npj | breast cancer

Article

Published in partnership with the Breast Cancer Research Foundation

https://doi.org/10.1038/s41523-025-00781-4

Genomic landscape of breast cancer in elderly patients

Pier Selenica[1,9], Higinio Dopeso[1,9], Matteo Repetto[1,2,9], Thais Basili[1], Andrea M. Gazzo, Christopher J. Schwartz[1], Fatemeh Derakhshan[1,3], Antonio Marra[1,4], Lorenzo Ferrando[1,5], Regina G. H. Beets-Tan[6], Y. H. Wen[1], Dara S. Ross[1], Edi Brogi[1], Hong Zhang[1], Larry Norton[7], Sarat Chandarlapaty[7], Pedram Razavi[7], Diana Mandelker[1], Jorge S. Reis-Filho[1,8], Britta Weigelt[1] ✉ & Fresia Pareja[1] ✉

Breast cancer (BC) displays age-related histopathologic and transcriptomic heterogeneity. Whether BC in elderly patients differs genetically from that of younger individuals remains unclear. We re-analyzed sequencing data from 1918 BCs previously subjected to an FDA-cleared paired tumor-normal targeted sequencing assay across elderly (≥65 years), middle-aged (>45 and <65 years) and young (≥45 years) patients. BCs in elderly individuals exhibited fewer germline but were numerically enriched in somatic homologous recombination deficiency (HRD)/DNA damage response (DDR) genetic alterations. Primary ER+/HER2- BC in elderly patients showed shifts in the spectrum of actionable PI3K/AKT alterations, whereas metastatic cases were enriched in *FAT1* and *RB1* mutations and fewer *ESR1* mutations, suggesting age-dependent therapeutic resistance mechanisms. Metastatic ER+/HER2- lobular BCs were enriched in actionable *ERBB2* mutations. Resistance-associated alterations were more prevalent in metastatic vs primary BC in elderly patients. Our findings reveal distinct actionable genetic features in elderly patients, highlighting the importance of genomic profiling and treatment personalization in this population.

Breast cancer (BC) is vastly heterogeneous in its histopathologic and genomic features, clinical outcomes and responses to therapy[1–3]. There is growing evidence to suggest that the biology, pathogenesis and genetic landscape of BC might vary according to age[4]. Compared to BCs in elderly individuals, BCs in younger patients have been found to be enriched for the basal-like molecular subtype[5,6]. Moreover, responses to chemotherapy markedly vary by age group, as reported in randomized clinical trials[7].

For instance, patients with early-stage hormone receptor-positive/HER2-negative (HR+/HER2-) BC younger than 50 years old seem to derive greater benefit from the addition of adjuvant chemotherapy to endocrine therapy compared to their older counterparts, based on TAILORx and RxPONDER trial results[8,9].

Whether the repertoire of genetic alterations affecting cancer genes in BC differs between elderly and younger patients remains to be fully elucidated. Previous work indicates that BCs occurring in older patients harbor a higher number of copy number alterations (CNAs), including 18p and 6q27 losses, a higher tumor mutational burden (TMB), and are enriched for *KMT2D* and *FOXA1* somatic mutations, compared to younger patients[10]. Even though the frequency of *BRCA1* and *BRCA2* germline mutations in triple-negative BCs in patients over 65 years old is not negligible[11], it is lower than that observed in BCs occurring in younger individuals[12], that are enriched for genetic alterations affecting cancer predisposition genes[13].

Here, we sought to determine the spectrum of genetic alterations in cancer-related genes and mutational signatures in BC in elderly individuals (≥65), compared to middle-aged (>45 and <65) and young (≤45) patients. Age thresholds of 45 and 65 years reflect established epidemiologic and policy frameworks[14–16] and have been previously associated with distinct

[1]Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [2]Department of Medicine, Early Drug Development Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [3]Department of Pathology and Cell Biology, Columbia University, New York, NY, USA. [4]Division of Early Drug Development for Innovative Therapies, European Institute of Oncology IRCCS, Milan, Italy. [5]Department of Internal Medicine and Medical Specialties (DiMI), University of Genoa, Genoa, Italy. [6]GROW School for Oncology and Developmental Biology, University of Maastricht, Maastricht, The Netherlands. [7]Department of Medicine, Breast Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [8]Present address: AstraZeneca, Gaithersburg, MD, USA. [9]These authors contributed equally: Pier Selenica, Higinio Dopeso, Matteo Repetto.
✉e-mail: weigeltb@mskcc.org; parejaf@mskcc.org

tumor biology[17,18]. Furthermore, we aimed to define the genomic drivers of progression in elderly patients through a comparison of the genetic alterations affecting metastatic and primary BC samples in the elderly population. To this aim, we performed a re-analysis of a large series of BC previously subjected to targeted sequencing using an FDA-authorized multigene sequencing assay, and investigated their mutational signatures utilizing methods applicable to formalin-fixed paraffin-embedded (FFPE) samples profiled with targeted capture sequencing.

Results

Clinicopathologic characteristics of breast cancer differ according to age

We reanalyzed targeted sequencing data from 1918 BC samples, including 918 primary and 1000 metastatic BC samples, previously subjected to the Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT)[19], a paired tumor-normal targeted sequencing assay including up to 468 cancer genes. In this cohort, 318 patients were classified as elderly (≥ 65 years), 1009 as middle-aged (>45 and <65 years) and 591 as young (≤45 years).

The distribution across histologic subtypes was found to be different in primary BCs in elderly individuals compared to young patients ($P < 0.05$), with a higher proportion of invasive lobular carcinoma (ILC) in the elderly group (39/220, 18%), compared to the young subgroup (21/234, 9%) as shown in previous studies[20]. These findings are depicted in Supplementary Fig. S1, which displays the clinicopathologic characteristics according to age. Differences were also detected in the histologic grade, with BCs in elderly patients being of lower grade than those in young individuals ($P < 0.001$; Supplementary Fig. S1a). In primary BCs, the distribution of estrogen receptor (ER)/HER2 status was found to be distinct in the elderly population compared to middle-aged ($P < 0.05$) and young ($P < 0.05$) patients, with a higher prevalence of ER+/HER2- primary BCs in elderly patients (81%) than in the young group (73%), and a lower proportion of HER2+ cases in elderly individuals (6%) compared to young (14%) and middle-aged (9%) patients; (Supplementary Fig. S1a). Elderly patients with primary BC presented with a lower TNM stage at diagnosis compared to middle-aged ($P < 0.01$) and young patients ($P < 0.001$; Supplementary Fig. S1a), possibly reflecting earlier detection in this age group. Post-neoadjuvant therapy primary BC samples were less frequent in elderly (3.2%) vs young patients (10.7%; $P < 0.01$) and similar to middle-aged patients (5.4%; $P > 0.05$).

In metastatic BCs, we observed differences in histologic subtype between elderly and young patients ($P < 0.001$), with an enrichment in ILC in the elderly group (28% vs 10%; Supplementary Fig. 1b). No statistically significant differences in histologic grade/differentiation or ER/HER2 status were observed in the metastatic cohort (Supplementary Fig. 1b). We also found that elderly patients with metastatic BC more often presented with higher TNM stage at diagnosis ($P < 0.01$, vs middle-aged; $P < 0.001$, vs young; Supplementary Fig. 1b), possibly reflecting selection bias for advanced cases undergoing biopsy. In line with these findings, we observed a higher proportion of de novo metastatic disease in elderly (34.7%) vs middle-aged (21.7%; $P < 0.01$) and young patients (16.2%; $P < 0.001$), which may explain, at least in part, the TNM stage differences. Moreover, metastatic BC samples from elderly patients (72%) were more frequently collected prior to systemic therapy for metastatic disease compared to those from young patients (58%; $P < 0.05$), and at a comparable rate to those from middle-aged patients (66%; $P > 0.05$). Among pre-treated cases, samples from elderly patients were obtained after fewer lines of therapy compared to young patients (median 2, range 1–7 vs median 3 range, 1–4 lines; $P < 0.01$), with no differences compared to middle-aged patients (median 3, range 1–12; $P > 0.05$).

We next sought to determine the repertoire of somatic genetic alterations and mutational signatures in primary and metastatic BC in elderly patients, compared to middle-aged and young patients. To account for the molecular heterogeneity of BCs based on ER/HER2 status and histologic subtype[21–23], we conducted the subset reanalysis of genetic alterations and mutational signatures of the ER+/HER2- BC cohort, which provided a sufficiently large sample size to draw robust conclusions ($n = 1501$). We separately analyzed primary ($n = 739$) and metastatic ($n = 762$) ER+/HER2- BCs from elderly, middle-aged and young patients, and subsequently according to histologic subtype. Analyses of the ER-/HER2- and HER2+ cohorts were not performed given the limited number of elderly patients in these groups, that would preclude meaningful conclusions to be drawn.

Enrichment in *PIK3CA* and *CDH1* mutations and lower genomic instability in primary ER+/HER2- breast cancer in elderly patients

We conducted a comparative analysis in primary ER+/HER2- BC by age group. No statistically significant differences were observed in the histologic subtype distribution in elderly patients compared to the other two age groups among primary ER+/HER2- BCs (Fig. 1a and Supplementary Table S1), with most cases being invasive ductal carcinoma of no special type (IDC-NST) followed by ILC in elderly (74% and 20%), middle-aged (74% and 19%) and young (82% and 12%) individuals; (Fig. 1a and Supplementary Table S1).

In primary ER+/HER2- BCs from elderly ($n = 179$), middle-aged ($n = 390$) and young ($n = 170$) patients *PIK3CA* (52%, 43% and 35%, respectively) was the most frequently mutated gene across all groups, followed by *CDH1* (22%), *MAP3K1* (16%), *TP53* and *GATA3* (15%, each) in elderly patients (Fig. 1b). We observed a shift in the spectrum of PI3K/AKT/mTOR pathway genetic alterations according to age. *AKT1* mutations were less frequent in elderly individuals (1%) compared to middle-aged (7%: $P < 0.01$) and young (6%: $P < 0.01$), while *PIK3CA* mutations were (52%) were more frequent in elderly vs young patients (35%; $P < 0.01$; Fig. 1b–d). Notably, *AKT1* mutations were mutually exclusive (CoMet P = 0.031) with *PIK3CA* mutations in the whole cohort (Fig. 1b). In addition, mutations in *CDH1* (22% vs 10%; $P < 0.01$) and *TBX3* (8% vs 3%; $P < 0.05$), both characteristic of ILC[24–26], were enriched in elderly compared to young and middle-aged patients, respectively (Fig. 1b–d). This likely reflects the numerically higher proportion of ILC cases in elderly patients (20%) compared to young patients (12%; Fig. 1a). Other genes more frequently altered in primary ER+/HER2- BC in elderly patients were the chromatin remodelers *KMT2C* (3% vs 0%; $P < 0.05$) compared to young patients, and *KMT2B* (3% vs 0.3%; $P < 0.05$) compared to middle-aged patients (Fig. 1b–d). Conversely, *TP53* mutations were less frequent in elderly patients, compared to young (24%; $P < 0.05$) and middle-aged individuals (24%; $P < 0.05$; Fig. 1b–d), in line with their lower histologic grade (Fig. 1a and Supplementary Table S1).

The tumor mutation burden (TMB) of ER+/HER2- primary BC was comparable across age groups (Fig. 1e). No differences in the frequency of specific CNAs were observed as depicted in Supplementary Fig. S2a, that displays comparisons of CNAs across age groups. Nonetheless, the fraction of genome altered (FGA) was significantly lower in BCs in elderly (median, 0.12; range, 0–0.72) compared to young patients (median, 0.17; range, 0–0.85; $P < 0.05$; Fig. 1f). These findings indicate lower genome-wide genomic instability in elderly patients, in line with their lower frequency of rate of *TP53* mutations, a key driver of genetic instability in BC.

To assess age related differences in mutational processes, we inferred mutational signatures using Signature Multivariate Analysis (SigMA)[27], a bioinformatics tool optimized for targeted capture sequencing data from FFPE samples, in cases with ≥5 somatic single base substitutions (SBS), a threshold previously validated for MSK-IMPACT data by our group[28,29]. This resulted in a dataset of 79, 170 and 71 ER+/HER2- primary BCs from elderly, middle-age and young patients, respectively. BCs in elderly individuals showed a numerically higher frequency of aging/clock-like signature (49%) and numerically lower APOBEC signature (23%), compared to middle-aged (aging, 45%; APOBEC, 32%; $P > 0.05$) and young (aging, 41%; APOBEC, 38%; $P > 0.05$) patients (Fig. 1g). The lack of statistical significance observed could be due to the limited number of cases with ≥5 SBS per group (Fig. 1g).

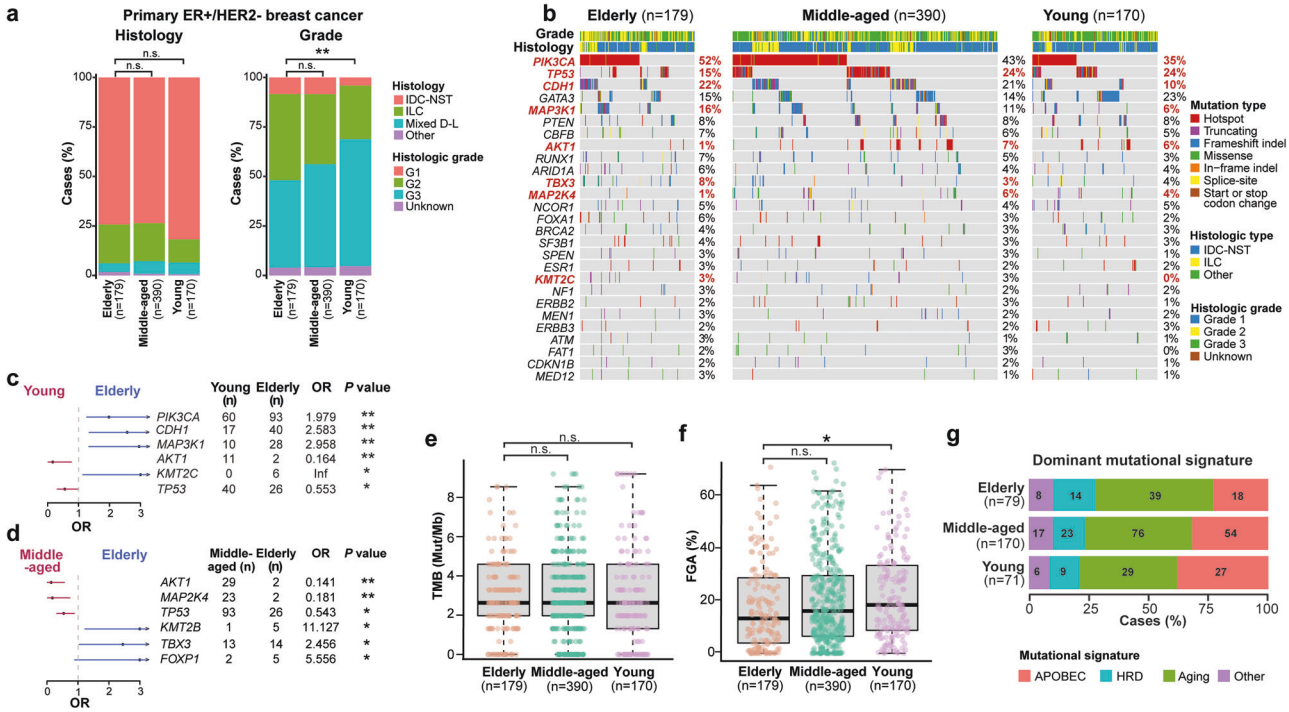

Fig. 1 | Clinicopathologic characteristics and repertoire of somatic genetic alterations in ER-positive/HER2-negative primary breast cancer in elderly patients. a Histologic subtype and histologic grade of primary ER+/HER2- breast cancer (BC) in elderly ($n = 179$), middle-aged ($n = 390$) and young ($n = 170$) patients. **b** Recurrent somatic genetic alterations in primary ER+/HER2- BC in elderly, middle-age, and young patients. Cases are shown in columns and genes in rows. Histological subtype, grade are shown in phenobars (top). Genetic alterations are color-coded according to the legend. Forest plots showing odds ratio (OR) of cancer genes altered at statistically significantly different rates in elderly individuals compared to young patients (**c**), and compared to middle-aged patients (**d**). Tumor mutational burden (TMB; **e**) and fraction of genome altered (FGA; **f**) of primary ER+/HER2- BC in elderly, middle-age, and young patients. **g** Dominant mutational signatures in primary ER+/HER2- BC in elderly, middle-age, and young patients. n.s., non-significant; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; Mann-Whitney *U* test and Fisher's exact test. IDC-NST invasive ductal carcinoma of no special type, HRD homologous recombination deficiency, ILC invasive lobular carcinoma, mixed D-L mixed ductal-lobular.

Enrichment of ILC-associated alterations in elderly patients and age-related shifts in resistance alterations in metastatic ER+/HER2- breast cancer

Our analyses in the metastatic ER+/HER2- cohort revealed the histologic subtype distribution to be different between elderly and young BC patients ($P < 0.001$) with a higher frequency of ILC and mixed ductal-lobular cases in elderly (31% and 10%), compared to middle-aged (22% and 5%) and young (12% and 3%) individuals (Fig. 2a and Supplementary Table S1). No differences in histologic grade/differentiation were observed (Fig.2a and Supplementary Table S1), In addition, the metastatic site distribution differed according to age. Elderly individuals had fewer metastases to the liver and ovary ($P < 0.05$, each) and more chest wall metastases ($P < 0.01$) sequenced, compared to young patients. Chest wall metastases were also more frequent in elderly vs middle-aged patients ($P < 0.01$; Supplementary Fig. S3a). Of note, prior exposure to CDK4/6 or mTOR inhibitors was similar across age groups, suggesting that these treatments are unlikely to confound the rates of resistance associated genetic alterations.

Analysis of metastatic ER+/HER2- BC in elderly ($n = 81$), middle-aged ($n = 408$) and young individuals ($n = 273$) revealed a higher frequency of mutations in *CDH1* in elderly (27%), compared to young patients (8%; $P < 0.001$; Fig. 2b, c). Moreover, in elderly patients, mutations in ILC associated genes were more frequent, including *ERBB2* (15%) and the transcription factors *NCOR1* (15%), *RUNX1* (6%) and *FOXA1* (10%), compared to young (*ERBB2*, 4%, $P < 0.01$; *NCOR1*, 3%, $P < 0.001$; *RUNX1*, 2%, $P < 0.01$) and middle-aged patients (*ERBB2*, 7%, $P < 0.05$; *NCOR1*, 4%, $P < 0.01$; *FOXA1*, 4%, $P < 0.05$; Fig. 2b–d). Accordingly, *GATA3* mutations, reported to have a lower prevalence in ILC[24] were less frequent in elderly vs young patients (12% vs 24%; $P < 0.05$; Fig. 2b, c), These findings likely reflect the enrichment of ILC observed in elderly compared to young individuals (Fig. 2a).

Metastatic ER+/HER2- BCs in elderly patients had a higher frequency of alterations linked to resistance to CDK4/6 inhibitors[30,31], such as in *FAT1* (11%) compared to young patients (4%; $P < 0.05$), and in *RB1* (8%) compared to young (4%; $P < 0.05$) and middle-aged (3%; $P < 0.05$) patients (Fig. 2b–d). Notably, *ESR1* mutations (10% vs 20%; $P < 0.05$), associated with resistance to endocrine therapy[32], were less common in elderly compared to young patients (Fig. 2b–d). Given that *ESR1* mutations have been reported to vary according to metastatic site[33], we further examined their distribution in liver metastasis, which were less frequent in elderly patients. None of the ER+/HER2- liver metastasis from elderly individuals (0%; 0/14) harbored *ESR1* mutations compared to 35% (30/85; $P < 0.05$) in middle-aged, and 28% (31/110; $P < 0.01$) in young patients (Supplementary Fig. S3b), suggesting that the differences observed cannot be explained solely by metastatic site distribution. These findings suggest age-related differences in therapeutic resistance mechanisms.

The TMB of metastatic ER+/HER2- BCs was higher in elderly (median 3.9, range 0–41) than in young patients (median, 3.9; range, 0–56; $P < 0.05$; Fig. 2e), but high TMB (≥ 10 mutations/Mb), an actionable biomarker for anti-PD1 immunotherapy benefit[34], was uncommon (Fig. 2e). FGA was lower in elderly patients (median 18%, range 0–100%) than in young individuals (median 24%, range 0–100%; $P < 0.05$; Fig. 2f), with no differences in the frequency of specific CNAs (Supplementary Fig. S2b).

Mutational signatures were inferred in 56, 261 and 165 metastatic ER+/HER2- BC samples with ≥ 5 somatic mutations in elderly, middle-aged and young patients, respectively. APOBEC mutational signature, linked to endocrine resistance[29,35,36], was the most prevalent in all groups (elderly, 38%; middle-aged, 41%; young, 35%), with no differences according to age (Fig. 2g).

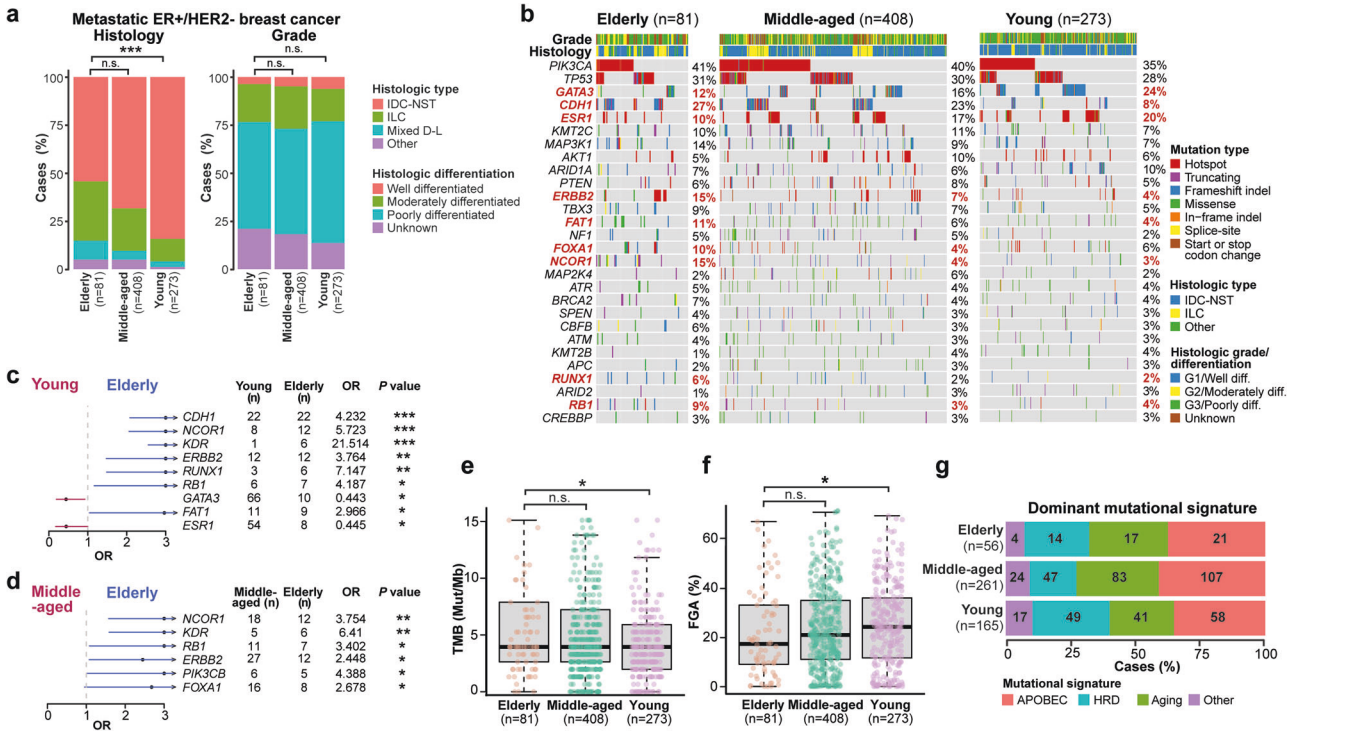

Fig. 2 | Clinicopathologic characteristics and repertoire of somatic genetic alterations in ER-positive/HER2-negative metastatic breast cancer in elderly patients. a Histologic subtype and histologic differentiation of metastatic ER+/HER2- breast cancer (BC) in elderly ($n = 81$), middle-aged ($n = 408$) and young ($n = 273$) patients. **b** Recurrent somatic genetic alterations in metastatic ER+/HER2- BC in elderly, middle-age, and young patients. Cases are shown in columns and genes in rows. Histological subtype and histologic differentiation are shown in phenobars (top). Genetic alterations are color-coded according to the legend. Forest plots showing odds ratio (OR) of cancer genes altered at statistically significantly different rates in elderly individuals compared to young patients (**c**), and compared to middle-aged patients (**d**). Tumor mutational burden (TMB; **e**) and fraction of genome altered (FGA; **f**) of metastatic ER+/HER2- BC in elderly, middle-age, and young patients. **g** Dominant mutational signatures in metastatic ER+/HER2- BC in elderly, middle-age, and young patients. n.s. non-significant; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; Mann-Whitney *U* test and Fisher's exact test. IDC-NST invasive ductal carcinoma of no special type, HRD homologous recombination deficiency, ILC invasive lobular carcinoma, mixed D-L mixed ductal-lobular.

Age related differences in PI3K/AKT/mTOR and MAPK/ERK pathways in invasive ductal carcinoma of no special type

We evaluated somatic genetic alterations in primary and metastatic ER+/HER2- BC by histologic subtype, conducting a separate analysis for IDC-NST and for ILC.

PIK3CA was the most frequently mutated gene in primary ER+/HER2- IDC-NST across all age groups (elderly, 47%; middle-aged, 41%; and young, 30%; Fig. 3a), which a higher mutational frequency in elderly than in young individuals (47% vs 30%; $P < 0.01$). Conversely, *AKT1* mutations were less frequent in elderly compared to middle-aged patients (2% vs 6%; $P < 0.05$; Fig. 3a–c). *MAP3K1* mutations were more prevalent in elderly vs young patients (17% vs 5%; $P < 0.01$), while *MAP2K4* mutations were less common than in middle-aged individuals (2% vs 5%; $P < 0.05$; Fig. 3a–c). These findings highlight age-related differences in targetable genetic alterations in PI3K/AKT/mTOR and MAPK/ERK pathways. No differences in TMB or FGA were observed according to age (Supplementary Fig. S4a, b). Although not statistically significant, we observed a higher proportion of cases with a dominant aging signature in elderly patients (49%), compared to middle-aged (45%) and young (41%) patients (Fig. 3d).

Comparisons of metastatic ER+/HER2- IDC-NST according to age were conducted and the findings depicted in Supplementary Fig. S5. In this subset, *PIK3CA*, *TP53*, and *GATA3* were the most frequently mutated genes and *ESR1* was frequently altered in all age groups (Supplementary Fig. S5a). We observed a higher frequency of *FAT1* alterations in elderly (16%) compared to middle-aged (4%, $P < 0.01$) and young patients (4%, $P < 0.01$; Supplementary Fig. 5a). Elderly patients had lower FGA as compared to young patients ($P < 0.05$), with no differences were observed in TMB or mutational signatures (Supplementary Fig. S5b–d).

Enrichment of *ERBB2* mutations in metastatic ER+/HER2- lobular breast cancer in elderly patients

Comparisons of primary ER+/HER2- ILC according to age were conducted and are shown in Supplementary Fig. S6. In primary ER+/HER2- ILC, as expected, *CDH1* and *PIK3CA* were the most frequently altered genes across all age groups. Other frequent alterations affected ILC enriched genes, such as *TBX3* and *PTEN* (Supplementary Fig. S6a). No significant differences were observed in mutational frequency, TMB, FGA and mutational signatures were identified between elderly and younger patients (Supplementary Fig. S6b–d).

Analysis of metastatic ER+/HER2- ILC in elderly ($n = 25$), middle-aged ($n = 90$) and young ($n = 32$) individuals showed *CDH1* as the most frequently altered gene in all groups (68%, 86% and 63%, respectively), with *PIK3CA*, *TP53* and *ESR1* also frequently mutated (Fig. 3e). ILC-related genes, such as *TBX3* (12%, 11% and 22%), *NCOR1* (20%, 8% and 3%) and *FOXA1* (12%, 8% and 9%) were frequently altered in all three groups (Fig. 3e). Notably, elderly patients had a significantly higher frequency of *ERBB2* activating mutations (44%) compared to middle-aged (8%; $P < 0.01$) and young patients (9%; $P < 0.001$; Fig. 3e–g). No significant differences were observed in TMB, FGA or mutational signatures (Fig. 3h and Supplementary Fig. S4c, d).

Seventy-two percent of *ERBB2* mutations identified across all age groups were oncogenic hotspot mutations (72%, 18/25 Fig. 3e). In elderly patients, most affected the L755 (33%, 4/12) and S310 (25%, 3/12) hotspot loci, both causing dysregulation of HER2 kinase activity[37–41] and classified as clinically actionable (OncoKB actionability level 3A and an ESCAT level IIb[42]). In contrast, only two out of six *ERBB2* mutations in young patients were oncogenic, which corresponded to Y772-A775 Exon 20

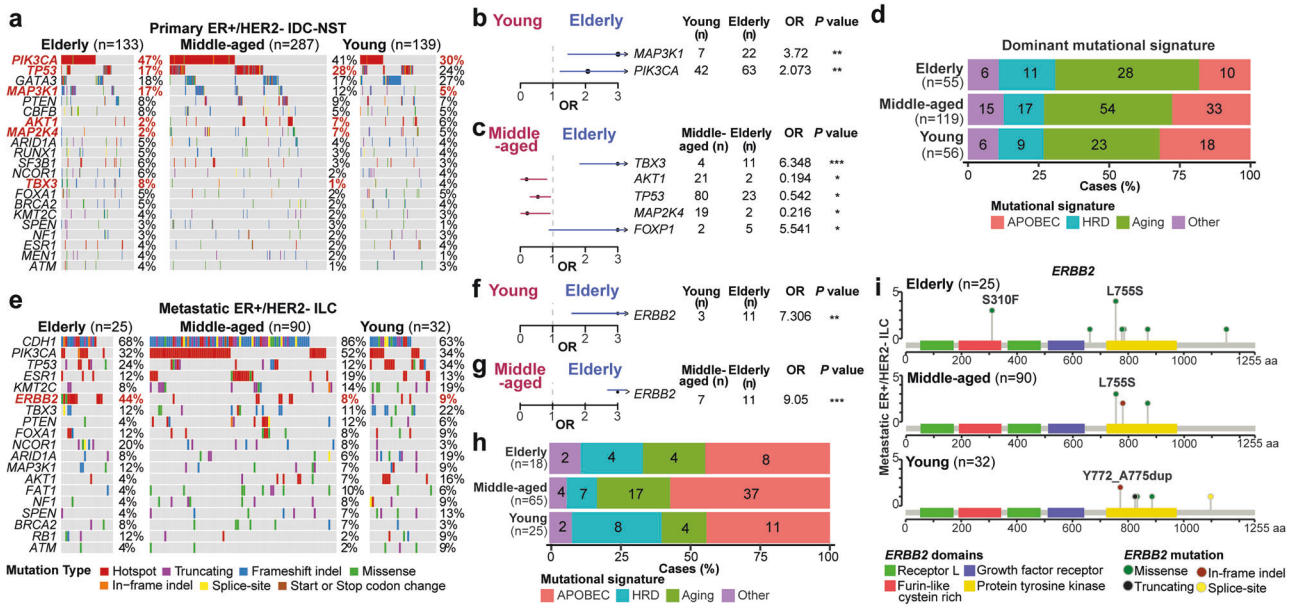

Fig. 3 | Repertoire of somatic genetic alterations in ER-positive/HER2-negative primary ductal and metastatic lobular breast cancer in elderly patients. Recurrent somatic genetic alterations in primary ER+/HER2- invasive ductal carcinoma of no special type (IDC-NST; **a**) and in metastatic lobular breast cancer (ILC; **e**) in elderly (primary IDC-NST, $n = 133$; metastatic ILC, $n = 25$), middle-aged (primary IDC-NST, $n = 287$; metastatic ILC, $n = 90$), and young (primary IDC-NST, $n = 139$; metastatic ILC, $n = 32$) patients. Cases are shown in columns and genes in rows. Genetic alterations are color coded according to legend. Forest plots depicting odds ratio (OR) of cancer genes altered at statistically significantly different rates between young and elderly (**b**, **f**) and middle-aged and elderly patients (**c**, **g**). Dominant mutational signatures in primary IDC-NST (**d**) and metastatic ILC (**h**) ER+/HER2- BC in elderly, middle-age, and young patients. (**i**) Lollipop plot of *ERBB2* mutations in metastatic ER+/HER2- ILC in elderly, middle-aged, and young patients. *ERBB2* domains and mutations are color coded according to the legend. n.s., non-significant; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; Mann-Whitney *U* test and Fisher's exact test. IDC-NST invasive ductal carcinoma of no special type, HRD homologous recombination deficiency, ILC invasive lobular carcinoma, mixed D-L mixed ductal-lobular.

duplications[43,44], that have OncoKB 3A and ESCAT IIb actionability levels[38,42].

APOBEC mutagenesis and resistance alterations in progression of ER+/HER2- breast cancer in the elderly

To identify genetic alterations involved in BC progression, we compared primary ($n = 179$) and metastatic ($n = 81$) ER+/HER2- BCs in elderly patients. Metastatic cases were enriched for genetic alterations linked to endocrine resistance, such as *ESR1* (10% vs 3%; $P < 0.05$) and *ERBB2* (15% vs 2%; $P < 0.001$) and CDK4/6 inhibitor resistance, such as *FAT1* (11% vs 2%, $P < 0.01$) and *RB1* (9% vs 2%, $P < 0.05$; Fig. 4a). This was in line with an enrichment of dominant aging signature and a numerically higher frequency of APOBEC-dominant cases in metastatic vs primary ER+/HER2- BC in the elderly cohort (Fig. 4b), consistent with the association of APOBEC mutagenesis with endocrine resistance[29,35,36]. In agreement with these findings, evaluation of the individual mutational signature exposures revealed a higher APOBEC mutagenesis contribution in metastatic compared to primary cases ($P < 0.05$), and a trend toward lower aging signature exposure in metastases vs primary cases (Fig. 4c, d). Both TMB (median, 5.6; range 0–24) and FGA (median, 19%; range 0–78%) in metastatic ER+/HER2- BC of elderly patients were higher than in primary BC samples (TMB, median 3, range 0–19, $P < 0.001$; FGA, median 12%, range 0–72%, $P < 0.01$; Fig 4e, f). These findings suggest that APOBEC mutagenesis, linked to high TMB and genomic instability, might play key roles in ER+/HER2- BC progression of in the elderly population.

Germline and somatic homologous recombination deficiency and DNA damage response in breast cancer in the elderly patients

Lastly, we assessed the frequency of pathogenic germline genetic alterations affecting homologous recombination-deficiency (HRD) and DNA damage response (DDR) genes, including *BRCA1*, *BRCA2*, *ATM*, *CHEK2, PALB2, RAD51B, BARD1, BRIP1, RAD51C* and *RAD51D* in the whole cohort, regardless of ER/HER2 status, comparing elderly patients to younger individuals. We observed that BCs in elderly patients harbored a numerically lower frequency of germline genetic alterations in HRD/DDR genes compared to BCs in young patients (3.9% vs 13.2%; $P > 0.05$; Fig. 5a). Conversely, BC in elderly patients had a numerically higher frequency of somatic alterations in HRD/DDR genes (4.7%) compared to middle-aged patients (3.1%; $P > 0.05$) and to young patients (2.9%; $P > 0.05$; Fig. 5a). The gene most frequently affected by pathogenic somatic alterations in the elderly was *BRCA2* (2.2%), while *ATM* was the most frequent somatically altered HRD/DDR gene in middle-aged (0.9%) and young patients (1.2%; Fig. 5a). Notably, our analyses revealed that, although not statistically significant, a lower proportion of genetic alterations in HRD/DDR genes were bi-allelic in elderly patients (36.8%), compared to middle-aged (53.7%) and young (57.9%) patients (Fig. 5b). When restricting this analysis to somatic HRD/DDR alterations, the proportion of bi-allelic inactivation remained lower in elderly (35.3%) vs middle-aged patients (54.8%) but was comparable to the one in young patients (38.9%), as shown in Supplementary Table S2, which summarizes the germline and somatic HRD/DDR alterations by age group. Notably, most germline HRD/DDR alterations in young patients (75%) and 50% of those in middle-aged individuals were bi-allelic. Only two elderly patients in our cohort had germline alterations in an assessed HRD/DDR gene, one had a bi-allelic *BRCA2* mutation, and the other a mono-allelic *BARD1* mutation (Supplementary Table S2). These findings suggest that albeit at a higher frequency, a subset of somatic alterations in HRD/DDR genes in elderly patients might not constitute drivers of the genomic instability in these cancers.

To further evaluate genomic instability, we assessed microsatellite instability (MSI) using MSIsensor scores derived from MSK-IMPACT data, available for 184 elderly, 743 middle-aged and 461 young patients. MSI-high status was observed in 1.1%, 0.8% and 2.2% of BC in elderly, middle-aged and young individuals, respectively. No statistically significant differences were observed across age groups (Supplementary Table S3).

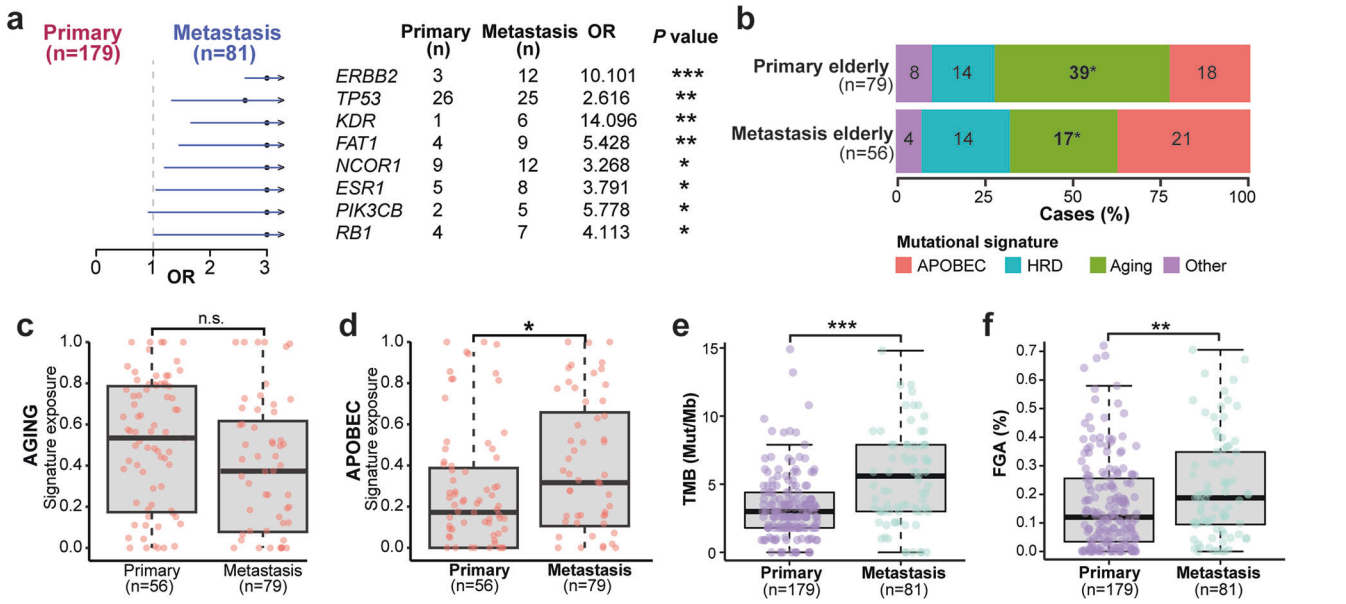

Fig. 4 | Comparison of primary and metastatic ER-positive/HER2-negative breast cancer in elderly patients and spectrum of germline and somatic alterations in HRD/DDR genes in the whole cohort. a Forest plots showing odds ratio (OR) of cancer genes altered at statistically significantly different rates between metastatic ($n = 81$) and primary ($n = 179$) ER+/HER2- breast cancer (BC) samples in elderly individuals. **b** Dominant mutational signatures in primary and metastatic ER+/HER2- BC in elderly patients. Boxplots depicting mutational signature exposures of aging (**c**) and APOBEC (**d**) signatures in primary and metastatic ER+/HER2- BC in elderly patients. Tumor mutation burden (TMB; **e**) and fraction of genome altered (FGA; **f**) or primary and metastatic ER+/HER2- BC. n.s., non-significant; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; Mann-Whitney *U* test and Fisher's exact test.

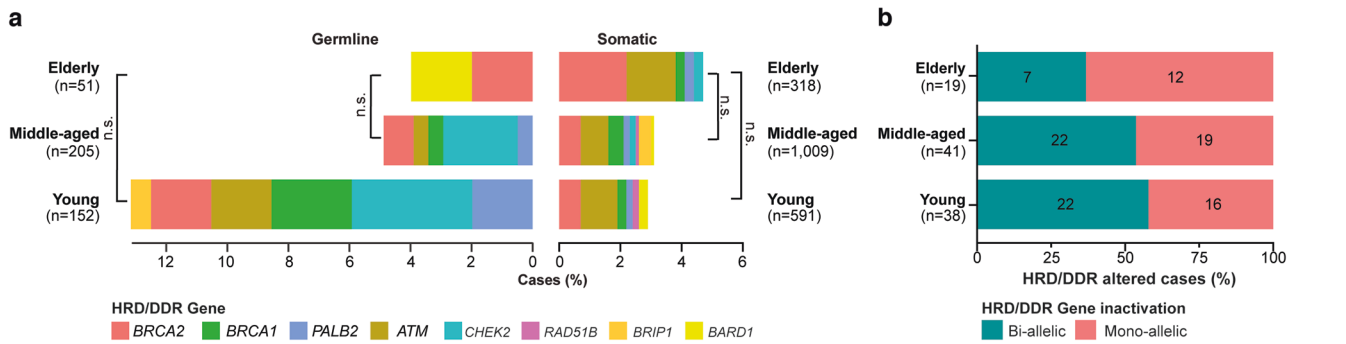

Fig. 5 | Genetic alterations in homologous recombination deficiency/DNA damage repair genes in breast cancer according to age. Frequency of germline and somatic genetic alterations in homologous recombination deficiency (HRD)/DNA damage response (DDR) genes in elderly, middle-aged and young patients (**a**), and proportion of mono- and bi-allelic inactivation in HRD/DDR-altered cases according to age (**b**). Analysis includes all breast cancer samples regardless of sample type or ER/HER2 status. n.s. non-significant; Fisher's exact test.

Discussion

Our re-analysis of curated MSK-IMPACT targeted sequencing data of primary and metastatic BC samples[22] in elderly patients compared to younger individuals showed an overall similar genetic make-up between the groups but also revealed important differences.

Metastatic ER+/HER2- BCs from elderly patients, compared to young patients were found to harbor a lower frequency of *ESR1* mutations, that are associated with resistance to aromatase inhibitors[32], and represent an actionable alteration with Elacestrant[45,46]. Conversely, metastatic ER+/HER2- BCs in elderly patients, compared to young patients, had a higher frequency in genetic alterations in *RB1* and *FAT1*, associated to resistance to CDK4/6 inhibitors in ER + BC[30]. These findings indicate that mechanisms of therapeutic resistance may exhibit differential activity according to age. Moreover, the emergence of resistance associated alterations, such as *RB1* and *FAT1* mutations may reflect the combined effect of a longer cumulative duration of endocrine and other therapies, and changes in tumor biology associated with age, such as the accumulation of somatic mutations over time[47], DNA methylation drift[48], and diminished immunosurveillance[49], which could help shape the evolution of BC in elderly patients.

Intratumor heterogeneity may also shape the genomic landscape of BC in elderly patients. While *ESR1* mutations have been reported to be enriched in liver metastases[33], the lower *ESR1* mutation rate we observed in metastatic ER+/HER2- BC in elderly patients persisted even in liver lesions, suggesting that metastatic site distribution alone does not fully explain the differences observed. Future studies using paired primary-metastatic and multi-site samples, as well as single cell, spatial or liquid biopsy approaches, will help resolve the BC heterogeneity in this population.

The spectrum of genetic alterations in the PI3K/AKT/mTOR and MAPK pathways appears to vary according to age group. A lower frequency of *AKT1* mutations and a higher prevalence of *PIK3CA* mutations were observed in primary ER+/HER2- BC in elderly patients compared to younger patients. Furthermore, a subset analysis of cases according to histologic type revealed that primary ER+/HER2- IDC-NST in elderly patients, had a higher frequency of *MAPK3K1* mutations, and a lower frequency of *AKT1* and *MAP2K4* mutations, than in younger patients.

These findings may reflect age-related differences in molecular subtype distribution, as previously reported[18], with *PIK3CA* mutations enriched in Luminal A and *AKT1* mutations in Luminal B tumors[50]. These differences were not seen in the metastatic BC studied here, possibly due to therapy induced changes that obscure age related or subtype specific mutational patterns.

Metastatic ER+/HER2- ILC affecting elderly patients, compared to middle-age and young patients, were found to harbor a higher frequency of *ERBB2* activating mutations. While not directly assessed in this study, these mutations are known to confer resistance to trastuzumab, pertuzumab and first generation anti-HER2 tyrosine kinase inhibitors (TKIs), such as lapatinib[37,51,52]. These findings suggest that elderly patients could be candidates to an alternative treatment with HER2 irreversible inhibitors such as neratinib, newer generation TKIs, such as tucatinib, antibody drug conjugates, that show activity in patients with *ERBB2*-mutant tumors[37,51–53]. We observed differences in the mechanism of *ERBB2* dysregulation according to age. While metastatic ER+/HER2- ILC in elderly patients were found to harbor predominantly *ERBB2* extracellular domain or Exon 18 activation mutations, these are not found in young patients, whose tumors harbored Exon 20 insertion mutations, that are associated with lower activity or resistance to neratinib and other HER2-TKIs[43]. Further studies are warranted to define the basis and biological implications of the different modalities of *ERBB2* mutations in this context.

Our comparison of ER+/HER2- metastatic and primary BC samples from elderly patients revealed important differences, such as higher rates of alterations in genes associated to resistance to CDK4/6 inhibitors (*FAT1* and *RB1*) and to endocrine therapy (*ESR1* and *ERBB2*). These findings suggest that the genomic landscape of metastatic ER+/HER2- BC in elderly patients is shaped in great part by therapeutic pressure. Notably, our analyses revealed greater exposure to APOBEC mutational signatures along with high TMB and FGA in metastatic vs primary BC in the elderly subgroup. These findings suggest that APOBEC mutagenesis, that induces hypermutation and genomic instability, and that might play roles in resistance to endocrine therapy, might be a major driver of progression in elderly patients.

In agreement with previous work[13], we observed a lower frequency of germline genetic alterations in HRD/DDR genes in elderly patients compared to young patients. Interestingly, we also observed a numerically higher frequency of somatic alterations in this group of genes in elderly patients; these were mostly mono-allelic, however, in contrast to the predominantly bi-allelic events identified in younger patients. These findings suggest a lower likelihood of functional HRD in elderly patients, potentially reflecting age-associated mutagenesis with an increase in likely passenger alterations in these cases, rather than true selection of HRD tumor clones. Although we did not assess epigenetic alterations as a second hit, and a formal HRD score could not be applied given that these analyses were based on a targeted sequencing panel, both limitations of our study, the lack of enrichment in HRD mutational signatures in BCs from elderly individuals, supports this interpretation. Nonetheless, a subset of somatic HRD/DDR alterations in BCs from elderly individuals may still have therapeutic relevance, which warrants further investigation. Although there is conflicting information on the actionability of somatic HRD/DDR genetic alterations other than *BRCA1*/2[54], somatic mutations in *BRCA2*, that we observed in the elderly population, have an OncoKB actionability level 3A, and an ESCAT level IIB for PARP inhibitors, based on the results of the TBCRC-048 clinical trial assessing olaparib in patients with metastatic BC with HRD mutations[54]. This highlights the importance of comprehensive functional and allelic characterization of HRD/DDR alterations in the elderly population.

Future studies should investigate the functional relevance of somatic HRD/DDR alterations in elderly patients, including the role of epigenetic silencing as second hit in mono-allelic cases, as well as the assessment of HRD genomic scars using whole-genome sequencing (WGS) data. WGS could also uncover the identification of large structural variants not detectable by targeted sequencing and allow the better characterization of APOBEC mutagenesis. In addition, given the association of APOBEC mutagenesis with resistance to endocrine therapies and targeted therapies[29], and actionability of HRD/DDR alterations[55,56], evaluation of these processes in BCs from elderly patients in clinical trials may inform their role as predictive biomarkers in this population.

Our study has limitations, such as the lack of gene expression data for BC subtype identification. In addition, our analysis was limited to 468 cancer genes present in the MSK-IMPACT panel. Moreover, the numbers of elderly patients were small in the ER-/HER2- and HER2+ cohorts, which precluded the analyses in those groups. Differences in the number of treatment lines across age groups in the metastatic cohort represent an additional limitation. Moreover, we observed age related differences in the proportion of primary BC samples collected post-neoadjuvant therapy, introducing further heterogeneity into the cohort. Despite these limitations, our analyses revealed important differences in the genomic landscape of BC in elderly patients compared to younger individuals, such as distinct rates of germline and somatic HRD/DDR alterations. We also observed differences in the spectrum of PI3K/ATK/mTOR and MAPK/ERK pathway actionable alterations in ER+/HER2- BC, and a higher frequency of actionable *ERBB2* hotspot mutation in metastatic ER+/HER2- ILC in elderly individuals.

These findings support routine genomic profiling of BC in elderly patients for the identification of actionable genetic alterations and to guide treatment personalization.

Methods

Subject and samples

This study was approved by an MSK Institutional Review Board (IRB) and as part of the study initially published by Razavi et al.[22], for which informed consent was provided. Targeted massively parallel sequencing data from 1918 BCs (primary, $n = 918$; metastatic, $n = 1000$) previously analyzed using the FDA-authorized MSK-IMPACT assay were retrieved from the study by Ravazi et al.[22]. Among 1918 samples, 82 were primary-metastasis pairs, (41 pairs), while 1836 were unpaired. Of the 1000 metastatic samples, 986 (98.6%) were distant metastases and 14 (1.4%) were local recurrences.

Patients were classified according to age at BC diagnosis as elderly (≥ 65 years), middle-age (between >45 and <65 years) and young (≤45 years)[10,57]. BCs were classified into subtypes according to ER expression defined by immunohistochemistry (IHC) and HER2 expression by IHC and/or fluorescence in situ hybridization (FISH), as previously described[22] and following the American Society of Clinical Oncology (ASCO) and College of American Pathology (CAP) guidelines[58,59].

Targeted massively parallel sequencing analysis

SBSs, insertions/deletions (Indels) and gene-specific CNAs of up to 468 cancer-related genes identified by clinical FDA-authorized MSK-IMPACT[60,61] sequencing were retrieved from the study by Razavi et al.[22]. Mutational hotspots were detected and annotated using a previously described algorithm[39,40] from Razavi et al.[22]. TMB was calculated as total number of mutations per megabase. The raw MSK-IMPACT sequencing data (i.e., BAM files) were reprocessed using our validated bioinformatics pipeline, as previously described[19,62,63], for the inference of genome-wide copy number gains and losses, loss of heterozygosity (LOH) of genes targeted by somatic mutations and mutational signatures. Mutational signatures were defined using the SigMA tool[27] in all cases with at least five SBSs, as previously described[64]. CNAs were detected using the FACETS algorithm[65], as previously described. FGA, calculated as the fraction of the genome that is not diploid divided by total genome, was retrieved from Razavi et al.[22]. Gene mutational frequencies were represented as elderly, middle-age and young, respectively. Germline variants for *BRCA1*, *BRCA2*, *ATM*, *CHEK2*, *PALB2*, *RAD51B*, *RAD51C*, *RAD51D*, *BRIP1* and *BARD1* were retrieved under a prospective IRB-approved protocol[66–68]. Variants with >1% frequency in the Genome Aggregation Database[69] were discarded. Remaining variants were classified based on the American College of Medical Genetics and Genomics guidelines[70]. Germline analysis included exon deletions and duplications. Splice-site variants targeting intronic positions up to 1–3 bp from exon-intron junctions were considered. MSI

status was assessed using MSIsensor[71] scores and categorized as MSI-high (score ≥ 10), MSI-indeterminate (score ≥ 3 and <10) or MSI-stable (score < 3)[72,73].

Statistical analysis

Statistical analysis was carried out using R v3.1.2. All tests were two-tailed. Fisher's exact test was performed for comparison of categorical variables. Chi-square tests were applied for larger contingency tables when expected cell counts were sufficient. Mann-Whitney *U* test was performed for continuous variables. Mutual exclusivity was calculated using CoMEt[74] in the maftools package[75], as previously described[76]. Statistical tests were performed to compare BC in elderly vs in middle-aged individuals, BC in elderly vs in young individuals, and metastatic vs primary BC in elderly patients, stratified by ER/HER2 status and/or histologic subtype. $P < 0.05$ (unadjusted) were considered statistically significant. Microsatellite instable cases were excluded from TMB comparative analysis.

Data availability

The MSK-IMPACT sequencing data supporting the findings of this study are publicly available in cBioPortal at the following accession: https://identifiers.org/cbioportal:breast_msk_2018.

Received: 6 January 2025; Accepted: 13 June 2025;
Published online: 10 July 2025

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Acknowledgements

Research reported in this publication was supported in part by a Cancer Center Support Grant of the NIH/NCI (Grant No. P30CA008748). A.M. is supported by the European Society for Medical Oncology (ESMO) José Baselga Fellowship for Clinician Scientists sponsored by AstraZeneca. S.C. and B.W. are supported in part by the Breast Cancer Research Foundation. S.C., B.W., and F.P. are supported in part by an NIH/NCI MSK SPORE on Genomic Instability in Breast Cancer P50CA247749 01 grant. F.P. is funded in part by an FDA 1U01FD007909-01A1 grant.

Author contributions

J.S.R.F., B.W., and F.P. conceived and designed the study. P.S., H.D., M.R., T.B., A.M.G., C.J.S., F.D., A.M., L.F. accrued and analyzed the data. J.S.R.F., B.W. and F.P. contributed to the interpretation of the results. P.S., H.D., M.R. and F.P. drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

A.M. reported consulting or advisory role for Menarini/Stemline and AstraZeneca, honoraria as speaker's bureau from Roche and Eli Lilly, received travel accommodation from Menarini/Stemline and Daiichi Sankyo, outside the submitted work. S.C. has received institutional grant/funding from Daiichi-Sankyo, AstraZeneca, and Lilly, Share options Totus Medicines, and consultation/Ad board/Honoraria from AstraZeneca, Lilly, Casdin Capital, Nuvalent, Blueprint, and SAGA Diagnostics. J.S.R.-F. is an employee of AstraZeneca and owns AstraZeneca stocks. Prior Conflicts of Interest in the last 2 yeas include the receipt of personal fees for the following activities: Board Membership at Grupo Oncoclinicas, consultant for Goldman Sachs Merchant Banking, consultant for Bain Capital, consultant for and SAB member of Paige.ai, consultant for and SAB member of Repare Therapeutics, Consultant of SAGA Diagnostics, Consultant of Personalis, and consultant at MultiplexDx. B.W. reports research grants from REPARE Therapeutics and SAGA Diagnostics paid to the institution, and employment of an immediate family member at AstraZeneca. F.P. reports membership on advisory boards for AstraZeneca and MultiplexDx, as well as receipt of consultancy fees from AstraZeneca. All other authors have nothing to disclose.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41523-025-00781-4.

Correspondence and requests for materials should be addressed to Britta Weigelt or Fresia Pareja.