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Basal-like subtype of breast carcinoma predicts poor clinical outcome in patients with high-risk breast cancer treated with high-dose (HD) or dose-dense chemotherapy: Results of multivariate analysis from the WSG-AM-01 phase III trial

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Introduction: Dose intensification strategies and especially adjuvant high dose chemotherapy in high-risk breast cancer are controversial. Prognostic and predictive factors identifying patient subgroups with maximal benefit from high-dose chemotherapy with peripheral blood stem cell transplantation (PBSCT) remain to be defined. We retrospectively evaluated the role of molecular markers especially those defining basal-like type of breast cancer (negative hormone receptors (HR), not overexpressing HER-2/neu, positive for basal cytokeratins such as Ck5, MIB and c-kit).
Methods: 403 patients were randomly assigned to dose-dense conventional chemotherapy (four cycles of EC, followed by three cycles of CMF q2w) or to two courses of EC followed by two courses of HD chemotherapy (EC-thiotepa) with PBSCT. Treatment arms were well balanced for age, menopausal status, tumour size, grading, number of involved lymph nodes, nodal ratio and hormone receptor status. A multivariate analysis was done in 239 patients, where paraffin-embedded tumors were assessable for central pathologic review including grading. Ck 5, HR, c-kit, p 53, MIB, HER-2/neu, bcl-2, Cyclin D1, p 16, E-Cadherin in these tumors were detected by immunohistochemistry.
Results: Within the WSG AM01 trial the average number of positive axillary lymph nodes was 17.6. At a median follow-up of 48.6 months there is a significant overall survival benefit for patients receiving HD. Ck5 status was available from 224 tumours (20 Ck5+/204 Ck5−). Ck5 positivity was associated with high grading ($p < 0.0001$), ER− ($p \leqslant 0.0001$), PR-($p = 0.0002$), p 53 ($p = 0.003$), c-kit ($p = 0.0007$), p16 ($p < 0.0001$) and MIB ($p = 0.0005$). In the multivariate analysis patients with Ck5 positive tumors had a poor outcome (HR for OS = 3,441 [CI: 1.51−7.67], $p = 0.0031$) independent of therapy, stage and nodal status. Other factors associated with poor outcome in the multivariate analysis were over expression of HER-2/neu ($p = 0.0089$), PR negativity ($p = 0.021$), tumor size ($p = 0.028$), MIB positivity ($p = 0.038$).
Interpretation: The retrospective evaluation of prognostic factors from other high-dose chemotherapy trials are highly controversial. HER-2/neu, age and grading are the best documented prognostic and/or predictive factors. Our data suggest that basal-type breast cancer correlates with poor outcome. Further analysis by treatment arm will be presented.

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Time to response – a comparison of fulvestrant with anastrozole

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Background: It has been speculated that pharmacokinetic differences between treatments may affect the observed time to response (TTR) in advanced breast cancer. Since fulvestrant is administered as a sustained release intramuscular (IM) injection, which takes 3 to 6 months to reach steady state, there is a concern amongst physicians that this may result in a delayed time to response compared with aromatase inhibitors.
Materials and methods: TTR data were collected from two Phase III trials (0020 and 0021) of fulvestrant 250 mg/month (intramuscular injection) versus anastrozole 1 mg/day (orally) in the treatment of postmenopausal women with advanced breast cancer who had recurred or progressed on prior tamoxifen therapy. Combined analysis of data from both trials was performed to determine median TTR with each treatment. Median TTR was calculated from randomisation to the observation of an objective response (complete or partial).
Results: The time taken to achieve an objective response was similar with fulvestrant and anastrozole (Table). Median TTR with fulvestrant was close to 3 months and almost identical to that of anastrozole, but values ranged between 0.9−33.1 months overall, suggesting that an objective response may still occur after a long period of stable disease with fulvestrant treatment.

	TTR (months)	
	Median	Range
Fulvestrant 250 mg/month (n = 82)	3.10	0.9−33.1
Anastrozole 1 mg/day (n = 70)	2.99	0.7−20.2

Data source: Combined data (Trials 0020 and 0021)

Conclusions: Median TTR was similar between fulvestrant and anastrozole. These data suggest that patients without rapidly progressive disease should be kept on endocrine treatment for at least 3 months to allow a response to be achieved prior to considering changing treatments. Furthermore, objective responses may occur following a long period of stable disease during fulvestrant treatment.

Poster presentations (Tue, 1 Nov)
Breast cancer – basic science, molecular predictive assays, translational research

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Co-expression of P27 kip1/p21 is an independent indicator of good prognosis in lymph node positive breast carcinomas

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Progression through the cell cycle is controlled by the cyclin-dependent kinases (Cdks), allowing repair of damaged DNA, and preventing its replication or the loss of genetic material. The kinase inhibitor proteins (KIPs) p27 kip1 and p21 Waf1 negatively regulate cell cycle progression by preventing the passage of cycling cells from G1 to S phase. This occurs via activation of G1 cyclin-dependent kinases, which are thought to play a role in tumour suppression. However, evidence is conflicting as to whether p27 kip1 and p21 Waf1 have a significant role to play in breast cancer.
Material and methods: The present investigation included 710 breast cancer cases with a median follow-up of 87 months, in order to determine the prognostic significance of p27 kip1 and p21 Waf1. Immunohistochemical analysis of tissue microarrays was used to assess expression of these markers. These specimens comprise a well characterized series of patients (70 years of age or less, mean: 54) with primary operable breast cancer diagnosed between 1987 and 1992.
Results: Univariate analysis showed a significant association between reduced p27kip1 expression and increasing tumour grade ($p < 0.001$), development of distal metastasis ($p = 0.012$) and tumour recurrence ($p = 0.014$). A further association was noted between reduced p27 kip1 and ductal/no special type tumours ($p = 0.008$). Survival analysis demonstrated that patients with tumours with high p27 kip1 levels had an improved survival compared with those with low expression (log rank = 0.006). Reduced p21Waf1 expression was noted in high grade tumours ($p = 0.02$) and poor prognosis tumour types ($p = 0.05$), however, no correlation was observed with survival. On multivariate analysis, using the Cox regression in lymph node positive patients, the combination of a p21(+)/p27(+) phenotype was independently associated with a good prognosis (HR 3.338, 95%CI: 1.215−9.168, $p = 0.019$).
Conclusion: It is concluded that lymph node positive breast cancer patients with a p21(+)/p27(+) phenotype demonstrate prolonged survival times, and that this is independent of other factors such as tumor size and grade. This may indicate a subset of patients for whom less aggressive adjuvant therapy is appropriate.

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The analysis of ATM mutations in high-risk breast cancer patients

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Background: Ataxia-telangiectasia (A-T), caused by mutations in the ATM (A-T mutated) gene, is an autosomal recesive disorder characterized by cerebellar ataxia, oculocutaneous telangiectasia, immunologic deficiency, hypersensitivity to ionizing radiation and predisposition to cancer. The finding that heterozygous mutation carriers (0.5−1% of the general population) have an increased risk for breast cancer (BC) was supported by numerous studies. The purpose of this study was to analyze the occurrence of ATM mutations with hereditary breast cancer.
Material and methods: Patients from high risk breast and/or ovarian cancer families that had previously been screened for mutations in BRCA 1 and BRCA 2 genes were analyzed for the presence of truncating ATM mutations. RNA was isolated from peripheral blood lymphocytes and reverse transcribed to cDNA. The coding sequence of ATM was amplified in 7 overlapping fragments. PTT was used for pre-screening of amplified fragments; the final analysis of identified gene alterations was done by sequencing.
Results: In a group of 70 high-risk families, the c.5932G>T nonsense mutation, which results in a termination of translation at codon 1978, was

identified. This pathogenic mutation, that had previously been shown to cause A-T in the homozygous state, was found in breast and ovarian cancer family (with one case of leukemia) in a patient with bilateral BC at the ages of 53 and 60 and ovarian cancer at the age of 61. Product of alternative splicing with deleted exon 36, which leads to a shift of the ATM reading frame, was the second identified sequence variant. To date, the mechanism for generation of this splice variant is not known.

Conclusions: Examination of a larger group of patients is currently under investigation to determine the incidence of ATM mutations in risk families and to test the hypothesis that mutations responsible for A-T cause in heterozygotes an elevated risk for breast cancer.

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p53 codon 72 and p21 codon 31 polymorphisms and susceptibility to breast cancer in the Turkish and Greek populations

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Background: The tumour suppressor gene TP53 and its downstream effector p21 are thought to play major roles in the development of breast cancer. Polymorphisms in TP53 are considered candidate risk factors because of the crucial role played by this gene in the maintenance of genomic integrity following genotoxic insult. p53 codon 72 polymorphism appears to be significantly associated with several cancers including that of the breast. Independent studies also provided evidence that the Arg and Pro alleles at codon 72 are structurally and functionally distinct and therefore may influence cancer risk or treatment. p21 cyclin dependent kinase inhibitor mutations proved to be extremely rare in a variety of cancer types investigated. Polymorphic variants of p53 at codon 72, and p21 at codon 31, have been found to be associated with cancer susceptibility, but few studies have investigated their effect on breast cancer risk. In this study, we aimed to investigate any possible association between increased susceptibility to breast cancer and p21 codon 31 and/or p53 codon 72 polymorphisms in the Turkish and Greek populations.

Materials and methods: In total, 478 breast cancer patients with breast cancer and 382 age-matched controls were genotyped by PCR-based restriction endonuclease digestion. The Minitab 13.1 software program was used for statistical analysis of the data. Binary logistic regression analysis was performed for odds ratio and 95% confidence interval calculations. Adjusted odds ratio calculations were carried out with the SPSS software program.

Results: The p53 Arg72 Arg inheritance was found to be significantly associated with breast cancer susceptibility in the Turkish (OR = 2.16; 95%CI = 1.08–4.31) as well as in the combined Greek-Turkish populations (OR = 2.35; 95%CI = 1.25–4.41). This association was further exacerbated with increased BMI (OR = 3.86; 95%CI = 1.12–13.26) in the Turkish population. p21 codon 31 was not associated with breast cancer susceptibility in either population. Most notably, combination of the two high-risk genotypes, p53 Arg72Arg and p21 Arg31Arg or Ser31Arg increases the risk to 2.66-fold (95%CI = 1.06–6.66).

Conclusion: These results let us to conclude that there is a strong association between the p53 Arg72Arg genotype and breast cancer risk in the Turkish and Greek populations and that the combination of the high-risk allelic variants of both p53 and its downstream effector protein p21 may have a role in breast cancer development.

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Silencing of LASP-1 influences zyxin localization, inhibits proliferation and reduces migration in breast cancer cells

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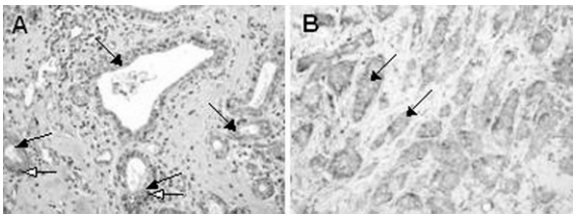
LIM and SH3 protein 1 (LASP-1), initially identified from human breast cancer, is a specific focal adhesion protein involved in cell migration. LASP-1 is an actin binding protein, which also interacts with the proline-rich domains of zyxin, a scaffolding protein required for cell movement and gene transcription.

In the present work we analyzed the effect of LASP-1 on different human breast cancer cell lines using the powerful small interfering RNA technique (siRNA) to silence protein expression in a sequence specific manner. Transfection with LASP-1 specific siRNA resulted in a reduced protein level of LASP-1 in BT-20 and MCF-7 cell lines. The siRNA-treated cells were arrested in G2/M phase of cell cycle and proliferation of the tumor cells was suppressed by 30–50% corresponding to around 50% of the cells being transfected successfully as seen by immunofluorescence. Tumor cells transfected with LASP-1 si-RNA showed a 50% reduced migration compared to control cells transfected with scrambled si-RNA. Overexpression of LASP-1 in non-tumor PTK-2 cells, which don't express endogenous LASP-1, resulted in a significant increase in cell motility. LASP silencing is accompanied with a reduced binding of the of LASP-1 binding partner zyxin to focal contacts without changes in actin stress fibre organisation as observed in immunfluorescence experiments.

The data provide evidence for an essential role of LASP-1 in tumor cell growth and migration, possibly through influencing the localization of zyxin.



Immunohistochemical staining of normal and cancerous breast tissue samples.

A): Normal breast tissue with two ducti in the centre and the acini at the left and right sides. LASP is positive in the myoepithelial cells (white head arrow) surrounding the LASP-negative luminal epithelia cells (black arrow). *B):* In breast cancer all cancer cells are intensively stained positive for LASP (arrows). Magnification × 400.

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Expression of the growth factor receptors HER2 and EGFR in primary tumors and in brain metastases of breast cancers

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Background: The EGFR-related growth factor receptors play an important role in breast cancer. Both HER2 and EGFR are targets for specific therapeutic interventions such as tyrosineinhibitors or Tratuzumab. Because of the unsatisfactory therapeutic results with brain metastases it is necessary to understand the expression of these growth factor receptors both in the primary tumor and in the brain metastases derived from them.

Material and methods: The expression of EGFR and HER2 in both the primary tumor and the derived brain metastases was investigated by means of immunohistochemistry. Further, the HER2 gene amplification was determined by fluorescence in situ hybridization (FISH).

Results: Immunohistologically 11 (37.9%) of the 29 primary tumors showed a clear positive (3+), 2 (6.9%) a medium-grade (2+), and 3 (10.3%) a weak (1+) HER2 expression. 13 patients (44.8%) showed no HER2 expression. Amplification of the HER2 gene was observed in all 2+ and 3+ cases. There was no amplification in the 1+ cases. Only one of the negative cases had a moderate amplification (5–10 gene copies). A total of 13 patients (45%) were evaluated as positive concerning a Trastuzumab-therapy. 28 (96.6%) of the 29 patients in this study showed the same HER2 expression both in the primary tumor and in the brain metastases. In only one patient the primary tumor revealed an overexpression of HER2, while the brain metastasis was HER2-negative. Only 2 of the 29 patients (7%) showed a clear EGFR expression in the primary tumor. In 28 of the 29 patients (96.6%) the expression pattern of EGFR in the primary tumor corresponded to that of the brain metastases. In only one patient who was EGFR-negative in the primary tumor, did the brain metastasis show a clear EGFR expression.

Conclusion: In the course of the development of a cerebral metastasis there is no essential change in the expression patterns for HER2 and EGFR between the primary tumor and the brain metastases. The expression patterns in the primary tumors are representative of the corresponding brain metastases. The failure of HER2 target therapy in cases of brain metastasis derived from HER2-positive tumors can not be attributed to a putative loss of the receptor.