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Comparative Genomic Profiling of Second Breast Cancers following First Ipsilateral Hormone Receptor–Positive Breast Cancers

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Purpose: We compared the mutational profile of second breast cancers (SBC) following first ipislateral hormone receptor–positive breast cancers of patient-matched tumors to distinguish new primaries from true recurrences.

Experimental Design: Targeted next-generation sequencing using the Oncomine Tumor Mutation Load Assay. Variants were filtered according to their allele frequency ≥ 5%, read count ≥ 5X, and genomic effect and annotation. Whole genome comparative genomic hybridization array (CGH) was also performed to evaluate clonality.

Results: Among the 131 eligible patients, 96 paired first breast cancer (FBC) and SBC were successfully sequenced and analyzed. Unshared variants specific to the FBC and SBC were identified in 71.9% and 61.5%, respectively. Paired samples exhibited similar frequency of gene variants, median number of variants per sample, and variant allele frequency of the reported variants except for *GATA3*. Among the 30 most frequent gene alterations, *ARIDIA*, *NSD2*, and *SETD2* had statistically significant discordance rates in paired samples. Seventeen paired samples (17.7%) exhibited common variants and were considered true recurrences; these patients had a trend for less favorable survival outcomes. Among the 8 patients with available tissue for CGH analysis and considered new primaries by comparison of the mutation profiles, 4 patients had clonally related tumors.

Conclusions: Patient-matched FBC and SBC analysis revealed that only a minority of patients exhibited common gene variants between the first and second tumor. Further analysis using larger cohorts, preferably using single-cell analyses to account for clonality, might better select patients with true recurrences and thereby better inform the decision-making process.

Introduction

Over the past decades, the improvement in surgical and radiotherapy techniques and the evolution in adjuvant and neoadjuvant systemic therapies have reduced the risk of locoregional and distant recurrences which allowed a more extensive use of breast conservative treatments (1). In comparison with older series, contemporary multimodality breast conservative treatments have substantially reduced the cumulative risk of locoregional recurrences below 4% to 10% at 10 years (2). Patients with younger age at diagnosis, larger tumor size, higher tumor grade, and presence of lymphovascular invasion remain at risk of developing locoregional recurrence (3–7). Upon local recurrence, mastectomy is considered the standard of care and conservative treatment may be an option in selected cases. Following surgery, the CALOR trial supported the use of adjuvant chemotherapy in patients with hormone receptor (HR)-negative locoregional recurrences, but not in those with HR-positive recurrences (6).

Women with HR-positive breast cancers exhibit the lowest risk of locoregional recurrence compared to other tumor subtypes with a 5-year local recurrence rate of 0.8% to 2.9% after breast conservative therapy (8–12). We have previously shown that the cumulative incidence of isolated local recurrences among women with HR-positive breast cancer increased steadily between 1 and 10 years from 0.2% to 2.5% (13). Local recurrences of HR-positive breast cancers are associated with a better prognosis and present a lower risk of synchronous or metachronous distant metastases in comparison with other breast cancer pathologies (6, 14). These clinical characteristics, in particular the late occurrence often 10 years after initial diagnosis, call into question whether these second breast cancers (SBC) are true recurrences or new primary tumors and ultimately the optimal systemic treatment after the local control (15). In clinical practice, the two entities are managed similarly although published data allude to biological and prognostic differences that can impact survival outcomes and treatment strategies (16–18). To address this question, we compared the genomic profile of HR-positive first breast cancers (FBC) with those of patient-matched ipsilateral SBC.

Materials and Methods

Patients

This retrospective analysis used individual-participant data from women treated at Gustave Roussy Cancer Center, France and Trieste University Hospital, Italy between 1992 and 2018. Eligible patients

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Translational Relevance

Using a targeted sequencing panel, we identified a number of genomic alterations that were present in the second breast cancer (SBC) but not in the ipsilateral first tumor. Patient-matched analysis revealed that around 18% of patients exhibited common gene variants in the first and SBC and can subsequently be considered true recurrences. We used copy-number alteration analysis to account for clonality emergence. Further analysis on larger cohorts, preferably using high-throughput molecular analyses (genome/exome sequencing) and single-cell analyses, might better select patients with true local recurrences from hormone receptor–positive breast cancer from those with new primary tumors and thereby better inform the decision-making process.

were women with histologically confirmed HR-positive (IHC estrogen or progesterone receptor expression of 10% and above) breast cancer that presented any type of ipsilateral SBC with locoregional involvement. Only patients with available tissue sample from the FBC and SBC, collected before any systemic therapies were included. The study excluded women that failed the study procedures detailed below. All participants have signed an institutional informed consent document for tissue biobanking of biological residual materials. The study respected the Declaration of Helsinki and European Good Clinical Practice requirements. The data generated in this study are available upon reasonable request from the corresponding author.

Tumor samples

Formalin-fixed, paraffin-embedded (FFPE) specimens of paired primary FBC and SBC of eligible patients before any systemic therapy were collected. Tumor sections of 3 to 4 μm were cut and stained using hematoxylin and eosin. Two experienced pathologists (I. Garberis and M. Lacroix-Triki) reviewed the slides to evaluate tumor cellularity. FFPE blocks with a tumor cell content of at least 50% underwent further processing for targeted next-generation sequencing (tNGS) and whole genome comparative genomic hybridization array (CGH).

Molecular analyses

Tumor DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and tNGS was performed, according to the manufacturer's instructions, on the IonTorrent S5 NGS platform with the Oncomine Tumor Mutation Load Assay covering 409 critical oncogenes or tumor suppressor genes. Briefly, 10 ng of each sample was used for manual library amplification. After the purification and dilution, the libraries were pooled together and then automatically charged on Ion 540 chip using Ion Chef Instrument (ThermFisher Scientific). The bioinformatics analysis was based on the Ion Reporter Suite software (v.5.14; Thermo Fisher Scientific). We retained the matched samples that passed the quality check and had a total mean depth coverage ≥ 300. Variants were filtered on their allele frequency ≥ 5% and a read count ≥ 5 reads, then subsequently selected according to their genomic effect by prioritizing the most relevant alterations and removing artifacts and benign polymorphisms. Only missense, non-sense, frameshifts, start/stop codon gain or loss, in-frame InDels and splice site variants not known as common polymorphisms (<0.1% in gnomAD and ESP database) were retained as variants of interest. Synonymous mutations were removed because their effect is commonly minimal and evolutionarily neutral. These alterations were then reviewed by a molecular geneticist, including visual inspection of sequencing data with Alamut Visual (version 2.15; SOPHiA GENETICS, Lausanne, Switzerland). We did not limit the filtering criteria to the known driver of breast cancer, despite their prominent functional consequences, because the other genes may be useful for identifying concordances between primary and secondary tumors and thus finding true recurrences.

The SNP array scanning was performed on the Affymetrix platform (ThermFisher Scientific) with OncoScan copy-number variation based on same FFPE DNA samples. Default parameters and quantification from the devices were used. Scans were then analyzed and annotated with our own bioinformatic pipeline as previously described (19). The SNP array analysis was mainly used to detect tumor gene amplifications as well as deletions (amplifications > $\times 0.7$ $\log_2$ ratio and deletions < 0.5 $\log_2$ ratio) and were reviewed molecular geneticist.

Statistical analysis

For the clinical analysis, we used descriptive statistics, specifically proportions for qualitative variables and median with range for quantitative variables to summarize patient, tumor and treatment characteristics. Disease-free survival (DFS) was defined as the time between FBC diagnosis and local recurrence with or without metachronous regional or distant metastases. Post-recurrence survival analyses included distant disease-free survival (DDFS) and overall survival (OS) and were defined as the time between SBC and distant recurrence, and death, respectively. Alive patients were censored at the date of the last follow-up update. DFS, DDFS, and OS functions were estimated using the Kaplan–Meier method, and the log-rank test was used to assess differences between groups. Median DFS and OS were reported as point estimates and 95% confidence intervals (CI) in round brackets. For the uni- and multivariable analyses, we used a Cox regression model to adjust for confounding factors and to compute the corresponding hazard ratio (HR and aHR, respectively) and corresponding 95% CI.

For the genomic analysis, we reported the number (proportions) and median (range) of recurrent and passenger variants. The concordance rate of the variants between the FBC and SBC was calculated by computing the ratio of the number of shared variants over the number of shared and unshared variants. The enrichment of unshared/shared variants for the whole cohort was also computed. To perform this analysis, we first calculated the proportion of all variants in our data set that were unshared ($850/868 = 0.98$). For a null model, we parametrized a binomial model with the probability of success equal to 0.98, the number of trials equal to the total number of mutations in a gene (counting each shared variant once), and the number of successes equal to the number of unshared mutations. We calculated a two-sided *P* value from this binomial model for each gene with at least ten mutations (R-package "MutationalPatterns"). We adjusted *P* values for multiple hypothesis testing using the Benjamini-Hochberg procedure and reported as q-values. To evaluate the similarity between FBC and SBC, the paired samples of each patient were examined to determine whether a statistically significant difference existed regarding variations in gene variants by the McNemar test, the median number of variants per sample by the paired Mann–Whitney test, and variant allele frequencies (VAF) of the reported variants between samples by the Student *t* test. Last, we evaluated the correlation of VAF of the detected variants between the two groups.

For the CGH analysis, the resulting data from the tumor matched pairs of the same patients were used to compute the likelihood ratio (LR2) using the package Clonality as described previously (20, 21). Briefly, LR2 was the sum of LR and LR1 where LR calculated the frequencies of gains/losses for each chromosome arm in the dataset

and LR1 calculated the concordance of gain/loss/normal profiles within each arm between pairs, taking into account the discordant events. Patients with LR2 > 1 were considered to have clonally related tumors thus true recurrence, otherwise the tumors were not related and considered new primary.

The statistical analyses were performed using R version 4.1 software. The P value < 0.05 was deemed statistically significant, although formal comparisons were only exploratory in the analysis of clinicopathologic characteristics and survival outcomes between new primary tumors and true recurrences.

Data availability

The data generated in this study are available upon request from the corresponding author.

Results

Patient characteristics and treatment

A total of 131 women were eligible for this study, 91 from Gustave Roussy Cancer Center and 40 from Trieste University Hospital. Ten samples were excluded because either tumor cellularity was below 50% or because their DNA yield was below the required quantity to be analyzed precisely by NGS. Hence, paired FBC and SBC samples of 121 patients underwent tNGS. Pairs from 96 patients were successfully sequenced and retained for analyses (**Fig. 1**). **Table 1** summarizes patient, tumor and treatment characteristics at baseline and recurrence. The median age at diagnosis was 54 years (range, 29–90 years). Tumors were predominantly invasive ductal carcinomas (n = 69; 71.9%) and grade I to II (n = 74; 77.1%). The median tumor size was 20 mm (range, 4.0–130 mm). The treatment strategy involved mainly breast-conserving surgery (n = 69; 71.9%) or mastectomy (n = 27; 28.1%), (neo) adjuvant chemotherapy (n = 38; 39.6%), radiotherapy (n = 70; 72.9%), and (neo)adjuvant endocrine therapy (n = 69; 71.9%). The median DFS was 7.0 years (95% CI, 5.3–8.7); 29 patients (30.2%) were diagnosed with SBC during endocrine therapy.

At the diagnosis of SBC, 7 patients had metachronous distant metastases. The median age was 68 years (range, 31–94 years). Tumors were mostly invasive ductal carcinomas (n = 62; 64.6%) and grade I to II (n = 44; 45.8%). The median tumor size was 15 mm (range, 3.0–80 mm). Patients underwent predominantly mastectomy (n = 55; 57.3%) and excision of the local relapse (n = 35; 36.5%) and did not receive neoadjuvant therapies. Systemic therapies included chemotherapy (n = 37; 38.5%), endocrine therapy (n = 73; 76.0%), and radiotherapy (n = 19; 19.8%). After a median follow-up of 4.8 years (95% CI, 3.7–5.9) from the local recurrence, 34 patients (34.4%) had died and the median OS was 9.6 years (95% CI, 4.0–15.1).

Variant analysis across first and SBC samples

Among the 409 sequenced genes, 886 variants including 810 SNV (non-synonymous, stop/gain) and 76 indels were retained, accounting for 851 different variants (**Fig. 2**). 143 samples exhibited at least one variation. 602 variants (553 SNV and 49 indels) were identified in FBC and 284 variants (257 SNV and 27 indels) in SBC. The most common alterations involved *KMT2D*, *MTOR*, *ATM*, *NOTCH1*, and *ATRX* in FBC and *KMT2D*, *PIK3CA*, *NOTCH2*, *ATM*, and *CDH1* in SBC. The concordance rate between FBC and SBC was 4%, with 36 shared variants and 850 unshared variants. The 886 variants included 10 recurrent variants (60%; 6/10) and 876 nonrecurrent variants (3%, 30/876). The concordance rate was higher for recurrent variants than for nonrecurrent variants (55% vs. 3%; **Table 2**). The recurrent variants concerned predominantly the driver gene *PIK3CA* (cDNA mutation *A3140G* and impact on protein synthesis H1047R). We further investigated investigate whether genes with at least 10 variants (*KMT2D*, *MTOR*, *ATM*, *KMT2A*, *NF1*, *NOTCH1*, *NOTCH2*, *ATRX*, *EP300*, *PIK3CA*, *AMER1*, *APC*, *ARID1A*, *FGFR3*, *NSD2*, *KDR*, *SETD2*, *KDM5C*, *ROS1*, *TP53*, *ABL1*, *CDH1*, and *SMARCA4*) had more unshared or shared mutation than expected by the chance, we used a binomial model. Among these, *ATM*, *NOTCH1*, *PIK3CA*, *FGFR3*, *NSD2*, *TP53*, *CDH1* were the genes with a shared variant between the two groups. Overall, the only statistically significant enrichment in unshared variants was for the gene *PIK3CA* (q-value = 0.0048).

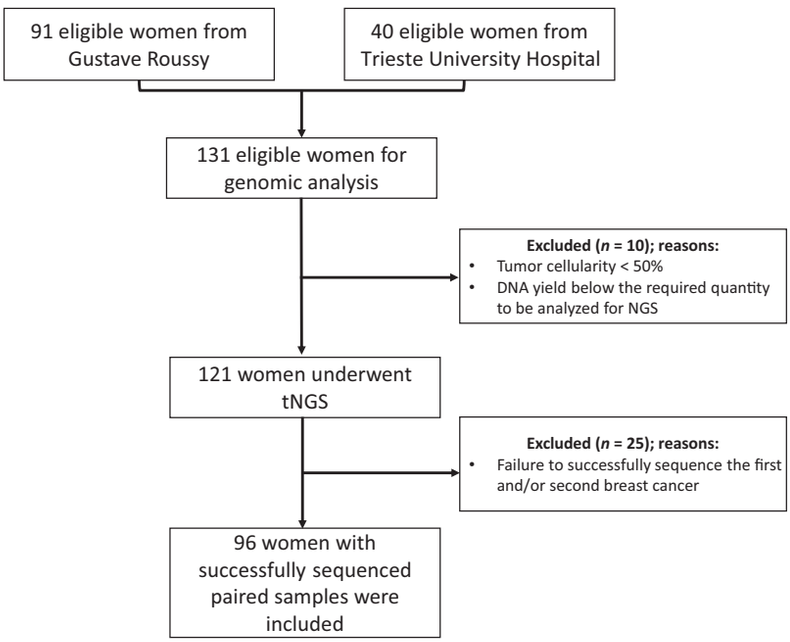


Figure 1.
Flowchart.

Table 1. Summary of the patient and tumor characteristics at first and SBC diagnosis.

Characteristics	At FBC $N = 96$ (%)	At SBC[a] $N = 96$ (%)
Age (years)		
Median	54	68
Range	29–90	31–94
Pathology of the tumor		
Invasive ductal carcinoma	69 (71.9)	62 (64.6)
Invasive lobular carcinoma	17 (17.7)	20 (20.8)
Other invasive carcinoma	10 (10.4)	14 (14.6)
Tumor size (mm)		
Median	20	15
Range	4.0–130	3.0–80
Missing	6	6
Grade		
Grade I	17 (17.7)	1 (1.0)
Grade II	57 (59.4)	43 (44.8)
Grade III	21 (21.9)	45 (46.9)
Missing	1 (1.0)	7 (7.3)
Estrogen receptor expression ≥ 10%		
Positive	84 (87.5)	87 (90.6)
Negative	5 (5.2)	7 (7.3)
Missing	7 (7.3)	2 (2.1)
Progesterone receptor expression ≥ 10%		
Positive	71 (75.0)	66 (68.8)
Negative	17 (17.7)	28 (29.2)
Missing	8 (8.3)	2 (2.1)
Proliferation index		
Ki67 ≥ 20%	15 (15.6)	NR
Ki67 < 20%	22 (22.9)	NR
Missing	59 (61.5)	NR
SLNB and/or axillary dissection	73 (76.0)	NA
Lymph node involvement	38 (39.6)	NA
Missing	4 (4.2)	NA
Surgery		
Mastectomy	27 (28.1)	55 (57.3)
Breast conserving surgery	69 (71.9)	NA
Excision of the local relapse	NA	35 (36.5)
Axillary dissection solely at relapse	NA	2 (2.1)
No surgery[b]	NA	4 (4.2)
(Neo)adjuvant systemic therapy		
(Neo)adjuvant chemotherapy	38 (39.6)	37 (38.5)[c]
(Neo)adjuvant endocrine therapy	69 (71.9)	73 (76.0)[c]
Adjuvant radiotherapy	70 (72.9)	19 (19.8)
Missing	1 (1.0)	1 (1.0)
Adjuvant endocrine therapy		
No adjuvant endocrine therapy	26 (27.1)	19 (19.8)
Tamoxifen ± analogue GnRH	35 (36.4)	9 (9.4)
Aromatase inhibitor ± analogue GnRH	24 (25.0)	54 (56.3)
Tamoxifen followed by aromatase inhibitor	7 (7.3)	2 (2.1)
Aromatase inhibitor followed by tamoxifen	2 (2.1)	0 (0)
Other	1 (1.0)	8 (8.4)
Missing	1 (1.0)	4 (4.2)

Abbreviations: NA, not applicable; NR, not reported; SLNB, sentinel lymph node biopsy.

[a]7 patients had distant metastases at the time of SBC diagnosis.

[b]4 patients did not undergo surgery for the SBC because they were diagnosed with stage IV before surgery ($n = 2$), had other cancers ($n = 1$), and were unfit for surgery ($n = 1$).

[c]These patients did not receive neoadjuvant therapies.

Sixty-nine samples (71.9%) had unshared variants specific to FBC samples and 59 (61.5%) had unshared variants specific to SBC samples.

Comparative analysis in patient-matched first and SBC

To compare FBC and SBC, we examined whether a statistically significant difference existed in the proportion of detected variants, the median number of variants per sample, and VAF of the reported variants in patient-matched samples. Paired samples exhibited similar frequency of gene variants [respectively 78.1% (75/96) vs. 70.8% (68/96); $P = 0.32$], median number of variants per sample [2 (range, 1–89) vs. 2 (range 1–33); $P = 0.08$] and VAF of the reported variants except for *GATA3* ($P = 0.04$). When paired samples were examined to variants in the 30 predominant gene alterations, statistically significant discordance rates were found for *ARIDIA*, *NSD2*, and *SETD2* ($P = 0.013$, 0.0012, and 0.0196 respectively) and a statistical trend towards significance in *ATM*, *KMT2A*, *ATRX*, *NOTCH1*, *PBMR1*, and *ROS1* ($P = 0.052$, 0.109, 0.083, 0.096, 0.078, and 0.059 respectively). Last, we did not detect a correlation between the VAF of the detected variants of the paired FBC and SBC. When evaluating the variants concordance between the paired samples, we noted that 17 pairs (17.7%) exhibited identical variants (**Fig. 3**). We could not evaluate the patterns of convergent evolution because the matched pairs carried only unshared mutations on the same individual gene without shared alterations.

In the following exploratory analysis, we considered the 17 patients that shared identical variants between FBC and SBC as true recurrence and the remaining patients as having new primary breast cancers. The patient and tumor characteristics at the diagnosis of the FBC and SBC as well as the treatment strategy are reported in **Table 3**. The median DFS after the FBC was similar among the patients assigned to the new primary breast cancer and true recurrence groups ($P = 0.988$). In comparison with patients with true recurrence, those with new primaries had numerically longer median OS (9.6 vs. 6.1 years; $P = 0.176$) and DDFS (not reached vs. 4.2 years; $P = 0.110$) following the diagnosis of the SBC (**Fig. 4**). The uni- and multivariable analyses showed that this categorization between true recurrence and new primary breast cancer was not associated with DDFS (HR, 0.543; 95% CI, 0.252–1.171 and aHR, 1.189; 95% CI, 0.566–2.50) and OS (HR, 0.595; 95% CI, 0.277–1.277 and aHR, 0.743; 95% CI, 0.341–1.623).

To further refine our categorization of the true recurrence and new primary breast cancer by taking into consideration clonally related tumors, we performed CGH analysis on the 9 patients with available samples. Using LR2 findings, 5 patients had true recurrences and 4 had new primaries. Among these patients, 8 were considered to have new primaries and one to present a true recurrence according to the aforementioned comparison of the mutational profiles by NGS. Thus, the 8 patients with SBC considered new primaries according to the comparison of the mutational profile by NGS included 4 true recurrences when analyzed for clonality using CGH. Notably one additional patient presenting two clonally unrelated tumors according to LR2 findings had focal rearrangement within chromosome 8 and 11, strongly suggestive of clonality.

Discussion

There are still controversies whether an ipsilateral SBC, often occurring many years after the initial diagnosis, should be considered a true recurrence or a new breast cancer. In the absence of specific guidelines, the choice of the most appropriate systemic treatment following the excision of local recurrence of breast cancer remains a difficult challenge. Adjuvant chemotherapy did not yield survival

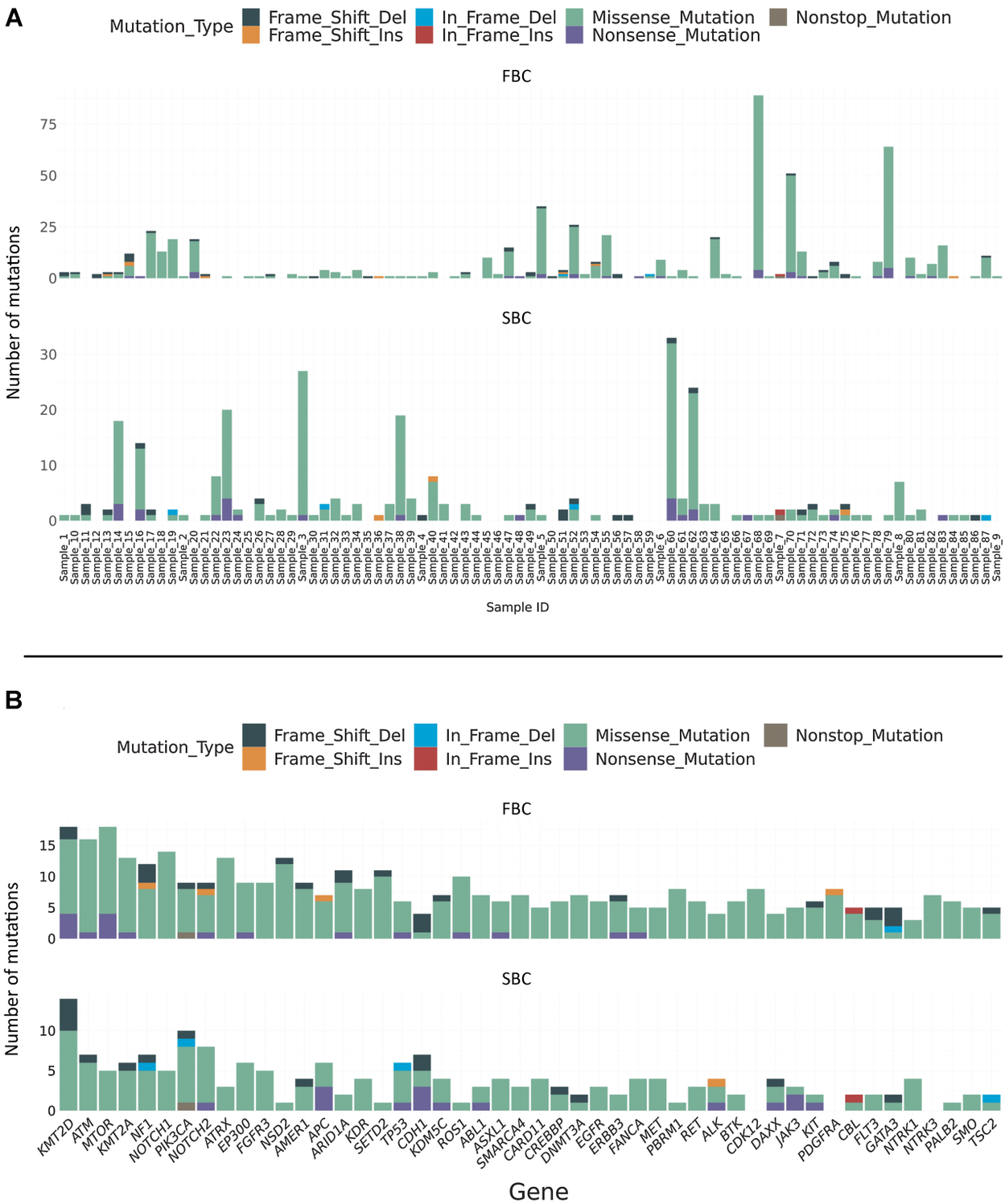


Figure 2.
Mutation analysis in first and second breast cancer: patient-specific mutation counts (**A**) and gene-specific mutation counts (**B**).

Table 2. Concordance between first and SBCs for all detected variants.

Types of variants	All variants (*N*)	Shared variants (*N*)	Unshared variants (*N*)	Concordance rate
All variants	886	36	850	4%
Recurrent variants	10	6	4	55%
Passenger variants	876	30	846	3%

Abbreviation: *N*, number of patients.

benefits among patients experiencing a local recurrence of HR-positive breast cancer, nevertheless, the adjuvant treatment in clinical practice is often individualized according to prior treatment exposure, time to recurrence, and endocrine sensitivity (6). The treatment duration is also unexplored and often established on assumptions used for adjuvant therapies after a new breast cancer diagnosis. Thus, it is essential to distinguish between new primary and true recurrence to better estimate patient's prognosis of patients and ultimately provide the most appropriate systemic treatment.

In our series of 96 patients with paired FBC and SBC analyzed using a targeted sequencing panel, we found similar similar proportions of the detected variants, median number of variants per sample, VAF of the reported variants between the paired FBC and SBC. 17.7% of patients can be confidently considered clonally related and the remaining cases likely reflect a mixture of true recurrences or new primaries. High-throughput molecular analyses using genome/exome sequencing and copy-number alteration analyses have shown higher concordance in comparing matched FBC and ipsilateral SBC (20, 22–24). The discordances between paired FBC and SBC may be attributed to many potential causes. First, there are theoretical differences in carcinogenic processes as true recurrence and distant metastasis enclose a regrowth of malignant tumors that are resistant to primary treatments whereas new primaries are *de novo* tumors arising from the residual breast tissue. Second, the low cellularity of tumor samples can generate false negative results, however, we only included samples with cellularity of at least 50%. Third, tumor heterogeneity and clonality may also account for these differences given that we selected the genes with VAF ≥ 5% and a read count ≥ 5 reads. Our evaluation of the concordance between single biopsy sites of FBC and SBC does not take into consideration the possibility that the two samples may arise from distinct subclones within the same tumor. Finally, the occurence of a divergent SBC profile (vs. FBC) was accounted for a new primary whereas it could be driven by a FBC clone, especially that the developpement of breast cancer is genomcially heterogeneous and subclones are frequently identified (25–29). For instance, SBC with novel gene alterations that were not identified in the paired FBC (14 unshared variants restricted to SBC) were considered new primaries but may indicate accumulations of alterations associated with the therapeutic resistant and clonal selections of true reccurences. Similarly, FBC with unshared variants (24 unshared variants restricted to FBC) may recurr via a clone that branchs off before the acquisition of this mutation in the FBC, herein SBC can also be wrongfully considered a new primary instead of true recurrence (25–29). Among the 8 patients with available samples for CGH analysis and considered to

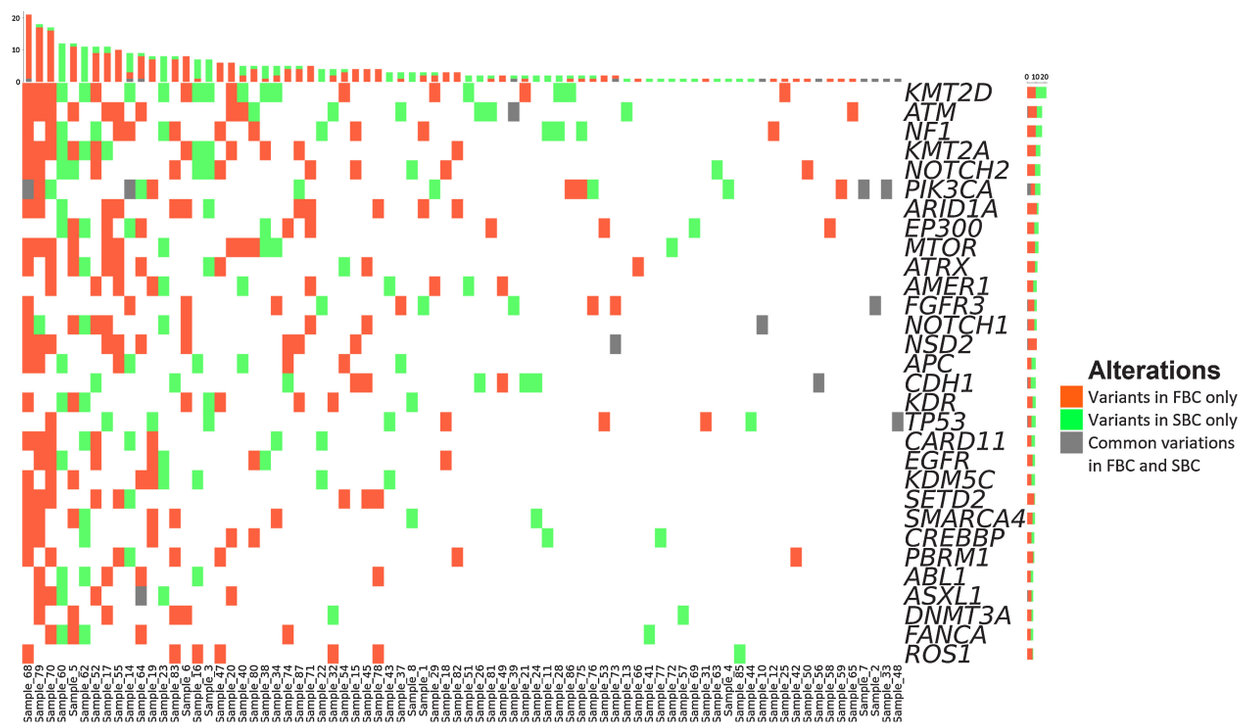


Figure 3.
Distribution of the mutations in the 30 most frequently mutated genes. Genes are ordered from top to bottom by decreasing frequency of gene-specific mutations. Samples are in random order. Common mutations are in gray. Discordant mutations present in the first breast cancer only are in red, and those present in the second breast cancer only are in green.

Table 3. Summary of the patient and tumor characteristics at first and SBC diagnosis according to the two subgroups "new primary breast cancer" and "true recurrence"[a]

	New primary breast cancer *N* = 79 (%)		True recurrence *N* = 17 (%)	
Characteristics	**At FBC**	**At SBC**	**At FBC**	**At SBC**
Age (years)				
Median	54	66	61	72
Range	29–86	31–94	39–90	50–94
Pathology of the tumor				
Invasive ductal carcinoma	57 (72.2)	51 (64.6)	12 (70.6)	11 (64.7)
Invasive lobular carcinoma	13 (16.5)	15 (19.0)	4 (23.5)	5 (29.4)
Other invasive carcinoma	9 (11.4)	13 (16.5)	1 (5.9)	1 (5.9)
Tumor size (mm)				
Median	18	15	18	20
Range	4–70	3–80	4–130	4–50
Grade				
Grade I	16 (20.5)	0 (0)	1 (5.9)	1 (6.7)
Grade II	45 (57.7)	35 (47.3)	12 (70.6)	8 (53.%)
Grade III	17 (21.8)	39 (52.7)	4 (23.5)	6 (40.0)
Estrogen receptor expression ≥ 10%				
Positive	70 (93.3)	71 (91.0)	14 (100)	16 (100)
Negative	5 (6.7)	7 (9.0)	0 (0)	0 (0)
Progesterone receptor expression ≥ 10%				
Positive	60 (80.0)	54 (69.2)	11 (84.6)	12 (75.0)
Negative	15 (20)	24 (30.8)	2 (15.4)	4 (25.0)
Tumor diagnosis during endocrine therapy	NA	23 (30.3)	NA	6 (35.3)
Surgery				
Mastectomy	22 (27.8)	47 (59.5)	5 (29.4)	8 (47.1)
Breast conserving surgery	57 (72.2)	NA	12 (70.6)	NA
Excision of the local relapse	NA	27 (34.2)	NA	8 (47.1)
Axillary dissection solely at relapse	NA	2 (2.5)	NA	0 (0)
No surgery	NA	3 (3.8)	NA	1 (5.9)
Chemotherapy	32 (40.5)	30 (39.0)	6 (35.3)	7 (41.2)
Endocrine therapy	61 (78.2)	60 (77.9)	8 (47.1)	13 (76.5)
Radiotherapy	60 (76.9)	15 (19.2)	10 (58.8%)	4 (23.5)

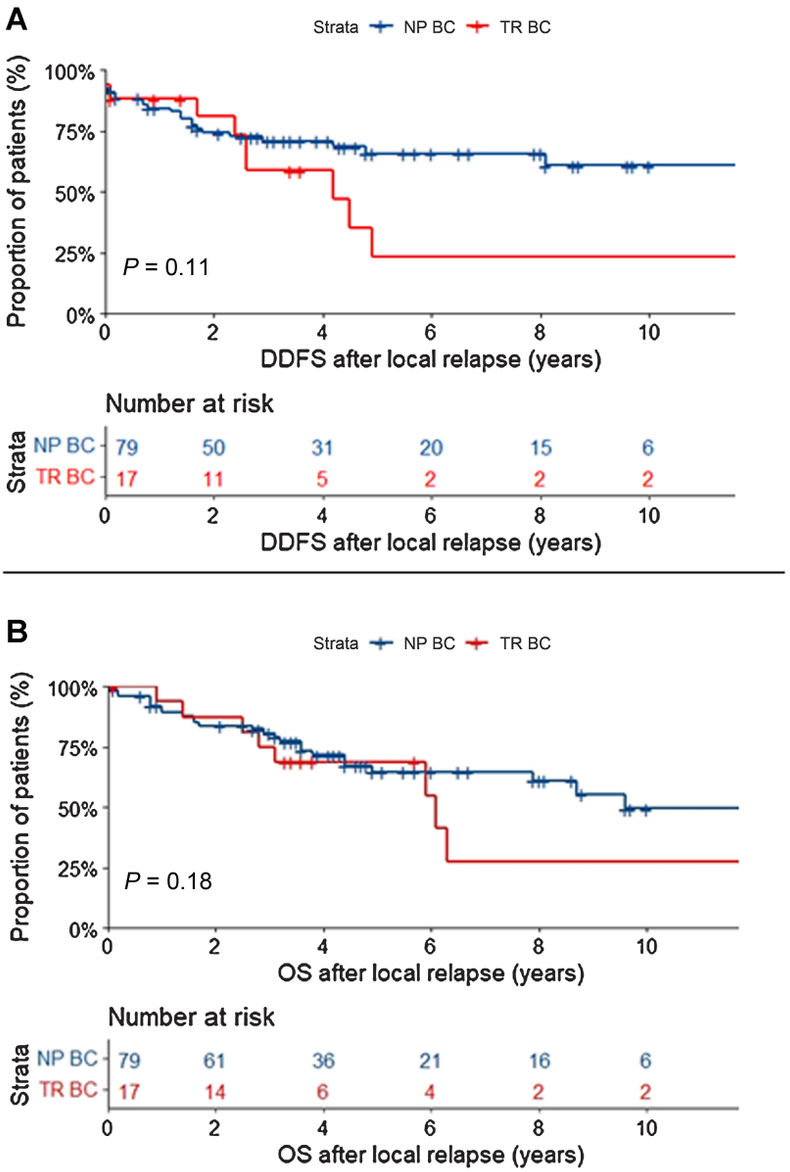
Abbreviations: *N*, number of patients; NA, not applicable.
[a]The patients who shared identical variants between first and SBC as true recurrence and the remaining patients as having new primary breast cancers.

present new primaries according to the comparison of the mutational profile by NGS, 4 patients had clonally related tumors and may be considered true recurrences instead of new primaries.

Recently, Wang and colleagues have reported on the outcomes of 5,413 patients with ipsilateral breast tumor recurrence from the SEER database (18). This study defined true recurrence by the occurrence of SBC in the same quadrant and with consistent histological subtype between the primary tumor and the subsequent tumor recurrence (16–18, 30–32); otherwise, the SBC was considered a new primary tumor. Using this definition, true recurrences and new primaries represented 46% and 54%, respectively. However, the accuracy of this ascertainment should take into consideration the limitations inherent to the SEER registry database. Moreover, the true anatomic site can be misinterpreted following breast conservative surgeries because of oncoplastic surgery and anatomical retractions (33) and the hormonal receptor expression may differ between primary and residual breast cancer after (neo) adjuvant systemic therapies (34). In our series, true recurrences and new primaries defined according to their genomic concordance by targeted NGS represented 17.7% and 82.3%, respectively. Nevertheless, the two studies showed a better prognosis among patients with new primaries. In the SEER analysis, patients with true recurrence had poorer outcomes than new primaries (HR, 0.73; 95% CI, 0.59–0.89) in line with previously published retrospective studies (18, 35). Our exploratory survival analysis also supports these findings showing less favorable survival outcomes among patients with TR, although caution in interpreting this data, given the small sample size in the two cohorts (17 patients with true recurrence and 79 with new primaries).

Although this study covered a knowledge gap in the understanding of local recurrence in a group of patients with HR-positive breast cancer using genomic analysis, several limitations should be acknowledged. The cohort was relatively small in size and therefore these findings need validation in a larger cohort of patients. Given the retrospective study design some additional information were not available and would have been interesting to evaluate such as the occurrence site of the SBC and its distance from the primary tumor bed. Moreover, the patients received heterogeneous treatment strategies after the FBC, mostly depending on the guidelines used at the time period of the first tumor occurrence. We used a bulk tNGS panel of cancer-associated genes instead of more exhaustive genome/exome sequencing and single-cell analyses that allow a comprehensive clonality analysis. Finally, chemical alterations induced by fixation procedures like fixative action and composition, pH, temperature and fixing time, are likely to influence the quality of nucleic acids during the process, as well as the posterior preservation of paraffin samples. The

Figure 4.
DDFS (**A**) and OS (**B**) according to the second breast cancer: new primary or true recurrence.



study of nucleic acids on tissue samples fixed by formalin or AFA (ethanol 75%, formalin 2%, acetic acid 5% and water 18%) is suboptimal as artefactual mutations can occur (36, 37).

Conclusion

As the understanding of the molecular biology of breast cancer and the impact of treatment advances continues to evolve, the identification of true recurrence and new primary would yield important information for future studies to personalize the treatment of patients with ipsilateral SBC. The differences in the genomic profile between FBC and SBC can theoretically correspond to different carcinogenic processes thus a new primary, but can also be attributed to intratumoral heterogeneity and clonal selection following the treatment of FBC. Our findings, if reproduced in larger cohorts preferably using multiregional or single-cell sequencing, would better guide treatment decisions in patients with ipsilateral SBC.

Authors' Disclosures

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Authors' Contributions

E. Rassy: Data curation, formal analysis, writing–original draft, writing–review and editing. **I. Garberis:** Data curation, formal analysis, validation, investigation. **A. Tran-Dien:** Software, formal analysis, validation. **B. Job:** Formal analysis, writing–review and editing. **V. Chung-Scott:** Data curation, validation. **I. Bouakka:** Data curation, validation, investigation. **J. Bassil:** Data curation, validation. **R. Ferkh:** Data curation, validation. **M. Lacroix-Triki:** Data curation, validation, investigation. **F. Zanconati:** Resources, data curation, investigation. **F. Giudici:** Data curation,

software, formal analysis, writing–original draft, writing–review and editing. **D. Generali:** Resources, data curation, investigation. **E. Rouleau:** Resources, data curation, formal analysis, methodology, writing–review and editing. **L. Lacroix:** Resources, data curation, formal analysis, methodology, writing–review and editing. **F. Andre:** Conceptualization, resources, supervision, validation, writing–original draft, project administration, writing–review and editing. **B. Pistilli:** Conceptualization, resources, supervision, validation, investigation, writing–review and editing.

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