Original Article

Integrated Molecular Profiling of Young and Elderly Patients With Triple-Negative Breast Cancer Indicates Different Biological Bases and Clinical Management Strategies

Ding Ma, MD[1,2,3]; Yi-Zhou Jiang, MD[1,2,3]; Yi Xiao, MD[1,2,3]; Meng-Dan Xie, MD[1,2,3]; Shen Zhao, MD[1,2,3]; Xi Jin, MD[1,2,3]; Xiao-En Xu, PhD[1,2,3]; and Zhi-Ming Shao, MD, PhD [1,2,3]

BACKGROUND: Age at the time of breast cancer diagnosis not only predicts clinical outcome but also indicates distinct molecular characteristics that provide the rationale for appropriate treatment strategies. However, to the authors' knowledge, little is known regarding the molecular profile and biological basis of triple-negative breast cancers (TNBCs) occurring in young and elderly patients. **METHODS:** Using the study institution's largest, single-center, multiomics TNBC data set, the authors analyzed the clinical and genomic features of young (aged ≤39 years) and elderly (aged ≥65 years) patients with TNBC. **RESULTS:** In the current study, a total of 50 patients, 354 patients, and 69 patients, respectively, were grouped as young, intermediate, and elderly patients with TNBC. Young patients with TNBC had worse short-term survival, upregulation of DNA repair, cell cycle and RNA metabolism gene sets, frequent pathogenic germline variants, and predominant homologous recombination deficiency-related mutational signatures. Several copy number alterations also were found to be enriched in young patients with TNBC. Nearly one-half of the TNBC cases in elderly patients were of the luminal androgen receptor subtype. TNBC in elderly patients was identified as being associated with severe fibrosis; a lower Ki-67 index; and somatic mutations in *PIK3CA, KMT2D*, *ERBB2*, *ERBB3*, and their corresponding pathways. Elderly patients with TNBC also were more likely to harbor targetable mutations. **CONCLUSIONS:** The findings of the current study indicated that young patients with TNBC had an enhanced cell cycle, which may have helped to explain their inferior short-term survival, whereas the homologous recombination deficiency and enriched pathogenic germline variants observed among young patients with TNBC suggested the need for genetic counseling and testing, as well as the potential use of DNA damage agents and poly(adenosine diphosphate ribose) polymerase (PARP) inhibitors. Molecular characteristics of elderly patients with TNBC, although suggesting less response to chemotherapy, provided a rationale for the routine detection of actionable somatic mutations. ***Cancer* 2020;126:3209-3218**. © *2020 American Cancer Society*.

KEYWORDS: age of onset, DNA copy number variations, gene expression, mutation, triple-negative breast neoplasms.

INTRODUCTION

Triple-negative breast cancers (TNBCs) are a group of breast cancers that lack expression of estrogen receptors (ERs) and progesterone receptors (PRs) and do not overexpress human epidermal growth factor receptor 2 (HER2).[1] In general, TNBCs are more aggressive, have higher proliferation activity, have more distant metastasis, and have lower responses to endocrine or anti-HER2 therapy, leading to an unfavorable prognosis.[2-4] However, as a diagnosis of exclusion, TNBCs are highly heterogeneous.[5,6] Over the past decade, studies have sought to subclassify TNBCs by either clinicopathological or molecular approaches, which have advanced our understanding and management of this complex disease.[7]

Patient age at the time of diagnosis has been shown to be valuable in prognosis prediction and therapeutic strategy optimization in patients with breast cancer.[8-10] The unfavorable prognostic role of younger age and the different response rates to therapeutics noted among age groups have suggested varying underlying biological characteristics. This idea already has been proven and expanded by molecular profiling studies.[11] Recent studies have shown that the clinical significance of age may differ among breast cancer subtypes,[12] and that the clinical significance of age among patients with TNBC remains controversial.[13-16] It is interesting to note that some studies have shown that TNBC occurring in young patients has higher proliferation scores, whereas TNBC diagnosed in elderly patients demonstrates varied expression of biomarkers such as Ki-67, Bcl-2, and cytokeratins.[17] These findings raise the hypothesis

Corresponding Authors: Yi-Zhou Jiang, MD, Department of Breast Surgery, Fudan University Shanghai Cancer Center, No. 270 Dong'an Rd, Shanghai 200032, China (yizhoujiang@fudan.edu.cn) and Zhi-Ming Shao, MD, PhD, Department of Breast Surgery, Fudan University Shanghai Cancer Center, No. 270 Dong'an Rd, Shanghai 200032, China (zhimingshao@yahoo.com).

[1]Key Laboratory of Breast Cancer in Shanghai, Department of Breast Surgery, Fudan University Shanghai Cancer Center, Shanghai, China; [2]Cancer Institute, Fudan University Shanghai Cancer Center, Shanghai, China; [3]Department of Oncology, Shanghai Medical College, Fudan University, Shanghai, China

The first 3 authors contributed equally to this article.

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that TNBCs occurring in young and elderly patients might be unique disease entities. Nevertheless, our knowledge of the underlying biological bases of these entities remains rather fragmented.

In the current study, using what to our knowledge is the largest single-center cohort of patients with TNBC to date who were diagnosed and treated at Fudan University Shanghai Cancer Center (FUSCC),[18,19] we analyzed the age-specific differences in prognosis, clinicopathologic variables, transcriptomic patterns, mutation profiling, and somatic copy number alterations (CNAs). The objective of the current comprehensive study was to provide clues regarding the unique biology of and potential therapeutic strategies for TNBCs arising in young and elderly women.

MATERIALS AND METHODS

The current study included all the patients who participated in the FUSCC TNBC project.[18,19] We classified these patients into 3 groups according to their age at the time of diagnosis: young (those aged ≤39 years), elderly (those aged ≥65 years), and intermediate (patients aged 40-64 years). The cutoff points were set based on other historical studies.[9,20,21] Detailed methods has been published previously,[18] and also can be found in the Supporting Information.

Data From External Cohorts

We obtained data from the Surveillance, Epidemiology, and End Results (SEER) database (18 population-based cancer registries [1973-2015]). A total of 21,217 women were identified using the following inclusion criteria: female aged 15 to 85 years; known time of diagnosis from January 1, 2010, to December 31, 2015; not diagnosed at the time of autopsy/death; presence of unilateral breast cancer; breast cancer as the first and only cancer diagnosis; presence of only 1 primary tumor site; pathologic confirmation of infiltrating ductal carcinoma; ER-negative, PR-negative, and HER2-negative status; American Joint Committee on Cancer stages I to III disease; and a follow-up ≥6 months (or the patient had died of breast cancer within 6 months). Patients diagnosed with breast cancer before 2010 were excluded because the SEER data did not include HER2 status until 2010. Breast cancer-specific survival (BCSS), which is defined as a net survival measure representing survival of a specified cause of death in the absence of other causes of death (https://seer.cancer.gov/causespecific/), was measured in the current study.

Both clinical and genomic data from the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) cohort were downloaded from the cBioPortal for Cancer Genomics (http://www.cbioportal.org/) on July 22, 2018.[22-25] Patients recorded as "-" for "ER status," "PR status," and "HER2 status" were defined as having TNBC in the current study. A total of 320 patients were enrolled.

Statistical Analyses

Frequency tabulation and summary statistics were used to characterize the data distribution. The Student *t* test, Mann-Whitney Wilcoxon test, and Kruskal-Wallis test were used to compare continuous variables and ordered categorical variables whereas the Pearson chi-square test or Fisher exact test were used for a comparison of unordered categorical variables. Survival curves were constructed using the Kaplan-Meier method and compared using the log-rank test to obtain results regarding recurrence-free survival (RFS) and BCSS. When a multiple comparison test was performed, the Bonferroni correction (log-rank test, Pearson chi-square test, and Fisher exact test), Nemenyi post hoc test (Kruskal-Wallis test), or Benjamini-Hochberg procedure (limma) were used for *P* value correction. All analyses were performed using R statistical software packages (R version 3.4.2; R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Prognosis and Clinical Features of Young and Elderly Patients With TNBC

A total of 473 patients with TNBC from the FUSCC TNBC project were included, of whom 50 patients, 354 patients, and 69 patients were aged ≤39 years (young group), aged 40 to 64 years (intermediate group), and aged ≥65 years (elderly group), respectively (Fig. 1A). The study cohort had a median length of follow-up of 47 months (interquartile range, 29.3-73.5 months) with 66 RFS events and demonstrated a similar age distribution compared with the patients with TNBC in the SEER database (Fig. 1B), which included thousands of cases with representative epidemiological characteristics.

We evaluated the clinical outcome of patients in the different age groups. In the current study cohort, young patients had a worse 2-year (24 months) RFS (log-rank $P = .031$) compared with patients in the intermediate age group (adjusted $P = .043$) (Fig. 1C), although no difference was observed throughout the entire follow-up

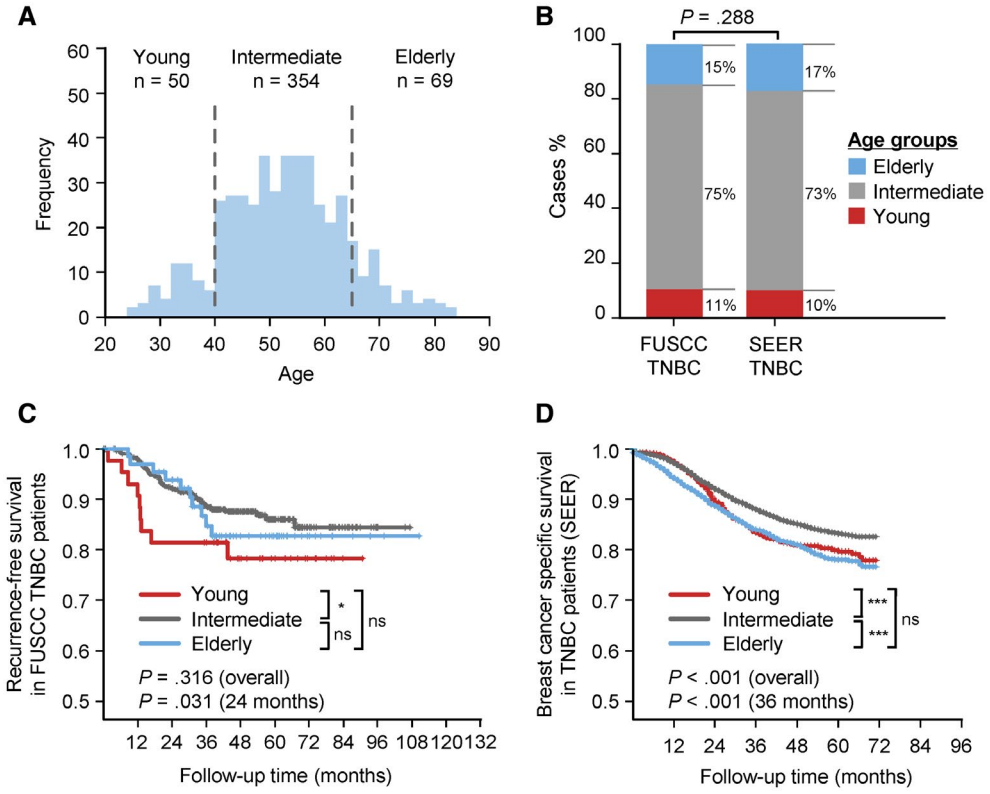

Figure 1. Distribution of age at the time of diagnosis among patients with triple-negative breast cancer (TNBC) and its clinical relevance. (A) Distribution of age at diagnosis and the definitions of age groups used in the study cohort. (B) Distribution of age groups in the Fudan University Shanghai Cancer Center (FUSCC) TNBC and the Surveillance, Epidemiology, and End Results (SEER) TNBC cases. (C and D) Prognosis of patients with TNBC in different age groups. (C) Overall and 24-month recurrence-free survival (RFS) in the FUSCC TNBC cases. (D) Overall and 36-month breast cancer-specific survival (BCSS) in patients with TNBC (data from the SEER database). Pairwise comparisons were performed for short-term survival (24 months for RFS and 36 months for BCSS) using Bonferroni correction, and annotated as $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$; or not significant (ns) ($P > .05$).

period (log-rank $P = .316$). We further investigated the prognostic value of age in patients with TNBC using the SEER database. Because the median life expectancy of patients with TNBC who experience disease recurrence or metastasis is approximately 1 year,[26] we defined 3 years (36 months) after surgery as a short-term period for BCSS. Both young and elderly patients with TNBC had worse RFS compared with the intermediate group within 3 years after surgery (adjusted $P < .001$) (Fig. 1D) (see Supporting Fig. 1A-C). This result lasted throughout the entire follow-up period.

Other clinicopathological features of these age groups also were studied. Although patients with TNBC who were diagnosed at different ages demonstrated similar tumor size, lymph node status, histology grade, and histology type ($P > .05$) (Table 1), elderly patients demonstrated a significantly lower Ki-67 index ($P < .001$) (Table 1), and were more likely to have severe fibrosis ($P = .005$).

Transcriptomic Features of Young and Elderly Patients With TNBC

Transcription-based subtyping has played a dominant role in untangling the heterogeneity of TNBC, and each messenger RNA-based subtype has been matched with unique biologic characteristics and therapeutic strategies. We found that TNBCs diagnosed at different ages have distinct subtype distributions ($P < .001$ for the FUSCC subtype and $P = .004$ for the Lehmann/Pietenpol subtype) (Fig. 2A,B).[7] It is interesting to note that we found that nearly one-half of the elderly patients had tumors that were of the luminal androgen receptor (LAR) subtype (49% of the FUSCC subtype and 42% of the Lehmann/Pietenpol subtype).

To further elucidate the biological features of young and elderly patients with TNBC, we performed gene set variation analysis to study their enriched gene sets. In the young patients with TNBC, 414 gene sets were found to be significantly upregulated (false discovery rate,

TABLE 1. Clinical Features of Patients With TNBC Who Were Diagnosed at Different Ages

Clinical Characteristics	Young (Aged ≤39 Years N = 50 (%)	Intermediate (Aged 40-64 Years) N = 354 (%)	Elderly (Aged ≥65 Years N = 69 (%)	*P*
Tumor size, cm				.891[a]
Median	2.3	2.5	2.5	
IQR	2.0-3.4	2.0-3.0	2.0-3.0	
Lymph node status				.921
Negative	29 (58.0)	219 (61.9)	41 (59.4)	
Positive	19 (38.0)	135 (38.1)	28 (40.6)	
Unknown	2 (4.0)	0 (0)	0 (0)	
Tumor grade				.510
1-2	6 (12.0)	67 (18.9)	15 (21.7)	
>2	36 (72.0)	256 (72.3)	49 (71)	
Unknown	8 (16.0)	31 (8.8)	5 (7.2)	
Histology				.173
Invasive carcinoma of no special type	47 (94.0)	311 (87.9)	57 (82.6)	
Other	3 (6.0)	43 (12.1)	12 (17.4)	
Ki-67 index, %				<.001[a]
Median	60	60	37.5	
IQR	48.8-80.0	30.0-70.0	20.0-62.5	
Necrosis				.233
No	14 (28.0)	155 (43.8)	24 (34.8)	
Yes	17 (34.0)	105 (29.7)	29 (42)	
Unknown	19 (38.0)	94 (26.6)	16 (23.2)	
Fibrosis				.005
No	13 (26.0)	96 (27.1)	15 (21.7)	
Low (score 1-2)	15 (30.0)	136 (38.4)	21 (30.4)	
High (score 3)	3 (6.0)	28 (7.9)	17 (24.6)	
Unknown	19 (38.0)	94 (26.6)	17 (24.6)	
Chemotherapy				<.001
Yes	49 (98.0)	343 (96.9)	55 (79.7)	
No	0 (0.0)	2 (0.6)	8 (11.6)	
Unknown	1 (2.0)	9 (2.5)	6 (8.7)	
Radiotherapy				.015
No	27 (54.0)	249 (70.3)	54 (78.3)	
Yes	23 (46.0)	104 (29.4)	15 (21.7)	

Abbreviations: IQR, interquartile range; TNBC, triple-negative breast cancer.
[a]Indicates *P* value was determined using the Kruskal-Wallis test, whereas others were derived using the Pearson chi-square test or Fisher exact test (when the Pearson chi-square test was not applicable).

$q < 0.15$) (see Supporting Table 1). Gene sets involved in DNA repair (eg, KEGG homologous recombination), cell cycle (eg, Kalma E2F1 targets), and RNA metabolism (eg, reactome mRNA decay by 3′ to 5′ exoribonuclease) were repeatedly observed (Fig. 2C,D)[7] (see Supporting Table 1). In the elderly patients, 1360 gene sets were found to be significantly enriched (false discovery rate, $q < 0.15$) (see Supporting Table 2), most notably the steroid hormone–related gene sets (eg, Nelson response to androgen up) (Fig. 2C,D)[7] (see Supporting Table 2). A similar pattern could be observed in the METABRIC TNBC cases (see Supporting Tables 3 and 4).

Mutation Profile of Young and Elderly Patients With TNBC

Somatic mutations can play a crucial role in human cancers, whereas some also may act as biomarkers and therapeutic targets. In the current study, we observed that elderly patients with TNBC harbored significantly more nonsynonymous somatic mutations in *PIK3CA* (young vs intermediate vs elderly patients: 0.0% vs 17.4% vs 31.0% [$P = .004$]), *KMT2D* (4.0% vs 2.4% vs 11.9% [$P = .020$]), *ERBB2* (0.0% vs 0.9% vs 7.1% [$P = .037$]), *ERBB3* (0.0% vs 0.5% vs 7.1% [$P = .015$]), and *NCOR2* (0.0% vs 0.9% vs 7.1% [$P = .035$]), whereas young patients with TNBC were found to have more *COL3A1* mutations (young vs intermediate vs elderly patients: 12.0% vs 4.7% vs 0.0% [$P = .005$]) (Fig. 3A) (see Supporting Table 5). To obtain a more comprehensive view of the mutation profile, we studied the mutational profile by pathways and gene groups (Fig. 3B). Elderly patients with TNBC harbored significantly more nonsynonymous somatic mutations in PI3K-AKT-mTOR signaling (young vs intermediate vs elderly patients: 8.0% vs 29.2% vs 54.8% [$P < .001$]), lysine methyltransferase (16.0% vs 11.8% vs 28.6% [$P = .022$]), and Notch signaling (4.0% vs 9.4% vs 21.4% [$P = .048$]), and marginally more mutations in the RTK signaling family (0.0% vs

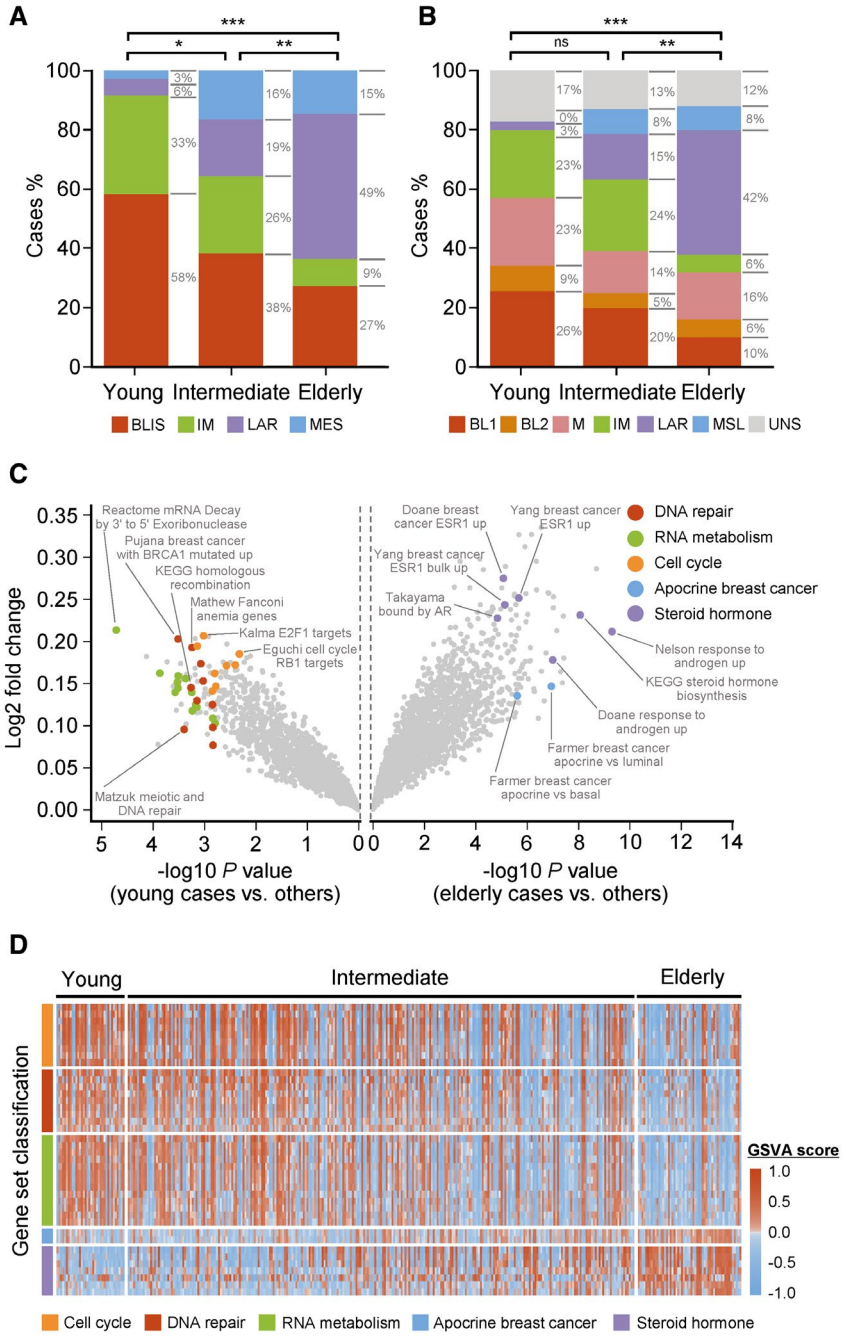

Figure 2. Transcriptomic characteristics of young and elderly patients with triple-negative breast cancer (TNBC). (A and B) TNBC subtypes in each age group. (A) The Fudan University Shanghai Cancer Center TNBC subtypes included: 1) luminal androgen receptor (LAR); 2) immunomodulatory (IM); 3) basal-like immune-suppressed (BLIS); and 4) mesenchymal (MES). (B) TNBC subtypes as defined by Lehmann et al[7] included: 1) LAR; 2) IM; 3) basal-like 1 (BL1); 4) basal-like 2 (BL2); 5) mesenchymal (M); 6) mesenchymal stem-like (MSL); and 7) an unstable group (UNS). (C) Enriched gene sets in young and elderly patients with TNBC. Gene sets that were upregulated in (*Left*) young TNBC patients and (*Right*) elderly TNBC patients were evaluated. The top 100 significant gene sets in each test were reviewed manually, in which recurrently altered gene set classifications were highlighted using different colors and representative gene sets were annotated further in the figure. (D) Heatmap according to gene set variation analysis (GSVA) scores of the gene sets highlighted in panel C. Pairwise comparisons regarding messenger RNA (mRNA) subtypes were performed using Bonferroni correction. *$P < .05$; **$P < .01$; ***$P < .001$; not significant (ns) $P > .05$.

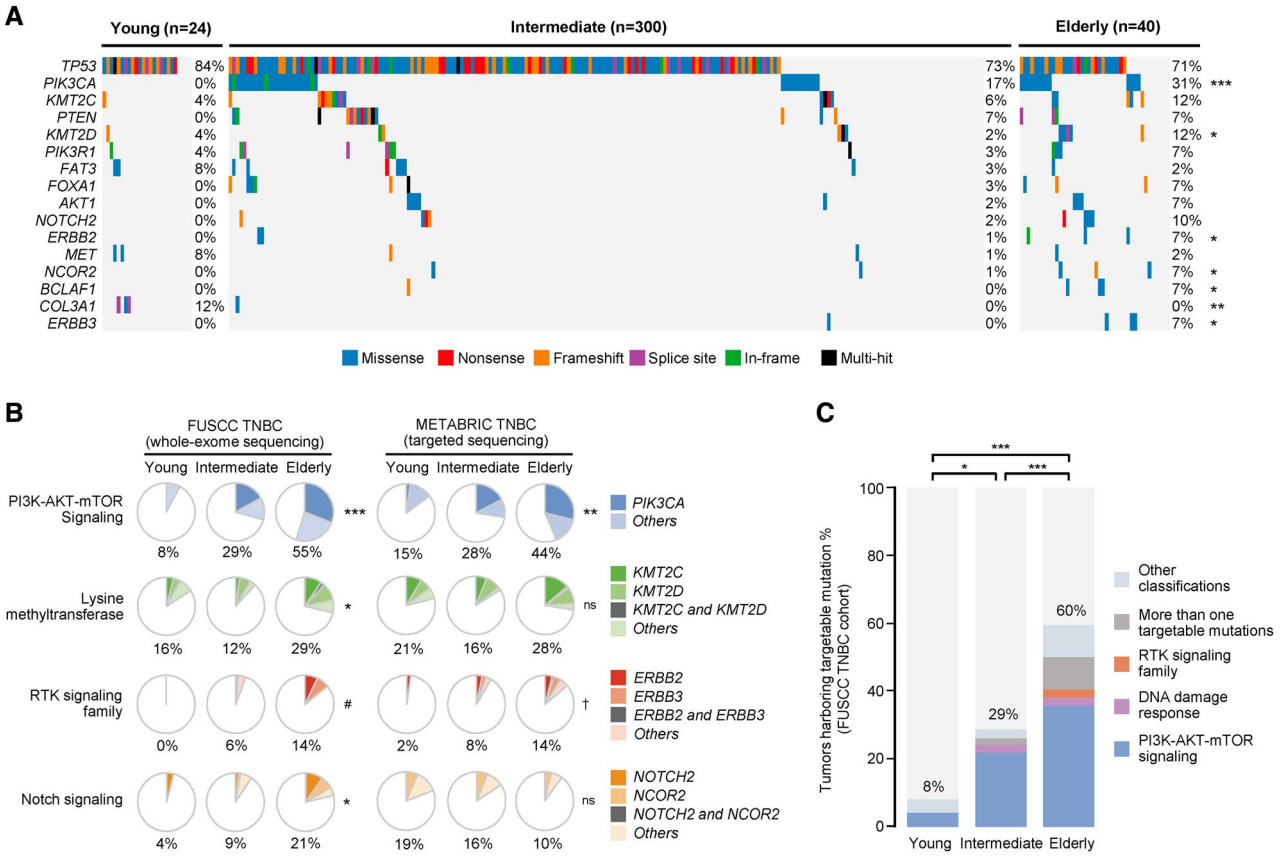

Figure 3. Somatic mutation profile in each age group of patients with triple-negative breast cancer (TNBC). (A) Somatic mutation profile of patients with TNBC diagnosed at different ages. Known cancer-related genes that were found to be mutated in at least 5% of patients in at least 1 age group were listed. (B) Differentially distributed mutation classifications among age groups in (*Left*) the Fudan University Shanghai Cancer Center (FUSCC) TNBC cohort and (*Right*) the Molecular Taxonomy of Breast Cancer International Consortium (METABRIC) cohort. (C) Actionable and/or potentially targetable somatic mutations among age groups. **P* < .05; ***P* < .01; ****P* < .001; # borderline with a *P* = .054; † borderline with a *P* = .071; not significant (ns) (*P* > .05). Pairwise comparisons regarding the percentage of cases with targetable mutations were performed using Bonferroni correction.

5.7% vs 14.3%). Similar trends could be observed in the METABRIC database (Fig. 3B).

To further investigate the potential impact of the somatic mutation profile on the clinical management of different TNBC age groups, we next focused on the targetable and/or potentially targetable mutations recorded in the OncoKB database. Elderly patients with TNBC more frequently were found to harbor at least 1 targetable and/or potentially targetable mutation (59.5% vs 28.8% vs 8% [*P* < .001]) (Fig. 3C).

Despite the above mentioned discrepancies regarding specific mutated genes, TNBCs occurring among patients of different age groups had comparable somatic mutation loads (median somatic mutation load: 82.0 vs 71.5 vs 73.5 [*P* = .668]) (see Supporting Table 6). To gain further insight into the underlying mechanism of mutation generation, we analyzed the mutation subtypes, which have been defined in our previous publication,[18] among different age groups. We found that patients with the homologous recombination deficiency (HRD) subtype were the predominant group among both the young and intermediate-aged patients with TNBC, but not in the elderly patients (HRD subtype percentage: 59.1% vs 49.7% vs 15.4%) (Fig. 4A). Conversely, the elderly patients with TNBC had a higher percentage of clock-like tumors (clock-like subtype percentage: 18.2% vs 20.1% vs 43.6%) (Fig. 4A). Investigation regarding specific mutational signatures validated these findings, in which elderly patients with TNBC demonstrated higher weights for clock-like signatures (median weight of signature 1 plus signature 5: 0.094 vs 0.118 vs 0.396; elderly vs young patients: *P* = .013 and elderly vs intermediate patients: *P* = .007) (Fig. 4B), and lower weights for HRD signatures (median weight of signature 3 plus signature 8:

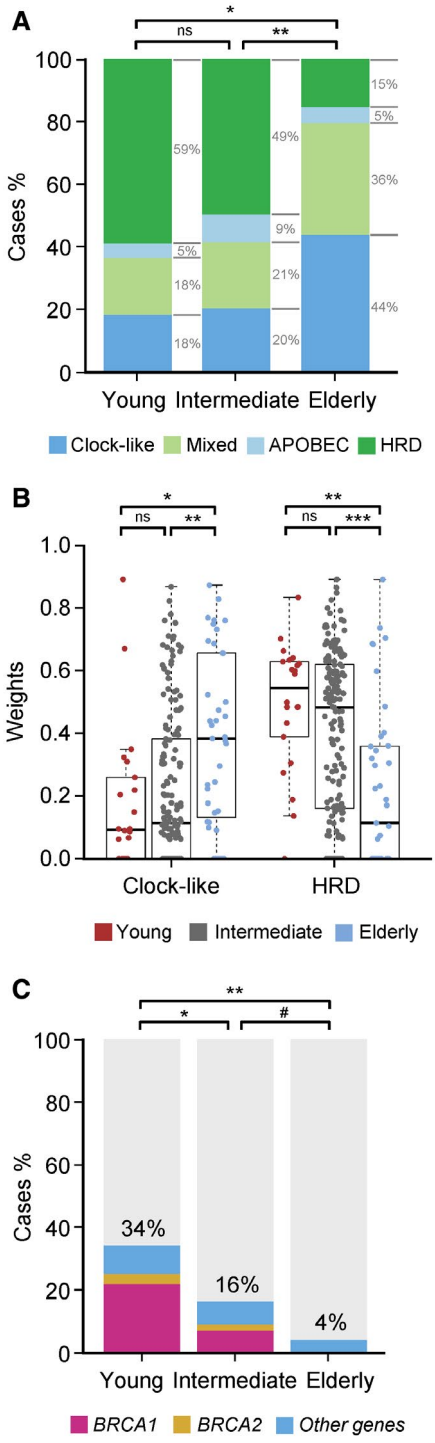

Figure 4. Somatic mutational signatures and pathogenic germline variants in each age group of patients with triple-negative breast cancer. (A) Mutational signature-based subgroups among different age groups. The upper part of the panel shows the somatic mutation load in each case denoted by bar plots; middle, known cancer-related genes mutated in at least 5% of patients in at least 1 age group; lower, weights of different mutational signatures. (B) Differentially distributed mutational signatures among age groups. (*Left*) Aging signature weights (Catalogue of Somatic Mutations in Cancer [COSMIC] mutational signature 1+ to COSMIC mutational signature 5). (*Right*) Homologous recombination deficiency (HRD) signature weights (COSMIC mutational signature 3+ to COSMIC mutational signature 8). (C) Pathogenic and/or likely pathogenic germline variants among different age groups. Pairwise comparisons were performed using either (A and C) Bonferroni correction or (B) the Nemenyi post hoc test. *$P < .05$; **$P < .01$; ***$P < .001$; † borderline with a P value between .05 and .07, not significant (ns) ($P > .05$). APOBEC, apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like.

0.556 vs 0.503 vs 0.118; elderly vs young: $P = .004$ and elderly vs intermediate: $P < .001$) (Fig. 4B).

Pathogenic germline variants in *BRCA1*, *BRCA2*, and other cancer susceptibility genes have been found to be related to the HRD subtype, and potentially indicate sensitivity to platinum-based chemotherapy and poly (adenosine diphosphate ribose) polymerase (PARP) inhibitors. We found that young patients with TNBC were more likely to harbor known pathogenic germline variants and likely pathogenic germline variants identified by Characterization of Germline Variants[27] (34.4% vs 15.8% vs 4.3%; young vs intermediate: $P = .031$ and young vs elderly: $P = .004$) (Fig. 4C). The *BRCA1* mutation demonstrated the most significant difference between the age groups (young vs intermediate vs elderly: 28.0% vs 7.6% vs 0.0% [$P = .002$]) (Fig. 4C), although the *RAD51D* mutation, which has been reported to be enriched in East Asian patients with breast cancer,[28] demonstrated a similar frequency among different age groups (3.2% vs 2.6% vs 2.2% [$P = .839$]). Furthermore, germline variants found in young patients were more likely to demonstrate biallelic inactivation in the corresponding tumor samples (see Supporting Table 7).

Somatic CNAs According to Patient Age

We first investigated the CNA loads between different age groups, and found that TNBCs among different age groups were similar with regard to the fraction of genome altered and CNA-derived HRD parameters, with the exception that young patients with TNBC had a higher level of large-scale state transitions compared with elderly patients with TNBC (adjusted $P = .043$) (see Supporting Fig. 2A-E). CNAs have been proven to have a significant impact on gene expression in patients with breast cancer. To search for more potential "driver" events in different age groups, we examined CNAs in TNBCs occurring among different age groups using focal amplification and deletion peaks identified by Geographic Information System (GIS) Technology Innovation Center (GISTIC).

With regard to amplifications, we observed that the Chr1p34 amplification was the only peak enriched in young patients with TNBC (26.6% vs 10.2% vs 16.1% [$P = .016$]) (see Supporting Fig. 2F) (see Supporting Table 8). The "peak" region of Chr1p34 contained 5 genes: *PTPRF*, *ST3GAL3*, *ARTN*, *KDM4A*, and *LOC100132774*, of which *ARTN* and *KDM4A* have been reported to be "drivers" in breast cancer.

Copy number deletion at Chr1p36, Chr4p14, Chr5q21, Chr10q23, and Chr15q13 was found to be significantly enriched in young patients ($P < .05$) (see Supporting Fig. 2F) (see Supporting Table 9). With the exception of Chr10q23, which had *PTEN* in the "peak" region, we noted that Chr15q13 loss or deletion might affect the *FAN1* gene in the "peak" region, which has been reported to be involved in DNA double-strand break repair. No GISTIC peak was found to be enriched in elderly patients with TNBC.

DISCUSSION

The results of the current study confirmed that TNBCs arising among young compared with elderly patients can be quite different entities. Young patients with TNBC were characterized by a higher incidence of early metastasis or disease recurrence, upregulated DNA repair, cell cycle and RNA metabolism gene sets, frequent pathogenic germline variants, a HRD-related mutational signature, and several focal CNAs. TNBCs in elderly patients were associated with the LAR subtype; severe fibrosis; a lower Ki-67 index; and somatic mutations in *PIK3CA, KMT2D, ERBB2*, *ERBB3,* and their corresponding pathways, as well as more frequent actionable mutations.

The prognostic effect of age at diagnosis in TNBC has been controversial. In the current study, a younger age at the time of diagnosis, although not necessarily leading to worse RFS in the long term, was significantly correlated with more early disease recurrence and metastasis in patients with TNBC. This might be explained by the upregulated cell cycle gene set analysis, because activated cell cycle and proliferation can give rise to the early recurrence of breast cancer.[29] We confirmed the relationship between unfavorable short-term prognosis and younger age in patients with TNBC using the SEER database. Furthermore, using this large cohort, we also found that both young and elderly patients with TNBC demonstrated inferior BCSS compared with intermediate-aged patients, and this result emphasized the significance of our efforts in trying to provide further insight into TNBC occurring in young and elderly patients. Other studies have found that when restricted to ER-negative or TNBC populations, elderly patients have an outcome that is much worse than that of others, which is different from our observation.[13] An important source of this discrepancy was that we only included patients aged <85 years. Our consideration was that the dismal BCSS of very old patients could have been caused by the underuse of curative treatment in these individuals,[30,31] which would not reliably reflect the biological behavior of the disease itself.

Although higher androgen receptor expression in postmenopausal women with TNBC has been reported in former studies, it still is impressive that nearly one-half of the elderly patients with TNBC in the current study were classified as having the LAR subtype. Studies have reported different response rates to neoadjuvant chemotherapy among patients with different TNBC subtypes, with lower response rates noted among those with the LAR subtype.[32] Furthermore, the Ki-67 index was found to be significantly lower in the elderly patient group.[33] It also has been reported that severe fibrosis in patients with breast cancer is associated with less response to chemotherapy, which might be the result of the poorer penetration of chemotherapeutic agents. All of these findings justified the more prudent use of chemotherapy in elderly patients with TNBC. Other therapeutic strategies, such as antiandrogen therapy targeting LAR tumors, should be considered in this age group.[34]

We further searched for potential drivers and therapeutic targets specific to elderly patients with TNBC using our genomic data. A significantly higher mutation prevalence of *PIK3CA*, *KMT2D*, *ERBB2*, and *ERBB3* was observed in elderly patients, and similar patterns could be observed in their corresponding pathways. It is important to note that approximately 59.5% of the elderly patients with TNBC harbored at least 1 actionable mutation. These results suggested that elderly patients with TNBC, rather than individuals of young or intermediate age, had a distinct pattern of oncogenic genomic alteration that can be easily captured by exome sequencing and even targeted sequencing. Taken together, although both clinical and molecular evidence demonstrated that elderly patients with TNBC might be less responsive to conventional chemotherapy, they might benefit from antiandrogen therapy and more personalized regimens based on actionable mutations.

Mutational signatures are genomic footprints that demonstrate the diversity of mutational progress, thereby suggesting cancer etiologies and underlying biological characteristics. In the current study, approximately 59%

of the young patients with TNBC were identified as having the HRD mutation subtype, whereas only 15% of the elderly patients with TNBC were classified into this group. Conversely, there was a preponderance of clock-like signatures among the elderly patients with TNBC. One interesting hypothesis is that young and elderly patients with TNBC might have experienced different evolutionary processes. Although mutations in elderly patients with TNBC may have undergone long-term selection and filtering, a significant number of the mutations observed in the young patients with TNBC simply might be the consequence of HRD. This hypothesis is consistent with the observation that TNBC among the elderly patients was associated with more known oncogenic mutations despite their similar mutation rate. Another interesting result is that in the current study cohort, approximately 34.4% of the young patients with TNBC had at least 1 pathogenic and/or likely pathogenic germline variant. Genetic counseling can be useful for young patients with TNBC rather than patients of other age groups. Pathogenic *BRCA1* and *BRCA2* variants have been related to the early onset of TNBC, as well as long-term sensitivity to platinum-based chemotherapy and PARP inhibitors.[35] The clinical significance of pathogenic variants in other genes and the HRD signatures deserves further study.

TNBC is a C-class tumor with prominent CNAs.[25,36] We observed several CNAs that were differentially distributed among age groups, including Chr1p34 amplification (*ARTN* and *KDM4A*) and Chr10q23 deletion (*PTEN*). We also identified that the Chr15q13 deletion, with *FAN1* in the peak, was enriched in young patients with TNBC. Because *FAN1* encodes an important DNA repair nuclease named FANCD2 and FANCI-associated nuclease 1, this recurrent alteration might have helped in promoting HRD in young patients with TNBC. It is interesting to note that although we have observed a clear association between age at diagnosis and HRD mutational signature in patients with TNBC, CNA-derived HRD parameters demonstrated little difference between these age groups. These "BRCAness" parameters should be used with caution until more definitive evidence has been obtained.

The current study has limitations. First, the age-specific clinical and molecular features found in this study were based on a retrospectively collected cohort. Second, although to our knowledge the current study cohort is the largest single-center, multiomic cohort of patients with TNBC presented to date, it was not large enough to achieve satisfying statistical power when investigating infrequent genomic events in specific age groups. Further validation in a prospective cohort with a larger sample size and higher statistical power will be useful. Specifically, these potential strategies require clinical validation before they can be used for the clinical management of patients with TNBC. Nevertheless, the results of the current study provide a clearer portrait of the heterogeneity of TNBC, and help to refine precision medicine for its treatment.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Conceptualization: **Ding Ma**, **Yi-Zhou Jiang**, and **Zhi-Ming Shao**. Methodology: **Ding Ma**, **Yi-Zhou Jiang**, **Yi Xiao**, and **Zhi-Ming Shao**. Data curation: **Ding Ma**, **Yi-Zhou Jiang**, **Yi Xiao**, **Meng-Dan Xie**, and **Shen Zhao**. Formal analysis: **Ding Ma**, **Yi-Zhou Jiang**, **Yi Xiao**, and **Meng-Dan Xie**. Writing-original draft: **Ding Ma**, **Yi-Zhou Jiang**, **Meng-Dan Xie**, and **Yi Xiao**. Writing-review and editing: **Ding Ma**, **Yi-Zhou Jiang**, **Yi Xiao**, **Meng-Dan Xie**, **Shen Zhao**, **Xi Jin**, **Xiao-En Xu**, and **Zhi-Ming Shao**. Project administration: **Ding Ma**, **Yi-Zhou Jiang**, **Yi Xiao**, and **Zhi-Ming Shao**. Supervision: **Yi-Zhou Jiang** and **Zhi-Ming Shao**.

REFERENCES

1. Gluz O, Liedtke C, Gottschalk N, Pusztai L, Nitz U, Harbeck N. Triple-negative breast cancer-current status and future directions. *Ann Oncol*. 2009;20:1913-1927.
2. Denkert C, Liedtke C, Tutt A, von Minckwitz G. Molecular alterations in triple-negative breast cancer-the road to new treatment strategies. *Lancet*. 2017;389:2430-2442.
3. Dent R, Trudeau M, Pritchard KI, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res*. 2007;13:4429-4434.
4. Foulkes WD, Smith IE, Reis JS. Triple-negative breast cancer. *N Engl J Med*. 2010;363:1938-1948.
5. Burstein MD, Tsimelzon A, Poage GM, et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. *Clin Cancer Res*. 2015;21:1688-1698.
6. Carey L, Winer E, Viale G, Cameron D, Gianni L. Triple-negative breast cancer: disease entity or title of convenience? *Nat Rev Clin Oncol*. 2010;7:683-692.
7. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest*. 2011;121:2750-2767.
8. Anders CK, Hsu DS, Broadwater G, et al. Young age at diagnosis correlates with worse prognosis and defines a subset of breast cancers with shared patterns of gene expression. *J Clin Oncol*. 2008;26:3324-3330.
9. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A. Breast cancer before age 40 years. *Semin Oncol*. 2009;36:237-249.
10. Fredholm H, Eaker S, Frisell J, Holmberg L, Fredriksson I, Lindman H. Breast cancer in young women: poor survival despite intensive treatment. *PLoS One*. 2009;4:e7695.

11. Azim HA Jr, Nguyen B, Brohee S, Zoppoli G, Sotiriou C. Genomic aberrations in young and elderly breast cancer patients. *BMC Med*. 2015;13:266.
12. Lian WB, Fu FM, Lin YX, et al. The impact of young age for prognosis by subtype in women with early breast cancer. *Sci Rep*. 2017;7:11625. doi:10.1038/s41598-017-10414-x
13. Freedman RA, Keating NL, Lin NU, et al. Breast cancer-specific survival by age: worse outcomes for the oldest patients. *Cancer*. 2018;124:2184-2191.
14. Radosa JC, Eaton A, Stempel M, et al. Evaluation of local and distant recurrence patterns in patients with triple-negative breast cancer according to age. *Ann Surg Oncol*. 2017;24:698-704.
15. Liedtke C, Hess KR, Karn T, et al. The prognostic impact of age in patients with triple-negative breast cancer. *Breast Cancer Res Treat*. 2013;138:591-599.
16. Partridge AH, Hughes ME, Warner ET, et al. Subtype-dependent relationship between young age at diagnosis and breast cancer survival. *J Clin Oncol*. 2016;34:3308-3314.
17. Syed BM, Green AR, Nolan CC, Morgan DAL, Ellis IO, Cheung KL. Biological characteristics and clinical outcome of triple negative primary breast cancer in older women-comparison with their younger counterparts. *PLoS One*. 2014;9:e100573.
18. Jiang YZ, Ma D, Suo C, et al. Genomic and transcriptomic landscape of triple-negative breast cancers: subtypes and treatment strategies. *Cancer Cell*. 2019;35:428-440.e5.
19. Xiao Y, Ma D, Zhao S, et al; AME Breast Cancer Collaborative Group. Multi-omics profiling reveals distinct microenvironment characterization and suggests immune escape mechanisms of triple-negative breast cancer. *Clin Cancer Res*. 2019;25:5002-5014.
20. Kan ZY, Ding Y, Kim J, et al. Multi-omics profiling of younger Asian breast cancers reveals distinctive molecular signatures. *Nat Commun*. 2018;9:1725. doi:10.1038/s41467-018-04129-4
21. Rottenberg Y, Naeim A, Uziely B, Peretz T, Jacobs JM. Breast cancer among older women: the influence of age and cancer stage on survival. *Arch Gerontol Geriatr*. 2018;76:60-64.
22. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Sci Signal*. 2013;6:pl1.
23. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2:401-404.
24. Pereira B, Chin SF, Rueda OM, et al. The somatic mutation profiles of 2,433 breast cancers refines their genomic and transcriptomic landscapes. *Nat Commun*. 2016;7:11479.
25. Curtis C, Shah SP, Chin SF, et al. The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. *Nature*. 2012;486:346-352.
26. Lobbezoo DJ, van Kampen RJ, Voogd AC, et al. Prognosis of metastatic breast cancer subtypes: the hormone receptor/HER2-positive subtype is associated with the most favorable outcome. *Breast Cancer Res Treat*. 2013;141:507-514.
27. Huang KL, Mashl RJ, Wu Y, et al. Pathogenic germline variants in 10,389 adult cancers. *Cell*. 2018;173:355-370.e14.
28. Chen X, Li Y, Ouyang T, et al. Associations between RAD51D germline mutations and breast cancer risk and survival in BRCA1/2-negative breast cancers. *Ann Oncol*. 2018;29:2046-2051.
29. Ribelles N, Perez-Villa L, Jerez JM, et al. Pattern of recurrence of early breast cancer is different according to intrinsic subtype and proliferation index. *Breast Cancer Res*. 2013;15:R98.
30. Freedman RA, Vaz-Luis I, Barry WT, et al. Patterns of chemotherapy, toxicity, and short-term outcomes for older women receiving adjuvant trastuzumab-based therapy. *Breast Cancer Res Treat*. 2014;145:491-501.
31. Schonberg MA, Marcantonio ER, Li DL, Silliman RA, Ngo L, McCarthy EP. Breast cancer among the oldest old: tumor characteristics, treatment choices, and survival. *J Clin Oncol*. 2010;28:2038-2045.
32. Masuda H, Baggerly KA, Wang Y, et al. Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. *Clin Cancer Res*. 2013;19:5533-5540.
33. Keam B, Im SA, Lee KH, et al. Ki-67 can be used for further classification of triple negative breast cancer into two subtypes with different response and prognosis. *Breast Cancer Res*. 2011;13:R22.
34. Parker JS, Peterson AC, Tudor IC, Hoffman J, Uppal H. A novel biomarker to predict sensitivity to enzalutamide (ENZA) in TNBC. *J Clin Oncol*. 2015;33S. doi:10.1200/jco.2015.33.15_suppl.1083
35. Robson M, Im SA, Senkus E, et al. Olaparib for metastatic breast cancer in patients with a germline BRCA mutation. *N Engl J Med*. 2017;377:523-533.
36. Ciriello G, Miller ML, Aksoy BA, Senbabaoglu Y, Schultz N, Sander C. Emerging landscape of oncogenic signatures across human cancers. *Nat Genet*. 2013;45:1127-1133.