Stiftelsen Kristian Gerhard Jebsen

K.G. Jebsen Centre for Translational Medical Research

Final report

for

K.G. Jebsen Centre for Breast Cancer Research

- **Principal Investigator:** Anne-Lise Børresen-Dale
- **Host institution:** University of Oslo
- **Host department:** Institute of Clinical Medicine
- **Project period:** 2011-2015
- **Grant number:** SKGJ-MED-004

Oslo 22/3-2016

Prepared by the centre leadership and signed by the Principal Investigator and the head of the host department. (See attached letter from host institution UiO)

1. Objectives

Main objectives of the centre as stated in the contract/ project description.

Vision for the Centre: *Towards personalized therapy for breast cancer; integrated molecular and epidemiological studies of breast cancer to reduce risk, improve prognosis, and tailor the treatment.*

The overall aim of the research Centre has been to open up for stronger collaboration between clinical and basic scientists, and to motivate exchange of ideas on how to best utilize the huge collection of patient materials and data generated for the benefit of the patients. By such synergism we have been able to explore genes/pathways/networks involved in basic processes like cell cycle, DNA repair, apoptosis, and immune response and their impact on breast cancer development, progression and response to therapy.

2. Brief summary (max. 1/2 page)

Progress and achievements with regard to the main objectives of the centre

We have performed longitudinal studies of samples at different stages of the disease and characterized such patient materials in full molecular details. The results have paved the way for initiating clinical trials to identify novel patient subgroups for tailored therapy and monitoring. The research project has utilized a number of previously and newly collected clinical cohorts. We have performed "state of the art" analyses of patient material, both on bulk tumours, single cells and liquid biopsies, at various stages of the disease, from consecutive series with up to 25 years follow-up (OsloVal, Oslo0, Oslo1, Oslo2). Several clinical trials have been instigated during the Centre period such as NeoAva, EMIT and IBCT. In the NeoAva trial (neoadjuvant treatment with/without bevacizumab) analyses at 3 different time-points at several molecular levels have been performed, and changes at all levels during treatment were observed. An overall strong correspondence in the shifts was seen at several molecular levels, but still substantial differences between the levels were observed for specific loci and biological functions. These results point to the importance of studying the correlated and non-correlated features at multiple molecular levels in order to develop integrated signatures for prediction and prognosis evaluation. In the EMIT study (Establishment of Molecular profiling for Individual Treatment decisions in Early Breast Cancer) analyses are ongoing and financing secured through other sources to complete the study. The IBCT study (Improved breast cancer therapy), a phase 2 clinical Trial, both in the neoadjuvant and metastatic setting, is aiming at identifying a biological rationale for optimal selection of treatment regimens). In this study patient recruitment has started, and funding has been secured through other sources to complete the recruitment and perform all molecular analyses.

3. Group leaders / senior investigators within the centre

The Centre has consisted of six research group

- Clinical groups: Dr. Ellen Schlichting, Prof. Em. Rolf Kåresen (surgery), Prof. Torill Sauer, Dr. Elin Borgen (pathologist, pHD), Dr. Hege G. Russnes (pathologist, pHD, consultant), Prof. Erik Wist, Dr. Olav Engebråten and Prof. Bjørn Naume (oncology)
- Molecular groups: Prof. Anne-Lise Børresen-Dale (director) and Prof. Vessela N. Kristensen (deputy director)
- Micro-metastases groups: Prof. Bjørn Naume, Prof. Em. Øystein Fodstad
- Model-systems and functional groups: Prof.Gunhild M. Mælandsmo and Prof. Em. Øystein Fodstad
- Metabolic profiling and imaging group: Prof. Tone F Bathen, Prof. Ingrid Gribbestad (diseased 2013), NTNU
- Bioinformatics/biostatistics group: Prof. Ole Christian Lingjærde

4. Research

- *Main research activities and achievements for each of the thematic areas of the centre*
- *Please account for any novel research opportunities (e.g. mechanisms / markers etc.) that may have been identified during the project period and describe how these have been pursued*

The Centre has focused on several well integrated activities 1) High throughput molecular characterisation of primary tumours; 2) Detection and characterization of occult tumour cells in bone marrow (BM) (DTC), blood (CTC) and sentinel lymph nodes (SLN) as well as detection of cell free tumour DNA (ctDNA) in blood as a new development; 3) Functional studies in experimental model systems; 4) Metabolic and physiological characterization and 5) Data integration.
Most of these activities will be prolonged in the OSBREAC consortium after termination of the Centre 1/1-2016. The OSBREAC consortium will continue to move the field forward, also through a Regional Research Network funded from HSØ to OSBREAC.

Activity 1

High throughput molecular characterisation of primary tumours

Combined analyses of molecular data (copynumber, mRNA, miRNA, methylation, protein expression, metabolic profiles) from > 2000 patients have identified novel biological functions and molecular pathways deregulated in breast cancer. These results have paved the grounds for initiating clinical trials to identify novel patient subgroups for tailored therapy and monitoring.

The NeoAva study. The molecular characteristics of responding and non-responding tumors from breast cancer patients when treated with antiangiogenic therapy were studied. Markers identifying patients with benefit for such therapy would introduce a new therapeutic strategy for such patients. The NeoAva study included patients with HER2 negative primary tumors of ≥25 mm that were randomized (1:1) to receive standard neoadjuvant chemotherapy with or without bevacizumab. Tumor material was obtained at screening, 12 weeks into treatment and at surgical removal at 25 weeks. mRNA expression profiling was performed. In this study, 132 patients were evaluable for tumor response, and pCR in breast and axilla were obtained in 14 (21%) patients in the chemo+bev arm, and in 7 (11%) patients in the chemo-only arm. The overall pCR rates were higher in the ER negative tumors compared to ER positive tumors, but addition of bevacizumab seemed to improve pCR in the ER positive patient group (9 vs 3) and not in ER negative patient group (5 vs 4). Elevated immune response was found to be a marker of good response in tumors treated with combination therapy in the whole patient population, but a stronger association was found in the estrogen receptor positive subgroup. Proliferation scores were reduced following treatment, in particular in the combination arm where the reduction in proliferation accelerated during treatment. In response to therapy, tumors achieved a better prognosis profile, i.e. Luminal A or Normal-like profile. The changes in gene expression in response to therapy were subtype–specific and the effect of adding bevacizumab, on gene expression, was most evident in Luminal B tumors. Analysis of copy number alterations demonstrated significant differences in genomic instability at the time of diagnosis. Differences in 25 specific loci were significantly different between good responders and poor responders. In addition, signs of subclonal reduction with the treatment were observed, that may indicate a differential effect on different tumor subpopulations. Protein (RPPA array) and DNA methylation analyses are finalized and data analyses are ongoing and several manuscripts under preparation. Analyses of metabolomics by magnetic resonance spectroscopy (HR-MAS) demonstrated clear metabolic changes as an effect of chemotherapy, with a decline in glucose consumption and a more normal metabolite profile, similar to the results in the gene expression studies. Patients with a low glucose and high lactate content were more likely to achieve a good response to therapy. (See also activity 4)

The insights provided in this study on molecular changes in tumors treated with bevacizumab in addition to chemotherapy may aid in identifying a patient group most likely to benefit from antiangiogenic therapy.

The EMIT study. The practical organisation and establishment of working groups of the EMITEBC study (Establishment of Molecular profiling for Individual Treatment decisions in Early Breast Cancer), headed by Bjørn Naume was initiated during 2015. RNA from formalin fixed paraffin embedded tissue from around 700 patients from the Oslo1 study has been isolated, followed by PAM50/ROR gene expression profiling using the Nanostring nCounter system. So far the classification results are available for 520 patients, and indicate that PAM50/ROR provides a better prognostic information and improved classification compared to today's routine standard analyses. The study is progressing as scheduled, and tissue from ca 100 patients belonging to the second, prospective, phase of the study are being collected. Collaboration within EMIT with Dr Aleix Prat (Medical Oncology Department, Hospital Clinic de Barcelona) primarily regarding the use of Nanostring nCounter analyses for molecular profiling has been initiated. Also a collaboration with Professor Åke Borg (University of Lund) has been established, primarily for parallel RNA sequencing analyses of the primary tumours from EMIT patients.

Deep sequencing of selected tumour samples has identified new genes involved in cancer evolution, and novel mutational processes that evolve across the lifespan of a tumour, with patient-specific signatures of point mutations and chromosomal instability. Advanced in-situ techniques such as immunoFISH (IFISH), and novel software we developed (GoIFISH) have now been used to objectively detect cell-to-cell variation within tumours. Estimation of intra tumour heterogeneity by IFISH has been performed on 40 patients with HER2-positive tumours. As anti-HER2 treatment was given in a neoadjuvant setting, tissue biopsies taken before and after the treatment have been analysed. The results show that patients that did not respond to therapy had an increased diversity prior to treatment. Further, the dynamics during therapy measured by a Kullback-Leibler divergence score predicted long term outcome. The software GoIFISH was published in 2014 and is shared on an open access site (https://sourceforge.net/projects/goifish/). The results from the study of the HER2 positive cohort have recently been defended in the PhD thesis by Inga H Rye (UiO) and the manuscript in process of being submitted.

Single-cell sequencing of bone-marrow epithelial-like cells, in parallel with intra-tumour genetic heterogeneity profiling from bulk DNA, is a powerful approach to identify and study DTCs, yielding insight into metastatic processes. Comparing DNA alterations of several single DTCs per patient to each other, to the corresponding primary tumour and lymph node, we observed dissemination of single tumour cells throughout the tumours evolution. By demonstrating subclonality in the lymph node metastasis, and their copy number profiles resemblance to primary tumour, we provide novel insight into the metastatic process. Evolutionary reconstruction analysis of bulk tumour and DTC genomes enabled ordering of CNA events in molecular pseudo-time and tracing the origin of the DTCs to either the main tumour clone, primary tumour subclones, or subclones in lymph node metastases. A heterogeneous population of CNA-positive cells of unknown origin was prominent in bone marrow. These analyses will be continued with combined genome/ transcriptome sequencing analyses of the single cells in other projects. (See also Activity 2)

DNA from blood leukocytes has been isolated and genotyped for the SNPs in the International Breast Cancer Association Consortium
http://ccge.medschl.cam.ac.uk/research/consortia/icogs. We have in the Centre performed and published a number of studies on the risk of genetic polymorphism (SNP). In the recent years the SNP studies have grown into more and more whole genome scans and most of our SNP analyses are now coupled to the Breast Cancer Association Consortium (BCAC). The advantages of performing SNP analyses in this format are numerous. Our contribution to the 80 000 breast cancer cases and similar number of controls is more than 2000 cases. In the first stage of the collaboration in the consortium we participated in the validation step after the identification of the first susceptibility GWAS loci. In the second phase, we participated in the so-called fine mapping, aiming at explaining the existing hits and to identify additional

susceptibility markers in the originally discovered genome loci. This work is now entering a third phase, where resequencing of the chromosomal regions around mutations identified by the ICGC (International Cancer Genome) consortium will be performed.

Activity 2:

Detection and characterization of occult tumor cells in bone marrow (BM) (DTC), blood (CTC), cell-free tumor DNA (ctDNA) and sentinel lymph node (SLN).

The SATT study aimed at analysing whether DTC status in breast cancer patients may be used as a surrogate marker for the effect of docetaxel given as a secondary adjuvant therapy. The patients were analysed for DTC after completion of standard antracyclin-containing chemotherapy, and given docetaxel if DTC-positive. In these patients new DTC analyses were performed also one and 13 months after completion of the docetaxel treatment. A total of 1128 patients were included. The results revealed that the patients with presence of DTC after standard chemotherapy receiving the secondary docetaxel regimen – and obtaining eradication of detectable DTC (79% of the patients) - had a particularly low risk of relapse (8.8% relapsed). The patients in which DTC could still be detected after the docetaxel therapy had a high risk of relapse (46.7 % relapsed) (Naume B et al, JCO 2014). This indicates that DTC analysis may be a valuable tool to identify patients who might benefit of changes in the additional therapy during follow-up after standard chemotherapy. In 2015 further analyses were started, aiming at identifying those among the patients that would have a particular benefit of having their DTC status determined. As part of this, isolation of RNA from the primary tumours (sections from the routine diagnostic formalin fixed paraffin embedded tissue blocks from a selection of 260-280 patients) are being performed and submitted to multi-gene expression analyses (PAM50/ROR profiling) using the Nanostring nCounter system recently established at OUS, Dept. of Pathology. Financing of continuation of this study has been secured through other sources.

The previously reported single-cell array high-resolution comparative genomic hybridization method for characterization of single DTCs was followed by analysis of the first two DTCs by next-generation sequencing as a proof-of-principle for the use of this technology (Møller et al., Frontiers in Oncology 2014).This work was continued in 2015, with whole genome sequencing of DNA from a total of 40 single DTC from 6 breast cancer Oslo1 study patients. The cells' DNA copy number aberration (CNA) landscapes were compared with those of the primary tumours and with an available lymph node metastasis. The data obtained enabled ordering of CNA events in molecular pseudo-time and tracing the origin of a quarter of the DTCs to either the main tumour clone, primary tumour subclones, or subclones in an axillary lymph node metastasis. The remaining cells, which are under further investigation, represented non-aberrant 'normal' cells and 'aberrant cells of unknown origin' having CNA landscapes discordant from the analysed parts of the primary tumour. Overall, these observations indicate that dissemination may happen both at an early time point of the tumour formation, as well as at later stages, and that the DTCs may also gain additional aberrations after leaving the primary site (Møller EK et al, submitted to Genome Biology January 2016). Further single cell analyses will be performed at the genome and transcriptome level to further elucidate the function of these cells with funding from other sources.

The DTC may persist in bone marrow in a dormant state for a long period, but may start dividing and give rise to further dissemination and tumour progress. The biological properties and characteristics of DTC are still largely unknown and the cells may be variably vulnerable or sensitive to cancer therapy. Efforts aiming at further characterisation of DTC from several patient cohorts are therefore being carried on in other projects. To assess the potential for cell proliferation and cell death (apoptosis) of DTC, a double immunofluorescence technique (Ki67/cytokeratin and M30/cytokeratin, respectively) was developed. Applied to bone marrow samples from the SATT study patients however, no clear correlation between the expression of these markers on DTC and docetaxel treatment effect could be revealed (Naume B et al, JCO 2014). To further supplement the picture, during 2015 a similar protocol for characterisation of DTC for the tumour dormancy marker NR2F1

was established and is currently being applied to patients belonging to the SATT, Oslo1 and the Neo-Tax studies (so far 90 patients analysed). This project is part of a collaboration newly initiated with professor Aguirre-Ghiso at Mount Sinai School of Medicine, New York. Preliminary results including, for some patients, both Ki67, the apoptosis and the dormancy marker, have been summarized in an abstract submitted to the International Symposium on Minimal Residual Cancer i Hamburg (March 2016).

The Neo-Ava study headed by Olav Engebråten aims at testing the addition of Avastin to neoadjuvant chemotherapy in locally advanced breast cancer patients.. Analyses for CTC, DTC and circulating endothelial cells (CEC) have been performed, and during 2015 a registration of the clinical status of the patients was performed. The CTC, DTC and CEC data will now be compared to other tumour analyses that have been performed (see Activity 1), in relation to clinical status. The first analysis related to CTC is planned as part of a large international pooled analysis (totally > 2000 patients; headed by Dr Bidard, Institute of Curie, Paris), to explore the prognostic value of CTC in comparison to therapy response (pathologic complete response) of the tumour. Data were transferred in 2015 to the Curie Institute for further processing, and presentation of final results are expected in first half of 2016.

In the Oslo2 study, in addition to the collection of primary tumour tissue, blood and bone are being collected and analysed for CTC and DTC. So far 1284 blood/bone marrow samples have been collected. In 2015 a registration of the clinical status of the first 600 Oslo2 patients were performed, planning to perform the first analyses on this study population during 2016.

Through the EU-funded collaboration Miracle (Grant agreement number 257743), a new system prototype for the detection and characterisation of CTC was developed and the project terminated in 2015. A new EU project, Cancer-ID (supported by IMI) was initiated in 2015, having as one of the aims to test and validate tools for detection of CTC in patients with HER2-positive breast cancer.

Methods for analysis of circulating tumour-DNA have been established during 2015 in a project headed by Hege Russnes. These are planned to be applied on patient samples for which DTC/CTC results are already available for comparison of the methods, and new funding will be sought.

Collaboration with University of Michigan (Russell Taichman) has been established to characterise breast cancer tumours (Oslo1 patients, TMA sections) for cancer stem cells and compare to DTC and clinical outcome.

Activity 3:

Model systems and functional studies

Model systems: We have put effort into establishment of patient-derived xenograft (PDX) models representing the different subtypes of breast cancer and are now equipped with seven orthotopic PDX models. The models have been characterized for histological, physiological, metabolic and molecular parameters (Moestue et al, 2010; Huuse et al, 2012; Kristian et al, 2013; Grinde et al, 2014; Kristian et al, 2014; Esmaeli et al, 2014), as well as functional heterogeneity (Skrbo et al, 2014), and they are found to resemble the clinical situation remarkably well. Thus, the orthotopic models constitute an extremely valuable resource to study cancer development and progression and for preclinical evaluation of drug responses (see Hidalgo, Mælandsmo et al, Cancer Discovery, 2014; Review by the EuroPDX Consortium).

In an experimental set-up resembling the OSL3/NeoAva clinical protocol, the effect of doxorubicin in combination with bevacizumab was investigated in the PDX models. The benefit of anti-angiogenic therapy in breast canceer is debated, and in attempts to identify predictive biomarkers the animals were examined by MR imaging (Moestue et al, 2013) and tumor tissue was subjected to multilevel molecular characterization (Lindholm et al, 2012; Borgan et al, 2013). Inhibition of the PI3K/AKT/mTOR pathway was suggested to sustain the anti-angiogenic effect (Moestue et al, 2013; Lindholm et al, 2014), and changes in phosphoglycerocholine (GPC) level were suggested as a predictive biomarker – a finding that also was seen in the clinical study.

Dynamic PET (dPET) is a non-invasive method that depicts the distribution of labelled glucose and may reflect treatment-induced responses on tumour physiology, vasculature and metabolism. We have demonstrated that anti-angiogenic treatment resulted in reduced perfusion and metabolism, and have shown that enhanced growth retardation was obtained when the cytotoxic drug was administered together, instead of delayed compared to the anti-angiogenic treatment (Kristian et al, 2013). We have also demonstrated that enhanced perfusion and permeability some days after treatment was associated with drug responses (Kristian et al, submitted), suggesting that dPET, similar to functional MRI, is useful to assess early treatment responses and for optimization of person-adapted treatment protocols.

Functional studies: In attempts to identify new strategies for inhibiting HER2-positive breast cancer, miRNAs negatively regulating the HER2 pathway was identified (Leivonen et al, 2013). miRNAs screens to identify regulators of the metastasis-promoting proteins B7H3 (Kveine et al, 2014) and S100A4 has also been performed with the aim of unravelling novel targets for anti-metastatic therapy. Drug sensitization screens utilizing HER2-postive cell lines identified dasatinib and AKT-inhibitors as potential promising drugs and in vivo experiments evaluating the combinations are in progress. Context-induced tumour cell plasticity has been investigated and EGFR, when expressed in HER2 overexpressing cells, was shown to act as a tumour suppressor by maintaining epithelial cell integrity (Ingthorsson et al, 2015). We aim to follow up this by studies of treatment-induced cellular plasticity with special emphasis on metabolic reprogramming. Recently, we demonstrated that B7H3 knockdown sensitises triple negative breast cancer cells to AKT/mTOR inhibitors by reducing the glycolytic capacity (Nunes-Xavier et al, 2015).

We have also demonstrated that S100A4-stimulated BC cells undergo a metabolic switch towards glycolysis and induce pro-inflammatory cytokines promoting differentiation of M2 macrophages (Bettum et al, 2013; 2015). Such activated macrophages have been shown to support BC cell migration and reduce the sensitivity for anti-cancer drugs (Tenstad et al, in prep). We hypothesize that S100A4 is a DAMP molecule (Damage Associated Molecular Pattern) that aids tumour cell growth during cancer progression and treatment, and that such DAMP-mediated signalling represents a potential target in cancer therapy to be tested in combination protocols. LXR, a master regulator of the innate immune system, cholesterol homeostasis and synthesis of inflammatory lipids, has also been linked to tumour cell proliferation. We have observed that LXR agonists reduce BC growth in vitro and in vivo, and are in the process of evaluating the effect in combination with conventional chemotherapy.

Activity 4:

Metabolic and tumor physiological characterization by MR

MR metabolomics is a widely used approach in translational research, aiming to identify clinically relevant metabolic biomarkers or generate novel understanding of the molecular biology of tumours. Generating robust and valid data using MR metabolomics requires close attention to experimental detail. Analysing tumour tissue obtained from the luminal-like and basal-like xenograft models (n=6) with HR MAS MRS, the metabolic changes caused by the time interval from surgical removal of a tumour until it is snap frozen for storage was investigated. The results showed that although time-dependent changes in concentration was observed for some metabolites, the metabolic profile was robust to freezing delay times up to 30 minutes (Haukaas et al, 2016). As metabolic reprogramming is emerging as an independent hallmark of cancer, these findings are important to consider during planning, collection and preparation of metabolomics studies.

Compared to genomic and transcriptomic characterization of breast cancer, metabolomics properties have been less explored. Combining these levels could contribute to identifying underlying mechanisms contributing to breast cancers complex heterogeneity. Metabolic profiles obtained from biopsies (228 patients) within the Oslo2-study were analysed using HR MAS MRS, revealing three novel metabolic clusters. Combination of these clusters with protein and gene expression data showed differences in breast cancer related proteins as well as genes related to extra cellular matrix and metabolic pathways known to be aberrant in cancer. Preliminary results of the metabolic subgrouping were

presented as a power pitch and an electronic poster at the 2015 ISMRM Annual Meeting and Exhibition in Toronto. A manuscript entitled “Metabolic clusters of breast cancer in relation to gene- and protein expression subtypes” was submitted to Cancer & Metabolism 15th of February 2016.

As part of the NeoAva study, tumour tissue samples (n=270) from 122 breast cancer patients were analysed using HR MAS MRS to investigate the metabolic response to neoadjuvant chemotherapy with and without bevacizumab in breast cancer. Principal component analysis pointed to a decrease in glucose consumption and a transition to normal breast adipose tissue as an effect of chemotherapy. Pathological minimal residual disease (pMRD) patients and pathological non-responders (pNRs) were successfully discriminated based on metabolic profiles after treatment, but not before or during treatment, with an accuracy of 77% ($p<0.001$). Furthermore, metabolic profiles of patients exhibiting a good response (≥90% tumour reduction) were discriminated from those with no response (≤10% tumour reduction) before and after treatment, with an accuracy of 76% ($p=0.001$) and 75% ($p=0.002$), respectively. Before treatment, the good response group exhibited lower glucose and higher lactate, while the opposite was observed after treatment. Using linear mixed models, a significant interaction between time and bevacizumab for glutathione showed higher levels of this antioxidant in chemotherapy-only patients after treatment compared to bevacizumab receivers. Preliminary results have been presented as a traditional poster at the 2015 Metabolomics Society Conference in San Francisco. A manuscript entitled ‘Evaluation of metabolomic changes during neoadjuvant chemotherapy combined with bevacizumab in breast cancer using MR spectroscopy’ is currently under the second round of revision at the ‘Breast Cancer Research’ journal. Based on previous studies predicting prognosis from metabolic profiles, one of the metabolic clusters is expected to be related to a worse prognosis. Available 5-year survival data will thus be used to evaluate the prognostic potential of the naturally occurring metabolic clusters, and this will be further validated by the metabolic data generated from the OSLOVAL cohort. In addition, data from other platforms, including DNA methylation, copy number aberrations and expression of miRNA will be used to further characterize the mechanisms for the metabolic reprogramming taking place in the individual clusters. Future work for the NeoAva study includes the investigation of differences in response to treatment between luminal A and basal-like tumours and relating metabolic differences to 5-year survival on the longer-term.

Activity 5:

Integrated analyses

The molecular processes that drive cancer initiation and progression involve a complex web of molecular interactions and only by studying multiple features and multiple molecular levels simultaneously can these effects be delineated. However, taking advantage of multi-level and multi-omics data frequently also require sophisticated mathematical and computational approaches (Kristensen et al, 2014, Nature Reviews Cancer). Key technical challenges include appropriate handling of very high-dimensional parameter spaces, partly unknown correlation structure between variables, appropriate handling of multiple comparisons in massively parallel and correlated experiments, and incorporation of diverse types of prior information in the models. As a consequence of this, a substantial number of projects have involved developing/refining and applying sophisticated statistical methodologies for handling high-dimensional data (e.g. Kristensen et al, 2012, PNAS; Nilsen et al, 2012, BMC Genomics; Nilsen et al, 2013, Statistical Applications in Genetics and Molecular Biology; Vollan et al, 2015, Molecular Oncology; Aure et al, 2015, Genome Medicine). An important conclusion from previous multi-level integrated analyses performed by us and others is that well-designed joint studies of several molecular levels can offer insights into tumorigenesis that extends far beyond the combined evidence from multiple single-level analyses. However, this often requires a deep integration of statistical theory and cancer biology and the replacement of easily available "off-the-shelf" statistical models with tailor-made biologically founded models (Russnes et al, 2014, Genome Biology).

For example, the in-cis relation between the DNA copy number and mRNA expression of a gene is commonly quantified by linear correlation, but we have demonstrated *in vivo* that non-linear dependencies are not infrequent in breast cancer. While the full biological significance of this is yet to be resolved (Solvang et al, 2011, BMC Bioinformatics), it implies that a standard linear model may not be appropriate to assess in-cis associations between copy number and mRNA. Accordingly, in several later studies focusing on the copy number-mRNA relationship in genes in breast cancer we have considered more general models allowing non-linearities, while still allowing the assessment of statistical significance. This was a crucial step in the development of an algorithm to search for genomic alterations leading to observable transcriptional perturbations in-cis, in-trans and on process-level (Aure et al, PLoS One 2013), which when applied to data from Oslo1 lead to a list of 56 candidate breast cancer driver genes. While many of the genes were already known to play a role in breast cancer, promising novel candidates were also found and these have been further studied within the Jebsen Centre. In another and more recent study, the coordinate behaviour of multiple miRNAs on protein expression of cancer genes was investigated, and inspired by the nonlinearities found between copy number and mRNA we developed theoretically a nonlinear mathematical model for the relationships between mRNA and protein as well as between miRNA and protein (Aure et al, 2015, Genome Medicine). The resulting miRNA-protein 'interactome map' strongly suggests the presence of nonlinearities, and this map will guide future breast cancer studies in narrowing down on the most relevant miRNA-protein interactions. In Tahiri et al (2013), we showed that key genes are likely to be dysregulated by several miRNAs (either alone or in concert) in both invasive but also benign tumors as well as in DCIS (the EUROCAN collaboration). This suggests that joint and possibly coordinated effect by several miRNAs may play a larger role in gene regulation than can be observed on the individual miRNA level.

Deep integration may also require explicit modelling of known or suspected interactions between variables. For example, in (Aure et al, 2015, Genome Medicine) the combined effect of miRNA expression and mRNA expression on protein expression was modelled by assuming that miRNA expression operates as a multiplicative modifier of the in-cis effect of mRNA on protein. While other appropriate model structures can also be envisaged, the comparison of the model with actual observations from Oslo2 in most cases supported the model hypothesis. In another study, the combined effect of DNA methylation and DNA copy number on miRNA expression was modelled by cross-classifying individual tumours and genes with respect to type of methylation (hypomethylation, hypermethylation or neither) and type of copy number alteration (loss, gain or neither) and assessing association to miRNA expression in each case (Aure et al, 2013, Genome Biology). Using this approach we demonstrated that several miRNAs are perturbed by DNA methylation in some tumours, by DNA copy number alteration in other tumours, and by both in some tumours. Furthermore, it was found that different members of five miRNA families were differentially affected by methylation and copy number, possibly a consequence of the miRNAs being situated in different (epi-)genomic contexts.

Elucidating the effect of changes in DNA methylation on mRNA expression in breast cancer has demonstrated to be a difficult problem, with local variations in methylation level in the vicinity of a gene having a potentially large effect on the expression of the gene. Alterations in DNA methylation patterns have been shown to be of biological and clinical importance, and we recently reported on the integrated analysis of molecular alterations including the methylation status of whole genome, validated by highly quantitative pyrosequencing (Fleisher et al Genome Biology 2014), and the association to gene expression, germline genotype and clinical parameters including survival (Fleischer et al 2013, Int Journal Cancer). Epigenetically regulated networks of three transcription factors involved in breast carcinogenesis, ESR1, GATA3 and FOXA1 were found prominent in the luminal types of cancer (Fleischer, Tekpli, manscript). In addition, meQTL was also able to identify and quantify lymphocyte infiltration of breast tumours. Furthermore, alterations in copy number are not independent of methylation changes, and the effect of the two on mRNA level may enforce or cancel each other out depending on context. A systematic study

of such interactions is currently being conducted and has revealed several potentially new targets for further study (Møller et al, manuscript). Yet another example of deep integration across molecular levels was performed in a study of the effect of DNA copy number and mRNA levels on protein expression (Myhre et al, 2013, Molecular Oncology). In this study, which involved samples from Oslo1 and Oslo2, it was found that the relation between DNA copy number and protein expression is context dependent. More specifically, the relation depended on the gene expression derived PAM50 subtype of the tumour, suggesting that the role of DNA copy number alterations in perturbing key proteins in breast cancer cannot be fully elucidated unless additional molecular levels (here, mRNA expression) are also considered.

Several integrative studies in the Jebsen Centre have also utilized other molecular levels than those described above, thus expanding the repertoire of models as well as insights into the biology and clinical features of breast cancer. An investigation into the combined role of the metabolome and transcriptome on the response to treatment with bevacizumab in breast cancer xenografts (Borgan et al, 2013, Molecular Oncology) found that the most prominent changes in gene expression were observed for the most efficient treatment, and the metabolome displayed a PAM50 subtype-specific effect on treatment response. In another study, we explored the presence of PAM50 subtype differences in expression of genes related to the process of glycosylation and their association to clinical parameters. Among other things it was found that most glycan-specific changes occur early in the carcinogenic process, and glycan-related alterations specific to breast cancer subtypes were found, including a prognostic signature for two basal-like subgroups. Investigations into more than three molecular levels at the same time are underway, including a systematic unsupervised exploration of existing and potentially novel tumor classifications in Oslo2 (Aure, Vitelli et al, manuscript) using data from four molecular layers (DNA, mRNA, miRNA and methylation). This analysis is based on consensus clustering as well as other recently developed clustering tools (Lingjærde et al, manuscript).

A different form of integration entirely has been pursued in the NeoAva serial biopsy study set. Here, the integration is performed across molecular levels as well as across time points (multiple biopsies per patient). Pilot studies have been conducted to reveal the time dynamics of copy number (Møller et al, manuscript) and miRNA and mRNA expression (Silwal-Pandit et al, submitted) as well as DNA methylation (Fleischer et al, manuscript) and protein (Haugen et al, manuscript). Common to all the above mentioned approaches is that we demonstrate or map the diversity of molecular mechanisms that may lead to altered expression of a miRNA, an mRNA, or a protein. A natural extension of this was to identify and catalogue the multitude of molecular events that may lead to aberrant behaviour of a biological process or pathway (Kristensen et al PNAS, 2012). This method (PARADIGM) together with another (PATHIFIER) is currently implemented to study the deregulated pathways in the time course clinical studies. Finally, our previously published method of allele specific analysis of copy number variation (ASCAT) (van Loo et al 2010, 2012) is now being extended to detect subclonality. In combination with mRNA expression data and novel characterizations of genomic architecture (including our previously published CAAI and WAAI analysis and the ongoing CARMA project) this will be particularly valuable for treatment course studies, such as the Neoadjuvant Avastin (NeoAva) treatment study (Oslo3).

Integration of yet another kind, involving molecular interactions within each molecular level, has been an integral part of many of the studies performed in the Jebsen Centre. We have developed a mathematical framework and accompanying computational tool for dissection of genome aberration patterns in allele-specific copy number profiles (Nilsen et al, manuscript). This framework, called Copy Aberration Regional Mapping Algorithm (CARMA), recognizes six variation patterns in individual copy number profiles which together constitute a compact, biologically relevant representation of the genomic architecture in a tumor. Application of the framework to > 2400 breast tumors from the Oslo2, METABRIC and OsloVal cohorts has revealed novel clinically relevant stratifications and predictors of patient outcome.

As a proof of principle that molecular endpoints can improve clinical assessment we helped organize a DREAM breast cancer competition, where participants competed to create an algorithm that could predict, more accurately than current benchmarks, the prognosis of breast cancer patients from clinical information (lymph node metastases, tumour size and histological grade), adding 2 layers of molecular data: genome-scale tumour mRNA expression data, and DNA copy number data. More than 1400 models were submitted. The winners used a mathematical approach based on co-expression gene networks associated with tumour phenotype and functional characteristics, to identify signature 'attractor' meta-genes, which outperformed other models to predict outcome. This competition showed that adding molecular data to classical clinical endpoints may be a powerful way to accelerate the translation of biomedical science to the clinic. Therefore this line of research will be continued in other projects by developing new models using further integration and refinement, with both statistical and bioinformatics tools towards "digital oncology" and science guided clinical decision.

5. Approach to translation and innovation

For the centre as a whole describe:

- *Its approach to / definition of translation*[1]
- *Its strategies for translation*
- *Any measures taken in order to: :*
    - *Establish and reinforce links and integration between research groups*
    - *Provide opportunities for clinical studies*
    - *Ensure that the competence and results achieved by the research are effectively transferred to and utilised by clinicians / other relevant users?*
    - *The involvement of patients / patient organisations*
- *Has the centre research generated additional concurrent R&D projects between research institutions and companies? Please specify.*
- *Have efforts been made to secure that results may be commercialised or made available for other users?*

The focus on therapy/prognosis-related biomarker/molecular analyses of tissue from prospectively collected study cohorts, validation studies, disease-monitoring studies, as well as intervention studies, has been strengthen during the K.G. Jebsen period. An analytical platform for profile-based molecular characterization of FFPE tissue has been purchased, installed and now tested on a larger series of patients (nCounter). In addition, molecular classifications developed within the Centre can further refine the tumour characterisation.To improve the general classification of breast cancer patients by higher level of accuracy and reproducibility, the molecular-based characteristics and classification-patterns identified during the Center period will be tested in the EMIT project. This project started in 2015 and includes initiation of a prospective intervention study based on a molecular profile classification algorithm for decisions of type and intensity of adjuvant treatment. The possibilities for initiating translational studies with complex logistics have indeed been improved, by the support of the K.G. Jebsen Center.
(see also point 4 and12).

6. International cooperation: where relevant, please describe briefly

- *Collaboration with international research groups and other ways of international collaboration both with academic researchers and industry*

[1] NIH definition:"Translational research" includes two areas of translation: One is the process of applying discoveries generated during research in the laboratory, and in preclinical studies, to the development of trials and studies in humans. The second area of translation concerns research aimed at enhancing the adoption of best practices in the community."

- *International exchange of researchers, both centre staff going abroad and visiting foreign researchers to the centre, including postdocs, research fellows and senior scientific staff from other institutions*
- *The role of the centre staff in international strategic forums*

The participation of the Centre in several EU projects has opened the doors to a diversity of large networks in Europe and worldwide, such as Eurocan, http://eurocanplatform.eu/ comprising of 28 European cancer Institutions, Glycohit, http://www.glycohit.eu/ including also scientist from China and Japan in addition to leading European groups, and The Breast Cancer Somatic Genetics study, BASIS, http://www.sanger.ac.uk/ research/areas/ humangenetics/basis.html, a leading next generation sequencing project financed by EU and the Welcome Trust. A recent EU project EpiMark ("An integrated approach for epigenetic risk assessment and biomarker development for breast cancer in prospective cohorts / Identifying early epigenetic predictors of breast cancer risk and their etiology determinants") within the ERA-Net TRANSCAN, with the participation of our Centre, started last year under the leadership of IARC, Lyon, France. Further, based on work from PI's in the Centre has made it possible for several of the groups to be engaged in a European Consortium on patient-derived xenograft models (EurOPDX) (headed by Dr. Sergio Roman-Roman, Institute Curie) aiming to conduct multi-Centre preclinical drug testing to unravel predictive biomarkers and optimal drug combinations for person-adapted treatment.

During the Centre period we have had close collaboration with exchange of personnel with the following Cancer Centres/ Universities:
MD Anderson Cancer Centre (Gordon Mills),
UCSF, Helen Diller, Family Cancer Centre (Allan Balmain),
Sanger Centre UK (Michael Stratton),
Cambridge Cancer Institute (Carlos Caldas, Florian Markowitz),
Chicago University (Kevin White),
Centre National de Génotypage, Paris (Jorg Tost)
Princeton University (Olga Troyanskaya)
Karolinska Institutet (Knut Steffensen and Janne Lehtiö),
Lawrence Berkley (Mina Bissell),
Finsen Laboratory (Ole W Pettersen),
University of Iceland (T Gudjunson),
Harvard Medical School/Dana-Farber Cancer Institute, US (Kornelia Polyak).
Radboud University Nijmegen, the Netherland (Lutgarde Buydens and Arend Heerschap)
Johns Hopkins Medicine, Radiology, Baltimore, USA (Kristine Glunde)

The Centre has facilitated the initiation and continuation of the following DTC/CTC-based collaborations:
MD Anderson Cancer Centre, TX, USA (Ricardo Alvares and James Reuben, planned CTC study); Department of Human Genetics, Leuven, Belgium and Wellcome Trust Sanger Institute, Hinxton, UK (Thierry Voet, Peter Van Loo, Kevin P. White, NGS of CTCs/DTCS), and The Cancer Institute in New Jersey (amplicon analyses; RAST study). Russel Taichman, Department of Periodontics & Oral Medicine, University of Michigan School of Dentistry, and Professor Aguirre-Ghiso, Mount Sinai School of Medicine, New York.

The Centre has facilitated the initiation and continuation of the following primary tumour-based collaborations related to the EMIT study: Aleix Prat, Medical Oncology Department Hospital Clínic de Barcelona and Åke Borg, University of Lund.

Scientists in the Centre have been involved in several International sequencing consortia:

- Using RNA seq to identify markers of diagnostic as well as therapeutic value, in collaboration with Ravi Shachaninandam Mt Sinai, NY.

- Using RNA seq to identify splice variants and alternative usage of promotors in a subtype specific manner, collaboration G. Bhanot, IAS, Princeton and S. Ganesan, Cancer Inst. of NJ. A PhD student from the Centre did perform the RNA seq analyses in US.
- Using exome sequencing to detect copy number variations (CNVs), rare single nucleotide variants (SNVs) and single nucleotide polymorphisms (SNPs) in relation to occurrence of disseminated tumour cells, KP White, Institute for Genomics and Systems Biology, University of Chicago. We had a visiting PhD student from the Centre that performed many of the analyses.
- Single cell sequencing of disseminated tumour cells, KP White, Institute for Genomics and Systems Biology, University of Chicago.

7. Cooperation within Norway: where relevant, please describe briefly

- *Collaboration with research groups and other ways of collaboration both with academic researchers and hospital clinics within Norway (including any research cooperation with other K.G. Jebsen Centres).*
- *Any exchange of researchers, including postdocs, research fellows and senior scientific staff with other institutions in Norway*
- *The role of the centre staff in national strategic forums.*

The Centre has had an important role in the breast cancer research collaboration between institutions in Norway. The main national collaborator has been NTNU Trondheim, where the group of Ingrid Gribbestad, that after her death in 2013 was taken over by Tone Bathen has been an integral part and a founding member of the Centre, with joint seminars being held every autumn at Kongsvoll Fjellstue, Dovre. Further, a joint seminar was held with the Stavanger breast cancer research group (led by Emiel Janssen, and Håvard Søiland) last fall, and many common activities were planned and have already been initiated (integrated analysis of miRNA/mRNA, mutual validation in the respective datasets).

Another axis of expansion nationally was the collaboration with Østfold Hospital (Fredrikstad) and Vestre Viken (Drammen), who became invaluable partners in sample collection and willing recipients of scientific projects streaming from the University hospitals. Also, our analytic approaches have been disseminated to similar studies of other cancer forms (lung, pancreas melanomas, and colorectal) through the close collaboration with other groups of the Department of Genetics, and within the MetAction project, not part of the K.G.Jebsen Center, studying these respective cancer forms.

Further, our progress has led us to revitalize an old collaboration with Haukeland Hospital, Bergen, which has been based on discovery of predictive markers for chemotherapy and targeted therapies (FUMI, doxorubicin, taxanes, B-raf inhibitors). Our recent studies on mRNA, miRNA and DNA methylation levels bring novel insights and will lead to a new and exciting phase of this collaboration.

A new collaboration during the last period in the Centre was initiated with Haukeland University Hospital and Stavanger University Hospital related to analysis of tamoxifen metabolites in serum of breast cancer patients treated with tamoxifen as adjuvant treatment in the Oslo1 cohort (Thomas Helland, (Hormonlaboratoriet) and Gunnar Mellgren, Haukeland University Hospital; Håvard Søiland, Dept of Surgery, Stavanger University Hospital, Stavanger).

8. Recruitment of early stage researchers

- *How many PhD candidates have completed their PhDs at the centre?*
- *How many postdocs have worked at the centre during the project period?*

In the period 2011-2016 there have been 23 PhD students affiliated to the Centre of which 18 have defended their thesis, and 5 that have completed their thesis and will

defend during 2016 (see list below). All projects use the datasets collected by the Centre. A total of 14 postdocs have been associated to the Centre. In addition the Centre has supervised 7 MSc from NTNU, Trondheim, UMBV, Ås and Høyskolen in Oslo, 4 of them have been recruited as PhD students or technical research associates into the project. Internationally we have hosted a MSc on the Erasmus program from Germany (2012) and from the NKI, Amsterdam (2013) and two of the PhD students also had affiliations abroad: one from the USCF, California and one from Rutgers University and Mt Sinai, USA. There have been three MD students, two "forskerlinje" students and one working on her science project in the 5th semester.

Defended&delivered PhDs:

1. Randi Ruud-Mathiesen, NFR. Analyses and clinical relevance of tumor-related single cells in blood and bone marrow in breast cancer. Defended 23.05.2013, main supervisor B. Naume
2. Margit Hesla Riis, UiO. Molecular Analysis of Pre- and Postoperative Biopsies in Breast Carcinomas. Defended 25.05.2013, main supervisor V.N. Kristensen
3. Evita Maria Lindholm, NFR/KF. Antiangiogenic treatment in breast cancer – identifying responders and mechanisms driving resistance. Defended 07.06.2013, main supervisor O. Engebråten
4. Fatemeh Kaveh, NFR. Genomic signatures in progression of breast cancer with reference to gynecological carcinomas. Defended 10.10.2013, main supervisor V.N. Kristensen
5. Miriam R Aure, NFR, From DNA to RNA to protein: Integrated analyses of high-throughput molecular data from primary breast carcinomas, Defended 27.11.2013, main supervisor V.N. Kristensen
6. Jovana Klajic, UiO. From normal breast to invasive carcinoma: DNA methylation profiling of stage and response to chemotherapy. Defended 17.06. 2014, main supervisor V.N. Kristensen
7. Himanshu Joshi, HSØ. Towards pathway and network- based medicine in breast cancer. Defended 03.04. 2014, main supervisor V.N. Kristensen
8. Thomas Fleischer, UiO. Epigenetic alterations in breast cancer: implications on classification and prognosis. Defended 20.06. 2014, main supervisor V.N. Kristensen
9. Marit Synnestvedt. NFR. Risk and intervention in early breast cancer: Studies of primary tumour and disseminated tumor cells. Defended June 2014. main supervisor B. Naume
10. Sandra Jernstrøm, HSØ. The search for therapeutic targets in breast cancer by genome analyses, Defended 09.12.2014. Co-supervisor A.L. Børresen-Dale
11. Gro Nilsen, UiO, Statistical learning in Genomics: Uncovering patterns and groups in high dimensions. Defended 07.05.2015, main supervisor O.C. Lindgjærde
12. Hans Kristian Moen Vollan, KGJ/OUS. Molecular Genetic analyses of DNA alterations in Tumors; Relevance for Prognosis in Breast Cancer, Defended 05.06.2015, main supervisor A.L. Børresen-Dale
13. Laxmi Silwal-Pandit, KF. TP53 mutations and molecular profiles of breast cancer – prognostic and therapeutic implications. Defended 04.05.2015, main supervisor A.L. Børresen-Dale
14. David A. Quigley, USCF/KGJ, Rewiring of Genetic Networks in Breast and Skin Cancer Progression, Defended 27.10.2014 main supervisors A.L. Børresen-Dale and VN Kristensen
15. Elen K Møller, KF. Genomic alterations and heterogeneity in progression of breast carcinomas. Defended 09.03.2015, main supervisor V.N. Kristensen
16. Shakila Jabeen, HSØ. Non-invasive monitoring of immune profiles in breast cancer patients, implications to targeted therapies. planned delivery 2016, main supervisor V.N. Kristensen
17. Andliena Tahiri, HSØ. Regulatory and functional genomic biomarkers in breast cancer and melanoma. Defended 03.03.2016. main supervisor V.N. Kristensen

18. Inga Hansine Rye, HSØ, Genomic alterations and heterogeneity in progression of breast carcinomas, Defended 10.03.16, main supervisor H. G. Russnes
19. Tonje Haukaas, KGJ, Metabolic profiling of breast cancer using ex vivo MR spectroscopy, to be defended April 2016. Main supervisor Tone F Bathen
20. Alexandr Kristian, KGJ, Dynamic positron emission tomography of breast cancer xenografts: tumor characterization and response assessment. To be defended June 2016. Main supervisor: Eirik Malinen
21. Leslie Euceda Wood, NFR, Evaluation of treatment response and sub-classification of breast cancer using MR metabolomics. To be defend December 2016, main supervisor T. Bathen
22. Sunniva Bjørklund, HSØ, Next-generation sequencing of non-canonical biological systems in breast cancer. To be defended 08.06.2016. main supervisors A.L. Børresen-Dale and VN Kristensen
23. Nirma Skrbo, HSØ. Molecular and functional analysis of tumor heterogeneity in breast cancer xenograft models. To be defended 17.06.2016, main supervisor T. Sørlie

The recruitment and "brewing" of young scientists in the Centre did culminated with new project groups established with funding from Helse SørØst to postdoc/scientist Hege G. Russnes and with a career stipend from Helse SørØst (4 years x 2mil/year) to one of the senior postdocs, Silje Nord.

9. Other funding opportunities

Has the centre participated in applications / obtained other competitive funding nationally and / or internationally during the project period? Please specify.

Helse SørØst (~Total 20 mill)
2011-2013- Towards personalized therapy for breast cancer through forming the Oslo Breast Cancer Research Center: OSBREAC
2012-2014 - The participation Ahus in the K.G.Jebsen Center
2013-2015 - Regional Networks. Exosomes and extracellular vesicles
2013-2016 - Cell-free tumor DNA in blood; non-invasive monitoring of therapy response and disease progression in breast cancer patients.
2014-2017 - Career stipend, Making sense of GWAS
2014-2017 – Intensifying breast cancer therapy (I-BCT) in the neoadjuvant and metastatic setting: A translational clinical study for selecting tumor therapy."

Helse Midt (~Total 2.8 mill)
2014-2016 -Molecular effects of exercise and chemotherapy on breast cancer

Norwegian Research Council (~Total 13 mill)
2013 – 2016(FRIPRO): "Met-NESTING; Metastatic Niche Establishment by Stromal-Tumor cell INteractions; Going towards novel therapies"
2013 – 2016(FRIPRO): Imaging the Breast Cancer Metabolome
2012-2016(The publicly-initiated clinical cancer studies program 2012-2016): "MetAction: Actionable targets in cancer metastasis – from bed to bench to byte to bedside

Kreftforeningen/Rosa Sløyfe (~Total 8,3 mill)
2011-2015: General Funding to the PIs in the center
2012-2016: Neoadjuvant Avastin in Breast Cancer (NeoAva)

EU (~Total 7 mill)
BASIS :Breast Cancer Somatic Genetics Study (2010-2014)
GlycoHIT: Glycomics by High-throughput Integrated Technologies (2011-2014)

EurocanPlatform: A European Platform for Translational Cancer Research (2011-2015)
MIRACLE (Magnetic Isolation and moleculaR Analysis of single CircuLating and disseminated tumor cElls on chip (2010-2015)
TRANSCAN: An integrated approach for epigenetic risk assessment and biomarker development for breast cancer in prospective cohorts / Identifying early epigenetic predictors of breast cancer risk and their etiology determinants(2014-2017

10. Research leadership and scientific advisory board

- *Describe how scientific leadership of the centre has been implemented*
- *Describe how gender equality has been advanced within the centre*
- *If relevant, please list the members of the scientific advisory board and comment on its contributions*

The Centre has been headed by Anne-Lise Børresen-Dale, and the Host institutions have been the Institute for Clinical Medicine, University of Oslo and Institute for Cancer Research (ICR), Oslo University Hospital, Radiumhospitalet (OUHR). The management team, headed by research coordinator Gry Geitvik, consisted of the director and co-director and all group-leaders in the Centre (see below). The role and the activities of the Principle Investigators and the management team have been well defined and these have functioned in good coordination. The PIs in the Centre have together with the leader of OSBREAC, Rolf Kåresen and from 2015 Kristine Kleivi Sahberg, ensured a smooth process of sample collection and collaboration between the different hospitals (see also National collaboration). The PsI of the Centre have also actively profiled the Centre internationally and set the road to multiple EU networks (see International collaboration).

The Centre has consisted of six research groups:

- Clinical groups: Ellen Schlichting, Rolf Kåresen (surgery), Torill Sauer, Elin Borgen, Hege G. Russnes (pathology), Erik Wist, Olav Engebråten and Bjørn Naume (oncology)
- Molecular groups : Anne-Lise Børresen-Dale (director) and Vessela N. Kristensen (deputy director)
- Micro-metastases groups: Bjørn Naume and Øystein Fodstad
- Model-systems and functional groups: Gunhild M. Mælandsmo og Øystein Fodstad
- Metabolic profiling and imaging group: Ingrid S. Gribbestad (deceased April 2013) and Tone F Bathen NTNU
- Bioinformatics/biostatistics group: Ole Christian Lingjærde

The molecular groups (ALBD and VNK) have successfully performed the molecular profiling at all levels and in close collaboration with the bioinformatics/biostatistic group (OCL) developed novel tools for integrated analysis. The molecular profiling from this stage has been integrated with the metabolic profiling and imaging (TFB, NTNU) and candidate markers from these studies been investigated by functional studies in model systems (GMM and ØF). The DTC and CTC research team has developed high competence in processing large number of samples for analysis, suitable for both large clinical studies and for future routine-based analyses. Furthermore, the team has established method for isolation of single tumour cells for high resolution molecular analyses, as well as oligo-marker analyses. Also, the team has succeeded in initiating multi-centre intervention-based study based on DTC analysis, and have been able to integrate these analyses in other studies, as exploratory objectives.

The Centre has had an external Scientific Advisory Board (SAB) that has given advice during the centre period and commented to the annual reports. The members of the SAB have been:

- Professor Gordon Mills, Chair, Department of Systems Biology, MD Anderson Cancer Center, Houston, TX, US
- Professor Carlos Caldas, Group Leader, Cancer Research UK Cambridge Institute, University of Cambridge, UK
- Professor Joaquin Arribas, Director of Preclinical Research, Vall D'Hebron Institute of Oncology, University Hospital Barcelona, Spain

11. Dissemination and public outreach. Please list the main:

- *Dissemination activities both within the centre and to the public at large*
- *Public outreach to specific groups (patient organisations, local health care, etc.)*

- Knowledge management, evaluation and dissemination of scientific results have been important aspects of the work of a multidisciplinary Centre like ours. Reshaping scientific knowledge dissemination and evaluation in the age of the internet web-based communication has been a major goal of our participation in the DREAM Competition project (see point 4) supporting the idea of free publication and interactive review of scientific data online. Communication of knowledge through the dissemination of research findings has been a key element of the work of the Centre.
- In addition to the over 165 Scientific publications from the Centre, listed in the attached publication list, several of them in high profiled/ indexed journals such as Nature, Cell, Science TM, PNAS, the researchers in the centre have been involved in writing 10 reviews and book chapters, and web tutorials for the developed methods. An active dissemination of results through scientific meetings and meeting research communities as well as public web sites and newspapers is in focus. The Centre has been profiled in two additions of the Public Service Review: European Union 23 in March 2012, and June 2013. We have also highlighted our activities through our own web page http://ous-research.no/kgjebsen/ in addition to the Foundations own Web page. In 2012 the Centre was co-organizer of the 7. International Symposium on Personalized Cancer Care, taking place in Oslo, 7-9 September, with >200 participants from all over the world and with speakers with the highest International reputation in the Cancer field. This symposium was a big success and will be organized again 18-20 May 2016 in Oslo, but this time not under the umbrella of the K.G. Jebsen Center since financing of the Centre was not prolonged into 2016. Yearly a seminar in the Academy of Sciences has been organised with the support of Thoresen Foundation with researchers from all walks of medical science, and where the Centre was profiled with several speakers.
- In 2013 the Centre was represented at the Forskningsdagene at the University Square in front of the University Aula downtown. The stand was very well visited and got a lot of attention.
- The director and the PI's of the Centre have been invited to give a large number of presentations at various International Meetings highlighting the Centre and presenting work based on all the projects in the Centre. Media attention of the work ongoing in the Centre has also been extensively.
- Lectures have been given by both the Centre director and/or the co-director every year to the public during the Pink Ribbon October month for breast Cancer awareness. The Foundation has been profiled during these events.
- The director of the Centre received in 2015 The Helmholtz International Fellow Award, in Germany and gave several lectures, presenting work from the Centre and acknowledging the Foundation. She also was elected to give the Elena Timofeeff-Ressovsky Lecture, Berlin 2015, and the Mildred Scheel Lectures (in Dresden, Berlin, Heidelberg and Cologne) in 2015. In all these lectures work from the Centre was presented and the K.G. Jebsen Foundation acknowledged.
- The Centre Director received in 2015 The Fritjof Nansen medal and award for Outstanding Research and Oslo University Hospital's Excellent Researcher Award.

- At the annual San Antonio Breast Cancer symposium in 2015 she was awarded The AACR Distinguished Lectureship in Breast Cancer Research, and the Centre was profiled.
- In summary, we have communicated the results through popular publications; scientific audiences, scientific journalism; posters with project information; press contacts; use of media and interactive web pages.

A complete list of publications from the K.G. Jebsen Centre is attached as a separate file. Several manuscripts based on work from the Centre are to be submitted during 2016. In these papers the K.G. Jebsen will be acknowledge and the list will be sent the Foundation in 2017.

12. How will the research / research collaborations be developed further after the SKGJ funding ends?

See also attached letter from the Host Institution

The K.J Jebsen Centre has been central in stimulating translation of research data into clinical studies. The Centre was based on the previous established OSBREAC (Oslo Breast Cancer Research Consortium) and will continue much of its activity through this consortium after the end of the K.G. Jebsen funding. OSBREAC (headed by Kristine Kleivi Sahlberg that took over after Rolf Kåresen in 2015) has status as a Regional Network with a grant from Helse SørØst in close collaboration and integration with the Breast Cancer Clinical Research Group (headed by Bjørn Naume and Erik Wist). OSBREAC will continue to work as a multidisciplinary group with basic researchers, bioinformaticians, biostatisticians, surgeons, pathologists and oncologists from the Oslo University Hospital, Akershus University Hospital, Vestre Viken Hospital, and University Hospital in Stavanger, working close together. Key clinical oncologists, surgeons and pathologists (Bjørn Naume, Olav Engebråten, Hege Russnes, Torill Sauer, Jurgen Geisler, Elin Borgen, Ellen Schlichting) are all active partners in OSBREAC. Our molecular and metabolic classification enter clinical trials that will improve treatment strategies like the EMIT trial, supported by Helse Sør-Øst. Our project, profiled world-wide as the Norwegian Breast Cancer Study (NBCS), in size and depth of the analyses can be compared to the three largest and most highly profiled similar studies worldwide: the TCGA, USA, (funded by the NIH), METABRIC (funded by Cancer Research-UK) and BASIS (funded by EU/UK), with whom we extensively have been collaborating.

One of the most profound effects of molecular classification on clinical studies is that it inevitably leads to a paradigm shift from large randomized clinical trials tailored for stratified patient groups, down to N-of-1 trials, where a single patient is the entire trial. Thus, we will expand our approach from evaluating the utility of molecular profiling in randomized arms, using the other arm as a control, to also include evaluation of response to experimental and control interventions in each individual using the same individual as a control. Two spin-off projects from the K.G Jebsen Centre and OSBREAC address new translational developments, 1) the N-of-1 concept in the MetAction project (PIs: Gunhild M. Mælandsmo and Anne-Lise Børresen-Dale, and funded by “Kreftsatstningen” in NFR); and 2) identification of patients with tumors sensitive to DNA targeting treatment by in-depth genomic aberration pattern analyses: the I-BCT-1 randomized study (PIs: Olav Engebråten, Ole Christian Lingjærde, Hege Russnes, and funded by Helse Sør-Øst). In the clinical N-of-1 trial in the MetAction project, patients with metastases from solid tumors, including breast cancer, are offered approved targeted therapy based on the findings from sequence analyses of biopsies from the metastatic lesion. This is the first clinical trial of this kind in

Norway and has been followed closely by our health authorities and received considerable interest from the public (eg. VG, April 5th 2013). The purpose of the I-BCT-1 (Improved Breast Cancer Therapy) trial is to determine whether aberration parameters can be used for the selection of additional therapeutic measures targeting the DNA instability found in BC. At the University of Oslo, the participating groups lead by professors Arnoldo Frigessi and Ole Christian Lingjærde will continue to work on data integration from these trials, and design stochastic models to study principles, dynamics and patterns of dependence of biological in a systems medicine approach.

Summary statement and advice to the foundation

Based upon your experience at the completion of the SKGJ program support, please briefly list:

3 areas in which the program could be improved

- The length of the program is too short. It takes a minimum of 5 years to really make a Centre well-functioning, and be able to get the very best out of the funding and to establish longterm-collaborations, in particular with international partners.
- The support from the host institution UiO has been limited. We were not prioritized in the first years for any additional funding for example to PhD students or postdocs. This seems to have improved in the more recent K.G. Jebsen Centers established at UiO.
- The administrative support from the host institution could have been better and more effective, and a closer and more direct link between the management team in the Center and the administrative team at Institute for Clinical Medicine at UiO.

3 positive characteristics of the program

- The Centre has led to a formalization of interdisciplinary collaboration between academic partners and clinicians, and this has resulted in that the translation of technology from development to clinical testing has become easier, exemplified with some of the latest studies in progress and the new ones initiated.
- The financing of the Centre has had a mark of quality, and this has indeed been of importance in the success in receiving funding from other sources, both national and international, and also to attract excellent partners from abroad.
- The Centre has been able to recruit and educate several young scientists that have got a unique possibility to start to build their own carrier, which has already taken place for several of them.