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MEETING ABSTRACTS

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Oral presentations

O1

Gene pathways associated with prognosis and chemotherapy sensitivity in different molecular subtypes of breast cancer

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Breast cancer consists of multiple different molecular subtypes and different biological processes, and consequently different molecular markers are associated with prognosis and chemotherapy sensitivity in the distinct disease subsets [1]. A large number of biological processes including cell cycle regulation, DNA replication, mitotic spindle checkpoint, and p53 function are strongly prognostic in ER^+ cancers but not among ER^- cancers [2,3]. Interestingly, the number of biological pathways, and therefore genes, that are associated with prognosis or treatment sensitivity are substantially larger and more consistent in ER^+ cancers than among ER^- tumors [1,4]. This implies that it is easier to discover prognostic and predictive markers for ER^+ than for ER^- cancers. In ER^- cancers, the single most consistent, but still modestly accurate, good prognostic predictor is the presence of immune cell infiltration [5]. Immune cell signatures are also associated with more favorable prognosis in highly proliferative ER^+ cancers but not in ER^+ cancers with low proliferation [6].

It is also increasingly clear that the same molecular marker can be associated with several different outcome endpoints in various and often opposing manners. For example, high Ki67 expression is predictive of worse prognosis in the absence of any systemic therapy in ER^+ cancers, but at the same time it is also predictive of higher sensitivity to chemotherapy. Similar opposing bidirectional associations with treatment response and prognosis exist for many other markers including histologic grade, Tau protein expression and almost all prognostic gene signatures [7].

It is important to be aware of these complex multi-directional interactions between molecular markers and various clinical endpoints that may also vary from breast cancer subtype to subtype. Ignoring these potential marker–disease subset–outcome interactions can lead to contradictory and confusing results across studies (due to differences in patient composition and heterogeneity of therapy between studies) and may also lead to the discovery of biomarkers that are clinically less useful (because of unrecognized subtype-restricted performance) [8,9].

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O2

Molecular classification of triple-negative tumors

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Triple-negative breast cancers (TNBCs) are among the most clinically challenging because of their poor prognosis and paucity of treatment options. In part through our genomic profiling studies, breast cancer is now appreciated as being composed of multiple diseases. One of these diseases, the basal-like breast cancer (BLBC) subtype, is now known to represent a unique disease entity with a distinct etiology and biology. Over the years, BLBC has become more commonly known as TNBC because the majority of these tumors lack expression of ER, PR and HER2; however, not all TNBC are BLBC, and not all BLBC are TNBC. Recently, we discovered that a significant subset of TNBC is comprised of a new subtype, the claudin-low, which is important because it is biologically distinct from BLBC and has a number of features reminiscent of mammary stem cells [1]. In addition, luminal A, luminal B, and HER2-enriched tumors are also identified within TNBCs in various small proportions, which highlights the complexity of the clinically based classification.

We have explored the treatment sensitivity of the various intrinsic subtypes to neoadjuvant anthracycline/taxane-based chemotherapy using a large publicly available dataset [2]. Across all patients, and within TNBC, basal-like tumors were found associated with a higher likelihood of achieving a complete pathological response (pCR) than the rest of the subtypes, including the claudin-low. In multivariate logistic regression models for pCR prediction, we observed that the intrinsic molecular subtypes virtually always make the final model, even if clinical variables and other genomic predictors are included. In addition, our analyses show that those tumors that achieve a pCR showed a better survival outcome than those that did not, regardless of their molecular subtype; this effect is much larger within the basal-like subtype, which is concordant with previous findings. This intriguing association between residual disease after therapy and poor outcome in basal-like and claudin-low tumors points to intratumor cell heterogeneity as a possible explanation, where resistant and aggressive cell clones might already exist in the pretreated tumor. Our preliminary

analyses using a combination of fluorescent activated cell sorting and global gene expression on numerous preclinical models of basal-like breast cancers including cell lines and primary tumor xenografts suggest the existence of at least two cell populations in many BLBC models. These different cell populations are currently being tested for tumor-initiating cell activities, and additional studies focusing on these populations changing with treatment are also being performed.

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O3

Poly(ADP-ribose) polymerase inhibitor development: are we in the right direction?

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Poly(ADP-ribose) polymerase-1 (PARP-1) is a nuclear DNA-binding enzyme activated by DNA strand breaks and has a key role in the signalling of DNA single-strand breaks as part of the repair process. In anti-cancer therapy, many agents cause DNA damage as their mechanism of cytotoxicity, and repair of damage is a cause of tumour resistance. Additionally in tumours where double-strand break repair is defective (for example in BRCA-related cancers) PARP inhibitors have potential single-agent activity. Thus, PARP-1 was identified as a potential therapeutic target for cancer treatment and PARP inhibitors have entered the clinic both in combination with cytotoxic chemotherapy, as single agents in DNA repair deficient tumours, and more recently in combination with radiotherapy.

The first PARP inhibitor to be given to cancer patients in 2003 was AG014699 (rucaparib), a tricyclic indole, which is a potent intravenous inhibitor of PARP. This phase I study had a pharmacodynamic endpoint of PARP inhibition in PBMCs, demonstrating for the first time proof of mechanism of the class. Subsequently AZD2281 (olaparib) entered clinical trials as a single agent (2005), and demonstrated the proof of concept of synthetic lethality in BRCA defective tumours in two small phase II studies. Over the last 5 years seven further inhibitors have entered cancer clinical trials either as a single agent (MK4827) or in combination with various cytotoxic regiments (ABT888, veliparib; BSI-201, iniparib; CEP-9722; INO-1001; E7016, BMN 673) in late preclinical development.

Initial exciting data suggesting that iniparib improved outcome in patients with triple-negative breast cancer in combination with chemotherapy have not been confirmed in phase III studies, although there are clearly patients who benefit from this agent. In terms of mechanism of action, iniparib differs from all the other compounds in the class that are competitive inhibitors at the NAD^+ binding site of PARP. Iniparib is postulated to have a different mechanism of action and may not be a *bona fide* PARP inhibitor.

It has been a period of rapid clinical development of a new class of agents with exciting evidence of improved response rates in some tumour areas. This class of agents also presents some interesting challenges in clinical trial design, and mechanistic understanding. This presentation will overview the current clinical status of PARP inhibitors and will discuss these challenges and potential biomarker strategies.

O4

Immunity and autoimmunity in breast cancer

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Evading immune destruction should be considered an emerging hallmark of cancer [1]. Highly immunogenic cancer cells can be eliminated in immunocompetent hosts as a result of the immunoediting process. Weakly immunogenic variants can grow and generate solid tumours. Regulatory T cells (Tregs) were found to be involved in the maintenance of the immune tolerance both preventing autoimmune disease and curtailing antitumour immune response. Modulation of immune response in cancer patients is the result of a balanced activity of Tregs and T-effector cells [2]. In cancer patients, an increased number of Tregs was found in blood and tumour tissue: it was demonstrated that Tregs suppress T-cell response and natural killer cell proliferation and function, thus interfering both with acquired and innate immunity. Upregulation of Tregs in the tumour bed can be associated with worse prognosis [3]. Drugs blocking function of Tregs increase activity of T effectors and, as a side effect, induce an autoimmune disease. Issues of biology and prognosis of breast cancer in the presence of a deregulation of the immune system need to be studied. The identification of immunological and genetic features affecting immune response in patients with minimal tumour burden is the optimal background for development of clinical studies in the adjuvant setting. Research on tumour-associated antigens (TAAs) has identified a large collection of peptide epitopes that have been and are being used for vaccination of cancer patients [4]. Several potential advantages of using peptide-based vaccines include: easy and relatively inexpensive production of synthetic peptides; the easy administration of peptides in a clinical setting; the possibility of treating only those patients whose tumours overexpress the target antigens; and the availability of *in vitro* or *ex vivo* assays that can assess patients' immune response to vaccine epitopes. The aim of future studies will be to assess the immunoreactivity of several antigens in a large series of breast cancer samples classified according to molecular subtypes. Identification of potential targets in subpopulations of patients with breast cancer may allow identification of patients who are potential candidates for adjuvant therapeutic vaccines. It is our current thinking that patients with minimal residual disease after preoperative chemotherapy are the ideal setting to test the efficacy of a vaccination strategy. To date, vaccines for breast cancer have been mainly used in end-stage disease. TAA antigens offer a novel opportunity for fostering vaccine development and therapy.

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O5

Epithelial–mesenchymal transition as a mechanism for the progression of breast carcinoma

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Epithelial–mesenchymal transition (EMT) is a major process controlling multiple events during development. EMT has been conserved throughout evolution to control morphogenetic events, such as the formation of the three primary germ layers during gastrulation. Most interestingly, signal transduction pathways have been remarkably conserved in many different species. EMT pathways are also tightly connected to determination and differentiation programmes, and are reactivated in adult tissues following injury or exposure to toxic agents. EMT is likely to operate during the early stages of carcinoma invasion that lead to blood or lymph vessel intravasation. Mesenchymal-like carcinoma cells undergo a mesenchymal-to-epithelial transition in distant sites from the primary tumour and eventually become micrometastatic. We have characterised bone marrow micrometastases from breast cancer patients and found that the detection of micrometastatic carcinoma cells was associated with poorer distant metastasis-free survival, local relapse-free survival, and overall survival. Despite high rates of adjuvant systemic treatment and breast irradiation in this series, disseminated carcinoma cells remain a

prognostic factor, in favour of the resistance to treatment of locally or distant disseminated cancer cells in bone marrow-positive patients. In addition, we detected micrometastatic carcinoma cells in patients with T1 tumours, suggesting that dissemination occurs much earlier during tumour progression than is generally accepted. Thus, bone marrow micrometastases should become a very useful prognostic indicator for relapse, and an excellent surrogate marker for patient's response to treatment. The mesenchymal-like state of carcinoma confers stemness, protection from cell death, escape from immune response and, most importantly, resistance to conventional and targeted therapies. Current strategies based on the EMT concept are aimed at designing new therapeutic approaches that interfere with the plasticity of carcinoma cells. Our laboratory has devised a high-content, high-throughput screen for EMT. Several combinations of drugs have been shown to selectively inhibit EMT. This strategy may be used to interfere with tumour progression, particularly in breast carcinomas that have acquired resistance to conventional therapies.

O6

Epigenetics of breast cancer

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DNA methylation and histone modifications have important roles in normal mammary differentiation and the development of breast cancer. Epigenetically mediated silencing of tumor suppressor coding genes and microRNAs is a hallmark of human breast tumors. CpG island hypermethylation is starting to be used as a biomarker of the disease, such as BRCA1 hypermethylation as a predictor of response to PARP inhibitors. Most importantly, both DNA methylation and histone modifications are new targets for upcoming drugs.

O7

Insulin resistance in breast cancer: relevance and clinical implications

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Breast cancer risk is increased in women who have attributes of the insulin resistance syndrome, such as obesity (postmenopausal), central obesity (premenopausal and postmenopausal), high endogenous insulin levels, clinical diabetes and physical inactivity. There is a large body of evidence that obesity is associated with a 25 to 50% relative increase in risk of breast cancer recurrence or death, with adverse effects that appear to be independent of hormone receptor status. Obesity, particularly when it is central, is strongly associated with insulin resistance in healthy individuals and breast cancer patients. Several studies have shown that higher insulin and/or C-peptide levels (a marker of insulin secretion), both of which are linked to insulin resistance, are associated with an increased risk of recurrence and death in women with early stage breast cancer, even in the absence of diabetes. Risk is increased twofold to threefold in those with insulin levels in the highest (versus lowest) quartile. Data from our group suggest that these prognostic associations of insulin are most marked in the first 5 years post diagnosis. A role of insulin in breast cancer outcomes is biologically plausible given overexpression of insulin receptors (IR), most frequently the fetal form of the receptor (IR-A), by the majority of human breast cancers. IR-A often hybridizes with insulin-like growth factor 1 receptors to stimulate mitogenic signaling pathways; hybrid receptor activation has been associated with poor clinical outcomes. The current observational and preclinical evidence linking insulin to breast cancer is sufficiently compelling that neoadjuvant and adjuvant intervention studies have been initiated to evaluate clinical anti-cancer effects of metformin, an agent that lowers insulin levels and has other potential non-insulin-mediated anti-cancer effects (mainly through activation of adenosine monophosphate-activated protein kinase). Early results from window of opportunity neoadjuvant studies suggest short-term, single-agent metformin (2 to 3 weeks) lowers insulin levels, reduces proliferation and increases apoptosis. NCIC MA32, an ongoing randomized, multicenter, placebo-controlled, adjuvant trial involving 3,582 women with early stage breast cancer, will provide more definitive evidence regarding potential anti-cancer effects. Additional studies of metformin in the metastatic setting are underway and/or planned. Because other factors (such as inflammation, adipocytokines (leptin, adiponectin), higher estrogen levels) may also mediate prognostic effects of obesity and/or insulin resistance in breast cancer, additional research targeting these mediators as well as obesity *per se* is also needed.

O8

The phosphatidylinositol-3 kinase/mTOR pathway: new agents

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The phosphatidylinositol-3 kinase (PI3K) pathway is overall the most frequently mutated pathway in cancer, with mutation and/or amplification of the genes encoding the PI3K catalytic subunits p110α (*PIK3CA*) and p110β (*PIK3CB*), the PI3K regulatory subunit p85α (*PIK3R1*), receptor tyrosine kinases (RTKs) such as HER2 (*ERBB2*) and FGFR1, the PI3K activator K-Ras, the PI3K effectors AKT1, AKT2, and PDK1, and loss of the lipid phosphatases PTEN and INPP4B. PI3K is activated by growth factor RTKs and G-protein-coupled receptors (GPCRs). PI3K activates Akt, which, in turn, phosphorylates and inactivates Tuberin (TSC2), a GTPase-activating protein of the Ras homologue Rheb. Inactivation of Tuberin allows GTP-bound Rheb to accumulate and activate the mTOR/Raptor (TORC1) complex, which regulates protein synthesis and cell growth. mTOR also couples with Rictor to form the TORC2 complex, which phosphorylates and activates AKT.

Class IA PI3K isoforms are heterodimeric lipid kinases that contain a p110 catalytic subunit and a p85 regulatory subunit. The three genes *PIK3CA*, *PIK3CB*, and *PIK3CD* encode the homologous p110α, p110β, and p110δ isozymes, respectively. Expression of p110δ is largely restricted to immune and hematopoietic cells, whereas p110α and p110β are ubiquitously expressed. p110α is essential for signaling and growth of tumors driven by *PIK3CA* mutations, RTKs, and/or mutant Ras, whereas p110β lies downstream of GPCRs and has been shown to mediate tumorigenesis in PTEN-deficient cells. *PIK3CA* mutations are the most commonly known genetic alterations of this pathway in cancer, where ≥80% occur within the helical (E542K and E545K) and kinase (H1047R) domains of p110α. Such mutations confer increased catalytic activity through different mechanisms, but both induce characteristics of cellular transformation including growth factor-independent and anchorage-independent growth, and resistance to anoikis.

Several drugs targeting multiple levels of the PI3K network (that is, PI3K, AKT, mTOR) have been developed. A number of ATP-mimetics that bind competitively and reversibly to the ATP-binding pocket of p110 are in early clinical development. These include the pan-PI3K inhibitors BKM120, XL-147, PX-866, PKI-587, and GDC-0941, the p110α-specific inhibitors BYL719, GDC-0032, and INK-1117, the p110δ-specific inhibitor CAL-101, and the dual PI3K/mTOR inhibitors BEZ235, BGT226, PF-4691502, GDC-0980, and XL-765. The pan-PI3K and p110α-specific inhibitors are equally potent against oncogenic p110α mutants. The rationale for the development of isozyme-specific antagonists is to allow higher doses of anti-p110α and anti-p110β drugs to be delivered without incurring side effects caused by pan-PI3K inhibitors. Interim results from a phase I trial with the p110δ-specific inhibitor CAL-101 in patients with hematologic malignancies showed that treatment reduced P-AKT levels >90% in peripheral blood lymphocytes and induced objective clinical responses. Recently completed phase I trials with BKM120, BEZ235, and XL-147 showed that treatment partially inhibited PI3K as measured by levels of P-S6 and P-AKT in patients' skin or tumors, and 2-deoxy-2-[^{18}F]fluoro-D-glucose uptake measured by PET. Main toxicities were rash, hyperglycemia, diarrhea, fatigue and, mood alterations. Few clinical responses were observed in patients with and without detectable PI3K pathway mutations, although screening for genetic lesions in this pathway was not comprehensive.

Both allosteric and ATP-competitive pan-inhibitors of the three isoforms of AKT are also being developed. AZD5363, GDC-0068, GSK2141795, and GSK690693 are ATP-competitive compounds that have shown antitumor activity in preclinical models and recently entered phase I trials. Allosteric inhibitors such as MK-2206 bind to the AKT PH domain and/or hinge region to promote an inactive conformation of the AKT protein that is unable to bind to the plasma membrane. MK-2206 inhibits AKT signaling *in vivo*, and suppresses growth of breast cancer xenografts harboring *PIK3CA* mutations or *ERBB2* amplification. Phase I data showed that treatment with MK-2206 decreases levels of P-AKT, P-PRAS40, and P-GSK3β in tumor cells, peripheral blood mononuclear cells, and hair follicles.

The mTOR kinase is a component of PI3K-driven oncogenesis that functions within two signaling complexes: TORC1 and TORC2 (described above). The macrolide rapamycin and its analogs form complexes with FK506-binding protein (FKBP12). This complex then binds to mTOR and inhibits the kinase activity of TORC1 but not TORC2. Formulation problems of rapamycin prompted the development of analogs such as CCI-779 (temsirolimus), RAD001 (everolimus), AP-23573 (deferolimus), and MK-8669 (ridaferolimus). These rapalogs have shown cytostatic activity in preclinical models and clinical trials, particularly in patients with renal cell cancer, and in patients with mutations in the TSC complex (upstream of TORC1) who harbor renal angiolipomas. Compounds that target the ATP-binding cleft of mTOR (that is, OSI-027, AZD8055, INK-128), and are thus active against both TORC1 and TORC2, are also in phase I trials.

O9

DNA repair and breast cancer: therapeutic opportunities

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The discovery and cloning of BRCA1 and BRCA2 was accompanied by optimism that these achievements would usher in a new era of insight into sporadic breast cancer. This optimism was fueled by precedents in other cancer types, where tumor suppressor genes identified in rare hereditary cancer syndromes proved to be involved in some, if not all, of the cases of sporadic cancer of the same type. In sporadic breast cancer, sequencing efforts have failed to show significant numbers of cases of biallelic somatic mutation of either BRCA1 or BRCA2, dashing hopes of simply leveraging the understanding of BRCA1 and BRCA2 into a better understanding of sporadic breast cancer.

Laboratory-based studies of BRCA1 and BRCA2 demonstrated that loss of function of either gene resulted in significantly increased susceptibility to certain forms of chemotherapy, including interstrand DNA cross-linking agents such as the platinum drugs and mitomycin C. More recently, loss of BRCA1 or BRCA2 function has also been shown to increase sensitivity to PARP inhibition, a finding made possible as a result of increased understanding of the DNA repair implications of BRCA1 or BRCA2 loss. To a large extent, these laboratory-based observations have now been verified in clinical trials enrolling patients with hereditary breast cancer.

The implications of the discovery of BRCA1 and BRCA2 for treatment options in sporadic breast cancer are more complex. Based on a series of striking phenotypic similarities between the majority of sporadic triple-negative breast cancers and most cancers that arise in BRCA1 heterozygotes, the hypothesis arose that perhaps many of these sporadic cancers might also share a similar lesion in DNA repair (BRCAness) with the BRCA1-related tumors. This notion has now been put to the test in ongoing clinical trials that treat sporadic triple-negative breast cancer patients with platinum agents, PARP inhibitors, or combinations. The current evidence for and against this hypothesis will be discussed.

O10

NoncodingRNAs: from bench to bedside

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The newly discovered differential expression in numerous tissues, key cellular processes and multiple diseases for several families of long and short noncodingRNAs (ncRNAs, RNAs that do not codify for proteins but for RNAs with regulatory functions), including the already famous class of microRNAs (miRNAs), strongly suggest that the scientific and medical communities have significantly underestimated the spectrum of ncRNAs whose altered expression has significant consequences in diseases. miRNA and other short or long ncRNA alterations are involved in the initiation, progression and metastases of human breast cancer. The main molecular alterations are represented by variations in gene expression, usually mild and with consequences for a vast number of target protein coding genes. The causes of the widespread differential expression of ncRNAs in malignant compared with normal cells can be explained by the location of these genes in cancer-associated genomic regions, by epigenetic mechanisms and by alterations in the processing machinery. miRNA and other short or long ncRNA expression profiling of human breast tumors has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment. In addition, profiling has been exploited to identify ncRNAs that may represent downstream targets of activated oncogenic pathways or that are targeting protein coding genes involved in cancer. Recent studies proved that miRNAs and noncoding ultraconserved genes are main candidates for the elusive class of cancer predisposing genes and that other types of ncRNAs participate in the genetic puzzle giving rise to the malignant phenotype. Last, but not least, the shown expression correlations of these new ncRNAs with cancer metastatic potential and overall survival rates suggest that at least some member of these novel classes of molecules could potentially find use as biomarkers or novel therapeutics in cancers and other diseases.

O11

Targeting HER2 in breast cancer: beyond trastuzumab

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Trastuzumab has altered the natural history of $HER2^+$ breast cancer. In the metastatic setting, it has improved progression-free and overall survival. In patients with operable breast cancer, adjuvant trastuzumab, when added to chemotherapy, has improved disease-free and overall survival. Unfortunately, virtually all patients with metastatic breast cancer develop disease that is at least partially resistant to trastuzumab. In these patients, there is still value in continuing trastuzumab in combination with other treatments, but trastuzumab alone is unable to fully suppress tumor growth. Multiple mechanisms of resistance to trastuzumab have been suggested including activation of other growth factor receptors, preferential finding of HER2 to HER3, loss of the extracellular domain of HER2, and activation of the PI3 kinase pathway as a result of PTEN loss or a PIK3CA mutation. It is unknown to what extent these mechanisms are relevant in individual patients, but it is probable that many different mechanisms of resistance are clinically important. Over the past decade, a number of treatments have been developed for patients with trastuzumab-resistant disease. At present, only lapatinib, a small-molecule inhibitor of HER1 and HER2, is commercially available. It is active when administered with either chemotherapy or trastuzumab. A variety of other therapies are under investigation in phase III clinical trials. Pertuzumab, a monoclonal antibody that inhibits HER2–HER3 heterodimers, appears to be effective when combined with trastuzumab ± chemotherapy. T-DM1, an antibody–drug conjugate, has also displayed remarkable activity in the setting of refractory disease and has limited toxicity. It is presently under investigation in multiple randomized trials. Neratinib is an oral irreversible tyrosine kinase inhibitor that targets HER1, HER2, and HER4. As a single agent, it appears to be more active than lapatinib, but is associated with more significant toxicity. It, too, is under evaluation in phase III trials in the adjuvant and metastatic settings. A variety of other agents are under active study including the mTOR inhibitors, the PI3kinase inhibitors, angiogenesis inhibitors, and IGFR antagonists. It is likely that a number of new agents will be available for the treatment of $HER2^+$ breast cancer in the next several years, and outcomes for this group of patients will continue to improve.

O12

Design of RESILIENCE: a phase 3 trial comparing capecitabine in combination with sorafenib or placebo for treatment of locally advanced or metastatic HER2-negative breast cancer

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Introduction A double-blind, randomized, phase 2b screening trial (SOLTI-0701) of sorafenib, an oral multikinase inhibitor, in patients with HER2-negative advanced breast cancer (BC), showed a statistically significant improvement in progression-free survival (PFS) in the sorafenib + capecitabine arm versus the placebo + capecitabine arm: 6.4 versus 4.1 months (hazard ratio = 0.58; one-sided $P = 0.0006$). Grade 3/4 toxicities were comparable except G3 hand–foot skin reaction/ syndrome (HFSR/HFS) (44% vs. 14%). These results support a phase 3 trial of sorafenib + capecitabine in advanced BC.
Methods RESILIENCE is an ongoing multinational, double-blind, placebo-controlled, phase 3 trial designed to assess sorafenib + capecitabine as first-line or second-line therapy in advanced HER2-negative BC (ClinicalTrials.gov, NCT01234337). Eligibility criteria include: ≥18 years of age; ≤1 prior chemotherapy regimen for advanced BC; resistant to/failed taxane and anthracycline or no indication for further anthracycline; no prior VEGF treatment. Patients are randomized to capecitabine (1,000 mg/m^2 p.o. twice daily, days 1 to 14 of 21) with sorafenib (p.o. twice daily, days 1 to 21, total dose 600 mg/day) or placebo. Sorafenib 600 mg/day corresponds to the average daily dose during SOLTI-0701 that was effective and manageable. Doses can be escalated to 2,500 mg/m^2 and 800 mg/day or reduced to manage toxicity. Dose re-escalation after reduction is only allowed for sorafenib/ placebo. Guidelines detail prophylactic and symptomatic therapy for HFSR/HFS. Radiographic assessment is every 6 weeks for 36 weeks, then every 9 weeks. The primary endpoint is PFS. Secondary endpoints include overall survival, time to progression, overall response rate, and duration of response. Enrollment began in November 2010 and targets ~519 patients.
Conclusion RESILIENCE will provide definitive PFS data for sorafenib + capecitabine as first-line or second-line therapy in HER2-negative advanced BC and will better characterize the benefit-to-risk profile of this regimen.

O13

Molecular heterogeneity of luminal breast cancer

S Loi
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Luminal (or clinically defined as estrogen receptor (ER)-positive and Her2-negative) breast cancer has long been successfully treated with anti-estrogen therapy, the first targeted anti-cancer agent in breast cancer. Recently, molecular profiling approaches have allowed better identification of a poor prognostic subgroup; however, the biological mechanisms which contribute this phenotype are currently unclear.
With regards to defining prognosis, it is clear that proliferation markers can clearly separate ER$^+$/HER2$^-$ breast cancer into at least two prognostic groups. Immunohistochemistry using Ki67 at the protein level and prognostic gene signatures such as Mammaprint™, the 21-gene Recurrence Score, the two-gene ratio and genomic grade all provide quantitative measurements of proliferation activity. However, a biologically relevant cut-off does not exist. Molecular subtype definitions using PAM50 or other gene expression-based classifiers do not provide a more concordant or reproducible luminal A or B definition. Improved definition and clinical management of luminal subtypes will come from an increased understanding of the molecular drivers of the phenotype.
Lately, PIK3CA and AKT1 mutations have been shown to be associated with the good prognosis luminal A phenotype whilst FGFR1 and ZNF703 amplifications are responsible for about 25% of the luminal B phenotype. It is hoped that new genomic technologies such as next-generation sequencing will offer new insights into the biology of ER-positive breast cancer. Recent next-generation sequencing studies have identified MAP3K1, ATR and MYST3 mutations in around 10% of ER$^+$ breast cancer which may be associated with *de novo* endocrine therapy resistance. These, if shown to be driver oncogenes, may shed new light on the biology of endocrine nonresponsive breast cancer and inspire new treatment strategies. Finally, recent results from the BOLERO-2 trial suggest that metastatic ER$^+$ disease may be effectively treated with the addition of a mTORC1 inhibitor, which suggests for many patients with acquired endocrine therapy resistance, mTOR pathway activation plays a significant part in their tumor biology.

O14

Translational breast cancer research in luminal breast cancer

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Luminal breast cancer constitutes almost all ER-positive tumours and as such constitutes around 75 to 80% of the disease. The luminal group is highly heterogeneous in terms of genetic aberrations such as mutations, amplifications/deletions and translocations, and also phenotypic characteristics such as proliferation and the expression of oestrogen-dependent proteins such as PgR, TFF1 and GREB1. While assessment of some of these molecular characteristics at presentation can act as a guide to outcome, there remains substantial uncertainty in prognostic and predictive algorithms. Our approach has been to study the biological relationships by applying specific suppressants of the synthesis of oestrogen – that is, aromatase inhibitors (AIs) – in the presurgical setting. The changes in proliferation (Ki67) that occur are related to treatment benefit and the residual Ki67 to residual risk of recurrence. In addition, the molecular changes can be characterized as intermediate endpoints of response.
The POETIC trial of 2 weeks' AI or not in the window of time between diagnosis and surgery has now recruited over 2,000 patients (August 2011). Biopsies taken before and after the AI are providing a uniquely powerful set of data to understand the mechanisms of response and resistance to oestrogen deprivation. Pilot work has indicated that although luminal B tumours have higher initial Ki67 levels, their antiproliferative response to an AI is proportionally similar to luminal A tumours, indicating a similar initial responsiveness but higher residual risk of recurrence.

O15

Interpretation and molecular validation of biomarker studies

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The development and implementation of biomarkers for the diagnosis and classification of breast cancers and stratification of breast cancer patients into clinically meaningful groups are essential for the realisation of individualised medicine. The accurate, robust and reproducible assignment of patients into subgroups of therapeutic relevance is of utmost importance. Breast cancer patient treatment decision-making currently relies on the analyses of a few immunohistochemical markers (for example, oestrogen receptor, progesterone receptor and HER2), fluorescence and/ or chromogenic *in situ* hybridisation, protein analysis of lysates, and quantitative real-time PCR. It has become clear, however, that these markers are not sufficient for the potential of individualised therapy to be fully realised. The advent of high-throughput technologies and their use in fundamental and translational research endeavours have led to

the development of diagnostic markers, potential prognostic and predictive factors, and therapeutic targets, which ultimately will need to be incorporated in clinical practice. Some of the major challenges in this process are to determine the accuracy of the research hypothesis, the exclusion of potential biases, and to define whether the reagents and methodologies are fit for purpose. This requires not only a thorough assessment of the accuracy, robustness and reproducibility of the markers and the methods for their analysis, but also an adequate contextualisation of the validity of a given biomarker. For instance, immunohistochemistry has become one of the major tools for the identification of expression of potential markers in cancer tissues; albeit at first glance trivial to perform, immunohistochemical analysis can be affected by numerous parameters that can affect its accuracy. Likewise, several gene expression profiling approaches for the identification of molecular subtypes of breast cancer have been shown not to assign the same patients into the same molecular subgroups consistently. Interpretation of *in situ* hybridisation is also fraught with difficulties. Discrepancies in the assessment of biomarkers have often been attributed to intra-tumour heterogeneity, without exclusion of sources of technical variation. The challenges for the interpretation of biomarker studies and validation of biomarkers in human tissues will be discussed.

Poster presentations

P1

Clinical features and prognosis of tubular breast cancer

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Introduction The aim was to compare the clinical features and prognosis of tubular breast cancer with the rest of breast cancer grade I.
Methods We analysed all tubular breast cancer studied by the Breast Diseases Committee during the period 1990 to 2009, comparing the clinical features and prognosis of tubular breast cancer with the rest of breast cancer grade I. Disease-free survival was analysed with Kaplan–Meier curves.
Results We studied 170 cases, 41 (24.1%) tubular breast cancer and 129 (75.9%) the rest of breast cancer grade I. There were no differences in the average age of patients with tubular breast cancer and breast cancer grade I (51.9 vs. 52.7), family history, parity, fertility treatment, nulliparous, menopausal status, tumour size, and hormonal receptors. HER2 receptors are more frequent in breast cancer grade I. Two cases of tubular breast cancer (4.8%) less than 15 mm had nodal involvement. In tubular carcinomas, disease-free survival at 5 years was 97% and was 93% at 10 years. In the rest of grade I carcinomas, disease-free survival at 5 years was 95% and was 89% at 10 years.
Conclusion Tubular breast cancer has an excellent prognosis and survival, but is a necessary axillary node study in all cases.

P2

Abstract withdrawn

P3

Nestin and collagen triple helix repeat containing 1 in breast cancer progression

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Introduction We have shown that nestin expression is higher in breast carcinoma with a basal phenotype [1] and collagen triple helix repeat containing 1 (CTHRC1) and periostin may predict bone metastasis of breast cancer [2]. Our aim therefore was to examine the simultaneous role of nestin and CTHRC1 in breast cancer progression.
Methods Archival formalin-fixed paraffin-embedded 173 invasive breast cancer samples classified into WHO histotypes and luminal, triple-negative and Her2 subtypes were immunohistochemically stained with CTHRC1, periostin, nestin and vimentin antibodies. Staining was evaluated with histoscore and neoangiogenesis was assessed as the number of nestin-positive new vessels. The degree of inflammation was evaluated on HE-stained slides. Data were statistically processed by nonparametric Mann–Whitney U test, Spearman correlation coefficient and Pearson chi-square.
Results Both CTHRC1 stromal (P = 0.013) and nestin epithelial expression (P = 0.001) were higher in the triple-negative subtype. We found strong association between nestin expression in cancer cells and CTHRC1 stromal expression in advanced stage patients (r = 0.614; P = 0.007). Nestin expression was also associated with vimentin expression in breast cancer cells (r = 0.491; P <0.001). Both nestin and vimentin showed positive association with degree of inflammation, in particular in triple-negative patients (r_{φ} = 0.422; r = 0.521; both P <0.001). We observed higher nestin positivity in patients with lymph node metastases and high periostin stromal expression (P = 0.031).
Conclusion For the first time, we report an association between CTHRC1 and nestin expression in patients with advanced breast cancer. Further investigation is needed to better clarify their role in inflammation and breast cancer progression.
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P4

Prognostic value of a high level of circulating endothelial cells in patients with HER2-recurrent or metastatic breast cancer treated with bevacizumab in combination with paclitaxel and gemcitabine as first-line therapy

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Introduction Circulating endothelial cells (CECs) are shed from vessels and enter the circulation reflecting endothelial damage. Increased numbers of CECs have been documented in cancer, and appear to correlate with progression of the tumor. Bevacizumab (B) in combination with CT improves progression-free survival (PFS) of first-line treatments and may modify tumor cell intravasation and CEC/CTC levels.
Methods Patients received B (10 mg/kg/2 weeks) combined with paclitaxel (P) 150 mg/m^2 and gemcitabine (G) 2,000 mg/m^2 days 1 and 15 with a cycle each 28 days of therapy, until disease progression, unacceptable toxicity or withdrawal. CTC/CECs were measured in 7.5 ml blood at baseline and after the first cycle of treatment. Enumeration was performed by the CellSearch System (Veridex).
Results Median of follow-up was 16.28 months. Baseline CECs were available for 31 patients. Median value of baseline CECs was 130 (minimum 4 to maximum 1,407) and 60.3 (minimum 0 to maximum 349) in the second determination, P = 0.02. High levels of baseline CECs

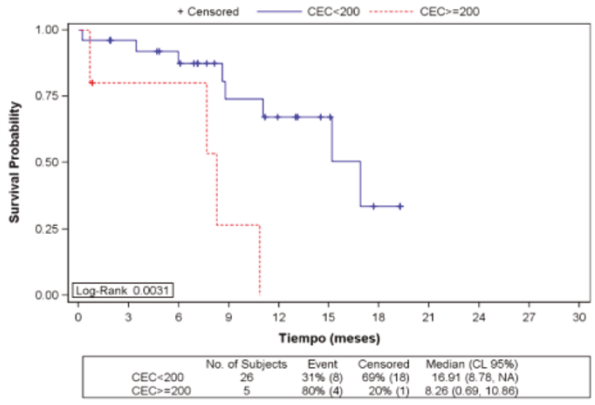

Figure 1 (abstract P4).

≥200 were associated with lower PFS of 8.2 months (95% CI = 0.6 to 10.8) compared with those with <200, PFS 16.9 months (95% CI = 8.78 to NA), $P = 0.003$. See Figure 1. No difference was observed in OS. Fourteen patients (74%) that had stable disease/partial response decreased or maintained their CEC value. Baseline CTCs ≥5 was associated with a median PFS of 15.2 months (95% CI = 7.6 to 16.9). Twenty-two patients (92%) that had stable disease/partial response decreased or maintained their CTC value. The CTC level was not correlated with the CEC level, $P = 0.74$.

Conclusion Our study suggests significant correlations between high levels of baseline CECs and poor prognosis. Addiction of B to first-line CT was related to a high reduction of CEC and CTC count.

P5

Oestrogen receptor status predicts for local recurrence following wide local excision for breast tumours

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Introduction Status of the surgical margins following wide local excision for breast cancer remains one of the strongest predictors of local recurrence. In our practice, a margin of 1 mm and more is considered adequate. In this study, we aim to determine whether clinicopathological factors other than surgical margins contribute to the risk of local recurrence.

Methods A retrospective review was performed of 548 consecutive patients who underwent wide local excision for invasive carcinoma or ductal carcinoma *in situ* (DCIS) from 1 January 2004 to 31 December 2008. Surgery was not routinely offered to patients with margins of 1 mm or more. All patients with wide local excision received postoperative whole breast irradiation, inclusive of a boost to the tumour bed.

Results Local recurrence developed in 20% of those with involved margins, as compared with 8.7% of those with close margins, and 5.4% of those with margins of 1 mm and more. Although local recurrence was more likely with an involved or close surgical margin, this reached only borderline significance ($P = 0.05$). Oestrogen receptor (ER) status was found to be an independent predictor of local recurrence, with ER-negative tumours being three times more likely to recur ($P < 0.01$, OR = 0.30, 95% CI = 0.13 to 0.66). There was no correlation with a triple-negative phenotype or other clinicopathological factors.

Conclusion A margin of 1 mm or more appears to be adequate following wide local excision. However, ER status emerged as a stronger predictor for local recurrence and alone remained significant on multivariate analysis.

P6

Getting deep in the luminal B breast cancer subtype and its ki67 cut-off value

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Introduction Inside the luminal breast cancer (BC) group, the B subclass carries a worse prognosis and is less responsive to hormonal treatment. Identification of the luminal B group, by Sørlie and colleagues [1], has been less consistent than other subclasses; and gene signatures based in estrogen-related genes or proliferation are better to identify this BC subclass. Cheang and colleagues genetically evaluated 144 luminal ER-positive HER2-negative tumors by IHC; they found a ki67 cutoff value of 13.25% to differentiate B from A subclasses [2]. No differentiation for PR status was done. The luminal B subgroup is usually defined as ki67 >13 if ER-positive, as well as HER2-positive or PR-negative. The target of this abstract is to evaluate behavior of different luminal B subsets.

Methods We reviewed early BC cases evaluated at Hospital 12 de Octubre between 1995 and 2007 and selected 710 initially operated luminal B BC. We divided this group into four subsets as shown in

Table 1 (abstract P6)

		HER2$^-$		
Variable	**HER2$^+$ER$^+$**	**ER$^+$PgR$^-$**	**ER$^-$PgR$^+$**	**ER$^+$PgR$^+$ ki67 >13**
Cases	189	126	10	385
Median age ($P = 0.0021$)	53.49	60.3	49.7	57.7
Ductal ($P = 0.002$)	173 (91.5%)	98 (77.8%)	10 (100%)	314 (81.5%)
HG III ($P = 0.03$)	41%	47%	40%	34.1%
Lobular	5.3%	17.5%	0	14%
Median ER	85%	83%	0	90%
Median PR	60%	0	45%	80%
Median ki67	20%	17%	12.5%	20%
Recurrences total (%)	52 (27.5%)	32 (25.4%)	2 (20%)	81 (21%)
Locoregional	9 (17.3%)	3 (9.4%)	0	15 (18.5%)
Bone	6 (11.5%)	14 (43.8%)	0	25 (30.9%)
Visceral ($P = 0.04$)	34 (65.4%)	11 (34.4%)	0	36 (44.4%)
Median (95% CI) DFS (years)	8.1 (6.5 to 8.7)	6.4 (5.4 to 7.2)	9.2 (7.6 to 11.9)	5.6 (5.2 to 6.1)
Median (95% CI) OS (years)	8.7 (8.1 to 9.0)	7.0 (5.8 to 7.9)	10.5 (7.8 to 13.6)	6.1 (5.6 to 6.6)

Table 1 and analyzed their clinical–pathologic features and outcomes. Additionally, we evaluated the prognostic behavior of lowering the ki67 cutoff in the $ER^+PR^+HER2^-$ group (820 patients).
Results The median ki67 value for the ER^+PR^- group was 17%. A ki67 cutoff at 14% discerns two groups of different prognosis inside the luminal group (extracting $HER2^+$ and PR^-); and comparison of ki67 cutoff between 14 and 11% found overlapped CI (median: 6.31 (5.99 to 6.62) vs. 6.49 (6.21 to 6.78)). Table 1 summarizes different characteristics and prognosis based on molecular features (statistical comparisons exclude the ER^-PR^+ subgroup).
Conclusion Subsets inside the luminal B subtype according to expression of HER2, ER, PgR and ki67 have different features and behaviors. In the $ER^+PR^+HER2^-$ subgroup, ki67 cutoff should be re-evaluated in order to avoid misclassification and subsequent under-treatment of poorer prognosis tumors as luminal A hormono-sensitive phenotype.
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P7

Lobular carcinoma of the breast: outcome of 205 patients

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Introduction Invasive lobular carcinoma (ILC) is the second most common type of invasive breast cancer (BC), which comprises approximately 10% of BC and appears to have distinct biologic and epidemiologic characteristics.
Methods We analyzed data of 205 BC patients diagnosed with ILC who were diagnosed between January 1994 and December 2007. The objective was to determine the clinicopathological features, treatment and patterns of recurrence of ILC.
Results Median age was 58.5 (range: 29.6 to 87.3). One hundred and thirty-six patients (66.3%) were postmenopausal, 131 patients (63.9%) underwent mastectomy and 74 (36.1%) a conservative surgical procedure. Pathological features were: T1: 79 patients (38.5%); T2: 84 patients (41%); T3: 19 patients (9.3%); T4: seven patients (3.4%); multifocal: 16 patients (7.8%). Nodal status N0: 131 patients (63.9%); N1: 41 patients (20%); N2: 16 patients (7.8%); N3: 17 patients (8.3%). Regarding phenotype, 90 patients (43.9%) were luminal A; 82 patients (40%) luminal B; 14 patients (6.8%) $HER2^+/RE^+$; two patients (1%) $HER2^+/RE^-$; and seven patients (3.4%) were triple negative. Sixty-seven patients (32.7%) did not receive adjuvant chemotherapy (CT). Most frequent adjuvant QT received was anthracycline-based (61 patients, 29.8%) followed by CMF (42 patients, 20.5%) and anthracycline + taxane-based CT (35 patients, 17.1%). A total of 185 patients (90%) received adjuvant hormonal treatment, the most commonly used being tamoxifen (111 patients, 60%) followed by up-front aromatase inhibitors (AI). With a median follow-up of 97.3 months, 47 patients (22.9%) had a relapse, with a median disease-free survival (DFS) of 184 months. Five-year and 10-year DFS rates were 81.8% and 69.1%, respectively. T1, N0 tumors that received CT/HT/RT had a significantly lower recurrence rate (P <0.05). The most frequent metastatic site at recurrence was bone (18 patients, 38%), followed by pleuropulmonar (seven patients, 15%), liver (five patients, 11%) and ganglionar (five patients, 11%). Median overall survival (OS) was not achieved; 5-year and 10-year OS rates were 94.4% and 81%, respectively. OS was significantly better (P <0.05) for T1, N0 tumors.
Conclusion In this review of ILC patients, the most common phenotype was luminal A. Recurrence and death rates were low, bone being the most common site of relapse.

P8

BCL2 is a predictive marker of adjuvant CMF regimen in triple-negative breast cancer patients

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Introduction Triple-negative breast cancers (TNBCS) are aggressive with poor prognosis. Patients cannot benefit from targeted treatment. Moreover, little is known of which TNBC patients will benefit from nontargeted adjuvant treatment. The objective was to search for predictors of adjuvant chemotherapy in TNBC.
Methods The study included 67 TNBC patients in clinical stages I to III, all but 22 had undergone adjuvant chemotherapy (CMF – 27 patients). FISH using p53, HER1, centromere 7 and 17 probes was performed on tumor tissue. Bcl2 was detected by immunohistochemistry.
Results HER1 amplification was found in 23.9%, p53 deletion was detected in 29.7% and bcl2 positivity was present in 32.8% tumors. A lower p53/chromosome 17 ratio correlated with higher grading (P = 0.003) and showed a strong trend toward HER1/chromosome 7 ratio (P = 0.053). Patients with a chromosome 17 copy number ≥1.9 had better overall survival than patients with a copy number <1.9 (Kaplan–Meier, P = 0.014). Bcl2-positive patients treated with adjuvant CMF had significantly better disease-free survival than bcl2-negative patients treated with adjuvant CMF (Kaplan–Meier, P <0.035).
Conclusion Initial data support the use of a classical CMF regimen in TNBC patients. However, the biomarker of CMF responsiveness is needed for clinical practice. We confirmed the bcl2 positivity as a predictor of CMF sensitivity in TNBC. Thus, validation of this marker in a larger study is needed. Higher chromosome 17 copy number was associated with better outcome, suggesting the importance of its assessment.
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P9

Role of CCND1 and C-MYC oncogenes in metastatic breast cancer patients treated by herceptin

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Introduction In breast cancer (BC), several cytogenetic markers are routinely investigated (Her2, progesterone and estrogen receptors) that distribute patients into different diagnostic groups, determine prognosis and predict effectiveness of therapy. Additional genetic markers can improve prediction or prognosis in BC patients. C-MYC and CCND1 gene aberrations frequently found in BC were chosen for this study.
Methods The status of C-MYC, CCND1 genes, chromosomes 8 and 11 were determined on 74 patients from a group of 103 BC patients treated with herceptin in a palliative regime, using FISH assay on formalin-fixed paraffin-embedded tissue samples. One hundred nuclei per sample were analyzed in each sample. Clinical data were correlated with our cytogenetic results.
Results Amplifications of CCND1 and C-MYC were occurring together in most cases (P <0.0001), especially in Her-2/neu-negative cases (P = 0.01, resp. 0.03). Progesterone receptor positivity was associated with CCND1, resp. C-MYC increased copy number (P = 0.039, resp. 0.038). Amplification (ratio gene/chromosome ≥2.2) of CCND1, resp. C-MYC was determined in 8.1% (6/74), resp. 12.2% (9/74) cases. Polysomy

(copy number ≥3.0) of chromosome 11, resp. 8 was determined in 6.8% (5/74), resp. 16.2% (12/74) cases.
Conclusion Increased copy numbers of CCND1 and C-MYC were associated with progesterone receptor positivity and Her-2/neu negativity. Clinical relevance of our findings will be presented.
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P10

microRNA and protein expression in breast cancer formalin-fixed paraffin-embedded samples

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Breast Cancer Research 2011, **13(Suppl 2):**P10 (doi: 10.1186/bcr3031)

Introduction microRNAs (miRNAs) constitute a new class of small noncoding RNAs that control post-transcriptionally the expression of gene products, either modulating directly protein translation or regulating the stability of messenger RNA. There is increasing evidence of the role that miRNAs play in regulating breast cancer gene expression. The main objective of this study is to evaluate the diagnostic utility of the expression of a panel of miRNAs in breast cancer and compare their expression with the expression of the proteins they regulate.
Methods miRNA expression was analyzed by RT-qPCR using TaqMan Arrays (Applied Biosystems). We compared the expression of 667 miRNAs on 19 fresh frozen (FF) and formalin-fixed paraffin-embedded (FFPE) matched breast cancer samples. Regarding protein expression, we have developed and evaluated different protocols for protein extraction from FFPE samples. Next, we studied the applicability of these protein extracts to classical and new high-performance proteomics techniques.
Results After proper normalization, 123 out of 671 miRNAs showed a good correlation of their expression data between FFPE and FF tissue, and sufficient analytical robustness (they are expressed in at least one-third of FFPE samples). In addition, we analyzed the expression of various markers with diagnostic value in breast cancer. As regards high-performance proteomics, the protocols developed generated over 6,000 MS/MS spectra, enabling the identification of hundreds of proteins in each sample.
Conclusion We have selected the most appropriate assays to study miRNA expression in breast cancer FFPE archived samples. The protocols developed allow proteome analysis of FFPE samples using the latest mass spectrometry equipment. The technologies implemented during the development of this project allow one to compare the expression data at both miRNA and protein levels to study breast cancer from an authentic system-biology perspective.

P11

Quantitative proteomics reveals novel proteins and central pathways associated with endocrine resistance in breast cancer

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Introduction Acquired resistance to endocrine therapies remains a major clinical obstacle in hormone-sensitive breast tumors. The complexity of the underlying biological mechanisms remains poorly understood and the purpose of this study was to identify low abundant proteins and central pathways associated with tamoxifen resistance.
Methods The global protein expression of the parental tamoxifen-sensitive MCF7S0.5 cell line and the tamoxifen-resistant TamR1 cell line were compared using SILAC labeling and quantitative mass spectrometry. Data were processed using MaxQuant, the global protein–protein interaction network was predicted using STRING and enriched pathways were identified with KEGG analysis. Selected proteins differentially expressed were validated using western blotting and immunocytochemistry.
Results Proteomic analysis identified 5,370 proteins based on at least one unique peptide of which 4,448 proteins could be quantified based on at least two peptides. Forty-one proteins were found to be differentially expressed more than threefold and eight proteins were validated by western blotting and immunocytochemistry as potential biomarkers associated with tamoxifen resistance. In total 539 proteins were differentially expressed 1.5-fold or more and could be subgrouped into kinases, transcription factors, receptor activity proteins, cell adhesion proteins, cell cycle proteins and stress responder proteins. We identified several regulated proteins to be important in subnetworks that among others are involved in focal adhesion, DNA replication, apoptosis, and insulin and HER2 signaling pathway.
Conclusion Novel low abundant proteins not previously associated with tamoxifen resistance have been identified and validated using biochemical methods. At present the proteins are being validated on a panel of primary breast cancer biopsies from patients treated with tamoxifen monotherapy and with known clinical outcome. Our data also revealed several pathways associated to tamoxifen resistance. The importance of these pathways needs to be studied further.

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The miRNA-200 family and miRNA-9 exhibit differential expression in primary versus corresponding metastatic tissue in breast cancer

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Introduction Metastases are the major cause of cancer-related deaths, but the mechanisms of the metastatic process remain poorly understood. In recent years, the involvement of microRNAs (miRNAs) in cancer has become apparent, and the objective of this study was to identify miRNAs associated with breast cancer progression.
Methods Global miRNA expression profiling was performed on 47 tumor samples from 14 patients with paired samples from primary breast tumors and corresponding lymph node and distant metastases using LNA-enhanced miRNA microarrays. The identified miRNA expression alterations were validated by real-time PCR, and tissue distribution of the miRNAs was visualized by *in situ* hybridization.
Results The patients in which the miRNA profile of the primary tumor and corresponding distant metastasis clustered in a unsupervised cluster analysis showed significantly shorter intervals between the diagnosis of the primary tumor and distant metastasis (median 1.6 years) compared with those that did not cluster (median 11.3 years) (P <0.003). Fifteen miRNAs were identified that were significantly differentially expressed between primary tumors and corresponding distant metastases, including miR-9, miR-219-5p and four of the five members of the miR-200 family involved in epithelial–mesenchymal transition. Tumor expression of miR-9 and miR-200b was confirmed using *in situ* hybridization, which also verified higher expression of these miRNAs in the distant metastases versus corresponding primary tumors.
Conclusion Our results demonstrate alterations in miRNA expression at different stages of disease progression in breast cancer, and suggest a direct involvement of the miR-200 family and miR-9 in the metastasis process.

Cite abstracts in this supplement using the relevant abstract number, e.g.:
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