Abstract

Progress has been made in the treatment of metastatic breast cancer in recent decades, but very few therapies use patient or tumor-specific characteristics to tailor individualized treatment. More than ten years after the publication of the reference human genome sequence, analysis methods have improved enormously, fostering the hope that biomarkers can be used to individualize therapies and offer precise treatment based on tumor and patient characteristics. Biomarkers at every level of the system (genetics, epigenetics, gene expression, micro-RNA, proteomics and others) can be used for this. This has led to changes in clinical study designs, with drug developments often only focusing on small or very small subgroups of patients and tumors. The screening and registration of patients and their molecular tumor data has therefore become very important for the successful completion of clinical studies. This new form of medicine presents particular challenges for patients and physicians. Even in this new age of genome-wide analysis, the focus should still be on the patients’ quality of life. This review summarizes recent developments and describes how the PRAEGNANT study network manages the aforementioned medical challenges and changes to create a professional infrastructure for patients and physicians.

Zusammenfassung

Introduction

As our understanding of breast cancer grows, it has become increasingly clear that it is possible not just to plan and assess the choice of therapy for patients with breast cancer but also the course of therapy using molecular and cellular tests [1]. Some therapies already treat patients based on the patient’s specific genetic mutations and amplifications or gene expression patterns. However, most of these therapies are still being carried out in the context of research or clinical studies. But new therapies for established patient and tumor characteristics are also emerging, with specific therapies developed for and aimed at particular subgroups of patients. The two most well-known examples of this are hormone therapy and anti-HER2 therapy.

The clinical and scientific basis of this approach are outlined below. The focus is on breast cancer patients with metastatic disease. The research and care initiative PRAEGNANT will also be described. This initiative aims to create a basis which will make this type of medicine available to patients with metastatic breast cancer and their physicians even outside clinical studies.

Prognostic and Predictive Factors in Metastatic Breast Cancer

The PRAEGNANT study program focuses on patients with advanced, incurable, metastatic breast cancer. There are a few clinical factors which could have a prognostic significance for patients with metastatic breast cancer, even if they are not yet used much in routine clinical practice. In routine practice, decisions on therapy tend to focus more on the overall clinical picture, the severity of symptoms according to the patient’s subjective assessment, and the subjective assessment of how rapid remission needs to be.

Factors described in some studies as prognostic factors for breast cancer patients with metastatic disease include age, tumor mass, grading, time from primary diagnosis to metastasis, and the site of metastasis [2]. Molecular factors have also been associated with prognosis. Most studies have focused on the prognostic characteristics of the primary tumor and the prognosis for the patient after metastasis. Hormone receptor status, HER2 status and Ki-67 are the most commonly investigated parameters [3]. However, tumor characteristics are known to change during the course of disease [4–11], and the recommendation to carry out biopict evaluation of metastases to determine their molecular characteristics has therefore been included in national therapy recommendations [12]. But although obtaining tumor tissue from the breast is relatively uncomplicated, for practical reasons and to avoid complications, physicians often object to taking biopsies of metastatic tissue. One of the aims of the PRAEGNANT study program is to carry out molecular tests in the setting of metastatic disease and to develop evaluation methods which can be carried out without requiring biopsies of metastatic tissue, for example by testing the patient’s blood. Circulating tumor cells, circulating nucleic acids or other biomarkers could be used for this type of analysis.

Molecular Patterns in Breast Cancer

Most of the current understanding of molecular patterns in breast cancer was gained from patients with primary breast cancer without metastatic disease. Most of the biomarkers listed below therefore refer to primary breast cancer.

As our knowledge of the human genome increases and the cost of genome-wide analysis decreases, the relationships between genomics, epigenetics, gene expression and protein functions are becoming clearer. The existence of molecular subgroups for breast cancer types and their prognostic relevance based on mRNA measurements were already discussed in the literature more than 10 years ago [13–15]. One classification differentiates between basal, luminal A, luminal B and HER2-enriched breast cancer subtypes. An attempt was subsequently made to classify these molecular subtypes using known histopathological characteristics [16–19]. Triple-negative tumors (ER negative, PgR negative and HER2 negative) were found to most closely resemble basal tumors. Slow-proliferating (e.g. Ki-67 < 14%) and hormone receptor-positive tumors generally correspond to luminal A tumors, while hormone receptor-positive tumors with high proliferation rates (e.g. Ki-67 > 14%) are most closely correlated with luminal B tumors [20–22]. These cut-offs mirror the biological subtypes. However, other cut-offs (for example 20%) are also being discussed in clinical practice [23].

The Human Genome Project and the publication of the human genome sequence [24–26] has created the basis for modern genome-wide analysis at various levels of systems biology. The biological interrelationships behind various diseases are gradually being – at least partially – uncovered. One of the next challenges in breast cancer research will be to obtain an understanding of why there are different patterns of breast cancer expression and why these may change during the course of disease. Genetic tumor factors probably play a role as could epigenetic patterns, micro-RNA and other, still unknown regulation mechanisms. Hereditary factors such as BRCA mutation or low-penetrance genetic variants are also known to affect gene expression in tumors [27].

Genetic Factors

Various genetic germline mutations and somatic genetic mutations have been associated with tumor biology and the prognosis of breast cancers. Both mutations and structural and numerical changes to the tumor genome and germline genome could be relevant for the tumor biology of breast cancer.

Mutations during pathogenesis

Genetic tumor mutations have already been investigated by comparing mutations to unchanged DNA. The most extensive investigation in this context was the Cancer Genome Atlas (TCGA) [28]. In the TCGA the genetic information of unchanged reference DNA was compared to the DNA of breast cancer tumors. This resulted in the identification of the most common mutations occurring in breast cancer. The most common gene mutations in breast cancer found in the TCGA investigations were TP53, PIK3CA, GATA3, MAP3K1, MLL3, CDH1, MAP2K4, RUNK1, PTEN and others. These initial investigations have already shown clear differences in mutation frequencies between different molecular subtypes classified according to their level of gene expression. Thus, a PI3K mutation was found in 32–49% of cases with luminal (A
and B) and HER2-positive breast cancer but only in 7% of cases with basal-like tumors [28]. This explains the current development of PI3K inhibitor drugs which focus on hormone receptor-positive and HER2-positive disease.

Changes in the number of gene copies
Another genetic tumor change can consist of an increase or decrease in the number of gene copies. In a large investigation carried out as part of the METABRIC study, the number of gene copies and the associated gene expressions were determined for every gene [29]. This allowed positions to be identified in the genome where changes in gene copy numbers and an associated change in gene expression occurred most commonly. Genes in which such changes occurred include ZNF703, PTEN, MYC, CCND1, MDM2, ERBB2, CCNE1, MDM1, MDM4, CDK3, CDK4, CAMK1D, PI4KB, and NCO1 (amplifications) and PPP2R2A, MTAP, and MAP2K4 (deletions). The strongest association was found between amplification in the genes HER2 (ERBB2) and cyclin D1 (CCND1).

Genetic variants as prognostic or predictive factors
Some studies have tried to link the most common mutations listed above and gene copy alterations to prognosis, in other words, to link prognosis to these new biomarkers. Gene copy alterations and the associated changes in gene expression could be used to improve the evaluation of prognosis for known molecular subgroups [29]. But with the exception of HER2, none of these loci have been found to have any clinical relevance for prognostic evaluation or therapy planning.

With regard to mutations, it was found that mutations in PI3K are associated with a poorer response to anti-HER2 therapy in patients with HER2-positive tumors. Only 19.4% of patients with PI3K mutation had complete pathological remission compared to 32.8% of cases without mutation in this gene [30]. Nevertheless, mutation in PI3K was not found to be correlated to a different prognosis [30]. Similarly, no association between prognosis and mutations and amplifications was found in patients treated with the mTOR inhibitor everolimus [31]. Some genes whose mutation affects the efficacy of neoadjuvant aromatase inhibitor therapy have been identified (MAP3K1, PIK3CA, TP53, GATA3, CDH1, TBX3, ATR, RUNX1 and others) [32]. Considerable overlap was found between these genes and the genes described by the Cancer Genome Atlas [28, 32].

A large-scale analysis of mutations in 83 genes done in a large population of hormone receptor-positive patients (n = 632) has provided more insight into the importance of mutations as prognostic markers [33]. The 83 genes were selected based on previous studies [28, 32, 34] and sequenced in the patient population. In this analysis, mutation in the DDR1 (discoidin domain receptor tyrosine kinase 1) gene [33] was found to be correlated with prognosis. It remains to be seen whether and which genes could be used as prognostic and predictive parameters based on their genetic alterations. Table 1 presents a number of such genes previously described in the literature [28, 32, 34].

**Table 1** Genes whose mutations could potentially play a role in the pathogenesis or prognosis of breast cancer or affect the therapeutic efficacy of breast cancer treatment [28, 32, 34].

<table>
<thead>
<tr>
<th>AFF2</th>
<th>CREBBP</th>
<th>JAK2</th>
<th>NCO1</th>
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<td>LYN</td>
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Significance of genetic germline variants
In addition to somatic variants, the relevance of hereditary germ-line mutations and variants for the prognosis of breast cancer are also being discussed. A patient’s genetic heredity can be associated with a specific molecular breast cancer subtype. The most famous examples of this are patients with the BRCA1 mutation. If a patient with the BRCA1 mutation develops breast cancer, the probability that this cancer will be triple negative is more than 50% [27, 35, 36]. Other low-penetrance genetic variants reported in the literature were also found to be associated with a specific molecular subtype [37–45]. Some of them are similar to BRCA1 and BRCA2 in that they play important roles in both breast cancer and ovarian cancer [46–48]. Unsurprisingly, genetic variants have also been associated with prognosis and the overall survival of breast cancer patients [49–52]. The causes can be so wide-ranging that even the
The presence of circulating tumor cells (CTC) in plasma has been consistently associated with prognosis in patients with metastasized breast cancer [66–69]. The presence of CTCs was found to be an independent prognostic factor even in the non-metastatic setting [70]. The next logical step was to determine the molecular properties of circulating tumor cells [71, 72]. Several clinical studies are currently looking at whether this could help with treatment planning and offer useful information for prognosis [73–76]. However, isolating the CTCs is still relatively expensive and time-consuming and requires large, cost-intensive equipment which is expensive to run.

A less expensive approach could be to analyze circulating nucleic acids. Tumor cells in the body release small amounts of DNA into the bloodstream, known as circulating DNA (ctDNA). This process was first described in 1948 [77, 78]. But it is only now that new analysis methods offer the opportunity to use these circulating nucleic acids to research and potentially treat tumor disease. Various analyses in serum become possible, they could provide early indications about the efficacy of and the response to treatment. A few small studies have reported promising results with concordance between mutations found in tumors and those found in ctDNA from the same patient [80].

**Table 2** Breast cancer studies which have integrated molecular tests in their study design or use them as predictors for the primary study goal (*relevant for therapy or study means that the results of biomarker tests affected the choice of therapy or the study design over and above stratification or subgroup analysis; **in some of these studies, inoperable locally advanced disease was sufficient for inclusion in the study).
Inclusion of Genetic Testing in Studies

Only two biomarkers are used in routine clinical practice to plan treatment for patients with breast cancer: HER2 status and hormone receptor status. Some clinical studies have included or prospectively will include the determination of biomarkers (usually testing for mutations or to measure gene expression) in the study design (Table 2). Some studies demand specific test results as a prerequisite for inclusion in the study. Other studies use the test results for prospectively planned subgroup analysis and/or stratification.

The current PARP inhibitor studies are one example of studies which require a specific testing result. Most of these studies demand evidence of BRCA mutation for inclusion into the study. Some studies additionally only include patients with triple negative breast cancer.

Others studies have prospectively integrated test results into the treatment concept and randomization algorithms. The NNBC3 study used invasion factors uPA/PAI1 to determine which patients should receive adjuvant chemotherapy. More recent studies have used multi-gene tests. The ADAPT study concept used a 21-gene test and serial analysis of tumors to identify patients with an excellent prognosis for whom it was postulated that they did not require chemotherapy.

PI3K mutation testing is an example of studies where an analysis of projected subgroups is planned. Although the biological importance of PI3K mutations, particularly in HER2-positive and hormone receptor-positive breast cancer, is well established and there are indications that tumor response to anti-HER2 treatment depends on mutation status [30], it is still unclear whether the mutation can predict efficacy when the enzyme itself is inhibited. In fact, there is some initial evidence that mutation status did not predict efficacy as measured by progression-free survival in patients treated with the PI3K inhibitor pictilisib [81].

The range of molecular tests included in clinical studies has increased in recent years. A number of studies completed or still underway in Germany have been listed in Table 2.

The PRAEGNANT Study (NCT02338167)

The PRAEGNANT study network (Prospective Academic Translational Research Network for the Optimization of Oncological Health Care Quality in the Advanced Therapeutic Setting) was set up in Germany to take account of some of the recent rapid developments in molecular medicine in the treatment of patients with metastatic breast cancer. Given all of the above, the PRAEGNANT study concept was set up for the following reasons:

- To carry out molecular tests under study conditions.
- To identify suitable breast cancer patients for clinical drug trials based on molecular testing.
- To identify breast cancer patients suitable for clinical drug trials based on conventional clinical inclusion criteria.
- To record treatment-induced toxicities and patient’s quality of life in routine clinical practice.
- To record, show and benchmark the reality of medical care provided to patients with advanced metastatic breast cancer.

Patient population, planned sample size and course of the study

Inclusion in this study concept is not limited to patients receiving specific treatment lines. All breast cancer patients who have either metastasis or inoperable loco-regional disease can be included in the study, irrespective of the treatment line they are receiving. Disease progression must be objectively evaluable. Analysis of the first 244 patients included in this study resulted in the distribution shown in Table 3. Almost 50% of patients were receiving first-line treatment for metastatic disease.

The study aims to include a total study population of around 3200 patients. This should ensure that approximately 150 patients receiving first-line and 150 patients receiving second-line treatment in the metastatic setting will be included in the study for every molecular subtype, even for rare subtypes with a prevalence of only 10%. This should provide a good insight into the prognosis and the quality of life of these patient groups.

Using research findings

Many current studies use specific clinical or molecular characteristics as inclusion criteria for patients in specific studies (cf. Table 2). Moreover, some studies require specific prior treatment and a specific progression over time before patients are included in the study. The PRAEGNANT study should be utilized to carry out investigations using the available biomaterials obtained in the study. As part of the analysis of this biomaterial, patients will be evaluated to see whether they are suitable for recruitment into particular studies. The patients will be informed of this at the start of recruitment into the PRAEGNANT study and can give their consent to being informed by their physician if they are found to be suitable for inclusion in other studies. This applies not only to molecular requirements but also to clinical parameters. Ideally, the patient is then referred to a center in the PRAEGNANT network where a specific study is being carried out.

It is planned that around 40 centers will be participating, which will allow prior treatment to be compared between centers. There are almost no guidelines about the order in which various therapeutic agents should be used in the metastatic setting. Clin-
ical studies require specific prior treatments as the prerequisite for inclusion in the study. The information which the PRAEGNANT study will collect can be used in various ways. Centers can be given feedback if the treatment they provide does not fulfil the conditions of current studies. Cooperating centers carrying out studies can be informed about treatments currently used in clinical practice, allowing the cooperating centers to adapt their inclusion and exclusion criteria – where possible – to standard treatment practice.

**Using the network to exchange knowledge**

The aim is to use the information obtained and collected to record differences in care between the participating centers and to encourage a discussion about these differences. Patients could be included in this evaluation through the use of Patient-reported Outcomes (PRO) and specific information should be shared with the patient. In this information age, data collection should not simply be used to generate study data but also to provide direct and immediate benefits to the patient and the participating centers through the documentation and collection of data.

**Using the study to show quality of life under real-life conditions**

In Germany and other countries, after approval has been granted, as part of the approval process the authorities often demand evidence based on standard treatment practice that the study results are confirmed with regard to efficacy and quality of life. This is precisely the type of data the PRAEGNANT study will collect and evaluate. Patients will complete validated questionnaires on quality of life at regular intervals. Substudies will continue to investigate issues of treatment compliance, nutrition and exercise and look at health economics and pharmacoeconomics. The data will be used for assessment, evaluated to allow comparisons with clinical studies and made publicly available. The recording of Adverse Events is of particular interest in this context because for many new medications physicians only learn the best way of recording possible side-effects during approval studies. A higher incidence of problems and more significant clinical problems are noted more often in the post-approval setting than in approval studies. The musculoskeletal symptoms experienced during treatment with aromatase inhibitors are a well-known example of this. Another example is stomatitis in patients receiving everolimus; the incidence of stomatitis initially appeared to increase in clinical practice but then decreased again as administration of...
everolimus continued, possible because of improved prophylactic measures [82]. It is effects such as these that the PRAEGNANT study aims to record.

Patient-reported Outcomes (PRO)
In clinical studies, assessment of the patient’s health is usually done by the physician. The patients themselves only complete validated questionnaires on their quality of life. Although this approach ensures a certain degree of objectivity when documenting side-effects, for example, the requirement of visits at regular intervals and the dependence on the motivation of the medical staff could also result in a poorer quality or otherwise affect the quality of the documentation. Modern, internet-based interaction portals can react flexibly to the documentation requirements of studies and patients. Various PRO modules covering areas such as quality of life, compliance to treatment, exercise, nutrition, Adverse Events and others will be tested and validated in the PRAEGNANT study.

Database concept
The PRAEGNANT study network uses an Oracle-based database with an eCRF format. It fulfills all the requirements for use by clinical studies (visit-based, recording of AEs and SAEs, audit trail...). Monitoring of all data is done using a professional query verification and source data verification system. What distinguishes the PRAEGNANT network from most other multicenter studies is that the participating centers can download their center-specific data at any time during the project and use it for scientific research themselves.

Biomaterial collection
The collection of biomaterials plays a central role in the PRAEGNANT study network. The obtained biomaterials will be used to carry out high-level analyses which are relevant for the patient. These include both testing of the primary tumor and the metastasis and testing of biomaterials obtained from blood (Figs. 1 and 2). Extensive blood samples will be taken from every patient on inclusion in the study, at each point of disease progression and/or every 3 months (Fig. 1). Patients will additionally be asked whether they will permit the analysis of archived tumor samples (primary tumor and metastasis) for research purposes (Fig. 1). Blood samples include cannula to obtain serum, plasma, micro-RNA, leukocyte RNA, CTCs, ctDNA and germline DNA. In addition, procedures which specify how biomaterials obtained in multicenter studies should be sent to a central laboratory without endangering the quality of the investigation will be optimized. Patients will additionally be requested to sign over part of the tumor material from the primary tumor and from the biopsies of metastases to the study. The tumor block will be requested by the respective pathologist and re-evaluated using H&E slides, and recuts will be made for archiving and RNA and DNA extraction. In addition, a tissue micro-array (TMA) will be created from the tumor block. The PRAEGNANT study network is thus ideally positioned to carry out the analyses described above for patients and for existing studies.

Conclusion
More than 10 years after the human genome was decoded, molecular analysis is being included in routine clinical practice and particularly in the design of clinical studies. The complexity of these analyses and the size of the subgroups defined by such analysis means that care networks, research networks and study networks must join forces to ensure that patients receive the best possible care and to ensure that it will continue to be possible to develop drugs even for small groups of patients. This is the goal that the PRAEGNANT study network has set itself for patients with metastatic breast cancer.

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Conflict of Interest

PAF received honoraria from Amgen, Celgene, Roche, Pfizer, Genomic Health, Novartis, Teva and carried out research for Novartis and Amgen. MPL received honoraria from Roche, Novartis and Celgene. HT received honoraria from Novartis, PH received honoraria from Amgen, Roche, Pfizer and Novartis. EB carried out research for Novartis, Roche, Celgene and Bristol-Myers Squibb. All other authors of this study declare no conflict of interest.

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References
22 Keam B, Im Sa, Lee KH et al. Ki-67 can be used for further classification of triple negative breast cancer into two subtypes with different response and prognosis. Breast Cancer Res 2011; 13: R22
30 Lohil S, von Minckwitz G, Schneevoss A et al. PIK3CA mutations are associated with lower rates of pathologic complete response to anti-human epidermal growth factor receptor 2 (her2) therapy in primary HER2-overexpressing breast cancer. J Clin Oncol 2014; 32: 3212–3220
31 Baselga J, Piccart M, Grunt M et al. Minimal molecular alteration in PIK3CA, FGFR1 and CCND1 is associated with increased benefit from everolimus in hormone receptor-positive, HER2 ‑advanced breast cancer: Insights from the BOLERO-2 trial. Eur J Cancer 2013; 49: S404–S405

Fasching PA et al. Biomarkers in Patients... Geburtsh Frauenheilk 2015; 75: 41–50


Robson ME, Tutt A, Balmaña J, et al. OlympiA, Neo-Olympia and OlympiA-D: randomized phase III trials of olaparib in patients (pts) with breast cancer (BC) and a germline BRCA1/2 mutation (gBRCAm). San Antonio Breast Cancer Symposium 2014; Abstract No. OT1–1–04


Fehm T, Becker S, Duerr-Stoesser S, et al. Determination of HER2 status using both serum HER2 levels and circulating tumor cells in patients with recurrent breast cancer whose primary tumor was HER2 negative or of unknown HER2 status. Breast Cancer Res Treat 2007; 9: R74


Fasching PA et al. Biomarkers in Patients... Geburtsh Frauenheilk 2015; 75: 41–50
81 Krop I, Johnston S, Mayer IA et al. The FERGI phase II study of the PI3K inhibitor pictilisib (GDC-0941) plus fulvestrant vs. fulvestrant plus placebo in patients with ER+, aromatase inhibitor (AI)- resistant advanced or metastatic breast cancer. San Antonio Breast Cancer Symposium 2014; Abstract No. S2–02
82 Fasching PA, Decker T, Schneeweiss A et al. Breast cancer treatment with everolimus and exemestane for ER+ women – Results of the 2nd interim analysis of the non-interventional trial BRAWO. European Society of Medical Oncology Meeting (ESMO). 2014; Abstract No. LBA9