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Implementation of precision medicine for patients with metastatic breast cancer in an interdisciplinary MTB setting

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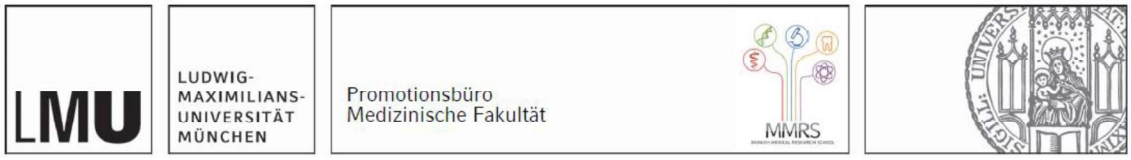

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Inhaltsverzeichnis

Abkürzungsverzeichnis

Ein Abkürzungsverzeichnis kann dem Leser dabei helfen, spezifische Abkürzungen besser zu verstehen und ggf. hier nachzuschlagen. Es empfiehlt sich vor allem dann, wenn zahlreiche Abkürzungen verwendet werden.

BC	Breast cancer
MTB	Metastatic breast cancer
MTA	Molecular Therapeutic Agents
NGS	Next Generation Sequencing
CGP	Comprehensive Genomic Profiling
MDT	Multidisciplinary Team
ITT	Intention-to-treat
IHC	Immunohistochemistry
FISH	Fluorescence in situ hybridization
PD-L1	Programmed death-ligand 1
TNBC	Triple-negative breast cancer
PARP	Poly (ADP-ribose) polymerase
gBRCA	Germline BRCA
CTC	Circulating tumor cell
LoE	Levels of evidence
GR	Grade
AGO	Arbeitsgemeinschaft Gynäkologische Onkologie (German Gynecological Oncology Group)
MTB	Molecular Tumor Board
ESCAT	ESMO Scale of Clinical Actionability
PFS	Progression-free survival
PFSr	Progression-free survival ratio
HR	Hormone receptor
ER	Estrogen receptor
PR	Progesterone receptor
HER2	Human epidermal growth factor receptor 2
NGS	Next-generation sequencing
MTA	Molecular targeted agents
OR	Overall response rate
OS	Overall survival
SD	Stable disease
TT	Targeted therapies
PR	Partial remission
FDA	Food and Drug Administration

Publikationsliste

1. **Sultova, Elena; Westphalen, C. Benedikt; Jung, Andreas; Kumbrink, Joerg; Kirchner, Thomas; Mayr, Doris; Rudelius, Martina; Ormanns, Steffen; Heinemann, Volker; Metzeler, Klaus H.; Greif, Philipp A.; Burges, Alexander; Trillsch, Fabian; Mahner, Sven; Harbeck, Nadia; Wuerstlein, Rachel (2021): NGS-guided precision oncology in metastatic breast and gynecological cancer: first experiences at the CCC Munich LMU.**
   Archives of gynecology and obstetrics, Vol. 303, Nr. 5: S. 1331-1345
   Impact Factor: 2.804 (2021)

2. **Sultova, E.; Westphalen, C.B.; Jung, A.; Kumbrink, J.; Kirchner, T.; Mayr, D.; Rudelius, M.; Ormanns, S.; Heinemann, V.; Metzeler, K.H.; Greif, P.A.; Hester, A.; Mahner, S.; Harbeck, N.; Wuerstlein, R. Implementation of Precision Oncology for Patients with Metastatic Breast Cancer in an Interdisciplinary MTB Setting.**
   Diagnostics 2021, 11, 733.
   Impact Factor: 3.992 (2021)

3. Cornelia Kolberg-Liedtke, Rachel Wuerstlein, Oleg Gluz, Florian Heitz, Muriel Freudenberger, Elena Bensmann, Andreas du Bois, Ulrike Nitz, Enrico Pelz, Matthias Warm, Monika Ortmann, Elena Sultova, Sara Y. Brucker, Ronald E. Kates, Tanja Fehm, Nadia Harbeck; Phenotype Discordance between Primary Tumor and Metastasis Impacts Metastasis Site and Outcome: Results of WSG-DETECT-PriMet. *Breast Care* 7 October 2021; 16 (5): 475–483. https://doi.org/10.1159/000512416
   Impact Factor: 1.77 (2021)

Ihr Beitrag zu den Veröffentlichungen

Beitrag zu Paper I

Zu den Aufgaben, die ich als Erstautor gehabt habe, gehörten:

- Teilnahme am MTB CCC München LMU;
- Erhebung und Zusammenstellung der Daten;
- Kommunikation mit behandelnden ÄrztInnen, PatientInnen und Angehörigen (zur follow-up Dokumentation);
- Analyse und Interpretation der Ergebnisse;
- Erstellung von Grafiken und Tabellen;
- Literatur recherchieren;
- Manuskript schreiben und Revision nach Kommentaren der Koautoren;
- Kommunikation mit Journal;
- Bereitstellung und Vorstellung von Poster und Präsentationen bei deutschen und internationalen Kongressen;
- Enge Zusammenarbeit mit der Arbeitsgruppe;

Alle Aufgaben wurden stets in Zusammenarbeit mit der Projektleitung durchgeführt.

Beitrag zu Paper II

Analog zu Paper I:

Meine Aufgaben umfassten folgende Punkte:

- Teilnahme am MTB CCC München LMU;
- Erhebung und Zusammenstellung der Daten;
- Kommunikation mit behandelnden ÄrztInnen, PatientInnen und Angehörigen (zur follow-up Dokumentation);
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Alle Aufgaben wurden stets in Zusammenarbeit mit der Projektleitung durchgeführt.

Introduction

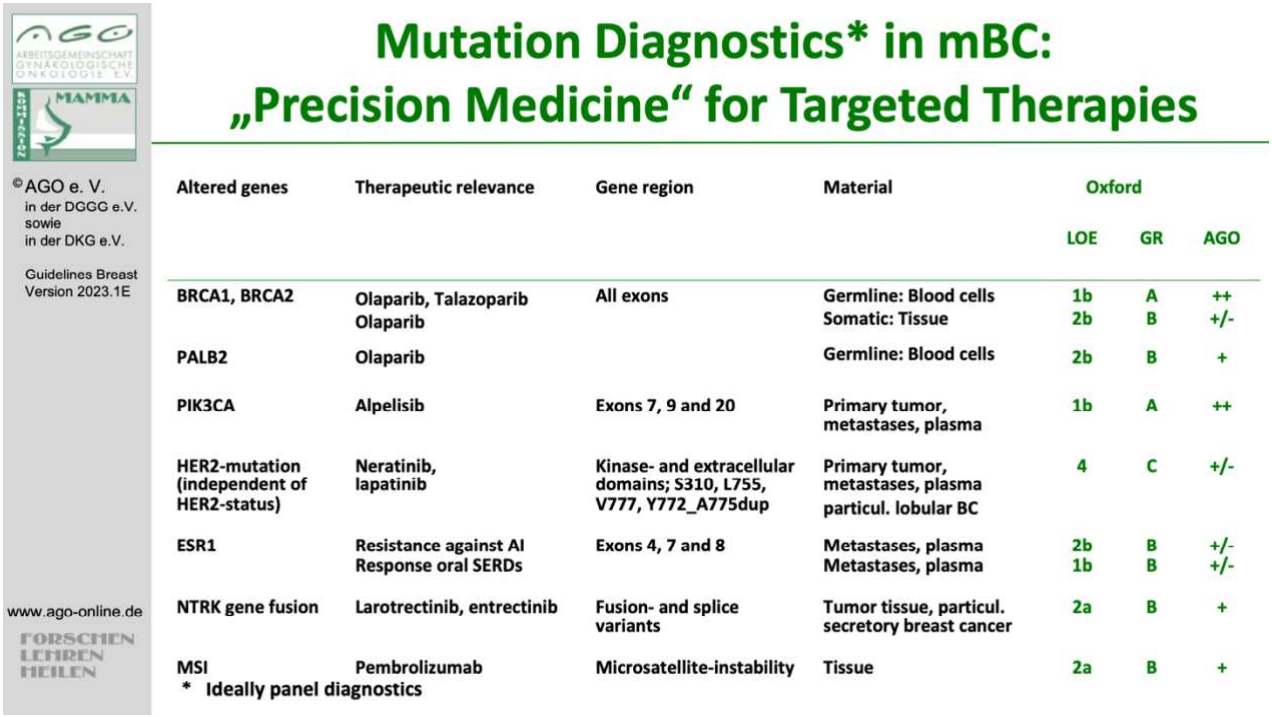
In women, breast cancer (BC) is the most commonly occuring cancer type, with a global estimate of 2.3 million newly diagnosed cases and almost 685 000 deaths as of 2020. (1) Incidence rates have been increasing due to several factors including early detection, better diagnostic technologies in the past few years, and an aging population. Despite a broad variety of standard of care treatment options as well as recent advances in oncology, there are still some BC patients, who do not profit from routine diagnostic and therapeutic approaches. Between 20% and 30% of the BC metastasize over time. (2) Metastatic breast cancer (mBC) patients have poorer survival rates with a 5-year survival probability of approximately 38% when compared to patients with an early BC and 5-year survival rate of 96%. (3, 4) In order to change the direction of these negative trends, new diagnostic and treatment strategies must be developed.

Standard treatment of metastatic breast cancer

Although guideline-based treatment options for mBC have essentially been expanding in the past few years, mBC management remains extremely challenging. Present standard treatments include most often surgery, radiation, and systemic drug therapies, comprising endocrine, cytotoxic and biological agents. The complex choice of systemic therapy depends on multiple factors such as tumor characteristics, prior therapies (and their toxicities), co-morbidities, disease-free interval after end of adjuvant treatment, estimated life expectancy and many others. While several biomarkers such as hormone receptors (estrogene and progesterone receptors) and Human Epidermal Growth Factor Receptor 2 (HER2) have long been utilized to guide therapy selection in mBC, the purpose of treatment remains palliative, aiming to prolong survival and optimally control symptoms. In the challenging task of choosing the right treatment, today, there is a variety of guidelines, both international and regional, providing key recommendations for managing mBC such as internationally the Metastatic Breast Cancer Clinical Practice Guideline by the European Society for Medical Oncology (ESMO) and, in Germany, interdisciplinary S3 and AGO (Arbeitsgemeinschaft Gynäkologische Onkologie) guidelines. (5)

Current approaches of treating mBC are primarily based on prognostic and predictive biomarkers, identified in a broad population of BC patients throughout the years. For example, the discovery of the importance of the hormone receptor status and HER2 expression revolutionized BC treatment by improving survival outcomes. Currently, the differentiation between luminal, HER2-enriched and triple negative BC is validated in the clinical practice and of great importance for predicting patient response to certain therapies. (6) Beside the immunohistochemical differentiation between hormone receptors such as estrogen and progesterone, as well as HER2, routine practice in mBC also includes determining tumor proliferation indicators, for instance Ki-67, and, recently, HER2-low, PD-L1 (programmed death-ligand 1), and germline BRCA1/2 genetic testing.

Although mBC is associated with low mutational burden, over the past few years, several molecular biomarkers in BC have been identified as well as implemented into clinical care as reliable predictors for tumor's response to tumor therapy as well as for disease prognosis. (**Fig. 1**)



Altered genes	Therapeutic relevance	Gene region	Material	Oxford LOE	Oxford GR	Oxford AGO
BRCA1, BRCA2	Olaparib, Talazoparib Olaparib	All exons	Germline: Blood cells Somatic: Tissue	1b 2b	A B	++ +/-
PALB2	Olaparib		Germline: Blood cells	2b	B	+
PIK3CA	Alpelisib	Exons 7, 9 and 20	Primary tumor, metastases, plasma	1b	A	++
HER2-mutation (independent of HER2-status)	Neratinib, lapatinib	Kinase- and extracellular domains; S310, L755, V777, Y772_A775dup	Primary tumor, metastases, plasma particul. lobular BC	4	C	+/-
ESR1	Resistance against AI Response oral SERDs	Exons 4, 7 and 8	Metastases, plasma Metastases, plasma	2b 1b	B B	+/- +/-
NTRK gene fusion	Larotrectinib, entrectinib	Fusion- and splice variants	Tumor tissue, particul. secretory breast cancer	2a	B	+
MSI	Pembrolizumab	Microsatellite-instability	Tissue	2a	B	+

Fig. 1. Predictive and prognostic molecular markers for mBC as defined by AGO Germany. (7) (Data accessed: 24.05.2023)

Despite of the fact that an increase in survival of mBC patients over time has been demonstrated in clinical trials, mBC remains still incurable with up to 70-80% of stage IV BC patients dying within the first 5 years. (8, 9) Recently, new treatment strategies incorporating immunotherapy and targeted treatments suggest a potential reduction of mortality and an improvement of the quality of life of mBC patients.

Precision medicine as the future of cancer medicine

The field of biomarker research is expanding, shifting the old “one-size-fits all” approach to a modern personalized one with the use of multigene sequencing. One of the newest innovative approaches to cancer treatment is precision oncology, which enables cancer patients to receive treatment specifically targeting their individual genomic alterations ultimately leading to better patient outcomes. Nowadays, the new advanced diagnostics like comprehensive genomic profiling (CGP) and next-generation sequencing (NGS) allow physicians to sequence DNA at unprecedented speed with the purpose of identifying driver and/or targetable alterations. This development of diagnostic technologies, combined with availability of new molecular therapeutic agents (MTA) could optimize disease management by establishing new patient databases. (10)

Better outcomes in metastatic breast and gynecological cancer, as well as other solid tumors, associated with such innovative personalized approach have been reported in several prospective trials. For example, in the SAFIR01 trial, 4 out of 43 mBC patients (9%) with implemented therapy recommendation based on genomic profiling showed an objective response and 9 of 43 (21%) demonstrated stable disease continuing over 16 weeks. (11) In the MOSCATO trial, 30% of the patients, who received multigene sequencing diagnostics, experienced an improved progression-

free survival (PFS) plus 11% had an objective response rate of 11%. (12) Recent data from the SAFIR02-BREAST trial shows that genomic analysis (and initiation of treatment based on these alterations) improves the outcome of mBC patients with tumor alterations classified as ESCAT I/II, confirming the importance of genomics for disease treatment. (13) The ESCAT ranking was established to support treatment recommendations based on NGS for patients with metastatic cancers. Also, in the BC experience of the MTB at San Diego Moores Cancer Center, conducted by Parker et al., 41% of the treated patients benefited from the personalized recommendation, suggesting that multidisciplinary MTBs could help optimize the management of patients with advanced, heavily pretreated BC. (14)

Table 1 shows a summary of recent studies concentrated on molecular diagnostics in BC.

Table 1. A summary of studies concentrated on precision oncology in BC. (15)

Author/Study	Tumor entity	Enrolled patients (n=)	MP patients	Actionable alterations	Implemented therapies - n (% of enrolled)	Results
André et al. (SAFIR01/UNI-CANCER)	breast	423	299 (71%)	195 (46%)	55 (13%)	ORR: 4 patients had a PR and 9 had SD >16 weeks (3% of all patients)
André et al. (SAFIR02/BREAST)	breast	1462	1222 (84%)	646 (44%)	238 (16%)	TT (ESCAT-recommendations - based) matched to genomics improve PFS
Parker et al.	breast	43	43 (100%)	40 (93%)	17 (40%)	7 patients (16% of all patients) achieved SD or PR
Van Geelen et al.	breast	322	234 (72%)	74 (23%)	No data	No data about implementation rate and outcome

MP = molecular profiled, PFS = progression-free survival, ORR = overall response rate, SD = stable disease, PR = partial response, n.a. = not available, TT = targeted therapies

Undoubtedly, the further development of precision oncology offers many new therapeutic opportunities. However, it also entails a number of scientific, clinical, and logistic challenges, which need to be addressed by close interdisciplinary teamwork. In Germany, such interdisciplinary platform to support and maintain cross-regional networking has already been founded, aiming to set standards for the use of precision medicine in clinical care, which are still missing.

Registry MTB

Considering the complexity of the NGS results because of the rising number of active targetable mutations, it's challenging for many oncologists to interpret complex genomic test results and incorporate them into clinical care or access early clinical trial units. (16) This problem has been addressed with the initiation of a molecular tumor board (MTB). This represents an innovative approach, designed to facilitate the use of precision medicine for oncology patients. To our

knowledge, we are the first research group of the MTB Project to provide data on our first experiences in gyneco-oncology and breast cancer with the establishment of an MTB at the Comprehensive Cancer Center (CCC) Munich LMU.

"The Informative patient" (local ethic committee registry number 284-10) is a prospective precision oncology registry with up to 240 enrolled patients (data accessed: June 2022), designed to prove the impact of MTB-guided diagnostic and treatment decisions on the progression-free survival of patients with progressive under routine therapy breast and gynecological malignancies. SMART PRO (local ethic committee registry number: 20-722), a following project of our study group, concentrates on patients with diverse tumors (138 patients till June 2022), who underwent molecular profiling and were then discussed by an MTB. All patients presented in this analysis signed an informed consent to participate in the presented work.

To evaluate the use of personalized treatment recommendations, clinical outcomes were compared by calculating PFS of study participants, who obtained the recommended therapy. PFS was defined as the time between initiation of a treatment and disease progression or death. In order to evaluate the effect of the treatment recommendations, we compared the PFS of patients on recommended therapy (PFS2) with PFS of patients on the last therapy prior to molecular profiling. (PFS1). The ratio PFS2/PFS1 was first proposed as endpoint by Von Hoff, who also concluded that a ratio > 1.3 would indicate a treatment benefit. This supports the observation that PFS decreases proportionally to the number of treatments over time during the disease course. (17) The first part of our analysis contains data on patients with advanced breast or other gynecological malignancy, whereas the second part demonstrates results only in mBC patients.

Patient recruitment and study design

The prospective studies, "The Informative Patient" and "SMART PRO", were performed at the LMU University Hospital Munich in cooperation with the CCC Munich in compliance with the Declaration of Helsinki and received an approval by the local ethics committee. All participants (n=195) signed an informed consent for us to analyze their genomic results, process their personal health information, and collect follow-up information regarding the disease's progression for research means and/or publication. All participating patients were discussed in the local MTB, in order to interpret and/or translate molecular profiling data into treatment or diagnostic recommendation. The data presented in this manuscript was collected between March 2017 and December 2019.

Trial's eligibility criteria included metastatic breast or gynecological cancer, at least one metastatic site or rare cancer type, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, as well as readiness to receive an off-label therapy or to participate in future clinical trials.

All patients and their disease history were initially presented in a breast and gynecological tumor board in the University Hospital LMU Munich, where the attending physicians discussed if the patient was eligible for an MTB case discussion following molecular profiling. After having their genetic profiling completed, all patients were discussed by the MTB team, comprised of experts in

oncology, molecular pathology, genetics, and other medical disciplines, with high knowledge of comprehensive molecular characteristics of various solid tumors. Eventually, based on the results of the molecular testing, considering the tumor profile, course of disease and other relevant radiological images, and after careful review of available literature and various databases, the MTB suggests potential diagnostical and therapeutic approaches including relevant clinical trials or molecular targeted agents. (Fig. 2)

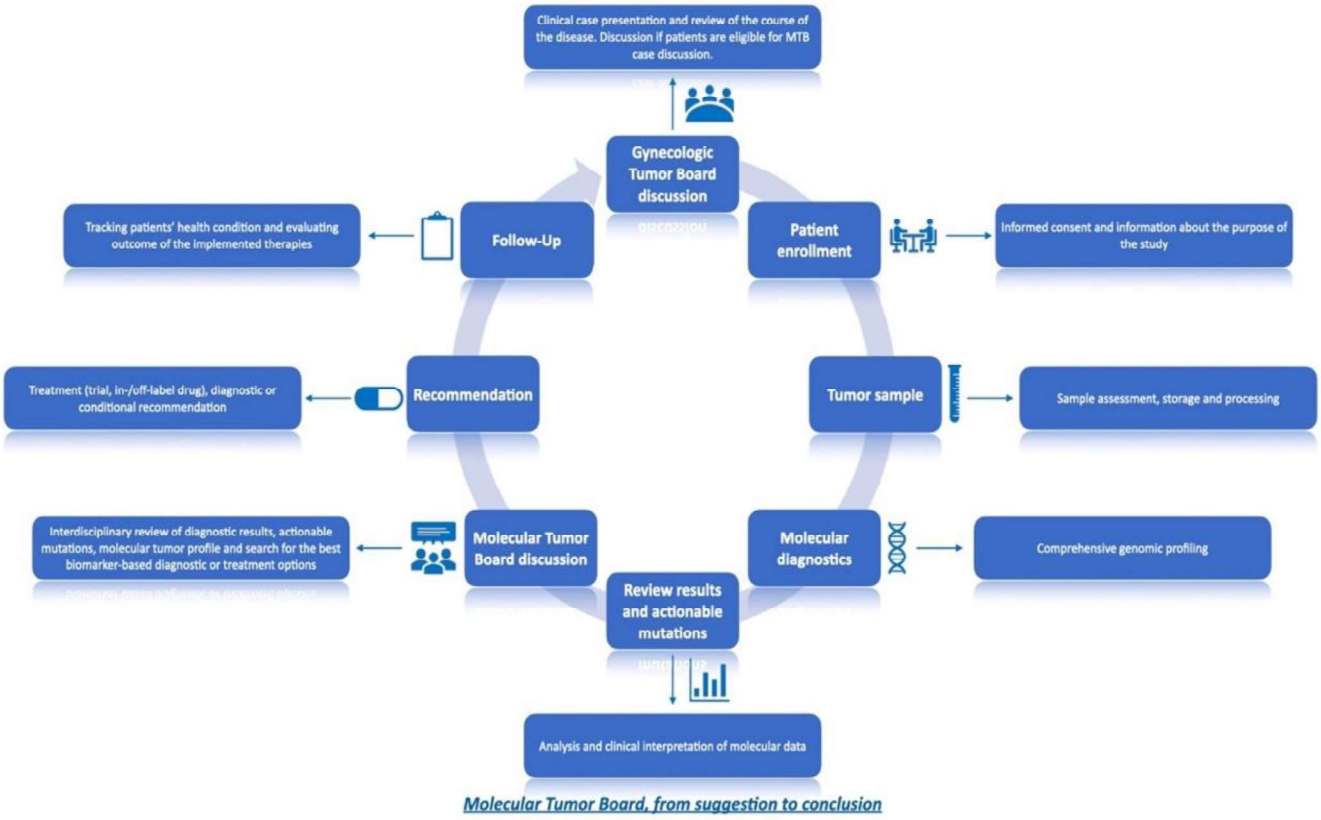


Fig. 2. MTB Project design. (18)

All molecular diagnostics was executed at the Pathology Department of LMU Munich using validated methods such as targeted NGS with Oncomine Focus Panel (comprising 52 cancer-related genes) until November 2018 and later with Oncomine Comprehensive v.3 assay searching molecular aberrations in 161 cancer-related genes.

All MTB recommendations were based on the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT) levels of evidence. (**Table 2**) (19)

Table 2. Actionable gene aberrations in BC as classified by ESCAT. (19) (Data accessed 01.04.2021)

Genomic Alterations	Prevalence
ESCAT Level I	
BRCA1/2 germline mutations	4%
ERBB2 amplifications	15–20%
Microsatellite instability-high	1%
PIK3CA hotspot mutations	30–40%
ESCAT Level II	
$AKT1^{E17K}$ mutations	5%
ERBB2 hotspot mutations	4%
ESR1 mutations	10%
ESCAT Level III	
BRCA1/2 somatic mutations	3%
ERBB3 mutations	2%
MDM2 amplifications	1%

Results

The first cohort (Cohort 1) comprised a total of 100 patients with mBC or advanced gynecological malignancies to be reviewed in an MTB setting from March 2017 to March 2019. However, five patients were excluded because of personal reasons such as withdrawal of consent or death prior to treatment recommendation. Then, considering the promising results of the first cohort, we decided to conduct a second study using larger sequencing panels, concentrating on the outcome of BC patients, because they were the biggest population of the first cohort and had demonstrated remarkable responses to personalized therapy recommendations. Between May 2017 to December 2019, 100 patients with mBC were included in the second part of the study. (Cohort 2) (15, 18)

In the first cohort, 95 patients with mBC or advanced gynecological malignant disease refractory to standard-of-care therapies were reviewed by the MTB (tumor entity: 68% breast, 20% ovary, 5% cervix, 3% endometrium and 4% other). Among all patients, PIK3CA (13.7%) and ERBB2 (10.5%) were the most common alternated genes. In total, 36% obtained a treatment recommendation based on the results of the molecular profiling. Treatment recommendations were pursued in nine cases (9%) and four of these patients (4%) demonstrated a clinical response in form of partial response (PR) or stable disease (SD) remaining for more than 16 weeks. (18)

Among the second cohort of 100 enrolled mBC patients, genomic alterations were detected in 72 cases (72%) (Median 2 per patient, range: 1 to 6), with the highest frequency in the PIK3CA (19%) and TP53 (17%) genes. 53% of all alterations found were actionable (ESCAT) and the MTB recommended a treatment in 49 (49%) cases. Overall, the suggested therapy was implemented in 16 (16%) cases. Nine out of sixteen patients (56%; 9% of all) demonstrated a progression-free survival ratio ≥ 1.3. (15)

Fig. 3 and Fig. 4 depict main results of our work for both cohorts.

Cohort 1 (n=95). Metastatic breast or gynecological cancer patients.

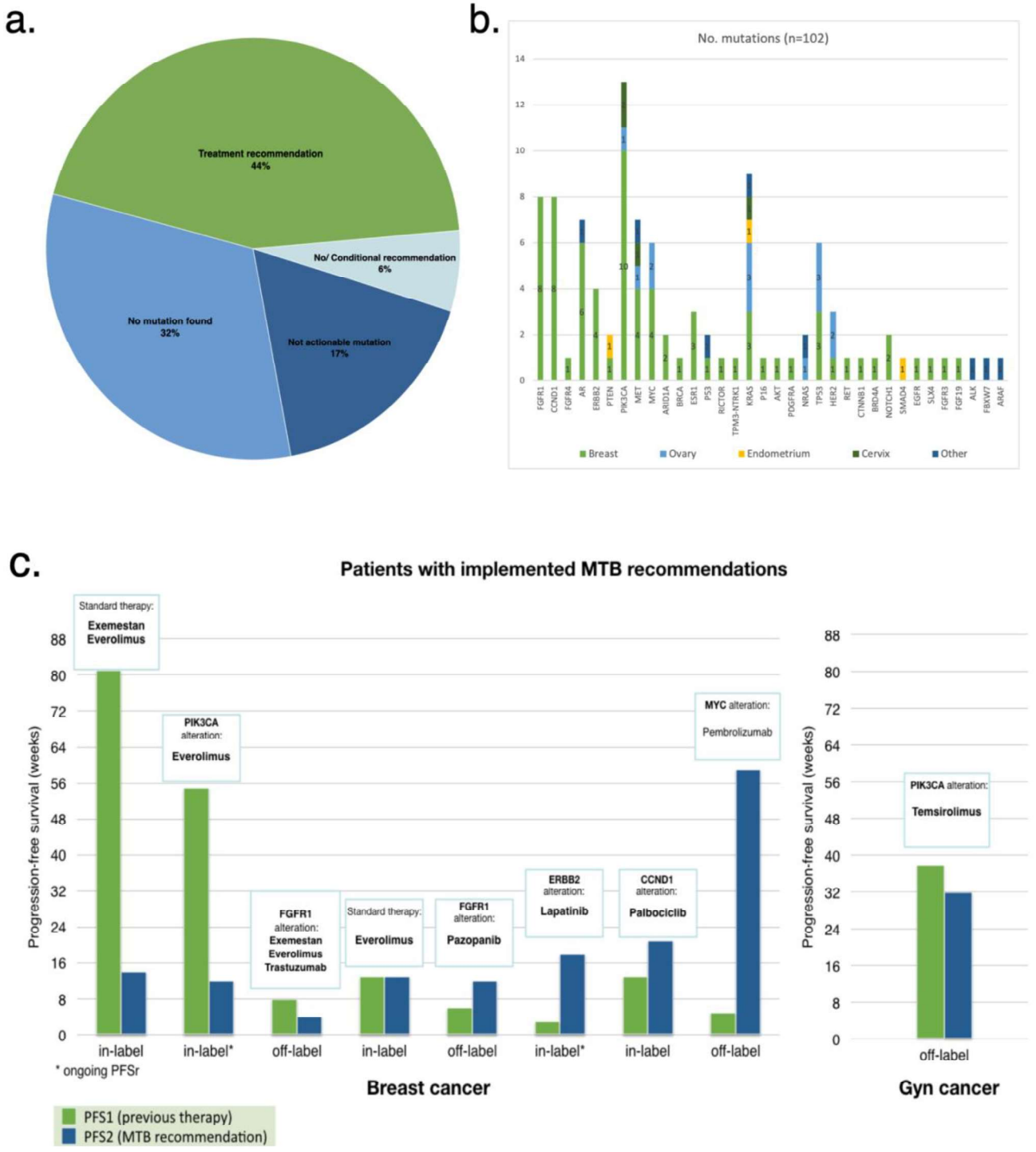


Fig. 3. Cohort 1. a) Results of the molecular diagnostic testing at the MTB CCC Munich LMU. (n=95) b) Frequency of genomic alterations. c) PFS comparison of the last treatment prior to molecular profiling (PFS1) and of implemented MTB-recommended treatment (PFS2). PFS was measured from the beginning of a treatment until disease progression or patient withdrawal. (18)

Cohort 2 (n=100). Metastatic breast cancer patients.

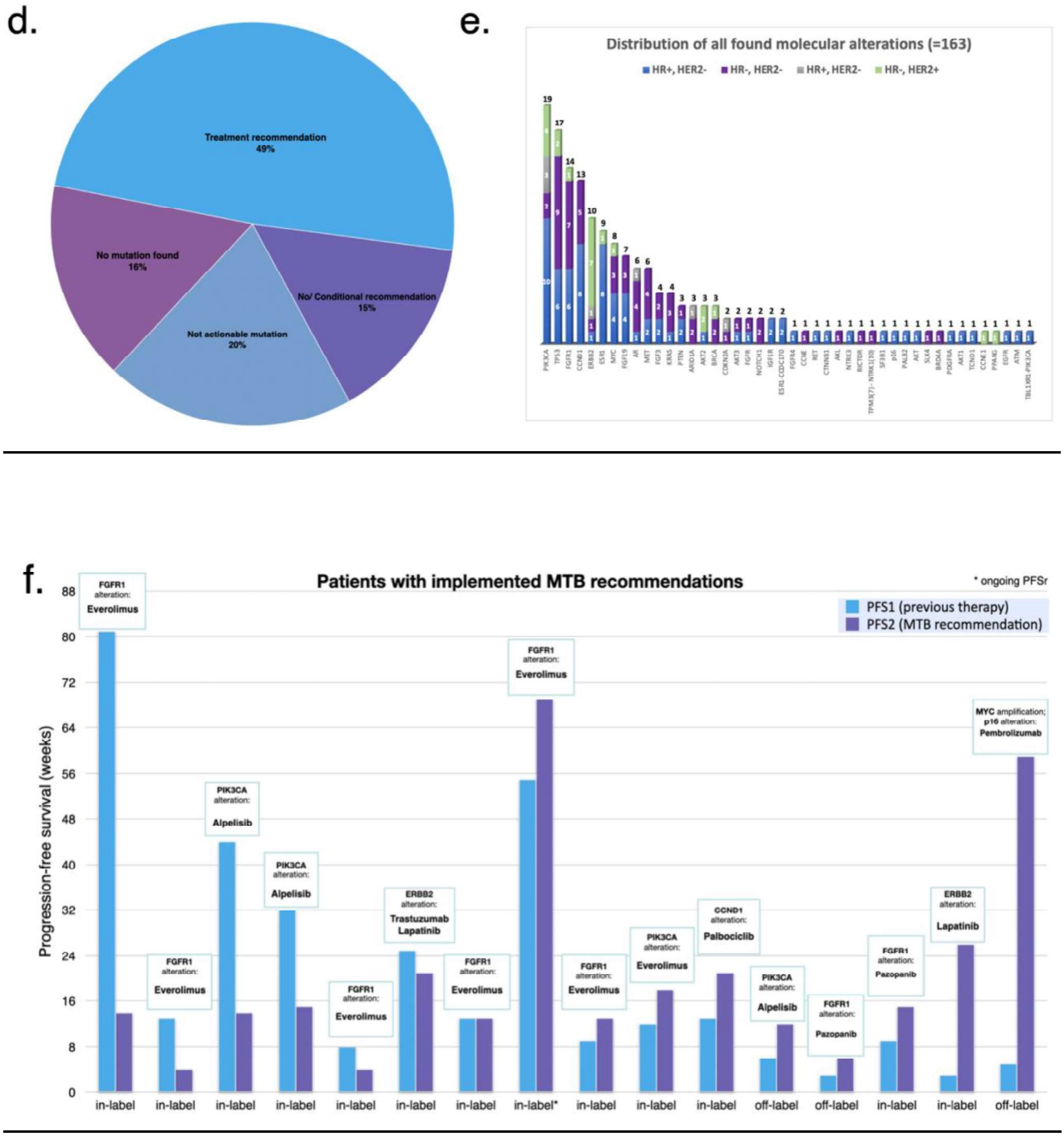


Fig. 4. Cohort 2. d) Results of the molecular diagnostic testing at the MTB CCC Munich LMU. (n=100) e) Frequency of genomic alterations. f) Progression-free survival (PFS) comparison of the last treatment prior to molecular profiling (PFS1) and of implemented MTB-recommended treatment (PFS2). PFS was measured from the beginning of a treatment until disease progression or patient withdrawal. (15)

Challenges and limitations of precision medicine

Although some small groups of cancer patients seem to benefit from precision medicine, critics argue that its clinical utility and significance are still not proven for larger patient populations, which- considering the high costs and other limitation-, makes its incorporation into clinical practice not reasonable at the moment. For instance, the SHIVA trial, one of the largest randomised trials, investigating the role of precision medicine by comparing patient-tailored therapies on the basis of molecular diagnostics with conventual therapies in patients with diverse cancer entities, failed to prove its primary objective. It failed to demonstrate statistically significant difference in PFS between patients treated with MTA and those receiving a standard-of-care treatment. (20) Other studies, such as WINTHER, TARGET, and I-PREDICT, showed that targeting identified molecular alterations is related to better disease control rates, PFS rates, and overall survival (OS) rates. (21-23) The biggest so far meta-analysis by Schwaederle et al. analyzed two patient groups, a total of 32 149 participants in 570 studies, who obtained a personalized therapy recommendation with those who didn't. The first group demonstrated a median response rate of 31% versus 10.5% in the second group, an extended median PFS (5.9 versus 2.7 months) and OS (13.7 versus 8.9 months), which showed the benefits of the personalized approach. (24) The results presented in this analysis are similar to results from internationally conducted studies in this area. For instance, in the CATCH study, also conducted in Germany, 64 of 128 enrolled mBC patients (50%) received a treatment recommendation by the local MTB, which eventually resulted in SD (13/64, 25%) or PR (8/64, 15%) in the treated patients. (25)

Why is precision medicine not a reality for every cancer patient? Even though there are more and more studies demonstrating the importance of precision medicine, as of today, it is still not implemented into routine clinical care for all patients because of some limitations, which hinder its development. On the one hand, high costs associated with personalized medicine represent a problem for patients and insurance companies. Not only do they come from the diagnostic methods and genetic testing, but also targeted drug agents are expensive, making their implementation into clinical practice a challenge for the health systems. Proving the cost-effectiveness of the testing methods, and of targeted drug therapies (for example by developing standard follow-up registries), could increase their availability in oncology care. Also, precision medicine could reduce costs associated with ineffective, often expensive therapies and hospitalizations for adverse drug reactions.(26)

On the other hand, although the list of actionable alterations is getting longer, there are not enough drugs on the market which could target the altered genes. This may be caused by the lack of appropriate clinical trials that could exploit the benefits of the new anticancer drugs. Therefore, improving clinical trial designs and investing in clinical trials is of great importance for the future development of precision oncology. Access to real-world data and patient outcomes is also a key-factor for developing genetically targeted therapies based on large databases. A recent example of the importance of new approved targeting drugs for the survival rates of BC patients was the approval of alpelisib, a PIK3CA inhibitor, in July 2020 in Europe. In the SOLAR-1 trial, the

experimental group treated with alpelisib achieved a PFS of 11 months versus 5.7 months in the control group. (27)
Also, on the basis of the outcome of the KEYNOTE-355 trial, late 2020, the immunotherapeutic agent pembrolizumab received an FDA approval for patients with non-resectable locally advanced or metastatic triple-negative, PD-L1-positive BC. Adding pembrolizumab to chemotherapy led to a significantly longer OS in comparison to chemotherapy alone. (28) Recognizing that PD-1 and PD-L1 play an essential role in the prognosis for TNBC has led to introducing immunotherapy as potential targeted treatment for MBC patients. (29)

There is not only a lack of molecular targeted agents, but the access to such treatments is also limited, as shown in the SAFIR01 trial. The reason for this is mostly because of missing reimbursement rather than regulatory issues. For example, the already mentioned alpelisib has faced a withdrawal from the German market in May 2021, almost a year after its approval, because of a failed attempt to reach a reimbursement agreement between pharmaceutical and health insurance companies. (30)

Today, in order to receive a medication beyond its labelled indication, most patients get referred to a clinical trial, if possible. However, most trials have a long list of inclusion and exclusion criteria, as well as short windows of patient recruitment, making the participation of patients with late-stage disease relatively challenging. So, addressing the cost and value of targeted therapies is crucial for enabling more patients to receive such treatments. Moreover, establishing more early-access-programs may serve as temporary solution to address logistic problems. Recent studies report an improved access to targeted therapies by implementing MTBs. (31)

An additional important aspect is the issue of the not fully designed concept of MTBs and the absence of guidelines and quality criteria. Their importance for establishing MTBs into clinical care is important mostly from a patient's perspective. For example, a great number of the patients in this study couldn't profit from personalized treatment strategies due to quick deterioration of the medical condition mainly caused by patient enrollment at a late stage of the disease. Therefore, MTBs' work should be optimized by defining key problems such as the right time to present patients for molecular profiling, determining actionability of molecular aberrations, and ensuring access to off-label therapies as well as to clinical trials. Moreover, the personalized treatment approach is of great clinical importance for therapy monitoring with the purpose of evaluating the efficiency of the drugs, in order to minimize adverse effects.

With the advancement and availability of molecular profiling comes the need to educate oncologists in this field and provide information about this topic for primary care providers. The lack of expertise in the field of precision medicine concerning actionable mutations and potential targeted agents is one of the primary barriers for its incorporation into routine clinical care. Because of the complex and broad results of molecular profiling, clinicians often face difficulties interpreting genomic data. Molecular tumor boards could serve as a platform of educating younger oncologists on identifying actionable molecular alterations and knowing how to target them with the right molecular agent. Moreover, this could also lead to the establishment of guidelines on using

personalized medicine for oncology patients, and to building personalized gene regulatory networks for precision medicine.

Furthermore, basing treatment strategies on a single molecular alteration found could be inappropriate because of the heterogeneity of solid tumors. Approximately two-thirds of the mutations found in a single biopsy could not be found in other biopsies across the same tumor. (32) Disease variability across cancers causes different responses to targeted therapies. The intratumor genetic differences could lead to tumors not responding to a therapy targeting a particular gene or cause an adverse reaction to the treatment, making the disease course still unpredictable. A good example of the role of certain pathomechanisms for responding to drug treatment is the resistance to endocrine therapy in BC, caused by ESR1 gene alterations. (33) Novel tailored treatments are emerging, offering promising results. In the EMERALD trial, elacestrant, a new selective estrogen receptor degrader, has been showed to improve significantly PFS compared to endocrine monotherapy not only in the overall population, but also in patients with ESR1 mutations and ER+/HER2- advanced BC and serves nowadays as a way to tackle resistance to endocrine therapies. (34)

Lastly, it is still unclear, whether tumor biopsies could be replaced by liquid biopsies, a new, non-invasive approach to utilize the detection of biomarkers in blood for prognostic and predictive purposes. Determining the best way to perform tumor molecular profiling is of great significance for the future of precision medicine. In order to compare both methods, Kesserer et al did simultaneously both liquid and tissue biopsies for various types of cancer and found a total of 45 mutations, but the overlap of those found by both tests was only 10, or 22%. (35) More research and advancement are needed to identify the most accurate diagnostic way of detecting actionable mutations in order to select the best personalized treatment.

All in all, precision medicine is a constantly evolving field combining new drug discoveries and approvals, innovative diagnostic approaches, and novel designed trials. Although precision medicine offers hope for treating previously incurable cancers, its full spectrum of evolving opportunities must be reviewed to derive maximal individual benefits.

Our work has several limitations. On the one hand, we did not include a large number of participants in the study. On the other hand, patients with late-stage disease have a limited number of treatment options, which can be used considering their side effects. Also, as previously mentioned, disease heterogeneity could also affect the outcome of the implemented treatments, making our results not applicable to other patients. Furthermore, the number of targetable actionable mutations is still rising, meaning some of the presented genetic may have been not actionable when being reviewed by the MTB. The molecular landscape of tumors may also vary during the disease course. Lastly, this manuscript represents a real-world data registry and doesn't represent a randomised controlled trial. However, the described multidisciplinary MTB structure aimed to provide data on standards, outcomes, and clinical utilization of precision oncology to further evaluate its potential benefits for patients with mBC and/or gynecological cancers.

Conclusion

Nowadays, precision medicine is believed to be the future of modern cancer medicine. It has transformed the oncology world by offering tools for understanding molecular mechanisms and their impact for treatment strategies, as well as by enabling cancer patients to receive treatments according to the individual characteristics of their tumor. Nevertheless, this type of tailored treatment is not yet being used in routine clinical care, because the clinical importance of personalized medicine is still controversial. Thus, the access to this innovative approach is still hampered either because of the low number of medical facilities offering this opportunity, or because of the small number of patients fitting inclusion criteria of trials enrolling such patients. Moreover, the costs associated with precision medicine, and the logistic problems that lead to limited access to targeted therapies, as well as the short list of actionable molecular alterations are some of the obstacles which hinder personalized medicine from becoming a reality for all patients. Therefore, real-world data proving the importance of implementing precision medicine for all suitable patients is needed.

In this single-center prospective registry, we demonstrated a positive impact of MTB-guided treatment recommendations based on molecular profiling on progression-free survival of breast and gynecological cancer patients. We detected at least one actionable mutation in 43% of the cases in cohort 1 (n=41), and 53% of the patients in cohort 2 (n=53), which proves the role of sequencing techniques in identifying key alterations in solid tumors. Based on these results, more than a third of the patients (n=34) in the first cohort and almost half of the patients (n=49) in the second cohort received a treatment recommendation. With four out of nine (44.4%, 4.2% of all) in the first cohort, and 16 out 49 (33%, 16% of all) implemented therapy recommendations in the second cohort, we demonstrated the clinical benefit (PFSr > 1.3) for over 16 weeks of the recommended targeted therapies. Also, it's important to point out that precision oncology constantly evolves. Comparing the results of our two cohorts, we have seen that therapy implementation rates have been improving (16% in the second cohort versus 12.5% in the first one). Moreover, the amount of recommendations given (49% vs 42%), as well as of alterations found (53% vs 48%) has also raised over time.

Therefore, we expect breast and gynecological cancer patients to benefit more from precision medicine over time and precision medicine to be increasingly implemented in the routine clinical care of this patient population, making the future of precision medicine more promising than ever. Its clinical use is of high importance and should be applied to all suitable patients. To truly unlock the potential of precision medicine approaches, new opportunities for educating healthcare staff, and broader access to precision medicine utilities and targeted therapies for patients, should be established.

Zusammenfassung

Die Entstehung von Krebs beruft auf Genveränderungen, die heutzutage immer detailierter erforscht werden können. Das Zusammenspiel aus umfassendem genomischen Profiling und individualisierten Therapieempfehlungen ist als Präzisionsonkologie bekannt. Ziel der Präzisionsonkologie am Comprehensive Cancer Center (CCC) München LMU im Zuge der Studie „Der Informative Patient“ und der darauffolgenden „SMART PRO“-Studie ist es, jedem/er Krebspatienten/in anhand einer detaillierten molekulargenetischen Diagnostik seines/ihres Tumors eine personalisierte Behandlung mit zielgerichteten Medikamenten anzubieten.

„Der Informative Patient” ist eine prospektive Studie des CCC München LMU im Bereich der personalisierten Medizin für diverse Malignome. Die daran anschließende Studie „SMART PRO“ konzentriert sich auf PatientInnen mit verschiedenen Tumorentitäten. Alle hier präsentierten PatientInnen nahmen an einer der beiden Studien teil. Ziel der hier gezeigten Analysen ist es, die Wirkung von personalisierten Therapieempfehlungen auf das progressionsfreie Überleben (PFS) von PatientInnen mit metastasierten Brust- oder gynäkologischen Malignomen im Vergleich zur Standardtherapie zu betrachten. Hier stellen wir unsere ersten Erkenntnisse im Bereich der Präzisionsmedizin vor.

Für die molekulardiagnostischen Untersuchungen wurde Next-Generation Sequencing verwendet. PatientInnen inklusive der Ergebnisse der erweiterten molekularen Diagnostik wurden im Rahmen von interdisziplinären Tumorboard Konferenzen vorgestellt und mögliche diagnostische und therapeutische Schritte besprochen.

Im ersten Teil unserer Analyse, von März 2017 bis März 2019, wurden 95 PatientInnen mit metastasiertem Brustkrebs oder anderen gynäkologischen Malignomen (Tumorentität: 68% Mamma, 20% Ovar, 5% Zervix, 3% Endometrium und 4% andere) ausgewertet. PIK3CA und ERBB2 waren die Gene mit den meisten Veränderungen. Das MTB hat in 36% der Fälle eine Therapie auf Basis des molekularen Tumorprofils aussprechen können. Eine Umsetzung von den Therapieempfehlungen folgte in neun Fällen, vier PatientInnen haben eine partielle Remission oder einer Stabilisierung der Krankheit von über 16 Wochen demonstriert. Kurz nach den MTB Diskussionen wurde die Zulassung von dem PIK3CA-Inhibitor Alpelisib bekanntgegeben, die in weiteren fünf Therapieempfehlungen resultiert hätte.

Im zweiten Teil unserer Analyse, von Mai 2017 bis Dezember 2019, haben 100 PatientInnen mit metastasiertem Brustkrebs eine erweiterte molekulargenetische Untersuchung bekommen, die in einer individuellen Therapieempfehlung bei 49 PatientInnen (49%) resultierte. Die meistmutierten Gene waren PIK3CA (19%) und TP53 (17%). Unter der empfohlenen Therapie, die bei 16 von 49 (16% von allen) PatientInnen umgesetzt wurde, haben neun (9% von allen) PatientInnen eine progressionsfreie Überlebensrate ≥ 1.3.

Die erste Analyse konzentrierte sich auf die Machbarkeit und den Nutzen eines MTB und der interdisziplinären Zusammenarbeit. Im Rahmen der zweiten Analyse wurde gezeigt, dass der Einsatz der personalisierten Medizin bei PatientInnen mit metastasiertem Brustkrebs eine Therapieoptimierung auch mit Verlängerung des PFS darstellt. Kurz zusammengefasst, von dem Ansatz der Präzisionsonkologie in Form einer personalisierten zielgerichteten Krebstherapie könnten einige PatientInnen mit Brust- oder gynäkologischen Tumoren profitieren. Daher sollte dies bei allen geeigneten PatientInnen angewandt werden.

Abstract (English)

With the introduction of next-generation sequencing (NGS), new opportunities of precision medicine have been opened up. This novel approach of matching targeted therapies to genomic aberrations aims to offer access to personalised therapy to oncology patients. In CCC Munich LMU, an interdisciplinary molecular tumor board (MTB) is established to enable patients to obtain tailor-based therapy recommendations based on genomic data. Here, we review the effect of MTB-based treatment recommendations on the progression-free survival of women with progressive under standard therapy metastatic breast or gynecological malignancies.

All tumor samples have been analyzed by performing NGS (Oncomine). From March 2017 through March 2019, the first cohort of 95 women with metastatic breast or gynecological cancer (tumor entity: 68% breast, 20% ovary, 5% cervix, 3% endometrium and 4% other) were included in a prospective local registry, received extended molecular profiling, and were then discussed in a multidisciplinary MTB. Most genomic alterations were detected in PIK3CA (14%) and ERBB2 (11%) genes. The MTB recommended biomarker-based targeted therapy for 34 patients (36%). Adherence to the MTB recommendation (9 /34, 10% of all) led to a partial response or stable disease for longer than 4 months in four patients (4 /34, 9% of all).

In the second cohort, between May 2017 and December 2019, 100 cases of women with metastatic breast cancer (mBC) were reviewed by the local MTB. A molecular alteration was detected in 72% of the mBC tumors, with PIK3CA (19%) and TP53 (17%) being the most frequently mutated genes. Furthermore, 53% of the found alterations were classified by the MTB as actionable and 49 patients (49%) obtained a therapy recommendation. Adherence to it (16/49,16% of all) demonstrated a positive effect with a progression-free survival ratio ≥ 1.3 in 9 patients (9/16, 9% of all).

Personalised therapy recommendations could result in a positive clinical response for patients with metastatic breast or gynecological cancer and should be given to all suitable patients.

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Paper I

NGS-guided precision oncology in metastatic breast and gynecological cancer: first experiences at the CCC Munich LMU

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Abstract

Purpose: Comprehensive genomic profiling identifying actionable molecular alterations aims to enable personalized treatment for cancer patients. The purpose of this analysis was to retrospectively assess the impact of personalized recommendations made by a multidisciplinary tumor board (MTB) on the outcome of patients with breast or gynecological cancers, who had progressed under standard treatment. Here, first experiences of our Comprehensive Cancer Center Molecular Tumor Board are reported.
Methods: All patients were part of a prospective local registry. 95 patients diagnosed with metastatic breast cancer or gynecological malignancies underwent extended molecular profiling. From May 2017 through March 2019, the MTB reviewed all clinical cases considering tumor profile and evaluated molecular alterations regarding further diagnostic and therapeutic recommendations.
Results: 95 patients with metastatic breast or gynecological cancers were discussed in the MTB (68% breast cancer, 20% ovarian cancer, 5% cervical cancer, 3% endometrial cancer and 4% others). Genes with highest mutation rate were PIK3CA and ERBB2. Overall, 34 patients (36%) received a biomarker-based targeted therapy recommendation. Therapeutic recommendations were implemented in nine cases; four patients experienced clinical benefit with a partial response or disease stabilization lasting over 4 months.
Conclusion: In the setting of a multidisciplinary molecular tumor board, a small but clinically meaningful group of breast and gynecological cancer patients benefits from comprehensive genomic profiling. Broad and successful implementation of precision medicine is complicated by patient referral at late stage disease and limited access to targeted agents and early clinical trials.

Introduction

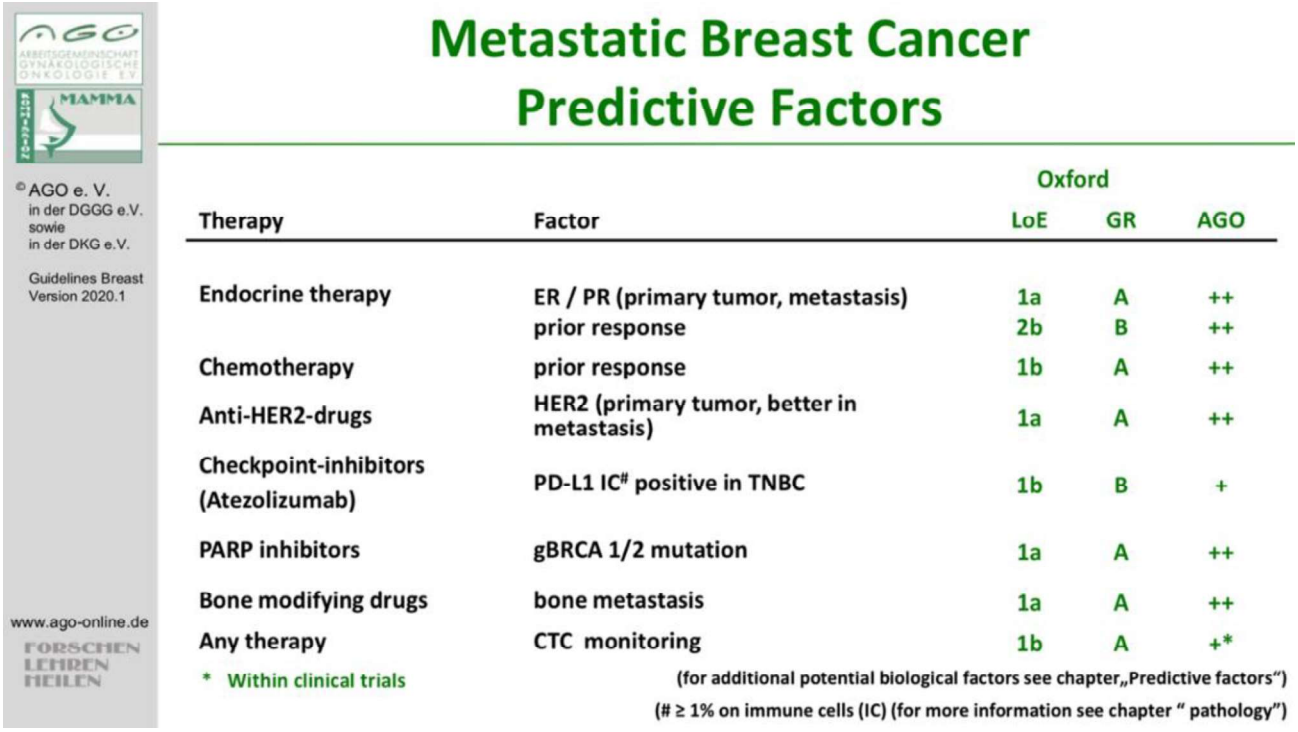
In women, metastatic breast cancer and gynecological malignancies are among the most frequent causes of cancer death. In 2018, there were an estimated 2 088 849 new cases of breast cancer and

626 679 deaths, 569 847 new cases of cervical cancer and 311 365 deaths, and 295 414 new cases of ovarian cancer and 184 799 deaths worldwide. [1] Despite rising overall incidence, mortality rate has steadily decreased owing to early detection and improvements in the therapeutic management of these patients. However, although the development of new drugs, vaccines, and systematic screening programs has improved patients' outcomes, effective measures to successfully treat metastatic cancer are still missing.

With the advent of molecular diagnostics, cancer treatment entered a new era. New techniques of sequencing DNA such as comprehensive genomic profiling (CGP) and hotspot next generation sequencing (NGS) provide tools for deciphering complete genes and later entire genomes at unprecedented speed. [2] These new approaches led to the development of a novel cancer treatment movement, known as precision medicine. By selecting the most effective treatment based on the molecular characteristics of tumor tissues or some other biologic parameters of the malignant disease, precision medicine aims to offer personalized treatment concepts to cancer patients with limited standard of care options. Molecular therapeutic agents (MTA) targeting individual actionable molecular alterations have been successfully developed in the past few years, showing the positive impact of using molecular-based therapy on the cancer patients' outcome. [3] – [6] These include the use of growth factor receptor 2 antibody trastuzumab in breast cancer, a tyrosine kinase inhibitor imatinib in myelogenous leukemia associated with the BCR-ABL fusion gene and EGFR tyrosine kinase inhibitors in lung carcinomas. [7], [8]

Breast and gynecological cancers constitute a heterogeneous group of malignant diseases associated with multiple genetic alterations. [9 - 11] In the past few years, a growing number of molecular markers in breast cancer for example have been investigated and some of them are now well-established as reliable predictors of prognosis and response to tumor therapy. (Fig.1a) Moreover, many different targeted therapies have been approved for use in breast cancer treatment. (Fig.1b) The recent approval of the PIK3CA specific inhibitor alpelisib has been the most recent example of targeted agents moving into routine care. [12] Treatment with alpelisib was shown to prolong PFS by more than 6 months compared to the control arm. [13]

a.



b.

Mutation diagnostics in mBC: „Precision medicine“ for targeted therapies

Altered genes	Therapeutic relevance	Gene region	Material	Oxford LOE	Oxford GR	AGO
BRCA1, BRCA2	PARP Inhibitors	All exons	Germline: Blood cells	1b	A	++
			Somatic: Tissue	2b	B	+/-
PIK3CA	Alpelisib	Exons 7,9 and 20	Primary tumor, metastases, plasma	1b	A	+
HER2-mutation (independent of HER2-status)	Neratinib, lapatinib	Kinase- and extracellular domains; S310, L755, V777, Y772_A775dup	Primary tumor, metastases, plasma	4	C	+/-
ESR1	Resistance against AI	Exons 4,7 und 8	Metastases, plasma	2b	B	+/-
NTRK gene fusion	Larotrectinib, entrectinib	Fusion- and splice variants	Tumor tissue, espec. secretory breast cancer	2a	B	+
MSI	Pembrolizumab	Microsatellite-instability	Tissue	2a	B	+

Fig.1 Predictive factors (a) and treatment-relevant genetic alterations (b) in metastatic breast cancer, German Gynecological Oncology Group. In 2018, AGO was the first international guideline-commission to make recommendations regarding precision medicine in breast cancer. (http://www.ago-online.de) [14]

In gynecologic malignancies, MTAs have also been successfully implemented into clinical care. For example, early data from a clinical phase II trial focusing on BRCA-mutated ovarian cancer showed that olaparib as maintenance treatment significantly improved progression-free survival (PFS) in relapsed platinum-sensitive ovarian cancer. [15] In 2018, these data could be transferred to the first line setting when treatment effects of the SOLO1 trial were presented. [16] Due to an impressive PFS improvement and a 70% lower risk of disease progression or death with olaparib compared to placebo, this effect led to the incorporation of PARP inhibitors into the primary treatment of ovarian cancer in 2019. [17] However when it comes to other gynecologic malignancies such as endometrial cancer, the development of MTA is delayed in comparison to other malignancies.
By detecting potential actionable pathways using molecular diagnostics, it is also possible to assess and treat various cancer types. For example, the ERBB2/PIK3/AKT/mTOR pathway is known for its relevance in breast cancer, but recently a relevant actionable mutation from the same pathway, PIK3R1W624R was also identified in ovarian cancer. [18] Another study suggested that some subtypes of cervical cancers may also benefit from existing ERBB2/PIK3/AKT/mTOR targeted agents. [19]
With the rising number of MTAs and considering the heterogeneous molecular profiles of breast cancer and gynecological malignancies, it is reasonable to expect that patients with these malignancies could potentially benefit from implementation of precision oncology based on comprehensive genomic profiling (CGP) into clinical care. Promising early data for such malignancies has been presented in multiple trials. In breast cancer, many reports of such driver alterations have emerged in the past few years, suggesting that patients could profit from precision medicine and targeted therapies. [20] For example, in the SAFIR01 multicenter prospective trial,

data of precision medicine benefitting breast cancer patients was presented. 9 out of 43 patients (21%) responded to the recommended targeted therapy with a stable disease lasting over 16 weeks. [21] In ovarian cancer, multiplatform molecular profiling, conducted in a commercially available profiling center, led to a significantly longer post-profiling survival in patients, who were treated with profile-guided targeted agents, in comparison to the control group. [22]
With the technical advances in molecular diagnostics and the continuous approval of many targeted therapies, the growing field of precision medicine is constantly expanding and requires optimization. Considering the complexity of precision medicine in oncology, it was reasonable to create a molecular tumor board (MTB) to leverage the knowledge of the many different disciplines involved in oncological treatment and to provide optimal treatment recommendations. In this manuscript, first experiences of the Comprehensive Cancer Center (CCC) LMU Munich Molecular Tumor Board are presented.
The aim of this project was to retrospectively measure the impact of MTB discussions and recommendations made by a multidisciplinary tumor board on outcome of patients with breast and gynecological cancers progressing under standard treatment. Detailed information including data on patient characteristics, diagnostic and treatment recommendations, implementation of the recommendations, and outcome of treated patients with breast and gynecological cancers (ovarian, endometrial, cervix and other type of cancer) are presented.

Materials and Methods

All patients reported here were discussed in the local MTB, which reviewed clinical cases and the respective tumor profiles with the associated actionable alterations. The final result of each MTB case discussion was a report, focused on NGS data and diagnostic and potential diagnostic and therapeutic alternatives. Thereby, the MTB presented itself as a multidisciplinary team (MDT), which was comprised of clinical oncologists, pathologists, molecular pathologists, genetic counselors, bioinformaticians and scientists with expertise in genetic and tumor profiling in diverse cancers. MTB-meetings were held every 2 weeks with the purpose of interpretation and/or translation of the molecular diagnostics' results into diagnostic and/or treatment recommendations. All patients' cases were firstly presented at organ-specific gynecology tumor boards by a team of experienced gyneco-oncologists, who reviewed all the clinical course of every individual patient and discussed if patients were eligible for a MTB discussion. Apart from recent tumor material, recent radiology images and other diagnostic tests were also required for the interdisciplinary setting of the MTB. All treatment recommendations were supported by levels of evidence by using the ESMO Scale for Clinical Actionability of molecular Targets (ESCAT). The process from enrolling the patient into the study till receiving a recommendation by the MTB is shown in Fig.2.

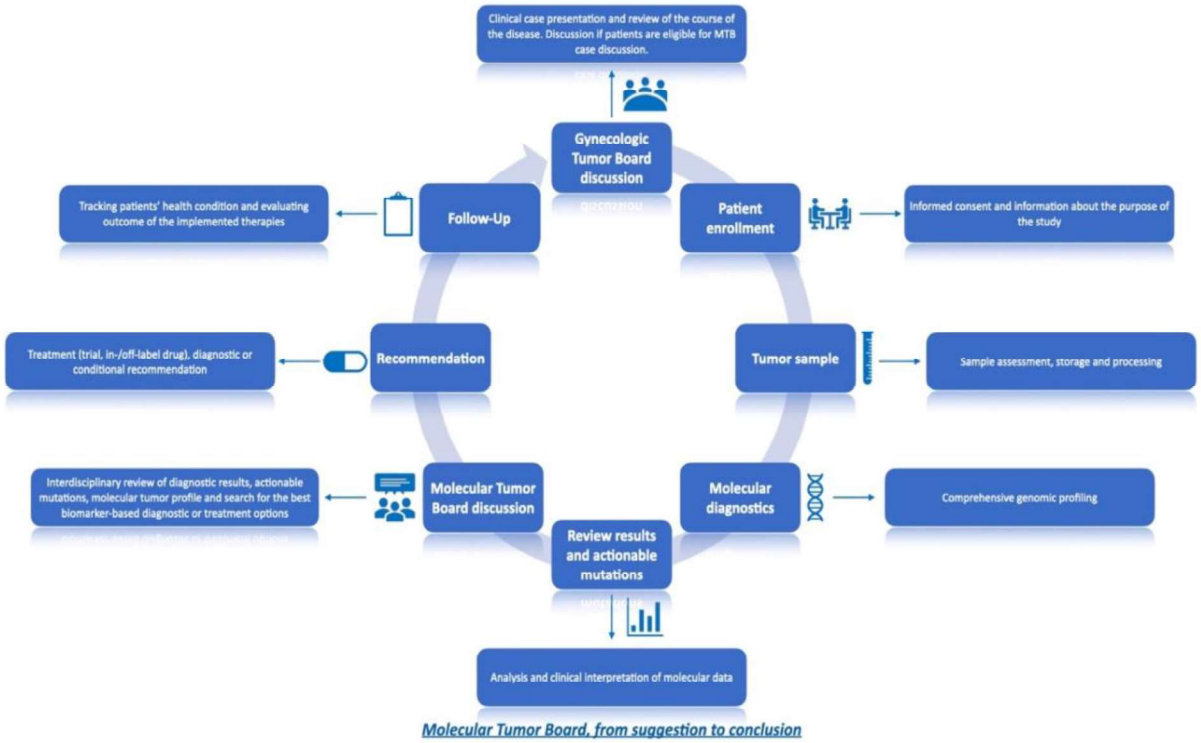


Fig.2 MTB, from suggestion to conclusion

Patients and Patient Informed Consent

All patients discussed (n = 95) were included in the prospective single-center case study, "The informative Patient", launched in March 2017 at the LMU University Hospital, Munich as a Munich-site part of the DKTK (German Cancer Consortium) program. All enrolled patients suffered from metastatic breast or gynecological cancer which had progressed after at least one line of prior standard treatment and who had no longer access to curative treatment. Prior to inclusion, all participants signed an informed consent that they were informed about potential and limitations that molecular diagnostics could offer for treatment selection and for analysis of their data, further discussion of their case by a multidisciplinary MTB, as well as for collecting follow-up data on the course of disease for research purpose (including requesting patient data from other physicians and institutions). The intention-to-treat (ITT) population consisted of 100 patients. Eventually, 5 patients were excluded, because of death prior to a treatment recommendation or withdrawal of consent. The data here is based on the results of an ITT population of 95 patients.

Molecular Pathology

Molecular analyses were performed at the Institute of Pathology of the LMU. Appropriate tissue regions were selected histo-morphologically from formalin-fixed paraffin embedded (FFPE)- or fresh frozen tissue. Moreover, liquid biopsies (blood, liquor) were included. In only four patients, analysis had to be repeated due to material constraints. Targeted NGS was performed with the Oncomine Comprehensive Cancer v.3 Panels (Agilent) thereby screening for changes in 161 genes on DNA (SNV, MNV, small ins, del, indels, CNV) and RNA (gene fusions) level. DNA and RNA were isolated using Qiagen's GeneRead DNA FFPE- or RNeasy FFPE-kits respectively. Nucleic acids (NA; DNA and RNA) from liquid biopsies were prepared by utilization of the QIAamp Circulating Nucleic Acid Kit. Subsequently, libraries were generated employing Ampliseq Library

Plus-, Ampliseq cDNA synthesis-, Ampliseq CD index, Ampliseq Equalizer- together with Ampliseq Comprehensive v3-kits (all Illumina) or DNA- and RNA-Oncomine Comprehensive Panels v3 and Ion AmpliSeq Library-, IonXpress Barcode Adapter-, Ion Library Equalizer-kits together with Ion Chip kits (mostly 550) (all Thermo Fisher) following for each step of the respective user manuals. Libraries were run on an Ion Torrent GeneStudio S5 Primer (Thermo Fisher) or Illumina 500 Next Seq (Illumina) NGS machine.
Analysis of results was performed with either the Ion-Reporter System (Thermo Fisher) followed by further variant and quality interpretation with a self-made excel tool or annotating VCF-files using wAnnovar (http://wannovar.wglab.org/) [23] together with the self-made python-script PathoMine filtering for clinically relevant mutations. Mutations were judged as relevant on the basis of the key 'interpretation' given in ClinVar. [24] Alterations were confirmed with the Integrated Genomics Viewer (IGV, Broad Institute). The resulting molecular pathological dataset together with data from immunohistochemistry, fluorescence in situ hybridization (FISH) and histo-morphology became part of a comprehensive pathological report which was sent out to the MTB.

Data assessment

For this analysis, electronic medical records were reviewed for patient characteristics and follow-up. If needed, medical oncologists, gynecologists, and general practitioners were contacted in order to collect follow-up data on treatment course and patient status. Patient characteristics were summarized using descriptive statistics. Follow-up of clinical outcomes was performed to track tumor response to recommended therapies and analyzed by measuring progression-free survival (PFS) of patients, who received the recommended treatment. PFS was calculated from the first day of treatment with the recommended in- or off-label targeted drug until the date of disease progression or death, whichever occurred first, analogous to the Johns Hopkins MTB study and to the Von Hoff et al. study. [25] In order to evaluate the benefit of the treatment recommendation, we then calculated the PFS ratio (PFSr) by comparing the PFS of the recommended treatment and the PFS of the previous therapy of the patients. Cut-off date for data analysis was August 1st, 2019.

Results

Patient characteristics

From March 2017 through March 2019, a total of 95 cases were submitted to the MTB. All patients (n=95) were female, had an underlying malignant condition, suffered from metastatic disease and had experienced disease progression under standard treatment. Patients with implemented therapy recommendations had received a median of 5 (range 2-6) prior therapies for metastatic cancer. The median age at time of the initial MTB presentation was 52 years (range, 19 to 82 years).
As shown in Fig.3, the most frequent tumor type was breast cancer (n=64, 68%), followed by ovarian cancer (n= 19, 20%). The majority of patients with breast cancer had triple-negative (ER, PR and HER2 negative; n= 30 ; 46.9%), followed by estrogen receptor (ER) -positive and/or progesterone receptor (PR) -positive, human epidermal growth factor receptor 2 (HER2) -negative (luminal-like) (n= 28 ; 43.8%), or HER2 positive, ER-negative, PR-negative disease (n= 5 ; 7.8%) at the time of the MTB case discussion; one patient (1.6%) had triple-positive disease (ER positive and/or PR positive, HER2 positive).
Characteristics of patients with a molecular profile are reported in Table1.

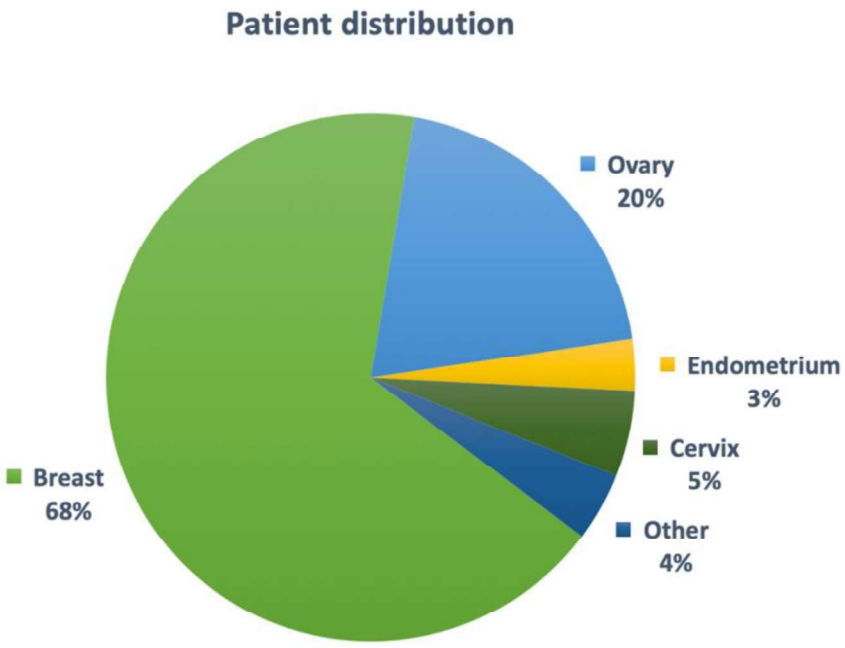


Fig.3 Distribution of the cases discussed at the MTB meeting by tumor entity (n=95)

Table1 Patient characteristics

Covariables	
Median age at diagnosis	47y (range: 12-80)
Age at diagnosis	
<30	5 (5.3%)
30-39	27 (28.4%)
40-49	21 (22.1%)
50-59	30 (31.6%)
60-69	8 (8.4%)
≥70	4 (4.2%)
Median age at MTB case presentation	52y (range: 19-82)
Age at MTB case presentation	
<30	2 (2.1%)
30-39	19 (20.0%)
40-49	20 (21.1%)
50-59	28 (29.5%)
60-69	18 (18.9%)
≥70	8 (8.4%)

Molecular Profiling

Molecular tests using NGS were performed for all 95 patients. Out of the set of mutations from the molecular pathological NGS-analysis, actionable mutations were defined as those matching or informing the use of available targeted agents.

Four patients had tumor sequencing performed twice during the course of disease. 81 (85.3%) patients had suitable tissues for multimodal molecular profiling (NGS). All in all, 103 molecular alterations were identified in 55 cases (57.9%). The median number of alterations observed in each sample was one (range 0-6). Out of the 55 patients, 41 (43.2%) had an actionable mutation, which the board reviewed as a potentially targetable. No genomic alterations in the 161 investigated genes were found in 40 (42.1%) analyses, in 14 (14.7%) of which the molecular diagnostics test was technically not successful because of poor DNA quality or insufficient material quality. Although 5 (5.3%) patients had an actionable mutation, they did not receive a therapy recommendation because of co-morbidities, not meeting trial inclusion criteria, or other requirements for receiving a specific targeted therapy.

We discovered mutations in over 30 different genes. Among the patients tested, the most common alterations were as follows: PIK3CA mutation (13/95; 13.7%); ERBB2 mutation (10/95; 10.5%); KRAS mutation (9/95; 9.5%) and CCND1 mutation (9/95; 9.5%). Incidences of genomic alterations by gene and the distribution of molecular alterations by tumor type are shown in Fig.4.

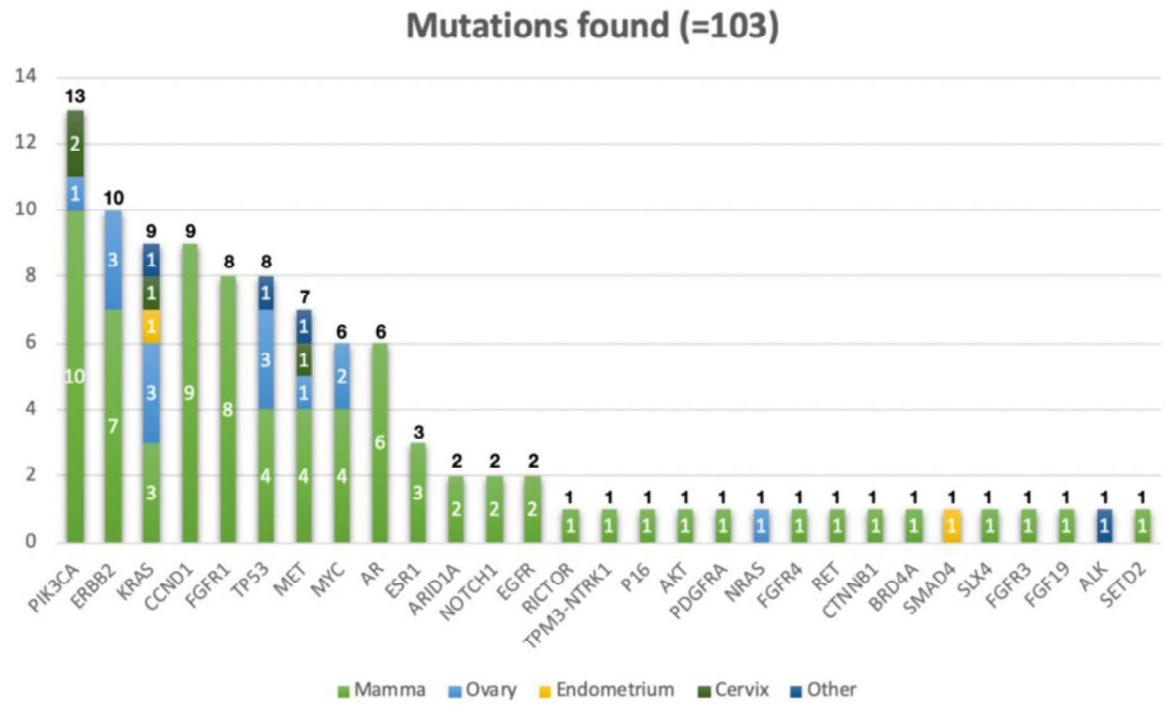


Fig.4 Frequency of genomic alterations for the different tumor entities (n=95)

Recommendations

Among the 55 (57.9%) patients with at least one molecular alteration identified, 41 patients (43.2%) had an actionable alteration, whereas 14 (14.7%) had only non-actionable variants. Eventually, this resulted in 15 diagnostic and 49 treatment recommendations for 45 patients (47.4%). Multiple recommendations were adjusted for 20 (21.1%) patients (multiple recommendation principle). 6 patients received a conditional recommendation, which required specific further diagnostics, 2 of which resulted in a treatment recommendation.

Diagnostic recommendations

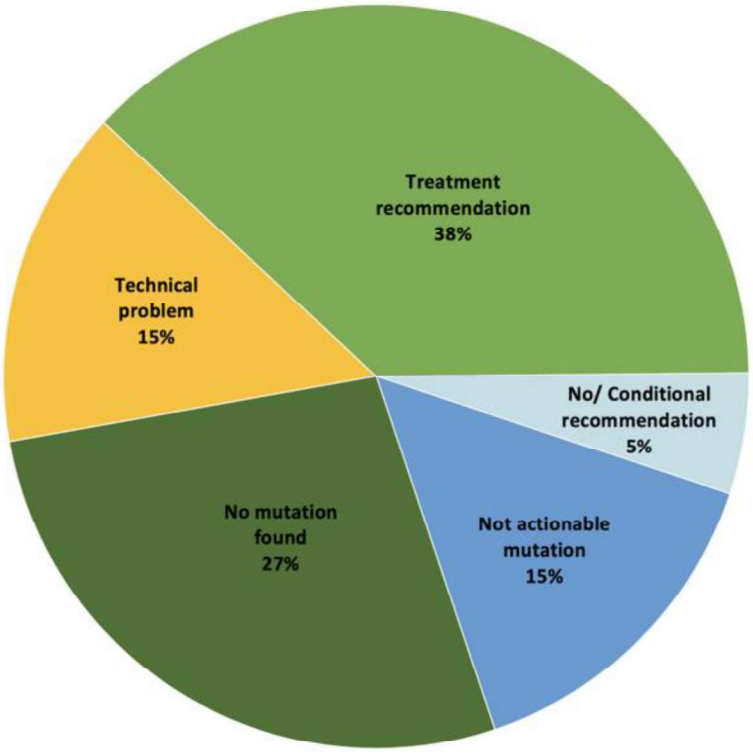
Out of 15 diagnostic recommendations, 10 were pursued. In 7 (7.4%) cases, extended genetic analyses were recommended and eventually 6 (6.3%) of them were performed. Re-biopsies were recommended in 14 cases, when the initial diagnostic tests were technically not successful, which we did not include in the evaluation of the final results.

Therapeutic recommendations

As shown in Fig.5, 36 (37.9%) patients were given a therapy recommendation, 14 (14.7%) of whom received more than one treatment suggestion, as their tumor molecular profile revealed more than one actionable mutation. Two (2.1%) patients were excluded from the evaluation of the clinical outcome, as they received the recommended therapy in the period between NGS analysis and MTB treatment recommendation.

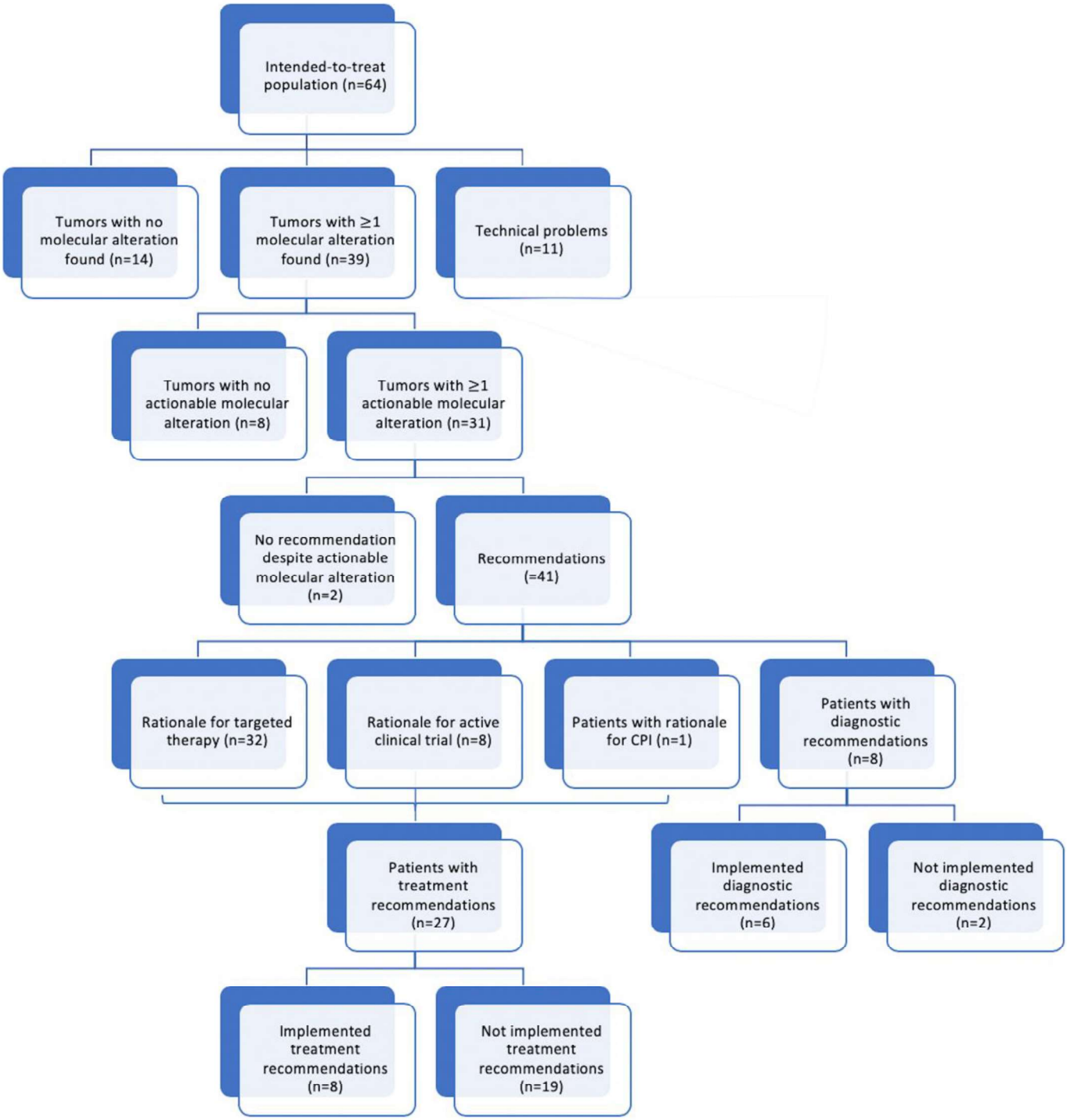
Overall, 9 of 34 therapeutic recommendations were pursued. Of note, in the present cohort, no patient pursued the recommended enrollment in a clinical trial. In-label therapy recommendations were implemented in 5 cases, whereas off-label recommendations were implemented in 4 patients. The most common reasons for non-administration of MTB-recommended therapy were deterioration of patients' physical health condition, early death, no access to the recommended drug therapy, declined reimbursement applications by payer, or patient decision.

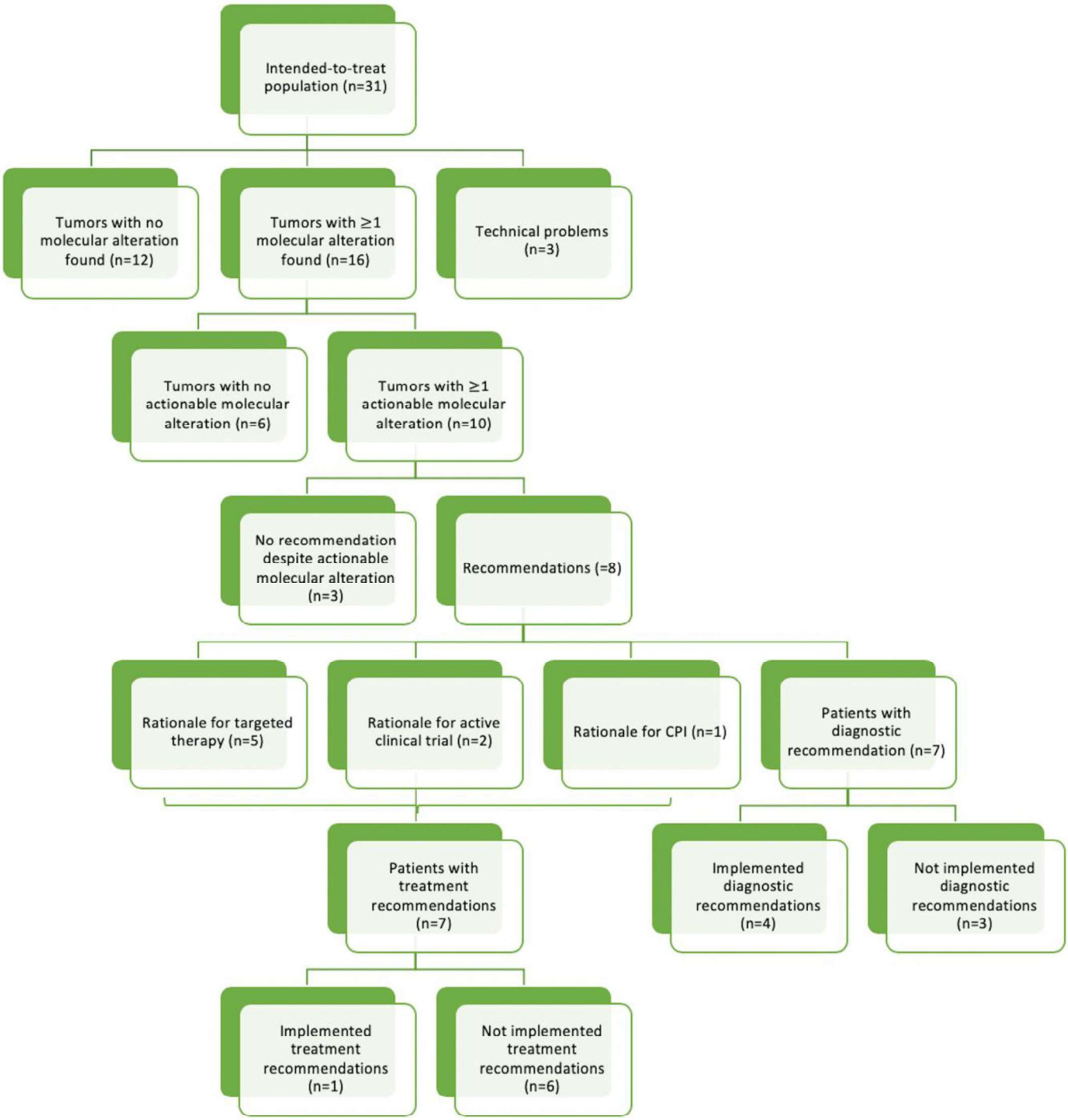
Table2 present a summarize of the MTB recommendations based on the molecular results.



a.

b.


Intended-to-treat population (n=64)
Tumors with no molecular alteration found (n=14)
Tumors with ≥1 molecular alteration found (n=39)
Technical problems (n=11)
Tumors with no actionable molecular alteration (n=8)
Tumors with ≥1 actionable molecular alteration (n=31)
No recommendation despite actionable molecular alteration (n=2)
Recommendations (=41)
Rationale for targeted therapy (n=32)
Rationale for active clinical trial (n=8)
Patients with rationale for CPI (n=1)
Patients with diagnostic recommendations (n=8)
Patients with treatment recommendations (n=27)
Implemented diagnostic recommendations (n=6)
Not implemented diagnostic recommendations (n=2)
Implemented treatment recommendations (n=8)
Not implemented treatment recommendations (n=19)



c.

Fig.5 Treatment or diagnostic recommendations. Note, all numbers do not add up because some patients are counted in more than one category (eg, had an actionable alteration for a treatment recommendation and also for diagnostic recommendation or received more than one treatment/ diagnostic recommendation).

a. Diagram representing the outcome of the molecular diagnostic testing (n=95)

b. Breast cancer patients

c. Gynecological cancer patients

Table2 Recommendations (Note, some patients received more than one diagnostic and/or treatment recommendation.)

	BC	GC
Patients with min. 1 recommendation	No.	No.
Diagnostic	8	7
Therapeutic	27	7
No treatment recommendation	30	20
Conditional recommendation	3	3
Referral to organ board		1
Diagnostic recommendations		
Extended genetic analysis	3	4
PD-L1 Test	2	
HR-Status	1	1
Other	5	3
Patients with diagnostic recommendations (n=15)		
Implemented	6	4
Non-implemented	2	3
Treatment recommendations		
Targeted therapy	32	5
Trial inclusion	8	2
Checkpoint inhibition	1	1
Patients with treatment recommendations (n=36)		
Implemented	7	1
Non-implemented	22	6

Clinical Outcome

All patients were included in the registry after multiple standard-of-care treatments. Out of 9 (9.5%) patients following therapy recommendation, 4 (4.2%) showed a state of partial remission or stabilization lasting more than 16 weeks, including 2 of them receiving off-label therapy recommendation. Comparing PFS of the recommended therapy with the PFS of the previously received systemic treatment, we estimated that 4 of 9 responders receiving MTB-recommended therapies displayed a progression-free survival (PFS) ratio (PFS2/PFS1; PFSr) > 1.3, showing the relevance of the suggested therapies. 2 patients responded with an ongoing PFSr. Fig.6 details the

actual comparison of PFS on implemented recommended treatment versus PFS on the patient's last prior treatment.
More information about the outcome of responding patients is shown in Table3.

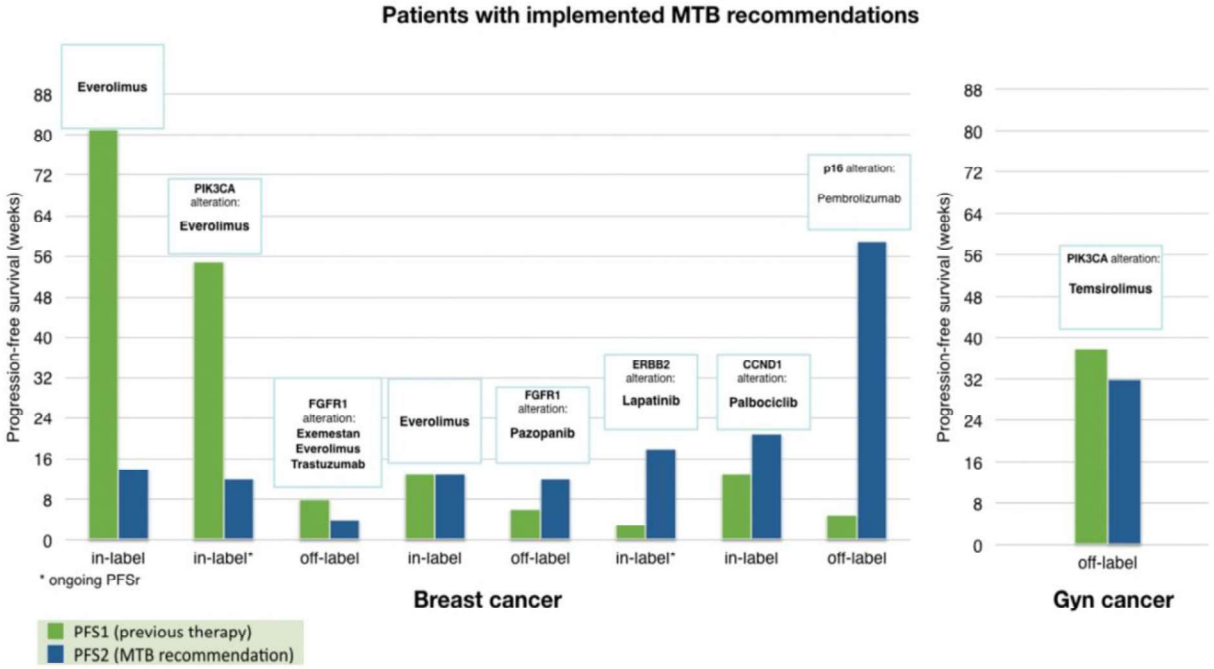


Fig.6 Comparison of PFS of previous line of therapy (PFS1) and implemented therapy recommendation (PFS2). PFS = the period of time between the start of treatment till disease progression/ death

Table3 PFS ratio (PFSr) = ratio of patients' PFS on the implemented recommended therapy (PFS2) (in this case the recommended in- or off-label targeted drug) to their PFS on the most recent previous line of therapy (standard of care) (PFS1). PFSr = PFS2/PFS1

#	Tumor entity	Treatment	Label	PFS2 (weeks)	PFS1 (weeks)	PFSr
1	breast	Everolimus	in	**14**	81	0.17
2	breast	Everolimus	in	**12**	55	0.22
3	breast	Exemestan + Everolimus + Trastuzumab	off	**4**	8	0.50
4	breast	Everolimus	in	**13**	13	1.00
5	breast	Pazopanib	off	**12**	6	2.00
6	breast	Lapatinib	in	**18**	3	6.00
7	breast	Palbociclib	in	**21**	13	1.62
8	breast	Pembrolizumab	off	**59**	5	11.80
9	cervix	Temsirolimus	off	**32**	38	0.84

See Appendix for details of identified actionable mutations and corresponded treatment recommendations made by the MTB.

Discussion

We evaluated the clinical consequences of actionable genetic alterations (by NGS) in 95 patients with metastatic breast cancer and gynecological malignancies, part of a pilot monocentric patient registry with the purpose of generating real-world data. 41 patients (43.2%) had at least one actionable molecular aberration. The total number of patients with a drug-targetable alteration was 34 (35.7%). Overall, 9 of 34 patients (9.5% of all) received the recommended drug treatment. A small, but significant group of patients, 4 out 9 with implemented therapy recommendations (44.4%) experienced a clinical benefit (PFSr >1.3) lasting over 16 months, a result similar to the one shown by Jameson et al in cases of patients with metastatic breast cancer, who received personalized therapy recommendations based on multi-omic molecular profiling. [26], [27]

Precision medicine offers not only personalized treatment concepts for patients, but also helps us optimize diagnostic and treatment options by identifying biomarkers that are linked to response and resistance to immunotherapy. For instance, in the past few years, the problem of resistance to endocrine therapy has been a point of research. Recently, the key role of the acquisition of ligand-independent ESR1 mutation in breast cancer as a common mechanism of resistance to hormonal therapy was discovered. [28]

So far, the precision medicine movement is controversial and has sparked multiple debates. On the one hand, the SHIVA trial (2015), one of the first randomized investigation of precision therapy, was negative for its primary endpoint (progression-free survival [PFS]), as no statistically significant difference in PFS between patients receiving molecularly targeted agents and the control arm was demonstrated. [29] On the other hand, studies recruiting large number of patients, such as MOSCATO 01 (2017) and ProfiLER (2017), suggested that high-throughput genomic analyses (i.e. next-generation sequencing, comprehensive genomic profiling) improve clinical outcome in patients with advanced cancers. However, this approach has only been proven to be beneficial to a small subset of patients so far. [30], [31] As shown in Table4, studies focusing on precision medicine show different, contradictory results. While in some studies more than 20% of the enrolled patients received the recommended according to molecular profiling treatment, in others the number of patients treated remains very low. These results suggest the need for large data collections in order to improve selection criteria and identify markers that discriminate patients that might benefit most from precision medicine.

Although molecular targeted agents themselves are more precise than standard cytotoxic agents, clinical evidence for a significant better outcome associated with MTAs is still missing, as the access to targeted therapies remains limited, making collecting data regarding their efficacy difficult. In order to achieve their implementation in clinical care, a re-assessment of the standards of evidence sufficient to prove the benefit of precision cancer therapies is needed. [32] New evidence suggests that appropriately conducted real world data studies have the potential to support regulatory decisions in the absence of RCT data. [33]

Based on initial results of the CCC LMU Munich, patients of various tumor entities benefit from extended molecular diagnostics and their implementation in clinical care. [34] Recently, many studies have described the positive effect of MTB case discussions for particular groups of patients with advanced solid cancers. However, there is not enough evidence for the utility of MTB decisions for patients with breast and gynecological malignancies.

The world of precision medicine is constantly evolving, and new targeted therapies are being developed and approved, enabling more and more patients (with up to this point of time not actionable mutation) to receive targeted therapies. For example, in spring 2019, the Food and Drug Administration of the U.S.A. (FDA) approved the PIK3CA inhibitor alpelisib in combination with endocrine therapy for patients with HR-positive, HER2-negative, PIK3CAmutated, advanced or metastatic breast cancer. The availability of this drug after start of the Managed Access Program in our clinic could have resulted in 5 further therapy recommendations in our MTB cohort, showing the need of identifying such alterations in cancer patients. The rising number of active targetable mutations affects the complexity of the results, making their interpretation a challenge for many oncologists. In 2014, Gray et al conducted a study, which evaluated cancer physicians' ability of using multiplex tumor genomic testing, and showed that many physicians lack confidence in interpreting complex genomic test results as well as in incorporating them into practice. [35] Thus, we see great potential in establishing the combination of molecular diagnostic tests and a subsequent case discussion by a multidisciplinary molecular board team not only as a routine for cancer patients but also as a training platform and a knowledge-expanding approach for oncologists to help guide their decisions.

However, precision oncology faces some challenges, which delay its widespread translation into clinical practice. Critics of the incorporation of NGS and similar methods into clinical practice express following concerns. First, the significant cost of molecular diagnostics and targeted drugs is still a great disadvantage. While prices of next-generation sequencing technologies are dropping from about $3 billion in the year 2000 and to $5,000 today, the selection of molecular targeted agents is still enormously expensive. [36] As the price of precision medicine is still rather high for most patients, it is now crucial to also evaluate its cost-effectiveness in order to support its translation into clinical practice, for example in the setting of clinical trials and research programs. [37]

Second, logistical problems causing limited access to targeted drugs and clinical trials for biomarker-positive patients represent another major problem. This is mainly due to the absence of reimbursement for drugs beyond their labelled indication. As a consequence, in order to receive the required, often off-label drug, patients need to be enrolled within active clinical trials or are required to cover the costs themselves or to file an application for reimbursement by the competent health insurance prior to treatment initiation. Clinical trials often have strict inclusion criteria and are therefore not easily accessible to many patients. As shown in the SAFIR01 trial, only a small number of patients benefit from personalized therapies mostly due to drug access problems. This problem could be solved by establishing a portfolio of early phase clinical basket trials or by early-access-programs. [38] Recent studies suggest that the implementation of a MTB improves access to targeted therapy. [39] As seen in our clinic, the early-access-program that we started in November 2019 enabled many patients with a PIK3CA mutation to derive benefit from the targeted drug alpelisib soon after its FDA approval in spring 2019. [40]

Third, another major limitation is the testing of tumors from patients with late-stage disease, which limits treatment options and hinders patients from receiving the recommended therapy or from enrolling in a clinical trial. As patients in an advanced cancer situation are often in an unstable health condition, obtaining biopsy material with a good quality of tissue is quite difficult. Our study had 14 (14.7 %) technically unsuccessful molecular diagnostics. Moreover, the time between enrolling patients in the study, processing tumor samples, followed by the molecular diagnostics and the MTB case discussion is still rather lengthy in view of the fact that malignancies in late stages tend to evolve at unprecedented speed, while causing deterioration of the general condition and hindering patients from receiving particular therapies, one of the main reasons for the relative low number of implemented therapies (9 out of 34). In this study, molecular profiling and discussion

were completed in a clinically reasonable time frame of approximately 4 weeks, which is comparable to the median turnaround times in other studies. Therefore, it is reasonable to expect that introducing molecular profiling at an earlier time point in a patient's disease trajectory could improve the quality of molecular diagnostics and allow patients to benefit more from a multidisciplinary tailored MTB-based treatment advice.
Forth, another concern is that the current trend of identifying single variables and matching it with an appropriate targeted therapy may be irrelevant for some patients because of the heterogeneous landscape of their cancer. Disease variability among individual tumors causes patients with tumors of similar histology to respond differently to targeted therapies. [41 - 43] For example, only 60% of lung cancer patients with the p.L858R mutation in the epidermal growth factor receptor gene (EGFR) respond to gefitinib, although all of them are carriers of the exact same mutation in the target gene, indicating that other, yet unknown genetic aberrations may influence the effect of targeted drugs and that the disease course is still unpredictable to a great extent. [44]
Fifth, the common use of medicines outside the approved label is controversial. Off-label drug use may represent a danger for patient safety in some cases, but it is sometimes justified from a clinical perspective. 4 out of 9 (44%) of the implemented recommended therapies in the study "The informative Patient" included off-label drugs; 2 of these patients (50%) experienced a clinical benefit with a partial response or stabilization lasting over 4 months, while having progressed under last standard treatment.

There were several limitations to our study. First, despite a relatively high number of breast and gynecological cancer, the overall number of included patients remains low. Second, our patient cohort presented had a heterogeneous tumor type, making general conclusions relatively difficult. Third, the number of patients with implemented therapies is limited, due to deterioration of patients' general condition or no access to the recommended targeted drug, as previously reported in other studies. Nevertheless, we do demonstrate feasibility of and patient benefit from a routine MTB at a large comprehensive cancer center.

Table4 Overview of studies focusing on molecular profiling

MP = molecular profiled, PFS = progression-free survival, ORR = overall response rate, SD = stable disease, PR = disease progression, n.a. = not available

Author/Study	Tumor entity	Enrolled patients (n=)	MP patients	Actionable alterations	Implemented therapies - n (% of enrolled)	Results
Le Tourneau et al. (SHIVA) [29]	solid tumors	741	496 (67%)	293 (40%)	96 (13%)	No significant difference in PFS (PFS: 2.3 vs 2.0 p = .41), hazard ratio for death or disease progression, 0.88 (95% CI, 0.65-1.19)
Stockley et al. (IMPACT/COMPACT) [45]	solid tumors	1893	1640 (87%)	187 (10%)	84 (5%)	ORR: 19% in genotype-matched group vs 9% in unmatched group, p = 0.61
Massard et al. (MOSCATO-01) [30]	solid tumors	1035	843 (81%)	411 (40%)	199 (24%)	ORR: 11%, SD 52%, PFSr > 1.3: 63/193 (33% of all treated patients or 7% of all enrolled patients)
Trédan et al. (PROFILER) [31]	solid tumors	2579	1980 (77%)	1032 (40%)	163 (6%)	ORR: 0.9% of all patients
Rodon et al. (WINTHER) [46]	solid tumors	303	303 (100%)	25 (89%)	107 (35%)	PFSr > 1.5: 22% of the patients with MP-based treatment
Hoefflin et al. [47]	solid tumors	198	n.a.	104 (53%)	33 (17%)	PR: 11/33 (33.3% of all treated patients or 5.5% of all enrolled patients) SD: 8/33 (24.2% of all treated patients or 4% of all enrolled patients)
André et al. (SAFIR01/UNICANCER) [21]	breast cancer	423	299 (71%)	195 (46%)	55 (13%)	ORR:4 patients had a partial response and 9 had SD >16 weeks (3% of all patients)
Parker et al. [48]	breast cancer	43	43 (100%)	40 (93%)	17 (40%)	7 patients (41% of all treated patients or 16% of all enrolled patients) achieved SD or PR

Conclusion

The landscape of molecular alterations in breast and gynecological cancers is heterogeneous. Advances in the quality and availability of molecular diagnostics and the number of targeted therapies increase rapidly, offering patients with advanced cancer a variety of new treatment options. MTBs try to bridge the gap in between molecular alterations and matching drugs in a structured manner.

The primary objective of the present monocentric study was to estimate, in a real-world setting, the impact of interdisciplinary MTB case discussions for patients with breast and gynecological malignancies. Altogether, on the basis of individual molecular diagnostics, diagnostic and treatment recommendations were made for 45 patients (47.4% of all). 9 out of 34 patients received the recommended treatment. 4 out of 9 patients responded with a PFSr > 1.3. Therefore, our results support the approach of matching specific drugs (in- and off-label) to particular genetic aberrations and demonstrate its relevance in breast and gynecological cancers for a small, but clinically relevant group of patients. By providing a multidisciplinary tailored-based treatment advice based on genetic tests, it is now possible for more patients with breast and gynecological malignancies to gain maximum clinical benefit and improve survival of patients with either advanced stage cancer or a rare tumor entity by applying personalized medicine.

The MTB strategy, however, needs to be standardized and optimized in order to eliminate major logistical problems such as limited access to targeted agents (often off-label) and clinical trials, as well as patient referral at stage disease that are too late for a beneficial therapeutic intervention.

Appendix

Table5 Data supplement

#	Mutation	Tumor entity	Treatment recommended in MTB	Followed treatment / Line of therapy	PFS (months) after start of treatment
1	FGFR1, androgen receptor and CCND1 amplifications	breast	1. CDK4/6 Inhibitor 2.Everolimus 3. androgen receptor blocker		
2	CCND1 amplification	breast	1. CDK4/6 Inhibitor 2. Palbociclib + Fulvestrant 3. Everolimus	Palbociclib	21
3	ERBB2 mutation	breast	Afatinib / Neratinib		
4	PTEN deletion; MET mutation	breast	1. NCT03337724 trial 2. Exemestan + Everolimus		
5	PIK3CA mutation	breast	Everolimus		
6	MET Exon 14 mutation	breast	Crizotinib		
7	MYC, FGFR1 and CCND1 amplifications	breast	Everolimus	Everolimus	13

8	androgen receptor amplification	breast	1. NCT01945775 / NCT02163694 trial 2. Bicalutamide / Tamoxifen		
9	PIK3CA mutation	breast	1. SOLAR-1 / IPATunity130 trial 2. Everolimus		
10	ERBB2 amplification	breast	Lapatinib, Trastuzumab, Emtansine and Pertuzumab		
11	ARID1A and PIK3CA mutations, LMB (4,16 muts/MB)	breast	Everolimus	Everolimus	12
12	ESR1 mutation, CCND1 amplification	breast	Fulvestrant + Everolimus		
13	TP53 and NOTCH1 mutations	breast	Cyclophosphamid		
14	TPM3(7) - NTRK1(10) gene fusion	breast	NCT02568267 trial		
15	MET Exon 2 mutation	breast	Cabozantinib		
16	KRAS and 2 PIK3CA mutations	breast	lipos. Doxorubicin / Bevacizumab + Temsirolimus/ Everolimus		
17	androgen receptor mutation, PIK3CA mutation	breast	Everolimus		
18	FGFR1, CCND1, EGFR, PIK3CA and PDGFRA amplifications	breast	Pazopanib		
19	ESR1 and PIK3CA mutations	breast	1. NCT03056755 trial 2. Everolimus		
20	p16 high expression and MYC mutation	breast	Checkpoint inhibitor	Pembrolizumab	59
21	androgen receptor amplification	breast	Androgen receptor blocker		
22	AKT mutation	breast	1.AKT inhibitors 2. IPATunity130 trial 3. Everolimus		
23	SLX4 and TP53 mutations;	breast	Pazopanib	Pazopanib	12

	amplifications: FGFR1, CCND1, FGF19, FGFR3				
24	ESR1 mutation	breast	Fulvestrant + CDK4/6 Inhibitoren		
25	CCND1 and FGFR1 amplifications	breast	1. Everolimus + antihormonal therapy; 2. Dovitinib		
26	PIK3CA and ERBB2 mutations, high expression ERBB2	breast	1.Pertuzumab/ Trastuzumab (+ Everolimus) 2. Neratinib	Lapatinib	18
27	FGFR1 amplification	breast	antihormonal therapy + Everolimus + Trastuzumab	Exemestan + Everolimus + Trastuzumab	4
28	CCND1 amplification	breast	1. Exemestan + Everolimus; 2. NCT-MASTER / TOP-ART trial		
29	CCND1 and FGFR1 amplifications	breast	1. Everolimus + Exemestan 2. NCT03517956 trial	Everolimus + Exemestan	14
30	KRAS and ERBB2 mutations	ovary	NCT02703571 trial		
31	ERBB2, MYC, PIK3CA amplifications	ovary	Everolimus + Letrozol		
32	PIK3CA alteration	cervix	Temsirolimus	Temsirolimus	32
33	PIK3CA and KRAS mutations, MET gene fusion	cervix	1.Crizotinib 2. Everolimus		
34	KRAS, SMAD4 and PTEN mutations	endometrium	Everolimus		
35	HTB (27 muts/MB)	other	1.Checkpointinhibitor 2. NCT Master trial		
36	EML4-ALK gene fusion	other	ALK inhibitor		

Declarations

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Paper II

Research Article

Implementation of precision medicine for patients with metastatic breast cancer in an interdisciplinary MTB setting

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Abstract:

The advent of molecular diagnostics and the rising number of targeted therapies have facilitated development of precision oncology for cancer patients. In order to demonstrate its impact for patients with metastatic breast cancer (mBC), we initiated a Molecular Tumor Board (MTB) to provide treatment recommendations for mBC patients who had disease progression under standard treatment. NGS (next generation sequencing) was carried out using the Oncomine multi-gene panel testing system (Ion Torrent). The MTB reviewed molecular diagnostics' results, relevant tumor characteristics, patient's course of disease and made personalized treatment and/or diagnostic recommendations for each patient. From May 2017 to December 2019, 100 mBC patients were discussed by the local MTB. A total 72% of the mBC tumors had at least one molecular alteration (median 2 per case, range: 1 to 6). The most frequent genetic changes were found in the following genes: PIK3CA (19%) and TP53 (17%). The MTB rated 53% of these alterations as actionable and treatment recommendations were made accordingly for 49 (49%) patients. Sixteen patients (16%) underwent the suggested therapy. Nine out of sixteen patients (56%; 9% of all) experienced a clinical benefit with a progression-free survival ratio ≥ 1.3. Personalized targeted therapy recommendations resulting from MTB case discussions could provide substantial benefits for patients with mBC and should be implemented for all suitable patients.

Keywords: precision medicine; personalized medicine; metastatic breast cancer; molecular tumor board; molecular diagnostics;

Introduction

Breast cancer is both the most commonly occurring malignant disease and the leading cause of cancer death among women worldwide, with an estimated 2 088 849 new cases and 626 679 deaths in 2018. (36) Diagnostic and treatment options have progressed substantially over the past few years,

which led to slightly increasing incidence rates and a decline in breast cancer mortality [2,3]. However, despite recent advances in oncology over the past few years, not all patients equally benefit from these improvements. Survival rates of patients with metastatic breast cancer (mBC) remain very poor compared to those of breast cancer patients at earlier disease stages. While patients with a localized or regionally confined breast cancer have a 5-year relative survival rate of 99% and 86%, mBC remains an incurable disease with a median overall survival of approximately 3 years and a 5-year survival of only 27% [4,5]. Moreover, as still 20–30% of breast cancer patients diagnosed at an early stage are likely to develop metastatic disease during the course of their disease [6], it is essential to develop new treatment concepts for this group of patients.

Recent technological advances in DNA sequencing have promoted discovery of biomarkers or oncogenic drivers that provide new treatment strategies for patients lacking other therapy alternatives. Biomarker analysis is a routine practice in breast cancer. Historically, estrogen receptor (ER) and progesterone receptor (PR) have been successfully used as predictive biomarkers for endocrine therapy [7]. Moreover, such biomarkers not only provide information about patients' response to a particular treatment but also have a prognostic value. For instance, several studies demonstrated that patients with ER or PR-positive tumors tend to have a better outcome than those lacking these receptors [8–11]. Recently, a heightened interest in the relevance of biomarkers in oncology has been witnessed, as their potential for guiding treatment decisions has been recognized. In the past years, impressive advances in cancer treatment outcomes through the combined use of molecular diagnostics and targeted therapies have been seen in various tumor entities [12–15]. For breast cancer patients, a rising number of predictive biomarkers have led to development of several new drugs designed for targeting specific genetic alterations, such as PARP (poly(ADP-ribose)-polymerase) inhibitors like olaparib or talazoparib for germline BRCA-mutated breast cancer.

[16] (Figure 1).

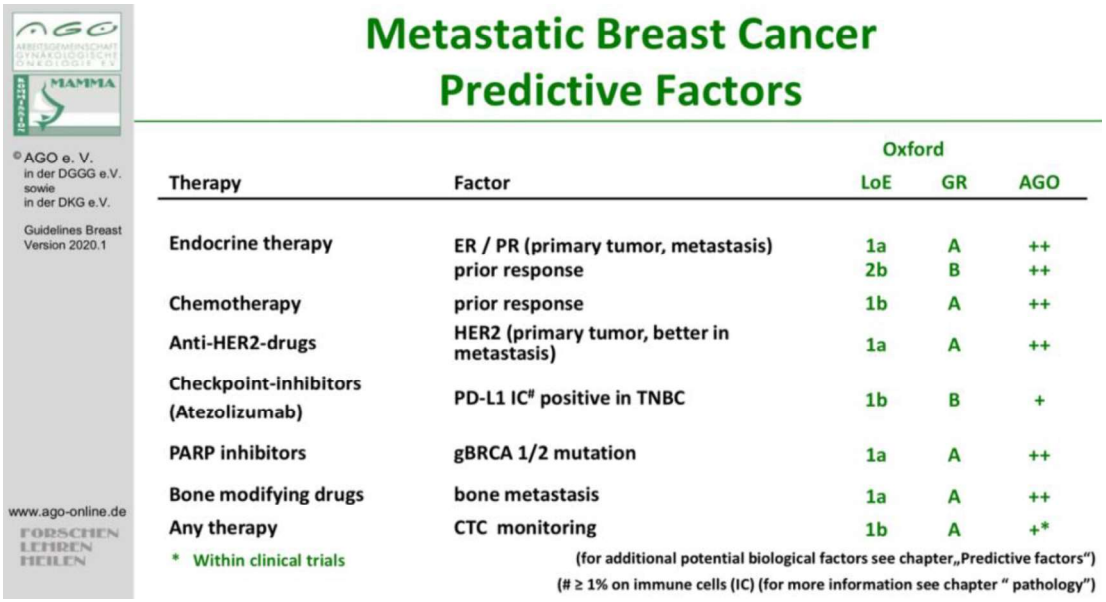

Figure.1 Predictive factors in metastatic breast cancer (mBC), German Gynecological Oncology Group (http://www.agoonline.de) Assessed on 21 Feb 2021. (ER = estrogen receptor, PR = progesterone receptor, HER2 = human epidermal growth factor, PD-L1 = programmed death-ligand 1, TNBC = triple-negative breast cancer, PARP = poly (ADP-ribose) polymerase, gBRCA = germline

BRCA, CTC = circulating tumor cell, LoE = levels of evidence, GR = grade, AGO = Arbeitsgemeinschaft Gynäkologische Onkologie (German Gynecological Oncology Group).

Moreover, since the introduction of anti-HER2 targeted agents, survival rates of patients with HER2-positive mBC have remarkably improved [17–19]. Patients with HER2- positive disease, who received the anti-HER2 agent trastuzumab, had a 44% decreased risk of death compared to the control group, which has turned trastuzumab into a routinely used drug today [20]. Besides HER2 amplification, HER2 mutations have become a predictive marker as well. Responses to neratinib, a tyrosine kinase inhibitor, were seen in about 30% of patients with HER2 mutations. Moreover, when combined with fulvestrant in previously treated hormone receptor-positive HER2-mutated tumors, it showed responses in the range of 40% [21]. Recently, multiple targeted agents have become available that have improved the outcomes of patients with breast cancer, with alpelisib being the most recent example. It proved to be beneficial for patients with a PIK3CA-mutated breast cancer, thus adding the PIK3CA gene to the list of ESCAT Level 1 actionable mutations. Patients treated with alpelisib had a progression-free survival (PFS) of 11 months (95% confidence interval [CI], 7.5 to 14.5), as compared with 5.7 months (95% CI, 3.7 to 7.4) in the control arm (hazard ratio for progression or death, 0.65; 95% CI, 0.50 to 0.85; $p < 0.001$) [22].

The advent of multiple targeted therapeutics and the promising advances in DNA sequencing techniques promoted research on molecular tumor characteristics and led to development of a new approach now known as "precision medicine". Its major aim is to use targetable molecular alterations for identification of specific subpopulation of patients, whose tumors express these markers and therefore could benefit from a certain treatment.

However, there is still a lack of clinical data on the impact of implementing precision medicine for patients with mBC (Table 1). In order to evaluate whether this subset of patients could benefit from this new approach, we initiated a molecular tumor board (MTB) to give personalized treatment recommendations based on comprehensive molecular tumor profiling. Here, we present the results of the first 100 mBC patients discussed at the Comprehensive Cancer Center, LMU Munich Molecular Tumor Board.

Table 1. Overview of studies focusing on molecular profiling in breast cancer.

Author/Study	Tumor Entity	Enrolled Patients (n =)	MP Patients	Actionable Alterations	Implemented Therapies-n (% of Enrolled)	Results
André et al. (SAFIR01/UNICANCER)	breast cancer	423	299 (71%)	195 (46%)	55 (13%)	ORR: 4 patients had a PR and 9 had SD > 16 weeks (3% of all patients)
Parker et al.	breast cancer	43	43 (100%)	40 (93%)	17 (40%)	7 patients (16% of all patients) achieved SD or PR
Van Geelen et al.	breast cancer	322	234 (72%)	74 (23%)	No data	No data about implementation rate and outcome

MP = molecular profiled, PFS = progression-free survival, ORR = overall response rate, SD = stable disease, PR = partial response, n.a. = not available.

Materials and Methods

3.2. Patient recruitment and study design

A total of 100 patients from Breast Center LMU were enrolled in a prospective single-center registry "The Informative Patient", conducted at the LMU University Hospital Munich in cooperation with the Comprehensive Cancer Center Munich. Informed consent was obtained from all individual participants. The registry was approved by the ethics committee of the LMU University Hospital Munich (reference number: 284-10). The study protocol was in accordance with the Declaration of Helsinki. The population presented here were accrued between May 2017 and December 2019. Key inclusion criteria were as follows: histological confirmation of breast cancer disease, at least one metastatic site, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, and willingness to take part in potential clinical trials or to start an off-label treatment. Molecular diagnostic testing was performed at the Pathology Institute of the LMU University Munich. The primary objective of the study was to use personalized recommendations made by a multidisciplinary tumor board to improve the progression-free survival compared to the previous treatment and to prove the impact of the MTB recommendation on the overall survival of mBC patients. Here, we present an organ-specific analysis of the first 100 patients with metastatic breast cancer, who took part of the "The Informative Patient" study. Details on progression-free survival, as well as on overall survival of all patients, who took part of the study, are yet to be presented.

3.3. Panel-guided next-generation sequencing

Molecular analyses were performed at the LMU Institute of Pathology. Sections from formalin fixed paraffin embedded (FFPE) tissue samples were prepared followed by hematoxylin-eosin staining of the first slide. Appropriate tissue regions were selected, and nucleic acids were extracted from subsequent sections using the GeneRead (DNA) and RNeasy FFPE kits (RNA) (both from Qiagen, Hilden, Germany). Targeted NGS was performed with the Oncomine Focus Panel (covering 52 cancer-associated genes) till November 2018 and then with the Oncomine Comprehensive v.3 assay screening for genetic alterations in 161 cancer-associated genes at the levels of DNA (single-nucleotide variants (SNV), multi-nucleotide variants (MNV), small ins, del, indels, copy number variation (CNV)) and RNA (gene fusions). Briefly, libraries were generated employing Ampliseq Library Plus-, Ampliseq cDNA synthesis-, Ampliseq CD index, Ampliseq Equalizer- together with AmpliSeq for Illumina Comprehensive Panel v3 (all Illumina) or Oncomine Comprehensive Assay v3 and Ion AmpliSeq Library-, IonXpress Barcode Adapter-, Ion Library Equalizer-kits together with Ion Chip kits (540 and 550) (all Thermo Fisher, Waltham, MA, USA) following each step of the respective user manuals. Libraries were sequenced on an Ion Torrent GeneStudio S5 Prime (Thermo Fisher) or Illumina 500 Next Seq (Illumina) next-generation sequencing (NGS) machine. Analysis of the results was performed with either the Ion Reporter System (Thermo Fisher) followed by further variant and quality interpretation with a home-made excel tool or the Illumina Local Run Manager with subsequent annotation of VCF-files using wAnnovar [23] and a home-made python-script filtering for clinically relevant mutations. Alterations were confirmed with the Integrated Genomics Viewer (IGV, Broad Institute, Cambridge, MA, USA) Mutations were judged as relevant on the basis of the interpretation criteria utilized in ClinVar [24]. Only likely pathogenic and pathogenic mutations as well as VUS (variant of unknown significance or not evaluated in ClinVar with a prediction trend of being likely pathogenic—majorly frameshift or truncating variants) with allele frequencies ≥3% were reported. A comprehensive pathological report comprising NGS results together with data from immunohistochemistry (used for HER2 and PD-L1 testing), fluorescence in situ hybridization (FISH) (used for confirming of the HER2 status) and histo-morphology was submitted to the MTB for further discussion of therapeutic options.

3.4. Study procedure

A flowchart of the trial "The informative patient" is shown in **Fig. 2**.

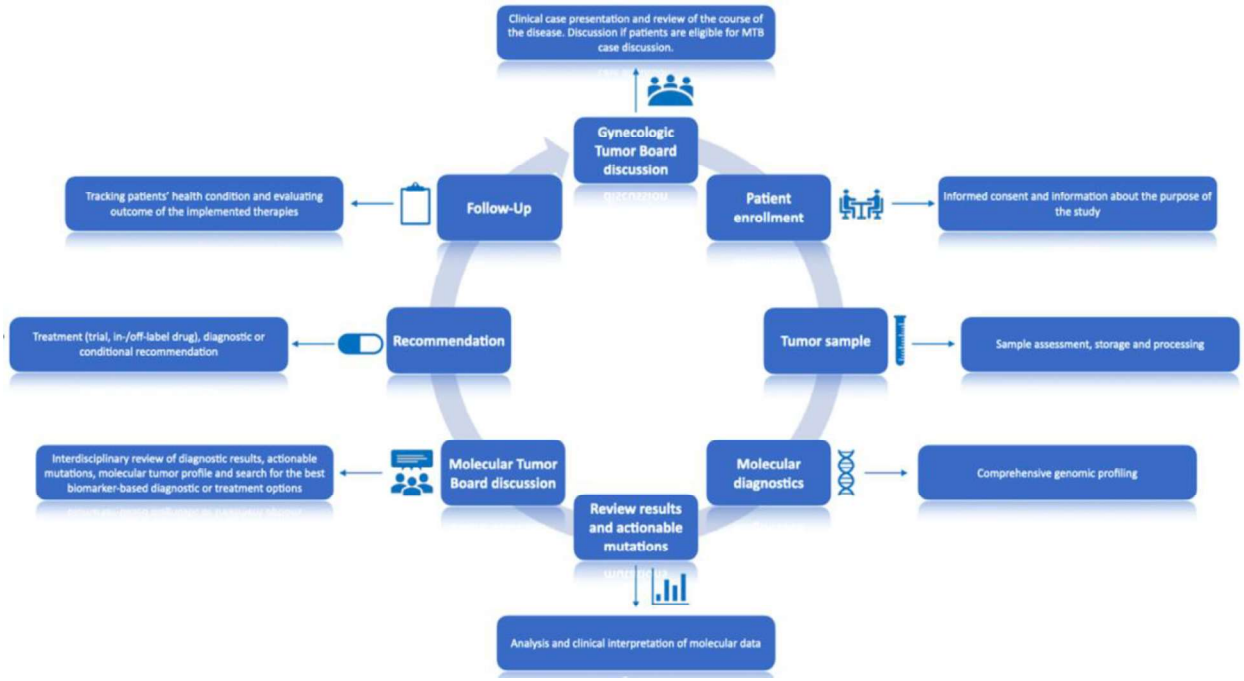

Fig. 2 "The informative patient" study design. All procedures were conducted in the LMU University Hospital, Munich.

All patients (n = 100) were first discussed in an organ-specific breast cancer tumor board (LMU, Department of Obstetrics and Gynecology), where the treating gyneco-oncologist presented the patient's case and requested case discussion at the Molecular Tumor Board (MTB). If eligible, after patient informed consent, all tumors underwent comprehensive molecular profiling and the results were then presented to the MTB. Each case was then discussed by the multidisciplinary MTB team, consisting of gyneco-oncologists with expertise in various cancer entities along with molecular pathologists, and genetic counselors. Each patient was presented by a moderator, who provided information about patient's course of disease, prior treatment history with response and comorbidities. After reviewing clinical history and molecular profile of each tumor, the MTB discussed actionability of the discovered mutations by reviewing literature and publicly available databases, such as PubMED, clinicaltrials.gov accessed on 10 April 2021, ClinVar, Varsome, OncoKB and CIViC [25]. The purpose of this research was to determine frequency of particular molecular alterations across patient populations as well as relevant pathways that may be affected, and then matching them to available drugs (in- and off-label) or clinical trials. For each patient, the MTB discussed possible diagnostic and treatment options and issued recommendations accordingly. Treatment recommendations were supported by levels of evidence for molecular targets by using the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT), defined according to their implications for patient management. **(Table 2)** [26].

Table 2. List of genomic alterations Level I/II/III in breast cancer as classified by the European Society for Medical Oncology (ESMO) Scale for Clinical Actionability of Molecular Targets (ESCAT) [27].

Genomic Alterations	Prevalence
ESCAT Level I	
BRCA1/2 germline mutations	4%
ERBB2 amplifications	15–20%
Microsatellite instability-high	1%
PIK3CA hotspot mutations	30–40%
ESCAT Level II	
$AKT1^{E17K}$ mutations	5%
ERBB2 hotspot mutations	4%
ESR1 mutations	10%
ESCAT Level III	
BRCA1/2 somatic mutations	3%
ERBB3 mutations	2%
MDM2 amplifications	1%

3.5. Analysis of results

In order to determine the clinical impact of panel-guided NGS adjusted therapies, we calculated progression-free survival ratio (PFSr) as previously described by Von Hoff et al. [28], by comparing progression-free survival on matched therapy (PFS2) with progression-free survival on the most recent therapy prior to NGS testing on which the patient experienced disease progression (PFS1). Progression-free survival (PFS) was calculated from start of recommended treatment to disease progression (as assessed by RECIST guidelines (version 1.1) or death whatever occurred first) [29]. Cut-off date for follow-up analysis was 1 August 2020.

Results

3.1. Patient characteristics

Between May 2017 and December 2019, 100 female mBC patients were included in the "The Informative Patient" study. The median age was 52 years (range: 30 to 82). Patients had a median of four therapies prior to inclusion (range: 1 to 13). The median number of metastatic sites per patient was 2 (range: 1 to 6). Regarding organ sites, the majority of patients had bone metastases (62%), followed by liver (51%), and lung (40%) metastases (Supplementary Table S2.) More information about patients' characteristics is listed in Table 3.

Table 3. Patient characteristics (n = 100).

Patient Characteristics	n =
Median age	52 (range 30–82)
Number of metastatic sites at time of presentation	
1	25
2	39
3	20
>3	16
Metastatic sites	
visceral	87
bone	62
brain	21
cutaneous	11
Number of previous therapies	
1	6
2	26
3	13
>3	55

The plurality of patients had triple-negative breast cancer (ER, PR and HER2 negative; n = 30; 46.9%), followed by estrogen receptor (ER)—positive and/or progesterone receptor (PR)—positive, human epidermal growth factor receptor 2 (HER2)—negative (luminal-like) (n = 28; 43.8%), or HER2-positive, ER-negative, PR-negative disease (n = 5; 7.8%) at time of the initial MTB presentation; one patient (1.6%) had triple-positive disease (ER-positive, PR-positive and HER2-positive) (**Figure 3).**

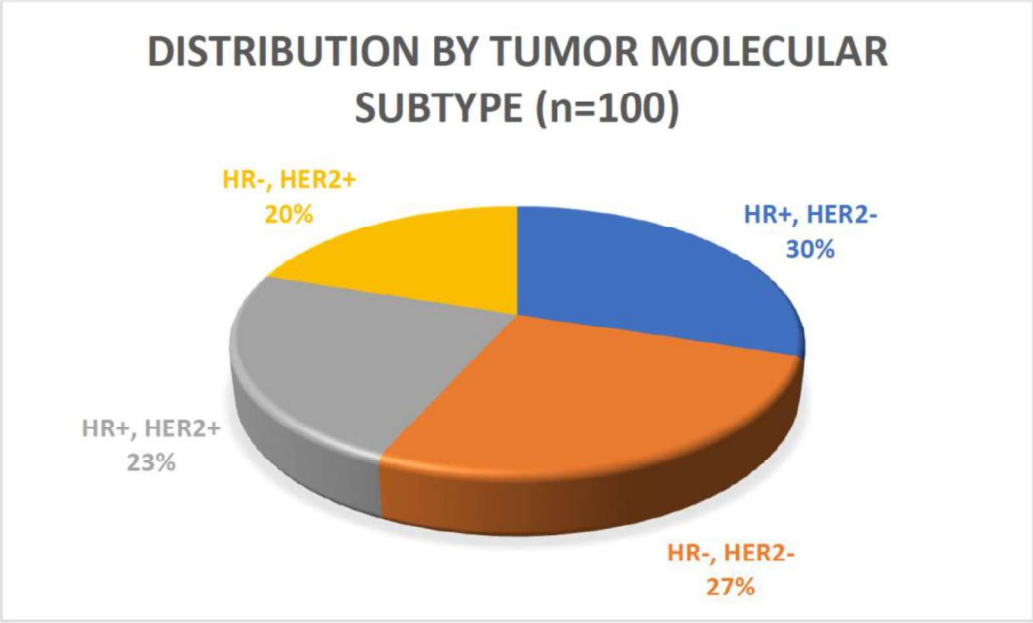

Figure. 3 Patient distribution by tumor molecular subtype (IHC) (n=100), (HR = hormone receptor, HER2 = human epidermal growth factor).

3.2. Molecular diagnostics

NGS was done for all patients. All tissue samples were collected either prior to molecular profiling or prior to the initiation of the last therapy a patient received. When selecting appropriate tissues,

we set the criteria of using samples that were not older than two years prior to initiation of molecular profiling, and when possible, collecting tissue samples after the last standard line of therapy, in order to provide the most accurate analysis of molecular profile data.
All tumor samples used for molecular profiling have been collected no more than 24 months prior to molecular profiling. The median turnaround time for completing molecular profiling was 19 days (range: 10–48). The median turnaround time between initiation of molecular profiling and MTB case discussion was 33 days, which is similar to reported median turnaround times in other studies [30]. In seven cases (7%) tumor sequencing was performed more than once. In 73 (73%) of the received samples, at least one molecular alteration was found. Among these 73 tumor samples, 53 (53%) had at least one actionable mutation, as classified by the MTB. More than one molecular alteration was found in 51 cases (51%). No genomic alterations were found in 27 samples (27%), 11 of which (11%) had insufficient material quality and therefore led to technically not successful molecular analysis.

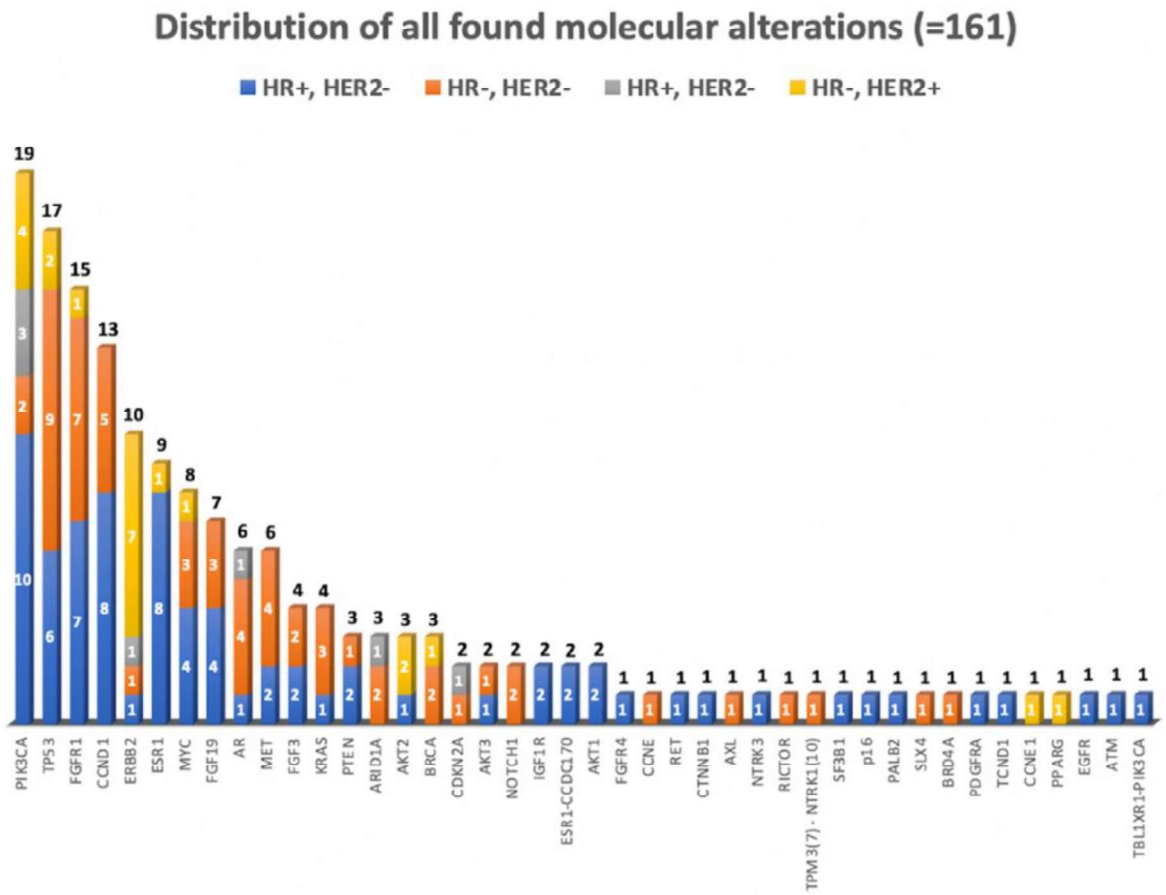

Figure 4. Distribution of genomic alterations sorted by tumor molecular subtype (n = 100) (HR = hormone receptor, HER2 = human epidermal growth factor).

All in all, we detected 161 molecular alterations, with a median of two alterations per sample (range: 0–6). In total, molecular changes in 42 genes were found. As shown in Figure 4, the most common molecular alterations across the sequenced samples were found in the PIK3CA gene (19/100; 19%); followed by TP53 gene (17/100; 17%), and FGFR1 gene (15/100; 15%) (Supplementary Table S1).

3.3 Recommendations

In total, 49 patients (49%) received at least one treatment recommendation from the MTB. Further, 18% of all patients obtained more than one treatment recommendation, as their samples contained more than one actionable alteration. The most common therapy recommendation (in 21 of 49 cases with at least one treatment recommendation) was everolimus, a mTOR inhibitor. Of note, five patients carrying a now actionable mutation (PIK3CA, found in 19% of patients in the presented cohort) received no therapy recommendation, as the drug targeting this mutation (alpelisib) was not approved at the time of MTB presentation.
In five of the cases (5%), the MTB suggested further diagnostic tests, three of which then resulted in a treatment recommendation. In the Appendix A, details on actionable mutations and following MTB treatment recommendations made by the MTB are provided.
All in all, 51 patients (51%) received no recommendation from the MTB. The main reasons for no recommendation were absence of molecular alterations in the NGS testing (27%), non-actionable mutations (20%), patient comorbidities or general condition by the time of MTB case discussion (3%). More information about the results of MTB case discussions is listed in Figure 5.

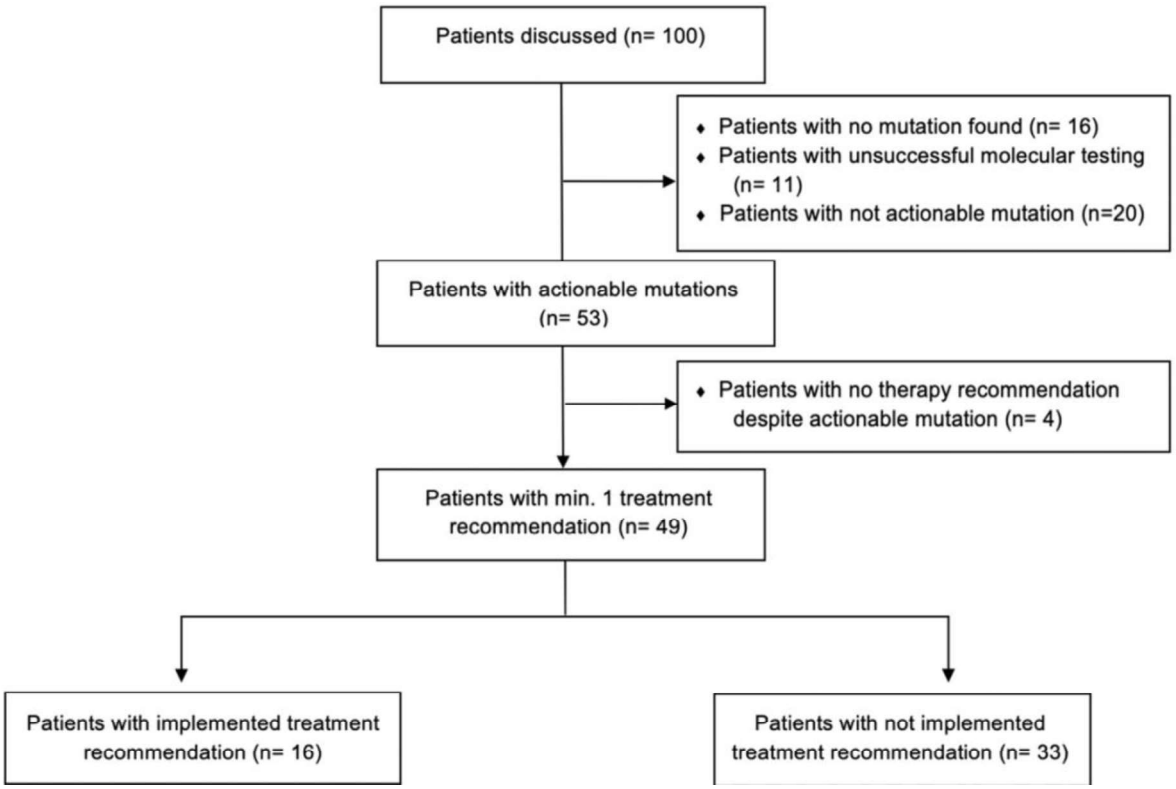

Figure. 5 Consort flow diagram showing the results of Molecular Tumor Board (MTB) case discussions based on molecular diagnostics results and implementation of treatment recommendations in our cohort (n = 100).

3.4. Progression free survival analysis

Follow-up information was available for 48 out of 49 patients with a treatment recommendation. In 16 out of 49 cases (16% of all patients), treatment recommendations were implemented. Lack of implementations was mostly caused by deterioration of the patient's health condition (10%),

inaccessibility to treatment recommendation (8%), not fulfilling trial inclusion criteria (6%), or patient preferences (5%).

The median turnaround time between the discussion in the molecular tumor board and the initiation of recommended therapies was 53 days. Among patients with implemented treatment recommendations, 13 (13%) received an in-label treatment, whereas three (3%) received an off-label drug. The most frequently implemented treatment recommendation was a mTOR inhibitor (mostly everolimus) in combination with endocrine therapy (mostly exemestane) in seven cases (7%). A total 9 of 16 patients (56%, 9% of all patients) with implemented treatment recommendations were found to have a PFSr ≥ 1.3, with a median of 1.3 (range: 0.2 to 11.8). Further, 6 patients (6%) achieved a state of partial remission or stable disease lasting over 16 weeks, with one patient having an ongoing PFS (Table 3).

#	Gene alteration	Implemented Therapy	Label	PFS2 (weeks)	PFS1 (weeks)	PFSr
1	FGFR1	Everolimus	in	**14**	81	0.2
2	FGFR1	Everolimus	in	**4**	13	0.3
3	PIK3CA	Alpelisib	in	**14**	44	0.3
4	PIK3CA	Alpelisib	in	**15**	32	0.5
5	FGFR1	Everolimus	In	**4**	8	0.5
6	ERBB2	Trastuzumab / Lapatinib	in	**21**	25	0.8
7	FGFR1	Everolimus	in	**13**	13	1
8	PIK3CA	Everolimus	in	**69**	55	**1.3**
9	FGFR1	Everolimus	in	**13**	9	**1.4**
10	PIK3CA	Everolimus	in	**18**	12	**1.5**
11	CCND1	Palbociclib	in	**21**	13	**1.6**
12	FGFR1	Pazopanib	off	**12**	6	**2**
13	FGFR1	Pazopanib	off	**6**	3	**2**
14	ERBB2	Lapatinib	in	**26**	3	**8.7**
15	p16	Pembrolizumab	off	**59**	5	**11.8**

Table 3. Patients with implemented treatment recommendations (in- and off-label).
PFS1 = progression-free survival on the most previous line of therapy (standard of care).
PFS2 = progression-free survival on the implemented recommended therapy.
PFSr = PFS ratio = PFS2/PFS1

Fig. 6 details the actual comparison of PFS on recommended therapy (PFS2) versus PFS on last therapy the patient received (PFS1).

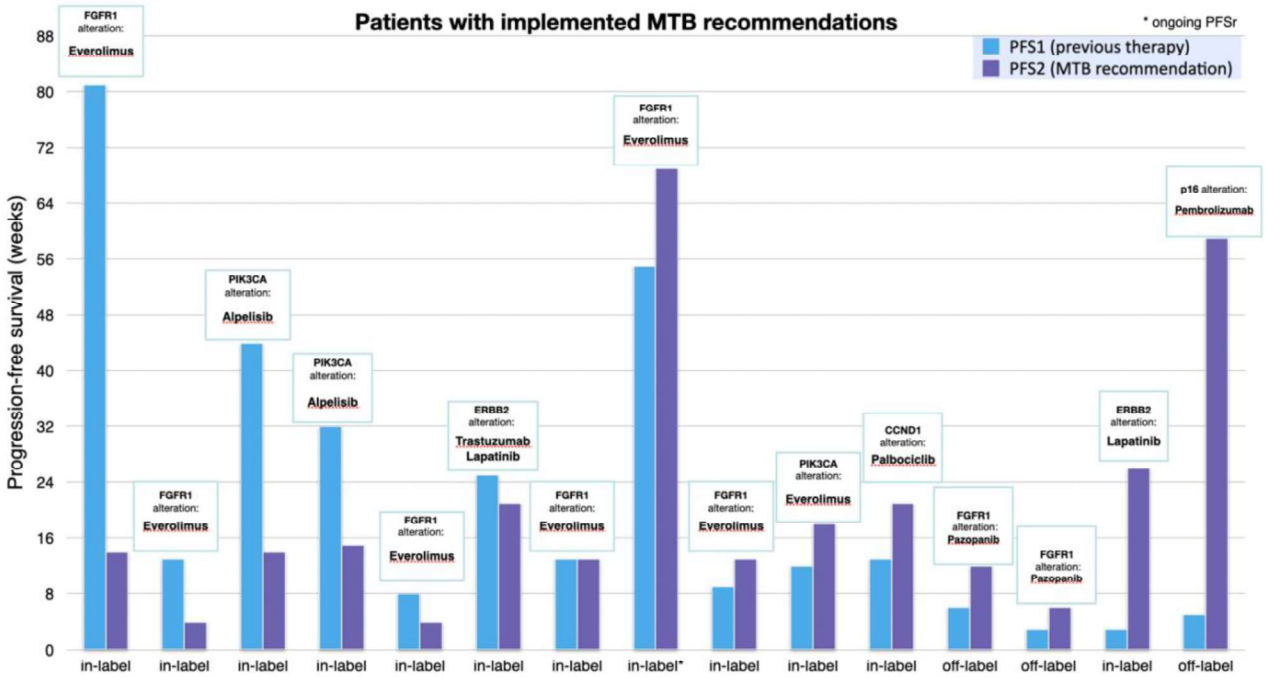

Fig. 6 Bar graph comparing progression-free survival (PFS) of previous line of therapy (PFS1) and of implemented therapy, as recommended by the MTB (PFS2). PFS was defined as the period of time between the start of treatment till disease progression or death.

Discussion

Modern sequencing techniques together with newly targeted therapies have revolutionized cancer medicine by providing substantial benefits for cancer patients in comparison to prior medical standards. However, the precision oncology movement remains controversial, as evidence supporting this approach is still missing. In 2015, a meta-analysis conducted by Schwaederle et al. compared results of 570 studies comprising 32,149 patients, divided in two groups of patients who received a personalized treatment strategy versus those that did not. The results supported the personalized approach, as it correlated with higher median response rate (31% vs. 10.5%), prolonged median PFS (5.9 vs. 2.7 months) and overall survival (13.7 vs. 8.9 months) [37]. Many other studies demonstrated similar positive results [38–41]. For instance, in the WINTHER trial, 22.4% of the patients receiving therapy based on molecular profiling had a survival ratio > 1.5. However, in the first randomized trial, SHIVA (n = 741 screened) no significant improvement in PFS was seen in the precision oncology arm compared to the standard-of-care arm, suggesting that off-label use of targeted therapies does not improve PFS compared with standard-of-care treatment [42]. All in all, over the past few years, many researchers have investigated the effect of using panel-guided molecular diagnostics on the PFS and OS of patients with advanced cancers. While some of them were able to demonstrate a clinical benefit and longer survival for patients with individualized therapies, the overall impact remains small, and therefore, a subject to discussions of the cost-effectiveness of this approach [43].

In this study, we demonstrated that individual treatment recommendation based on molecular profiling using NGS could improve PFS of mBC patients. Among those patients with implemented treatment recommendations, more than a half had a PFSr ≥ 1.3, which demonstrates the potential relevance of involving targeted NGS-guided therapies in mBC. Previous studies focusing on implementation of precision oncology in breast cancer care also showed similar results, demonstrating that this approach is feasible and of great importance—at least for a subset of patients [44]. For instance, in the SAFIR01 trial, 9% of the patients with implemented treatment recommendation had an objective response, while 21% responded with stable disease lasting more than 16 weeks [45]. Other recent studies, such as the one by Geelen et al., which accrued 357 breast cancer patients of whom 74% had a potentially actionable alteration, also demonstrated feasibility of using molecular diagnostics to detect actionable molecular alterations. This suggests that clinical utility of genomic profiling in combination with more available targeted therapies will expand over time [46]. Within the context of mBC, there are various applications of molecular diagnostics, which could potentially improve patient outcome. Apart from identifying oncogenic driver mutations, it is also possible to define genomic alterations, associated with secondary resistance, another major clinical problem in mBC. For example, ESR1 mutations, occurring in 10–30% of pre-treated ER-positive mBC, are known to cause resistance to aromatase inhibitors [47]. Thus, detecting such alterations could provide valuable information about signaling pathways causing resistance to certain treatments. As tumor biological factors of breast cancer often tend to differ in the primary and in the distant metastatic tissue, affecting patient prognosis, there is a need of understanding the tumor biology of these malignancies at a higher level [48].

In the presented study, approximately 25% of the patients had a Level 1 actionable alteration, corresponding to ESCAT levels of evidence (LOE) I/II genes, with PIK3CA being the most frequently altered gene. The latest breakthrough in breast cancer oncology was the approval of alpelisib in combination with fulvestrant. Of note, some patients in our cohort, harboring a PIK3CA mutation, did not receive a treatment recommendation, if the PIK3CA gene was not classified as "actionable" at time of their initial MTB presentation. Thus, considering the high frequency of PIK3CA gene alterations in breast cancer (more than 25% of all breast malignancies), the discovery of alpelisib was of great importance for many patients, proving that detecting genomic alterations is crucial, as research in the past decade has been mainly focused on developing new drugs targeting such molecular aberrations [49].

However, although the number of MTAs (molecular targeted agents) is constantly rising, there is still a lack of drugs targeting many genes, commonly expressed in breast cancer, like TP53 mutations (17% of our patients expressed this alteration) for example, making matching genomic alterations with targeted therapies still a great challenge for the majority of patients and one of the greatest limitations for precision oncology. Developing newly targeted therapies represents a major issue, as it requires a large number of patients to be screened in order to perform a clinical trial. Accruing many patients for this purpose appears to be problematic, as the cost for high-throughput genomic profiling to identify patients carrying particular mutations is still relatively high. However, with the advent of NGS technologies and prices of this innovative approach constantly decreasing, it has now become easier than ever to incorporate molecular diagnostics into clinical routine [50].

Nevertheless, the cost of molecular profiling accounts for a very small amount of the whole therapy. Molecular-guided treatment still represents the main cost driver, accounting for more than 50% of all costs [51]. Undeniably, the high costs associated with molecular profiling and targeted therapies, and limited drug access represent more barriers for successful translation of precision oncology into clinical breast cancer practice [52,53]. In our study, the cost of the recommended targeted therapy was one of the most common reason why patients did not receive the recommended treatment.

Unfortunately, with the rising number of approved targeted drugs, their costs have increased during the same time [54].

As shown in this study, clinical trials unfortunately often remain unavailable for patients, mainly because of deterioration of patients' physical condition or existing exclusion criteria for a given trial. Considering the fact that breast cancer accounts for one of the highest uses of targeted therapies, we need to find a way to ensure access to targeted therapies for patients with actionable mutations. One possible solution is to develop basket trials, testing the effectiveness of a single drug against a molecular alteration in various cancer entities. Another option is to create umbrella trials, which focus on the effect of different drugs targeting different gene alteration in a single cancer entity [55]. MTBs could serve as a platform to improve access to targeted therapies by constantly reviewing relevant clinical trial options for particular groups of patients. As other authors already suggested, the access to a MTB increases the chance for application of genetics-guided cancer care [56]. According to the recently published 5th ESO-ESMO international consensus guidelines for advanced breast cancer (ABC 5), suitable patients, ready to participate in clinical trials of novel therapies, should undergo NGS testing in centers with relevant trial options [57].

Another major benefit of implementing MTBs into clinical care is that they also improve clinicians' knowledge about molecular oncology [58]. The complexity of the large amounts of data generated by genomic profiling requires expert review for maximum clinical benefit. MTBs provide a system to guide clinical decision-making in precision oncology, while also training physicians who are still inexperienced in this topic and improving their confidence in understanding this new field.

However, the concept of MTBs is not fully defined, as guidelines and quality criteria are still missing, which is the reason why there are great discrepancies in outcomes of clinical trials focusing on precision oncology. Different centers tend to have different patient selection criteria and also differ in selection of multigene panels used for molecular profiling. The right time of enrolling patients into trials enabling access to precision cancer care is still a matter of debate. As seen in our cohort, patients' disease stage at time of enrollment is of great importance for evaluating the impact of personalized treatment recommendations for cancer patients. Rapid deterioration of the physical condition was one of the main reasons why patients did not receive the recommended treatment. The median turnaround times from indication for molecular profiling to MTB case discussion are still in some cases quite long for cancer patients at a late disease stage. This suggests a need to evaluate which patient groups would benefit most from implementing of precision oncology in standard oncology care. Defining actionability of genomic alterations, providing access to clinical trials and off-label drugs and quality assurance of molecular diagnostics also seem to vary from center to center. In the constantly changing world of precision oncology, there is a need for standardizing and optimizing the work of MTBs and for developing international guidelines and real-world databases to guide clinician decision-making in precision oncology.

The precision oncology field is constantly evolving. In our clinical center, we managed to evaluate this trend over the past two and half years. Comparing the results of the presented study with those of our first study where we presented results of the first 100 patients with mBC or gynecologic malignancies, we have observed an improvement in the therapy implementation rates (16% in the presented study vs. 12.5% in the previous study), in the number of recommendations given (49% vs. 42%), and in the number of mutations found (53% vs. 48%) [59]. In addition, the number of technical problems occurring in the molecular diagnostics was significantly lower in the presented trial as compared to our earlier experiences (11% vs. 17% in our last presented study). These results demonstrate the importance and potential of developing precision oncology access programs in academic centers.

In view of our results and recently published experiences, we expect molecular profiling and molecular tumor boards to become increasingly implemented in breast cancer care over the next few years. In order to maximize clinical benefit for more patients, it is essential to optimize MTB structures, reconsider selection patient criteria for tumor molecular profiling, and to determine new biomarkers and associated targeted therapies by improving access to clinical trials. In addition, it's important to consider using liquid biopsies for molecular profiling, a revolutionary but still limited new tool for precision medicine. As an important diagnostic tool, it has advantages such as providing representative analysis in the presence of multiple tumor foci and being less invasive compared to traditional tumor biopsy analysis, but also disadvantages such as high costs and questionable sensitivity.

Within the setting of our molecular tumor board, liquid biopsies were only considered in a minor part of the patients (other tumor entities) where no recent tumor biopsy was available or performable. Reasons for this were the potential false negative rates, high analyses cost due to high sensitivity systems combined with a very low chance of health insurance reimbursement.

The presented study has several limitations. First, the cohort presented, comprising 100 mBC patients, is relatively small. Moreover, our patients already had advanced stage disease and a therefore limited number of available, previously not implemented treatment options. Thus, it is possible that our findings may not be applicable to patients exposed to comprehensive molecular profiling and MTB discussion at an earlier disease stage. Second, defining actionable mutations is challenging and also depends on approved targeted therapies at time of case presentation. As the field of molecular oncology is rapidly evolving, the importance of specific biomarkers may vary. Third, as tumors tend to evolve during the disease course, it is possible that the molecular landscape of the tumor may have changed by the time of molecular profiling. Furthermore, some studies suggest a possibility of cancers evolving under cancer therapy [60]. As some of the tissue samples were collected prior to the last systemic treatment, this may have caused inaccuracy in the matching of targeted therapies and actionable mutations. Lastly, the presented study was not designed as a randomized controlled trial, but rather as a real-world data registry.

Conclusion

Although the number of patients is still low, our experience shows that patients with mBC may benefit from implementation of MTB recommendations based on targeted panel-guided sequencing into clinical care. MTBs have proven to be a helpful tool for patient care, as they combine clinical expertise in several oncology areas in order to improve patient outcome by providing a personalized tailored-based treatment advice. They also encourage interdisciplinary knowledge transfer and are a great platform for expanding experience in precision oncology. In order to maximize the clinical utility of precision oncology, logistical support to ease access to drugs and clinical trials is needed.

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Abbreviations

MTB Metastatic breast cancer
PD-L1 Programmed death-ligand 1
TNBC Triple-negative breast cancer
PARP Poly (ADP-ribose) polymerase
gBRCA Germline BRCA
CTC Circulating tumor cell
LoE Levels of evidence
GR Grade
AGO Arbeitsgemeinschaft Gynäkologische Onkologie (German Gynecological Oncology Group)
MTB Molecular Tumor Board
ESCAT ESMO Scale of Clinical Actionability
PFS Progression-free survival
PFSr Progression-free survival ratio
HR Hormone receptor
ER Estrogen receptor
PR Progesterone receptor
HER2 Human epidermal growth factor receptor 2
NGS Next-generation sequencing
MTA Molecular targeted agents
OR Overall response rate
SD Stable disease
PR Partial remission

Appendix

#	Alteration found	Treatment recommendation	Follow-up
1	PIK3CA, ESR1 and TP53 mutations	alpelisib	lost to follow up
2	FGFR1, androgene receptor and CCND1 amplifications	1. CDK4/6 inhibitor 2. everolimus 3. androgene receptor inhibitor	not implemented
3	PIK3CA and ESR1 mutation, TBL1XR1-PIK3CA gene fusion	1. alpelisib 2. everolimus	implemented
4	PIK3CA mutation	alpelisib	implemented
5	ERBB2 amplification	HER2 inhibitor	not implemented
6	CCND1 amplification	1. CDK4/6 inhibitor 2. palbociclib + fulvestrant 3. everolimus	implemented
7	PIK3CA, PTEN and AKT3 mutations	mTOR inhibitor	implemented
8	FGFR1 and AKT2 amplifications; TP53 mutation	FGF1 inhibitor	not implemented
9	ERBB2 mutation	afatinib / neratinib	not implemented
10	PTEN deletion	1. IPATunity130 trial (NCT03337724) 2. exemestane + everolimus	not implemented
11	PIK3CA mutation	everolimus	not implemented
12	FGFR1, FGF19 and FGF3 mutations	1. mTOR inhibitor 2. pazopanib	implemented

13	MET mutation	crizotinib	not implemented
14	MYC, FGFR1 and CCND1 amplifications	everolimus	implemented
15	BRCA mutation; androgene receptor amplification	1. trial (NCT01945775) 2. trial (NCT02163694) 3. bicalutamide / tamoxifen	not implemented
16	PIK3CA mutation	1. SOLAR-1 trial 2. IPATunity130 trial 3. everolimus	not implemented
17	AKT2 amplification; SF3B1 mutation	everolimus + hormone therapy	not implemented
18	PIK3CA mutation; MET amplification	crizotinib	not implemented
19	FGFR1 mutation	1. pazopanib 2. MASTER trial	not implemented
20	ESR1 and PALB2 mutations; ESR1-CCDC170 fusion	1. platin-based chemotherapy 2. olaparib	not implemented
21	FGFR1 and MYC amplifications; TP53 mutation	1. FGFR1 inhibitor 2. mTOR inhibitor	not implemented
22	ERBB2 amplification	lapatinib, trastuzumab emtansine and pertuzumab	not implemented
23	ARID1A and PIK3CA mutations	everolimus	implemented
24	ESR1 mutation	fulvestrant + everolimus	not implemented
25	FGFR, CCND1, FGF19 and IGF1R amplifications; ATM mutation	mTOR inhibitor	implemented

26	TP53 mutation; FGFR1, TCND1, FGF19 und FGF3 amplifications	FGFR1 inhibitor	not implemented
27	PIK3CA, HER2, CDKN2A mutations	1. TDM1 + alpelisib 2. neratinib 3. CDK4/6 inhibitor 4. everolimus; in combination with trastuzumab	not implemented
28	TPM3(7) - NTRK1(10) fusion	trial (NCT02568267)	not implemented
29	MET mutation	cabozantinib	not implemented
30	KRAS and PIK3CA mutations	peg-Doxorubicin / bevacizumab and temsirolimus / everolimus	not implemented
31	androgene receptor and PIK3CA mutations	everolimus	not implemented
32	MET, CCND1, FGF19, FGFR amplifications	FGF1 inhibitor	implemented
33	FGFR1, CCND1, EGFR, PIK3CA und PDGFRA amplifications	pazopanib	not implemented
34	PIK3CA mutation; ERBB2 amplification	1. HER2 inhibitor 2. HER2 inhibitor + neratinib 3. PIK3CA inhibitor	not implemented
35	ERBB2 mutation; CCNE1, AKT2, ERBB2 amplifications	trastuzumab + lapatinib	implemented
36	ESR1 and PIK3CA mutations	1. trial (NCT03056755) 2. everolimus	not implemented
37	p16 high expression and MYC mutation	checkpoint inhibitors	implemented
38	AKT3 and TP53 amplifications	MASTER trial	not implemented

39	androgene receptor amplification	androgene receptor inhibitors	not implemented
40	AKT mutation	1. AKT inhibitors 2. IPATunity130 trial (NCT03337724) 3. everolimus	not implemented
41	SLX4 mutation; FGFR1, CCND1, CCND1, FGF19, FGFR3 amplifications	pazopanib	implemented
42	ESR1 mutation	fulvestrant + CDK4/6 inhibitors	not implemented
43	CCND1 and FGF19 amplifications; AKT1 mutation	1. IPATunity130 trial (NCT03337724 2. mTOR inhibitor	not implemented
44	PIK3CA and TP53 mutations	alpelisib	implemented
45	CCND1 and FGFR1 amplifications	1. everolimus + hormone therapy 2. dovitinib	not implemented
46	PIK3CA and ERBB2 mutations; ERBB2 high expression	1. dual HER2 inhibitors 2. Neratinib	implemented
47	FGFR1 amplification	everolimus + hormone therapy	implemented
48	CCND1 amplification	exemestane + everolimus	not implemented
49	CCND1 and FGFR1 amplifications	1. exemestane + everolimus 2. trial (NCT03517956)	implemented

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