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GBG-108

Molecular mechanisms of therapy resistant breast cancer (MOMENTUM)

– Study Protocol –

Principal Investigator:

Prof. Dr. med. Sibylle Loibl

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2 Approval Signatures

Molecular mechanisms of therapy resistant breast cancer (MOMENTUM).

Study code.: GBG 108

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Protocol Version Date: 10.11.2023

I, the undersigned, have read this protocol and confirm that to the best of my knowledge it accurately describes the planned conduct of the study.

Signature: 

Date: 22.11.23

Prof. Dr. Sibylle Loibl
Principal Investigator
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4 Glossar

eBC	Early Breast Cancer
mBC	Metastatic Breast Cancer
ERα	Estrogen-Receptor alpha
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HER-2	Human Epidermal Growth Factor Receptor 2
HR	Hazard Ratio
IHC	Immunohistochemistry
NACT	Neoadjuvant chemotherapy
NGS	Next-Generation-Sequencing
OR	Odds Ratio
pCR	Pathological Complete Response
PR	Progesterone Receptor
SOP	Standard Operating Procedure
TCGA	The Cancer Genome Atlas
TNBC	Triple-Negative Breast Cancer
WES	Whole-Exome-Sequencing
WGS	Whole-Genome-Sequencing

5 Deutsche Kurzfassung

Studientitel	Molecular Mechanisms of Therapy resistant breast cancer (MOMENTUM) Molekulare Mechanismen von therapieresistentem Brustkrebs
Studienleiterin	Prof. Dr. Sibylle Loibl
Hintergrund	Ungeachtet der therapeutischen Fortschritte haben Patientinnen mit Brustkrebs im Frühstadium (eBC), die nach einer neoadjuvanten Chemotherapie (NACT) keine pathologische Komplettremission (pCR) erreichen, immer noch ein höheres Rückfallrisiko. Insbesondere Patientinnen mit triple negativem Brustkrebs (TNBC) und/oder anderen biologischen oder klinischen Risikofaktoren haben eine schlechtere Prognose. In der fortgeschrittenen Erkrankungssituation erhalten Patientinnen in der Regel mehrere Behandlungslinien, um das Fortschreiten des Tumors zu verhindern. Bedingt durch Resistenzentwicklungen generieren spätere Therapielinien allgemein kürzer werdende Progressions-freie Überlebenszeiten als frühere Therapielinien. Einer der möglichen Gründe dafür ist die Tumorheterogenität. Next generation sequencing (NGS) hat zu einem besseren Verständnis von Tumorheterogenität bei Brustkrebs geführt, welche mit Resistenzentwicklung und Überleben assoziiert ist. Mehrere Mutationen wurden identifiziert, die Drivermutationen für Tumorprogression sein könnten (Stephens Nature 2012, Koboldt Nature 2012, Curtis Nature 2012, Arnedos Nat. Rev. Clin. Oncol. 2015). Weitere Studien sind dringend erforderlich, um die Tumorheterogenität eingehend zu charakterisieren, insbesondere die molekularen Veränderungen, die unter/nach Einfluss von medikamentösem Selektionsdruck auftreten. Ein besseres Verständnis von Resistenzmechanismen ist notwendig, um neue Therapiestrategien und ggfs. potenzielle, neue Therapie Targets zu identifizieren. MOMENTUM ist eine prospektive, nichtinterventionelle, translationale Registerstudie. Sie hat zum Ziel Patientinnen/Patienten mit eBC und relevanten, klinischem Residualtumor nach NACT sowie Patientinnen/Patienten mit metastasiertem Brustkrebs (mBC) und Progression unter/nach Systemtherapie über den zeitlichen Verlauf zu begleiten. Die registrierten Informationen aus dem klinischen Verlauf sollen mit Untersuchungen von longitudinalen Biomaterialien (Tumorgewebe/Blut) korreliert werden. Dabei sollen klinische Daten zu Therapien und Überleben unter dem Gesichtspunkt von Tumorheterogenität und Veränderungen von molekularen Biomarker Profilen über den zeitlichen Verlauf der Erkrankung systematisch analysiert werden.
Hypothese	Die Analyse von residualem Tumorgewebe nach NACT bei eBC sowie Tumorgewebe von Patientinnen/Patienten mit mBC und Progression unter/nach Systemtherapie ist relevant für die Identifizierung molekularer

	Mechanismen von Therapiesensitivität und Resistenzentwicklung sowie zur Identifizierung von neuen Therapie Targets.
Studienziele	**Studienziele** 1. Untersuchen von Tumorheterogenität anhand der Analyse longitudinaler Tumorproben 2. Definieren von Biomarkern zur Vorhersage von Therapieresistenz in der neoadjuvanten, adjuvanten oder metastasierten Therapiesituation 3. Beschreiben von Veränderungen von Brustkrebssubtyp und Veränderungen molekularer Marker nach Systemtherapie 4. Identifizieren von neuen, prognostischen Biomarkern, die mit schlechterem Langzeitüberleben korrelieren (krankheitsfreies Überleben, progressionsfreies Überleben und Gesamtüberleben) 5. Identifizieren von neuen, prognostischen Biomarkern für Therapiesensibilität für post-neoadjuvante und palliative Systemtherapien 6. Identifizieren von neuen Therapie Targets, die für den Einsatz als post-neoadjuvante und palliative Systemtherapien untersucht werden können.
Studiendesign	MOMENTUM ist eine prospektive, nichtinterventionelle, translationale Registerstudie. Die Registerstudie wird klinische Daten zu Patientinnen/Patienten mit eBC und residualem Tumor nach NACT, Patientinnen/Patienten mit Rückfall sowie Patientinnen/Patienten mit mBC erheben, bei denen mindestens zwei longitudinale Proben von Tumorgewebe (eine vor und eine nach Therapieeinfluss) verfügbar sind. Bei einer Studienteilnahme werden nur Gewebeproben gesammelt, die bei Maßnahmen der klinischen Routine gewonnen wurden (Diagnosestellung, Brustoperation, etc.) und daran Veränderungen der Tumorheterogenität und Entwicklung von Resistenzmechanismen im zeitlichen Verlauf untersucht. Zusätzlich werden Blutproben zum Zeitpunkt des Studieneintritts sowie bei Indikation zur Veränderung der Systemtherapie aufgrund von Progression gesammelt, um molekulare Mechanismen der Tumorprogression auf zirkulierender Tumor DNA (ctDNA)-Ebene zu überwachen (diese werden bei geplanten Blutabnahmen der klinischen Routine angeschlossen). Die Studie soll weitere Einblicke in die Tumorheterogenität von Brustkrebs zu entscheidenden Zeitpunkten der Erkrankung generieren und molekulare Mechanismen für Therapieresistenz analysieren.
Einschlusskriterien	1. Patientinnen/Patienten mit eBC und relevantem klinischem Residualtumor nach NACT oder Fernrezidiv unter/nach (neo-)adjuvanter Therapie oder mit primär mBC und Progression nach mindestens einer Linie Systemtherapie 2. Alle Patientinnen/Patienten müssen ein Paar bestehend aus zwei longitudinalen Tumorgewebeproben zur Verfügung stellen können: • Eine Probe vor NACT und eine nach NACT für

	Patientinnen/Patienten mit eBC • Eine Probe vor und eine nach Krankheitsprogression für Patientinnen/Patienten mit mBC 3. Weibliche Patientinnen wie männliche Patienten ≥ 18 Jahre alt 4. Teilnahme ist unabhängig von histologischen Eigenschaften (duktale, lobuläre, andere Differenzierungen) und unabhängig vom Brustkrebssubtyp (luminal A, luminal B, HER2-positiv, TNBC) möglich 5. Unterschriebene Einverständniserklärung mit Einwilligung zur Sammlung klinischer Daten und Biomaterialien 6. Die Teilnahme an anderen, auch interventionellen Studien ist möglich.
Ausschluss-kriterien	1. Patientinnen/Patienten mit anderer maligner Vorerkrankung sind ausgeschlossen mit den folgenden Ausnahmen: a. Erkrankungsfreiheit von mindestens 5 Jahren (letzte erkrankungsbezogene Untersuchung muss innerhalb von 3 Monaten vor Studieneinschluss erfolgt sein) und die die Primärerkrankung muss ein niedrig Risiko Profil aufweisen b. Carcinoma in situ der Zervix Uteri, Basalzellkarzinom oder Plattenepithelkarzinom der Haut 2. Patientinnen/Patienten ohne histologische Sicherung der Brustkrebsdiagnose 3. Patientinnen/Patienten mit eBC oder primär mBC, die noch keine Systemtherapie für die Brustkrebserkrankung erhalten haben 4. Eine Entnahme von Gewebeproben durch Biopsie oder Operation nicht möglich 5. Jegliche physische oder psychische Beeinträchtigung oder schwerwiegende Komorbiditäten, die die eine adäquate Studienteilnahme nicht erlauben 6. Bekannte, signifikante, neurologische oder psychiatrische Erkrankungen, die das Verständnis der Studienziele und die informierte Einwilligung in die Studie nicht zulassen.
Studien-maßnahmen	MOMENTUM ist eine nicht-interventionelle Registerstudie mit dem Fokus auf der Erfassung der Behandlung in der klinischen Routine und deren Korrelation mit molekularen Eigenschaften von Biopsien der klinischen Routine. Es werden nur Gewebeproben verwendet, die im Rahmen von Maßnahmen der Routine Versorgung (z.B. Biopsie bei Diagnosestellung, Operation, etc.) gewonnen wurden. Die Blutabnahmen für Vollblut- und Plasmaproben sollen zu Zeitpunkten von Blutabnahmen der Routine erfolgen. Es sind keine studienspezifischen Interventionen oder Untersuchungen vorgesehen. Der retrospektive Einschluss verstorbener Patienten mit sekundär mBC und Fernmetastasierung nach anfänglicher neoadjuvanter und/oder adjuvanter Therapie mit archivierten Biomaterialien und klinischen Daten ist in Einklang mit der “Empfehlung für die Bewertung forschungsbezogener Biobanken durch Ethik-Kommissionen empfohlen vom Arbeitskreis Medizinischer Ethik-

	Kommissionen Version 2.1. gemäß Beschluss vom 24.6.2022“ möglich.
Patientenanzahl	Circa 2000
Follow-up	Das Follow-up beträgt 5 Jahre
Zentren	Geplant circa 50 Studienzentren in Deutschland
Finanzierung	Die Finanzierung der Studie wird durch die GBG Forschungs GmbH sichergestellt

6 Synopsis

Study Title	Molecular Mechanisms of Therapy resistant breast cancer (MOMENTUM)
Coordinating Investigator	Prof. Dr. Sibylle Loibl
Study Background	Despite recent therapeutic advances, early breast cancer (eBC) patients without pathologic complete response (pCR) after neoadjuvant chemotherapy (NACT) still have a high risk of relapse. Especially patients with triple-negative breast cancer (TNBC) and/or other biological or clinical high-risk features have a worse prognosis. In the advanced breast cancer setting, patients usually received multiple treatment lines to face tumor progression. However, each subsequent therapy line is less effective than earlier lines in prolonging patients’ progression-free survival, and drug resistance develops. One of the reasons is the development of tumour heterogeneity. Next generation sequencing has contributed significantly to the better understanding of the tumour heterogeneity of breast cancer, which is linked to treatment resistance and outcome. Several gene mutations were discovered as likely drivers of disease progression (Stephens Nature 2012, Koboldt Nature 2012, Curtis Nature 2012, Arnedos Nat. Rev. Clin. Oncol. 2015). Further investigations are needed to deeply characterize breast cancer heterogeneity, in particular molecular alterations which occur during or after therapeutic pressure exerted through drug therapy. A better understanding of drug resistance mechanisms is crucial to the discovery of new potential treatment targets and the development of new therapeutic strategies with impact on survival outcomes. MOMENTUM is a prospective registry trial with a non-interventional and translational design that aims to follow-up patients with residual disease after NACT or patients with metastatic breast cancer (mBC) to record the administered post-(neo)adjuvant respective palliative therapies and to collect information from routine longitudinal biomaterial samples (tissue and blood) to systematically assess tumor heterogeneity features over time by molecular profiling and biomarker analyses.

Hypothesis	The analysis of the residual tumor after NACT in eBC and of metastasis in mBC is relevant for identification of molecular mechanisms of treatment resistance and treatment sensitivity as well as to the identification of potential new treatment targets.
Study Objectives	1. To assess tumor heterogeneity by analyzing longitudinal tumor samples. 2. To define biomarkers to predict resistance to systemic therapy, in the neoadjuvant, adjuvant and metastatic setting. 3. To describe the change in breast cancer subtype and in molecular markers after systemic therapy. 4. To identify new prognostic biomarkers associated with worse long-term outcome (e. g. disease-free, progression-free and overall survival). 5. To identify possible markers predictive for tumor sensitivity for post-neoadjuvant and palliative systemic therapies. 6. To identify new druggable targets which could be investigated for post-neoadjuvant and palliative treatment
Study Design	MOMENTUM is a prospective registry trial with a non-interventional and translational design. The study will collect clinical data on patients with eBC and residual disease after NACT, and at the time of relapse as well as on patients with mBC. Longitudinal tumor tissue samples taken during routine assessments (diagnosis, surgery, etc.) will be collected to assess changes in tumor heterogeneity over time. Additionally, blood samples will be collected at the time of patient enrollment and later when oncologic treatment change due to progression is indicated to monitor molecular mechanisms of progression on ctDNA level (during routine visits). The study aims to gain further insights into the genomic heterogeneity of breast cancer at critical timepoints of the disease and to analyze mechanisms of resistance to breast cancer treatment.
Inclusion Criteria	1. Patients with eBC and clinically gross residual tumor after NACT or distant recurrence of breast cancer during/after (neo-)adjuvant therapy or mBC with progression after at least one line of therapy for mBC. 2. All patients must be able to provide a pair of longitudinal tissue samples namely: • one sample before NACT and one sample after NACT for patients with eBC • one sample before and one sample after disease progression occurred for patients with mBC 3. Female and male breast cancer patients aged ≥ 18. 4. Participation is allowed independently of breast cancer histology (ductal, lobular, others) and of breast cancer subtype (Luminal A, luminal B, HER2-

	positive, triple-negative) but will focus on high-risk tumors such as TNBC and luminal B. 5. Informed consent for data collection and biomaterial collection. 6. Participation in other interventional clinical trials is allowed.
Exclusion Criteria	1. Patients with a history of any malignancy beside breast cancer are ineligible with the following exceptions: a. Disease-free for at least 5 years (last assessment must have been performed within 3 months before inclusion) and the features of the primary tumor are considered low risk. b. CIS of the cervix, basal cell and squamous cell carcinomas of the skin. 2. Patients with no histological verification of the diagnosis of breast cancer. 3. Patients with eBC or de novo mBC that have not received any systemic therapy. 4. Tissue acquirement either by biopsy or during surgery not possible. 5. Any physical or mental condition or severe comorbidities that would compromise the adequate cooperation of the patient. 6. History of significant neurological or psychiatric disorders that would prohibit the understanding of the study purpose and giving of informed consent.
Study Procedures and Examinations	MOMENTUM is a non-interventional registry trial with a focus on real world patients. Clinical information on oncologic/medical history are collected at baseline, and follow up information on subsequent cancer therapies and oncologic outcome will be collected. To analyze association of molecular parameters with the clinical outcome, only tissues will be collected that were obtained during routine procedures (e. g. diagnostic core biopsy, surgery, etc.). The blood collection for whole blood and plasma sampling should be scheduled during routine visits. No study-specific interventional procedures or examinations are planned. The retrospective inclusion of deceased patients with secondary mBC and distant progression after initial neoadjuvant and/or adjuvant therapy and archived residual biomaterial and clinical data is possible in accordance with the “Empfehlung für die Bewertung forschungsbezogener Biobanken durch Ethik-Kommissionen empfohlen vom Arbeitskreis Medizinischer Ethik-Kommissionen Version 2.1. gemäß Beschluss vom 24.6.2022“
Patients	Approx.2000
FU	Patients will be followed-up for 5 years.
Sites	Ca. 50
Funding	The funding will be guaranteed by the GBG Forschungs GmbH.

7 Background

7.1 Introduction

Breast cancer in female patients has been the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases. Breast cancer is the fifth leading cause of cancer mortality worldwide, with 685,000 deaths.[1] Five intrinsic breast cancer subtypes have been identified in the early 2000s based on different gene expression patterns and are currently used to select different targeted treatments.[2,3]

In eBC, neoadjuvant chemotherapy (NACT) is a potential standard treatment option whenever chemotherapy is considered. A meta-analysis of nine randomized studies found that neoadjuvant and adjuvant therapy are equivalent in terms of survival and overall disease progression.[4] However, the neoadjuvant approach allows for an in vivo sensitivity testing and permits to identify tumors, that are resistant to standard treatment. That way, patients who can potentially benefit from further therapy after surgery can be selected as well.[5,6,7] Patients with a pathologic complete response (pCR) after NACT have a better prognosis than those without a pCR. The Food and Drug Administration (FDA) conducted a meta-analysis of 11,955 patients treated in twelve randomized NACT trials. Results showed that pCR in breast and axilla was significantly associated with improved event-free survival (EFS; ypT0 ypN0: HR 0.44; 95% confidence interval (CI) 0.39-0.51) and overall survival (OS; HR 0.36; 95% CI 0.30-0.44). This correlation was more pronounced in patients with aggressive breast cancer subtypes such as TNBC (HR [EFS] 0.24; HR [OS] 0.16) and in those with HER2-positive, HR-negative tumors who received trastuzumab (HR [EFS] 0.15; HR [OS] 0.08). The GBG meta-analysis conducted prior to the FDA analysis, demonstrated similar results. There was no correlation between pCR and EFS, but certain trials of the GBG such as the GeparSixto and GeparSepto trials demonstrated that an increase in the pCR rate can translate to an improved iDFS on a trial level.[8,9,10,11]

Within the group of patients not achieving a pCR after NACT, prognosis can vary greatly, but is generally less favorable. A more precise information regarding prognosis is needed to facilitate decision making regarding adjuvant treatment strategies. A potential strategy to improve the outcome of patients without pCR after NACT, is the administration of additional therapy after surgery (post-neoadjuvant therapy).[12, 13,14,15,16,17,18] Despite the available treatment options, patients without pCR after NACT, especially having triple negative breast cancer (TNBC) or hormone receptor-positive BC with high CPS-EG score, still have a high risk of relapse but limited treatment options. The analysis of the residual tumor after NACT might help in understanding the mechanism of therapy resistance, identifying new markers of treatment sensitivity and new treatment targets allowing to better allocate patients in need for further treatment after NACT.

However, despite considerable advances in the treatment of eBC, treatment selection for patients, who did not achieve a pCR after NACT remains a clinical challenge. Available treatment agents from the metastatic setting (e.g., platinum salts, capecitabine, immune checkpoint inhibitors, cyclin-dependent kinase 4/6 inhibitors, PARP inhibitors, antibody-drug-conjugates) are emerging options as post-neoadjuvant strategies. Some of these medications are indicated in clearly defined situations, and even when selected properly, not effective for a great part of those patients. Based on these premises, selection criteria are critical to identify patients who may benefit from post-neoadjuvant

therapies, through the validation of prognostic and predictive biomarkers for a reliable risk assessment and estimation of benefit.
In the advanced breast cancer setting, patients usually receive multiple treatment lines, as consequence of tumor progression. However, each subsequent therapy is less effective in prolonging patients' progression free survival as drug resistances accumulate. Further investigations are of paramount importance to characterize breast cancer heterogeneity in therapy-resistant tumors and hopefully lead to a better understanding of resistance mechanisms and eventually to new target molecules.

7.2 Breast cancer heterogeneity and drug resistance

Several mutations were identified to be likely drivers of disease progression. The International Cancer Genome Consortium (ICGC) examined the genomes of 100 breast cancer tumors and revealed their substantial heterogeneity. Putative driver mutations were discovered in several new cancer genes including *AKT2, ARID1B, CASP8, CDKN1B, MAP3K1, MAP3K13, NCOR1, SMARCD1* and *TBX3*.[19] The Cancer Genome Atlas (TCGA) showed that high frequency somatic mutations were relatively few.[20] In early breast tumors single nucleotide variations or genomic alterations (i.e. amplifications, deletions) in known breast cancer genes such as *PIK3CA, TP53, GATA3, MAP3K1, MLL3, CDH1* and *PTEN* were found.[20] Additional mutated genes were identified, such as *CCND1, CCNE1, MDM2, CDKN2A, RB1* and *FBXW*.[21,22] Furthermore, the role of the PI3K/AKT/mTOR, p53 and CCND1/CDK4/6/Rb signaling pathways activation was defined.[25,23] All the analyses highlighted the heterogeneity of breast cancer, as assumed cause for the different treatment response and prognosis.

SATURN[3] is a multidisciplinary consortium aiming to characterize intra-tumoral heterogeneity and its spatiotemporal evolution with single cell resolution longitudinally in patients using innovative tissue sampling schemes, then functionally explore the underlying mechanisms driving therapy resistance and metastasis, and eventually validate biomarkers and novel therapeutic strategies within clinical settings. Three high prevalent tumor entities will be analyzed, harboring common biological principles: Triple negative and luminal-B breast cancer, colorectal cancer, and pancreatic ductal adenocarcinoma. The data generated from these tumors will provide the basis for identifying generalizable rules and mechanisms of tumor evolution. To ensure identification of promising targets for clinical use, SATURN[3] will take advantage of samples collected within the CATCH and COGNITION trials conducted under the responsibility of the university of Heidelberg. COGNITION is a prospective, non-interventional register and translational diagnostic study aiming to assess clinical features, genomics and molecular markers to identify patients with eBC for enrolment on marker driven trials. CATCH serves as companion program for COGNITION. While COGNITION focusses on eBC patients, CATCH focuses on mBC. Therefore, patients presenting initially eBC (enrollment in COGNITION) might be also enrolled into CATCH in case they present with metastatic disease throughout the course of the diseases.

MOMENTUM will collect samples that will be analyzed within SATURN[3]. MOMENTUM is a prospective registry trial with a non-interventional and translational design aiming to follow-up patients with residual disease after NACT or metastatic disease to record the administered post-(neo)adjuvant and palliative therapies and collect the information from longitudinal routine biomaterial samples (tissue and blood) to systematically assess tumor heterogeneity features over time by molecular profiling and biomarker analyses. Additionally, mechanism of treatment resistance

will be analyzed. Therefore, patients are included in MOMENTUM at the time of disease-progression after neoadjuvant, adjuvant or metastatic therapy, and longitudinal samples before and after therapy are collected. Clinical information on oncologic/medical history are collected at baseline, and follow up information on subsequent cancer therapies and oncologic outcomes will be collected. To analyze association of molecular parameters with the clinical outcome, only tissues will be collected that were obtained during routine procedures. The new molecular approach might enhance the precision of treatment decisions. Optimal allocation to existing post-neoadjuvant/palliative treatment options on an individual basis is one possible achievement, discovery of innovative targets for post-neoadjuvant/palliative treatments and foremost identification of existing drug resistances thereby safeguarding patients from non-beneficial yet detrimental therapies would be most desirable.

8 Study Objectives

1. To assess tumor heterogeneity by analyzing longitudinal tumor samples.
2. To define biomarkers to predict resistance to systemic therapy, in the neoadjuvant, adjuvant and metastatic setting.
3. To describe the change in breast cancer subtype and in molecular markers after systemic therapy.
4. To identify new prognostic biomarkers associated with worse long-term outcome (e. g. disease-free, progression-free survival and overall survival).
5. To identify possible markers predictive for tumor sensitivity for post-neoadjuvant and palliative systemic therapies.
6. To identify new druggable targets which could be investigated for post-neoadjuvant and palliative treatment.

9 Study Design

MOMENTUM is a prospective registry trial with a non-interventional translational design. The goal of the trial is to obtain longitudinal biomaterial pairs (at least two tumor samples) representing different clinical situations, to assess molecular changes during disease progression. Eligible early breast cancer patients should preferably be enrolled in the study prior to surgery (after NACT) to allow for collection of fresh tumor tissue in addition to formalin-fixed paraffin embedded (FFPE) breast tumor tissue. Patients might also be enrolled after surgery to provide FFPE tumor tissue only.
Patients with metastatic breast cancer may be enrolled only if they have received oncologic treatment and have experienced tumor progression during/after this therapy irrespective of the clinical situation in which therapy was applied (neoadjuvant/adjuvant or metastatic setting). Clinical information on oncologic/medical history are collected at baseline,and follow up information on subsequent cancer therapies and oncologic outcomes will be collected. To analyze association of molecular parameters with the clinical outcome, only tissues will be collected that were obtained during routine procedures. Archived samples from the primary tumor (untreated and/or after NACT) will be collected to assess molecular changes during tumor progression via longitudinal comparisons.

10 Study Population

10.1 Inclusion Criteria

1. Patients with eBC and clinically gross residual tumor after NACT or distant recurrence of breast cancer during/after (neo-)adjuvant therapy or mBC with progression after at least one line of therapy for mBC.
2. All patients must be able to provide a pair of longitudinal tissue samples namely:
   - one breast tumor sample before NACT and one after NACT for patients with eBC
   - one sample before (breast tumor or metastasis) and one sample after (metastasis) disease progression occurred for patients with mBC.
3. Female and male breast cancer patients aged ≥ 18.
4. Participation is allowed independently of breast cancer histology (ductal, lobular, others) and of breast cancer subtype (Luminal A, luminal B, HER2-positive, triple-negative).
5. Informed consent for data collection and biomaterial collection.
6. Participation in other interventional clinical trials is allowed.

10.2 Exclusion Criteria

1. Patients with a history of any malignancy beside breast cancer are ineligible with the following exceptions:
   a. Disease-free for at least 5 years (last assessment must have been performed within 3 months before inclusion) and the features of the primary tumor are considered low risk.
   b. CIS of the cervix, basal cell and squamous cell carcinomas of the skin.
2. Patients with no histological verification of the diagnosis of breast cancer.
3. Patients with eBC or de novo mBC that have not received any systemic therapy.
4. Tissue acquirement either by biopsy or during surgery not possible.
5. Any physical or mental condition or severe comorbidities that would compromise the adequate cooperation of the patient.
6. History of significant neurological or psychiatric disorders that would prohibit the understanding of the study purpose and giving of informed consent.

11 Collection of Biomaterials

11.3 Patient recruitment and consenting

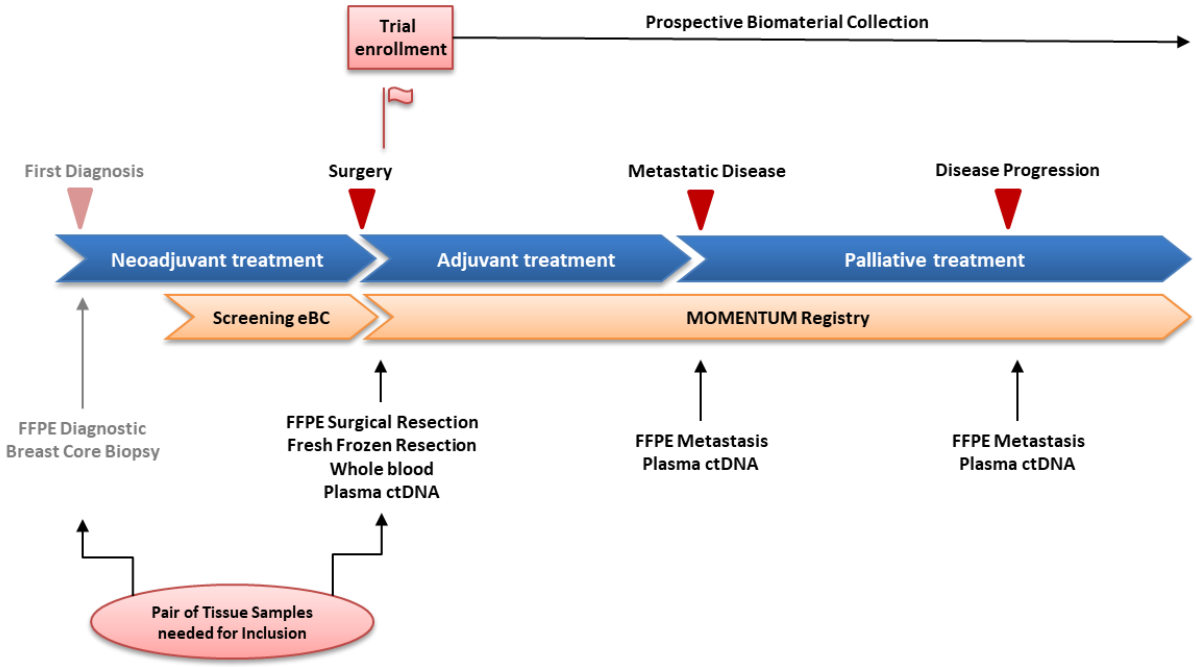
Patients will be screened for eligibility during interdisciplinary oncologic conferences (preoperative or metastatic) at the participating sites. During consulting with the treating physician/investigator the purpose of the study, obligations, risks and potential benefits will be discussed with the patient. After a check for compliance with the inclusion/exclusion criteria, written informed consent will be obtained. Details for informed consenting are described in part 16.4.

The retrospective inclusion of deceased patients with secondary mBC and distant progression after initial neoadjuvant and/or adjuvant therapy and archived residual biomaterial and clinical data is possible in accordance with the “Empfehlung für die Bewertung forschungsbezogener Biobanken durch Ethik-Kommissionen empfohlen vom Arbeitskreis Medizinischer Ethik-Kommissionen Version 2.1. gemäß Beschluss vom 24.6.2022“.

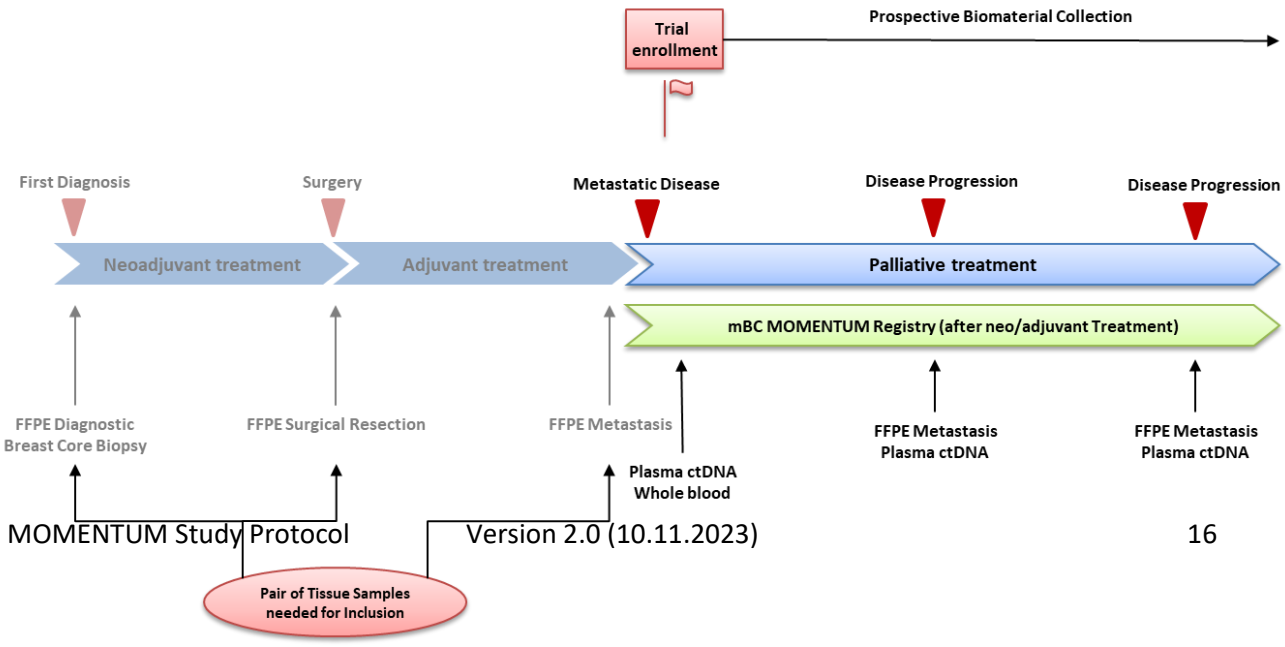
The use shall be restricted to breast cancer research. The identification of molecular markers for drug resistance has the power to protect patients from ineffective but side-effect-prone therapies, to identify potentially superior therapies and to optimize the allocation of resources in the healthcare system. Hence the scientific interest in identifying these mechanisms represents an enormous value that serves the common good of health care research, the interests of individual patients as well as the population and society as a whole.

11.1 Overview

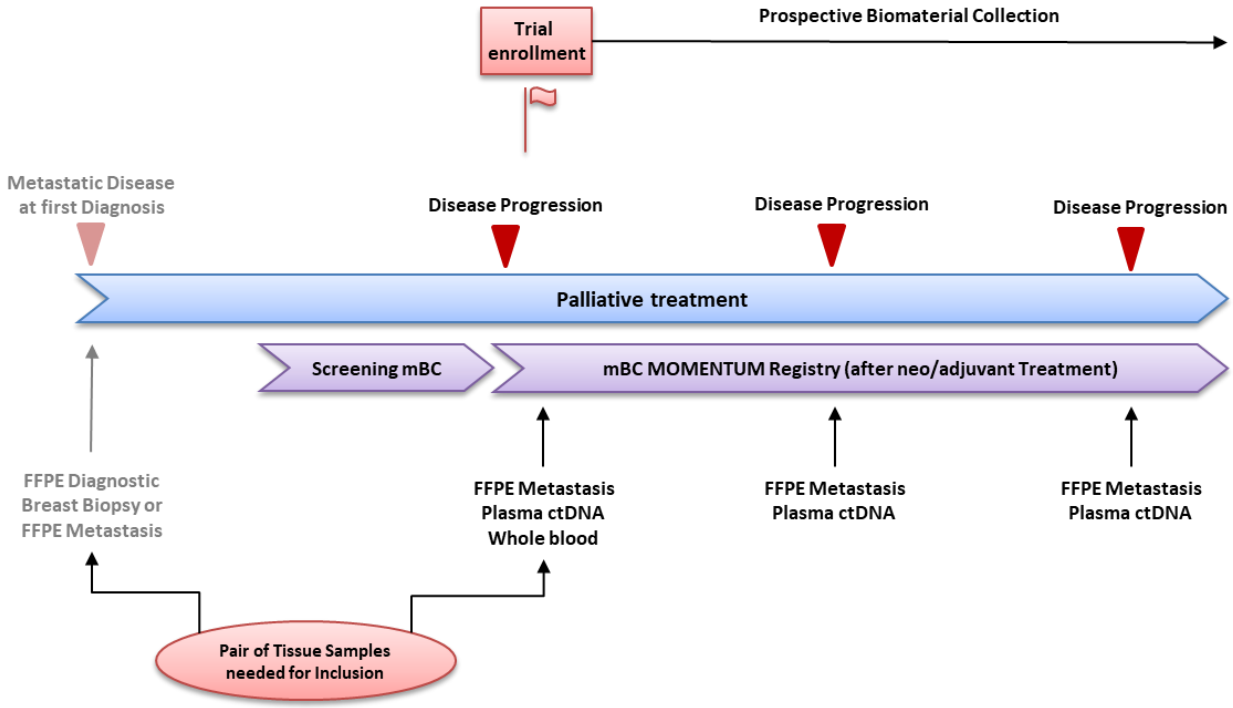
11.1.1 eBC patients with clinically gross residual tumor after neoadjuvant chemotherapy



11.1.2 Enrollment of patients with secondary mBC and distant progression after initial neoadjuvant and/or adjuvant therapy



11.1.3 Enrollment of patients with de-novo mBC and progression after at least 1 line of metastatic therapy



11.2 Tumor Tissue

Early breast cancer:

- FFPE breast tumor tissue must be collected from primary tumor, before NACT and at surgery after NACT. If recurrence occurs, FFPE from metastasis will be collected, whenever feasible. If no biopsy of first recurrence is possible/available, then also material from later recurrences may be collected.
- Optional collection: Fresh tumor tissue from the residual tumor should be collected at surgery. The collected tissue needs to be shock frozen in liquid nitrogen and then stored at -80°C or in liquid nitrogen. GBG will organize transportation of the fresh frozen tissues to the GBG biobank.
- To note: Tissue of lymph nodes may be sent additionally to breast tumor tissue, if available

Metastatic breast cancer:

- After neoadjuvant therapy: FFPE from metastasis must be collected; additionally, FFPE from core biopsy of the primary breast tumor prior to NACT and/or surgical tissue in case of

residual tumor after NACT will be collected. FFPE from metastasis of following biopsies during disease progression will be collected whenever feasible.

- After adjuvant therapy: FFPE from metastasis must be collected; additionally, FFPE from primary breast tumor (core biopsy or surgical tissue) will be collected. FFPE from metastasis of following biopsies during disease progression will be collected whenever feasible.
- In de novo mBC FFPE from metastasis and or breast tumor prior to any therapy and after progression after at least one line of systemic treatment must be collected. FFPE from metastasis of following biopsies during disease progression will be collected whenever feasible.
- To note: Tissue of lymph nodes may be sent additionally to breast tumor tissue, if available.

11.3 Blood

- 10 mL EDTA blood will be collected after enrollment.
- 20 mL EDTA blood will be collected after enrollment, and at the beginning of subsequent therapy lines (if oncologic treatment was changed due to disease progression) for plasma preparation and subsequent analysis of circulating tumor DNA (ctDNA). Especially in case of biopsy of metastasis during treatment progression a timely EDTA blood sample should be collected.

11.4 Storage and Logistics of Biomaterial

- FFPE tissue will be sent to the GBG Biobank at Marburg (Prof. Denkert, Institute of Pathology, UKGM Marburg).
- Fresh Frozen Tissue will be stored at -80°C at the site, until shipment is organized by GBG.
- EDTA blood and plasma will be stored at -20°C/-80°C at the site, until shipment is organized by GBG.

12 Discontinuation patient, site or study

12.1 Treatment Discontinuation of Individual Patient

If a patient shows one of the following reasons the study participation must be discontinued:

- Patient's death
- Patient's request or non-compliance.

The reason and date of discontinuation for all patients will be documented on the eCRF (e.g. death, withdrawal of consent, lost to follow-up, etc.).

12.2 Premature Termination of Study at a Particular Site

The GBG Forschungs GmbH as the sponsor has the right to close this study at a particular study site which may be due to but not limited to the following reasons:

- Non-compliance with the protocol
- Insufficient number of recruited patients
- Inadequate co-operation with GBG Forschungs GmbH or its representatives
- The Investigator requests to close of his/her study site
- Quality issues

12.3 Premature Termination of the study

The GBG Forschungs GmbH as the sponsor has the right to close this study, e.g., if the study conduct (e.g., recruitment rate; drop-out rate; data quality; protocol compliance) does not suggest a proper completion of the study within a reasonable time frame.

13 Statistics

This explorative and descriptive approach will generate novel hypotheses. The analyses will generate heterogeneous individual molecular datasets, which do not allow planning of classical statistical procedures. Hence, this explorative trial will not use a fixed framework of statistical assumptions, which determines sample size and power since there will be also no specific treatment arms at the beginning. Analysis will be descriptively.

14 Data Management

14.1 Data Management and Documentation

Data management will be carried out by the GBG Forschungs GmbH using a web-based EDC (Electronic Data Capture) system that is FDA 21 CFR Part 11 compliant. Data management activities include CRF design, database creation, data processing and data validation.

14.2 Data Entry and Queries

All CRF data will be entered into the trial database using a web-based EDC system, which will perform automated plausibility and value range checks before accepting the data into the database. CRF data will be reviewed; data fields that do not match the trial guidelines will be queried. These queries are forwarded to the center for resolution. The data will be checked by a data manager and either closed or re-queried.

14.3 Data Validation

Visual and computerized methods of data validation are applied in order to ensure accurate, consistent and reliable data.

14.4 Pseudonymization

In order to protect patient data confidentiality and for safeguarding the privileged doctor patient relationship, each participating patient is assigned a unique GBG reference number. Instead of the true patient identity the pseudonym is used in all communication between the trial site and the GBG

Forschungs GmbH. Each patient will receive a study subject ID, for which a subject log needs to be stored at the site.

14.5 User Access Control

Every user is provided with a unique personal username and unique password which defines their access rights as well. Access control is based on the User Role in the web-based EDC system. Therefore, users can only access and amend those datasets necessary for them to fulfil their tasks.

14.6 Data Collection

The following data will be collected:

- Baseline demographic and tumor characteristics
- Neoadjuvant Chemotherapy
- Surgery
- Post-neoadjuvant therapy
- Adjuvant therapy
- Clinical follow up on oncologic medications for treatment of metastasis and survival
- Biomaterial data (biopsies, blood samples)

15 Follow up

Data will be collected within the MOMENTUM study during recruitment phase. After LPI the data collection will be continued for all included patients for 5 years.

16 Legal and Ethical Considerations

16.1 Declaration of Helsinki

This study is to be performed in accordance with the Declaration of Helsinki (Fortaleza, October 2013).

16.2 Beratung nach §15 Berufsordnung der Bundesärztekammer

The study will be assessed by the Ethik-Kommission der Landesärztekammer Hessen, Hanauer Landstraße 152, 60314 Frankfurt. The study will only be started after being approved by the ethics committee.

16.3 Funding

The funding will be guaranteed by the GBG Forschungs GmbH. Additionally, the study will be supported by funding from Deutsche Krebshilfe (INTEGRATE-TN) and BMBF (SATURN3).

16.4 Patient Informed Consent and Patient Insurance

Prior to enrollment and tissue/blood sample collection according to protocol, the patient is informed about the intended purpose, possible benefits, and possible adverse experiences.

An approved informed consent (ICF) statement will then be read and signed by the patient, and, if required, a witness, as well as the investigator. The patient will be provided with a copy of the signed ICF. The patient may withdraw from the study at any time in any way without prejudicing future medical treatment.
Patients are informed that pseudonymized data from their case may be stored electronically and that such data will not be revealed to any unauthorized third party. Data will be reviewed by the monitor, an independent auditor and possibly by representatives of regulatory authorities and/or ethics committees. The terms of the local data protection legislation will be applied as appropriate.

A patient insurance for the compensation of patients for possible study-related injury is not required as no study specific interventions are planned. Only biomaterial, which is collected during standard of care procedures, will be acquired and used for study specific analyses.

17 Publication

Publication will be performed according to the SOPs of the GBG Forschungs GmbH.

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