Male breast cancer: genomic profiling

The Genomic Landscape of Male Breast Cancers

Salvatore Piscuoglio1*, Charlotte K. Y. Ng1*, Melissa Murray1, Elena Guerini-Rocco1,2, Luciano G. Martelotto1, Felipe C. Geyer1,3 Francois-Clement Bidard1,4, Samuel Berman1, Nicola Fusco1,5, Rita A. Sakr8, Carey A. Eberle1, Leticia De Mattos-Arruda1, Gabriel S. Macedo1, Muzaffar Akram1, Timour Baslan7,8,9, James B. Hicks7, Tari A. King6, Edi Brogi1, Larry Norton10, Britta Weigelt1, Clifford A. Hudis10, Jorge S. Reis-Filho1

1Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, NY.
2Department of Pathology, European Institute of Oncology, Milan, Italy.
3Department of Pathology, Hospital Israelita Albert Einstein, Instituto Israelita de Ensino e Pesquisa, São Paulo, Brazil.
4Department of Medical Oncology, Institut Curie, Paris, France.
5Division of Pathology, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy.
6Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY.
7Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
8Department of Molecular and Cellular Biology, Stony Brook University, NY.
9Department of Cancer Biology and Genetics, Memorial Sloan Kettering Cancer Center, New York, NY.
10Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY.

*These authors contributed equally to this work

Running Title: Male breast cancer: genomic profiling

Key Words: Massively parallel sequencing, male breast cancer, copy number alterations, somatic mutations

Correspondence: Dr. Jorge S Reis-Filho, Department of Pathology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, NY 10065, USA. Phone: 212-639-8054; Fax: 212-639-2502; E-mail: reisfilj@mskcc.org

Conflict of interest: The authors have no conflicts of interest to declare.

Word count: 4,999; Figures: 5, Tables: 1
Male breast cancer (MaBC) is an uncommon disease accounting for <1% of all invasive breast cancers. Given its rarity, there have been no prospective clinical trials for MaBC patients, and critical treatment decisions are generally based on extrapolations from prior knowledge obtained through studies of patients with female breast cancer (FBC). Here we found that although overall MaBCs and FBCs appeared to be characterized by similar patterns of somatic genetic alterations, they differ in their mutational repertoire and the mutational frequency of the most commonly mutated genes. MaBCs less frequently harbor PIK3CA and TP53 mutations than FBCs of the same immunohistochemical profile and display an enrichment for potentially pathogenic mutations affecting DNA repair-related genes. Our data suggest that caution should be exercised in the extrapolation of biological and therapeutic findings from studies performed in FBCs to MaBCs, and that further studies to define targetable alterations in the latter are warranted.
Male breast cancer: genomic profiling

ABSTRACT

Purpose: Male breast cancer (MaBC) is rare and its genomic landscape has yet to be fully characterized. Lacking studies in men, treatment of MaBC patients is extrapolated from results in females with the disease (FBC). We sought to define whether MaBCs harbor somatic genetic alterations in genes frequently altered in FBCs.

Experimental Design: All MaBCs were estrogen receptor-positive and all but two were HER2 negative. 59 MaBCs were subtyped by immunohistochemistry and tumor-normal pairs were microdissected and subjected to massively parallel sequencing targeting all exons of 241 genes frequently mutated in FBCs or DNA-repair related. The repertoires of somatic mutations and copy number alterations of MaBCs were compared to that of subtype-matched FBCs.

Results: 29% and 71% of MaBCs were immunohistochemically classified as luminal A-like or luminal B-like, respectively. MaBCs displayed a heterogeneous repertoire of somatic genetic alterations that to some extent recapitulated that of estrogen receptor (ER)-positive/HER2-negative FBCs, including recurrent mutations affecting PIK3CA (20%) and GATA3 (15%). ER-positive/HER2-negative MaBCs, however, less frequently harbored 16q losses, and PIK3CA and TP53 mutations than ER-positive/HER2-negative FBCs. In addition, MaBCs were found to be significantly enriched for mutations affecting DNA repair-related genes.

Conclusion: MaBCs less frequently harbor somatic genetic alterations typical of ER-positive/HER2-negative FBCs, such as PIK3CA and TP53 mutations and losses of 16q, suggesting that at least a subset of MaBCs are driven by a distinct repertoire of somatic changes. Given the genomic differences, caution may be needed in the application of biological and therapeutic findings from studies of FBCs to MaBCs.
INTRODUCTION

Male breast cancer (MaBC) is an uncommon disease, accounting for <1% of all breast cancers (1), with approximately 2,350 new cases diagnosed per year in the U.S. (2). In contrast to female breast cancers (FBCs), MaBCs are usually diagnosed at later stage and older age (3). Risk factors of MaBC are largely related to physiologic changes in estrogen levels, hereditary syndromes (e.g. Klinefelter syndrome) and \textit{BRCA2} or \textit{PALB2} germline mutations (4, 5). The majority of MaBCs are estrogen receptor (ER)-positive invasive ductal carcinomas of no special type (IDC-NSTs). Unlike FBCs, MaBCs rarely display \textit{HER2} gene amplification or a triple-negative phenotype (6). Given its rarity, there have been no prospective clinical trials for patients with MaBC (7) and critical treatment decisions for these patients are generally extrapolated from small single-institution retrospective studies and prior knowledge obtained from studies carried out in FBC patients (6).

From a clinico-pathologic standpoint, MaBCs are similar to estrogen receptor (ER)-positive/ luminal FBCs. Patients with MaBC and FBC appear to have similar prognosis when matched according to age at diagnosis and clinico-pathologic characteristics (9, 10). Even when prognostic differences were documented (i.e. FBCs had better 5-year overall survival (OS) than MaBCs in the early but not in the late stages) (11), these differences could be attributed to the lack of mammographic screening in men, higher likelihood for non-breast-cancer-related mortality in men and deficiencies in data collection and reporting by cancer registries (11).

The genetic landscape of MaBCs has yet to be fully characterized. Massively parallel sequencing (MPS) studies have provided a comprehensive portrait of the genetic landscape of FBCs and demonstrated that breast cancers are characterized by complex genomes (reviewed in (12)). These studies have identified \textit{bona fide} driver genetic alterations in breast cancer, in particular recurrent mutations affecting \textit{PIK3CA}, \textit{TP53}, \textit{GATA3}, \textit{MAP3K1}, \textit{MAP2K4}, \textit{MLL3} and \textit{CDH1} in ER-positive breast cancers (12, 13). It should be noted, however, that only a few MaBCs were included in these studies. In The Cancer Genome Atlas (TCGA) project, six MaBCs were included, of which all were of luminal.
Male breast cancer: genomic profiling

subtype, two harbored PIK3CA somatic mutations and none had TP53 mutations (13). The repertoire of genetic alterations affecting 48 common cancer genes has been analyzed in familial MaBCs (BRCA1, BRCA2 and BRCAX patients) (14); these tumors were found to display a mutational landscape similar to that of luminal A FBCs, with PIK3CA being the most commonly mutated gene (17%) in addition to infrequent TP53 and PTEN mutations (4% and 2%, respectively) (14). Substantial differences were detected, however, between BRCA2 and ‘BRCAX’ patients. Whilst TP53 mutations were only found in BRCA2 cases (14), PIK3CA mutations were significantly more prevalent in BRCAX patients (15), suggesting that BRCA2-associated MaBCs may have distinct genomic features, possibly due to the DNA repair defect caused by BRCA2 mutation.

Recent studies have shown that MaBCs and FBCs of luminal molecular subtype harbor similar copy number alterations (CNAs), including recurrent 1q gain and 16q loss (16, 17). Of note, these two CNAs (1q gain/ 16q loss), which often co-occur with PIK3CA mutations, define a genetic signature present not only in low-grade/ luminal A-like, but also in a subset of high-grade/ luminal B-like breast cancers (13, 18, 19). By integrating CNAs and gene expression, Johansson et al. (20) identified 30 candidate driver genes in MaBCs, of which only a minority included known cancer genes (MAP2K4, LHP, ZNF217), suggesting that MaBCs may display a constellation of candidate driver genes distinct from that of FBCs (20).

Given the limited genetic characterization of MaBCs and the apparent clinico-pathologic similarities between ER-positive MaBCs and FBCs, here we sought i) to define the repertoire of somatic genetic alterations in MaBCs using a platform containing the genes most frequently mutated in FBCs as well as genes involved in DNA repair, given the reported enrichment for BRCA2 and PALB2 mutations in MaBCs, and ii) to compare the mutational and CNA profiles of MaBCs with FBCs and post-menopausal FBCs using this platform, given the similarities in the hormonal milieu and age of presentation between MaBCs and post-menopausal FBCs.
MATERIAL AND METHODS

Cases

Fifty-nine MaBCs were retrieved from the pathology archives of Memorial Sloan Kettering Cancer Center (MSKCC). Only patients diagnosed and managed at MSKCC, whose tumors were >1cm in size and for which representative histologic slides and blocks were available for review were included. Patients for whom not all histologic slides were available for review were excluded, as well as samples from patients who received neoadjuvant therapy. Samples were anonymized prior to analysis and the study was approved by the Institutional Review Board of MSKCC. All cases were independently reviewed by four pathologists (MM, EGR, NF and JSR-F) who classified and graded the tumors following the World Health Organization criteria (21) and the Nottingham grading system (22), respectively. All clinico-pathologic features are summarized in Table 1 and Supplementary Table S1.

Immunohistochemistry and intrinsic subtyping

Immunohistochemical profile of the included cases (n=59) was assessed on 4μm-thick sections, using antibodies against ER, progesterone receptor (PR), HER2 and Ki-67 as previously described (23). Positive and negative controls were included in each experiment. The ER, PR, and HER2 immunohistochemical results were evaluated by three pathologists (MM, EGR and NF) according to the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) guidelines (24, 25). Ki-67 score was defined as the percentage of cells with nuclear staining in at least 1000 invasive neoplastic cells randomly selected from the periphery of the tumor over 10 high-power fields (magnification, 400X) (26). Immunohistochemistry with antibodies against E-cadherin and p120 catenin was performed in selected cases and interpreted by two of the authors (FCG and JSR-F). Antibody clones and dilutions are described in Supplementary Table S2. The intrinsic (molecular) subtype of MaBCs were defined using the immunohistochemical surrogate according to the St. Gallen criteria for FBCs (27).

Microdissection and DNA extraction
Male breast cancer: genomic profiling

Eight-μm-thick sections from representative formalin-fixed paraffin-embedded histologic blocks of MaBCs were stained with Nuclear Fast Red in RNase-free condition and subjected to microdissection with a sterile needle under a stereomicroscope (Olympus) to ensure a percentage of tumor cells >90%, as previously described (28). Microdissection was also performed for matched normal tissue to ensure that normal samples were devoid of neoplastic or atypical cells. DNA was extracted from microdissected tissue using the DNeasy Blood and Tissue Kit (Qiagen), according to the manufacturer’s guidelines, and quantified using the Qubit Fluorometer assay (Life Technologies). All samples yielded DNA of sufficient quantity and quality for sequencing analysis.

Targeted capture massively parallel sequencing (MPS)

Tumor and germline DNA of 59 MaBCs were subjected to targeted capture MPS using a platform containing baits targeting all exons of 241 genes that are either recurrently mutated in breast cancer or involved in DNA repair pathways (28). Custom oligonucleotides (NimblegenSeqCap) were designed for hybridization capture of the 241 genes (Supplementary Table S3) (28). Barcoded sequencing libraries were prepared (New England Biolabs) using 50ng to 250ng DNA and pooled at equimolar concentrations into a single capture reaction as previously described (23, 28). Paired-end 76bp reads were generated on the Illumina HiSeq2000. Sequence reads were aligned to the human reference genome GRCh37 using the Burrows-Wheeler Aligner (BWA, v0.7.10) (29). Local realignment, duplicate removal and base quality recalibration were performed using the Genome Analysis Toolkit (GATK, v3.1.1) (30). Sequencing data have been deposited in the NCBI Sequence Read Archive under the accession SRP055003.

Somatic single nucleotide variants (SNVs) were identified using MuTect (v1.0) (31), Strelka (v2.0.15) (32) and VarScan 2 (v2.3.7) (33) using a consensus approach, retaining those detected by at least 2 mutation callers. Small insertions and deletions (indels) were detected using Strelka (v2.0.15) (32) and VarScan 2 (v2.3.7) (33). We filtered out SNVs and indels outside of the target regions, those with mutant allelic fraction (MAF) of <1% and/or those supported by ≤5 reads (34).
Male breast cancer: genomic profiling

Further excluded SNVs and indels for which the tumor MAF was <5 times that of the matched normal
MAF, as well as SNVs and indels found at >5% global minor allele frequency of dbSNP (build 137). All
candidate mutations were subsequently reviewed manually using the Integrative Genomics Viewer
(IGV) (35).

Copy number alterations (CNAs) were identified using FACETS (36) (Supplementary Methods). A
combination of Mutation Taster (37), CHASM (breast) (38) and FATHMM (39) was used to define the
potential functional effect of each missense SNV. Missense SNVs defined as non-
deleterious/passenger by both MutationTaster (37) and CHASM (breast) (38), a combination of
mutation function predictors shown to have a high negative predictive value (40), were considered
passenger alterations. The missense SNVs considered not to be passengers using this combination of
mutation function predictors were defined as likely pathogenic if predicted by CHASM (breast classifier)
and/ or FATHMM (39) as “driver” and/ or “cancer” alterations, respectively. For in-frame indels, those
defined as “deleterious” by MutationTaster or PROVEAN (41), and targeted by LOH of the wild-type
allele or affected cancer genes included in the cancer gene lists described by Kandoth et al. (127
significantly mutated genes) (42), the Cancer Gene Census (43) or Lawrence et al. (Cancer5000-S
gene set) (44) were considered to be likely pathogenic. In addition, frameshift, splice-site and truncating
mutations in the presence of LOH of the wild-type allele or affected genes of at least one of the three
cancer gene datasets were also considered likely pathogenic.

The cancer cell fraction (CCF) of each mutation was inferred using the number of reads supporting the
reference and the alternate alleles and the segmented Log_2 ratio from MPS as input for ABSOLUTE
(v1.0.6) (45). Solutions from ABSOLUTE were manually reviewed as recommended (45, 46). A
mutation was classified as clonal if its clonal probability, as defined by ABSOLUTE, was >50% (46) or if
the lower bound of the 95% confidence interval of its CCF was >90%. Mutations that did not meet the
above criteria were considered subclonal.
Male breast cancer: genomic profiling

**Sequenom MassARRAY**

The fifty-nine MaBCs included in this study were also subjected to hotspot mutation screening of eight known cancer genes (i.e. PIK3CA, GATA3, CTNNB1, KRAS, NRAS, CDH1, AKT1 and ERBB2) using a validated screening panel (Sequenom) (47). The experiments were carried out as previously described in duplicate (47).

**Sanger sequencing**

PCR amplification of 10ng of genomic DNA was performed using the AmpliTaq 360 Master Mix Kit (Life Technologies) on a Veriti Thermal Cycler (Life Technologies) as previously described (48) (Supplementary Methods; for primers see Supplementary Table S2).

**Pathway analysis**

Genes included in the targeted sequencing assay were mapped to KEGG pathways to identify pathways recurrently altered. Significantly regulated pathways and networks were determined using the Ingenuity Pathway Analysis (IPA) program as previously described (49). For the analysis, genes harboring likely pathogenic mutations in MaBCs, and not mutated in FBCs, were mapped to networks available in the Ingenuity database and ranked by a score indicating the likelihood of finding those genes together by chance. Using a 99% confidence level, scores of ≥3 were considered significant.

**Statistical analysis**

Genes significantly mutated in MaBCs were defined using Mutational Significance in Cancer (MutSigCV) version 1.4 as previously described (46). Genes of q<0.1 were considered significantly mutated. Mutational frequencies and GISTIC copy number data for the ER-positive/HER2-negative, ER-positive/HER2-positive and luminal A/B FBCs from TCGA were obtained from the TCGA Data Portal (13). Comparisons of the number of non-synonymous mutations between MaBC and FBC/ post-menopausal FBC were performed using the Wilcoxon rank-sum test. Fisher's exact tests were employed to compare the frequency of specific non-synonymous mutations or the frequency of a given
Male breast cancer: genomic profiling

gene being affected by non-synonymous mutations in MaBC vs. FBC. Two-tailed p-values were adopted for these analyses. For the statistical analysis comparing the copy number profiles between subtypes of MaBC, copy number states were collapsed on a per-gene basis and compared using Fisher’s exact tests corrected for multiple comparisons using the Benjamini-Hochberg method. p-values <0.05 were considered statistically significant. Univariate survival analysis (disease-free and overall survival) was performed using the Kaplan-Meier method and analysed using the log-rank test. Disease-free and overall survival were expressed as the number of months from diagnosis to the occurrence of an event (local recurrence/metastasis and disease-related death, respectively). All tests were two-sided. P-values <0.05. The above statistical analyses were performed with the software suites GraphPad Prism 6 (GraphPad Software Inc.), SPSS 20.0 (IBM) and R v3.0.2.

RESULTS

Clinico-pathologic features of MaBCs

The median age at diagnosis of the 59 patients with MaBC was 66 years (range 33-88 years). The median tumor size was 2.0 cm (range 0.6-4.7 cm). Ten of the patients consented to germline genetic analysis and three were found to carry \textit{BRCA2} germline mutations.

All 59 MaBCs included in this study were subjected to central histologic review and review of the immunohistochemical results, which revealed that most MaBCs were IDC-NSTs (91%) and that 7%, 49% and 44% of cases were of histologic grades 1, 2 and 3, respectively (Table 1, Supplementary Table S1). 57 MaBCs were ER-positive/HER2-negative and two cases were ER-positive/HER2-positive.

The surrogate immunohistochemistry definitions according to the St. Gallen criteria (27) for FBCs were used to define the intrinsic subtypes of the MaBCs included in this study. Briefly, luminal A-like tumors were defined as ER-positive (>1%), PR-positive (>20%), HER2-negative and Ki-67-low (<14%), whereas the remaining ER-positive tumors were classified as luminal B-like. Using this approach, we classified our cohort of MaBCs into luminal A-like (n=17; 29%) and luminal B-like (n=42; 71%, Table 1,
Male breast cancer: genomic profiling

Supplementary Table S1). The higher prevalence of luminal B-like MaBCs is in contrast with the higher frequency of the luminal A subtype among FBCs. It should be noted, however, that the differences observed in the intrinsic subtype frequencies between MaBCs and FBCs may in part be due to the moderate correlation between immunohistochemistry and gene expression for intrinsic molecular subtyping.

Somatic genetic alterations in MaBCs

To define the repertoire of somatic genetic alterations in MaBCs, we subjected the series of 59 cases to MPS of the coding regions of 241 genes (Supplementary Table S3), including the genes most frequently mutated in FBCs (13) and genes associated with DNA repair (28). Our MPS analysis (median depth of 626x, range 135x to 1881x; Supplementary Table S1) revealed somatic mutations in 102 of the 241 genes investigated, with a median of three non-synonymous mutations per case (range 0-24; Supplementary Table S4). As a validation, all samples were subjected to hotspot mutation screening of eight known cancer genes using Sequenom MassARRAY, which confirmed the genotype of all SNVs tested (n=26, Supplementary Fig. S1A), demonstrating the accuracy of the MPS performed. In addition, all mutations selected for Sanger sequencing were validated (Supplementary Fig. S1B).

The genes most frequently mutated in MaBCs were PIK3CA (20%) and GATA3 (15%, Fig. 1, Supplementary Figure S2, Supplementary Tables S4 and S5). All but one PIK3CA mutation affected the hotspots E542, E545 or H1047 (Supplementary Tables S4). Of the 241 genes sequenced, MutSigCV demonstrated that PIK3CA, GATA3, TP53 and MAP3K1 were significantly mutated in MaBCs (q<0.1; Supplementary Table S6). Cancer genes belonging to the Cancer Gene Census (43), or the 127 genes significantly mutated in cancer (42) and the Cancer5000-S pan-TCGA datasets (44), including PIK3CA, GATA3, MLL3 and TP53, were also mutated in MaBCs (Supplementary Table S4). All PIK3CA (12/12) and all but two GATA3 (7/9) mutations were found to be clonal by ABSOLUTE analysis. By contrast, only 2/5 TP53 mutations were considered to be clonal using the same...
Male breast cancer: genomic profiling

methodology (Supplementary Figure S3). One case displayed a CDH1 missense mutation coupled with loss of heterozygosity of the wild-type allele; this case was found to be a mixed ductal lobular carcinoma. Immunohistochemical analysis revealed that in this case, the ductal areas displayed strong E-cadherin and p120 catenin membranous expression, whereas the lobular carcinoma areas either lacked or displayed discontinuous E-cadherin membranous expression and displayed cytoplasmic expression of p120 catenin (Supplementary Figure S4).

Analysis of the CNAs revealed that MaBCs harbored recurrent gains of 1q, 8q, and 16p, and losses of 1p, 16q and 17p (Fig. 2). High-level amplifications of 8p11.23 (14%, including FGFR1 and ZNF703), 8q24.21 (17%, encompassing MYC), 17q23.2 (3%, including PPM1D) and 20q13.2 (3%, encompassing AURKA) were observed, as were homozygous deletions in CDKN2A (2%) and ATM (2%, Fig. 2).

As an exploratory, hypothesis-generating analysis, we sought to define whether the presence of mutations affecting highly recurrently mutated genes or affecting pathways whose genes were recurrently affected by somatic mutations would be associated with the disease-free or overall survival of MaBC patients. Univariate survival analysis revealed that mutations in GATA3 were found to be associated with worse disease-free survival in MaBC patients (p=0.038, Supplementary Figure S5) and mutations affecting DNA repair pathway-related genes were associated with disease-free survival and overall survival (p=0.036 and p=0.041, respectively, Supplementary Figure S5). None of these associations were found to be statistically significant on multivariate models including age, size and lymph node status (data not shown).

Genomic landscape of luminal A-like and luminal B-like MaBCs

As an exploratory, hypothesis-generating analysis, we compared the repertoires of somatic genetic alterations between luminal A-like (n=17; 29%) and luminal B-like (n=42; 71%) MaBCs. The most frequently mutated genes in luminal A-like MaBCs included PIK3CA, HERC2 and MAP3K1 (all at 12%), whereas the most frequently mutated genes in luminal B-like MaBCs were PIK3CA and GATA3 (24%
Male breast cancer: genomic profiling

and 21%, respectively, Supplementary Table S7). GATA3 mutations were found only in luminal B-like MaBCs (21% vs 0% in luminal A-like MaBCs, Fisher's exact test p=0.0482, Supplementary Table S7 and Fig. 1). Interestingly, the pattern of mutations found in GATA3 did not resemble that of FBCs by TCGA (Supplementary Fig. S5) (13), in which hotspot GATA3 mutations differed according to luminal subtype (at residues S308 and S407 in luminal A and luminal B subtypes, respectively). In fact, seven out of nine GATA3 mutations found in MaBCs were frameshift mutations and none of these affected the aforementioned hotspots.

We next mapped the mutated genes to KEGG pathways, including DNA repair, homologous recombination, PI3K/ATK/mTOR and classical MAPK pathways and compared the number of luminal A-like and luminal B-like MaBCs harboring mutations in these pathways/nets (Supplementary Table S8). We found that DNA repair-related genes were more frequently mutated in luminal B-like (33%) than in luminal A-like MaBCs (6%, Fisher's exact test p=0.04, Supplementary Table S8).

In contrast with the distinct repertoires of somatic mutations among luminal A-like and B-like MaBCs, the two cohorts exhibited remarkably similar CNA profiles, both subtypes displaying recurrent gains of 1q, 8q and 16p and losses of 1p and 16q (Fig. 3).

MaBC is different from FBC and post-menopausal FBC

We next compared the mutational landscape of MaBCs with that of FBCs from TCGA (13). Given that the MaBCs analyzed here were consistently ER-positive, we specifically compared their repertoire of somatic genetic alterations to that of ER-positive FBCs and to that of post-menopausal ER-positive FBCs (13). The latter comparison was performed given the similarities between MaBCs and post-menopausal FBCs in terms of hormonal exposure and age at presentation. Given that only two of the MaBCs were ER-positive/HER2-positive, we have restricted the comparisons to ER-positive/HER2-negative MaBCs and ER-positive/HER2-negative FBCs and post-menopausal ER-positive/HER2-negative FBCs. We observed that the number of somatic non-synonymous mutations in the 241 genes
Male breast cancer: genomic profiling

profiled in ER-positive/HER2-negative MaBCs (mean 3.4 per case, range 0-24) was similar to that of both sets of ER-positive/HER2-negative FBCs/ post-menopausal FBCs (means 2.9 and 3.0, respectively; Wilcoxon test p>0.05; Supplementary Table S9) (13). Genes reported to be recurrently mutated in ER-positive/HER2-negative FBCs and post-menopausal ER-positive/HER2-negative FBCs, including PIK3CA, GATA3, MAP3K1 and TP53, were also altered in ER-positive/HER2-negative MaBCs, but at significantly different frequencies. In fact, we found a lower frequency of PIK3CA mutations in ER-positive/HER2-negative MaBCs compared to ER-positive/HER2-negative FBCs/ post-menopausal FBCs (18% vs 42% and 42%, respectively, Fisher’s exact tests p=0.0005 and p=0.0014, respectively; Fig. 1 and Supplementary Table S5), as well as a higher frequency of mutations affecting the E3 ubiquitin-protein ligase gene HERC2 (7.0% vs 0.8% and 0.8%, respectively; Fisher’s exact tests p=0.0121 and p=0.0296, respectively; Supplementary Table S5). Lower frequencies of mutations in HUWE1 and TP53 were detected when ER-positive/HER2-negative MaBCs were compared to germline BRCA2-mutant ER-positive/HER2-negative FBCs (HUWE1: 0% vs 25%, TP53: 7% vs 38%, Fisher’s exact tests p=0.0135 and p=0.0348, respectively; Supplementary Table S5). Of note, additional genes identified as significantly mutated genes in FBCs, including RUNX1, AKT1 and NCOR1, were not found to be mutated in this cohort of MaBCs (Supplementary Table S5). Given that none of the MaBCs included in this study were classified as invasive lobular carcinomas, we repeated the analyses comparing the repertoire of somatic mutations in ER-positive/HER2-negative MaBCs with that of ER-positive/HER2-negative FBCs/ post-menopausal FBCs, after the exclusion of all cases classified as “infiltrating lobular carcinoma” or “breast lobular adenocarcinoma” in the TCGA dataset. The differences reported above were confirmed (Supplementary Table S5).

Next we compared the proportion of cases harboring mutations in selected pathways. This analysis revealed an enrichment of ER-positive/HER2-negative MaBCs with mutations in genes affecting DNA repair pathway when compared to FBCs/ post-menopausal FBCs of ER-positive/HER2-negative subtype (DNA repair: 30% vs 16% and 15%, respectively, Fisher’s exact tests p=0.0211 and p=0.0274, respectively; Supplementary Table S8) but lower mutation rates in genes affecting the
Male breast cancer: genomic profiling

PI3K/AKT/mTOR pathway (37% vs 58% in ER-positive/HER2-negative FBC and 58% in post-menopausal ER-positive/HER2-negative FBC, Fisher’s exact tests p=0.0049 and p=0.0073, respectively; Supplementary Table S8).

Of the 241 genes sequenced, 27 genes were found to be mutated exclusively in ER-positive/HER2-negative MaBCs but not in FBCs of ER-positive/HER2-negative phenotype. A pathway and networks analysis of these genes using the IPA software revealed an enrichment of genes involved in DNA repair mechanisms (network score=17, Fig. 4). Of note, the genes enriched for DNA repair mechanisms, such as FANCM and PALB2, were also associated with loss of heterozygosity in the ER-positive/HER2-negative MaBCs harboring mutations in these genes (Supplementary Table S4). Furthermore, using The Drug Gene Interaction Database (50), we investigated if any of these 27 genes exclusively mutated in ER-positive/HER2-negative MaBCs would be actionable. This analysis revealed that ten genes, including PRKCA and RICTOR, may be actionable by a variety of anti-neoplastic drugs (Supplementary Table S10).

In terms of CNAs, ER-positive/HER2-negative MaBCs and FBCs/ post-menopausal FBCs of ER-positive/HER2-negative subtype shared many of the recurrent amplifications, gains and losses, including gains of 1q, 8q and 16p, losses of 1p and 16q and amplifications of 8q. Losses of 16q and 17p were significantly less frequent in ER-positive/HER2-negative MaBCs than in ER-positive/HER2-negative FBCs/ post-menopausal FBCs (Fisher’s exact test p<0.05; Fig. 5 and Supplementary Fig. S6). Given the high frequency of 16q losses and 16p gains in lobular carcinomas and that none of the MaBCs included in this study were of invasive lobular carcinoma histologic type, we compared the repertoire of CNAs in MaBCs and FBCs/ post-menopausal FBCs, after the exclusion of all cases classified as “infiltrating lobular carcinoma” or “breast lobular adenocarcinoma” in the TCGA dataset. The differences reported above were confirmed (Supplementary Fig. S7).
Luminal A-like and luminal B-like MaBCs are distinct from luminal A and luminal B FBCs/ post-menopausal FBCs

As another exploratory, hypothesis-generating analysis, we compared MaBCs stratified into luminal A-like and luminal B-like by immunohistochemistry with FBCs/ post-menopausal FBCs stratified into luminal A and B intrinsic subtypes by PAM50 (13). Similar to the previous analysis, luminal A-like MaBCs displayed significantly fewer PIK3CA mutations than luminal A FBC/ post-menopausal FBCs (12% vs 46% and 50%, respectively, Fisher’s exact tests p=0.005 and p=0.003, respectively; Supplementary Table S11). Accordingly, within the luminal A subtype, the number of MaBCs harboring mutations in the PI3K/AKT/mTOR pathway was significantly lower than in both FBCs and post-menopausal FBCs (29% vs 61% and 65%, respectively, Fisher’s exact tests p=0.02 and p=0.01, respectively; Supplementary Table S8). An enrichment of mutations in genes involved in the classical MAPK pathway was observed in luminal A-like MaBCs as compared to FBCs of luminal A molecular subtype (24% vs 7%, Fisher’s exact test p=0.04; Supplementary Table S8). Within the luminal B subtype, MaBCs had a significantly lower frequency of TP53 mutations than FBCs/ post-menopausal FBCs (10% vs 34% and 43%, respectively; Fisher’s exact tests p=0.002 and p=0.0004, respectively; Supplementary Table S12). Furthermore, genes related to DNA repair pathways were more frequently mutated in luminal B-like MaBCs than in luminal B FBCs (36% vs 18%, Fisher’s exact test p=0.03; Supplementary Table S8).

CNA analysis within luminal A and luminal B subtypes evaluated separately demonstrated similar copy number profiles between MaBCs of luminal A-like or luminal B-like subtype and FBCs/ post-menopausal FBCs of luminal A or B subtype, respectively, with notable differences in the frequency of 16q and 17p losses (Fisher’s exact test p<0.05; Supplementary Fig. S8). These differences remained significant after the exclusion of invasive lobular carcinomas from the TCGA dataset (Supplementary Fig. S9). No differences were observed in the pattern of gene amplifications upon stratification according to luminal subtypes (Supplementary Fig. S10).
DISCUSSION

MPS approaches have provided evidence that breast cancers have complex and heterogeneous genomes and have offered means to study specific subsets of rare breast cancers (12). Here we characterized a series of 59 MaBCs, which were found to display a luminal phenotype and to be largely of intermediate-to-high grade. Of the ten patients who consented to genetic testing, three harbored germline BRCA2 mutations and none carried a germline PALB2 mutation. Germline genetic analyses were not performed in the remaining 49 patients. Our targeted sequencing analysis of somatic genetic alterations affecting 241 genes either frequently mutated in FBC or directly related to DNA repair revealed that overall MaBCs are heterogeneous at the mutational and CNA levels, and bear resemblance to ER-positive/HER2-negative FBCs and post-menopausal ER-positive/HER2-negative FBCs.

Despite the similarities between ER-positive/HER2-negative MaBCs and ER-positive/HER2-negative FBCs, important differences were observed in their repertoire of somatic genetic alterations. Similar to FBCs, we demonstrated that MaBCs constitute a heterogeneous disease, with a small number of significantly recurrently mutated genes, namely, PIK3CA, GATA3, TP53 and MAP3K1, as well as numerous genes harboring potentially pathogenic mutations at lower frequency. Compared to ER-positive/HER2-negative FBCs, MaBCs less frequently harbored mutations in PIK3CA and genes involved in the PI3K/AKT/mTOR pathway. MaBCs also less frequently harbored TP53 mutations, but more frequently displayed mutations in genes associated with DNA repair pathways. Further studies are warranted to investigate whether the lower frequencies of TP53 mutations in MaBCs may be compensated by the increased frequency of mutations in DNA repair related genes in these tumors.

The landscape of CNAs found in MaBCs closely recapitulates that of ER-positive/HER2-negative FBCs, with recurrent copy number gains of 1q, 8q and 16p, and losses of 1p and 16q, as well as recurrent amplifications of the 8p11.23 and 8q24.21 loci. MaBCs, however, displayed 16q and 17p losses significantly less frequently than ER-positive/HER2-negative FBC. Of note, the infrequent 17p losses,
Male breast cancer: genomic profiling

encompassing the **TP53** gene locus, coupled with infrequent mutations in **TP53** suggests that complete inactivation of **TP53** may not be as prevalent in MaBCs compared to FBCs. These findings in conjunction with the lower prevalence of 16q loss and **PIK3CA** mutations in MaBCs than in ER-positive/HER2-negative FBCs suggest that at least a subset of MaBCs may be driven by a distinct repertoire of genetic alterations, and most likely, follow a different evolutionary pathway. Given that the adult male breast, outside of systemic hormonal imbalance, is not as fully developed as the adult female breast, and may be composed of cell populations distinct from those of the adult female breast, it could be posited that the genetic differences in FBC and MaBC may be in part related to differences in their cell of origin or the differentiation status of the cell of origin of these tumors.

Actionable somatic genetic alterations, although present in MaBCs, are limited compared to FBCs. For instance, **PIK3CA** mutations, which are present in approximately 35% of FBCs, were found in only 20% of the MaBCs analyzed here. Importantly, however, we have identified potentially pathogenic somatic mutations affecting DNA repair-related genes, including **PALB2** and **FANCM**, which may also be exploited therapeutically. Given that the majority of breast cancer patients enrolled in breast cancer-specific clinical trials are female and that the DNA repair-related genes mutated in MaBCs are rarely altered in FBCs, studies investigating the potential use of therapeutic agents targeting DNA repair-defects caused by the loss of function of these genes are warranted.

The survival analysis performed in this retrospectively accrued cohort of MaBCs revealed that mutations affecting **GATA3** and DNA repair pathway-related genes were associated with disease-free and both disease-free and overall survival, respectively. These differences were not significant when tested in a multivariate survival model including known prognostic parameters (e.g. age, size and nodal status). Further studies investigating the prognostic impact of mutations affecting **GATA3** and DNA repair pathway-related genes in larger cohorts of MaBCs are warranted.
Male breast cancer: genomic profiling

Our study has important limitations, including the retrospective nature of the study design, its relatively small sample size, and the fact that only 17% of the patients consented for germline genetic analysis. Although we cannot exclude that the differences between MaBCs and FBCs may have stemmed from differences in the prevalence of BRCA2 mutation carriers in the cohort of MaBCs analyzed here, a previous study (15) demonstrated that MaBCs in BRCA2 mutation carriers have a low frequency of PIK3CA mutations (6%), a gene whose mutation frequency was relatively high in our cohort (20%). Given that BRCA2 mutations have been reported in 2% to 8% of all MaBCs (1), the overall frequency of 5% of BRCA2 germline mutations in the cases included in this study suggests that it is unlikely that a substantial number of BRCA2 germline mutations were missed in the cohort included in this study. Moreover, the exploratory, hypothesis-generating analysis MaBCs stratified into luminal A-like and luminal B-like was performed using a validated immunohistochemical surrogate for luminal A and luminal B breast cancers as defined by PAM50 (27); albeit validated, the agreement between this immunohistochemical surrogate and the PAM50 results for the classification of the same cases is by no means perfect. Despite these limitations, our data suggest that although overall MaBCs and FBCs appear to share similar patterns of genetic alterations, their mutational landscapes differ, with notable differences in the distribution and frequency of the most frequently mutated genes. In particular, a significant enrichment for mutations in DNA repair-related genes was observed in MaBCs. Given these important differences, further investigation to define potentially targetable genetic alterations and the optimal clinical management of MaBC is warranted. On the basis of this work, we would contend that caution should be exercised in the extrapolation of biological findings and potential targeted therapies for MaBCs from prior studies performed in FBCs.

AUTHORS' CONTRIBUTIONS


Development of methodology: S. Piscuoglio, C.K.Y. Ng, B. Weigelt and J.S. Reis-Filho.
Male breast cancer: genomic profiling

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.):
M. Murray, E. Guerini Rocco, F-C. Bidard, N. Fusco, R.A. Sakr, C.A. Eberle, G.S. Macedo, M. Akram, T. Baslan, J.B. Hicks, T.A. King and E. Brogi.

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis):

Writing, review, and/or revision of the manuscript:

FINANCIAL SUPPORT
SP is funded by a Susan G Komen Postdoctoral Fellowship Grant (PDF14298348) and GSM by CAPES (#BEX 5714/14-1). Research reported in this publication was supported in part by a Cancer Center Support Grant of the National Institutes of Health/National Cancer Institute (Grant No. P30CA008748). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
REFERENCES


Male breast cancer: genomic profiling


Male breast cancer: genomic profiling


Male breast cancer: genomic profiling


**Table 1:** Clinico-pathologic features and immunohistochemical subtypes of the 59 male breast cancers included in this study.

<table>
<thead>
<tr>
<th>Clinico-pathologic features</th>
<th>Male breast cancers (n=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median age at diagnosis (years)</strong></td>
<td>66 (range: 33-88)</td>
</tr>
<tr>
<td><strong>Histologic grade</strong>*</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>2</td>
<td>29 (49%)</td>
</tr>
<tr>
<td>3</td>
<td>26 (44%)</td>
</tr>
<tr>
<td><strong>Median size (cm)</strong></td>
<td>2.0 (range: 0.6-4.7)</td>
</tr>
<tr>
<td><strong>Histologic type</strong></td>
<td></td>
</tr>
<tr>
<td>IDC-NST</td>
<td>54 (91%)</td>
</tr>
<tr>
<td>IPC</td>
<td>2 (3%)</td>
</tr>
<tr>
<td>IMC</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>IMuC</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Mixed ductal lobular carcinoma</td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>ER status</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Positive</td>
<td>59 (100%)</td>
</tr>
<tr>
<td><strong>PR status</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>7 (12%)</td>
</tr>
<tr>
<td>Positive</td>
<td>52 (88%)</td>
</tr>
<tr>
<td><strong>HER2 status</strong></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>57 (97%)</td>
</tr>
<tr>
<td>Positive</td>
<td>2 (3%)</td>
</tr>
<tr>
<td><strong>Ki-67 labeling index</strong></td>
<td></td>
</tr>
<tr>
<td>High (≥14%)</td>
<td>39 (66%)</td>
</tr>
<tr>
<td>Low (&lt;14%)</td>
<td>20 (34%)</td>
</tr>
<tr>
<td><strong>Intrinsic subtype</strong>**</td>
<td></td>
</tr>
<tr>
<td>Luminal A-like</td>
<td>17 (29%)</td>
</tr>
<tr>
<td>Luminal B-like</td>
<td>42 (71%)</td>
</tr>
</tbody>
</table>

ER, estrogen receptor; IDC-NST, invasive ductal carcinoma of no special type; IMC, invasive micropapillary carcinoma; IMuC, invasive mucinous carcinoma; IPC, invasive papillary carcinoma; PR: progesterone receptor.

* according to the Nottingham grading system (22); ** defined by immunohistochemical analysis according to St. Gallen criteria (27).
Male breast cancer: genomic profiling

FIGURE LEGENDS

Figure 1: Repertoires of mutations and copy number alterations in male breast cancer, ER-positive/HER2-negative female breast cancer and female breast cancer of luminal subtype.

Somatic mutations in the 241 genes, either recurrently mutated in female breast cancer (FBC) or related to DNA repair, included in the targeted capture massively parallel sequencing assay employed in this study. The results for (A) ER-positive/HER2-negative male breast cancer (MaBC) and ER-positive/HER2-negative female breast cancer and (B) male breast cancer and FBC of luminal subtype are ordered from top to bottom in decreasing order of mutational frequency in MaBCs (Top). Patterns of copy number alterations in MaBCs and FBCs in selected genes (Middle). Expression of ER, PR, HER2 and Ki-67 (MaBC only) as defined by immunohistochemistry (IHC) in the samples analyzed. In (A), MaBCs and FBCs were classified into clinical subgroups based on ER and HER2 status and in (B), MaBCs were classified into luminal A-like and luminal B-like subtypes defined based on their immunohistochemical profiles following the St Gallen’s criteria (27), and FBCs are classified into luminal A and luminal B molecular subtypes based on PAM50. Sequencing and copy number data of the FBCs were retrieved from The Cancer Genome Atlas (13).

Figure 2: Patterns of copy number alterations in male breast cancer.

Repertoire of copy number alterations as defined by targeted capture massively parallel sequencing in male breast cancers classified into luminal A-like and luminal B-like subtypes. Samples are distributed along the y-axis; the 232 genes mapping to autosomes included in the targeted capture massively parallel sequencing assay are distributed according to their genomic position on the x-axis. Dark blue: amplification, light blue: copy number gain; white: neutral; light red: copy number loss; dark red: homozygous deletion.

Figure 3: Comparative genomic profiling of luminal A-like and luminal B-like male breast cancers.

Frequency plots and multi-Fisher’s exact test comparisons of chromosomal gains and losses in luminal
Male breast cancer: genomic profiling

A-like (top) and luminal B-like (middle) male breast cancers (MaBCs). The frequency of gains (green bars) or losses (purple bars) for each gene is plotted on the y-axis, according to their genomic position on the x-axis. Inverse Log₁₀ values of the Fisher’s exact test p-values are plotted according to genomic location (x-axis). Note the lack of any significant differences in the copy number profiles between luminal A-like and luminal B-like MaBCs.

**Figure 4: Ingenuity Pathway Analysis of 25 genes mutated in ER-positive/HER2-negative male breast cancers but not in ER-positive/HER2-negative female breast cancers from The Cancer Genome Atlas study.**

Likely pathogenic mutations present in ER-positive/HER2-negative male breast cancers but not targeted by likely pathogenic mutations in ER-positive/HER2-negative female breast cancers from The Cancer Genome Atlas (TCGA) study were annotated using Ingenuity Pathway Analysis (IPA). The ‘DNA replication, recombination and repair’ network was significantly enriched for the 25 genes affected by likely pathogenic mutations present in ER-positive/HER2-negative male breast cancers but not in ER-positive/HER2-negative female breast cancers from TCGA (IPA score 17).

**Figure 5: Patterns of copy number alterations in male breast cancers and ER-positive/HER2-negative female breast cancers/ post-menopausal ER-positive/HER2-negative female breast cancers from The Cancer Genome Atlas study.**

Frequency plots of chromosomal gains and losses in 57 ER-positive/HER2-negative male breast cancers (MaBCs) compared to 250 ER-positive/HER2-negative female breast cancers (FBCs) (A) and 132 post-menopausal ER-positive/HER2-negative FBCs (B) from The Cancer Genome Atlas study. The frequency of gains (green bars) or losses (purple bars) for each gene is plotted on the y-axis, according to their genomic position on the x-axis. Inverse Log₁₀ values of the Fisher’s exact test p-values are plotted according to genomic location (x-axis). Copy number data of FBCs were retrieved from The Cancer Genome Atlas (13).
**Figure 1**

**A**

**ER-positive/HER2-negative male breast cancer (n=57)**

- **Mutations**
  - PIK3CA (16%)  
  - GATA3 (16%)  
  - TP53 (7%)  
  - MAP2K1 (7%)  
  - KMT2C (6%)  
  - ATM (4%)  
  - CDH1 (2%)  
  - MAP2K4 (2%)  
  - PTEN (2%)  
  - TBX3 (2%)  
  - CTCF (2%)  
  - CDKN1B (2%)  
  - RUNX1 (0%)  
  - AKT1 (0%)  
  - CBF2 (0%)  
  - NCOA1 (0%)  
  - FAM157B (0%)

- **Copy number alterations**

**IHC**

- ER  
- PR  
- HER2  
- Ki-67

**B**

**Male breast cancer (n=59)**

**Luminal A-like**

- **Mutations**
  - PIK3CA (20%)  
  - GATA3 (15%)  
  - TP53 (12%)  
  - MAP2K1 (10%)  
  - KMT2C (5%)  
  - ATM (3%)  
  - CDH1 (2%)  
  - MAP2K4 (2%)  
  - PTEN (2%)  
  - TBX3 (2%)  
  - CTCF (2%)  
  - CDKN1B (2%)  
  - RUNX1 (0%)  
  - AKT1 (0%)  
  - CBF2 (0%)  
  - NCOA1 (0%)  
  - FAM157B (0%)

- **Copy number alterations**

**IHC**

- ER  
- PR  
- HER2  
- Ki-67

**Luminal B-like**

- **Mutations**
  - PIK3CA (41%)  
  - GATA3 (14%)  
  - TP53 (19%)  
  - MAP2K1 (11%)  
  - KMT2C (7%)  
  - ATM (2%)  
  - CDH1 (9%)  
  - MAP2K4 (6%)  
  - PTEN (4%)  
  - TBX3 (3%)  
  - CTCF (3%)  
  - CDKN1B (1%)  
  - RUNX1 (4%)  
  - AKT1 (3%)  
  - CBF2 (2%)  
  - NCOA1 (4%)  
  - FAM157B (0%)

- **Copy number alterations**

**IHC**

- ER  
- PR  
- HER2  
- Ki-67

**Luminal female breast cancer (n=315)**

**Luminal A**

- **Mutations**
  - PIK3CA (41%)  
  - GATA3 (14%)  
  - TP53 (19%)  
  - MAP2K1 (11%)  
  - KMT2C (7%)  
  - ATM (2%)  
  - CDH1 (9%)  
  - MAP2K4 (6%)  
  - PTEN (4%)  
  - TBX3 (3%)  
  - CTCF (3%)  
  - CDKN1B (1%)  
  - RUNX1 (4%)  
  - AKT1 (3%)  
  - CBF2 (2%)  
  - NCOA1 (4%)  
  - FAM157B (0%)

- **Copy number alterations**

**IHC**

- ER  
- PR  
- HER2  
- Ki-67

**Luminal B**

- **Mutations**
  - PIK3CA (41%)  
  - GATA3 (14%)  
  - TP53 (19%)  
  - MAP2K1 (11%)  
  - KMT2C (7%)  
  - ATM (2%)  
  - CDH1 (9%)  
  - MAP2K4 (6%)  
  - PTEN (4%)  
  - TBX3 (3%)  
  - CTCF (3%)  
  - CDKN1B (1%)  
  - RUNX1 (4%)  
  - AKT1 (3%)  
  - CBF2 (2%)  
  - NCOA1 (4%)  
  - FAM157B (0%)

- **Copy number alterations**

**IHC**

- ER  
- PR  
- HER2  
- Ki-67

**ER-positive/HER2-negative female breast cancer (n=250)**

- **Mutations**
  - PIK3CA (42%)  
  - GATA3 (12%)  
  - TP53 (22%)  
  - MAP2K1 (12%)  
  - KMT2C (6%)  
  - ATM (1%)  
  - CDH1 (9%)  
  - MAP2K4 (6%)  
  - PTEN (4%)  
  - TBX3 (3%)  
  - CTCF (3%)  
  - CDKN1B (2%)  
  - RUNX1 (4%)  
  - AKT1 (4%)  
  - CBF2 (2%)  
  - NCOA1 (4%)  
  - FAM157B (0%)

- **Copy number alterations**

**IHC**

- ER  
- PR  
- HER2  
- Ki-67
Figure 2

![Genome-wide copy number landscape for Luminal A-like and Luminal B-like breast cancer subtypes](image)

- **Red**: Homozygous deletion
- **Dark blue**: Copy number gain
- **Light blue**: Copy number neutral
- **Light orange**: Copy number loss
- **Black**: Amplification

Chromosome numbering from 1 to 22.
Figure 3

Luminal A–like male breast cancer (n=17)

Luminal B–like male breast cancer (n=42)

Fisher's exact test (log scale)

% of cases

% of cases

p-value

Chromosome
Clinical Cancer Research

The Genomic Landscape of Male Breast Cancers
Salvatore Piscuoglio, Charlotte K. Y. Ng, Melissa P. Murray, et al.

Clin Cancer Res  Published OnlineFirst March 9, 2016.

Updated version
Access the most recent version of this article at:

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2016/03/09/1078-0432.CCR-15-2840.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.