Prospective Breast Cancer Biobanking

(PBCB)

Project description -

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Long-term molecular monitoring of operable breast cancer patients

Project description

Content

1. Introduction

1.1 Main challenges in breast cancer

Breast cancer remains the most frequent female malignancy in the Western world[1]. Although the incidence still increases, mortality has declined the last two decades due to early detection by screening programs and improved adjuvant systemic treatment[2]. Presently, treatment algorithms comprise surgery and radiation combined with adjuvant systemic treatment, which consists of chemotherapy, endocrine and targeted biological modalities directed towards potential systemic spread of cancer cells[3]. The clinical decision making process is based upon stratification of patients into high-risk and low-risk groups, based on various clinical and tumor-biological markers measured in the resected primary tumors[4,5]. According to this risk assessment, high-risk patients are currently offered adjuvant systemic chemotherapy[3]. Despite this, both early (< 5 years after diagnosis) and late relapses (> 5 years after diagnosis) occur, and as a consequence 586 women died from breast cancer in Norway in 2017[2]. Thus, there is a need for more accurate diagnostics to guide treatment and more sensitive methods for detection of both early and late recurrences. Primary tumor assessment restricted to thin tissue sections do not necessarily reflect the characteristics of residual cancer cells, either because of primary tumor heterogeneity or evolutionary changes in residual tumor cells during therapy[6]. Thus, a more comprehensive assessment of the tumor cell phenotype both before treatment, during therapy (residual tumor material) and in the subsequent 10-year follow-up window may enhance administration and personalization of systemic treatment.

Current follow-up during and after treatment is primarily focused on detection of local relapse by various imaging methods and by clinical examination[3]. Unfortunately, high-resolution imaging technologies can only detect larger groups of tumor cells (i.e. 1-5 mm) and not minimal residual disease or micrometastases[7]. Thus, it is a paradox that current practice does not include searches for early systemic relapse even though the distant metastatic lesions will kill the patient if they become clinically overt. In this respect, there is a need for new and enhanced biomarkers to sensitively monitor the disease, with the intention of detecting disease recurrence much earlier. New or prolonged systemic treatment (i.e. chemotherapy or endocrine therapy) may be adapted accordingly. Recent evidence suggests that residual tumor cell material can be found in peripheral blood samples and provide the requested biomarkers, as described in further detail below (recently reviewed[8]).

1.2 Circulating tumor DNA

Circulating tumor DNA (ctDNA) is cell-free tumor-derived DNA present in the plasma fraction of a cancer patient's blood[9]. It may originate from apoptotic and necrotic tumor cells in the primary tumor, metastases or circulation, as well as being directly released from viable tumor cells[10]. Tumor-specific mutations have been utilized to distinguish ctDNA from other circulating DNA[11]. Furthermore, substantial evidence supports the idea that ctDNA detection may provide therapeutically relevant, predictive and prognostic information in breast cancer[12,13]. It has also been shown that mutation tracking in serial blood samples from patients with early breast cancer results in increased sensitivity for detection of systemic relapse, with a median lead-time of 8 months over radiological imaging[13]. In a recent report, the same group even demonstrated a median lead time of 10.7 months for ctDNA-based detection of recurrence in an enlarged cohort[14]. A potential for monitoring of treatment response during chemotherapy has also been demonstrated[12].

Further, striking genetic disparities between primary tumor, metastases and disseminated tumor cells for the same patients have been reported for several solid cancers[15]. This further strengthens the use of ctDNA analysis as it is expected that the ctDNA pool will reflect the total tumor burden in a cancer patient and consequently contain DNA from all tumor subclones. Accordingly, ctDNA analysis is a non-invasive and more exhaustive alternative to metastatic tissue biopsies for mutational analysis in breast cancer[16], that can also be feasible for serial sampling in disease monitoring and tumor evolution

studies. Moreover, ctDNA assessment is a central part of the recently published CancerSeek technology for early detection of solid cancers[17].

1.3 Circulating tumor cells

Circulating tumor cells (CTCs) are tumor cells that have detached from the primary tumor or metastases either by passive tumor cell shedding, or by an active mechanism involving the epithelial-to-mesenchymal transition (EMT)[18]. EMT is presumed to be required for invasion and metastatic dissemination as the tumor cells acquire mesenchymal features during this process, resulting in increased motility, invasiveness and resistance to apoptosis[19]. Due to the very low frequency of CTCs in blood (1 CTC per billion blood cells), it is technically challenging to identify and distinguish them from normal epithelial cells. Detection of CTCs is therefore usually preceded by an enrichment procedure based on selection of CTCs expressing a specific surface marker (eg. EpCAM, positive selection) or by depletion of blood cells (eg. CD45, negative selection). The most frequently used method for CTC detection, the CellSearch system, is dependent upon the presence of Epithelial Cell Adhesion Molecule (EpCAM) on the cell surface. There is evidence that EpCAM is downregulated in a subset of CTCs due to the EMT process, encouraging the use of EpCAM-independent enrichment methods in future studies[26,27].

Around 15-25% of operable breast cancer patients will develop metastatic disease in their future, and there is good evidence that the detection of CTCs, both before and after adjuvant therapy, is of prognostic value in breast cancer patients[20–23]. In 2016, a large pooled analysis demonstrated an independent prognostic value of CTC detection in operable breast cancer[21]. Recently, the presence of CTCs two years after chemotherapy was also found to be associated with decreased survival in the SUCCESS trial[23], while other studies report that CTCs can be detected even 5 years after primary diagnosis and are predictive of later relapse[24,25].

1.4 Metabolomics

Metabolomics is the study of small molecules comprising substrates, intermediates and end products of cellular metabolism, such as amino acids, sugars and small organic acids. The metabolic state of cancer cells is substantially altered compared to normal cells[26,27], a fact that can be utilized for diagnostic purposes. Specific metabolic signatures from tumor tissue provide additional information for determination of breast cancer subtypes and prediction of outcome[28–31]. For example, increased tumor lactate and glycine levels are related to poor prognosis in patients with estrogen receptor (ER) positive cancer[32]. Metabolomic analyses of primary tumors have also demonstrated predictive value in relation to neoadjuvant treatment of patients with locally advanced disease[31].

Circulating metabolites have also been shown to provide prognostic information in operable breast cancer[33–35] and further stratify risk within existing genetically determined risk categories[34]. Importantly, metabolomic alterations may arise directly or indirectly from micrometastatic disease, rather than primary tumor. Recently, we have shown that systemic lactate and pyruvate levels predict inferior outcome in patients with operable ER-positive breast cancers[36]. Tumor-adjacent tissue and immunological responses may also contribute to an altered metabolomic profile[35]. There is also evidence that metabolic profiling can be used for patient monitoring in some cancers[37], although such evidence is still lacking in breast cancer. Therefore, we here intend to investigate whether postoperative monitoring by means of metabolic profiling in blood is useful for early detection of breast cancer recurrence.

1.5 microRNA in extracellular vesicles

microRNAs are small, noncoding RNA molecules that regulate gene expression. They offer great potential as biomarkers for cancer detection because of their remarkable stability in blood and characteristic expression in different diseases (reviewed in [38]). microRNAs have also been found in a

subclass of extracellular vesicles now referred to as "small extracellular vesicles"[39] (sEV; previously called exosomes; reviewed in [40]). There is evidence that sEVs play a role in signaling within and between cells and tissues[40]. Recent data also suggests that sEVs may act as messengers of metastasis, and assist in the formation of metastasis by "priming" the metastatic site to create a microenvironment that is conducive for cancer cells[41]. In addition to microRNAs, sEVs have been found to contain RNA, lipids, proteins, and metabolites. Analyses of the molecular content show that sEVs derived from tumor cells reflect the content of their ancestor cells[42]. Many studies have shown that the content and especially microRNAs from these sEVs can be transferred and mediate biological effects in recipient cells[43]. Recent studies have also demonstrated different sEV miRNA profiles in peripheral blood from patients with breast cancer in different disease stages[44], suggesting that sEV miRNAs may be promising biomarkers in breast cancer.

1.6 Integrative molecular monitoring for recurrence detection

New technology has revolutionized the level of biological information that can be obtained from clinical samples, represented by the new "omics" terms genomics, transcriptomics, metabolomics, etc. The availability of such big datasets, even in the public domain, has encouraged the development of integrative methods that can extract vital information from multiple combined data sources[45]. Surprising new connections between omics-datasets have been revealed by such approaches, exemplified by a link between cell-free DNA fragmentation and gene expression[46]. Accordingly, our knowledge about breast cancer has also been extended by integrative approaches, resulting in a more comprehensive understanding of the disease[47]. The prognostic subclassification of breast cancers has for instance been refined and novel tumor-specific antigens identified[47–50]. Integrated molecular data have even been shown to have a higher prognostic power than separate molecular levels in breast cancer[39]. Thus, a combined analysis of the genetic (ctDNA), transcriptomic (miRNA) and metabolomic data levels in peripheral blood samples is therefore planned in the current project to maximize their joint biomarker potential in operable breast cancer.

2. Impact on Patient Care

Breast cancer patients have to live with a continuous risk of relapse for the rest of their lives, as occurrences can appear more than 20 years after surgery. This cruel fact is a major concern for both patients and clinicians. Therefore, endocrine treatment has been extended to last for 10 years after surgery. Standard follow-up of breast cancer patients during adjuvant chemotherapy is primarily focused on detection of local relapse (i.e. mammogram/ultrasound and clinical examination). Paradoxically, there is no search for systemic relapse, even though a poor outcome is unavoidable when systemic relapses are clinically manifested. Thus, there is a need for enhanced non-invasive surveillance that is **cost-effective** and **easy** to perform. Radiological imaging and PET scans have low sensitivity for small metastases, are resource-demanding and expensive. By investigating new circulating biomarkers in the current project, we want to provide new tools for **more sensitive** and **less resource-demanding** surveillance that can be offered systematically to all patients with operable breast cancer. Earlier detection of systemic relapses may allow for earlier therapeutic interventions which may improve long-term survival and reduce side effects from unnecessary treatment. Thus, the current project provides several potential positive benefits for breast cancer patients and their next of kin.

3. Objectives and expected results

Primary objective: To develop new diagnostic tools to detect breast cancer recurrences at the earliest possible time point, both by analysis of single molecular data sources and by integrative analysis of combined data.

Specific sub-aims (figure 1):

1. To identify recurrence markers in peripheral blood by measuring circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), metabolites and sEV miRNA in peripheral blood samples obtained every 6 months from patients with operable breast cancer who have experienced disease recurrence during follow-up (WP1- 4).

2. To investigate DNA aberrations in biopsies from primary tumor and metastasis (WP5) for comparison with the blood sample results.

3. To integrate the combined molecular markers of the blood and tissue samples to further refine the monitoring potential (WP6).

Our hypotheses will be investigated in a case-control design within the Prospective Breast Cancer Biobank (PBCB) project in the current study and validated in the overall cohort in a later phase when the follow-up times are longer. Validation in an independent patient cohort is the ultimate future goal. The long-term goal of the project is to implement our new diagnostic tools in an interventional study, where recurrence therapy is offered earlier than today, aiming at longer patient survival.

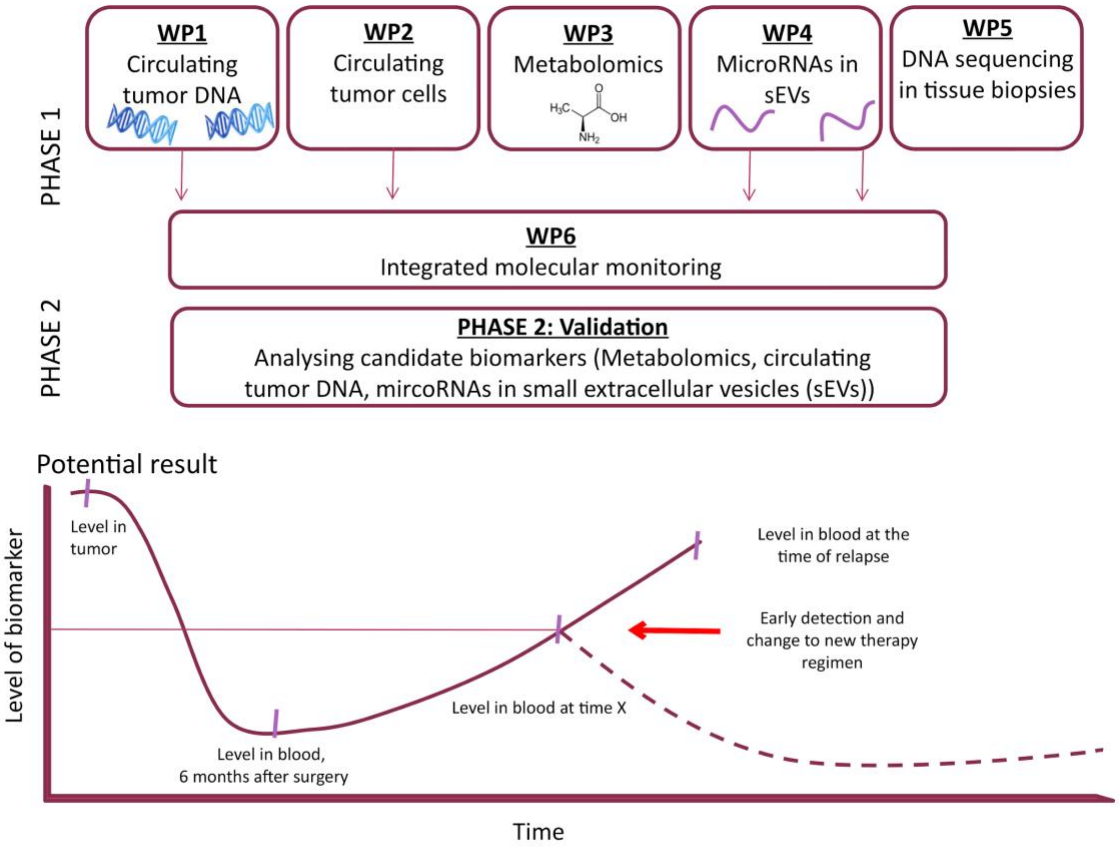

Figure 1: Overview of the project design and aims.

4. Experimental approach

4.1 Study Design

The current project application is based on the Prospective Breast Cancer Biobank (PBCB), which is a regional collaboration between Haukeland and Stavanger University Hospitals. In the PBCB project blood samples are collected from operable breast cancer patients at the time of diagnosis and every 6

(Stavanger)/12 (Haukeland) months until disease recurrence or for a total of 11 years. In total, 1253 patients have been recruited to this biobanking project.

The current study is designed as a retrospective case-control follow-up study (figure 2), where PBCB patients who have already developed systemic metastases (n=50, group A in figure 2) are included as cases. These patients will be matched with a control group of patients who are recurrence-free at least 5 years after surgery (n=50, group B in figure 2). A group of age-matched healthy individuals (n=50, group C) will also be included in the analyses for comparison. Having collected 6 blood samples in average from the patients in group A means there will be approximately 400 blood samples to analyze. Biopsies from the primary tumor biopsies and eventual metastases are available from the Departments of Pathology at both hospitals (Stavanger and Bergen).

The study is organized in six work packages (WP) as described below (figure 1). Our hypothesis is that some of our proposed biomarkers will detect recurrence earlier than radiological imaging. If successful, the resulting biomarkers will be analyzed in the overall PBCB cohort in a future study and later validated in an independent patient cohort.

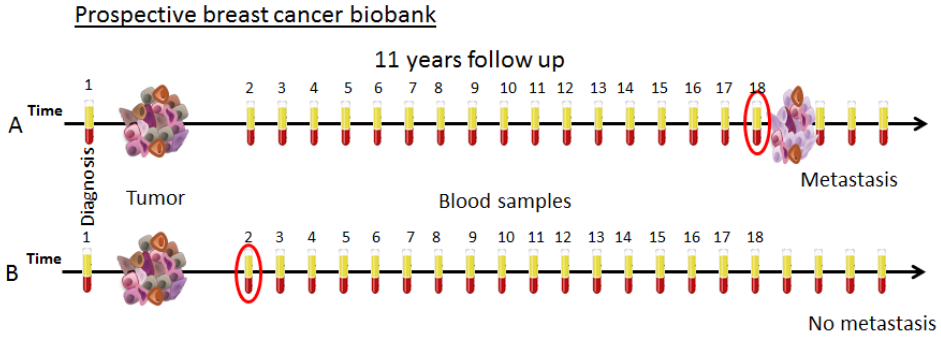

Figure 2: Study design. Samples from patients (A) with recurrent disease will be compared to early post-operative samples from patients without evidence of recurrence (B).

4.2 Choice of Methodology and Analysis

WP 1: Circulating tumor DNA (ctDNA)

Tumor-specific mutations can be utilized as markers for ctDNA because they are not present in normal cells and normal plasma DNA. The oncology group has previously demonstrated the clinical relevance of ctDNA measurements in pancreatic cancer[51], and they will now measure ctDNA levels in plasma samples from operable breast cancer patients using targeted deep sequencing. By this approach the plasma sample will be examined for point mutations and copy number aberrations in ten frequently mutated genes using the "Oncomine Breast cfDNA Research Assay v2" (Thermo Fisher). The assay is based on molecular barcoding of templates and allows reproducible detection of mutations down to 0,1% allele frequency. We have tested the assay and confirmed this sensitivity with commercial control samples. In a pilot study we also detected ctDNA in 5 of 16 patient plasma samples from operable patients, with a relative concentration as low as 1% compared to normal cell-free DNA (cfDNA). We also detected ctDNA in plasma from 3 of 4 breast cancer patients with recurrent disease. Analyses of serial blood samples further showed that mutations found in the primary tumors and metastases are present in plasma before and at the time of recurrent disease in these 3 patients. These are very encouraging results, and we will confirm variants with low allele frequencies by analysis of more plasma cfDNA and a preamplification-based setup for digital droplet PCR (ddPCR) to enable analysis of multiple variants in the same cfDNA volume[52]. The sequencing will be performed on our Ion Proton deep sequencing instrument (Thermo Fisher), whereas the ddPCR analysis will be accomplished with

our QX200 instrument (Bio-Rad). Both technologies have been applied for ctDNA detection in breast cancer in previous studies with success[12,13]. In addition, we will also sequence DNA from white blood cells from the same patients to confirm that the variants we find originate from tumor cells and not from white blood cells[53].

In WP1, plasma samples from the included patients will be assessed by this approach, and the 50 patients in the case group (A, section 4.1) will be compared to the plasma samples from the first postoperative sample from group B and the 50 control samples from healthy individuals (group C). ctDNA levels significantly above the levels in the control group (if measurable) will be interpreted as disease recurrence and the time for ctDNA-based recurrence detection compared to the time of radiological detection. The mutational profile from the primary tumor biopsy and the plasma samples will also be compared in order to reveal potential tumor heterogeneity. In addition, longitudinal changes in the mutation profile of ctDNA will be compared with disease development to shed light on the biological mechanisms causing disease relapses.

WP2: Circulating tumor cells

In our ongoing PBCB project, we enrichCTCs from peripheral blood samples by density centrifugation and subsequent immunomagnetic depletion of leukocytes using a new EpCAM-independent depletion strategy, termed MINDEC (Multi- marker Immuno-magnetic Negative Depletion Enrichment of CTCs), developed in our lab[54]. This method has superior recovery and enrichment rates[54]. Using EpCAM-independent CTC enrichment, we expect to isolate both mesenchymal- and epithelial-like CTCs, in contrast to what is possible with the more conventional CellSearch method. RNA is then isolated from the enriched fraction, to allow for CTC detection using specific mRNA markers being expressed at high levels in tumor cells and low levels in normal leukocytes as surrogate markers. Both epithelial-specific and markers related to the epithelial-mesenchymal transition are included in the marker panel. The mRNA markers are pre-amplified before they are quantified by real-time PCR. Elevated marker levels compared to the healthy control group reveal presence of CTCs in the patients' blood samples. We have performed preliminary analyses of blood samples from six patients with recurrent disease and compared the data with analysis of blood samples from 163 recurrence-free patients and 30 healthy control samples. This analysis was encouraging as it revealed a much higher rate of positive samples from the patients with recurrent disease than those without known relapse. The presence or level of CTCs in the analyzed samples will later be related to known prognostic factors, treatment effect and disease outcome.

WP 3: Metabolic analyses

Blood (serum) samples will be analyzed for metabolites within glycolysis-, citric acid-, amino acid and phospholipid metabolism; and detailed analysis of the lipid subfractions in serum will be performed. The concentrations of these metabolites will be determined by High Resolution Magnetic Resonance Spectroscopy (HR MRS). MRS offers precise identification of metabolites and high throughput, automated quantitative analysis. HR MRS will be performed at the MR Core Facility, Dept. of Circulation and Medical Imaging, NTNU. The MR Core Facility hosts state-of-the-art spectrometers with ultrashielded magnets operating at 600MHz for proton detection. Patient serum will be transported to the MR Core Facility at NTNU for analysis. Subsequently, the samples will be refrozen and transported back to Stavanger University Hospital (SUH) for future further analyses. Metabolomic analyses yield large amounts of data which will be analyzed by specialized multivariate statistical methods (chemometrics). Modelling biological pathways will be done by integrating multivariate statistics with biological databases (Human Metabolome Database (HMDB), Kyoto Encyclopedia of genes and genomes (KEGG)), using available tools (Metaboanalyst, integrated Pathway Analysis (IPA)). In the first phase, a more exhaustive statistical analysis will be performed to identify metabolic profiles associated with recurrent disease. In a validation phase, these profiles will be sought in all previous follow-up samples available from the relapsed patients.

WP 4: Circulating microRNA from sEVs

Total RNA will be isolated from sEVs in plasma samples using the exoRNeasy Serum/Plasma kit (Qiagen), which isolates sEVs and sEV-contained RNA in a two-step process. In a first phase, microRNA profiling will be performed on the isolated RNA using PureLink™ miRNA Isolation Kit (Invitrogen) and Ion total RNA-seq kit V2 (Thermo Fisher) on our Ion Proton deep sequencing instrument. This experimental pipeline has already been established in our lab. MicroRNAs that are differentially expressed in the blood samples obtained from 50 patients at the time of recurrence (group A, figure 2), in comparison to the blood samples from patients in the control arm (group B, figure 2) and the normal control (C) group, will be identified. The most promising candidates will be analyzed in blood samples taken from patient group A at earlier time points by quantitative reverse transcription PCR (RT-qPCR). In a preliminary analysis, sEVs and microRNA have been isolated and analyzed in four patients with systemic relapse that were disease-, age- and BMI-matched with non-recurrent patients; these patients have been analyzed for eight miRNAs by RT-qPCR (miR-10a, miR-1246, miR-16-5p, miR-18b, miR-99, miR-451 and miR-505-3p). These preliminary results show that our methods work and that these microRNAs are present in sEVs at measurable levels. Differences in expression levels are observed as well, but currently no statistics have been applied due to the low number of samples.

WP 5: DNA sequencing of tissue biopsies

DNA from the primary tumors of 100 operable breast cancer patients (group A and B, figure 2) will be analyzed by deep sequencing using the Oncomine Comprehensive Assay. This is an amplicon-based approach, analyzing hotspot SNVs, indels, and CNVs. Furthermore, we will also analyze DNA from the 50 metastatic lesions, if tissue is available. Identified variants will be compared with the variants found in ctDNA. In addition, these analyses will provide us with a biological understanding and background knowledge for each of the individual tumors

WP 6: Analyses of Healthy Controls

Objective: Collection of blood samples and questionnaires from 200 healthy women who attend mammography at Old Stavanger Hospital, for comparison with women who have breast cancer, in various research projects. Inclusion takes place on Wednesdays from the morning of. We expect to be able to include about 36 participants per inclusion day (6 per hour x 2 employees x 3 hours = 36 participants)

Research question: What is the contribution of the cancer disease itself on various analyses in the various WPs we have planned for the patients?

Strategy: To answer this question, we will need blood samples from healthy normal women to compare them with the patients. The healthy women should be representative for the age of our breast cancer patients. This will be achieved by accrue healthy women attending the mammography screening program.

Inclusion:

- Will only take place in Stavanger University Hospital.
- Women entering the waiting room are informed about the research project by the host. Receives a consent form and questionnaire
- Participants sit in waiting rooms and fill out a questionnaire, write during the consent
- Participants submit questionnaires to the host or in the mailbox
- Host refers to blood sampling / PBCB staff retrieves from waiting room
- Mammography personnel pick up mammograms at their convenience (are flexible in order of their patients).

Blood sampling procedure:

Performs as ordinary a PBCB (REK 2010/1957) inclusion as possible.
1) Ask about the date of birth and sign the lab requisition. Ask if they have menstruation, possibly the first day last while, if they have fasted (both food and medicine).
2) Take blood tests:
a. 1 x serum 9mL
b. 4 x plasma 9mL (one set cold, three kept at room temperature)
c. 1 x plasma 4 mL (whole blood)
d. 1 x CPT
3) Turn the tubes 8 times
4) Label the tubes with labels from fresh controls kit

Analyses: The analyses on the healthy normal women will be the same as in the various WPs in this project description.

WP 7: Integrative analysis
As a consequence of all our different analyses in WP1-5, large datasets of complementary molecular information will be available for combined analyses. As a first step, a multivariable logistic regression will be performed using the candidate biomarkers or variables suggested in the different WPs, in addition to clinical, histopathological and tumor molecular information from the primary tumor. Candidate variables will be analyzed for independent information content and co-variability. This analysis will provide a combined risk score with a cutoff for early detection of recurrence in breast cancer patients, which may be validated in other biobanks. Furthermore, an exploratory combined analysis of the datasets will be performed in order to extract corresponding biological information between the different molecular levels. In this way we may discover potentially novel subtypes by combining weak yet consistent alteration patterns across data types[55]. In this analysis, the full datasets from each analysis will be combined. Nonlinear classification methods (PLSDA, K-Nearest Neighbors classification, Random Forest, Elastic net variable selection) and clustering methods (iCluster, intNMF)[55,56] will be performed and compared to obtain the best predictive performance for recurrence detection and to develop models which describe the biological systems. Finally, we will apply supervised machine learning techniques (artificial intelligence) for optimal distinction between recurrence and non-recurrence [57].

4.3 Statistical power

Calculations demonstrated that 50 cases (patients with recurrence) and 50 controls (patients without recurrence) are sufficient to give a matched case-control study power of 0.80. This sample size is based on the assumption that the probability of a positive biomarker score is 20% in the control group, with an odds ratio of 3.41 for developing recurrence in patients with biomarker positive scores. The estimated Sample size calculations were performed using STATA 15.1 software (power mcc command). We are also testing whether our new biomarkers can detect disease progression earlier than conventional radiological imaging. This can be assessed by a one-sample t test of the time difference between ctDNA and imaging compared to the null hypothesis that there is no difference. We obtained estimates for the population mean time difference (median 10.7 months) from a recent report presenting similar data for 144 patients with cancer[14]. The test power of a single-sample t test with a population difference of 10.7 months, standard deviation 20 months, and 50 included patients is 96% (power.t.test function in R).

4.4 Organization and Collaboration

This project is based upon a multiregional collaboration, in which the blood samples are provided from the regional PBCB-biobank headed by Prof. Gunnar Mellgren in Bergen and Prof. Håvard Søiland in

Stavanger. The tissues samples are provided by the departments of Pathology from both Stavanger and Haukeland University Hospitals. Metabolic analyses are performed at NTNU and bioinformatic analyses are performed in close collaboration with the University in Oslo. Breast cancer research at SUH is based upon a close collaboration between the three research groups from the Department of Breast & Endocrine Surgery, Department of Hematology & Oncology and the Department of Pathology; this collaboration is also part of the national breast cancer research network (www.breastcancerresearch.no).

The applicant, senior researcher and chief engineer Kjersti Tjensvoll (PhD), is a member of the research group for Cancer and Medical Physics, led by Dr. Bjørnar Gilje at the Department of Hematology and Oncology, SUS. She works in close collaboration with Professor Oddmund Nordgård and together they have extensive experience in the field of liquid biopsies. Dr. Tjensvoll will in this project be the principal investigator of WP1, and also participate in WP2. Professor Nordgård will be responsible for the bioinformatic analysis of WP1 as he has considerable experience in bioinformatics related to cfDNA sequencing. Professor Nordgård will also be the PI for WP2, and participate to the computational analyses of WP5 and WP6. Kristin Løge Aanestad (MD), who applies for a PhD grant in a separate application, will also be responsible for laboratory analyses in WP1 and WP2 (see PhD application). Professor Emiel Janssen from the Department of Pathology will be the PI of WP4 and 5 and supervise postdoc Marie Austdal in her work with WP3-6. Dr Austdal will also be responsible for WP3 and WP6 in the current project. She was awarded a postdoc grant from Helse Vest last year, based on the same work packages as described here.

Key collaborators for this project are: Prof. Håvard Søiland (leader of Stavanger Breast Cancer Study Group and founder of the PBCB study), head of department Bjørnar Gilje (group leader Research group for Cancer and Medical Physics), Prof. Tone Frost Bathen (Leader of the MR Cancer group, Dept. of Circulation and Medical Imaging, NTNU). The MR Cancer group is internationally recognized as one of the most experienced groups in large scale metabolomics analyses of intact cancer tissue and biofluids. Prof. Lars A. Akslen (head of the Center for Cancer Biomarkers, Dept. of Pathology, Haukeland University Hospital) will be participating in WP5 and will provide tissue samples from the patients in Bergen that will be analysed in WP5. He has extensive experience in biomarker discovery and breast cancer research. Prof. Håvard Søiland together with Prof. Gunnar Mellgren at Haukeland University Hospital is responsible for the PBCB material. Kristin Jonsdottir, PhD, is constituted leader for Stavanger Breast Cancer Study Group and will be co-PI for the miRNA profiling of sEVs (WP3). Tone Lende (MD), is head of the Department of Breast and Endocrine Surgery at SUS. Professor Ole Christian Lingjærde is the leader of the Research group for biomedical informatics at the UiO. He has been involved in several "omics" integration projects. He will be co-supervisor for the postdoc, with special focus on the integrated molecular monitoring in WP5. Miram Ragle Aure, PhD, is experienced in miRNA/mRNA analyses in breast cancer and integration of 'omics' data. She will be an important collaborator in WP3 and a future collaborator for validation of our results.

4.5 Communication

We plan to communicate the results in peer-reviewed international journals and in a popular form in local newspapers and the web sites www.brystkreftforskning.no and www.forskning.no. The following tentative scientific titles are planned:

1. Relapse detection in operable breast cancer by assessment of circulating tumor DNA
2. The prognostic relevance of circulating tumor cell detection in pre-operative blood samples from high-risk patients with operable breast cancer.
3. Metabolomic monitoring of operable breast cancer patients for recurrence, pre- and post-operative

4. Pre- and post-operative vesicular microRNA detection in operable breast cancer in relation to clinicopathological factors and preliminary survival data
5. Monitoring of operable breast cancer patients by detection of microRNA from sEVs
6. Integrated data analysis of liquid biopsies in monitoring of operable breast cancer patients

4.6 Plans for Implementation

We want to design new studies that link our circulating biomarkers to clinical interventions, such as treatment choices and change of therapy. These studies will be organized through the national breast cancer network that we already participate in. If the interventional studies are successful, the next step will be to organize systematic surveillance of operated breast cancer patients through the Norwegian Breast Cancer Group, based on yearly assessment of the new circulating biomarkers in peripheral blood samples.

5. Expected significance to the field

This project may change the way patients with operable breast cancer are followed after surgery. We anticipate a new surveillance scheme consisting of yearly blood samples examined for the new markers provided by the current study. We also foresee a change in therapy regimen in response to early evidence of relapse. This change of therapeutic regimen will, however, require future studies in which the survival benefit of such interventions is demonstrated.

6. Relevance to the call

This project is a collaboration between three research groups at Stavanger University Hospital (SUH: Department of Hematology and Oncology, Department of Pathology and Department of Breast and Endocrine Surgery) in addition to professor Gunnar Mellgren (Haukeland University Hospital), who is among one of the initiators of the ”Prospective Breast Cancer Biobank” (PBCB). Although the collection of samples to this unique biobank is still ongoing, our focus is now on utilization of the samples and a large integrated analysis were we will combine data from analysis of both tissue, plasma and serum samples stored in the PBCB biobank. This project has the potential to allow for a more extensive follow-up through personalized monitoring as well as development of more sensitive diagnostic tools for detection of systemic disease at an early stage. If successful, new biomarkers may reduce the use of resource-demanding radiological imaging. This again will hopefully expedite a change of treatment and thereby result in more effective treatment of breast cancer patients leading to an improved long-term survival.

7. User Involvement

The PBCB group at Stavanger University Hospital has an active collaboration with the Regional Breast Cancer Society representing all breast cancer patients in our region. Two members of this society are included in our regular PBCB meetings as active partners of the user involvement program and have provided valuable input into several aspects of the PBCB project. The user representatives have contributed to project meetings by presenting their experience with breast cancer diagnostics and treatment, and their participation in research projects. They have also evaluated the information letters, the questionnaires and our interaction with patients in the PBCB project.

8. Ethical Considerations

The Prospective Breast Cancer Biobank project is approved by the regional ethical committee (no. 210.04 and 127.97). PBCB is also approved in REK-Nord (no.2010/1957-4) and HOD (no.2007- 07/758). The current biomarker project is approved by REK Vest (2015/2010).

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1. Project Title

Prospective Breast Cancer Biobanking (PBCB)

– Towards personalized treatment and monitoring of breast cancer

2. Introduction

Presently, there is no monitoring of early systemic relapse in the follow-up care for breast cancer patients, despite the knowledge that distant metastatic lesions will eventually kill the patient should they become clinically overt. Breast cancer is the most frequent and deadly form of cancer among women worldwide. [1] In 2015, 3324 new cases of breast cancer and 663 deaths due to breast cancer were registered in Norway. Moreover, there are currently 42 000 breast cancer survivors in Norway who must cope with various phases of the disease. [2] Disease incidence in Norway is doubled over the last 50 years[3] in line with the pattern that has been observed across most of the Western world.

In essence, our project portfolio provides new knowledge towards a more personalized monitoring and treatment approach. Due to the potential systemic spread of breast cancer cells from the primary tumor at the point of diagnosis, current treatment algorithms combine surgery and adjuvant systemic treatment such as chemotherapy, endocrine and targeted biological modalities.[4] Unfortunately, despite this multipronged strategy both early and late relapse still occur, leading to deaths from breast cancer. Our overarching aim is therefore to improve existing treatment and to identify new biomarkers in order to avoid both over- and under-treatment of breast cancer patients through detection of robust novel predictive biomarkers for better classification of the tumor itself, combined with long-term monitoring of patients in order to increase future survival.[5]

Based on drawbacks with our retrospective study from 2006, where lack of available serum and reliable clinical data jeopardized the final analyses[6] we felt encouraged to start the Prospective Breast Cancer Biobank (PBCB) in 2010. This is an **ongoing** regional collaboration between Haukeland University Hospital (HUS) and Stavanger University Hospital (SUS), supported by Helse Vest from the start. In our prospective biobank we have, since 2011, consecutively recruited operable breast cancer patients and have now enrolled 1017 patients from the catchment area of the two hospitals. A broad panel of tissue samples, liquid biopsies and Patient Reported Outcome Measures (PROM) are being collected per operatively, biannually and annually respectively. Combined with longitudinal follow-up over 11 years PBCB will create a solid base for numerous projects with over 10 000 person-years in various analyses. National and international collaborators have given the PBCB-project their scientific recognition. Importantly, this collaboration platform is now producing clinically embedded studies, based on key clinical issues with an innovative approach.

The concomitant increment of breast cancer and obesity across Western populations over the past 50 years suggest a causal relationship.[7] Interaction between the cancer cell and the tumor adjacent adipocytes may explain the malicious phenotypical behavior of some tumors through paracrine signaling and by inducing inflammation.[8] Signal interactions between the adjacent adipose tissue and the primary tumor offer possibilities of detecting novel biomarkers that can enable better definition of breast cancer sub types. This micro environmental interaction may also be used to explore new molecular and metabolic profiling in cancer cells and in liquid biopsies, to establish novel biomarkers for an early warning of systemic relapse.

Tamoxifen is still an extensively used Estrogen Receptor (ER) antagonist and the drug of choice in endocrine adjuvant therapy for pre-menopausal patients and, in concert with aromatase inhibitors for postmenopausal patients.[9] Tamoxifen is a pro-drug which is metabolized by liver enzymes to various serum levels of active metabolites with a more than 10-fold inter-individual difference. [6,10] As of today, no method has been established to deal with this problem. Controversies exist over whether *CYP2D6* genotyping or serum levels of active metabolites can be used to evaluate ER inhibition and its effect on survival. Recently, our group has showed that metabolite-guided therapy is a promising approach to avoid under-treatment of breast cancer patients receiving

long-term endocrine tamoxifen treatment.[11] As 75% of all breast tumors are ER positive a personalized monitoring of tamoxifen treated patients may result in a significant impact on the clinical management of breast cancer patients.

Moreover, the high discontinuation level (up to 50%) over a 5-year period of tamoxifen outside clinical trials[12] impacts on survival.[13] Sound prospective studies are therefore needed to identify risk factors relating to patients' non-adherence to tamoxifen. Both these tamoxifen related topics have become highly relevant due to the recent extension from 5 to 10 year adjuvant tamoxifen treatment in clinical guidelines.[13] Our findings will contribute to personalize treatment and increase the quality in clinical management of these patients.

As blood represents the biological interface between the primary tumor, the micro environment and the distant metastases, they form an ideal medium as "liquid biopsies" to monitor the patient`s cancer biology. [14] There is a need for identifying novel circulating biomarkers which can enable clinicians to detect and target the relapse with various treatment modalities while it is still at a microscopic level — thereby having a better chance to cure the patient.[15] Circulating biomarkers that are considered promising in this context are circulating tumor cells (CTC), circulating tumor DNA (ctDNA) and microRNA (from exosomes and from Tumor Educated Platelets (TEPs), which all are stable tumor derived compounds feasible for collection, storage and analysis. However, there is a gap in knowledge of how these biomarker groups can be utilized in monitoring of patients.

3. Project Objectives and Goals/Milestones

Project objectives

Primary objective:

Our primary objective is to continue the unique biobanking of tissue and liquid biopsies from women with early breast cancer (Stage I and II) in Western Norway through biannual follow-up in an 11-year perspective. This is a large undertaking, which will ultimately establish the basis for early detection of systemic relapse in these women and therapeutic drug monitoring of tamoxifen, both to improve long term survival of breast cancer patients.

Secondary objectives:

To achieve the primary objective, the PBCB project portfolio is organized into 4 work packages (WPs) reflecting the secondary objectives**.**

1. To continue the prospective accrual and follow up (until 11 years) of 1200 operable breast cancer patients collecting blood, urine, tissue and PROM data. (WP-1)

2. To establish the Master Database for PBCB – containing both HUS and SUS data (WP-1).

3. To validate the clinical relevance of direct measurement of active tamoxifen metabolites in serum (therapeutic drug monitoring (TDM)) in breast cancer patients adjuvantly treated with tamoxifen (WP-2).

4. To identify risk factors for patients' low adherence to tamoxifen (WP-2)

5. To establish the necessary biological material for investigation of the clinical relevance of *new circulating tumor biomarkers* in plasma for treatment monitoring and early detection of systemic relapse (WP-3).

6. To validate our results and implement them in clinical trials (WP-4)**.**

4. Feasibility

4.1. Study Design, Choice of Methodology and Analysis

WP-1. Population based collection of liquid biopsies, tissue and PROM-data from patients with early stage breast cancer in Western Norway.

Population

At the regional level, approximately 700 new breast cancer patients are diagnosed annually in Helse Vest. Of these, more than 500 belong to the catchment area of HUS and SUS (approx. 310 and 220 respectively). Women diagnosed with operable breast cancer (Stage I and II) at HUS and SUS are consecutively invited to participate in the PBCB- project, i.e. to be followed-up prospectively for 11 years. One unique population feature is that people who live in this catchment area rarely move home or seek care elsewhere. Hence, a near-complete follow-up of patients is possible, creating population based cohorts of highly committed patients. To illustrate, the drop-out rate is only 6% at SUS. Furthermore, every Norwegian citizen has a unique personal social security number allowing coupling of information to various nationwide registries including the Norwegian Cancer Registry, the Cause of Death Registry, and Statistics Norway. Therefore, a complete follow up of patients is facilitated, creating a robust population-based prospective biobank of women with early stage breast cancer.

Liquid biopsies

The "liquid biopsy kit" comprises various blood sample tubes (Serum, EDTA plasma, EDTA whole blood, PAX-gene and CPT-tubes) and urine (Figure 1). The protocol is relatively time consuming as it involves both adequate "patient side" drawing of the various blood samples and immediate further processing in the lab. All samples are aliquoted and stored at -80 C. It takes about one hour to fulfil the sampling protocol for one patient. Importanly, plasma is sampled at +4°C for assessment of cytokines and CPT-tubes make DNA isolation from lymphocytes possible. This has made research on chronic fatigue together with **Prof. Timothy Lash** and **prof. Mylin Torres**, Atlanta, US (see 4.2.3.) possible. Definitely, the liquid biopsies represent a gold mine for future research on breasr cancer.

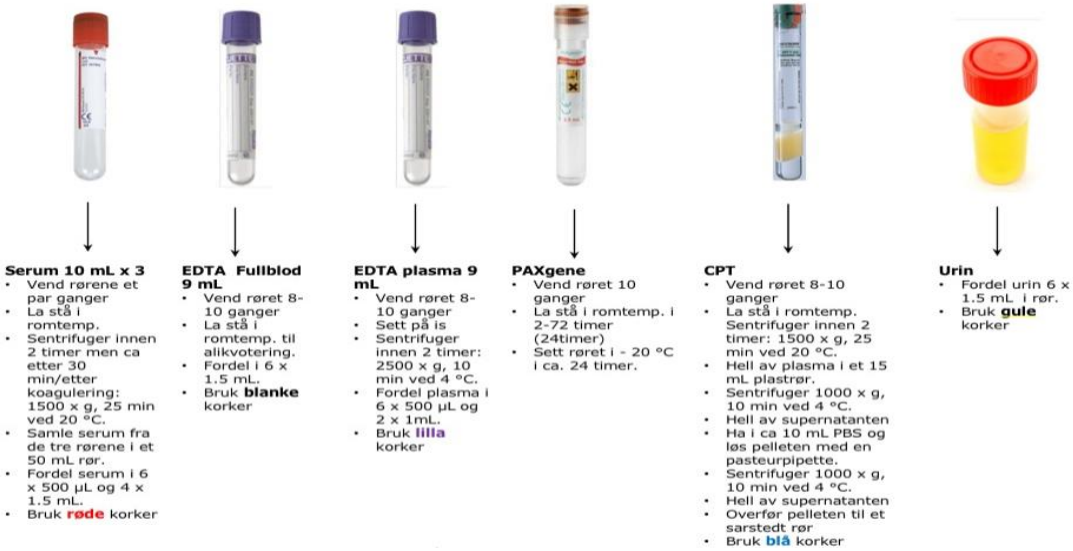

Figure 1. Sampling protocol in PBCB

Tissues

At SUS, fresh frozen tumor tissue is obtained from the surgical specimens where the tumor > 10 mm in diameter. Otherwise, paraffin embedded tumor tissue is available from the diagnostic biobanks at both HUS and SUS for all patients. Moreover, we aim to improve the definition of breast cancer by studying the role of inflammation at the invasive front of the tumor.

Recently, this phenomenon has been recognized as one of the new hallmarks of cancer.[16] There is also a clear link between obesity (BMI above 30kg/m2) and breast cancer incidence.[17] It consists of two parts and is powered to enroll 75 patients from the PBCB-cohort. We have developed an original protocol for tumor and adipose tissue sampling (Figure 2) and a pilot study showed excellent yield of RNA from adipocytes included morphological control. Presently, 22 patients are enrolled at SUS and accrual is ongoing. This study is also closely linked to WP-3 and will serve as bases for a PhD project when all samples are obtained.

Patient reported outcome measures (PROM)

To enable identifying patients who are at high risk of discontinue tamoxifen various Patient Reported Outcome Measures (PROM) are collected once a year (Fig. 3). The PROM-data are obtained at baseline and thereafter yearly from **all** patients at SUS. The PROM-data consists of 1. Basic health and clinical data; 2. HRQoL instruments *(EORTC QLQ-C30, EORTC QLQ-BR23 and*

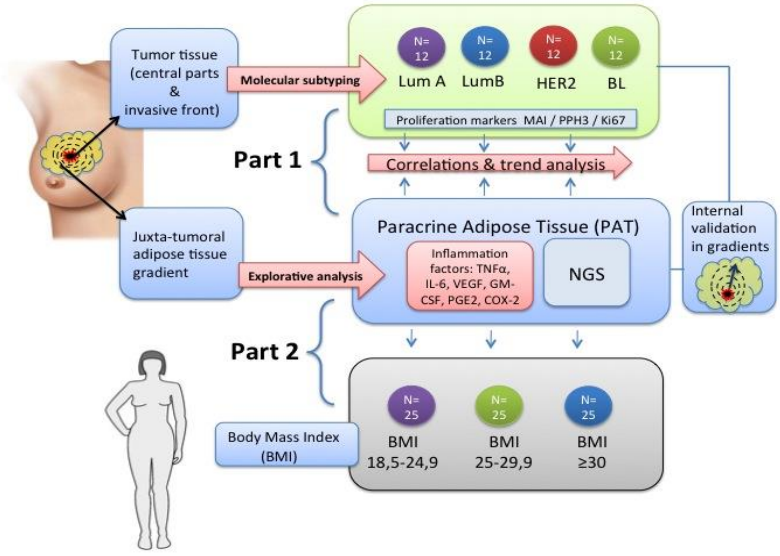

Figure 2. Study design of the "Paracrine-study".
Part 1. Analysis of gene expression to identify inflammatory factors involved in the paracrine signaling between the invasive front in 12 Luminal A (LumA), 12 Luminal B (LumB), 12 HER-2 and 12 Basal Like (BL) breast cancer subtypes and the juxta-tumoral paracrine adipose tissue (PAT) (n=48 all together). The adipose tissue is sampled from the edge of the tumor + 3 biopsies in radial gradients out in the adipose tissue from this point.
Part 2. Characterization of the potential correlation between t factors from part 1 and related to three Body Mass Index (BMI) groups with 25 patients in each group (n=75 all together)
NGS; Next generation sequencing

FACT B); 3. Hospital Anxiety and Depression Scale, *HAD*; 4. Fatigue instruments=*Fatigue Impact Scale, FIS*; Fatigue Severity Scale (FSS) and VAS (visual analog scale)-fatigue; 5. Side effect questionnaires; 6. Joint-pain questionnaire; 7. The Mishel Uncertainty in Illness Scale, *MUIS;* 8. Food habits questionnaire and the 9. ROMA III questionnaire for bowel complaints (IBD and IBS). Some of the PROMs require a trained study nurse /research coordinator to guide the patient in their completion. At HUS, PROM data are obtained annually from 160 patients.

Sampling schedule in the PBCB protocol

At SUS the liquid biopsies are biannually and annually at HUS. PROM-data are collected yearly at SUS from all patients. At HUS, the PROM data is collected once a year from a cohort of 160 patients. The total follow-up is 11 years (Figure 3).

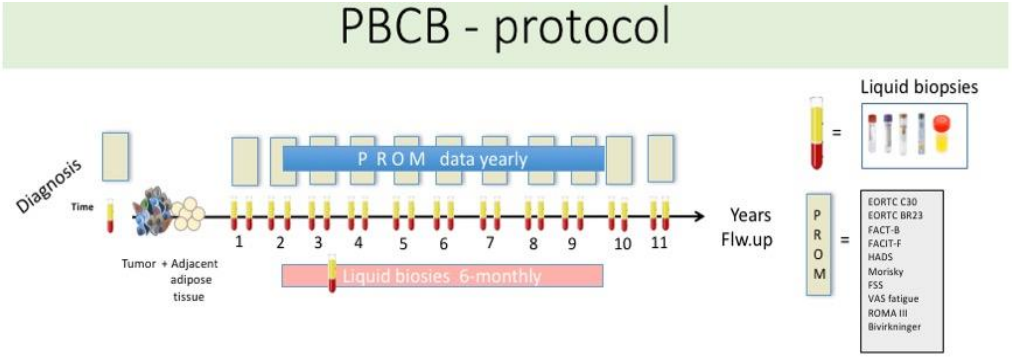

Figure 3.

Sampling schedule in the PBCB protocol

Master Database

The PBCB biobank has generated a huge amount of clinical-, PROM- and laboratory analysis data that needs to be digitalized to be of use in our prospective research. Since the start of PBCB only data from a few patients have been plotted. The amount of data to be processed and inserted into the database increases weekly. An example: 1,200 patients x 10 PROM x 11 years would create 132,000 PROM entities. In addition, a large amount of laboratory data from the various WPs will soon be available. We have developed a database for PROM-data and started plotting the data. Therefore, there is an urgent need for qualified personnel who can continue the handling of this vast amount of PROM-data and clinical data. This co-worker will take care of both HUS and SUS data in the coming 3-year period. This work will be done under supervision of prof. Jan Terje Kvaløy at UiS.

Work load and organizing of the work force

Presently, the number of enrolled patients is 1017. The final accrual aim is N=1200, and these patients will be followed for a period of 11 years with blood and urine samples obtained every six months. Presently, approximately 30% of the patients have completed Year 3 assessments. When fully enrolled, PBCB will create up-to 1200 x 2 = 2400 visits every year, 50-60 patients a week, 10-12 patients a day. **To take care of one patient takes 1 hour**. In addition, if a patient experiences disease recurrence, a tissue biopsy of the metastases and monthly blood samples (for three months) are collected after recurrence discovery. Currently, ten patients have been diagnosed with a recurrence with monthly liquid biopsies taken for 6 months.

The accumulated amount of blood samples that have to be drawn, spinned and stored needs dedicated research coordinators/study nurses. Today, the sampling is organized partly from Helse Vest ("overførte midler fra 2016") and by allocation of internal resources. However, this is not sufficient to cover the growing workload of sampling and biobanking. Work on the master database has commenced but we need human resources for it to be completed and remain up-dated during the project period.

Since PBCB is the largest biobank project on liquid biopsies at SUS (i.e. with 200 000 expected aliquots), we are invited to participate in the pilot phase of the new robot based biobank system (LabVantage) in Helse Vest and the integration of the new biobank identification/tracking software. Moreover, our unique prospective sampling design has attracted attention from Biobank Norway offering a collaboration for future projects of mutual interests.

WP-2. Optimizing tamoxifen treatment: Therapeutic Drug Monitoring (TDM) and increased adherence

A.Therapeutic Drug Monitoring

The two most active tamoxifen metabolites are Z-4OHtam and Z-4OHNDtam (Z-endoxifen) have 30-100 times higher affinity for ER than tamoxifen. These metabolites ultimately constitute the blocking effect at the ER-level aiming to eradicate micro metastatic disease and are responsible for the improved survival following the establishment of this adjuvant systemic treatment. Direct measurement of these metabolites bypasses all disturbances from the diversity of CYP2D6 activity i.e. : alternative metabolic pathways, adherence to the drug and inhibiting drug interactions. Our novel LC / MS-MS methodology[10] takes into account all of the above-mentioned variables and provides a functional read-out report of the serum level of the active tamoxifen metabolites in the individual patient. This method can also distinguish between the inactive and active isomers of Endoxifen and 4-OHtam, which are the most active ER-blocking metabolites of tamoxifen.[18] In collaboration with the Oslo Breast Cancer Research Group (OSBREAC; see 4.2.2), we have recently shown in a **retrospective observational study**[11] that patients with low serum concentrations of Z-4OHtam < 3.26 nM or Z-Endoxifen < 9.00 nM (about 12% of all patients) have significant worse breast cancer specific and total survival than patients with serum concentrations

above these thresholds(adjusted HR = 4.3; CI95 = 1.9-13.6) (red curves in Figure 4). Patients with a very high level of these metabolites (approximately 12% of patients) had no breast-specific endpoints (green curves in Figure 4) . [11]

Now, we need to validate this discovery in independent patient materials. **If validated, this will be of direct clinical benefit for 25% of ER + breast cancer patients planning adjuvant tamoxifen treatment.** Such therapeutic drug monitoring (TDM) could identify risk patients with inadequate levels of active tamoxifen metabolites. This could lead to a dose increase or switch to an alternative endocrine treatment form in these patients. Patients with very high metabolite levels can continue on tamoxifen and do not need to switch to an aromatase inhibitor.

Thus, TDM may turn out to be a paradigmatic shift in endocrine tam treatment of ER-positive breast cancer. Importantly, the distance from "bench to bed" in this study is very short due to our recent findings, a feasible method and over 30 years of experience with tamoxifen in the clinical setting. **A revised version of this paper [11] will be submitted to *Breast Cancer Research* within 20. Sept. 2017.**

Validation Cohort 1

In an OSBREAC study, Secondary Adjuvant Treatment with Taxanes (SATT), [19] with over 1,000 patients enrolled, there are 750 patients who have started tamoxifen. 11% of them have recurrence or died of breast cancer. Here we have bone marrow plasma samples from most of the participants 1 year after this treatment. The number of patients who have taken Tamoxifen and are relevant for studying tamoxifen metabolites will be 453 patients from this study. Of these, 11.7% have relapsed or died of breast cancer during the observation period (i.e. 60 endpoints). This material constitutes an appropriate material validation as there is an adequate number of patients, follow-up time and sufficient amount of endpoints.

Validation Cohort 2

We have established a collaboration with Prof. Helena Jernström at the University of Lund, Sweden. Here, serum samples from adjuvantly tamoxifen treated breast cancer patients are available for analysis. The clinical follow-up is completed and the database is established. The median follow-up time is 7 years and there are 30 breast cancer related endpoints.

Power analysis

If we anticipate 12 % of the patients belong to the low metabolite group, HR = 4.3 , α= 0.05 and β=0.20 the number of needed endpoints = 35. If we pool the OSLO and LUND cohorts (i.e. 90 endpoints) we will have 80% power to detect a HR of 2.5 or larger.

B. Adherence to endocrine treatment

The underlying causes of low adherence to endocrine treatment are complex and consist of both biological (metabolic differences in tam/estradiol levels) and psychosocial factors.[20] We aim to design an algorithm helping clinicians to identify risk factors for low adherence in breast cancer patients in the PBCB-cohort. This algorithm will identify patients with a high risk of becoming non-adherent and make the patient follow-up more focused and individualized. The 10 PROMs collected in WP-1 (e.g. HRQoL, Depression, uncertainty in Illness, fatigue, side effects) are used to map the psychosocial factors and side effects. Blood samples from the PBCB biobank will be used to map the metabolite profile; Furthermore, we have access to the Norwegian Registry of prescription (Reseptregisteret) to control for use of prescribed drug. In addition to the anti-ER effect, the tam metabolite profile may also be associated with side effects, specifically to the concentration of tam and its de-methylated metabolites in serum. [21]

WP-3. Personalized Monitoring of Breast Cancer Patients (PerMoBreCan) novel biomarkers for early detection of early systemic relapse

PBCB serves as a crucial basis for the **PerMoBreCan** (Personalized Monitoring of Breast Cancer Patients) project. Now, we need funding to cover the operating costs for PBCB to serve this project portfolio. PerMoBreCan offers original contributions with regard to detection of new biomarkers of systemic relapse in early breast cancer. This **prospective observational study** will characterize the tumor-biological line from the primary tumor, and circulating biomarkers from the metastasis. This study is powered to enroll 125 high-risk breast cancer patients from the PBCB-cohort at SUS. Funding will also be sought through other applications for the PerMoBreCan project.

Primary tumor analyses

Genome-wide expression profiles have shown that breast cancer patients could be divided into subgroups with different prognosis.[22] The PBCB material will be used to isolate and sequence total RNA from the primary tumor of high-risk patients by the Ion Proton system for Next Generation Sequencing (NGS). The primary tumor samples will be checked for mutations especially in ER and other known oncogenes, and characterization of differentially expressed microRNAs and mRNAs between patients with and without recurrences under treatment. As proliferation is the most important prognostic and predictive in breast cancer it will be assessed by the novel "**K**i-67 **A**djusted **M**itotic **S**core" (KAMS) approach. KAMS and centrosome amplification will be performed in collaboration with Dr. Aneja at Georgia State University (Atlanta, USA) (See 4.2.3).

Circulating tumor markers

a) Circulating tumor cells **(CTCs)** have been shown to provide prognostic information in metastatic and non-metastatic breast cancer patients, and have been used successfully for monitoring the effects of chemotherapy in the metastatic setting.[23] The PBCB biobank will be used to enrich circulating tumor cells (CTCs) from peripheral blood. **Presently, 133 patients from the PBCB-cohort have been analyzed. Unpublished data confirm high feasibility of the method in our cohort.**

b) Circulating tumor DNA (ctDNA) is extracellular tumor-derived DNA that originates from apoptotic and necrotic cells in the primary tumor, metastatic lesions or in circulation (CTCs). [7] ctDNA detection may also provide therapeutically relevant, predictive and prognostic information in breast cancer.[24] The PBCB material will be used to establish a gene panel including 50 gene regions/genes highly mutated in breast cancer and to perform sequencing of multiple target genes/gene regions by NGS.

c) MicroRNAs are promising biomarkers for cancer detection and treatment response monitoring in breast cancer.[25,26] The PBCB material will be used to perform microRNA profiling on RNA isolated from exosomes and Tumor Educated Platelets (TEPs). We will compare tissue microRNA profiles from tumors sensitive to adjuvant chemotherapy with profiles from resistant metastatic tumors.

III. Analyses of the metastatic lesion.

In patients who experience relapse, biopsies from the lesions will be taken as well as liquid biopsies every month to monitor the treatment. All markers obtained from the primary tumor analysis, CTCs, ctDNAs and microRNAs will be compared with the metastases.

WP-4. Validation and implementation in clinical trials

PBCB is part of an inter-regional network on breast cancer (with Oslo University Hospital and NTNU, Trondheim).[27] This collaboration allows for the necessary validation of all statistically relevant biomarkers in large patient cohorts.

- PBCB Bergen: N=685 patients with annual blood samples.
- OSLO-2: N=2000, a consecutive study collecting tissue/blood from breast cancer patients with primary operable disease (Stage I and II) in Southeastern Norway.[28] This network will provide the necessary infrastructure for performing interventional studies with randomized controlled design.

Funding for this WP is not included in this application. Long term planning and funding will be

sought in due time through collaboration with Oslo Breast Cancer Research Group (see 4.2.2).

4.2 Organization and Collaboration

4.2.1 The regional PBCB-platform

PBCB has expanded during the last 7 years, forming a basis for important regional research based on collaboration between Haukeland University Hospital and Stavanger University Hospital. Regional meetings are held twice a year, with a research group consisting of 22 members who represent a wide spectrum in the field of breast cancer research (breast cancer surgeons, pathologist, oncologist, radiologists, endocrinologists, molecular biologists, nurses, cultural studies researcher and lab personnel). We have complete organization, procedures, personnel, and policies for recruiting patients, collecting biological specimens, data, questionnaires, and a plan for establishing the Master Database. Steering committee: Håvard Søiland, Gunnar Mellgren*, Turid Aas, Jørn V. Sagen, Jennifer Gjerde, Ernst Lien and Tone Hoel Lende. The PBCB study group has published 68 papers over last 5 years (see attachment). ***Prof. Mellgren is head of PBCB-Bergen. He has published 49 papers over the last 5 years (mean IF=6.19). H-index=27. He has supervised 28 master students, 13 PhD candidates and 10 postdoctoral fellows.**

4.2.2 National collaboration

A network collaboration South-East Norway (funded by the South-Eastern Norway Regional Health Authority) is established (Head of the OSBREAC group is **Kristine Kleivi Sahlberg)**, which allows for validation of our data in the OSLO-2 study (WP-4). In addition, we participate in a large national neo-adjuvant study with 8 different treatment arms (PETREMAC). In this study we will analyze proliferation and microRNA expression (Pathology/SUS) and ctDNA in blood from participating patients (Oncology/SUS). Furthermore, collaboration with **Prof. Bjørn Naume** (OSBREAC) secures access to the SATT-study for validation of the tamoxifen metabolites study in WP-2. He is also head of the Norwegian Breast Cancer Group (NBCG). **Prof. Jan Terje Kvaløy**, statistician at UiS/SUS, will supervise the process of establishing the master database in PBCB (WP-1).

4.2.3 International collaboration

Prof. Timothy Lash is a highly experienced medical epidemiologist with a great international reputation in cancer epidemiology and studies on cancer biomarkers. He is head of Department of Epidemiology, Rollins School of Public Health, Emory University, Atlanta GA, USA. His expert knowledge in molecular biology and testing of the validity of various cancer biomarkers (PROBE) will be important for WP-2 and 3. **Prof. Mylin Torres** at Emory University, Atlanta, US, is the director of the Winship's Glenn Family Breast Center. Together with Timothy Lash and Mylin Torres we have started a project on biological mechanisms for chronic fatigue in breast cancer survivors with a basis in the PBCB-patients and the PBCB biobank material. The PBCP-group also has a long-term collaboration with the European Institute of Oncology in Milano, Italy **(Dr. Bernardo Bonanni).** Recently, we have published several papers in collaboration with this group. Dr. Bonanni will also contribute in WP-2 and WP-3. The pathology group at SUS currently collaborates with **prof. Ritu Aneja** (Atlanta) on a large cohort of triple negative breast cancer patients, investigating centrosome amplification/proliferation/leucocyte infiltration and exosome sequencing in correlation to disease outcome (WP-3). Moreover, the PBCB regional study group has a close collaboration with Importantly, we have also established a collaboration with **Prof. Helena Jernström** in University of Lund, Sweden where serum samples from tamoxifen treated patients are available to validate the findings of active tamoxifen metabolites in WP-2.

4.3 Budget

The budget plan is a continuation of the former support from Helse Vest in 2010 and 2013. In the present application, we apply for 1,500 000 NOK annually for 3 years, a total sum of 4,500 000

NOK in this 3-year period (See e-application). Importantly, the PBCB project is in urgent need of two study nurses/lab personnel divided between HUS and SUS who can handle the growing amount samples to be collected from accrued patients and patients in follow-up. Just as urgent is the need of a qualified person who can serve both HUS and SUS in order to continue building and maintaining the Master Database during this project period under supervision of Prof. Kvaløy. Notably, WP-1 is the basis of WP-2 and WP-3 in this application. Therefore, the budget is given priority to this in the present application. A separate application will be submitted for the PerMoBreCan study in WP-3.

4.4. Plan for Milestones and Dissemination

Milestones of the WPs in the present application of the PBCB-project portfolio 2017 – 19	Estimated Timeline
Completion of 1st time visit of 1200 breast cancer patients (WP-1)	Q4 2019
Collect liquid biopsies and PROM data from ~1000 patients in follow-up (WP-1)	Q4 2020
Building the Master Database in PBCB at SUS and HUS (WP-1)	Q4 2020
Finish of tissue collection in 75 patients in the paracrine study (WP-1)	Q1 2020
Finish anlysis of PROM- & adhernce data for risk assessment of non-adherence (WP-1)	Q1 2020
Analyses of tamoxifen metabolites in the SATT- study /Oslo (WP-2)	Q3 2018
Analyses of tamoxifen metabolites in Lund-material /Sweden (WP-2)	Q2 2020
Isolate microRNA from TEPs in the PBCB material (WP-3)	Q4 2019

Dissemination

Scientific findings will be reported in reputable international peer review journals and presented at conferences regionally, nationally and internationally. Results will also be communicated directly to user organizations and clinical milieus. The hospital's website (www.sus.no) provides information about the biobank for general public. Results will be communicated to the public though social media channels (Facebook & Twitter) and through mass media (regional & national newspapers, radio & television).

4.5 Plans for Implementation

Tamoxifen is an established and cheap drug without any special economical conflict of interests to analyze. The LS/MS/MS method for measuring tamoxifen metabolites is established in the core facility at University of Bergen and the Hormonlaboratoriet at HUS. The method is accurate, feasible and has capacity to analyze tamoxifen metabolites in serum from all breast cancer patients in Norway allocated to this treatment. We have established contact with Norwegian Breast Cancer Group (NBCG) for evaluation of the results from the validation study in WP-2. Hence, we have all the resources and contacts needed for a rapid implementation of therapeutic drug monitoring of tamoxifen in Norway. Circulating biomarkers of early systemic relapse can easily be obtained from a simple blood sample in the follow-up of the patients. A second adjuvant treatment may then be given. Implementation of these results will be performed through internal validation between our centers and external validation through our inter-regional/national network on breast cancer. Importantly, forthcoming novel treatment options (e.g. immune therapy) will demand novel disease markers to meet the requirement for sufficient clinical management of the future breast cancer patient. The PBCB-project portfolio **represents a bold and highly innovative** approach to meet this coming need. The innovation potential will be evaluated through the project period and contact will be made with *Bergen Teknologioverføring (BTO)* in Bergen and *Validé TTO* in Stavanger when relevant.

5. User Involvement

PBCB has an active collaboration with two user representatives from the regional breast cancer society (Brystkreftforeningen), a breast cancer advocacy group in our region. Both serve as active partners in the user involvement program (Brukermedvirkning) and take part in our project meetings. They are both breast cancer survivors and have provided valuable input into several

aspects of the PBCB projects. For the WPs, the end-users will be *the future breast cancer patient*. They will get a more personalized follow-up program and hopefully a much more sophisticated and appropriate surveillance and treatment of systemic relapse.

6. Ethical Considerations

All patients have given written consent to participate in the various projects. PBCB got the main formal biobank approval (WP-l) in 2010 by REK-Nord #2010/1957). The collection of PROM data are in REK-Nord #2011/2161. The PerMoBreCan project (WP-2) is approved in REK-Nord #2015/2010. The paracrine project (WP-3) in REK-Nord #2013/25. The validation and implementation of validated biomarkers will have to be approved by the regional ethical committee and the Norwegian Breast Cancer group (NBCG).

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Overall research protocol for the study of work life participation in Breast Cancer Survivorship

Background

Treatment of breast cancer employs all surgery, chemotherapy, radiation therapy and various targeted therapy options like antiestrogen and anti HER-2 therapy.These treatment options create bothersome side effects both in a short term and long term perspective. Importantly, more complex relationships between oncological therapy modalities and subjective health complaints in breast cancer patients are recently discovered (1). Fatigue, anxiety and depression scores are probably functional read outs of the total treatment burden. The anti cancer treatment has also a substantial impact on Quality of Life and social aspects for the cancer survivors which extends to work life participation. Sick leave (SL) five years after the breast cancer diagnosis is more dependent on social factors than on illness (2). Hence, breast cancer hits the women twice; first the disease burden then the sosio-economic worries. Breast cancer survivors have almost 3 x higher risk of receiving disability pension (DP) compared to cancer –free patients in a retrospective register based study (3). However, this study was based on mostly abandoned oncological treatment regimens.

Aims

The aim of this study is to identify risk factors for becoming a long term SL, WAA or DP receiver by an in-depth qualitative analysis and mixed-method approach by triangulating PROM data, clinical/biological data and the NAV-data. Use of NAV-data as endpoints will provide valuable information to clinicians and general practitioners who may optimize the follow-up also in regards to work-life participation. Hence we have a robust study design to identy fisk factors for high SL, WAA and DP rates. Ultimately, we aim to lower the financial expenses breast cancer generates to society.

Project description - 200720

Study design / material & methods.

The study design is an observational study where we will in the present study map the rates of and denty fisk factors for high SL, WAA and DP among breast cancer survivors with the up-to date treatment schedules. We will follow a 4-step translational approach:

1. We will first conduct an exploratory qualitative study using document analysis of health and work life policy in the Norwegian context, combined with empirical data from semi- structured interviews and focus group with 20 high risk patients) to be analyzed thematically. The theoretical framework for this qualitative project is derived from symbolic interactionism in which uncovering and understanding meaning in specific contexts is a goal. Since meaningful social and professional interactions are important for psychosocial rehabilitation of breast cancer individuals symbolic interactionism is an appropriate methodological framework. This qualitative approach will create the bases of the approach in the PROM based and NAV-database oriented studies below.

2. NAV- data

   We currently collaborate with vice director Anneline Christine Teigen and senior consultant Günter Olsborg in NAV-Rogaland and the Mikrodata section at Statistics Norway (SSB). The aim for this collaboration is gain more knowledge about the use of social security services among breast cancer patients together with the NAV-Rogaland experts and together with SSB use the FD-trygd database to obtain reliable prospective sociodemographic data on SLs (both short & long term), Work Assessment Allowance (WAA) (Arbeidsavklaringspenger) and DPs (both time limited & life long) for each patients in the present PerMoBreCan study.

3. Patient reported outcome measure (PROM) data is obtained at baseline and thereafter yearly from all PerMoBreCan patients. The PROM data consists of 1.HRQoL instruments *(EORTC QLQ-C30,EORTC QLQ-BR23 and FACT B)*, 2.*Hospital Anxiety and Depression Scale, HAD*, 3. Fatigue instruments *Fatique Impact Scale, FIS*; Fatugue Severity Scale (FSS) and VAS-fatigue, 4.Side effects questionnaires, 5. Joint-pain questionnaire, 6. *The Mishel Uncertainty in Illness Scale, MUIS;* and 7. Food habits questionnaire and the 8.ROMA III questionnaire of bowel complaints (IBD and IBS).

4. Biomarker identification.

   All patients attending this study will be screened on circulating biomarkers releated to fatigue. These biomarkers are on the protein level, genetic expression level and also on the epigenetic level. Our long-established pleasant cooperation with prof. Roald Omdal at Stavanger University Hospital, who is a clinical immuologist and transaltional fatigue researcher, is committed to conduct these analyses in his research lab.

Collaborations

In addition to the collaboration with NAV, we have a close collaboration with Prof. Roald Omdal who is a clinical immunologist and translational fatigue researcher, and will perform the analyses of various biomarkers related to fatigue. Moreover, an important collaboration is established with Prof. Elisabeth Severinsson (University College of Southeast Norway) and Ass. Prof. Kari Nyheim Solbrække (University of Oslo) to include qualtitative and mixed-method designs. Coworkers in the Breast Cancer Surgery group and the Oncology group will work together to elaborate on this WP.

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