# Breast Cancer Genomics

# 15

Maurizio Scaltriti

## 15.1 Introduction

Recent large-scale next-generation sequencing (NGS) studies defined the landscape of genomic aberrations in breast cancer. Copy number variations, missense mutations, and small insertion/deletions of certain genes can be associated with carcinogenesis and tumor progression. These so-called cancer drivers supposedly confer either growth advantages or protection from therapeutic stress.

Some of these genomic alterations can be inherited, but the vast majority occurs in somatic cells by stochastic events in DNA editing/repair and environmental mutagens.

In primary breast tumors, the most frequently altered genes are *TP53*, *PIK3CA*, *MYC*, *CCND1*, *PTEN*, *ERBB2*, *ZNF703/FGFR1* locus, *GATA3*, *RB1*, and *MAP3K1* [1]. This scenario, however, can change in the metastatic setting and/or in tumors subjected to pharmacological pressure. An archetypal example of this divergence is the mutation rate of *ESR1*, the gene encoding for estrogen receptor alpha (Fig. 15.1). While mutations in this gene are found in less than 1% of primary tumors, up to one-third of patients relapsing from anti-endocrine therapy have tumors harboring *ESR1* mutations [2].

Adding another layer of complexity, it is now possible to use mathematical approaches to define mutational signatures associated to specific genomic rearrangements, gene expression patterns, or clinical features [3, 4]. Although these signatures may define more precisely the genomic status of the tumors and can provide some prognostic information, their exploitability in the clinic is debatable.

In this chapter, I will focus on the possibilities that practice oncologists currently have to offer rational therapeutic options based on genomic analysis.

M. Scaltriti
Pathology and Hyman Oncology and Pathogenesis Program (HOPP), Memorial Sloan Kettering Cancer Center, New York, NY, USA
e-mail: scaltrim@mskcc.org

## 15.2 Change in Mentality

It is generally thought that genomic testing is appropriate only for patients who have exhausted standard therapy or have orphan tumors that lack a standard therapy. However, I contend that with the current possibilities of analyzing tumor samples by targeted exome sequencing (see below), each patient with advanced disease or with high-risk prognostic situations should at least be given the opportunity of being informed about genomic approaches other than following the standard protocols. Not having genomic infrastructures or not being a recruiting center for clinical trials is not a valid argument to refute this possibility. As I will describe below, anybody can easily submit ordinary formalin-fixed paraffin-embedded (FFPE) samples to certified companies that can sequence the tumor DNA in matter of weeks. Although the cost of this procedure can be burdensome, it may be affordable for the majority of the patients.

Patients should at least be informed about this possibility, explaining very clearly that this analysis will not provide a "magic pill," but it can potentially uncover genomic vulnerabilities in the tumor, and in turn help in choosing the specific targeted therapies. Obviously, the oncologist also has to explain that, should this be the case, the matching therapeutic option may be either available as standard of care or, conversely, part of experimental studies that are not necessarily available in the same geographic area. The patient has to decide whether undertaking this path is worthwhile, with very little influence from the treating physician.

No real advances in medicine (or in science in general) have ever been made by just following the existing paradigms and not thinking outside the box. The word "conventional" in medical care is a very dangerous one. Without reaching "Newtonian" extremes, I believe there is a need for a radical change in the way cancer patients are managed and treated. The gap between the first lines of research and the current clinical practice is too wide and too often is seen as the difference between science fiction and the real world. This has to change.


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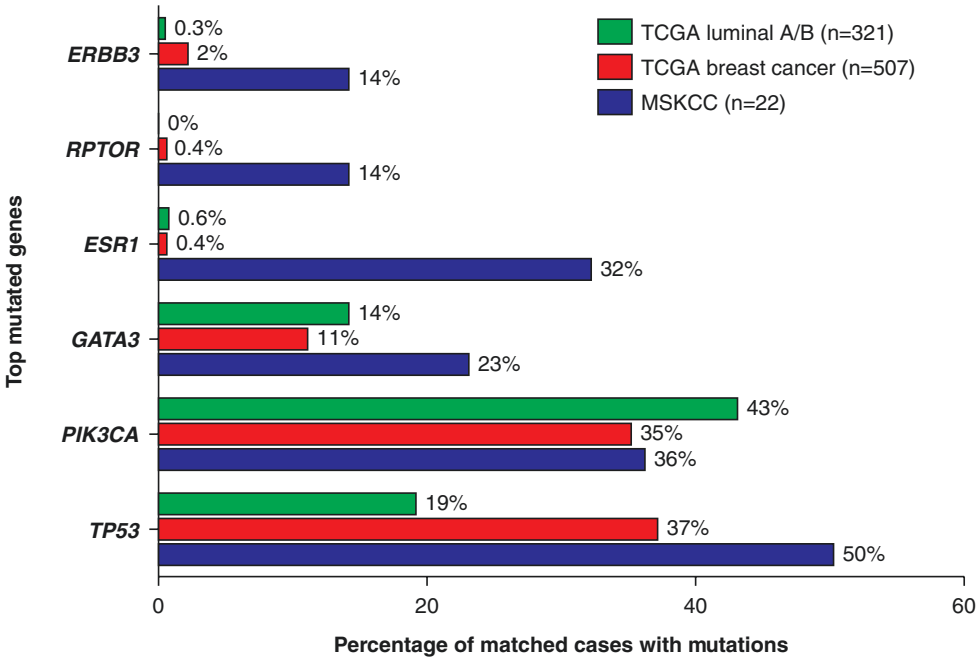


**Fig. 15.1** Different prevalence of mutations between tumors relapsed to anti-endocrine therapy (MSKCC cohort) and tumors from the TCGA

## 15.3 What We Can Realistically Do

The genome of the tumors can be analyzed in several ways. Whole genome sequencing (WGS) provides a detailed map of single nucleotide variations (SNVs), insertions and deletions (indels), gene translocations, and copy number changes. WGS is certainly the most complete approach, but has a number of important caveats. It generates an enormous amount of information, but most of it is of unknown clinical importance. It requires a relatively high amount of tissue, not always available from tumor biopsies. Moreover, besides the still prohibitive costs for its standard application in the clinical setting, WGS entails complex informatics analysis and big data storage. Whole exome sequencing (WES) provides the same map of genomic aberrations (with the exception of gene translocations) present in the genes that are expressed in mRNA. WES requires less material and cost is lower compared to WGS, but, although the amount of information generated is also reduced, it still involves a level of analytical work not wanted (and not needed) in the clinical practice. A more targeted approach to sequence the genome seems to be more reasonable for the widespread clinical implementation of this technology.

The field started with the detection of single gene mutations that allowed some patient stratification for certain therapies (e.g., KRAS detection in colon cancer) or the detection of germinal alterations linked to increased risk of developing cancer (e.g., BRCA1/BRCA2 mutations in both ovarian and breast cancer). Subsequent advances included the detection of well-known gene mutations (so-called hotspots) associated with tumor onset and/or resistance to therapy. Although merely diagnostic, these tests were useful to better stratify patients for clinical trials testing novel targeted agents.

The real revolution in the field was the development of targeted (or capture-based) exome sequencing platforms, in both research and clinical settings. From ~200 ng of DNA or less it is now possible to gather genomic information that is easily interpretable and can strongly influence the practice of treating medical oncologists. This technology does not require specific sample preparation and is commercially available and usually friendly to order online. Samples can be shipped at room temperature, and results are returned in the form of an easily interpretable report in 4–6 weeks.

This approach ensures deep exome sequencing (high-gene coverage) of a selected number of genes (usually a few hundred) considered important for tumor progression and resistance to therapy. A comprehensive analysis of the coding sequences of these genes may inform on the intrinsic genomic vulnerabilities of the tumor and therefore its possible sensitivity toward a given targeted therapy (e.g., *PIK3CA* mutations for PI3Kα inhibitors or *BRCA1/BRCA2* mutations for PARP inhibitors). Moreover, targeted exome sequencing can in some instances inform on the genomic instability of the tumor, perhaps rendering it more likely to respond to DNA-damaging agents or provide prognostic information (e.g., *TP53* mutations). Moreover, thanks to the quantification of the allele frequencies of each mutated genes, it can reveal the presence of different subclonal populations within the tumors and roughly estimate the level of tumor heterogeneity. Similarly, targeted exome sequencing can estimate the mutational load of the tumor, based on the number of driver and passenger mutations, especially when associated with mutations of genes involved in DNA repair.

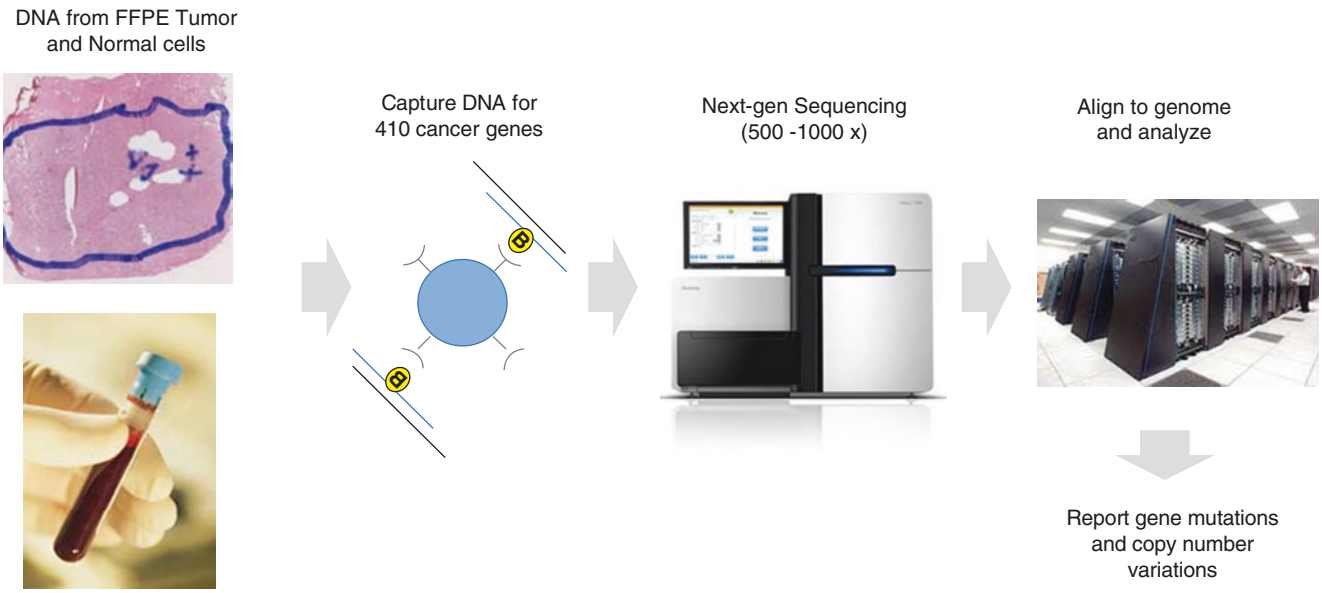


**Fig. 15.2** MSK-IMPACT targeted exome sequencing platform. DNA is extracted from either tissue or blood and only protein-coding exons of 410 cancer-associated genes are sequenced

Not ignorable is also the possibility to uncover possible not-so-obvious drivers present at a relatively low frequency. Two valid examples in breast cancer are HER2 and AKT1 mutations, which strongly predict for response to pan-HER kinase inhibitor neratinib [5] and AKT kinase inhibitors [6], respectively.

In addition to Clinical Laboratory Improvement Amendments (CLIA)-certified companies that provide this service, several cancer centers worldwide have developed their in-house platforms. One of the most successful examples is the MSK-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT), a targeted exome sequencing platform developed at Memorial Sloan Kettering Cancer Center (MSKCC) [7, 8]. The MSK-IMPACT platform has a track record of performing in small FFPE biopsies and cell blocks and ensures a deep sequencing coverage of 410 key cancer-associated genes (Fig. 15.2). To date, more than 10,000 patients (more than 1000 breast cancer patients) had their tumor sequenced with this platform at MSKCC, with ~60% of the cases having metastatic disease. Thanks to this effort, an unprecedented percentage of patients are entering in clinical trials and being treated with rationale-based targeted therapies. Some of these patients are experiencing exceptional responses unlikely to be seen with "canonical" therapies.

The accumulation of sequencing reports from breast cancer patients also allows identifying novel correlations between certain genomic alterations and sensitivity to given therapy. Moreover, this high number of cases permits to uncover new hotspot mutations and/or new drivers of disease.

## 15.4 Tumor Heterogeneity

Most tumors are thought to originate from a parental clone that accumulates genetic aberrations during carcinogenesis. As a result, these aberrations are present in the majority of, if not all, tumor cells in the neoplasm. During tumor progression, however, spatially distinct subclones characterized by heterogeneous somatic mutations and chromosomal imbalances may arise from the parental clone [9]. The aberrations that arise during subclonal evolution are thought to be the result of the selective pressure exerted by the tumor environment and/or as an adaptive response to antitumor therapy.

It is widely accepted that the sensitivity to a given drug depends on tumor heterogeneity. The proportion of tumor cells that express the target of interest or harbor the mutation determinant of sensitivity may vary among the different metastatic sites (inter-tumor heterogeneity) or within single lesions (intra-tumor heterogeneity). Therefore, tumor heterogeneity can predict the degree of drug sensitivity and possibly the selection of resistant clones.

Such heterogeneity of subclones within the primary tumor or metastases represents a major challenge in the management of cancer for a number of reasons. Tumor biopsies are invasive procedures and may be associated with complications [10] and significant costs. Additionally, a single biopsy may be subject to tumor sampling bias and thus fail to capture the therapeutically relevant mutations [9, 11, 12]. Furthermore, in those tumors with several metastatic sites, the sampling bias is surely amplified as there is well-described genomic branching of the tumors [9, 11–15]. As a

result, intra-tumoral heterogeneity may explain the difficulties encountered in the validation of oncology biomarkers and for prediction of therapeutic resistance.

A number of studies have shown that ctDNA may be isolated from blood [16–18]. Early studies of ctDNA demonstrated that polymerase chain reaction (PCR)-based assays could accurately detect the mutations that have already been identified in the tumor bulk [19–24]. In preliminary studies it has been shown that targeted and genome-wide NGS of ctDNA is a feasible approach and can be employed to identify genomic alterations in the ctDNA [20, 25–30]. NGS ctDNA assays can potentially provide an easily obtainable and minimally invasive surrogate for tumor tissue biopsies that will markedly facilitate identifying potential targets to guide treatment decisions. It may also provide a potentially sensitive and specific biomarker that can be monitored in real time during therapy.

Collectively, understanding the extent to which the genetic heterogeneity among subclonal populations in the same patient converges to a similar and targetable phenotype may contribute to more rationale-based therapeutic approaches.

## 15.5 Sequencing to Understand Drug Resistance

Resistance to therapy can be pre-existing (intrinsic) due to the presence of concurrent aberrations. In breast cancer the presence of certain genomic aberrations can predict the response to given therapeutic agents. HER2 amplification, for example, is a determinant of sensitivity to drugs such as trastuzumab, pertuzumab, lapatinib, or trastuzumab emtansine (T-DM1). The presence of *PIK3CA*-activating mutations, on the contrary, is associated with resistance to these agents (with the exception of T-DM1 [31]). However, harboring these *PIK3CA* mutations is required to respond to PI3K p110α inhibitors [32, 33].

More frequently, drug resistance can arise upon therapeutic pressure via either loss of the therapeutic target [34] or positive selection of resistant clones [35, 36]. In fact, the constant pharmacological pressure may favor the fitness of tumor cells harboring certain genomic features and result in acquisition of drug resistance.

This occurrence is very well known, for example, in EGFR-mutant lung cancer, where the acquisition of the T790M gatekeeper mutation in EGFR or the amplification of other receptor tyrosine kinases such as MET or HER2 represents the majority of the mechanisms of resistance to the anti-EGFR molecule erlotinib. In breast cancer, several genomic mechanisms of acquired therapy resistance have been recently validated in the clinic. The selection of cells harboring *ESR1* mutations following endocrine therapy in ER-positive tumors is perhaps the most obvious example [2]. There are also evidences that the regeneration of a functional *BRCA2* upon therapy with PARP inhibitors leads to emergence of drug resistance [37]. Another example is the discovery of parallel genomic evolution occurring in patients treated with a PI3K p110α inhibitor where PTEN expression was lost over time via six different genetic mechanisms [12]. In this work, we discovered that different genomic aberrations but leading to the same convergent resistance phenotype can coexist in different metastatic lesions. The tumor genomic evolution during pharmacological stress was studied in a patient with metastatic breast cancer treated with the PI3Kα inhibitor BYL719 achieving a lasting clinical response, after which drug resistance emerged and died shortly thereafter. A rapid autopsy was performed and a total of 14 metastatic sites were collected and sequenced. When compared to the pretreatment tumor, all metastatic lesions had a copy loss of *PTEN*, and those lesions that became refractory to PI3Kα inhibition had additional and different *PTEN* genetic alterations (either copy number loss or missense mutations), resulting in the loss of PTEN expression (Fig. 15.3). Acquired biallelic loss of *PTEN* was found in one additional patient treated with BYL719, whereas in two patients *PIK3CA* mutations present in the primary tumor were no longer detected at the time of progression. These findings were functionally characterized in the laboratory using both in vitro and in vivo preclinical models that confirmed the causative role of *PTEN* knockdown in inducing resistance to PI3Kα inhibition.

This work is an archetypical example that access to metastatic lesions of patients who initially responded and then progressed to a given targeted therapy is crucial to elucidate the role of tumor heterogeneity and genetic evolution in the acquisition of drug resistance. Moreover, it also underscores the power of rapid autopsies of particularly informative patients (e.g., exceptional responders—see below) in uncovering novel genomic aberrations associated with drug sensitivity.

Without relying on these extreme cases, however, it is reasonable considering to re-biopsy at disease progression for genomic sequencing. In addition to confirm the origin, morphology, and the HER2/ER status of the tumor, this practice can inform of genomic vulnerabilities lost or gained during the treatment. This is of pivotal importance as it may uncover mechanisms of acquired resistance to therapy and set the stage for the next therapeutic option.

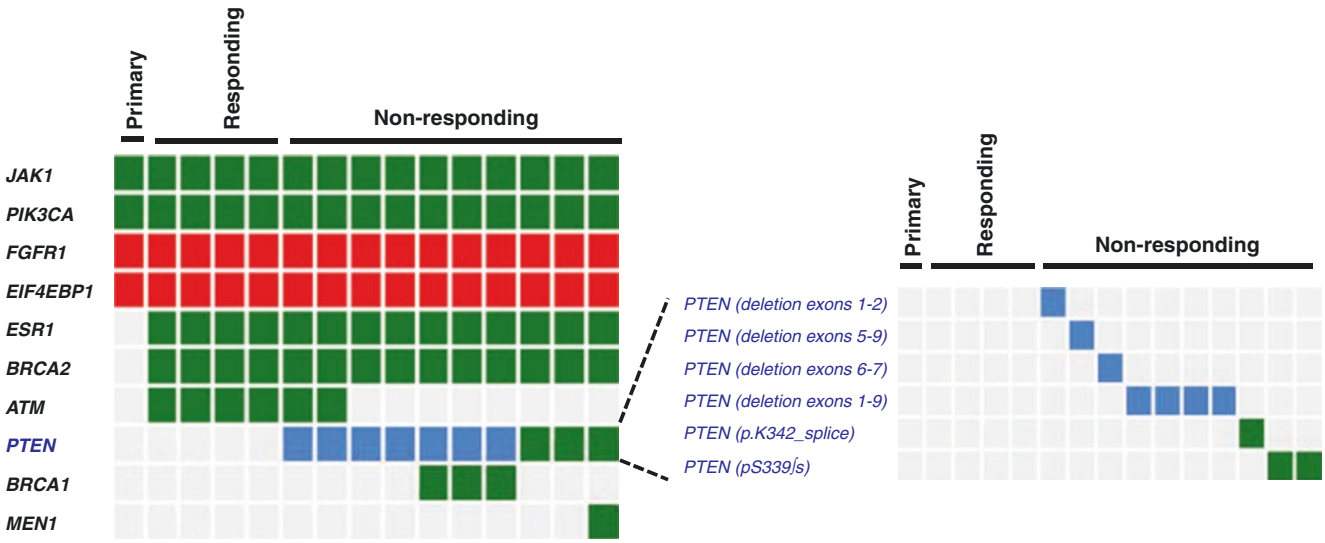


**Fig. 15.3** Convergent evolution resulting in genomic loss of *PTEN* in metastatic lesions from a breast cancer patient treated with a selective PI3Kα inhibitor

## 15.6 Exceptional Responders

Among the population of patients treated with either chemotherapy of targeted agents, there are rare cases that, unpredictably, show much higher-than-expected (or lower-than-expected) clinical responses. Examples of exceptional responders can be HER2-positive metastatic breast cancer patients that respond to trastuzumab-based therapy for a decade or show durable pathological complete response to trastuzumab without concomitant chemotherapy [38] or triple-negative breast cancer patients with metastatic disease that show durable response to cytotoxic chemotherapy. But perhaps the best examples are those patients that already progressed to every standard of care options but still achieve dramatic and/or durable response to investigational agents.

The tumors from these patients are likely to have particular molecular/genomic characteristics that render them exquisitely sensitive to the therapy. Samples obtained from patients catalogued as exceptional responders (or exceptional resistant) represent an invaluable source of material to study the intrinsic determinants of drug sensitivity and/or tumor evolution upon therapeutic pressure. These samples should be analyzed as thoroughly as possible, with all the available resources. In some of these cases, oncologists chose (wisely) to collaborate with academic centers that have the possibility to perform both WES and WGS. Besides mutations or changes in gene copy numbers, these tumors may harbor gene rearrangements or alterations in noncoding DNA (e.g., gene promoters, enhancers). If tissue procurement is not a limiting factor (i.e., surgical removal of the primary tumor or distant metastases), RNA sequencing or other platforms that consent to evaluate the gene expression profile of these lesions are strongly advised. Results from these analyses can potentially identify genomic or transcriptomic markers of response (or resistance) that, if confirmed in larger cohort of patients, will allow a more rational patient stratification.

Unfortunately, also exceptional responders may recur to therapy and develop drug resistance. As mentioned above (and if the onset of a different tumor is discarded), these lesions are likely to be the results of a selection of cells bearing genomic aberrations that confer fitness under the pharmacological pressure. Thus, they should be analyzed as deeply as possible and confront their genomic landscape with the one of the matched therapy-sensitive tumors. Each individual case may indicate possible mechanisms of drug resistance that can be confirmed using publicly available data and/or by testing the hypotheses in the laboratory.

## 15.7 Therapeutic Plan of Action

Once we have gathered the sequencing data of our tumor samples, we need to interpret the results and explore possible therapeutic options. The ability to identify patients with tumors harboring specific genomic vulnerabilities and match

them to the most appropriate therapy is central. At least four different scenarios may be in front of us:

1. We discover genomic aberrations that are targetable and for which there are FDA-approved drugs for that indication. This is obviously the best possible situation.
2. The genomic alterations present in our tumor sample indicate that one or more FDA-approved agents may be effective in this particular case but these agents are not registered for their use in breast cancer patients. In these circumstances it is generally feasible to ask for compassionate use of these drugs. This requires some extra work and ultimately depends on the positive response of the pharmaceutical companies, but it would be unethical not to attempt it.
3. The sequencing results uncover actionable mutations or gene amplifications that would justify the inclusion of the patient into an existing clinical trial testing the activity of a compound still under investigation. This scenario is likely the major deterrent for the broad use of genomic testing as routine diagnostic practice. As a matter of fact, one common argument made against the genomic characterization of tumor samples is the lack of available clinical trials in the geographic area of interest. In other words, the oncologist may wonder why the patient should be sequenced if there are no options to offer a therapeutic strategy based on the genomic data. This point deserves some considerations. First of all, it is very unlikely that every oncologist is aware of every clinical trial open and enrolling at a given time in their geographic area. Secondly, the term "geographic area" is very subjective. Some patients may be intimidated of traveling hundreds of kilometers or even consider a clinical study abroad, but some others may think that the chance of receiving a rationale-based therapy is worth the hassle.
4. The last scenario is the most frustrating. Actionable genomic aberrations are identified, but only experimental drugs under investigation for other tumor types could potentially be used to achieve clinical benefits.

Despite these premises, it is undeniable that a major limitation in developing precision medicine approaches is the fact that only few cancer drivers are represented at a relatively high frequency. As a matter of fact, we calculate that 85% of all hotspot mutations affect <5% of any cancer type in which they are found [39]. This problem can be partially overcome by enrolling patients based on genomic alterations rather than tumor type, following the concept of the "basket" clinical trial. This formula consents to test investigational drugs to a variegated patient population harboring the same genomic aberration. An example is the recently published results from patients with BRAF-mutant solid tumors treated with the small molecule BRAF inhibitor vemurafenib [40]. In this study, they found that the BRAF V600 mutation is a targetable oncogene in several cancer types other than melanoma. Dramatic responses were observed in cancers that would have had no therapeutic options if the patients were not part of this study. A patient with a rare case of BRAF-mutant breast cancer, for example, could be treated with vemurafenib by either compassionate use or by being enrolled in such trial (scenario n. 2). Similarly, breast tumors with a *NTRK* fusion (rare but often extremely dependent on this gene translocation) could be treated with NTRK inhibitors as part of an existing basket trial testing these compounds (scenario n. 3 or 4).

Other examples are the ongoing clinical trials testing the activity of the pan-HER inhibitor neratinib and the AKT inhibitor AZD5363 in HER2-mutant and AKT1-mutant tumors, respectively. Recent large-scale NGS studies have revealed recurrent activating *ERBB2* (the gene-encoding HER2) mutations across a wide variety of cancer types. In breast cancer, these activating mutations are relatively rare (~2%) and typically mutually exclusive with amplification of the gene [41]. These patients, therefore, are excluded from receiving conventional anti-HER2 therapy. Similarly, *AKT1* E17K mutations arise in ~3% of breast cancers, and despite compelling preclinical data that supports a central role for oncogenic AKT1 in the pathogenesis of many cancers, it remains unknown whether mutant *AKT1* is a rational therapeutic target. In both cases, heavily pretreated metastatic breast cancer patients are experiencing impressive responses to inhibitors of these kinases are given in combination with the ER degrader fulvestrant. These studies are currently being carried out only in selected institutions, but they represent proofs of concept that targeted therapies based on genetic characterization may provide clinical benefit even in patients that exhausted any other therapeutic options. The long-term objective is to expand these studies to more hospitals and bring these targeted agents to early-phase treatments in selected patient populations.

A parallel approach to predict the most effective therapy based on genetic data is the use of patient-derived xenografts (PDXs) harboring the same genomic aberrations found in the analyzed tumor. In some cases the xenograft model may be derived from the same tumor lesion that has been sequenced. Although this practice is widely used in translational research in cancer centers via academic collaboration, it is not as diffused in the clinical practice. It is, however, possible to ship fresh tumor samples to specialized companies that can provide this service. The use of PDXs is particularly useful in those cases where the rationale for the use of a given drug is not very strong or there are multiple therapeutic options available.

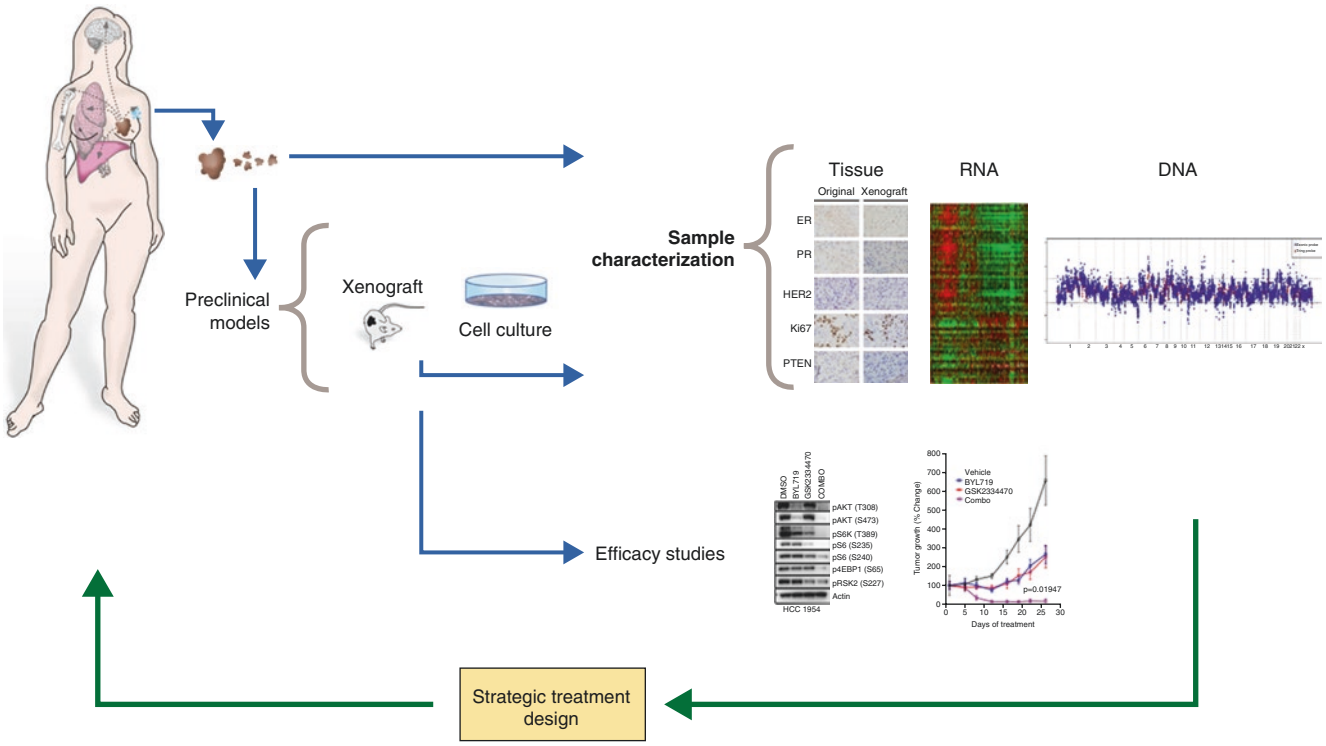


**Fig. 15.4** Personalized medicine approach. Tissue from either primary tumors or metastatic lesions is characterized by canonical techniques and next-generation sequencing. In parallel, preclinical models from the same samples can be established to test rationale-based therapeutic options

## Conclusions

Increased accessibility to targeted platforms and the expansion of clinical studies that enroll patients based on the genetic vulnerabilities will be key to move toward personalized medicine. An interesting pilot experiment to increase the number of patients undergoing genetic testing followed by matched targeted therapy is the Spanish study AGATA (NCT02445482). To be enrolled in this study, patients belonging to a defined geographic area have to consent that a committee of both clinical and translational investigators will suggest their enrollment in the most appropriate clinical studies based on their sequencing data. In principle, the more participating centers, the more available clinical trials that can recruit these patients. Moreover, this approach may speed the accrual of many of these studies with patients that, supposedly, are more likely to respond to the investigational therapies.

Another benefit of genomic sequencing that should not be underestimated is the detection of gene aberrations predictive of lack of response to given therapies. Perhaps the genomic results will not identify an actionable gene or pathway, but will indicate which compound should not be chosen for that patient, avoiding the toxicity and economic burden of treatments that will most likely fail. KRAS mutations, for example, are well known to limit the sensitivity to cetuximab in colon cancer. In breast cancer, the presence of *ESR1* mutations is indicative of resistance to tamoxifen and/or aromatase inhibitors [2], the loss of *PTEN* is sufficient to discourage the therapy with specific PI3Kα inhibitors [12], and the loss of *RB* renders CDK4/6 inhibitors ineffective [42].

It is tempting to imagine that soon every breast cancer patient will have their tumor DNA sequenced, perhaps multiple times, in order to monitor disease progression, thus enabling a rational use of molecularly guided therapies (Fig. 15.4). Similar to the antibiogram that is normally done for bacterial infection to choose the most effective antibiotic, the genomic aberrations of each tumor may one day be used to routinely indicate the most appropriate antitumor therapy for each patient.

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