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REVIEW

New generation of breast cancer clinical trials implementing molecular profiling

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ABSTRACT The implementation of molecular profiling technologies in oncology deepens our knowledge for the molecular landscapes of cancer diagnoses, identifying aberrations that could be linked with specific therapeutic vulnerabilities. In particular, there is an increasing list of molecularly targeted anticancer agents undergoing clinical development that aim to block specific molecular aberrations. This leads to a paradigm shift, with an increasing list of specific aberrations dictating the treatment of patients with cancer. This paradigm shift impacts the field of clinical trials, since the classical approach of having clinico-pathological disease characteristics dictating the patients' enrolment in oncology trials shifts towards the implementation of molecular profiling as pre-screening step. In order to facilitate the successful clinical development of these new anticancer drugs within specific molecular niches of cancer diagnoses, there have been developed new, innovative trial designs that could be classified as follows: i) longitudinal cohort studies that implement (or not) "nested" downstream trials, 2) studies that assess the clinical utility of molecular profiling, 3) "master" protocol trials, iv) "basket" trials, v) trials following an adaptive design. In the present article, we review these innovative study designs, providing representative examples from each category and we discuss the challenges that still need to be addressed in this era of new generation oncology trials implementing molecular profiling. Emphasis is put on the field of breast cancer clinical trials.

KEYWORDS Clinical trial; molecular profiling; breast cancer; study design

Introduction

Personalized medicine, alternatively known as precision medicine, corresponds to "a form of medicine that uses information about a person's genes, proteins and environment to prevent, diagnose and treat disease" according to the National Cancer Institute (NCI) definition[1]. Taking into account the highly complex nature of cancer diagnoses, with extreme underlying tumor molecular heterogeneity even among cancers of the same type, there is a pressing need to implement this concept in the field of oncology, providing tailored treatment approaches to patients with cancer. Lately, we have been witnessing success stories of personalized medicine in oncology, through the registration of highly potent molecularly targeted agents for patients with tumors bearing specific molecular aberrations. This is exemplified by the successful development of targeted agents blocking epidermal growth factor receptor (EGFR) for patients with metastatic non-small cell lung cancer (NSCLC) with tumors bearing *EGFR* mutations, or the one of MEK and BRAF inhibitors for patients with *BRAF* mutated metastatic melanoma (**Table 1**).

In the field of breast cancer (BC), the earliest successful application of personalized medicine took place through the clinical development of tamoxifen and subsequent other endocrine agents for patients with metastatic and early-stage disease on the basis of hormone receptor (HR) positivity, changing the natural history of the so called luminal BC[2]. It must be noted that the first clinical trials assessing tamoxifen in the setting of BC did not implement HR status assessment and were conducted in all comers, irrespective of HR status[3,4]. This means that the antitumor efficacy noted was diluted by the patients having no HR positivity; however, thanks to the high HR overexpression rates seen in BC, potent antitumor activity of tamoxifen was documented[5]. The subsequent refinement of patients enrolled in such trials, based on the biological rational of the molecular mechanism of action of endocrine agents being estrogen receptor (ER)

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Table 1 Molecular aberrations defining administration of approved targeted agents in different solid tumor diagnoses

Cancer type (alphabetically)	Molecular target	Assessment technique	Molecular aberration	Approved targeted agent (chronologically)
Breast cancer	ER and/or PgR	IHC	Overexpression	Tamoxifen, AIs fulvestrant
	HER2	IHC FISH	Overexpression and/or amplification	Trastuzumab, lapatinib pertuzumab T-DM1
Colorectal cancer	KRAS	DNA	Mutation	Cetuximab, panitumumab
Gastric cancer	HER2	IHC FISH	Overexpression and/or amplification	Trastuzumab
GIST	KIT	IHC	Mutation	Imatinib
Lung cancer	EGFR	DNA	Mutation	Gefitinib, erlotinib
	ALK and/or ROS	FISH	Rearrangement	Crizotinib
	RET	FISH	Rearrangement	Vandetanib
Melanoma	BRAF	DNA	Mutation	Vemurafenib, dabrafenib

AI: aromatase inhibitor, ALK: anaplastic lymphoma kinase, EGFR: epidermal growth factor receptor, ER: estrogen receptor, FISH: fluorescent *in situ* hybridization, GIST: gastrointestinal stromal tumor, HER2: human epidermal growth factor receptor 2, IHC: immunohistochemistry, PgR: progesterone receptor, T-DM1: trastuzumab DM1.

signaling inhibitors revealed the true magnitude of benefit from these agents for patients with luminal BC.

Another success story of personalized medicine's implementation in BC oncology is the development of trastuzumab, a monoclonal antibody, for patients with human epidermal growth factor receptor 2 (HER2) positive BC[6]. In that case, the scientific community had learned its lesson, thus the clinical development of this first-in-class HER2 blocking agent followed a different route: in particular all trials assessing trastuzumab were conducted among patients showing HER2 positivity, defined as protein overexpression and/or gene amplification, with a parallel development of robust HER2 status assessment methodology, to optimize patient identification[7,8]. It must be noted that the clinical development of trastuzumab in the setting of HER2-positive BC revolutionized the field in several ways, namely: i) it changed the natural history of HER2 positive BC, significantly improving the clinical outcome of patients with this-before trastuzumab's availability- notoriously hard to treat and clinically/biologically aggressive BC subtype, 2) it identified HER2-positivity as an "Achilles heel" of BC, rendering it vulnerable to several HER2 blocking agents that were developed subsequently, 3) it exemplified the importance of conducting clinical trials assessing molecularly targeted agents within rationally pre-selected molecular niches of the disease, and iv) it underlined the importance of having a robust companion diagnostic for a specific predictive biomarker that can expedite the clinical development of targeted agents and refine the patient selection once a new drug has been approved. This success story continues expanding further, in particular with the recent successful implementation of dual HER2 blockade as a potent therapeutic strategy for patients with HER2-positive BC[9].

To the present day, an abundance of studies implementing high-throughput molecular profiling techniques such as gene-expression profiling and next-generation sequencing (NGS) have been conducted in the setting of BC, resulting in molecular fragmentation of this group of diseases[10,11]. Such studies deepen our understanding of the molecular mechanisms underlying malignant progression in BC, identifying specific oncogenic signaling pathways and their respective molecular components being deregulated and functioning as drivers of the disease. It must be noted that a more "holistic" approach of BC has been implemented, with extensive molecular characterization conducted not only for the bulk tumor cells, but also for a subset of tumor initiating cells and the tumor microenvironment. Importantly, these efforts open new possible therapeutic avenues, since there is an expanding list of molecularly targeted agents undergoing clinical development in the field of BC, with many of them developed for patients with specific molecular aberrations[12].

There is an important shift in the field of clinical trials needed that will transition us from the era of conventional clinico-pathologic disease characteristics dictating patient eligibility, into a new era of clinical trials conducted within specific molecular niches of any given cancer diagnosis[13,14]. This paradigm shift necessitates an important molecular

profiling pre-screening step, prior to patient enrollment, adding logistical and other challenges in the conduct of clinical trials. Additionally, this expanding list of investigational anticancer agents undergoing clinical development, coupled with the identification of increasingly smaller molecular fragments of BC on the basis of rare, yet functionally important molecular aberrations, render the conventional study designs outdated and suboptimal. New, innovative and more efficient study designs are urgently needed to facilitate the successful clinical development of targeted agents within specific molecular fragments of BC[15]. In the next sections, we will provide an overview of such innovative study designs, once we have provided a summary of the molecular landscapes of breast cancer.

Molecular landscapes of breast cancer

Gene expression profiling analysis studies

The implementation of high-throughput gene expression profiling analysis through DNA microarrays in the seminal studies conducted by the Stanford group, already 15 years ago, led to the identification of the so-called four intrinsic BC subtypes; these subtypes are associated with different prognosis, ranging from favorable to poor clinical outcome, as well as with different therapeutic vulnerabilities, and even different patterns of metastatic dissemination[10], namely: i) luminal A BC, showing high levels of HR expression, with low proliferation rates and indolent clinical behavior, coupled with sensitivity to endocrine therapeutic manipulations, 2) luminal B BC, showing also HR-positivity, associated however with higher proliferation rates and more aggressive clinical behavior than their luminal A counterparts, with patients having this BC subtype in need of cytotoxic chemotherapy on top of endocrine therapeutic manipulations[16], 3) HER2-like BC, characterized at the molecular level by *ERBB2* gene amplification, affecting also other genes in the same amplicon, with a respective sensitivity to HER2 blockade therapeutic manipulations[17], and lastly iv) basal-like BC, largely showing a triple negative phenotype with lack of expression of ER, progesterone receptor (PgR) and HER2, resulting in aggressive clinical course and lack of molecularly targeted therapeutic options[18].

Interestingly, subsequent studies that coupled gene expression profiling analysis with that of genome copy number provided evidence for distinct profiles of copy number aberrations among the aforementioned BC intrinsic subtypes[19,20]. More recent studies, applying gene expression profiling analysis to larger sets of primary BC samples indicate extensive intertumor heterogeneity in BC, with increasing molecular fragmentation being identified. In particular, there was an important integrated analysis of almost 2,000 primary BC samples coupling copy number and gene expression data from the METABRIC (Molecular Taxonomy of Breast Cancer International Consortium) group. This initiative identified a total of 10 different BC subgroups, associated with different clinical outcomes and prognoses[21]. The basal-like intrinsic BC subtype has been studied extensively, with data supporting further molecular fragmentation through the identification of: i) the claudin-low tumors, corresponding to the most undifferentiated tumors along the mammary epithelial hierarchy, having a gene expression profile resembling that of mammary stem cells as well as mesenchymal features, and showing enrichment for epithelial-to-mesenchymal transition (EMT) markers[22], and 2) molecular-apocrine tumors, being a subset of ER-negative BC, which is characterized by expression of androgen receptor (AR) and downstream signaling, with AR blockade being assessed as possible targeted therapeutic option[23].

NGS studies

During more recent years, we have witnessed many research studies applying NGS (alternatively known as massive parallel sequencing - MPS), mostly for primary BC samples, expanding further our knowledge about the molecular landscapes of BC and fueling the evidence of both inter- and intra-tumor molecular BC heterogeneity[24-28]. This evolving technology represents a powerful molecular profiling technique, able to decipher DNA and RNA sequences and to interrogate different types of molecular aberrations, ranging from nucleotide substitution mutations, and insertions/deletions, to copy number variations (CNVs) and structural rearrangements[11]. Additionally, NGS can provide quantitative information about the allelic frequency of any mutational event detected, thus allowing the discrimination between clonal and subclonal molecular aberrations and enabling the formation of cancer samples phylogenetic trees; the latter one provides valuable insight for the clonal architecture and life cycle of any given tumor analyzed[29,30].

Another important finding originating from NGS studies in BC is the supporting evidence for the extensive inter-tumor heterogeneity underlying primary tumor samples sequenced, as shown by the study of Stephens et al; in this study among the 100 sequenced BC primary samples, 73 different combination possibilities of mutated cancer genes

were identified[28]. Furthermore, one additional main message from these studies is that there is only a handful of commonly mutated cancer-related genes, exemplified by *TP53* and *PIK3CA*, with the rest forming a long tail of mutated genes found in less than 10% of the BC samples analyzed[31,32]. Lastly, the ability of NGS to quantify the levels of allelic frequencies of molecular aberrations indicates that not all detected gene mutations constitute clonal molecular events, since most of them are detected within subclonal populations of cancer cells in the samples analyzed[33].

Of note, steeper than what was initially anticipated, decreases in the financial costs of NGS, rendered this powerful tool for molecular characterization widely available across the scientific community, nowadays being used in laboratories and hospitals around the world[34]. To the present day, different approaches of NGS-based molecular profiling have been developed, that can be summarized as follows:

Whole-Genome Sequencing (WGS), with the first reported case of this approach being that of a cytogenetically normal acute myeloid leukemia (AML), a highly malignant hematopoietic tumor[35], with several studies having used subsequently this approach in the setting of BC[28,36,37].

It must be noted that factors such as restrictions posed by archived formalin-fixed paraffin-embedded (FFPE) tumor material that are subsequently subjected to WGS, as well as considerable financial costs and highly complex and laborious bioinformatic tools needed for the analysis of the results, limit the clinical implementation of WGS[38].

Targeted sequencing, being either whole-exome sequening (WES), or targeted-gene sequencing (TGS), represents an alternative approach based on panels of selected cancer-related genes. Despite the obvious advantages of such approaches as compared to WGS, namely reduced financial costs and less complex bioinformatic analysis approaches needed for data analysis and interpretation, there is an innate compromise with this approach, in terms of not being able to detect translocations and other structural rearrangements on the one hand, as well as remaining "blinded" towards many genes that could bear (relevant) molecular, still unknown, aberrations [39].

Innovative clinical trial designs

Currently, there is an increasing use of the aforementioned molecular profiling for patients with BC, in particular in the setting of high-volume, tertiary academic institutions, where extended profiling programs are being developed, sometimes in a CLIA (Clinical Laboratory Improvement Amendments) environment [40]. Such initiatives are usually set up, with the objective being to guide patients in clinical trials assessing targeted agents for specific genotypes of cancer[41]. However several challenges can be identified, in regard to the success of trials assessing such experimental anticancer compounds, matched to specific molecular abnormalities (**Table 2**). To address these ever more frequently met challenges, new transformative clinical trial designs are needed. In these new generation innovative clinical trials, eligibility is based on the molecular profile and/or genotype of BC, rather than the classic clinicopathologic characteristic of the disease. These study designs hold the promise to facilitate the clinical development of anticancer drugs, as well as to keep the numbers of patients recruited in the respective trials at reasonable levels, since the expected antitumor efficacy will not be diluted by patients with inherently resistant disease towards the targeted agent undergoing clinical assessment. To this end, several innovative study designs are being developed, which we present in the following sections.

Longitudinal cohort studies with or without downstream clinical trials

This is a study design corresponding to the enrollment of patients in a program of extensive molecular profiling coupled with prospective follow-up for the clinical outcome of the enrolled patients; they can be treated either with standard of care, or be directed to downstream clinical trials.

Table 2 Challenges faced in current clinical trials assessing targeted anticancer agents and proposed mechanisms to circumvent them

Challenge	Potential solution
Coupling of molecular aberrations to targeted agents	Preclinical cancer research findings indicating potential therapeutic opportunities
Selection of specific mutations based on their predicted functional output	"All-comers" approach or evaluation of functional output of aberrations through functional experiments and/or bioinformatic tools
Subclonality of molecular aberrations	"All-comers" approach and retrospective look or arbitrary selection of threshold
Lack of "actionable" aberrations	Therapeutic agents that do not rely on molecular aberrations, e.g. chemotherapy
Concurring "actionable" aberrations	Second randomization or prioritization based on allelic frequency or prioritization based on frequency of aberration or physician's choice or algorithm combining the above

The AURORA(Aiming to Understand the Molecular Aberrations in Metastatic Breast Cancer) study, initiated by Breast International Group (BIG), represents a recent example of longitudinal cohort study that aims to elucidate the molecular landscapes not only of primary but also of metastatic BC, as well as to generate knowledge about the life cycle of the latter one (NCT02102165)[42]. In particular, AURORA is an academic initiative conducted in leading European hospitals of the BIG network that will enroll 1,300 patients with metastatic BC and will perform NGS and RNA sequencing of matched primary and metastatic tumor samples (**Figure 1**).

The patients entering AURORA will be followed prospectively for up to a maximum of 10 years with rigorous collection of treatment and clinical outcome information that will be then associated with the extensive molecular background information generated through AURORA. Furthermore, additional blood/plasma samples will be collected at several timepoints, with the intention being to have a future molecular profiling analysis for the identification of plasma-based putative biomarkers. This molecular profiling can support the conduct of "nested" or "downstream" clinical trials that can be either genotype-driven or not[43], with the goal being to: i) guide molecularly pre-screened patients to "nested" or "downstream" clinical trials conducted within specific molecular niches of BC, and 2) identify putative predictive biomarkers, since the clinical outcome of patients enrolled in the "downstream" clinical trials can be coupled with the molecular profile information generated through such a program. In the case of AURORA, a downstream trial called PYTHIA (Palbociclib in Molecularly Characterized ER-positive/HER2-negative Metastatic Breast Cancer) is about to open enrollment, aiming to assess the efficacy of palbociclib in the setting of endocrine resistant metastatic luminal BC and assess putative predictive biomarkers for CDK4/6 inhibition coupled with endocrine treatment (NCT02536742).

Further benefits can be expected by such initiatives, through the educational effect they bring to clinicians, since they get familiarized with the reporting of genomic data and

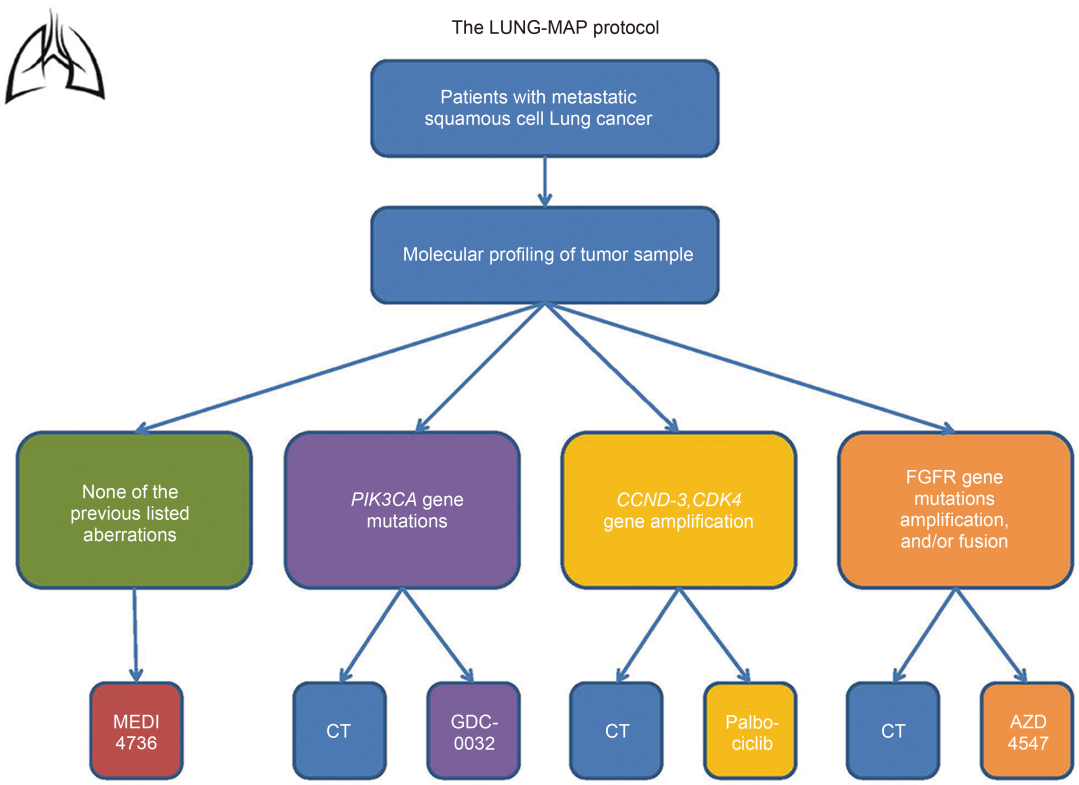

Figure 1 The lung cancer master protocol (LUNG-MAP). CCND1-3: cyclin D1-3, CDK4: cyclin-dependent kinase 4, CT: Chemotherapy, FGFR: fibroblast growth factor receptor, LUNG-MAP: lung cancer master protocol, PIK3CA: phosphatidylinositol-4, 5-bisphosphate 3-kinase, catalytic subunit alpha.

their clinical interpretation. Another similar initiative that has been conducted in France by UNICANCER is the SAFIR01 research program conducted among more than 400 patients with metastatic BC, recruited across 18 French centers[44]. These patients had metastatic lesion biopsy, with subsequent molecular characterization through comparative genomic hybridization (CGH) as well as Sanger sequencing for the *PIK3CA* and *AKT1* genes[44]. The same research group is currently conducting a new open label multicentric phase 2 randomized trial called SAFIR02_Breast and sponsored by UNICANCER, recruiting patients with HER2-negative metastatic BC, whose metastatic lesion biopsies will be subjected to NGS (NCT02299999). These patients, who will be pretreated with no more than 1 line of systemic chemotherapy, will receive 6-8 cycles of cytotoxic chemotherapy; thereafter patients that did not develop disease progression will be randomized to receive standard of care or a targeted agent matched to their molecular profile according to a list of 51 molecular alterations, as a "maintenance" therapy. The primary endpoint of this trial, which aims to recruit 460 patients, is the progression free survival (PFS) in the targeted drug arm compared to the standard maintenance therapy arm.

Despite the obvious advantages of this study design, deriving from the coupling of clinical with molecular information, along with the possibility to assess different experimental drugs within this patient population, there are certain challenges as well. In particular, the wide spectrum of molecular aberrations found in patients with BC render the interpretation of their potential clinical significance difficult. Additionally, this wide repertoire of aberrations seen, inserts statistical restrictions related to multiple testing issues. Consecutively, findings reported from such cohorts of patients should be more viewed as hypothesis-generating that need to be confirmed by subsequent studies.

Studies assessing the clinical utility of molecular profiling

The new powerful molecular profiling tools have provided much information about the molecular landscapes of BC and they promise to guide patients to targeted treatment based on the molecular profile of their disease[45]. However, their clinical utility needs still to be proven, with a newly emerged study design specifically trying to address this issue. In particular, this study design attempts primarily to reply to the question whether there is clinical benefit for patients with cancer to receive targeted agents guided by molecular profiling as compared to conventional treatment. It must be noted that such studies are "proof-of-concept" not assessing individual treatment options, but the whole concept of molecular profiling guiding treatment selection. In particular, these studies implement and assess treatment algorithms that match patients to specific anticancer (targeted) agents based on specific molecular aberrations of their tumors.

Such a study has been reported by Tsimberidou et al,[41] through a non-randomized phase 1 clinical trial program with promising results conducted at the University of Texas MD Anderson Cancer Center. In the context of this program, 1,144 patients with advanced solid tumors of several different histologies were enrolled, with their tumor tissue being molecularly profiled and then directed to one of several phase 1 trials assessing targeted compounds: patients that had one molecular aberration and received targeted treatment based on the molecular profile of their disease (n=175) showed increased overall response rate (ORR, 27% *vs*. 5%, P<0.0001), longer time-to-treatment failure (TTF, 5.2 months *vs*. 2.2 months, P<0.0001) and longer overall survival (OS, 13.4 *vs*. 9.0 months, P=0.017) as compared to patients receiving conventional treatment (n=116)[41].

Similarly, the SHIVA trial (NCT01771458) is a recently completed randomized proof-of-concept phase 2 trial, conducted at eight French academic centers that compared the two conceptually different approaches among patients with several different types of solid tumors, namely conventional treatment versus targeted therapy based on metastatic tumor tissue molecular profiling results[46]. In particular, SHIVA assessed 10 different regimens consisting of 11 available molecularly targeted agents (erlotinib, lapatinib plus trastuzumab, sorafenib, imatinib, dasatinib, vemurafenib, everolimus, abiraterone, letrozole, tamoxifen), on the basis of identification of molecular alterations within three oncogenic signaling pathways. The SHIVA trial had a feasibility part in its protocol, which demonstrated the feasibility and safety of incorporating biopsy of metastatic disease for the first 100 enrolled patients[47]. SHIVA's full results were recently reported: out of 741 patients with different tumor types screened, there were 293 (40%) with at least one molecular aberration, conferring eligibility to one of 10 available targeted regimens[48]. A total of 195 (26%) patients were randomly assigned, with 99 in the experimental arm and 96 in the control arm. After a median follow-up of 11.3 months for both arms, there was no difference in terms of PFS among the two arms (experimental arm, 2.3 months *vs* control arm, 2.0 months, HR 0.88, 95% CI 0.65-1.19, P=0.41). These results indicate that the matching of molecularly targeted agents with molecular aberrations, outside their registered indications, did not improve clinical

outcome of heavily pretreated patients with advanced solid tumors as compared to conventional treatment.

Master-protocol trials

This type of study design enables the assessment of several targeted agents on parallel. After one molecular prescreening step, patients are being directed to one of several downstream treatment arms, receiving a targeted agent matching the molecular profiles of their disease[49]. The main advantage of this innovative type of study design is the reduction in the percentage of screening failure rate, since patients undergoing the molecular prescreening can have more options for subsequent matched targeted treatment in one of the downstream trials. Additionally, there is increased efficiency in some of the important preparatory steps to activate such a trial, such as the need to have one common ethics committee approval that will allow the clinical assessment of several different investigational agents.

An important initiative implementing study design, recently launched by the NCI (National Cancer Institute) in collaboration with SWOG, the Foundation for the National Institutes of Health, and the Friends of Cancer Research and the FDA, is the master protocol for second-line treatment of patients with squamous NSCLC; according to this protocol NGS of tumor samples will be performed using a panel of 250 cancer-related genes and patients will be guided to one of the five integrated study strata, with a total of 10 treatment arms[50]. Within each one of these strata, a phase 2/3 study design has been incorporated, with predefined thresholds of efficacy that need to be demonstrated prior to the phase 3 component activation (**Figure 2**). In the setting of BC, BIG is currently designing such a master protocol trial, aiming to assess several molecularly targeted agents for patients with aggressive metastatic triple negative breast cancer (TNBC).

Basket-trials

This is an innovative, histology-independent trial design, where patients with cancer diagnoses of different histologies can be enrolled in the study protocol based on the presence of a specific molecular aberration. There is a currently ongoing clinical trial that aims to develop a small molecule HER2 blocking agent within patients with *ERBB2* mutated cancers that exemplifies this approach[51]. The main disadvantage in this innovative design is a biology-driven one; in particular this is the issue of the potentially different functional outputs that a specific molecular aberration could have among different types of cancer. This has been reported in studies documenting lack of antitumor efficacy of vemurafenib, a BRAF small molecule inhibitor, in the setting of *BRAF* mutated metastatic colorectal cancer; these findings are in direct contradiction with the dramatic antitumor activity seen among patients with metastatic melanoma bearing the V600E *BRAF* mutation[52].

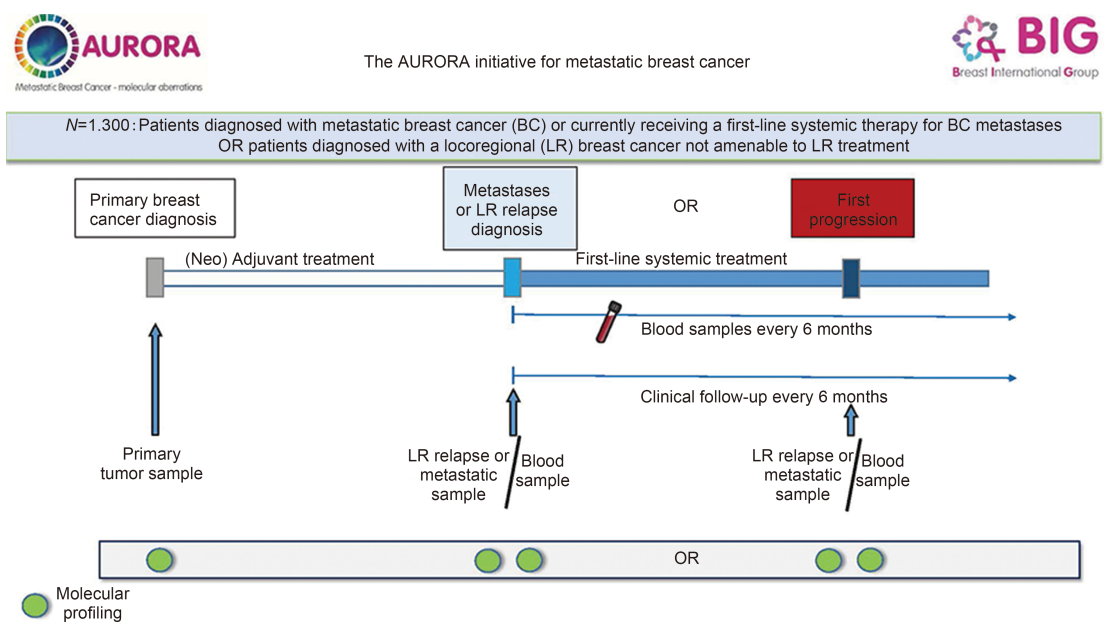

Figure 2 The AURORA initiative for metastatic breast cancer of Breast International Group.

Adaptive-trials

Another innovative study design that has entered the arena of clinical trials in oncology are the so-called adaptive trials[53]. This type of study designs corresponds to trials that allow modifications in the study during its conduct, related among other parameters to the study population, or the statistical framework. The initial conceptual development of this study design dates back to 1970s, when the concept of adaptive randomizations was firstly introduced[54]. Adaptive trials can be conducted in different phases, namely phase 1 adaptive dose finding studies, or phase 1/2 adaptive seamless studies for early clinical development of experimental compounds, as well as phase 2/3 adaptive seamless trials for late clinical development. Such a study design has been recently exemplified by the BATTLE (Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination)-1 and -2 clinical trials, conducted among patients with metastatic NSCLC as an effort to personalize treatment of patients with lung cancer[55], or the I-SPY (The Investigation of Serial Studies to Predict Your Therapeutic Response with Imaging and Biomarker Analysis)-1 and -2 trials conducted among patients with early-stage BC, in the neoadjuvant setting[56-58]. These constitute trials that evolve and dynamically shape literally during their conduct, on the basis of ongoing generated results from the actual trial.

During the initial phase of an adaptive trial, the enrolled patients are being recruited at equal ratio across the different arms; however, as more patients are being enrolled and efficacy data begin to emerge from the trial and are pooled together from the different arms, the adaptive phase starts. During this adaptive phase, randomization ratios can be adapted according to these results, with the additional flexibility of either closing or opening new treatment arms, on the basis of either futile results or new data indicating promising results for other investigational agents[53]. Studies following an adaptive design are more laborious in the sense that clinical trials' simulations are needed, with different statistical scenarios needed to be developed by highly skilled biostatisticians and trialists[59]. This characteristic could discourage expansion of the use of this innovative study design; however, recent guidance provided by regulatory authorities, i.e. FDA and EMA, about the adaptive trial designs they deem acceptable supports the further embracement of adaptive trial designs[60].

Conclusions

Personalized/precision cancer medicine has demonstrated its potential in the field of BC, with the successful implementation of endocrine treatment as well as HER2 blockade therapeutic strategies in preselected patients on the basis of HR and HER2 status, respectively. In the new era of molecular profiling with powerful, high-throughput techniques, we witness the identification of several molecular aberrations that could be used to direct patients with BC to specific molecularly targeted agents undergoing clinical development. Before the implementation of either molecular profiling and/or new targeted agents, prospective clinical trials are needed to establish their clinical utility and antitumor activity, respectively. Given the increasing molecular fragmentation of BC and the expanding numbers of investigational compounds that enter clinical development, it becomes apparent that new, innovative clinical trial designs are needed[61]; such study designs must take into account the extensive molecular heterogeneity underlying BC[62].

These designs must implement efficiently the integration of validated molecular profiling techniques as pre-screening step to guide patients with specific molecular profiles to their respective targeted agents. Of note, despite the current predominance of NGS in the arena of molecular characterization of BC that has already contributed significantly to the elucidation of the genomic landscapes of this common disease, other techniques of molecular profiling undergo development, such as proteomics-based methods. Such methods, when coupled with the former ones, can prove to be very informative about the functional output of specific molecular aberrations, thus refining the patients' selection for new clinical trials. These new trials provide further research opportunities through the formation of extensive biosamples' collections that can be mined for subsequent translational research. Of note, the successful implementation of these new, innovative study designs reviewed above necessitates further deepening of the collaboration between different academic BC research groups and with pharma companies developing targeted agents, or even between different companies.

Conflict of interest statement

No potential conflicts of interest are disclosed.

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