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Progression of precursor and pre-invasive lesions to invasive cancer

A.L. Børresen-Dale[1]. [1]*Institute for Cancer Research Oslo Univerity Hospital Radiumhospitalet, Department of Genetics, Oslo, Norway*

In order to better understand the transition of normal breast epithelial cells through hyperplasia, DCIS and to invasive cancer we have analyzed a series of breast tissues, normal and malignant, sampled from healthy women and women with breast cancer at various stages of the disease at DNA and RNA level using various microarrays. Expression analyses revealed a distinct pattern of gene expression in normal vs malignant breast tissue. Focusing on genes involved in synthesis, degradation and binding of N-linked and O-linked glycans, Lewis antigens, glycosaminoglycans and glycosphingolipids demonstrated a unique glycan gene expression signature indicating that synthesis, degradation and adhesion mediated by glycans are altered drastically in mammary carcinomas. These genes are involved in regulation of growth factors/growth factor receptors, cell-cell adhesion and motility as well as immune system modulation, demonstrating that altered glycan structures are of importance in malignant transformation, and that a more comprehensive understanding of the glycobiology are of importance to elucidate the whole carcinogenic process.

Expression analyses also revealed heterogeneity within the different premalignant/malignant subgroups, identifying the five main intrinsic subtypes in all cohorts. Within the DCIS group a distinct subgroup with gene expression characteristics more similar to advanced tumours were identified, reflecting activated processes related to re-organisation of the microenvironment. This raises interesting possibilities for identification of DCIS lesions both with and without invasive characteristics.

DNA from the various tissue cohorts were submitted to quantitative DNA methylation analysis of 12 *selected genes* by pyrosequencing. Aberrant hypermethylation was observed in all malignant diagnosis groups for *ABCB1, FOXC1*, *GSTP1*, *MGMT, MLH1*, *PPP2R2B*, *PTEN* and *RASSF1A*. For most of these genes, methylation was already present in DCIS with the same frequency as within IDCs. The average DNA methylation levels were significantly higher in the pure IDCs and IDCs with DCIS compared to pure DCIS ($p = 0.007$ and $p = 0.001$, respectively). For *FOXC1* significant differences in methylation levels were observed between normal breast tissue and invasive tumours ($p < 0.001$). Low *FOXC1* gene expression in both methylated and unmethylated DCIS and IDCs indicates that the loss of its expression is an early event during breast cancer progression.

References

Potapenko et al. Mol Onc, in press 2010.
Muggerud et al. BCR in press, 2010.

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Should all pre-invasive lesions be excised and irradiated?

B. Cutuli[1]. [1]*Polyclinique de Courlancy, Radiation Oncology, Reims, France*

In situ malignant lesions cover a wide spectrum of cell proliferations with different potential evolutions to invasive tumours. We focused our analysis on ductal carcinoma in situ (DCIS), sometimes also called ductal intra-neoplasia (DIN) [4], and lobular carcinoma in situ (LCIS), also called lobular intra-epithelial neoplasia (LIN).

Both lesions can be graded in there categories: DIN 1, 2 and 3, and LIN 1, 2 and 3. With the wide use of mammographic screening, these lesions reach 15−20% of all breast cancers.

1. Ductal Carcinoma In Situ (DCIS): DCIS represents 85−90% of all in situ lesions. DCIS treatments are mastectomy, breast conserving surgery alone (BCS) and BCS with whole breast irradiation (BCS + RT). There is a risk of progression to invasive carcinoma, with a 15% long-term risk of metastasis occurrence.

1.1. Mastectomy: Mastectomy provides approximately 98% of local control rate.

1.2. Breast Conserving Surgery (BCS): BCS, both in retrospective studies and randomized trials, leads to a 20−25% local recurrence (LR) rate at 7 years, including 40−45% invasive. Young age and small margins increase LR rates. In a recent very much selected study (only 6 mm median size lesions) by ECOG including 565 low-intermediate grade DCIS and 105 high-grade DCIS, the 7-year LR rates were 10% (53% invasive) and 18% (35% invasive).

1.3. Breast Conserving Surgery With Radiotherapy (BCS+RT): In several large series, the 7-year LR rate was approximately 10%, but with large heterogeneities due to selection criteria, extent of surgery and RT modalities. Four randomized trials (NSABP B-17, EORTC 10583, UK-DCIS and SweDCIS) confirmed a significant benefit with whole breast RT (50 Gy/25 fractions or equivalent), with a 50−60% LR reduction (both in situ and invasive). The results are shown in table 1. Both in NSABP and EORTC trials, RT efficiency was observed in all clinical or histopathological subgroups. Young age (under 40) and incomplete/doubtful excision remain the most important LR risk factors. Therefore, BCS+RT is considered the standard treatment with a 1% annual risk of LR and 98% 15-year specific survival. In a recent meta-analysis, a 2 mm (minimal) margin excision was required to optimize the long-term results of BCS+RT. Despite these results, RT usefulness was questioned by some authors.

Table 1: Results of trials comparing breast conserving surgery alone (BCS) or with radiotherapy (BCS+RT): local recurrence rates in %.

	NSABP B-17		EORTC 10583		UK-AZ DCIS		SWE DCIS	
	BCS (403)	BCS+RT (410)	BCS (500)	BCS+RT (502)	BCS (502)	BCS+RT (522)	BCS (520)	BCS+RT (526)
LR	31.7	15	26.4	14.9	20.7	7.7	27.1	12.2
In situ LR	14.1	7	13.4	7.2	10.4	4	14.8	4.9
Invasive LR	16.6	8	13	7.7	10.3	3.7	12.3	7.3
Follow-up	129 mo		120 mo		152 mo		101 mo	

2. Lobular Carcinoma In Situ (LCIS): Lobular carcinoma in situ (LCIS) represents 1−2% of all breast cancers and the exact significance remains uncertain, ranging from subsequent carcinoma marker to real precancerous lesion. LCIS is now revealed by several radiological features, especially microcalcifications.

The literature data on LCIS treatment are rare, but there are the same DCIS options, although BCS+RT was only reported in details in one study. Mastectomy gives an almost 100% cure rate. BCS alone gives an average 15% of subsequent invasive LR at 10−15 years. In a recent French retrospective study including 255 cases treated by BCS (with a 9.4-year median follow-up), 49 LR occurred (21 in situ and 28 invasive), corresponding to 12% and 23% of LR rates at 5 and 10 years respectively. Moreover, among 37 women treated by BCS+RT, only two LR (5.4%) were observed. LCIS is not always an “indolent” disease and, in several cases, it looks like DCIS, but with a longer relapse length. Several aggressive subtypes (i.e. pleiomorphic and some other LIN-3 patterns)) are now defined by pathologists. Both mastectomy and CS+RT could be seen as possible options instead of simple lumpectomy. In the NSABP P-1 chemoprevention trial, Tamoxifen reduces the subsequent risk of invasive BC by 56%.

Friday, 26 March 2010 15:30–17:00

CLINICAL SCIENCE SYMPOSIUM

Gene profiling and treatment of disease

441 Invited

Why Adjuvant! Online remains a useful tool in the era of multigene expression signatures

P.M. Ravdin[1]. [1]*University of Texas Health Science Center, Cancer Therapy and Research Center, San Antonio Texas, USA*

Adjuvant! is a tool that was developed to present numerical estimates of outcome to breast cancer patients as part of shared decision making with a patient. As part of this effort in the 1990’s we studied the numerical knowledge that patients and their doctors had about prognosis without adjuvant therapy and the relative efficacy in terms of proportional risk reductions of adjuvant therapy. We found that there was a wide variation in these estimates and that many of them were very unrealistic. Guidelines were of no help in this regard as they were amount always non-numerically based prescriptive recommendations.

Adjuvant! draws from information from large registries to make estimates of patient outcome. These registries such as the United States SEER registry have classical information about patient outcome. The current version uses information about tumor size, nodal status, estrogen receptor status, and histologic grade to make estimates of outcome at 10 years of follow-up. Efficacy estimates for different classes of the hormone and chemotherapy are drawn directly or indirectly from the Early Breast Cancer Clinical Trialist’s meta-analyses. Estimates of completing mortality come from United States mortality tables.

The goal of the output of this tool is to provide to the patient and physician good general estimates of patient outcome with the strongest endpoint being survival and the program providing estimates of survival at 10 years, death due to breast cancer at 10 years (with or without adjuvant therapy), and an estimates of death due to competing mortality. The overall accuracy of this tool has been validated in Canadian and Dutch registries. Some much sought after estimates such as how adjuvant trastuzumab affects 10 year outcomes cannot be made because of the short follow-up of the trials assessing prognostic estimates.

Can such a tool be improved upon? When new technologies become available is it still relevant?

Certainly! But it too will have to evolve. In this presentation the new multigene expression signatures will be reviewed. There are certainly signatures based on both protein and nucleic acid technologies that can refine estimates of patient prognosis beyond the estimates that can be made by classic pathologic information. Some of these signatures have also shown promise in making estimates of treatment efficacy. Adjuvant! was specifically designed so that estimates for prognosis and efficacy from other sources and can be entered either to be combined with or to override estimates based on newer technologies.

No tool can ever be claimed to be perfect. This is because many factors evolve with time. Screening, exogenous exposures, general medicine and salvage therapy all in a state of change. Much of what we "know" in terms or treatment efficacy is based on short term follow-up. We are inevitably developing new models for 10 year outcome based on population of patients from at least 10 years ago, and making projections of outcome for 10 years from now. Given the rapid evolution of technology these will always be approximations. Nonetheless prognostic and treatment efficacy tools that produce numerical estimates have moved us beyond the era of vague non-numerical statements to an era of shared decision making where patients and their health care team can discuss options in a more complete way.

443 Proffered paper oral

PARP is expressed in all subtypes of early breast cancer and is a predictive factor for response to neoadjuvant chemotherapy

G. von Minckwitz[1], B. Müller[2], S. Loibl[3], J.U. Blohmer[4], A. duBois[5], J. Huober[6], R. Kandolf[7], J. Budczies[2], C. Denkert[2]. *[1]GBG Forschungs GmbH, Managing Director, Neu-Isenburg, Germany; [2]Charité Universitätsmedizin Berlin, Institut für Pathologie, Berlin, Germany; [3]German Breast Group, Medicine & Research, Neu-Isenburg, Germany; [4]St. Gertrauden Krankenhaus, Gynecology, Berlin, Germany; [5]HSK Wiesbaden, Gynecology, Wiesbaden, Germany; [6]UFK Tübingen, Internal Medicine, Tübingen, Germany; [7]University of Tübingen, Institut für molekulare Pathologie, Tübingen, Germany*

Background: The polyadenosine diphosphate [ADP]-ribose polymerases (PARPs) are a large family of multifunctional enzymes. PARP-1 plays a key role in the genomic stability. Increased expression is considered to be associated with resistance to DNA damage-inducing therapeutic agents. Combining these cytotoxic agents with a PARP inhibitor showed improved activity in patients with triple negative metastatic breast cancer.

We investigated PARP expression in various hormone (HR)/HER2 receptor subtypes of early breast cancer and evaluated its predictive value for pathological complete response (pCR; defined as no invasive residuals in breast and nodes).

Methods: Tissue microarrays from core biopsies of 582 patients recruited to the phase III GeparTrio trial, who received neoadjuvant 6−8 cycles TAC/NX chemotherapy, were centrally stained immunohistochemically for PARP, ER, PgR and HER2 expression. Cytoplasmatic and nuclear staining of PARP was assessed with regard to intensity and percentage of positive cells and scored as low, medium or high expression.

Results: Overall, cytoplasmatic PARP expression was high in 24.4%, medium in 52.4% and low in 23.2% of patients. High expression was found in 19.9% of 286 HR+/HER2−, 20.2% of 129 HR+/HER2+, 36.0% of 50 HR−/HER2+ and 35.6% of 101 HR−/HER2− tumours ($p = 0.001$). High PARP expression was significantly correlated with undifferentiated tumour pattern ($p < 0.001$), non-lobular cancers ($p < 0.001$), negative HR ($p < 0.001$). Correlation was only of borderline significance for tumour size and nodal status, no correlations were found for HER2 status and age. Patients with high PARP expression showed a pCR rate of 25.7% compared to 18.8% and 6.1% in patients with medium or low expression ($p < 0.001$). In univariate logistic regression, pCR rate was different between PARP high and low expressing tumours with OR = 5.3 (95% CI 2.4–12.0). This result remained significant when corrected for tumour stage, nodal status, histological type, tumour grade, molecular subtype and age, OR = 2.6 (1.1−6.4). No such correlations were found regarding nuclear PARP staining.

Conclusions: Cytoplasmatic PARP expression can be detected by immunohistochemistry in all subtypes of early breast cancers and is correlated with an aggressive biological tumour pattern. Cytoplasmatic PARP expression predicts pCR to neoadjuvant taxane-anthracycline-based chemotherapy. Clinical investigation of PARP inhibitors should not be limited to triple negative tumours alone.

444 Proffered paper oral

The EORTC 10041/BIG 03−04 MINDACT trial is feasible: first results of the pilot phase

E. Rutgers[1], M. Piccart[2], S. Delaloge[3], L. Van 't Veer[4], I. Rubio[5], G. Viale[6], J. Bogaerts[7], F. Cardoso[2]. *[1]The Netherlands Cancer Institute, Department of Surgery, Amsterdam, The Netherlands; [2]Institut Jules Bordet, Department of Medical Oncology, Brussels, Belgium; [3]Institut Goustave Roussy, Department of Surgery, Villejuif, France; [4]Netherlands cancer institute, Department of Molec. pathology, Villejuif, The Netherlands; [5]Hospital Vall d'Hebrón, Department of Breast Pathology, Barcelona, Spain; [6]European Institute of Oncology, Department of Pathology, Milano, Italy; [7]EORTC, EORTC Breast Cancer Group, Brussels, Belgium*

The MINDACT trial (Micro array In Node negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy) investigates whether the 70-gene profile (Mammaprint™) selects the right pts for adjuvant chemotherapy (CT) as compared to standard clinicopathological criteria. All pts have the 70-gene test (genomic high vs low risk) and clinical-pathological prognostic risk, the latter assessed through a modified version of Adjuvant! Online (low risk defined as >88% 10-years breast cancer specific survival for ER-positive and >92% for ER-negative disease). Genomic (G) and clinical (C) high risk pts are proposed adjuvant CT and may be randomized between an anthracycline-based regimen and the combination docetaxel-capecitabine. G-low and C-low risk patients do not receive CT. All ER-positive pts are offered an endocrine therapy randomization between 7 years of letrozole and 2 years of tamoxifen followed by 5 years of letrozole. Discordant patients (G-low/C-high or G-high/C-low) are randomized between decision of adjuvant CT based on G or C risk assessment. The study aims to enroll 6000 pts and has a predefined "pilot phase" of 800 pts to ensure its feasibility. The first pt was enrolled in March 2007, and in November 2008 accrual passed the 800 enrolled pts (i.e. pts with treatment assigned); these first pts are all node negative. The IDMC reviewed data from this pilot phase and endorsed communication of these results:

- The accrual is currently around 140 enrolled pts/month.
- 46% of screened pts were enrolled; 73% of screened pts had their sample shipped; of the shipped samples, 67% went through successful hybridization and testing by the 70-gene array.
- Reasons for non-eligibility: 28% of cases node positivity (before amendment), 29% for sample quality problems, 43% for failure to enrol within timelines or other reasons.
- C/G risk allocation: C/G low risk: 386 pts (48%); C/G high risk 198 pts (24.8%); C low risk/G high risk: 75 pts (9.4%); C high risk/G low risk 141 pts (17.6%). Total proportion of discordant cases: 27%.
- A statistically significant difference of 8.25% (C. I 4.7–11.8) is observed between pts that have a high C risk (42%) and those with a high G risk (34%) showing that, in the accrued population, more pts are assigned low risk by the G test than the C one.
- Compliance to randomization: within the key group of 69 pts with high C risk/low G risk assigned to no CT, 3 pts still received CT. In the 39 pts C low/G high risk assigned to CT, 5 pts did not receive it. Overall compliance >92%.

Conclusions: (1) The logistically complex MINDACT trial is feasible in an multinational setting. (2) The proportion of discordant pts, the expected reduction in CT in the 70-gene low risk group, and the compliance to treatment assignment in the discordant groups are according to plan. (3) The trial continues to accrue with new centers joining, and it was amended to include node positive disease, decreased tumor cellularity needed and increased timelines; these measures have already substantially decreased the number of ineligible pts.

445 Proffered paper oral

Beta-blocker treatment is associated with a reduction in tumour metastasis and an improvement in specific survival in patients with breast cancer

D.G. Powe[1], M.J. Voss[2], H.O. Habashy[3], K.S. Zänker[2], A.R. Green[3], I.O. Ellis[1], F. Entschladen[2]. *[1]Nottingham University Hospitals Trust, Cellular Pathology, Nottingham, United Kingdom; [2]University of Witten/Herdecke, Institute of Immunology, Witten, Germany; [3]University of Nottingham, Molecular Medical Sciences, Nottingham, United Kingdom*

Background: Breast cancer (BC) is the most common cause of cancer death in women and usually results from metastatic events. Recent studies suggest that neurotransmitters induce cancer cell migration mediated by beta2-adrenergic receptors (β_2AR). Therapeutic treatment with beta-blockers could protect against metastasis development giving improved clinical outcome in BC.

Materials and Methods: An epidemiologic study of beta-blocker treatment and its associations with metastasis and BC-specific survival