SYNOPSIS

Study Title	AURORA: Aiming to Understand the Molecular Aberrations in Metastatic Breast Cancer
Study number	BIG 1-14
Sponsor	Breast International Group (BIG)
Indication	Patients with locally recurrent/advanced breast cancer not amenable to treatment with curative intent or metastatic breast cancer (MBC)
Treatment	None
Study Type	Exploratory
Background and Rationale	The current era of molecular oncology offers the technology to characterize, at the base pair level, the complete molecular landscape of cancer. This heralds great promise with regards to understanding driving genetic aberrations, elucidating tumor genetic heterogeneity, discovering new therapeutic targets, and ultimately improving outcomes for cancer patients. For BC in particular, recent studies using massively parallel sequencing have uncovered a large number of candidate "driver" mutations that occur at a low frequency. In some cases, these driver mutations and/or other molecular aberrations are potentially targetable by agents currently approved in the clinical settings or in various stages of clinical development. There is increasing evidence to demonstrate that BC metastases often acquire new molecular aberrations compared to their matched primary tumors, and that different treatment-resistant clones may emerge over time. While the clinical relevance of these phenomena is not yet well understood, obtaining biopsies from the metastatic lesions could help uncover mechanisms of resistance and thus help refine treatment decisions. Furthermore, a non-invasive method using circulating tumor deoxyribonucleic acid (ctDNA), harboring somatic mutations and gene copy number aberrations, has emerged and is being developed as a biomarker with a quantitative correlation to tumor burden and response to therapy. It also holds the promise for monitoring patients, tracking acquired resistance to therapies and offering the possibility of early therapeutic interventions. Finally, ctDNA analysis could help

	capture the spatial and temporal genomic heterogeneity already demonstrated in metastatic BC. There is currently an exponential growth of molecular screening initiatives, at the national, single hospital or even at the private laboratory level, aimed at sequencing tumor DNA from BC patients in order to identify "actionable mutations" that could be targeted in the clinical setting. However, such isolated approaches have major limitations as they generate fragmented results that might lose their potential and impact if not contextualized in a proper, structured clinical setting. Moreover, the use of modern techniques is likely to result in BC being further reclassified into smaller molecular subpopulations. Clinical trials for these molecularly defined small subpopulations are likely to require international collaboration in order to meet recruitment objectives. In order to exploit the potential of tumor molecular characterization, the Breast International Group (BIG) has set up AURORA - a large, multinational, collaborative MBC molecular screening program.
Objectives	The objectives of the Program are: 1. To improve the understanding of locally recurrent/advanced BC and MBC by using high-throughput technologies on primary, metastatic, as well as plasma ctDNA samples, to explore tumor heterogeneity, clonal evolution and transcriptional changes associated with mutational and copy number variation (CNV) patterns. 2. To discover biomarkers of response and/or resistance to systemic therapy using genomic and transcriptomic data of "exceptional responders" and "rapid progressors" (collectively referred to as "outliers", as defined in the AURORA protocol, section 3). 3. To provide evidence that can contribute in assessing the feasibility of implementing a global molecular screening platform of MBC. 4. To identify patients with candidate driver alterations in their tumors that can be matched to biomarker-driven clinical trials.

	5. To build new therapeutic hypotheses based on findings generated by Targeted Gene Sequencing (TGS). 6. To evaluate the prognostic relevance of genomic alterations detected in plasma ctDNA samples, tumor metastatic biopsies and archived primary tissue. 7. To correlate molecular alterations in patients with the efficacy endpoints (response rate, progression-free survival and overall survival).
Study Population	Patients with locally recurrent/advanced BC not amenable to treatment with curative intent or MBC, either newly diagnosed or treated with no more than one line of systemic therapy in the metastatic setting from several European hospitals affiliated with groups from the BIG network will be followed within the context of the AURORA program. One thousand (1000) patients with successfully analyzed primary tumor and/or metastatic lesion will be included in the AURORA program.
Inclusion criteria	1. Female or male with diagnosis of locally recurrent/advanced BC not amenable to treatment with curative intent or MBC. 2. Eastern Cooperative Oncology Group (ECOG) Performance Status 0 or 1. 3. Written informed consent prior to registration into the program. 4. Patient aged ≥ 18 years. 5. Availability of primary tumor tissue for research purposes. 6. Patient must have a metastatic lesion accessible for biopsies and must agree with the biopsy procedure. Both FFPE and fresh frozen samples are mandatory for inclusion. Brain tissue is accepted if provided through surgical excision not planned for the AURORA program, but as part of the routine clinical practice. Up to 100 patients with bone-only disease will be included without a metastatic biopsy provided that a plasma sample is collected at registration in the program for ctDNA analysis and that the patient meets all other eligibility criteria including the availability of primary tumor tissue. Of note, no metastatic lesion biopsies will be collected for patients with bone-only disease. 7. The biopsy of the metastatic lesion must be conducted either at the initial diagnosis before the initiation of 1st line systemic therapy of the BC relapse or at the 1st disease progression before initiation of a second line systemic treatment. Biopsies obtained as part of routine clinical practice are accepted if both

	formalin-fixed paraffin-embedded (FFPE) and Frozen Tissue (FT) blocks were collected concurrently from the same metastatic lesion and if collected at the pre-specified timelines. 8. Availability of a whole blood, serum and plasma samples collected at the time of screening. 9. There is no restriction in the type of therapeutic modality considered as 1st line systemic treatment, which can consist of any type of treatment administered after the diagnosis of the BC relapse till the 1st disease progression thereafter. 10. Patient agrees to provide blood samples at regular intervals, both at screening as well as during the follow-up (FU) phase of the program.
Exclusion criteria	1. The patient has received more than 1 line of systemic therapy (any type) in the metastatic setting. 2. Patients who have received prior palliative radiotherapy to the only site that is accessible to biopsy. 3. Bone biopsy as the only available metastatic sample. Note that up to 100 patients with bone-only disease will be accepted without a metastatic biopsy provided that a plasma sample is collected at screening in the program and that the patient meets all other eligibility criteria. 4. Presence of severe hematopoietic, renal, and/or hepatic dysfunction, including but not restricted to albumin < 3 g/dl. 5. Known increased risk of hemorrhage during biopsy procedure, as evaluated by the treating physician. 6. Previous or current malignancies of other histologies within the last 5 years, with the exception of in situ carcinoma of the cervix, and adequately treated basal cell or squamous cell carcinoma of the skin.
Program workflow	• Candidate participants will sign the AURORA Informed Consent Form (ICF). • The local Principal Investigator (PI) or his/her designee will access the secure AURORA Molecular Screening IT Platform, register the patient and enter baseline patient clinical data necessary for the verification of the eligibility criteria of the program.

	• If the patient meets the eligibility criteria of the program, the secure AURORA IT platform will then provide instructions related to biosamples collection and transportation. • Mandatory sample collection at program entry: 1. Two core needle biopsies [no Fine Needle Aspiration (FNA) accepted] or an excisional biopsy (surgical excision of metastatic lesion) from one metastatic tumor (either collected prospectively or obtained during routine procedures). One biopsy should be frozen (at -80°C) in Optimal Cutting Temperature (OCT) compound, while the other should be submitted as FFPE block. In case the metastasis is excised, a part of the tumor should be frozen in OCT and FFPE tissue should be prepared from the remaining part. If an entire FFPE block cannot be submitted, FFPE sections are acceptable (25 sections of 5µm each). It is strongly recommended that an entire FFPE block be submitted in order to ensure that sufficient tumor material is available. Please note that FFPE and frozen tissue samples must have been taken from the same lesion at the same time to minimize heterogeneity. Metastatic biopsies should be performed either at the relapse diagnosis or at the time of 1st disease progression before starting a new treatment line. The choice of the site is left to the judgment of the treating team, who will assess the feasibility of the procedure according to the accessibility and volume of the metastasis. The biopsy will be carried out in accordance with local recommendations. Of note, archived metastatic tumor tissue is allowed, provided the biopsy/excision was performed up to 6 months prior to patient's registration in AURORA, both FFPE and frozen tissue blocks are available and were collected concurrently from the same metastatic lesion at the same time; such metastatic tumor tissue must have been taken either at the time of disease recurrence or at the time of first disease progression before having started a new treatment line. Up to 100 patients with bone-only disease will be included without a metastatic biopsy provided that a plasma sample is collected at registration together with the primary tumor sample in the program and that the patient meets all other eligibility criteria. 2. Whole blood (9 ml) collection. Whole blood should be stored (at -80°C) within one hour after collection.

	3. Plasma (2 X 9ml of whole blood) collection. Plasma should be prepared immediately (within 1 hour) after drawing whole blood, and stored (-80°C) immediately after preparation. 4. Serum (1 X 9 ml of whole blood) collection. Serum should be prepared immediately (within 1 hour) after drawing whole blood, and stored (-80°C) immediately after preparation. 5. An (archived) FFPE block of the primary tumor (collected at the time of diagnosis or definitive surgery). If an entire FFPE block cannot be submitted, FFPE sections are acceptable (25 sections of 5µm each). It is strongly recommended that an entire FFPE block be submitted in order to ensure that sufficient tumor material is available. Of note, for patients undergoing neoadjuvant therapy, a block containing the diagnostic pre-treatment biopsy is the preferred option. If this sample is not available, a sample from residual disease is acceptable. On the contrary, a block containing invaded lymph node(s) is not acceptable. • The local PI or his/her designee will record the collection of these biosamples in the secure AURORA IT platform and dispatch them (primary tumor, metastatic lesion (FFPE and fresh frozen), whole blood and some plasma) to the Central Laboratory (CL). Two aliquots from the screening plasma samples will be sent to the CL with the other mandatory samples. The remaining screening plasma and all serum aliquots will be stored on site and will be sent in batches every year to the AURORA bio-repository. • At the CL, tumor cellularity and composition will be checked on the FFPE (from both primary and metastatic tissue) and frozen tissue samples. If tumor cellularity and composition reach the pre-established cut-off, histopathology review and Estrogen Receptor (ER), Progesterone Receptor (PgR), Human Epidermal Growth Factor Receptor 2 (HER2), and Ki67 staining will be performed on the FFPE samples, and results will be uploaded onto the secure AURORA IT platform If either the primary sample or metastatic tumor biopsy fails the molecular tests (either not enough tumor cellularity, not enough DNA of good quality or sequencing failure), the patient will remain eligible for AURORA provided that all mandatory samples were sent to the central lab. Patients will be considered as screening failures if all tissue samples fail the molecular tests. • DNA shall be extracted at the CL on a single sample basis from FFPE primary lesion, metastatic lesion (except for the 100 included

	patients with bone-only disease), plasma samples and from the whole blood. • Remaining frozen, FFPE, plasma and blood samples will be temporarily stored at CL, and periodically shipped in batches to the AURORA bio-repository (see below). • The CL will perform high coverage next-generation TGS of 411 cancer-related genes on the DNA extracted from primary and metastatic lesion, as well as whole blood. A high coverage next-generation TGS of 27 cancer-related genes on the DNA extracted from the screening plasma sample will also be performed. Patients could be considered as screening failures also at this stage, if technical problems occur during processing and/or if sequencing data of all tissue samples are not usable. The TGS results will be uploaded onto the secure AURORA IT platform. A germline aberration could be detected and in case the patient consented to the reporting of heritable genetic factors, they will be reported back through the AURORA IT platform. • An email alert is sent to the involved investigator when the molecular results are available for each included patient. The investigator will then log onto AURORA IT platform and obtain the results as a PDF report. Through the IT platform, the results of the TGS will be reported for all included patients, for those genes where an aberration is found. The aim is to have the results of the molecular tests available to the Investigator ideally within 15 working days from the reception of the samples at the CL. • A body of individuals with expertise in clinical oncology, genetics, genomics, molecular biology, bio-ethics and pathology will function as Molecular Advisory Board (MAB) that will advise on general reporting, will provide through the IT platform comments about patients' molecular aberrations and comments/information not meant to be comprehensive about eligibility for clinical trials assessing molecularly targeted agents and will answer patient and/or treating physicians-specific questions arising during the conduct of the program. A MAB Charter details the composition and responsibilities of the MAB. However, suitable resources must be available at each institution in order to complement and act upon reports made available on the IT platform.

	In case the patient consented to the reporting of heritable genetic aberrations, a germline aberration could be detected and reported back through the AURORA IT platform. Please note that these molecular results are generated in a research environment. The local PI should ensure that the results are confirmed in the context of genetic counseling using conventional/approved genetic tests, prior to the introduction of any clinical action (such as prophylactic surgery). Furthermore, the results should be confirmed before they are used for pre-symptomatic testing of unaffected relatives. • When the molecular test results are available to the local PI, he/she will discuss with the patient and either treat the patient as per local practice or enroll her/him into an available clinical trial, at that time or in the future, depending on trial specific eligibility criteria, and the patient's wishes. Those clinical trials will have their own approval process and patients will have to sign a study-specific ICF, provided they meet the trial's eligibility criteria. • Irrespective of whether the patient is treated with standard of care or enrolled in a clinical trial, he/she will be followed by the local PI and will have follow-up data collected every 6 months and at every new disease progression for determination of disease status and survival endpoints. • Clinical data will be collected and stored on a specific AURORA **electronic case report form** (e-CRF). • Irrespective of whether the patient is treated with standard of care or enrolled in a clinical trial, blood samples of 2 X 9 ml for plasma preparation and blood samples of 1 X 9 ml for serum preparation (in addition to the ones collected at the same time as metastatic biopsy or program entry) must be taken at the following time points: 1. Every 6 months +/- 1 month. 2. At every new disease progression before starting a new treatment line. • The biosamples and their derivatives (located at the CL) as well as the screening and follow-up plasma and serum samples (located at the sites) will be transferred on a regular basis (once per year) to the AURORA bio-repository, under the guardianship of the AURORA Steering Committee (SC). • Data and bio-specimens (and their derivatives) may be used for future research and additional molecular testing (whole

	exome/genome and RNA (ribonucleic acid), sequencing technologies), as allowed by the ICF signed by the patient. • Proposals for access to such samples and data will have to be approved by the AURORA SC as well as the appropriate Ethics Committee(s), following a procedure outlined in detail in a separate document called Policy on Access to Study Data and Biological Samples. This policy will be made available to all investigators/sites and Groups participating in AURORA. Please note that the molecular screening results obtained from AURORA are generated in a research environment. Results from the screening tests may eventually require confirmation by other validated techniques or diagnostic tests before any clinical action is taken. The local PI together with the patient will take the final treatment decision.
Research Aspects	To complement the TGS and RNA sequencing performed on all AURORA patients, additional molecular characterization of patients considered by the SC as outliers (i.e. either "exceptional responders" or "rapid progressors") will be performed. In the event that patients are on investigational therapy, only "exceptional responders" will be eligible for the in-depth molecular characterization of their biospecimens. Outlier patients shall undergo additional tests (including but not necessarily limited to Whole Exome Sequencing (WES) on primary and metastatic lesions, plasma and whole blood).
Safety	A Data Monitoring Committee will be established to provide periodical safety assessment of adverse events due to metastatic lesion biopsy procedure in order to safeguard the interest and safety of the participating patients. The membership, key responsibilities of the DMC and the specific procedures to be followed will be defined in a DMC Charter.
Study duration	Recruitment period will be assessed according to the yearly patient accrual. Inclusion in AURORA will be based on competitive recruitment from the participating centers. Follow-up: performed on a patient basis, until death or 10 years or withdrawal of consent or lost to follow-up, whichever occurs first.