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KIT Genetic Alterations in Breast Cancer

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Abstract

AIMS: Activating somatic mutations or gene amplification of *KIT* result in constitutive activation of its receptor tyrosine kinase, which is targetable in various solid tumors. Here, we sought to investigate the presence of *KIT* genetic alterations in breast cancer (BC) and characterize the histologic and genomic features of these tumors.

METHODS: A retrospective analysis of 5,575 BCs previously subjected to targeted sequencing using the FDA-authorized MSK-IMPACT assay was performed to identify BCs with *KIT* alterations. A histologic assessment of *KIT*-altered BCs was conducted, and their repertoire of genetic alterations was compared to that of BCs lacking *KIT* genetic alterations, matched for age, histologic type, estrogen receptor (ER)/HER2 status and sample type.

RESULTS: We identified 18 BCs (0.32%), including 9 primary and 9 metastatic BCs, with oncogenic/likely oncogenic genetic alterations affecting *KIT*, including activating somatic mutations (n=4) or gene amplification (n=14). All *KIT*-altered BCs were of high histologic grade, although no distinctive histologic features were observed. When compared to BCs lacking *KIT*

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COMPETING INTERESTS

JSR-F reports receiving personal/consultancy fees from Goldman Sachs, REPARE Therapeutics and Paige.AI, membership of the scientific advisory boards of VolitionRx, REPARE Therapeutics, Personalis, Bain Capital and Paige.AI, membership of the Board of Directors of Grupo Oncoclinicas, and ad hoc membership of the scientific advisory boards of Roche Tissue Diagnostics, Ventana Medical Systems, Merck, Daiichi Sankyo and Astrazeneca, outside the scope of this study. BW reports ad hoc membership of the scientific advisory board of REPARE Therapeutics, outside the scope of the submitted work. All other authors declare no conflicts of interest.

ETHICS APPROVAL STATEMENT

This study was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center.

CONTRIBUTORSHIP STATEMENT

JSR-F and FP conceived and planned the study. MV, FD, JRS-F and FP reviewed the cases. MV, FD, ADCP, HD, AM, AMG, DNB, PS, DR, PR, HZ, BW, HYW, EB, JSR-F and FP analyzed and interpreted the data. MV, FD and FP wrote the first manuscript, which was reviewed by all coauthors. MV, FD and ADCP contributed equally to this study.

genetic alterations, no distinctive genetic features were identified. In two metastatic *KIT*-altered BCs in which the matched primary BC had also been analyzed by MSK-IMPACT, the *KIT* mutations were found to be restricted to the metastatic samples, suggesting that they were late events in the evolution of these cancers.

CONCLUSIONS: *KIT* genetic alterations are vanishingly rare in BC. *KIT*-altered BCs are of high grade but lack distinctive histological features. Genetic alterations in *KIT* might be late events in the evolution and/or progression of BC.

Keywords

Breast cancer; *KIT*; Receptor tyrosine kinase; Massive parallel sequencing; MSK-IMPACT

INTRODUCTION

KIT maps to 4q11–q12 and encodes c-KIT (CD117), a type III transmembrane receptor tyrosine kinase [1]. Constitutive activation of *KIT* by hotspot activating mutations targeting its cytoplasmic juxta-membrane domain, extracellular region and tyrosine kinase domains have been reported in gastrointestinal stromal tumors (GIST), acral and mucosal melanomas, renal cell carcinomas, dysgerminomas and malignant gliomas [2–9], whereas *KIT* gene amplification has been described in GISTs [10] and melanomas [7,11]. Importantly, *KIT* genetic alterations are well established key therapeutic targets, as highlighted by the effectiveness of imatinib and ripretinib in GISTs [12,13]. Early preclinical studies suggested a potential efficacy of KIT inhibition in breast cancer (BC) models [14–16]. Nonetheless, clinical trials evaluating KIT inhibition in BC patients, either alone or in combination with endocrine therapy and/or chemotherapy, showed no evidence of clinical efficacy [17–20]. These disappointing results led the discontinuation of anti-KIT drug development in BC.

Previous studies focused on BCs displaying c-KIT overexpression rather than *KIT* genetic alterations. Increased c-KIT protein expression has been documented in a small subset (1–13%) of BCs [21,22], and to be rather prevalent in adenoid cystic carcinomas (AdCCs) of the breast [23]. The role of *KIT* oncogenic alterations in BC remains to be investigated. Hence, here, we sought to determine the frequency of *KIT* activating somatic genetic alterations in primary and metastatic BC and to describe the clinicopathologic and genomic features of these tumors.

MATERIALS AND METHODS

Cases

This study was approved by the Institutional Review Board of Memorial Sloan Kettering Cancer Center (MSK). We retrospectively investigated the presence of oncogenic/likely oncogenic somatic mutations affecting *KIT* in targeted sequencing data from 5,575 BCs previously subjected to the FDA-authorized MSK Integrated Mutation Profiling of Actionable Targets (MSK-IMPACT) assay [24] in the clinical setting.

Targeted sequencing analysis

Non-synonymous somatic mutations, amplifications, and homozygous deletions for the cases included in our cohort were retrieved from cBioPortal [25]. The fraction of genome altered (FGA; i.e. the number of copy number segments which are not copy neutral divided by the total number of copy number segments [26]), and the non-synonymous tumor mutation burden (TMB) (i.e. the number of non-synonymous mutations divided by the total genomic region assessed by MSK-IMPACT, per megabase), were retrieved from cBioPortal. Mutational signatures were inferred using SigMA [27], using all synonymous and non-synonymous somatic mutations in BCs with at least five single nucleotide variants (SNVs), as previously described [28,29]. In addition, we retrieved the raw MSK-IMPACT sequencing data (i.e., FASTQ files) and reprocessed them using our validated bioinformatics pipeline [30,31] for two cases with paired primary and metastatic samples to infer the copy number alterations and cancer cell fraction (CCF) using ABSOLUTE [32].

Histopathologic assessment

The histopathologic review and classification of BCs harboring *KIT* oncogenic/likely oncogenic alterations was conducted by four pathologists (MV, FD, JSR-F, FP) following the criteria put forward by the World Health Organization (WHO) [33]. Tumors were graded according to the Nottingham grading system [33,34]. Estrogen receptor (ER) and HER2 status, determined according to the American Society of Clinical Oncology/College of American Pathologists guidelines [35,36], were retrieved from the pathology reports.

Comparison with BCs lacking *KIT* genetic alterations

We compared the frequency of non-synonymous somatic mutations, amplifications and homozygous deletions, non-synonymous TMB, FGA and mutational signatures of the *KIT*-altered BCs (n=18) to those of BCs lacking genetic alterations affecting *KIT* from the study by Razavi et al [37], matched by age, menopausal status, sample type, histologic type and ER/HER2 status to the KIT-altered cases at a 3:1 ratio (n=54).

Immunohistochemistry

c-KIT expression was assessed by immunohistochemistry (IHC) using a Benchmark ULTRA system (Ventana, Oro Valley, AZ). Following heat-based antigen retrieval with the CC1 buffer for 32 minutes, tissue sections were incubated with the anti-CD-117 polyclonal antibody (catalog number: A4502) from DAKO (Glostrup, Denmark) at a 1:2000 dilution for 20 minutes. Subsequently, the primary antibody was detected with a polymer-based secondary kit. Positive and negative controls were included in each slide run. Only c-KIT membranous expression was considered.

Statistical analysis

Statistical analyses were conducted using R (v3.1.2). Comparisons of categorical and continuous variables were performed by using Fisher's exact and Mann-Whitney *U* test, respectively. Multiple testing correction using the Benjamini-Hochberg method was applied to control for the false discovery rate whenever appropriate. $P < 0.05$ was considered as statistically significant. All tests used were two-tailed.

RESULTS

Following a retrospective query of 5,575 BCs previously subjected to targeted sequencing using the FDA-authorized MSK-IMPACT [24], we identified 18/5,575 (0.3%) cases harboring V560_Y578del in-frame deletion (case KIT2; Fig. 1A–1B, Table 1). In addition, to validate the frequency of *KIT* genetic alterations observed, we interrogated the whole-exome sequencing data of 1,108 BCs from The Cancer Gene Atlas (TCGA) [12] for the presence of oncogenic/likely oncogenic alterations in this gene and identified 9/1,108 (0.8%) BCs harboring *KIT* gene amplification (Supplementary Table 1). No *KIT* oncogenic/likely oncogenic mutations were identified in the Breast TCGA cohort.

Clinicopathologic characteristics

We sought to determine whether BCs harboring *KIT* oncogenic genetic alterations would display distinctive histologic features. All primary (n=9) and most (8/9, 89%) metastatic *KIT*-altered BCs from the MSK-IMPACT cohort were invasive ductal carcinomas of no special type (IDC-NSTs). One metastatic BC harboring a *KIT* R634Q missense mutation was an invasive lobular carcinoma. Notably, all primary and metastatic *KIT*-altered BCs identified were of histologic grade 3/ poorly differentiated (Figure 1C–1D, Table 1). Most primary *KIT*-altered BCs were either ER-negative/HER2-negative or HER2-positive (4/9, 44%, each), whereas most metastatic *KIT*-altered BCs were ER-negative/HER2-negative (5/9, 56%; Table 1). Likewise, all *KIT*-altered primary BCs from TCGA (n=11) were IDC-NSTs and were of histologic grade 3. In agreement with our observations in the MSK-IMPACT cohort, most *KIT*-altered primary BCs from TCGA were ER-negative/HER2-negative or HER2-positive (3/9, 33%, each; Supplementary Table 1). Immunohistochemical analysis of c-KIT expression in four *KIT*-altered BCs (primary, n=1; metastatic, n=3) with available material revealed moderate to strong membranous expression in all cases interrogated (Figure 1C–1D, Table 1). Taken together, our findings indicate that BCs harboring *KIT* oncogenic genetic alterations display aggressive histologic features, but no distinctive histologic features.

Repertoire of somatic genetic alterations in *KIT*-altered BCs

We next sought to determine whether *KIT*-altered BCs would genetically differ from cases lacking alterations affecting this gene. We compared the repertoire of non-synonymous somatic genetic alterations in primary and metastatic *KIT*-altered BCs (n=9, each) with that of primary and metastatic *KIT*-wild type (WT) BCs (n=27, each) from the study by Razavi et al [37], matched for age, menopausal status, histologic type and ER/HER2 status at a 3:1 ratio, respectively. *TP53* was the gene found to be most frequently mutated in primary (8/9; 89%) and metastatic (7/9; 78%) *KIT*-altered BCs. Compared to *KIT*-WT BCs, no gene was found to be affected in a statistically significantly different frequency in the *KIT*-altered BCs (Fig. 1A–1B), besides *PDGFRA* and *KDR* that map to the same amplicon as *KIT* and showed frequent co-amplification with this gene in both primary and metastatic BCs (*PDGFRA,* 67% vs 0%, $P<0.01$; *KDR*, 56% vs 0%, $P<0.01$; Fig. 1A–1B). Whilst no differences were detected in the non- synonymous TMB between the different groups (Fig. 1E–1F), the FGA of primary (median, 0.49; range, 0.15–0.6) and metastatic (median, 0.63; range, 0.2–0.8) *KIT*-altered BCs was significantly higher than that of primary (median,

0.26; range, 0.01–0.57; $P<0.05$) and metastatic (median, 0.23; range, 0–0.8; $P<0.01$) BCs lacking alterations in this gene matched by clinicopathologic characteristics, respectively (Fig. 1E–1F). Due to the limited sample size, however, type II or β errors cannot be entirely ruled out. Akin to their matched controls, most primary (6/9; 67%) and metastatic (4/7; 57%) *KIT*-altered BCs displayed a dominant aging (clock-like) mutational signature (Fig. 1A–1B).

Comparative analysis of paired primary and metastatic samples of *KIT*-altered BC

To investigate the role of *KIT* genetic alterations in BC progression, we analyzed two *KIT*-altered metastatic BCs from our cohort for which paired primary BC samples had also been subjected to targeted sequencing using MSK-IMPACT. Case KIT-4 corresponded to a woman in her late 70s who presented with an ER-positive/HER2-negative pleomorphic invasive lobular carcinoma (Fig. 2A). Following mastectomy, despite receiving two lines of therapy including everolimus in combination with an aromatase inhibitor (4 months) and palbociclib plus tamoxifen (12 months), sixteen months later, the patient progressed with a metastatic outgrowth in the skin (Fig. 2B). Our analysis of the paired primary (KIT4-P) and metastatic (KIT4-M) BC samples reveals a clonal *CDH1* frameshift mutation associated with loss-of-heterozygosity (LOH) of the wild-type allele and truncal mutations affecting other genes classically enriched in lobular carcinomas [38,39], including *TBX3* and *KMT2C* loss-of-function mutations, as well as *FOXA1* (I176V) and *ERBB2* (L755S) hotspot mutations (Fig. 2C-2D). We observed a clonal *RB1* frameshift mutation associated with LOH and a likely oncogenic *KIT* R634Q missense mutation restricted to the metastatic sample (Fig. 2C). *RB1* mutations have been shown to be enriched in ER-positive metastatic BC compared to early BC and to be associated with resistance to CDK4/6 inhibitors [40,41]. Nonetheless, it is possible that the p.R634Q *KIT* mutation identified in the metastatic sample of this case may have contributed, at least in part, to BC progression in this case. Both primary and metastatic BC samples displayed a dominant aging mutational signature (Fig. 2D).

Case KIT-1 corresponded to a woman in her mid 40s who presented with an ER-positive/HER2- negative IDC-NST (Fig. 2E). Following surgical excision of the primary tumor, the patient was treated with adjuvant chemotherapy and endocrine therapy. Five years later, the patient relapsed with metastatic BC involving liver, which lacked expression of ER and HER2 (Fig. 2F). Our analysis of the paired primary and metastatic BC samples revealed a truncal *GATA3* frameshift mutation as well as a *KIT* hotspot M552_Y570 inframe deletion, absent in the primary BC sample (Fig. 2G-2H). Taken together, these findings demonstrate that *KIT* genetic alterations may occur as relative late events in BC evolution and suggest a potential role for *KIT* in disease progression and/or acquired treatment resistance in a small subset of BCs.

DISCUSSION

Through the reanalysis of targeted sequencing data of a large cohort of primary and metastatic BCs, we demonstrated that oncogenic alterations affecting *KIT* are vanishingly rare in BC, in contrast to other cancer types such as GIST, melanoma, seminoma, ovarian

dysgerminoma and gliomas [26,42–44]. Approximately 75–80% of GISTs harbor gain-of-function mutations in *KIT*, whereas only <3% of GISTs have a *KIT* gene amplification [10,45]. In contrast, in our cohort of *KIT*-altered BCs only a small subset of cases were found to harbor gain-of-function *KIT* mutations, whereas most cases displayed *KIT* gene amplification, akin to what has been reported for melanoma [11,46], dysgerminoma[2], medulloblastomas and primitive neuroectodermal tumors (PNET) [47].

Although *KIT* genetic alterations have been successfully targeted in other tumor types, as exemplified by the success of imatinib in GISTs [12], clinical trials investigating imatinib monotherapy or combined with chemotherapy and endocrine therapy in BC have yielded disappointing results [17–20]. Although *KIT* gene amplification is considered potentially targetable similarly to *KIT* activating mutations, the efficacy of imatinib in *KIT*-amplified tumors remains contentious [11,48,49]. Given that the selection of cases in previous studies in BC were conducted based on overexpression of c-KIT, rather than on *KIT* genetic alterations, it is possible that the limited efficacy of pharmacologic KIT inhibition observed in BC might be due to the fact that *KIT*-altered BCs are mainly *KIT*-amplified and only minority harbor activating mutations. Three of the BCs studied here harbored mutations targeting the exon 11 of *KIT*, encoding for the juxtamembrane domain, that confer sensitivity to imatinib in GISTs [50], while one of them harbored an exon 13 mutation, frequently associated to resistance to this drug [51]. Whether the rare BCs harboring *KIT* oncogenic mutations would respond to Imatinib remains to be determined.

AdCCs express c-KIT, which is used as ancillary diagnostic tool for this entity [23]. None of the *KIT*-altered cases we identified here were AdCCs, they were all IDC-NSTs instead. These data are in agreement with our previous findings indicating that AdCCs, which are underpinned by *MYB-NFIB* fusion gene, *MYBL1* rearrangements or *MYB* gene amplifications [52], do not harbor *KIT* genetic alterations [23,53]. The mechanism by which c-KIT is upregulated in AdCC is unknown.

Our cohort included two BC in which paired primary and metastatic samples were analyzed, and in which oncogenic/likely oncogenic mutations in *KIT* were restricted to the metastasis, suggesting that, at least in a subset of cases, genetic alterations in this gene might constitute a late event in the evolution and/or progression of BC. Further studies aimed at evaluating the role of *KIT* alterations in progression and in determining resistance to standard treatments in BC, such as endocrine therapy, are warranted.

Our study has important limitations. The small size of the cohort given the rarity of *KIT* genetic alterations in BC did not allow for the comparison of clinical and genomic features with adequate statistical power. Moreover, we were not able to assess the expression of c-*KIT* in all cases systematically due to unavailability of material. Despite these limitations, our findings indicate that genetic alterations affecting *KIT* are exceedingly rare in BC, but detectable in a subset of cases. Although *KIT*-altered BCs were found to be uniformly of high histologic grade, they do not display a distinctive histologic phenotype. In at least a subset of cases, genetic alterations targeting *KIT* might represent a late event in BC evolution or progression and may even might play roles in the acquisition of resistance to standard BC treatments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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KEY MESSAGES

What is already known on this topic

- *KIT* activating mutations or gene amplification, which result in tyrosine kinase activation, are well known therapeutic targets in various tumors.

What this study adds

- Breast cancers harboring oncogenic alterations affecting *KIT* are rare and display aggressive histologic features, but not a distinctive phenotype

How this study might affect research, practice or policy

- *KIT* oncogenic alterations might represent late events in breast cancer progression in a subset of cases

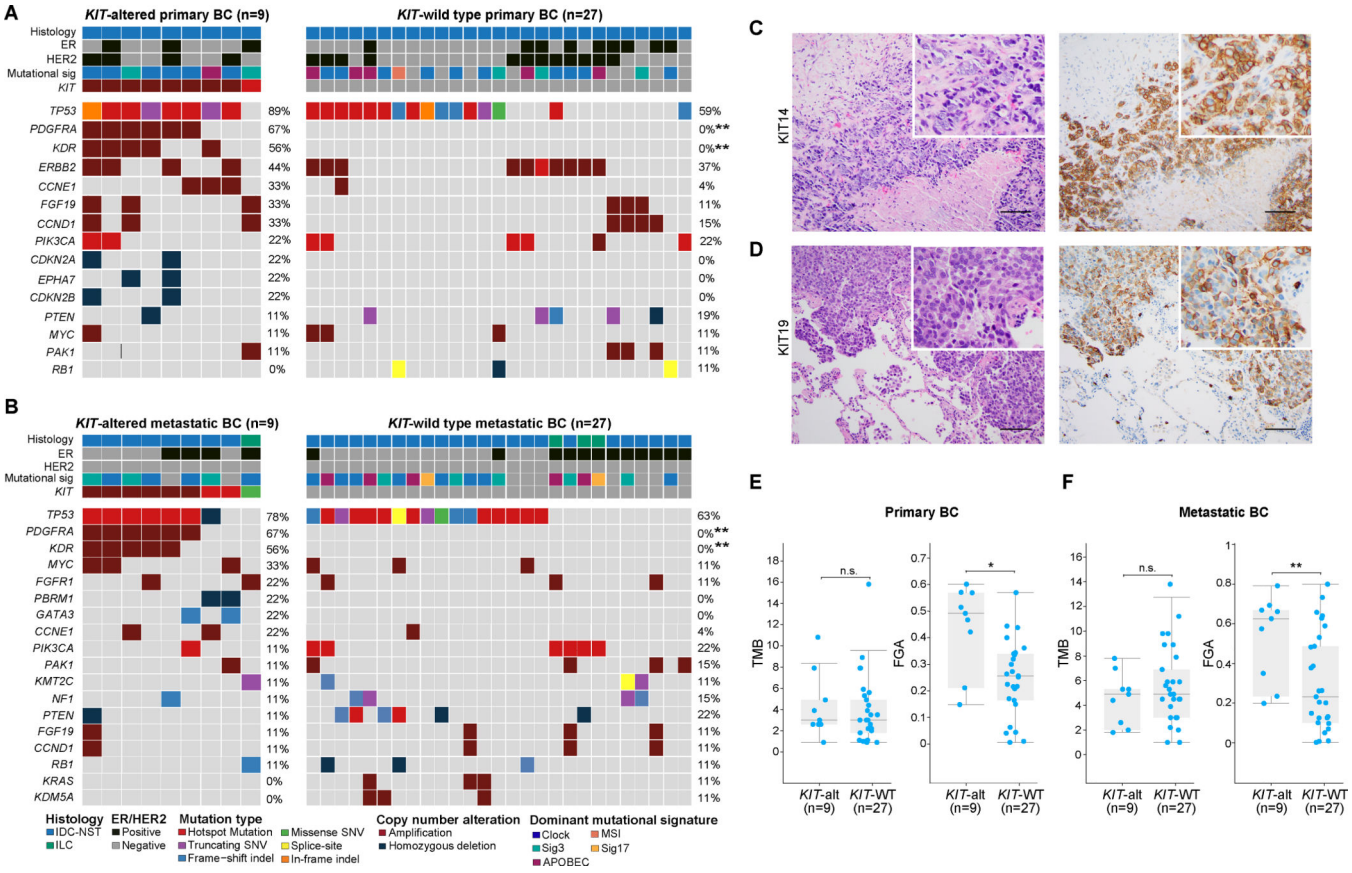

Figure 1. Repertoire of genetic alterations in breast cancers with *KIT* genetic alterations.

(**A-B**) Heatmaps depicting the non-synonymous somatic mutations, amplifications and homozygous deletions in (**A**) primary (n=9) and (**B**) metastatic (n=9) *KIT*-altered breast cancers (BC) compared to BCs lacking *KIT* genetic alterations matched by clinicopathologic features at a 3:1 ratio (n=27, primary and metastatic BC, each). Recurrently affected (≥2 cases) genes in *KIT*-altered BCs and the most frequently altered (≥3 cases) genes in *KIT*-wild type (WT) BCs are shown. Fisher's exact test, FDR adjusted *P* value, **, <0.01. (**C-D**) Representative hematoxylin and eosin (H&E) micrographs (left) and corresponding KIT protein expression micrographs (right) assessed by immunohistochemistry for the **(C)** primary BC case KIT14, and **(D)** metastatic BC case KIT19. Scale bar, 50 microns. (**E-F**) Box-plots displaying the non- synonymous tumor mutation burden (TMB; left) and fraction of genome altered (FGA; right) in **(E)** primary and **(F)** metastatic BCs harboring *KIT* genetic alterations (n=9, each) compared to *KIT*-WT BCs matched by clinicopathologic features at a 3:1 ratio (n=27, each). Mann-Whitney *U* test, *, *P*<0.05; **, *P*<0.01; n.s., non-significant.

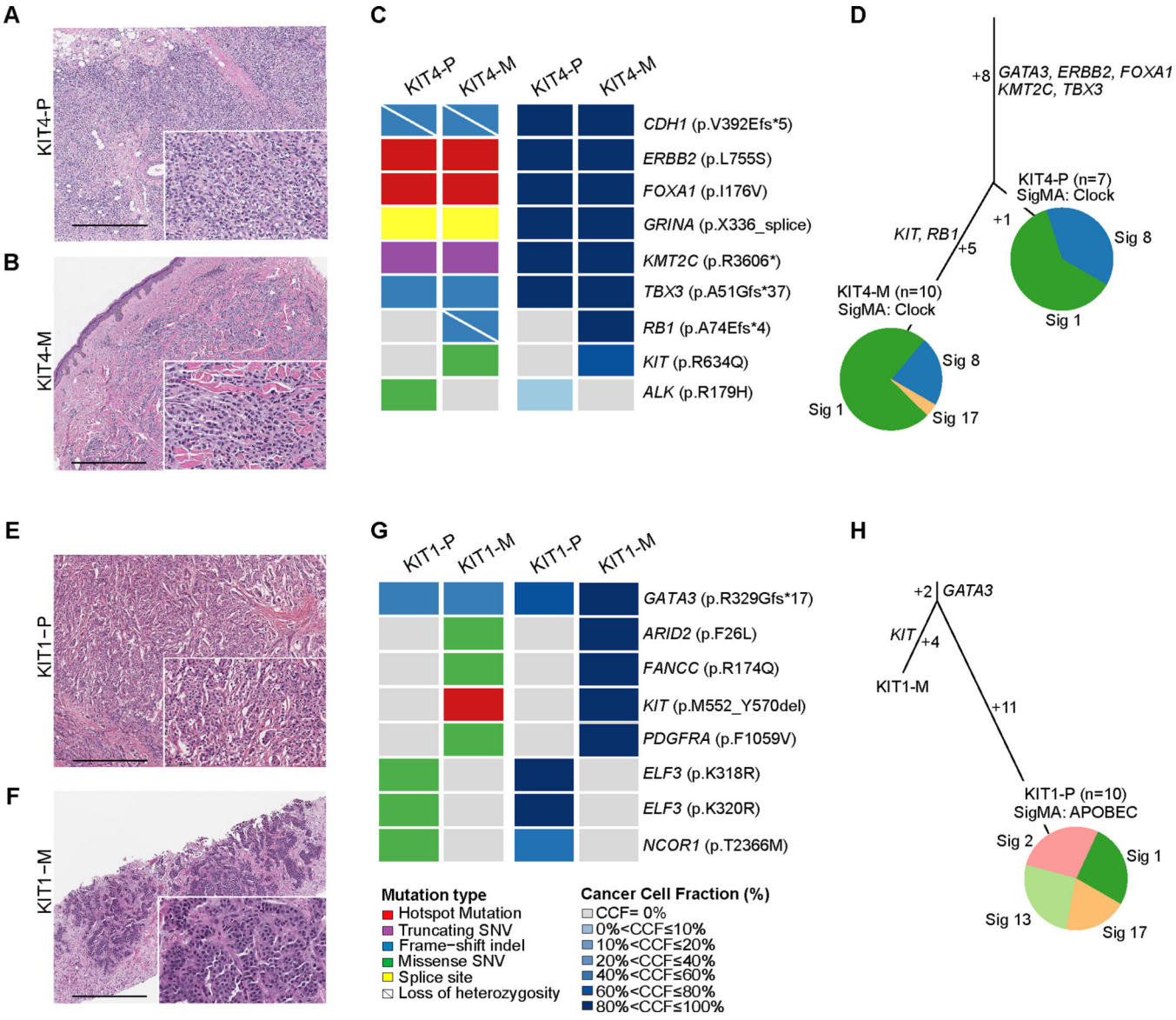

Figure 2. Clonal decomposition and mutational signatures of paired primary and metastatic *KIT*-altered breast cancers.

(A,B,E,F) Representative hematoxylin and eosin (H&E) micrographs of the paired primary (P) and metastatic (M) breast cancer (BC) samples of case KIT4 including **(A)** KIT4-P and **(B)** KIT4-M, and of case KIT1, including **(E)** KIT1-P and **(F)** KIT1-M. Scale bar, 500 microns. **(C,G)** Heatmaps depicting the non-synonymous somatic mutations (left) and cancer cell fraction (right) of the paired primary and metastatic BC samples of cases **(C)** KIT4 and **(E)** KIT1. **(D,H)** Mutation based phylogenetic trees depicting the clonal evolution of the paired primary and metastatic BC samples of **(D)** case KIT4 and **(H)** case KIT1. The length of the trunk and branches of the trees is proportional to the number of shared and private mutations in the primary and metastatic BC samples. Mutational signatures identified in the primary and metastatic BCs with ≥5 SNVs as inferred by SigMA are depicted in pie charts.

Table 1.

Clinicopathologic characteristics of breast cancers harboring oncogenic/likely oncogenic alterations affecting *KIT* from the MSK-IMPACT cohort

Sample ID	Sample type	Histology	Grade/ Differentiation	ER	HER2	*KIT* genetic alteration	Hotspot mutation (*KIT*)	KIT expression (IHC)
KIT1	Metastasis	IDC-NST	Poorly differentiated	Negative	Negative	M552_Y570del	Yes	NP
KIT2	Metastasis	IDC-NST	Poorly differentiated	Positive	Negative	V560_Y578del	Yes	Positive
KIT3	Primary	IDC-NST	Grade 3	Positive	Negative	V559G	Yes	NP
KIT4	Metastasis	Pleomorphic ILC	Poorly differentiated	Positive	Negative	R634Q	No	NP
KIT5	Metastasis	IDC-NST	Poorly differentiated	Positive	Negative	Amplification	NA	NP
KIT6	Primary	IDC-NST	Grade 3	Positive	Positive	Amplification	NA	NP
KIT7	Metastasis	IDC-NST	Poorly differentiated	Positive	Negative	Amplification	NA	NP
KIT9	Primary	IDC-NST	Grade 3	Negative	Positive	Amplification	NA	NP
KIT10	Primary	IDC-NST	Grade 3	Negative	Negative	Amplification	NA	NP
KIT11	Primary	IDC-NST	Grade 3	Positive	Positive	Amplification	NA	NP
KIT12	Metastasis	IDC-NST	Poorly differentiated	Negative	Negative	Amplification	NA	NP
KIT13	Primary	IDC-NST	Grade 3	Negative	Positive	Amplification	NA	NP
KIT14	Primary	IDC-NST	Grade 3	Negative	Negative	Amplification	NA	Positive
KIT16	Primary	IDC-NST	Grade 3	Negative	Negative	Amplification	NA	NP
KIT17	Metastasis	IDC-NST	Poorly differentiated	Negative	Negative	Amplification	NA	Positive
KIT18	Metastasis	IDC-NST	Poorly differentiated	Negative	Negative	Amplification	NA	NP
KIT19	Metastasis	IDC-NST	Poorly differentiated	Negative	Negative	Amplification	NA	Positive
KIT20	Primary	IDC-NST	Grade 3	Negative	Negative	Amplification	NA	NP

ER, estrogen receptor; IDC-NST, invasive ductal carcinoma of no special type; IHC, immunohistochemistry; ILC, invasive lobular carcinoma; NA, not applicable; NP, not performed.