedt C, Dirix L, Dronov S, Fatima A, Foekens JA, Gerstung M, Hooijer GK, Jang SJ, Jones DR, Kim HY, King TA, Krishnamurthy S, Lee HJ, Lee JY, Li Y, McLaren S, Menzies A, Mustonen V, O'Meara S, Pauporte I, Pivot X, Purdie CA, Raine K, Ramakrishnan K, Rodriguez-Gonzalez FG, Romieu G, Sieuwerts AM, Simpson PT, Shepherd R, Stebbings L, Stefansson OA, Teague J, Tommasi S, Treilleux I, Van den Eynden GG, Vermeulen P, Vincent-Salomon A, Yates L, Caldas C, Veer L, Tutt A, Knappskog S, Tan BK, Jonkers J, Borg A, Ueno NT, Sotiriou C, Viari A, Futreal PA, Campbell PJ, Span PN, Van Laere S, Lakhani SR, Eyfjord JE, Thompson AM, Birney E, Stunnenberg HG, van de Vijver MJ, Martens JW, Borresen-Dale AL, Richardson AL, Kong G, Thomas G, Stratton MR (2016) Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature 534:47–54. https://doi.org/10.1038/nature17676
22. Eroles P, Bosch A, Perez-Fidalgo JA, Lluch A (2012) Molecular biology in breast cancer: intrinsic subtypes and signaling pathways. Cancer Treat Rev 38:698–707. https://doi.org/10.1016/j.ctrv.2011.11.005
23. Wang Y, Waters J, Leung ML, Unruh A, Roh W, Shi X, Chen K, Scheet P, Vattathil S, Liang H, Multani A, Zhang H, Zhao R, Michor F, Meric-Bernstam F, Navin NE (2014) Clonal evolution in breast cancer revealed by single nucleus genome sequencing. Nature 512:155–160. https://doi.org/10.1038/nature13600
24. Yoshida T, Ozawa Y, Kimura T, Sato Y, Kuznetsov G, Xu S, Uesugi M, Agoulnik S, Taylor N, Funahashi Y, Matsui J (2014) Eribulin mesilate suppresses experimental metastasis of breast cancer cells by reversing phenotype from epithelial-mesenchymal transition (EMT) to mesenchymal-epithelial transition (MET) states. Br J Cancer 110:1497–1505. https://doi.org/10.1038/bjc.2014.80

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