The Influence of Cancer Molecular Subtypes and Treatment on the Mutation Spectrum in Metastatic Breast Cancers

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ABSTRACT

Next-generation sequencing has sparked the exploration of cancer genomes, with the aim of discovering the genetic etiology of the disease and proposing rationally designed therapeutic interventions. Driver gene alterations have been comprehensively charted, but the improvement of cancer patient management somewhat lags behind these basic breakthroughs. Recently, large-scale sequencing that focused on metastasis, the main cause of cancer-related deaths, has shed new light on the driving forces at work during disease progression, particularly in breast cancer. Despite a fairly stable pool of driver genetic alterations between early and late disease, a number of therapeutically targetable mutations have been found enriched in metastatic samples. The molecular processes fueling disease progression have been delineated in recent studies and the clonal composition of breast cancer samples can be examined in detail. Here we discuss how these findings may be combined to improve the diagnosis of breast cancer to better select patients at risk, and to identify targeted agents to treat advanced diseases and to design therapeutic strategies exploiting vulnerabilities of cancer cells rooted in their ability to evolve and drive disease progression.

Background

The past decade was marked by the huge endeavor of the scientific community to define the genomic landscape of cancers (1–5). This work has laid the foundations of precision medicine and has improved the understanding of cancer biology and tumor diversity. For breast cancer, these findings refined the definition of the molecular subtypes previously characterized by gene expression profiling. These different subtypes (luminal, basal, Her2-enriched) are associated with different outcomes, prognoses, and responses to treatment (6–12).

The main limitation of all these studies is the focus on primary tumor without analysis of advanced disease or metastases. The choice of studying early-stage cancers was logical, as histologic material is readily available. Surgery is indeed the only way to cure early-stage cancer. Furthermore, exploring primitive tumor biology gives a direct access to the discovery of major driver genes involved in carcinogenesis and the link between clinical and molecular subtypes. However, metastasis is the main cause of death for patients with cancer and the next step was to analyze advanced and pretreated disease. Recent studies in the field of cancer genomics have shown that cancers present spatial heterogeneity, and metastases do not present the same genomic landscape as their matched primaries (13–16). Furthermore, treatments create a selection pressure on tumor cell populations and can lead to clonal selection/expansion and occurrence of new major driver mutations. In liquid tumors such as chronic myeloid leukemia where longitudinal sampling is possible (17, 18), emergence of resistance mutations in patients receiving imatinib is well described (19–21). Examples of clonal expansion can be found in solid tumors as well. In patients receiving first-generation EGFR inhibitors (22), *EGFR* T790M mutations can occur and in turn be targeted by second-generation EGFR inhibitors (23). Similar observations have been made in gastrointestinal stromal tumor (GIST), where secondary *KIT* mutations involving either the ATP binding pocket of the kinase domain (exons 13 and 14) or the kinase activation loop (exons 17 and 18) occurred after resistance to imatinib (24). Altogether, these data suggest that the genomic alterations observed in lethal, advanced cancers could differ from those of primary tumors, and could be the result of clonal expansion, especially in cancers treated by oncogene de-addiction (25). Similarly, breast cancer' genomes display evolution as the tumor progresses. Indeed, around 20% of patients presenting hormone receptor-positive (HR^+)/$Her2^-$ breast cancer acquire activating estrogen receptor 1 (*ESR1*) mutations during the course of endocrine therapy (26), leading to ligand-independent activation of the receptor, and resistance to endocrine therapy.

Beside the therapy-induced *ESR1*-activating mutations, which have been the focus of a wealth of studies, little is known about the molecular alterations associated with breast cancer progression. Fumagali and colleagues reported on the targeted sequencing of 11 genes in 182 patients with ER-positive (ER^+) metastatic breast cancer (mBC; 88 metastatic samples and their matched primitive tumors and 94 primitive tumors alone), but did not find any drivers enriched in the metastatic setting (25). We previously reported whole-exome sequencing (WES) in 216 metastatic biopsies from patients with advanced breast cancers and could not identify any drivers that were significantly enriched in metastatic samples, with the exception of *ESR1* (27). Yales and colleagues aimed to compare different stages of the disease progression including metachronous metastasis and locoregional relapse (148 from locoregional relapse and 79 from metastatic samples and 72 not reported). They concluded to the absence of metastasis-specific genomic alterations, even though some mutations were enriched in metastatic and relapsed samples, such as Jak2–Stat3 pathway and SWI/SNF genes (16). Finally, Nayar and colleagues have recently reported primarily results from an ongoing WES study of 168 metastatic biopsies from HR^+/$HER2^-$ mBCs. They found *ERBB2*

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activating mutations in 12 patients. Interestingly, *in vitro* analysis found that *ERBB2* mutation was associated with resistance to endocrine therapy (28).

Overall, these pioneer studies raised the possibility that the sample size should be increased for mBC drivers to be detected. Several groups have recently sequenced whole exomes or large panels of genes to characterize the genomic landscape of mBCs from large sample cohorts. Three major studies have recently reported the genomic characterization of very large cohorts of patients with mBC. Razavi and colleagues have described the genomic landscape of 1,501 HR+ mBCs including 1,000 samples from distant metastases and 692 tumors previously exposed to hormonal therapy. This study reported that despite the fact that *ESR1*, MAPK pathway genes, and Myc-associated transcription factors were mutated in 18%, 13%, and 9% of relapsed samples, respectively, around 60% of those sampled displayed no obvious resistance-associated genomic alterations. The authors also reported that alterations in several genes including *TP53, ESR1, ARID1A, ERBB2*, and *NF1* were found more frequently in mBC than in early breast cancer (eBC). *ESR1*-mutant and MAPK pathway alterations (including *NF1* loss) were associated with a shorter duration of response to subsequent hormonal therapies (29).

Some studies not only searched for metastasis and/or resistance-associated gene alterations, but also examined the molecular basis of cancer genome evolution. Angus and colleagues for example have analyzed 442 samples from metastatic lesions of breast cancer (279 $HR^+/HER2^-$ mBCs) by whole genome sequencing (WGS). The five most frequent metastasis-enriched gene alterations were in the *TP53* (42.8%), *PIK3CA* (42.3%), *ESR1* (14.3%), *GATA3* (11.3%), and *KMT2C* (11.3%) genes. Mechanistically, the APOBEC (Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) signature enrichment in mBC could drive the metastatic mutational landscape (30). In another study by Bertucci and colleagues (31), WES of 617 mBCs and germline DNA was performed. Samples were collected from secondary lesions ($n = 543$) or breast tumors ($n = 74$). The main findings of these studies (listed in **Table 1**) are detailed in the following sections.

Identification of driver genomic alterations enriched in mBCs

In Bertucci and colleagues, 21 genes were identified to present a "driver gene alteration" in their dataset of 617 mBCs. This included *TP53, PIK3CA, GATA3, ESR1, KMT2C, CDH1, PTEN, NF1, MAP3K1, NCOR1, AKT1, FOXA1, KRAS, MAP2K4, RB1, TBX3, RIC8A, PIK3R1, RUNX1, BXW7*, and *CBFB*. In patients with $HR^+/HER2^-$ breast cancer ($n = 381$), 9 driver genes were more frequently mutated in the metastatic setting, as compared with eBC (TCGA cohort). This included *TP53* (29%), *ESR1* (22%), *GATA3* (18%), *KMT2C* (12%), *NCOR1* (8%), *AKT1* (7%), *NF1* (7%), *RIC8A* (4%), and *RB1* (4%). We could not find any driver alterations enriched in metastatic $HER2^+$ ($n = 30$) or triple-negative breast cancer (TNBC; $n = 182$).

Among the 381 $HR^+/HER2^-$ samples, 337 (88%) previously received endocrine therapy and 42 (11%) did not. *ESR1* mutations were detected in 24% of patients who had been treated with endocrine therapy, and in 2% of patients who had not received endocrine therapy ($P < 0.0005$, Fisher exact test). *KMT2C* mutations were present in 14% of patients pretreated with endocrine therapy and 2% of patients who did not receive endocrine therapy ($P < 0.04$, Fisher exact test). Similarly to other reports (29), *RB1* and *NF1* mutations were enriched in $HR^+/Her2^-$ mBCs, as compared with primary tumors. Alterations in these 2 genes were associated with poor outcome (HR = 2.37; 95% CI, 1.15–4.81; $P = 0.019$ and HR = 1.91; 95% CI, 1.09–3.35; $P = 0.024$).

Mutational processes and homologous recombination deficiency

Because metastatic samples were enriched in several drivers, we further assessed which mutational processes were involved in tumor evolution (32). Signatures S3 (homologous recombination deficiency [HRD]), S10 (POLE-associated signature), S13 (APOBEC), and S17 (unknown origin) were increased in $HR^+/HER2^-$ mBCs compared with $HR^+/HER2^-$ eBCs ($P < 0.001$). The detection of signatures 13, 10, 17 was associated with poor outcome. When a sample presented two or three signatures among signatures 13, 10, 17, the median overall survival (OS) was 18 months, as compared with 37 and 27 months when 0 or 1 signature was detected, respectively.

To assess HRD, we combined signature 3 and large-scale state transitions (LST), a genomic trace of HRD (33). In the $HR^+/HER2^-$ cohort, there were more cases with HRD in mBCs than in eBCs (15% vs. 8.0%, $P = 0.005$, Fisher exact test), but no difference was found between early and late tumors of the $HER2^+$ and TNBC groups. Yet, an increase in the frequency of somatic biallelic loss-of-function (LOF) mutations located on the homologous recombination pathway was observed in metastatic TNBC (mTNBC; 7%) as compared with early TNBC (eTNBC; 2%).

Since these data suggest that mutational processes drive tumor evolution toward more complexity at the genetic level, we assessed tumor mutational burden and clonality in metastatic samples. Tumor mutational burden was higher in metastatic $HR^+/HER2^-$ breast cancer and TNBC compared with eBC ($P < 0.001$). Clonal diversity was also assessed according the Shannon index (34), and was found to be increased in all subtypes of mBCs compared with eBCs. All these results show a higher level of genomic complexity in metastatic disease and confirm a genomic evolution of breast cancer alongside disease progression. **Figure 1** summarizes key findings from the large-scale sequencing studies of mBCs and their potential therapeutic impact.

Implications and Future Directions: Which Molecular Mechanisms Drive the Metastatic Process?

Although the number of samples in this analysis was high, the study failed to identify a genomic alteration that could drive the metastatic process. Indeed, most of the driver alterations enriched in metastatic samples relate to drug resistance rather than metastatic process, with the possible exception of *RIC8A*, a guanine nucleotide exchange factor. There are several possible explanations for this.

First, it could be that genomic drivers of dissemination occur at very low incidence and that we need more than 600 samples to address this question. It must also be emphasized that most of the metastatic samples in this disease were obtained several months or years after the primary tumor samples were collected. Identifying molecular mechanisms of the metastatic process would require the analyses of metastases that are synchronous with primary tumors, to avoid the noise related to genome evolution over time. Alternatively, the metastatic process may not be mediated by genomic alterations of coding regions, but instead rely on epigenetic and transcriptional deregulations.

Epigenetics and the metastatic phenotype

The emergence of cancer and its therapeutically targetable traits are mostly looked at from a genetic point of view. One of the main results of early large-scale cancer genome studies therefore came as a surprise, revealing that half of the cancers bear mutations in epigenetic genes (1–5).

Table 1. Studies on metastatic breast cancer genomics.

Study	Patients	Samples	Subtypes	Sequencing Approach	Depth
Yates and colleagues (16)	170	148 locoregional 79 metastatic biopsies 72 unknowns (purity >70%)	87 $ER^+/HER2^-$ 34 $HER2^+$ 37 TNBC 12 Unknowns	Multiregion targeted gene screen (365 genes)	—
Fumagalli and colleagues (25)	182	182 primary tumors 88 with match metastases	182 ER^+ 10 $ER^+/HER2^+$	PCR-based MUT-MAP assay (11 genes) NanoString nCounter Analysis System (400 genes)	—
Nayar and colleagues (28)	168	168 metastatic biopsies.	168 $ER^+/HER2^-$	WES	—
Angus and colleagues (30)	442	442 metastatic biopsies (purity >30%)	279 $ER^+/HER2^-$ 77 $HER2^+$ 58 TNBC 28 Unknown	WES	107×
Bertucci and colleagues (31)	617	543 metastatic biopsies (purity >30%) 74 breast tumors	381 $ER^+/HER2^-$ 30 $HER2^+$ 182 TNBC 24 Unknown	WES (∼20,000 genes)	120×
Razavi and colleagues (29)	1918	1,000 metastatic biopsies (purity >30%) 918 primary biopsies	1364 $HR^+/HER2^-$ 224 $HER2^+$ 168 TNBC	Targeted sequencing (MSK-IMPACT)	771×
MBC project (https://mbcproject.org/)	237	189 breast tumor 48 metastases biopsy	113 HR^+ 34 $HER2^+$ 9 TNBC 81 Unknown	WGS	—
AURORA programme (85)	381	—	228 $ER^+/HER2^-$ 51 $HER2^+$ 71 TNBC 31 Unknown	Targeted sequencing	

Broadly speaking, epigenetic regulation impacts the chromatin accessibility to transcription factors and gene expression regulators and relies on a number of mechanisms, among which, DNA methylation and histone tail posttranslational modifications are the most studied in cancer. In fact, it is the distribution of these permissive or repressive chromatin states along the genome, the so-called epigenetic landscape, that sets the repertoire of genes expressed in a cell—the transcriptional program—that defines a cell type's identity. Epigenetic deregulation can therefore result in phenotypic cell plasticity that is semistable and can be passed on over several cell divisions.

Metastatic spreading typically requires a transient phenotypical change including epithelial-to-mesenchymal transition, survival in the circulation and homing to distant sites. Once seeded, most of these phenotypical traits can be, or even need to revert. Permanent genetic modification is not needed and instead, might be a hurdle for the metastatic process to then proceed successfully.

Epigenetic regulations can exert a semitransient control of entire transcriptional programs impinging deeply on cell identity and phenotype. Epigenetic alterations therefore stand as an obvious candidate mechanism to enable cancer cells to metastasize and provide them with phenotypical plasticity to adapt to coming environmental changes.

Accordingly, early studies of DNA methylation profiles could identify metastasis-specific methylation signatures across breast cancer subtypes (35). Epigenetic imbalance can also be associated with resistance to therapy, as clearly shown with targeted therapy in melanoma (36). In prostate and breast cancer, epigenetic reprogramming was similarly shown to promote endocrine therapy resistance (37, 38). More recently, the epigenome of 47 primary and metastatic HR+ breast cancer samples were examined. They identified key *cis*-regulatory elements showing widespread deregulation in metastatic samples. Perhaps more importantly, "phenotypical/epigenetic clones" could be identified and traced in primary and metastatic samples documenting their selection during disease progression (39). The selection of epigenetic clones was also documented in the context of resistance to hormone therapy by single-cell epigenetic profiling on patient xenograft models of breast cancer (40). Also, the elevated activity of FOXA1, which was previously linked to endocrine resistance in mBC, has recently been shown to drive genome-wide enhancers reprogramming (hypoxia-inducible factor-2α top target) through epigenetic dysregulation (41).

Finally, the suspected role for epigenetic alterations in breast cancer progression is also corroborated by several studies of metastatic-specific genomic alterations showing alterations of SWI/SNF components (16, 30) and of the *KMT2C* (30, 31) genes.

Clinically actionable driver gene alterations

Most of the driver alterations detected in the study by Bertucci and colleagues have previously been reported to be associated with resistance to therapy. Although much has already been reported about *ESR1* mutations, there are many clinical implications related to other drivers involved in resistance.

RB1 mutation and resistance to CDK4/6 Inhibitors

RB1 gene product controls the G_1–S transition through transcriptional repression of the proliferation-associated target genes of E2F1, among which, the CDK4/6 cyclin-dependent kinase. The predictive value of *RB1* mutation regarding the therapeutic response to CDK4/6 inhibitors was therefore evaluated (42). Next-generation sequencing on ctDNA from the MONALEESA trials enabled the detection of *RB1*

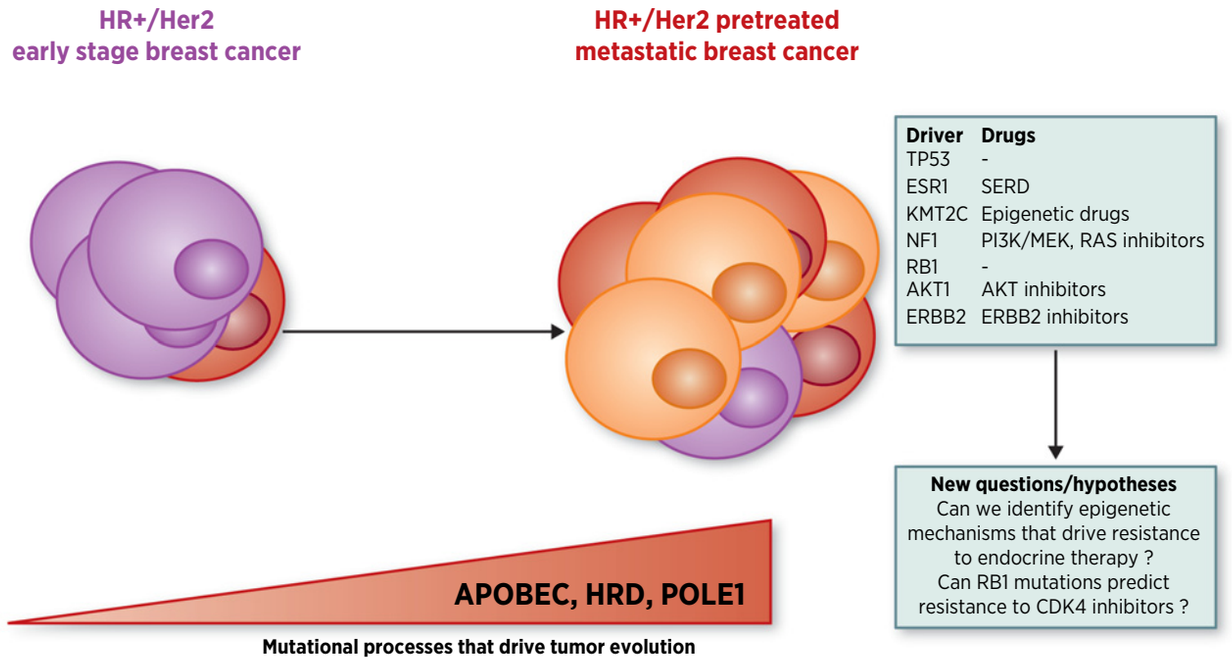

Figure 1.
Genomic evolutions alongside tumor progression in breast cancer.

mutation in 1% of patients at baseline. Median PFS was 3.7 months in these patients, showing that most of them presented a primary resistance to CDK4/6 inhibitors combined with endocrine therapy, as reported previously (43, 44).

In the Bertucci and colleagues report, we could find 4% of samples that presented *RB1* mutations in patients who did not receive CDK4/6 inhibitors. These mutations were associated with resistance to CDK4/6 inhibitors in a pooled analysis of three randomized trials. The finding that *RB1* mutations are associated with resistance to CDK4/6 is corroborated by Li and colleagues (45). In this latter study, patients who presented *RB1* mutations have 3.6 months median PFS under CDK4/6 inhibitors and endocrine therapy. Because CDK4/6 inhibitor improves PFS and OS in $HR^+/HER2^-$ mBC at frontline or after failure of aromatase inhibitor-based endocrine therapy (46–51), this class of drugs is becoming a standard of care and is widely prescribed. Interestingly, to our knowledge, no predictive factor of CDK4/6 inhibitor efficacy has been identified. Although *RB1* mutation cannot yet be considered as level 1 evidence, the finding is very strong and will have direct clinical implications once confirmed. First, there is a need to test alternative therapies to CDK4/6 inhibitors in these patients, and one could argue that given the resistance to endocrine therapy, these patients could directly be selected for chemotherapy. Second, there is a need to develop drugs that target *RB1* deficiency. In this regard, preclinical data suggest synthetic lethality with aurora kinase inhibitors in *RB1*-deficient cell and murine models (52, 53), and therefore a phase I/II trial is ongoing in *RB1*-deficient solid tumors including breast cancer (NCT03092934).

NF1 mutation and MEK inhibitors

NF1 mutations have been consistently reported to be enriched in $HR^+/Her2^-$ mBCs (29, 54). NF1 inhibits Ras activation, and, when mutated, leads to the activation of the RAS pathway. In Bertucci and colleagues, *NF1* mutations were observed in 7% of patients, and were associated with poor outcome and resistance to MEK inhibitors. Several strategies are being developed to target NF1, including cotargeting of MEK and PI3K pathways. Nevertheless, the most promising approach in this genomic segment could be the targeting of Ras protein. There are several efforts to turn Ras into a druggable target, as illustrated by recent studies on opportunities to target Ras pathway through upstream flux inhibition with targeting SHP2, SOS, or GRB2 (55).

The KMT2C case

Finally, perhaps one of the most exciting findings from the genomic profiling of mBCs is the observation that around 15% of metastatic lesions present a *KMT2C* mutation. Mutations of *KMT2C* have been found enriched in $HR^+/HER2^-$ mBCs; *KMT2C* (*MLL3*) encodes a histone methyl transferase and has been previously involved in estrogen-independent proliferation of luminal cell lines *in vitro* (56). The observation that *KMT2C* mutations are associated with resistance to endocrine therapy highlights the role of epigenetic alterations in the resistance to endocrine therapy. This finding provides a rationale to characterize epigenetic landscapes of mBC and to develop the clinical use of epigenetic drugs to reverse resistance to endocrine therapy. Interestingly, preclinical data have suggested that KTM2C-containing COMPASS (Complex of proteins associated with SET1) complex interacts with the BAP1 complex to facilitate transcription of tumor suppressor genes such as *FRZB, GRHL2*, and *DACT2*. In cancers bearing mutations in *KTM2C*, COMPASS fails to properly localize at *cis*-regulatory elements, leading to an increased activity of the PRC2 polycomb repressive complex and hence the silencing of target genes. *In vivo*, treatment of *KTM2C*-deficient cells with an EZH2 (a catalytic subunit of the PRC2 complex) inhibitor decreases proliferation (57). Moreover, another recent work indicated

an alternative way to target *KTM2C*-deficient tumor. *KMT2C* was shown to localize at *cis*-regulatory elements controlling the expression of DNA damage response and repair genes such as *BRCA1/2, RAD51, ATM, ATR*. Consequently, in a murine model of *KTM2C*-deficient breast cancer, treatment with PARP inhibitor leads to decreased proliferation and apoptosis (58).

Turning Cancer Mutator Phenotypes into Therapeutic Opportunities?

APOBEC

One of the key findings of the large-scale mBC sequencing is the high rate of samples presenting an APOBEC signature. APOBEC mediates genomic C to T mutations (32). The finding that HR^+/$Her2^-$ metastatic lesions present an enrichment of APOBEC signatures led to the hypothesis that APOBEC could mediate resistance to endocrine therapy. Interestingly, this hypothesis is supported by preclinical data showing that estrogen deprivation results in APOBEC activation, and that blockade of APOBEC synergizes with endocrine therapy (59). These data lead to the hypothesis that targeting APOBEC by pharmacologic agents could delay resistance to endocrine therapy. Drugging APOBEC remains a major challenge for the future. Two main strategies for the targeting of APOBEC in cancer have been proposed. The first one is based on a synthetic lethality-like rationale, in which triggering a further increase in the mutation load by interfering with the DNA damage response with PARP or ATR inhibitors (60–62) would lead to lethal levels of DNA damage and result in cell death. A second more direct strategy aims to pharmacologically inhibit the DNA deaminase activity of APOBEC (63–65).

Signature 17 (unknown origin)

The increased levels of signature 17 in metastatic samples could be related to previous exposure to 5-Fluorouracil (5-Fu) or capecitabine. Christensen and colleagues have shown that 5-Fu treatment induces characteristic T>G mutations in human cancer (66). This would therefore suggest that signature 17 observed in metastatic samples could be a genomic scare, rather than the activation of a new mutational process.

Signature 10 (POLE-associated)

The increased levels of signature 10 are not explained and need further investigations. Previous studies have shown that signature 10 is associated with altered activity of the error-prone polymerase *POLE* and with the presence of *PIK3CA/ARID1A/PTEN/P53* mutations in colon and endometrial cancer, even though the causal relationship is unclear (67).

Somatic biallelic mutation in homologous recombination genes

Finally, an increase in somatic biallelic LOF mutations on genes located on the homologous recombination pathway was observed in mTNBC (30, 31). Interestingly, this is inversely correlated with germline LOF mutations, which decreased dramatically between eTNBC and mTNBC. The decrease in germline LOF mutations could be explained by the higher sensitivity to cytotoxic agents for patients presenting such alterations (68–70). The increased frequency of somatic LOF mutations suggest that homologous recombination deficiency is involved in the genome evolution of mTNBC. Several studies are currently testing whether PARP inhibitors could delay tumor evolution in this group of patients (71).

From Simple to Complex Disease: Deconvoluting the Clonal Dynamics at Work during Disease Progression

The findings that advanced cancers display increased genomic and clonal diversity raise the question of the phylogenic relationships between the cellular components of the various stages of cancer. Addressing this aspect of cancer progression requires regionally as well as temporally resolved samplings. A handful of studies have tackled these complex issues using longitudinal primary tumor and progressed disease samples (72, 73) or in the context of autopsy programs (74, 75). Overall, examining longitudinally collected samples or spatially distributed lesions including primary and locally recurring tumors as well as lymph node and distant metastases, these studies have contributed to delineate highly complex and variable routes for metastatic spreading and disease progression. Philogenically, multiple metastatic lesions sometimes originate from the same tumor cell subclone but can also evolve in parallel from multiple subclones. Alternatively, metastatic lesions can originate from cross-seeding. Examining the clonal make-up of regionally distributed lesions have also shown monoclonal but also multiclonal origin of metastatic lesions, the mode of spreading being seemingly biased by the genomic complexity of the primary tumors of different molecular subtypes. Of note, the involvement of synchronous axillary lymph node metastasis in the metastatic spreading was ruled out, in favor of a hematogenous dissemination of distant metastasis (73).

Targeting the complexity of metastatic genomes and clonality

Overall, all sequencing efforts of mBCs report consistently that mBCs acquire a high mutational burden and polyclonality. This is also observed in the tumor evolution of other cancers such as lung or prostate cancer (76, 77). The increased genetic complexity may stimulate drug resistance because it increases the likelihood that a resistant genetic subclone emerges. Also, it is a surrogate of active mutational processes leading to genome evolution. These findings represent a strong rationale to move drug development earlier in the disease course thanks to the identification of patients with high risk of relapse. As mentioned earlier ("Mutational processes"), the evolution process can itself be targeted (PARP inhibitors, deaminase inhibitors, etc.).

Consequence of mutational burden on immunotherapy

To what extent mBCs with high mutational burden are sensitive to immunotherapeutics is unclear. Most of the mutations detected in mBCs with high mutational burden are subclonal and generated by APOBEC activity and should not lead to high sensitivity to immunotherapy. Nevertheless, a recent report suggests that these patients could derive some benefit from such treatment modalities (78). Ongoing trials testing atezolizumab, an anti-PD-L1 antibody restoring antitumor T-cell activity, in mBCs with high mutational load will address this question (NCT02458638).

Moving toward prediction of outcome based on mBC tumor evolution

Gene alterations acquired during tumor evolution could predict poor outcome in patients with mBC. This includes *TP53, NF1, RB1* mutations, but also detection of signature 13, 10, and 17. This leads to the hypothesis that combining these six parameters could enable the early detection of patients with very poor outcome and select them for innovative therapies. The vision is to implement diagnostic methods to detect genome evolution as early as possible during the disease course,

with the aim of offering access to innovation early in the disease course to patients whose tumor genome starts to evolve. Optimally, tumor evolution could be detected through the sequencing of circulating DNA, therefore allowing a monitoring of genome evolution.

The "Strange Case" of TNBC

A striking finding of mBC sequencing is the absence of consistent tumor evolution observed in mTNBC, as compared with $HR^+/Her2^-$ mBC. It must be emphasized that this observation could be related to the small sample size associated with mTNBC, since, in Bertucci and colleagues, only 186 samples presented a triple negative phenotype. Nevertheless, there are several hypotheses that could explain this observation. First, metastatic relapse occurs very early after treatment of primary tumor in TNBC, and usually in cancers where a macroscopic residual disease is observed after chemotherapy. Second, as opposed to endocrine therapy, chemotherapy is not a chronic therapy that blocks an oncogene, so there is a much less focused pressure selection under this class of drugs. Finally, several studies have suggested that the most genetically complex TNBCs could be the ones that are more sensitive to chemotherapy, suggesting that genomic complexity is actually counter-selected by chemotherapy. As a matter of fact, in the SWOG S9313 trial, HRD was associated with better disease-free survival (HR = 0.72; 95% CI, 0.51–1.00; $P = 0.049$) in patients with TNBC receiving adjuvant chemotherapy (79).

What About Other Cancer Types?

A very large genome analysis of metastatic solid tumors from several primitives has been recently reported (80). In this study, the authors performed WGS of 2,520 pairs of metastatic lesions and normal tissue from different types of solid tumors. As already reported, mutational load was higher in skin and lung carcinoma and low in neuroendocrine tumor or sarcoma. Interestingly, when compared with the primary tumor-orientated Pancancer Analysis of Whole Genomes (PCAWG) dataset (81), there was no difference between early and metastatic disease except for breast and prostate cancer. This observation induces several hypotheses, breast and prostate cancer having in some point of view, a similar natural history. Indeed, both are very common in the general population and endocrine pathways play an important role in carcinogenesis and tumor progression. Furthermore, endocrine therapy is a standard of care for both and selective pressure induced by long-term treatment could influence genome evolution. Interestingly, as shown in mBCs with *ESR1* mutation, metastatic prostate cancers harboring androgen receptor amplification occurred in 70% of cases. Similarly to mBC, several mutational signatures were found enriched in metastatic versus early prostate cancer, including potentially clinically relevant signatures such as HRD or microsatellite instability (MSI) (82). All these results support the hypothesis of an increase in genomic complexity and heterogeneity along tumor progression.

Conclusion

In $HR^+/HER2^-$ mBCs, strong evidence for genome evolution of $HR^+/Her2^-$ metastasis has been generated, particularly under the pressure of endocrine therapy. On the one hand, the assessment of tumor evolution could identify a subset of patients with very poor outcome who would be eligible to innovative treatments. On the other hand, several genomic alterations acquired during genome evolution of metastatic lesions are targetable and could lead to drug development and changes in patient management in the next few years.

One of the major stakes in the future will be to detect these alterations earlier in the disease course to define those patients with poor outcome. However, these genomic alterations are observed at very low frequency in eBC, making them difficult to detect by sequencing approaches classically used in a clinical context. Even when sequencing can be performed deeply enough, core biopsies most likely do not sample the whole complexity of a tumor.

Because studies on WES did not fully address the questions related to mBCs, there is a need to further explore this entity, thanks to new technologies including single-cell sequencing, proteomics, and assay for transposase-accessible chromatin using sequencing (ATAC-seq). Preliminary studies have enabled to specify immune ecosystem of TNBC (83) and suggest that subclonal resistant cells preexist and can be selected by treatment (84). This approach is nevertheless dependent on the sampling, just as bulk sequencing approaches. As temporal and special sampling of cancerous lesions is clinically challenging and even not feasible in most clinical situations, liquid biopsy can be a promising alternative approach. Although the spatial resolution is lost by this procedure, longitudinal monitoring of "cancer DNA" might enable both the broad genome evolution and the occurrence of specific, targetable lesions to be monitored in a relatively noninvasive procedure. Adequate therapeutic interventions can then be proposed early and outcomes potentially improved.

Disclosure of Potential Conflicts of Interest

F. Mosele is a researcher at Institut Gustave Roussy. F. André is a consultant/advisory board member for AstraZeneca, Lilly, Novartis, Pfize, Daiichi Sankyo, and Roche. No potential conflicts of interest were disclosed by the other authors.

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