for p53(−) vs 78.7% for p53(+); P = 0.002, intermediate-risk group; 96.5% for p53(−) vs 90.7% for p53(+); P = 0.003).

Conclusions: This study demonstrates that p53 accumulation based on IHC has prognostic impact in LNN-BC, and it gives the additional prognostic information for intrinsic phenotypes and the St Gallen consensus.

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Nuclear p53 protein and cell cytosol LDH expression as prognostic indicators to monitor FEC treatment in triple negative breast cancer

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Background: The p53 tumor suppressor is involved in the control of cell growth and programmed cell death. p53 mutations are most commonly seen in human cancer, with some estimate 25% of primary breast carcinomas. Approximately 10−17% of triple negative (lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) invasive breast cancer is significantly associated with mutant p53 overexpression. This type of cancer displays more aggressive clinical behaviour, distinctive metastatic patterns and poorer prognosis when compared with other breast cancer subtypes. 5-Fluorouracil, Epirubicin and Cyclophosphamide (FEC) are usually combined to treat as the neo-adjuvant or adjuvant chemotherapy for breast cancer. Lactate dehydrogenase (LDH) is a stable cytoplasmic enzyme that is present in all cells. It is rapidly released into the cell-culture supernatant upon damage of the plasma membrane. In this study breast cancer cells were treated with single and combination use of FEC followed by the measurement of the level of nuclear p53 protein, cytosol LDH and DNA synthesis in 5-bromo-2'-deoxyuridine (BRDU).

Material and Methods: High levels of mutant p53 breast cancer cell line MDA-MB-231 was selected. The cells were cultured in flasks and 96 well plates with L-15 medium in assigned control, 0.6ug/mL 5-Fluorouracil, 0.5ug/mL Epirubicin, 0.6ug/mL Cyclophosphamide and FEC combination groups, respectively for 24 hours. At the harvest day, the cultured medium and cell homogenates of different groups were quantified by a photometric enzyme immunoassay, ELISA kit. The LDH released from damaged cells and DNA synthesis labelled with BRDU were measured by the nonradioactive colorimetric immunoassay.

Results: All four test groups demonstrated a statistically significant difference from control group and the most significant result was from the FEC group.

Group	p53 (pg/ml)	LDH (OD)	BRDU (OD)
Control	1.89±:0.04	1.35±:0.10	1.27±:0.15
5-Fluorouracil	*0.71±:0.001	*1.93±:0.07	*0.94±:0.03
Epirubicin	*0.65±:0.006	*1.87±:0.02	*0.86±:0.02
Cyclophosphamide	*0.8±:0.001	*2.12±:0.02	*1.14±:0.02
FEC	*0.32±:0.001	*2.44±:008	*0.57±:0.03

Values are mean±:SD (standard deviation); OD: Optical Density. *p < 0.05.

Conclusion: FEC regimen suppresses cell proliferation, nuclear p53 mutations and LDH. These three indicators may predict how triple-negative breast cancer patients would respond to various chemodrugs or regimens.

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New recurrence prediction model for breast cancer by data mining

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Background: Many studies were published to predict recurrence of breast cancer. The most frequently used methods to predict recurrence are the statistical technique of regression. However, Cox regression does not allow non-linear relations between the independent and dependent variables and low accuracy. A new technique based on machine learning has recently been proposed as a supplement or alternative to Cox regression. Our study aims to develop more accurate prediction model for recurrence of breast cancer.

Material and Methods: Data from 1480 patient with breast cancer from the department of surgery of Ajou university hospital were collected and recorded during the period 1994–2007 years. This study used 631 patients to be excluded in case of other cancer, man, and metastasis to other organ, stage IV, and follow up period under 5 years. Eight among 64 variables were selected with Pearson chi-square test. To obtain a reliable estimate of model accuracy applied the holdout method that divided into 438 patients for training and 193 patients for testing. Since Cox regression is the most popular algorithm to build a predictive model for time-to-event data, this study compared accuracy of two algorithms; Cox regression and Support Vector Machine (SVM).

Results: The results of the univariate analysis used to determine the correlation between clinicopathologic variables and recurrence of breast cancer and showed a significant association between recurrence of breast cancer and variables such as histological grade ($p < 0.001$), local invasion of tumor ($p < 0.001$), HER2 ($p < 0.05$), number of tumor ($p < 0.001$), tumor size ($p < 0.001$), lymphovascular invasion ($p < 0.001$), estrogen receptor ($p < 0.05$) and number of metastatic lymph node ($p < 0.001$). For both model (Cox regression and SVM), a recurrence probability for each patient in the test set was calculated. The predictive accuracy of two models was computed using the area under Receiver Operation Curve (ROC) curve (AUC). SVM: AUC=0.842) was higher AUC than Cox regression (AUC=0.648). As compared by Adjuvant! Online software program, The AUC and accuracy of purposed model (0.842, 80.3%) was slightly higher than the adjuvant! online (0.7, 70.5%).

Conclusions: This study predicted recurrence of breast cancer which is as important as early detection of breast cancer. A parallelism of adequate treatment and follow-up by recurrence prediction prevent the recurrence of breast cancer. This study compared accuracy of models; Cox regression and SVM. SVM showed higher AUC than Cox regression. Our new model can predict more accurately recurrence of breast cancer than previous models.

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Ki-67 as a long-term prognostic factor in lobular breast cancer

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Background: Ki-67 has recently been introduced in the St Gallen consensus as an important prognostic factor in breast cancer. The aim of the present study was to specifically investigate the prognostic bearing of Ki-67 in a subgroup of lobular breast cancer.

Material and Methods: This population-based non-screening material consists of 212 patients (pts) with lobular breast cancer diagnosed between 1980 and 1991. The median follow-up time was 12 years and for those still living, 20 years (range 0.6−30 years). Clinical stage was recorded. The expression of Ki-67 in the tumour was assayed by immunohistochemistry (Mib-1 antibody).

Results: With this long follow-up time, 72 pts (34%) have died of breast cancer, 73 pts (34%) have died of other causes and 67 pts (32%) are alive. Among those alive there were five with local recurrences, one with regional recurrence and one with distant recurrence. At diagnosis the clinical stage was stage I in 90 pts (42%), stage II in 70 pts (33%), stage III in 41 pts (19%), stage IV in 7 pts (3.3%) and stage was undefined in 4 pts (1.9%). Ki-67 expression was categorized into four groups: 0 (0%), 1 (1−10%), 2 (11−30%) and 3 (>30%). A logrank test for trend shows that the null hypothesis of equal overall survival for these four groups could be rejected in favour of the trend alternative (p = 0.037). High Ki-67 expression gives negative impact on survival.

Conclusions: Ki-67 expression seems to give long time prognostic information in lobular breast cancer and might thus be a useful tool in the adjuvant decision making.

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Body mass index is associated with breast cancer of large size and positive lymph nodes in pre-menopausal but not post-menopausal women

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Background: Obesity is known to be a risk factor for breast cancer. While many studies suggested that overweight would be associated with higher risk of breast cancer with higher grade tumours, greater tumour burdens and poorer prognosis, the association between body mass index (BMI) and breast cancer outcome is controversial. There is recent suggestion that the prognostic outlook of Chinese breast cancers might be somewhat different from those in the Western population. Western studies have shown that body mass index may affect important biological mechanisms related with breast cancer prognosis but there is limited data with regard to the impact of BMI upon breast cancer features in the Hong Kong Chinese population.

Methods: We conducted a preliminary retrospective study on 166 Hong Kong Chinese primary breast cancer patients seen as new cases during

January 2006 and December 2006 in a teaching hospital. Histopathological features of the primary tumour including tumour size, tumour grade, nodal status, estrogen receptor (ER), progesterone receptor (PgR), and HER-2 status and patients features including menopausal status, age at diagnosis, body weight and body height were recorded. Multivariate analysis was used to identify independent factors affecting primary tumour features.

Results: The mean age at diagnosis was 49.6 with mean BMI being 23.78. Mean size of the primary tumour was 2.42 cm with 2.86 positive axillary lymph nodes. There were altogether 70 post-menopausal breast cancer women (42.2%), 89 pre-menopausal women (53.6%) and 7 peri-menopausal women (4.2%). Within the pre- and peri-menopausal population, higher BMI was associated with larger tumour size ($p < 0.01$) and higher number of positive lymph nodes ($p = 0.03$) but it had no effect on the tumour size or nodal status within the post-menopausal population. The body weight (BW) also demonstrated the same observation within the 2 subgroups of patients. On the other hand, body height was associated with younger age at diagnosis for both pre-menopausal ($p = 0.02$) and post-menopausal women ($p = 0.03$) but no effect on the tumour size or nodal status. Further analysis using ANOVA showed no statistically significant difference of the BMI for different breast cancer subtypes such as HER-2 positive breast cancer, triple-negative breast cancer and the hormone positive breast cancer.

Conclusions: Body mass index is associated with breast cancer with large size and positive lymph nodes in pre-menopausal but not post-menopausal women. Different elements of body weight and body height may have different roles affecting the primary tumour features. Further large-scale study is warranted to confirm the above findings.

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Correlation between cyclin D-1, estrogen and progesterone receptors in breast carcinoma after a short period treatment with tamoxifen and anastrozole in a prospective placebo double blind study

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Background: Hormone therapy (HT) plays an important role in breast cancer mortality reduction. Predictive biomarkers of early cellular response have being studied with the intention to predict the hormone resistance. The positivity of cyclin D-1, estrogen and progesterone receptors (ER/PR) is being related to the resistance to the tamoxifen treatment. We proposed this trial to evaluate the variation of the cyclin D-1, ER and PR in postmenopausal patients with ER positive invasive ductal carcinomas (IDC) prior and after short period (26 days) of treatment with tamoxifen, anastrozole and placebo.

Material and Methods: Fifty-eight patients with palpable ER-positive invasive ductal carcinoma (stage II and III) were double-blind randomized in a prospective placebo controlled trial with 3 neoadjuvant HT groups in the pre operatory phase (26 days): P (placebo, N = 25) T (tamoxifen 20 mg/day, N = 15) and (anastrozole, 1 mg/day N = 18). Pre and post HT samples were disposed in tissue micro array blocks and submitted to immunohistochemical assay. Biomarkers status (cyclin D-1, ER and PR) was obtained comparing each immunohistochemical evaluation of pre and post-surgery samples using semi-quantitative Allred's method. Statistical analyses were performed using the parametric test of Anova. ($p \leqslant 0.05$).

Results: There was a reduction in Allred's PR score from 4.22 (pre-treatment) to 1.94 (post-treatment) only in patients treated with anastrozole ($p = 0.01$). There was a linear positive correlation between cyclin D-1 and PR in the group A ($p = 0.0001$), negative in the T ($p = 0.0001$) without varying in the placebo group ($p = 0.35$)*.

Conclusion: It is possible that PR and cyclin D-1 could be a good predictor of early response for aromatase inhibitors in breast cancer.

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The validity of combination analysis of subtype classification and genomic DNA amplification of Decoy Receptor 3 (DcR3) for estimating prognosis in breast cancer

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Background: Decoy receptor 3 (DcR3) shows inhibitory effect to Fas-mediated apoptosis (Nature 1998; 396:699–703). We have reported the positive relationship between DcR3 mRNA expression and the gene amplification (DcR3-amp) in breast cancer tissues (the 23rd SABCS; abstract #380), and the positive relationship between DcR3-amp in breast cancer tissue and both lymphatic invasion and lymphnode metastasis (The 12 th ECCO; abstract #406), and also the relationship of DcR3-amp and poor outcome especially in ER negative patients (The 28th SABCS; abstract #6021). On the other hand, recently, breast cancer has been classified into subtypes such as Luminal A or B, Her2, Basal-like, and Unclassified, by the status of ER/PgR and Her2 expression. These subtypes have been reported to correlate with prognosis. In the present study, we examined the relationship between DcR3-amp and the subtypes to clarify their validity for estimating breast cancer prognosis.

Materials & Methods: Ninety-three patients who underwent operation for primary breast cancer at Niigata University Hospital during 1996–2000 were selected for the present study. Patients without axillay dissection or with distant metastasis at operation were excluded. Genomic DNA of 93 breast cancer tissues extracted from paraffin embedded sections of surgical specimens by microdissection under light microscope. Real-time quantitative PCR was performed to measure DcR3-amp by standardizing with b-globin gene.

The ER/PgR and Her2 status of each specimen were examined immunohistochemically, and the patients were categorized into 3 subtypes; Luminal type of ER and/or PgR positive group, Her2 type of ER/PgR negative and Her2 positive, and Basal-like type of ER/PgR/Her2 negative group. The effects of DcR3-amp, subtype, and the combination of both on disease free survival (DFS) and overall survival (OS) were analyzed.

Statistical analysis was performed by Breslow-Graham-Wilcoxon test, and the statistical significance was defined as $P < 0.05$.

Results: Both DFI and OS showed significantly difference among the 3 subtypes; best in Luminal, and worst in Basal-like group. DcR3-amp positive group showed significantly lower DFI, and lower OS (not significant), compared with DcR3-amp negative. The combination analysis showed that DcR3-amp status did not affect on DFI and OS in Luminal group. However, in Her2 group and Basal group, DcR3-amp positive showed lower DFS and OS.

Conclusion: Our results suggest that the DcR3-amp status seems to be valid for estimating prognosis in Her2 type and Basal-like type breast cancer.

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'Triple negative' receptor status as a risk factor for recurrence and death in cancer of the breast

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Introduction: Invasive breast cancers that do not show significant staining for oestrogen receptor (ER), progesterone receptor (PR) or c-erb-B2/HER2 ('triple negative') are perceived to have a poor prognosis with an increased risk of relapse. We aimed to determine whether 'triple negative' status was a significant risk factor for both recurrence and death.

Methods: We reviewed a consecutive series of 464 patients who underwent surgery for primary invasive breast cancer between January 2002 and July 2004. Follow up data was available for 455 patients. Minimum follow up was 5 years. Immunohistochemical staining was used to determine ER, PR and c-erbB2/HER2 status. HER2 negative status was defined as scoring 0 or 1+ for staining. Eighty patients subsequently recurred and 44 died of their disease. Multivariate analysis was performed using logistic regression, with recurrence and then cancer specific survival as the dependent variables and 'triple negative' status, young age at diagnosis (<50), tumour size (>3 cm), high tumour-grade and lymph node (LN) status (>3 LN involved) as the explanatory variables.

Results: On multivariate analysis, recurrence was predicted independently by young age at diagnosis (Odds Ratio (OR) 1.79, 95% Confidence Interval (CI) 1.02–3.15; $p = 0.04$) and lymph node (>3 LN involved) status (OR 4.2, 95% CI 2.22–7.94; $p < 0.0001$) but not by tumour size, grade or 'triple negative' status. The median interval before relapse, when it occurred, was 16 months (Interquartile range (IQR) 15−50) for 'triple negative' tumours, compared with 34 months (IQR 22−48) for all other receptor combinations ($p = 0.06$). The median time to cancer specific death following recurrence was 17 months (IQR 13−25) for 'triple negative' cancers compared with 37 months (IQR 22−56) for the other receptor groups ($p = 0.0247$). Lymph node (>3LN involved) status (OR 2.9, 95% CI 1.28–6.13; $p = 0.0051$) and tumour size >3 cm (OR 5.29, 95% CI 2.56–10.49; $p \leqslant 0.0001$) were independent predictors of cancer specific death.

Conclusion: Patients with 'triple negative' breast cancer were no more likely to relapse, or die of cancer, than patients with other receptor profiles, but when relapse occurred it tended to be earlier and subsequent death sooner, than other receptor groups.