CDK4/6 Inhibition – Therapy Sequences and the Quest to Find the Best Biomarkers – an Overview of Current Programs

CDK4/6-Inhibition: Sequenztherapien und die Suche nach den besten Biomarkern – ein Überblick über die aktuellen Programme

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ABSTRACT

In recent years, new targeted therapies have been developed to treat patients with hormone receptor-positive (HR+)/human epidermal growth factor receptor 2-negative (HER2−) breast cancer. Some of these therapies have not just become the new therapy standard but also led to significantly longer overall survival rates. The cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) have become the therapeutic standard for first-line therapy. Around 70–80% of patients are treated with a CDK4/6i. In recent years, a number of biomarkers associated with progression, clonal selection or evolution have been reported for CDK4/6i and their endocrine combination partners. Understanding the mechanisms behind treatment efficacy and resistance is important. A better understanding could contribute to planning the most effective therapeutic sequences and utilizing basic molecular information to overcome endocrine resistance. One study with large numbers of patients which aims to elucidate these mechanisms is the Comprehensive Analysis of sPatial, TempORal and molecular patterns of ribociclib efficacy and resistance in advanced Breast Cancer patients (CAPTOR BC) trial. This overview summarizes the latest clinical research on resistance to endocrine therapies, focusing on CDK4/6 inhibitors and discussing current study concepts.

ZUSAMMENFASSUNG

Für Patientinnen wie auch Patienten mit hormonrezeptorpositivem (HRpos)/Human epidermal growth factor receptor 2-negativem (HER2neg) Mammakarzinom wurden in den letzten Jahren einige neue, zielgerichtete Therapien eingeführt. Einige dieser Behandlungen konnten sich nicht nur als neuer Therapiestandard etablieren, sondern führten auch zu einem signifikant verlängerten Gesamtüberleben. Insbesondere die Cyclin-dependent Kinase 4 and 6 Inhibitors (CDK4/6i) haben sich als Therapiestandard in der ersten Therapielinie etabliert. Insgesamt 70–80% der Patientinnen werden mit einem CDK4/6i behandelt. Sowohl für die CDK4/6i als auch für die endokrinen Kombinationspartner wurden in den letzten Jahren zunehmend Biomarker beschrieben, die mit einem Progress oder einer klonalen Selektion oder Evolution assoziiert sind. Vor diesem Hintergrund ist die Kenntnis um Effektivitäts- und Resistenzmechanismen von besonderer Bedeutung. Dieses Wissen könnte wegweisend sein, um die effektivsten Sequenzen zu planen und molekulare Grundlagen für das Überwinden der endokrinen Resistenz zu nutzen. Eine der Studien, die mit einer großen Fallzahl dazu beitragen soll, diese Mechanismen zu erforschen, ist die Comprehensive Analysis of sPatial, TempORal and molecular patterns of ribociclib efficacy and resistance in advanced Breast Cancer patients (CAPTOR BC)-Studie. Diese Übersichtsarbeit fasst den aktuellen Stand der klinischen Forschung zur Resistenz gegen endokrine Therapien mit Fokus auf CDK4/6-Inhibitoren zusammen und erörtert aktuelle Studienkonzepte.

Introduction

In 2009, a preclinical trial first noted that cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) could play a special role in the treatment of breast cancer patients with hormone receptor-positive (HR+)/human epidermal growth factor receptor 2-negative (HER2−) disease [1]. Since then, the three CDK4/6is ribociclib, palbociclib and abemaciclib have become part of the standard first-line treatment provided to HR+/HER2− patients with advanced disease [2–4]. Two studies have looked at the use of CDK4/6i in the adjuvant setting and reported a benefit with regards to invasive recurrence-free survival [5,6]. Abemaciclib has already been approved for use in the adjuvant setting while ribociclib is still awaiting approval.

The widespread use of these substances in the treatment algorithms of patients with breast cancer clearly shows that understanding the modes of action of and resistance mechanisms to CDK4/6i is an important precondition for developing therapies for this group of patients.

In this context, both retrospective analyses and prospective studies are investigating whether and how patient groups can be identified who would particularly benefit from or be disadvantaged by therapy with CDK4/6i.

Some concepts have focused on the efficacy of the endocrine combination partner by determining mutations in the estrogen receptor gene (ESR1). Others are studying the molecular changes which occur during CDK4/6i therapy or investigating the overall effect of molecular or immunological patterns on their efficacy. A number of study programs have been set up in recent years which specifically focus on identifying these mechanisms.

This overview presents the current status of clinical and translational research. Current national and international study pro-
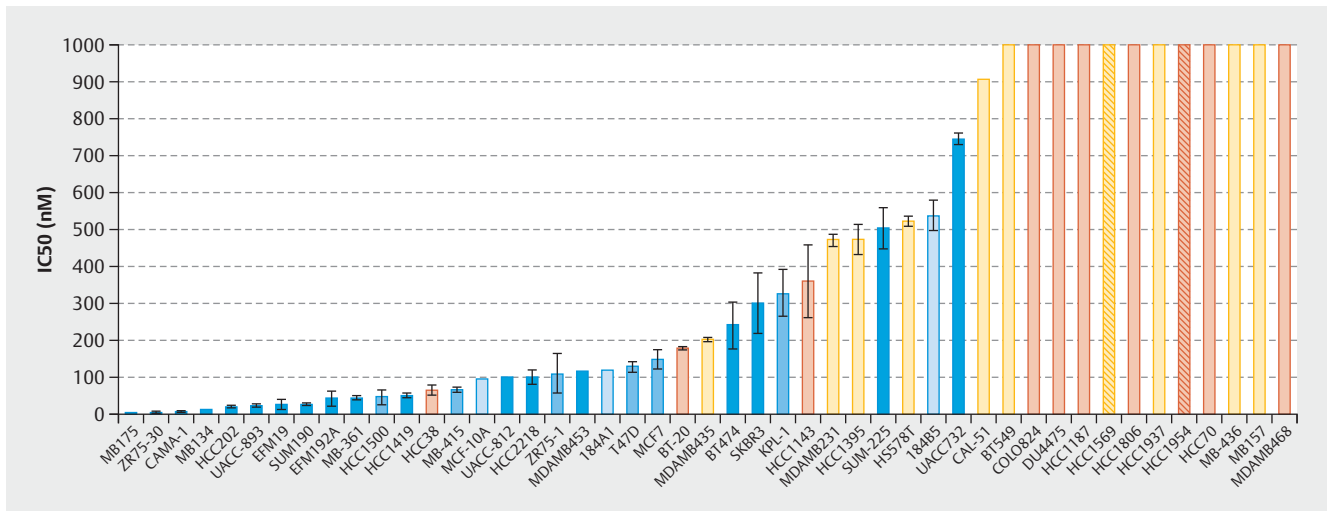
▶ **Fig. 1** Concentrations of palbociclib required to inhibit 50% of cell growth [1].

grams investigating the modes of action of CDK4/6i are also described. In Germany, for example, attention has focused on the CAPTOR BC trial, which is extensively collecting biomaterials to identify markers for both efficacy and resistance.

CDK4/6 Inhibitors

High preclinical activity in HR+/HER2− cell lines

The efficacy of treatment with palbociclib was initially investigated in a number of cell lines [1]. A total of 47 cell lines were studied to identify the different molecular subtypes of breast cancer and the variability between patients. ▶ **Fig. 1** shows that particularly with HR+, luminal cell lines responded well to treatment with palbociclib [1]. This study and other preclinical studies were followed by early clinical trials with palbociclib, in particular the PALOMA-1/TRIO-18 trial which led to the approval of palbociclib for the treatment of metastatic breast cancer [7]. During the initial preclinical studies with cell lines [1], extensive tests were already being carried out to determine which biomarkers correlated with response in addition to hormone receptor positivity. Microchip technology was used for genome-wide evaluation to determine which genes correlated preclinically with palbociclib efficacy. Around 450 genes were identified where expression varied between cell lines which responded to palbociclib and cell lines that did not [1]. In sensitive cell lines, the expression of genes which coded for the retinoblastoma protein (Rb) and cyclin D increased but the expression of *CDKN2A* decreased [1]. These early studies already showed that the mode of action of CDK4/6i therapy depends on molecular markers. Following this and other studies, large randomized studies of advanced breast cancer were carried out which led to the approval of CDK4/6i for this indication.

Introduction of CDK4/6 Inhibitors into Clinical Practice

Studies to establish the use of CDK4/6 inhibitors

Studies with the three CDK4/6is palbociclib, ribociclib and abemaciclib were carried out in different clinical scenarios and with different endocrine combination partners. The findings of the studies which investigated the use of CDK4/6i as first line-therapy can be grouped together. These trials mainly included patients in whom the probability of endocrine resistance was low. The PALOMA-2 (palbociclib), MONALEESA-2 (ribociclib) and MONARCH-3 (abemaciclib) trials therefore chose an aromatase inhibitor as the combination partner. But there are also a number of studies which included patients with a high probability of endocrine resistance. These were usually patients who were receiving more advanced therapy lines or with rapid progression under an aromatase inhibitor. Fulvestrant was selected as the combination partner in the PALOMA-3 (palbociclib) and MONARCH-2 (abemaciclib) trials. Two other studies investigated additional questions. The MONALEESA-3 trial which included patients receiving both early and advanced therapy lines also provides results for ribociclib + fulvestrant as a first-line therapy. The MONALEESA-7 trial provides results for ribociclib + an aromatase inhibitor in exclusively premenopausal patients.

All studies showed comparable effects with regards to progression-free survival. Median progression-free survival (PFS) almost doubled in all of the studies. With regards to overall survival, however, the studies showed clinically relevant differences. The two palbociclib studies did not achieve a statistically significant prolongation of overall survival, whereas the remaining studies showed a statistically significant reduction in the relative mortality risk of around 25%. Although the recent interim analysis of the MONARCH-3 trial seems to suggest an overall survival benefit, the

final analysis has not yet been published. ▶ **Table 1** provides an overview of the most important randomized phase III trials with CDK4/6i.

The data were so convincing that since the approval of CDK4/6i in 2016 and 2017 in Germany around 70–80% of patients with advanced HR+/HER2− breast cancer receive first-line therapy with CDK4/6i (▶ **Fig. 2**). Understanding the resistance and efficacy mechanisms is particularly important in this context to establish effective therapy sequences for these patients.

In the therapy recommendations issued by the Breast Commission of the AGO (▶ **Fig. 3**), three sequential therapies which are initiated after failure of CDK4/6i therapy are now based on predictive molecular markers. Poly (ADP-ribose) polymerase inhibitors (PARPi) are indicated in patients with confirmed *BRCA1/2* germline mutation; the phosphatidylinositol 3-kinase inhibitor (PI3Ki) alpelisib is prescribed to patients with *PIK3CA* mutation, and the oral selective estrogen receptor degrader (SERD) elacestrant has been approved in the USA for patients with confirmed mutation in the estrogen receptor gene *ESR1*. Elacestrant has not yet been approved for use in Europe (as at 09/2023), although the EMA has already issued a positive opinion for elacestrant. The identification of mutations using plasma-based circulating tumor DNA (ctDNA) is expected to become increasingly important in this context. Both *PIK3CA* mutations and *ESR1* mutations can be determined using ctDNA. The scope of ctDNA analysis in clinical care and research will increase even further in the coming years. ▶ **Fig. 4** shows the process, influencing factors and possible clinical applications.

Biomarker Investigations in Large Randomized Studies

### Initial findings on clonal evolution in the PALOMA-3 trial

Most large randomized studies now also include extensive translational research programs. These programs have collected important information which can help to predict treatment efficacy and resistance based on the use of biomarkers.

The PALOMA-3 trial was one of the first studies which extensively investigated the clonal evolution of disease under CDK4/6i therapy [8, 9]. Using ctDNA obtained from blood samples, investigations were carried out to determine which mutations are most commonly found at the end of therapy in potentially relevant genes compared to the start of therapy. The most common mutations were in the two genes *ESR1* and *PIK3CA*. Mutations in *RB1* occurred more frequently in the palbociclib arm of the study [9]. Mutations in *PIK3CA* (relating to the PI3Ki alpelisib) and probably, in the near future, also mutations in *ESR1* (relating to the SERD elacestrant) are actionable mutations. The PALOMA-3 trial shows the importance of mutation analysis carried out immediately before the start of therapy as it provides the basis for treating patients in accordance with their identified mutation status.
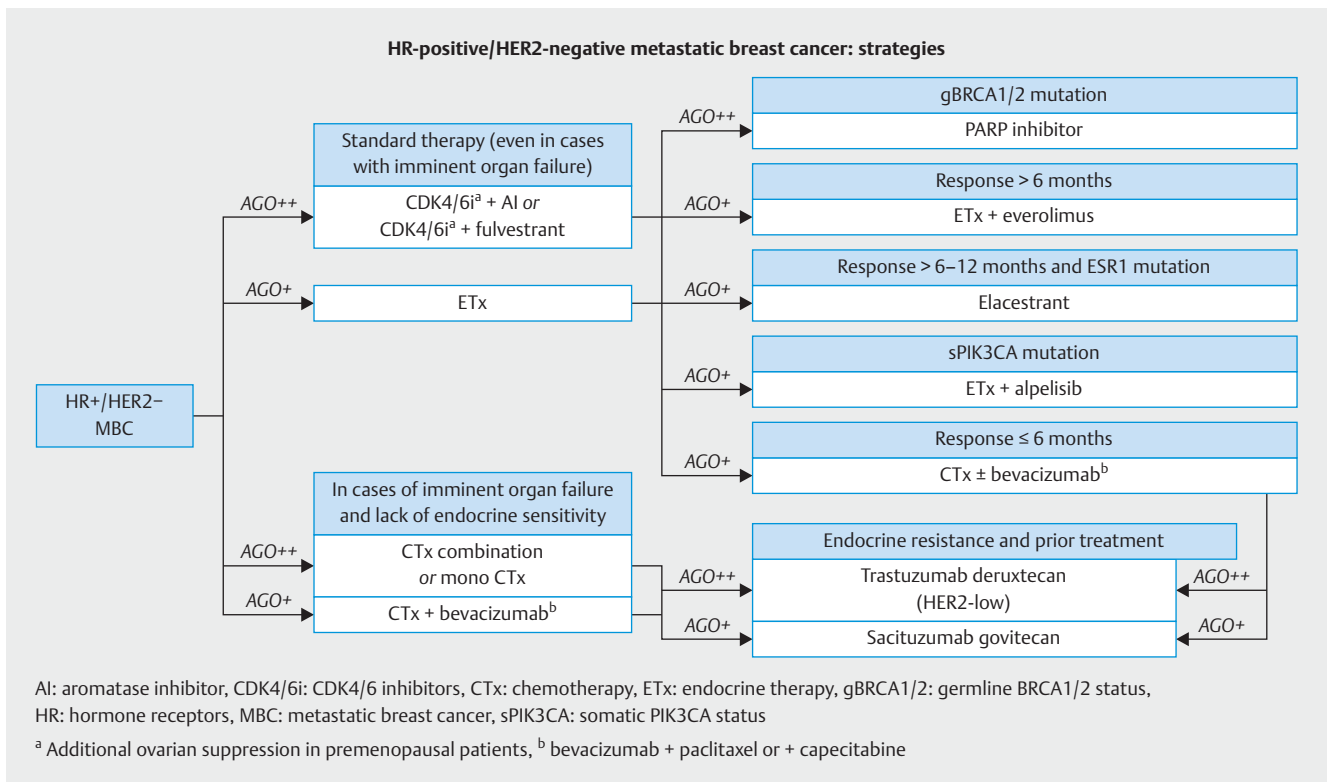
▶ **Table 1** Overview of randomized phase III trials with palbociclib, abemaciclib and ribociclib for the treatment of patients with advanced breast cancer.

|	N	Therapy	Last patient in	PFS HR (95% CI)	Median PFS CDK4/6i | Placebo*	OS HR (95% CI)	Median OS CDK4/6i | Placebo*	Percentage with de novo metastasis	Percentage of patients with DFI < 12 months	References
MONALEESA-2	668	ribociclib ± letrozole	03/2015	0.56 (0.43–0.72)	25.3 | 16.0	0.76 (0.63–0.93)	53.9 | 51.2	34%	18%	[41, 42]
MONARCH-3	493	abemaciclib ± NSAI	11/2015	0.54 (0.41–0.72)	28.2 | 14.8	0.76 (0.58–0.97)	67.1 | 54.1	40%	**	[43, 44]
PALOMA-2	666	palbociclib ± letrozole	07/2014	0.58 (0.46–0.72)	24.8 | 14.5	0.96 (0.78–1.18)	63.9 | 51.4	37%	22%	[45, 46]
MONALEESA-7	672	ribociclib ± ET	08/2016	0.55 (0.44–0.69)	23.8 | 13.0	0.71 (0.54–0.95)	58.7 | 48.0	19%	4.3%	[47–49]
MONALEESA-3	726	ribociclib ± fulvestrant	06/2016	0.59 (0.48–0.73)	20.5 | 12.8	0.72 (0.57–0.92)	53.7 | 41.5	40%	5.4%	[50–53]
MONARCH-2	669	abemaciclib ± fulvestrant	12/2015	0.55 (0.45–0.68)	16.9 | 9.3	0.76 (0.61–0.95)	46.7 | 37.3	NA	NA	[54, 55]
PALOMA-3	521	palbociclib ± fulvestrant	08/2014	0.46 (0.36–0.59)	9.5 | 4.6	0.81 (0.64–1.03)	34.8 | 28.0	NA	NA	[8, 56, 57]
DAWNA-1	361	dalpiciclib* ± fulvestrant	09/2020*	0.42 (0.31–0.58)	15.7 | 7.2	not yet reported	not yet reported	NA	NA	[58]

* = interim analysis, ** = not reported, DFI = disease-free interval, HR = hazard ratio, NA = not applicable, OS = overall survival, PFS = progression-free survival
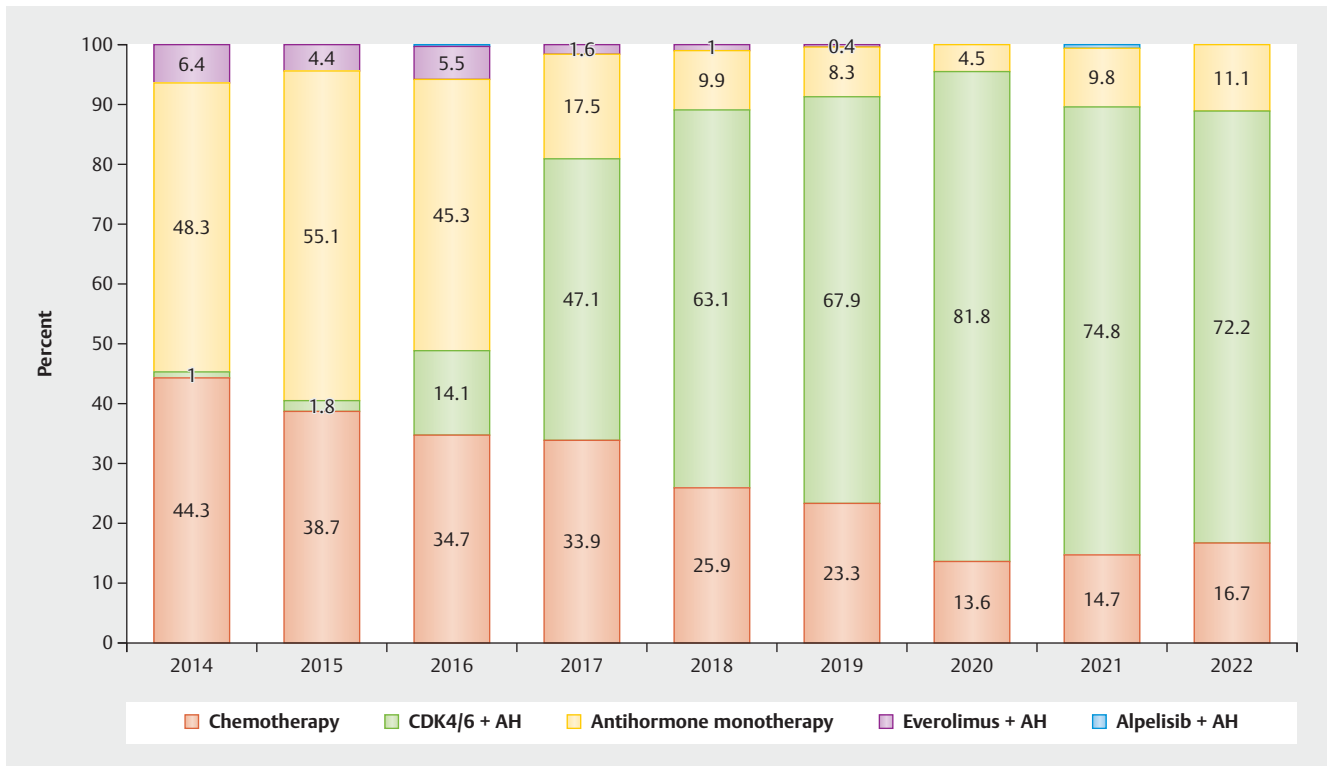
▶ **Fig. 2** Use of different therapeutic options as first-line therapy in patients with advanced HR+/HER2− breast cancer [2].

AI: aromatase inhibitor, CDK4/6i: CDK4/6 inhibitors, CTx: chemotherapy, ETx: endocrine therapy, gBRCA1/2: germline BRCA1/2 status, HR: hormone receptors, MBC: metastatic breast cancer, sPIK3CA: somatic PIK3CA status

[a] Additional ovarian suppression in premenopausal patients, [b] bevacizumab + paclitaxel or + capecitabine

▶ **Fig. 3** Treatment algorithm of the Breast Commission of the AGO outlining sequential therapies for patients with HR+/HER2− breast cancer in an advanced therapy setting.

▶ **Fig. 4** Possible course and clinical application of ctDNA analysis.
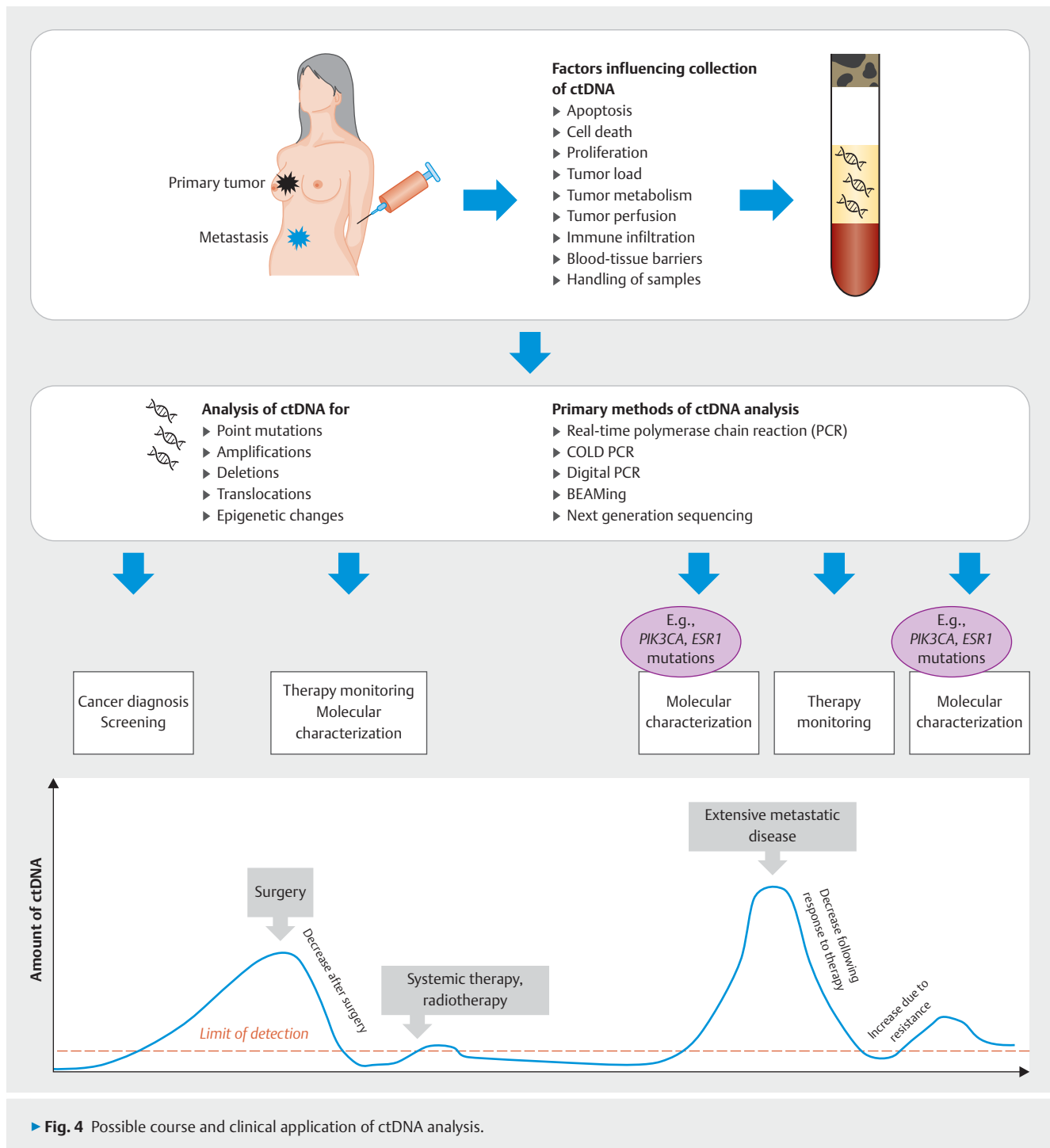
PIK3CA and *ESR1* mutations in the MONARCH-2 trial

In the MONARCH-2 trial, patients with endocrine resistance were treated with abemaciclib and fulvestrant or fulvestrant alone. Samples of ctDNA taken just before starting therapy were investigated for mutations in *PIK3CA* and *ESR1* [10]. A mutation in *PIK3CA* was found in 44% of patients and an *ESR1* mutation was confirmed in 59% of patients. In this trial, however, these biomarkers had no impact on patients' prognosis [10].

Biomarker examinations in the MONALEESA study program

In the MONALEESA trials 2, 3 and 7, extensive pooled analyses of gene expression and other genomic analyses were carried out in a large group of patients. More than 1150 patients were categorized into their respective intrinsic subtypes using gene expression analysis with PAM50. Even though these breast cancers are often described as luminal-like, this clinical assessment is only an
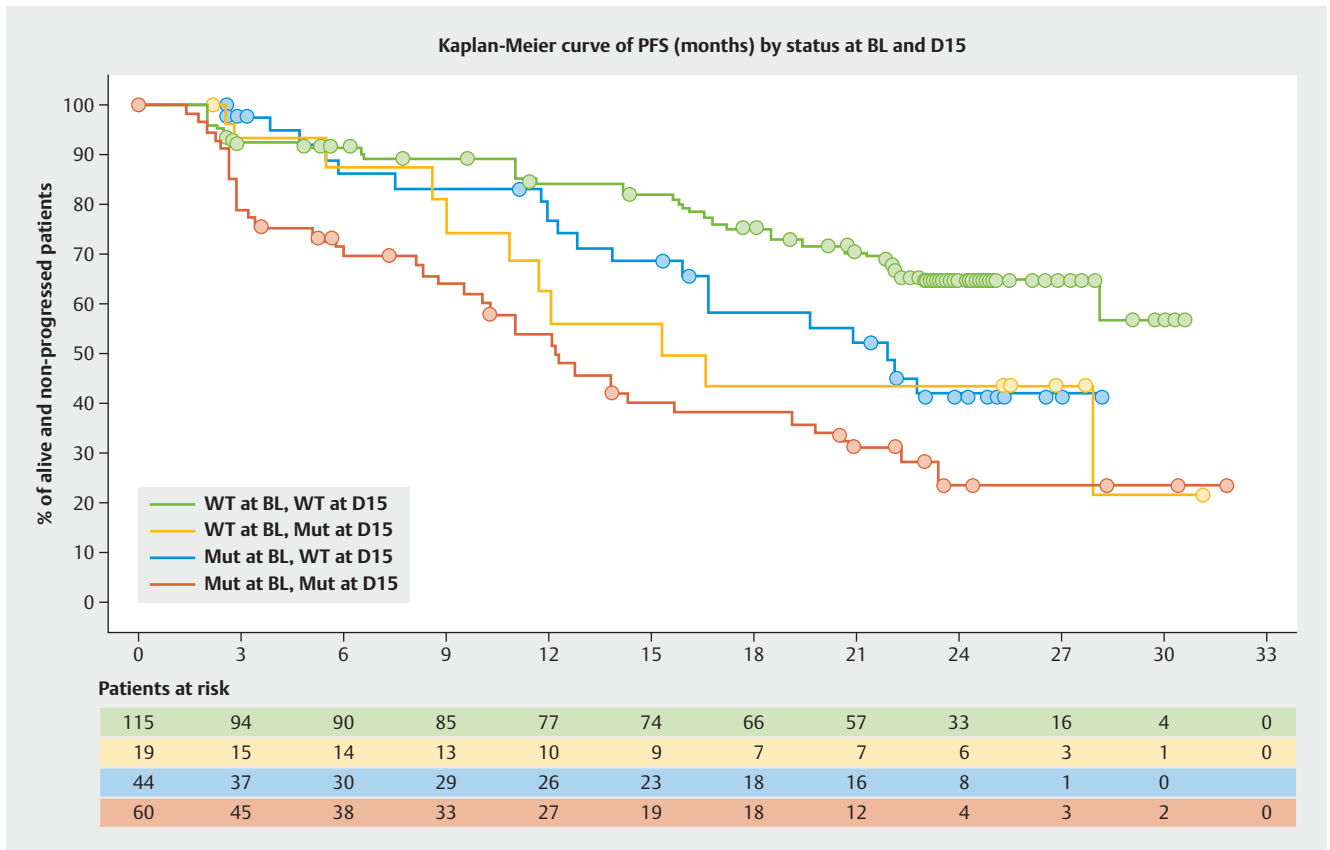
Fig. 5 Progression-free survival (PFS) in the BioItaLEE trial depending on changes in ctDNA between the start of therapy (BL) and 15 days after the start of therapy (D15) [13]. Mut = mutated, WT = wildtype.

estimation. In addition to luminal-A (46.8%) and luminal-B tumors (24.0%), the pooled MONALEESA-2, 3 and 7 analyses also found tumors with HER2-enriched (12.6%), normal-like (14.1%) and basal-like (2.4%) intrinsic subtypes. What was interesting was that the therapeutic effect of ribociclib was found to differ across the different subgroups. With a hazard ratio (HR) of 0.40 (95% CI: 0.26–0.62), the effect appeared to be greatest in the group with HER2-enriched tumors. This is probably due to the pronounced endocrine resistance in this group, which became evident when results were compared to outcomes with monotherapy. More than one third of patients experienced primary progression under endocrine monotherapy. The addition of ribociclib probably overcame resistance in a majority of these patients. Similarly, a majority of patients with basal-like tumors demonstrated early progression under endocrine monotherapy which was resolved by the addition of ribociclib (HR = 1.14; 95% CI: 0.46–2.83) [11].
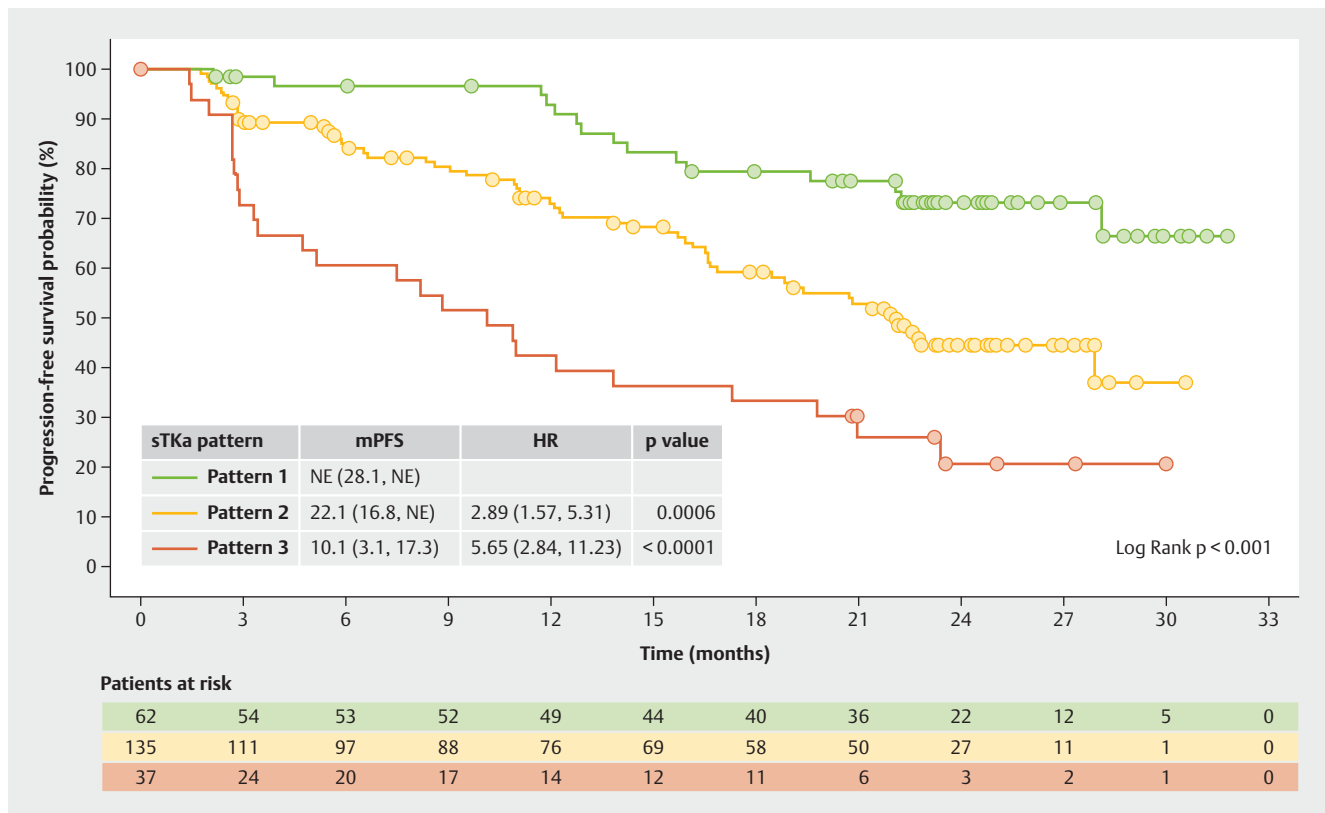
Extensive analysis of tumor mutations and gene amplifications was also carried out in a pooled patient group (n = 1703) from the MONALEESA-2, 3 and 7 trials [12]. At the start of treatment with ribociclib and endocrine therapy, ctDNA was analyzed with regards to mutations and amplifications of around 550 genes using next generation sequencing. The most common finding was an alteration in *PIK3CA* (33%), which in most cases was a mutation. The most commonly amplified genes were *FDF3, FDF4* and *FGF19* (8–9%). Some genes were identified if a mutation under endocrine

monotherapy was associated with a very short median PFS which was then significantly improved by additonally administering ribociclib. These genes were *FRS2, MDM2, PRKCA, AKT1, BRCA1/2* and *ERBB2*. Conversely, no improvement in median PFS following the addition of ribociclib was found for mutations in *CHD4, CDKN2A/B/C* and *ATM*. These analyses have given rise to a number of hypotheses, but they show that gene mutations and amplifications play a role in the context of endocrine resistance which has not yet been scientifically investigated. It is therefore clear that a clinically simple method (blood sampling and ctDNA analysis) can help to determine predictive markers which will then be used to direct further therapy (► **Fig. 4**). This is also being offered to patients with progression in the CAPTOR BC trial in the context of a scientific subproject (► **Fig. 8**).

Therapy Monitoring with ctDNA Analysis

Monitoring of CDK4/6i therapy with ribociclib – the BioItaLEE trial

The BioItaLEE trial is a potentially pioneering study into the monitoring of ribociclib plus endocrine therapy using ctDNA. In this study, the amount of tumor-specific ctDNA was determined prior to the start of therapy and 15 days after starting therapy and correlated with the median PFS [13]. Patients were divided into four

sTKa pattern	mPFS	HR	p value
Pattern 1	NE (28.1, NE)		
Pattern 2	22.1 (16.8, NE)	2.89 (1.57, 5.31)	0.0006
Pattern 3	10.1 (3.1, 17.3)	5.65 (2.84, 11.23)	< 0.0001

Log Rank p < 0.001

Patients at risk

62	54	53	52	49	44	40	36	22	12	5	0
135	111	97	88	76	69	58	50	27	11	1	0
37	24	20	17	14	12	11	6	3	2	1	0

▶ **Fig. 6** Progression-free survival in the BioItaLEE trial depending on the changes in serum thymidine kinase activity under therapy with ribociclib [15]. HR = hazard ratio, mPFS = median progression-free survival, sTKa = serum thymidine kinase activity

groups based on the amount of ctDNA. After a median follow-up time of 26.9 months, clear prognostic patterns with regards to median PFS were identified:

- no ctDNA at the start of therapy → no ctDNA after 15 days: median PFS not achieved
- no ctDNA prior to starting therapy → ctDNA newly present after 15 days: median PFS 15.9 months
- ctDNA present before the start of therapy → decrease in ctDNA after 15 days: median PFS 21.9 months
- ctDNA present before the start of therapy → ctDNA present after 15 days: median PFS 12.3 months

The Kaplan-Meier curves are shown in ▶ **Fig. 5**. Modern tumor-specific biomarkers obtained through ctDNA analysis could allow the success of therapy to be estimated after just a short treatment period.

The BioItaLEE trial also analyzed other biomarkers such as serum thymidine kinase (sTK), a proliferation marker which correlates with the effect of CDK4/6i [14]. Of particular interest were patients where sTK was found to be suppressed under the limit of detection (LOD) after both 15 days of therapy and 28 days of therapy (▶ **Fig. 6**, Pattern 1). This group had the best prognosis. With a median follow-up time of 26.9 months, this group did not achieve the median PFS. Patients in whom suppression of sTK under the LOD was initially achieved after 15 days of therapy but who then showed an increase again after 28 days of therapy (▶ **Fig. 6**, Pattern 2) had a

median PFS of 22.1 months. Patients who showed no decrease in sTK under the LOD after 15 days of therapy (▶ **Fig. 6**, Pattern 3) had the poorest median PFS with just 10.1 months [15].

Homologous Recombination and Endocrine Resistence

New molecular patterns associated with endocrine resistence

Data from the MSK-IMPACT cohort on prognosis under CDK4/6i therapy has already been presented at the 2021 San Antonio Breast Cancer Symposium (SABCS). *BRCA2* germline mutation was found to have an unfavorable prognostic effect in patients under CDK4/6i therapy. Compared to patients with *BRCA2* wild-type, patients with *BRCA2* mutation had a higher risk of progression (HR = 2.32; 95% CI: 1.38–3.91) [16].

Other analyses in this cohort investigated the effect of complex mutation patterns on prognosis under CDK4/6i therapy [17]. The differentiation of tumors according to complex mutation patterns (signatures or profiles) is an attempt to determine different prognostic categories based on these mutation patterns. During the pathogenesis of tumors, different irritants and circumstances lead to characteristic mutation profiles. [18]. Such mutation profiles can be developed for different types of mutations (single base
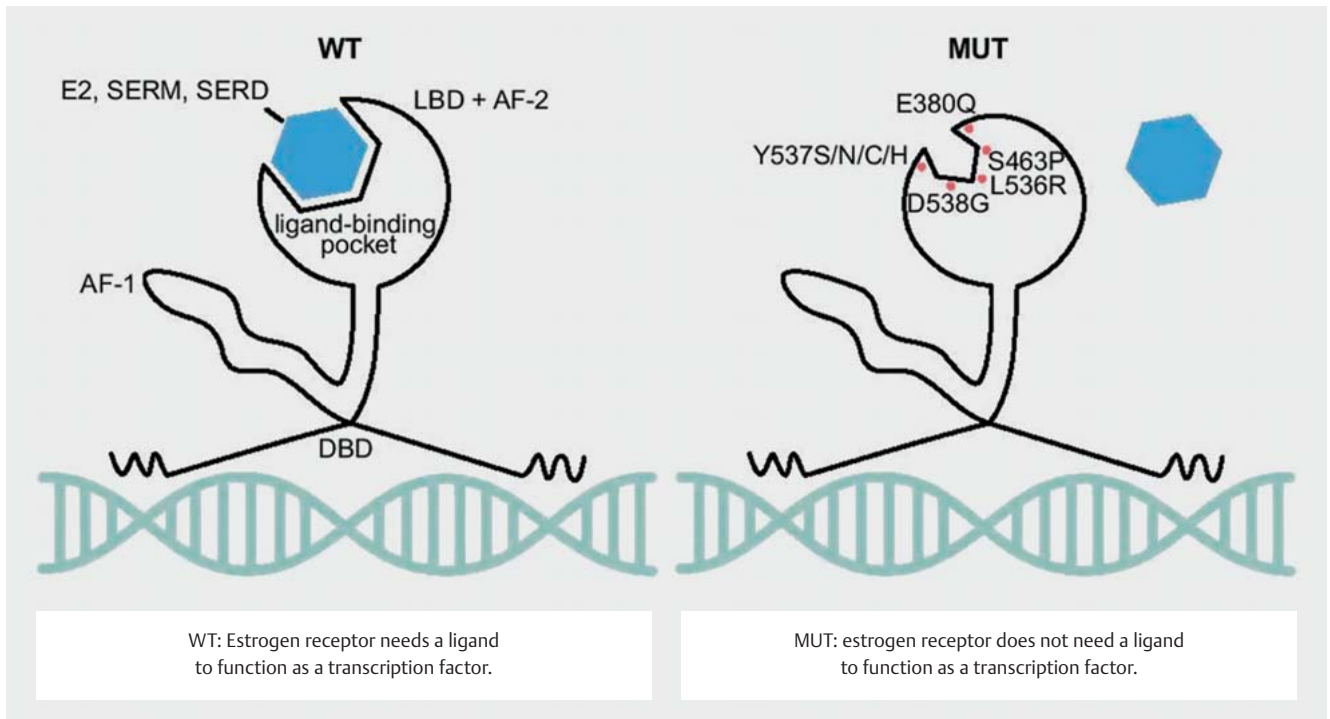
► **Fig. 7** Effect of *ESR1* mutation on the function of the estrogen receptor (Creative Commons Licence 4.0, [59]). LBD: ligand-binding domain, AF-2: activating factor-2 domain, DBD: DNA-binding domain, AF-1: activating factor 1 domain, E2: estradiol, SERM: selective estrogen receptor modulator, SERD: selective estrogen receptor degrader, WT: wildtype, MUT: mutated (Source: Brett JO, Spring LM, Bardia A et al. ESR1 mutation as an emerging clinical biomarker in metastatic hormone receptor-positive breast cancer. Breast Cancer Res 2021; 23: 85. doi:10.1186/s13058-021-01462-3.) © 2021. The Author(s). Licensed under a Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/). Changes: Addition of explanations for WT and MUT in the image.

pair, doublet base pair, insertion-deletion mutations). The analysis of the MSK-IMPACT cohort focused on single base pair mutations (SBS) [17] for which a recently published work described 96 different signatures [19]; these signatures are also available from the Catalogue of Somatic Mutations in Cancer (COSMIC) [20].

Some of these SBS signatures are found more often in breast cancer and can be divided into the following etiological groups: clock-like signatures, apolipoprotein B mRNA editing enzyme, catalytic polypeptide (APOBEC) signature, homologous recombination deficiency (HRD) signature, smoking-related and mismatch repair-related signatures.

The presented clinical data refer to the change in the mutation profile of the primary tumor when it metastasizes and also to the impact of the mutation profile on the prognosis of patients treated with CDK4/6i as their first-line therapy. The two mutation profiles which were found to have increased most during the progression of disease from early-stage HR+/HER2− breast cancer to metastatic disease were the APOBEC and HRD signatures [17]. Significant differences were found with regard to the prognostic importance of the mutation profiles under first-line therapy with a CDK4/6i. Patients with few mutations had a median PFS of 17.8 months, patients with an APOBEC signature had a median PFS of 12.3 months and patients with an HRD signature had a median PFS of only 7.6 months [17].

To what extent this knowledge can be used to determine subsequent therapies or therapy sequences must be elucidated in future studies. Currently, treatment with a CDK4/6i is and remains the standard first-line therapy for patients with advanced HR+/HER2− breast cancer. However, given the short median PFS of patients with an HRD signature, the question arises whether these patients should not rather be treated with PARPi. The HRD group consisted of only 10.5% of patients treated with CDK4/6i in the MSK-IMPACT cohort, representing only a small number of the patients in the study, meaning that further studies or real-world data are needed for a conclusive assessment.

ESR1 Mutations and Endocrine Combination Partners

Around 30–40% of tumors in patients with advanced endocrine-resistant breast cancer were found to have a mutation in the estrogen receptor gene *ESR1* [21 – 23]. These mutations can be determined with a relatively high sensitivity using serum ctDNA [24, 25]. It should be easy to determine these mutations over the clinical course of disease. The mutations in the gene segments of *ESR1* coding for the ligand-binding domain were correlated with a poorer clinical outcome [24 – 26], presumably because these

mutations lead to changes in protein structure in the sense of a constitutively active form of the estrogen receptor [27, 28] (▶ **Fig. 7**). Their frequency and the clonal selection of *ESR1* mutations under endocrine therapy have focused interest in endocrine resistance on this mutation.

ESR1 mutations and the oral SERD elacestrant

The SERD fulvestrant which is administered intramuscularly has been approved to treat patients with advanced breast cancer for more than 20 years. With the development of oral SERDs, research activities into this class of substances have increased significantly. In the USA, the oral SERD elacestrant has already been approved to treat patients with hormone therapy-resistant advanced breast cancer if *ESR1* mutations are confirmed in the patient's ctDNA. In the EMERALD approval study [29], patients with *ESR1* mutation who received elacestrant had a longer median PFS than patients who received standard endocrine therapy (HR 0.55; 95% CI: 0.39–0.77). Elacestrant did not improve median PFS in patients without *ESR1* mutation (HR 0.86; 95% CI: 0.63–1.19) [29].

ESR1 mutations and fulvestrant as first-line therapy

In addition to the approval studies, studies have also focused on biomarkers. The PADA-1 trial is particularly important in this context. In this study, ctDNA was used to determine whether patients who were receiving first-line therapy with an aromatase inhibitor and palbociclib had an *ESR1* mutation. If the presence of *ESR1* mutation in serum was confirmed and the patient showed no clinical progression, patients were randomized into groups: in one group the endocrine combination partner of the aromatase inhibitor was switched to fulvestrant while the other group continued to receive treatment with an aromatase inhibitor and palbociclib. 172 patients were randomized. The median PFS under continuation of the aromatase inhibitor therapy was 5.7 months; the median PFS if therapy was switched to fulvestrant was 11.9 months (HR 0.61; 95% CI: 0.43–0.86) [30].

The CAPTOR Study Program

CAPTOR BC study design

The CAPTOR BC study is a phase IV trial which investigates resistance and the mechanisms of action for combination therapy with ribociclib and endocrine therapy. The study is also investigating several new research concepts to find new genes and pathways which could identify a priori patients for whom ribociclib would be effective as well as those for whom ribociclib will not be effective. This could provide additional information on which therapy sequences will be most appropriate. The CAPTOR BC trial also records all subsequent therapies initiated after the end of therapy with ribociclib.

As the study is hoping to include large numbers of patients, the aim has been to simplify the practical implementation of the study while simultaneously achieving the extensive study goals (▶ **Fig. 8**).

Patients are included if routine clinical examination finds that ribociclib is indicated as part of their first-line therapy. Patients will be followed up until death, for a maximum period of 4 years, or until the end of the overall study (November 2027). Blood samples are taken on inclusion in the study and each patient is requested to provide the study with paraffinized tumor samples. Up to 7 more blood samples are taken over the course of the study (▶ **Fig. 8**). The patient's quality of life is also recorded using patient-reported outcome tools and a paper-based questionnaire or app.

This means that extensive information and biomaterials can be obtained with a relatively simple study design. Some of the scientific objectives of the CAPTOR BC trial are described in more detail below.

Detection of agnostic biomarkers in the CAPTOR BC trial

Despite numerous studies which have already investigated the mechanisms of action of CDK4/6i-based therapies, many methods have not yet been exhausted. Up to now, the use of agnostic approaches was largely ruled out, purely because of the rather small case numbers in the studies carried out to date. Modern methods of analysis are being used to carry out genome-wide identifications of gene expression, genetic mutations and gene copy alterations in a short space of time. If the case numbers are sufficiently high, new genes and signalling pathways which were not previously taken into consideration will be identified. A good example of this was the discovery of more than 300 gene loci which explain more than 40% of familial breast cancer risks [31–38]. The first big association studies were able to discover and validate the breadth of the genetic basis of the risk of breast cancer. Other examples are the immunomodulatory genes identified using a similar approach in the context of the SUCCESS trial which correlate with neutropenia and prognosis [39]. The large number of patients (n = 2000) in the CAPTOR BC study paves the way to carry out several such genome-wide analyses.
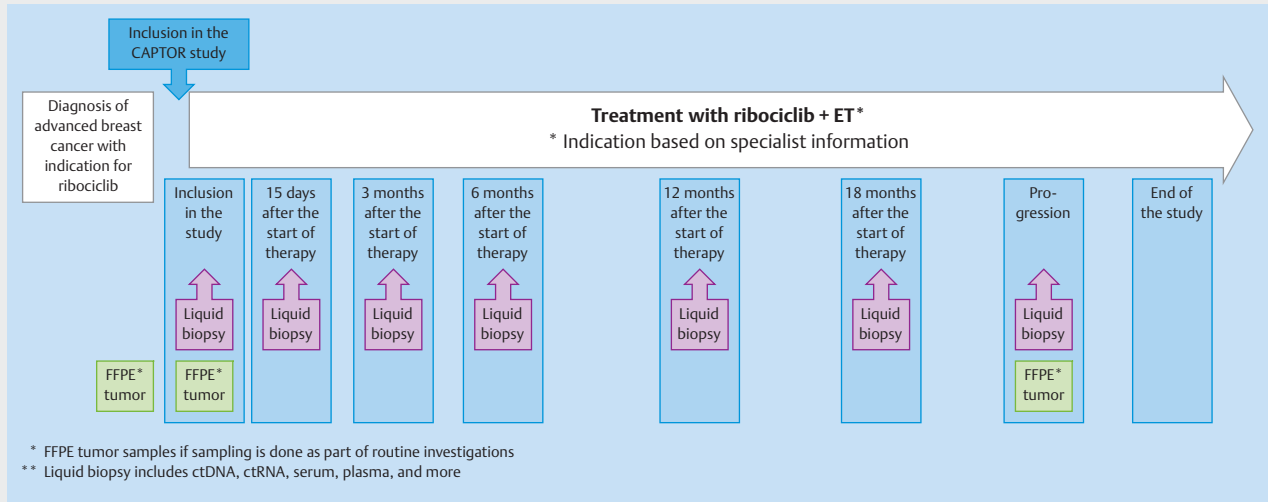
ctDNA in scientific and healthcare research in the CAPTOR BC trial

Just how varied the options for ctDNA analysis are has been described. A number of scientific and healthcare applications are conceivable, both in the context of therapy monitoring and for the molecular characterization of diseases. Serial blood sampling is being carried out as part of the CAPTOR BC trial, and the samples are prepared and processed in accordance with the latest state of knowledge of ctDNA analysis. One of the scientific objectives is to learn as much as possible about the course of disease progression under CDK4/6i therapy. The basis for this will be the analysis of ctDNA obtained at the time of progression. As part of the CAPTOR BC study, patients who have received treatment with a CDK4/6i and for whom marker-guided therapy is now indicated will be offered a ctDNA analysis under study conditions at the time of progression. Research is currently focused on investigating whether there are other molecular genomic markers in addition to the genes *PIK3CA, ESR1* and *BRCA1/2* that could help to identify appropriate subsequent therapies. One of the programs

Study design and study objectives

- N = 2000 patients with advanced breast cancer and an indication for ribociclib
- Liquid biopsy at the start of the study and sequentially over the course of the study
- FFPE tumor samples if sampling is done as part of routine investigations
- Collection of all digitally available information (histological, radiological images...)

- Co-primary study endpoints: PFS and OS rates after 12 months
- Secondary study endpoints: PFS, OS, quality of life, tolerability
- Exploratory study endpoints: genome-wide biomarker detection and validation

Inclusion in the CAPTOR study

Diagnosis of advanced breast cancer with indication for ribociclib

Treatment with ribociclib + ET*
* Indication based on specialist information

Inclusion in the study	15 days after the start of therapy	3 months after the start of therapy	6 months after the start of therapy	12 months after the start of therapy	18 months after the start of therapy	Pro-gression	End of the study
Liquid biopsy	Liquid biopsy	Liquid biopsy	Liquid biopsy	Liquid biopsy	Liquid biopsy	Liquid biopsy	
FFPE* tumor						FFPE* tumor	

FFPE* tumor

* FFPE tumor samples if sampling is done as part of routine investigations
** Liquid biopsy includes ctDNA, ctRNA, serum, plasma, and more

Germline DNA: HR genes, genome-wide SNPs

ctDNA changes over time to monitor disease

ctDNA for molecular tumor characterization
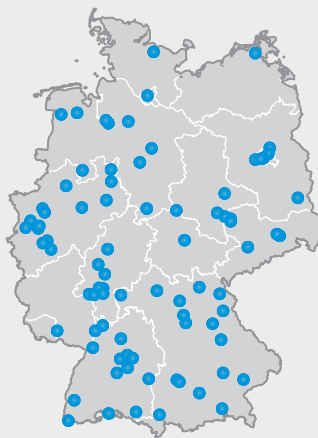
ctDNA for molecular tumor characterization

Leukocyte RNA for immunological characterization

Leukocyte RNA for immunological characterization

FFPE for spatial multiomics

FFPE for spatial multiomics

Participating centers

Involved study groups/professional associations

- AGO-B Breast Study Group
- Gynecological oncology working group (AGO)
- German Society of Senology

▶ **Fig. 8** Study design of the CAPTOR BC trial and planned molecular analyses. ET = endocrine therapy, FFPE = formalin-fixed, paraffin-embedded tissues, HR = homologous recombination, OS = overall survival, PFS = progression-free survival, SNP = single-nucleotide polymorphism

▶ **Fig. 9** Technical procedure used for molecular characterization in the CATCH program.
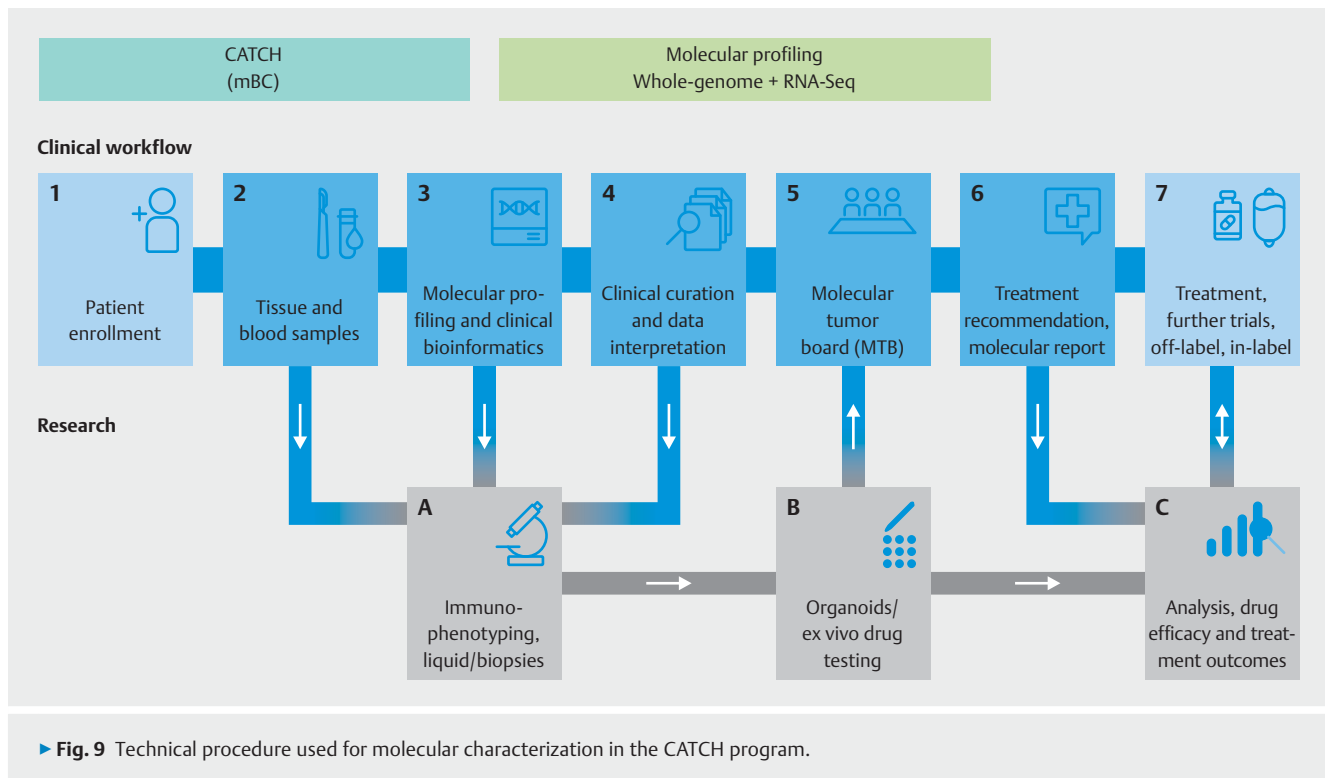
in this field of study is the multicenter program "Comprehensive Assessment of clinical feaTures and biomarkers to identify patients with advanced or metastatic breast Cancer for marker-driven trials in Humans" (CATCH) of the National Center for Tumor Diseases (NCT).

New Therapeutic Approaches in Clinical Studies

The CATCH study program

The speed of drug development processes has increased significantly in recent years. As the understanding of how the human genome functions and the importance of relevant mechanisms on the development of breast cancer has increased, more targeted drugs have been approved, i.e., drugs which address a target partly determined by molecular testing. Examples include CDK4/6i (palbociclib, ribociclib, abemaciclib), the PIK3CAi alpelisib, PARPi (olaparib, talazoparib), immune checkpoint inhibitors (ICIs) (atezolizumab, pembrolizumab), antibody-drug conjugates (ADCs) (sacituzumab-govitecan, trastuzumab-deruxtecan), the AKT inhibitor capivasertib, the oral SERD elacestrant and others.

As drug developments have occurred in parallel, the current therapy standard is usually not reflected in study populations. One example of this is alpelisib and the SOLAR-1 trial. This study included almost no patients who had received pretreatment with CDK4/6i, even though treatment with CDK4/6i is now the standard first-line therapy. This makes it clear how important it is to carry out therapies and, specifically, sequential therapies in molecularly informed trials.

The multicenter CATCH program is a platform which aims to provide a basis to test the efficacy of individualized cancer drugs (precision oncology), specifically drugs for metastatic breast cancer, and to provide comprehensive access to controlled evidence-based precision oncology structures.

To do this, the individual genetic makeup of therapy-resistant metastasis in breast cancer patients is analyzed using modern molecular biological procedures to identify molecular points of attack (▶ **Fig. 9**). Based on specific molecular changes, patients will be offered targeted therapies in further clinical studies, either in the context of approval studies or as off-label therapies (▶ **Fig. 10**). These drugs should delay disease progression while maintaining patients' quality of life. The pilot phase of the trial showed that it was possible to delay disease progression for longer in around one third of patients than is currently possible with standard therapies, and that a positive impact on disease progression could be expected even for patients who had received many prior therapies [40]. The information obtained in this study will be used over the longer term in subsequent studies to develop innovative targeted effective substances and modern diagnostic processes which will be transferred to standard care. The CATCH program is independent of the CAPTOR BC trial, but for patients, both study concepts may be closely connected. After disease progress is identified in the CAPTOR BC trial and the relevant molecular characterization is carried out, studies such as CATCH will be used to offer new and innovative therapies to patients based on their molecular characteristics.
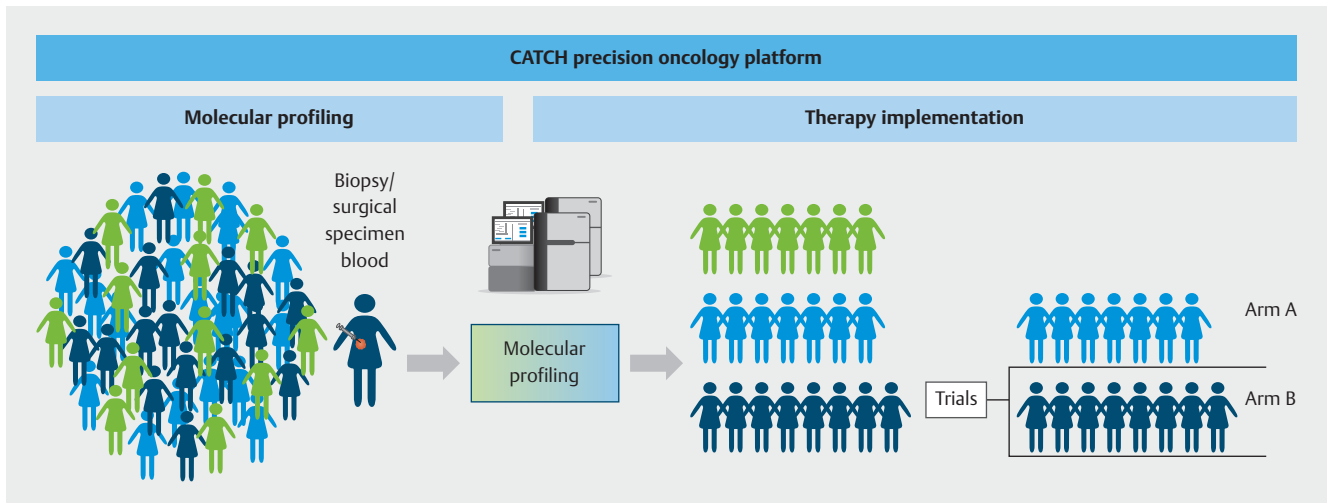
▶ **Fig. 10** Therapy allocation based on molecular characteristics in the CATCH program.

Outlook

The large number and the quality of analyses of molecular biomarkers show ever more clearly that molecular characterization of breast cancer offers benefits to patients. The use of some markers is already standard when determining the indications for approved therapies. Large study programs such as the CAPTOR BC and CATCH studies, which include high numbers of patients, provide the basis for the creation of future treatment concepts which will expand this body of knowledge for the benefit of patients.

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Conflict of Interest

B. A. received honoria and travel grants from AstraZeneca, Gilead, Genomic Health, Roche, Novartis, Celgene, Lilly, MSD, Eisai, Teva, Tesaro, Onkowissen, Daiichi Sankyo and Pfizer.
C. B. is an employee of Novartis Deutschland GmbH.
E. B. received honoraria from Gilead, Ipsen, Sanofi, Sandoz, SunPharma, AstraZeneca, Novartis, Hexal, BMS, Lilly, Pfizer, Roche, MSD, B Braun and onkowissen.de for clinical research management and/or medical education activities.
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T. E. has received honoraria from AstraZeneca, Eli Lilly, Daiichi Sankyo, Gilead, GSK, MSD, Novartis, Pfizer, Roche, Stemline.
T. N. F. has participated on advisory boards for Amgen, Daiichi Sankyo, Novartis, Pfizer, and Roche and has received honoraria for lectures from Amgen, Celgene, Daiichi Sankyo, Roche, Novartis and Pfizer.
A. D. H. received speaker and consultancy honoraria from AstraZeneca, Genomic Health, Roche, Novartis, Celgene, Lilly, MSD, Eisai, Teva, Tesaro, Daiichi Sankyo, Hexal and Pfizer.
C. C. H. has received honoraria from Pfizer, Novartis, Roche, AstraZeneca, Eisai and Daiichi Sankyo.
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H. N. received honoraria for lectures and/or consulting from Amgen, AstraZeneca, Daiichi Sankyo, Exact Sciences, Gilead, Lilly, MSD, Mylan, Novartis, Pierre Fabre, Pfizer, Roche, Sandoz, Seagen.
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