pPR = persistence of less than 10 microscopic foci of invasive tumor cells in the breast or in the axilla; pNR = any larger amount of residual cancer cells in the breast or in the axilla.

Results: Gene expression was plotted toward pathological response. Low tubulin levels in isoforms III, IVa, IVb were associated with pathological response.

Conclusion: The patterns of beta-tubulin isoforms distribution may predict for pathological response to paclitaxel and paclitaxel/radiation regimens.

544 POSTER

Proliferation, apoptosis and related markers in invasive ductal breast carcinoma

P. Buytaert[1], W. Tjalma[1], J. Bogers[2], M. Dewulf[1], J. Weyler[3], G. Voet[1], P. Hellemans[1], P. van Dam[1], E. Van Marck[2]. *Departments of [1]Gynecology; [3]Epidemiology; [2]Department of Pathology, University Hospital Antwerp, Belgium*

Purpose & Methods: Expression of bcl-2, bax, p53 and PCNA genes was studied immunohistochemically in 170 invasive ductal breast carcinomas (median follow-up time 91 months, range 24–186 months). In addition the mitotic activity index (MAI) and apoptotic cell death (Tunnel technique) were scored. Classic histopathological features and steroid receptor status of the tumours, and clinical patient characteristics were incorporated in the database.

Results: No relationship could be observed between bcl-2, bax or p53 status and tumour grade, pTNM staging and menopausal status. A strong positive relationship was demonstrated between bcl-2 immunoreactivity and steroid receptor status (ER and PR: $p < 0.001$). There was an inverse relationship between bcl-2 expression and p53, but not with bax or PCNA. Multivariance analysis demonstrated absence of bcl-2 expression and the MAI to be independently related to shortened disease-free survival ($p < 0.01$) and shortened overall survival ($p < 0.001$).

Conclusions: Our data suggest that bcl-2 expression plays a crucial role in the behaviour of invasive ductal carcinoma and may be an important modulator of response to adjuvant therapy.

545 POSTER

Incidence of breast cancer associated with use of hormone replacement therapy and other risk factors in 1709 patients

S. Gehani, Z. Khan, S. Parbhoo. *Breast Unit & Cancerkin, University Department of Surgery, Royal Free Hospital & School of Medicine, London NW3 2QG, UK*

Background: Various studies have suggested an association between hormone replacement therapy (HRT) and breast cancer but the reports have been conflicting and controversial. A meta-analysis of 52 epidemiological studies in 21 countries by the Imperial Cancer Research Fund (ICRF) in 1997 concluded that the relative risk for breast cancer was 1.023 for each year of use.

Aims:

(1) To assess the incidence of HRT related breast cancer in our patient population.
(2) To study other risk factors in the women who developed breast cancer while on HRT.

Patients & Methods: A retrospective study of 1709 patients seen between January 1987 and December 1997 was made. The mode of presentation, duration of HRT, age at diagnosis, other "risk" factors (alcohol, smoking, family history, past history of breast biopsy) and pathological features of the cancer were analysed.

Results: Sixty-five per cent of the 62 patients (mean age 60 years) with HRT associated breast cancer presented with symptomatic disease while 19% were detected within the UK Breast Screening Programme. The duration of HRT intake was <2 years in 9, 2–5 years in 24, 6–10 years in 17, 10–15 years in 5 and >15 years in 5 patients. In 38.7% a family history of breast cancer (11 first degree and 13 second degree relatives) was noted. Smoking and alcohol intake was average in 87% of patients. Twenty patients had a history of a previous benign breast biopsy. Histological review showed that 55.8% had a T1 carcinoma and 57% were N0. Invasive ductal carcinoma was found in 82.3% with 19.5% being Grade I (Bloom & Richardson) and 61% Grade II.

Conclusion: The highest risk in relation to HRT appears to be in the first five years suggesting that HRT may stimulate the growth of an undetected cancer. The high association with a strong family history and previous benign biopsies has implications for women seeking advice about starting HRT.

546 POSTER

Breast cancer and missense mutations in the transactivation region of the BRCA1 gene

J.M. Garcia, G. Dominguez, J.M. Silva, R. Gonzalez, M.A. Navarro[1], C. Corbacho[1], G. Martinez[1], M.J. Villanueva, F. Navarro, F. Bonilla. *Departments of Medical Oncology; [1]Pathology, Clinica Puerta de Hierro, Madrid, Spain*

Purpose: The implication of missense mutations in germline of BRCA1 gene in the pathogenesis of breast cancer is not well understood. When a missense mutation occurs in a specialized region of the gene, the functional impact of this change can be meaningful.

Methods: We investigated missense mutations located in the transcriptional transactivation gene region (amino acids 1528–1863), and correlate them with clinical and familial characteristics of the patients. We studied 192 patients, 87 with a definite family history of the disease, and 105 without antecedents and considered to have sporadic breast cancer. The entire coding region of the BRCA1 gene was analyzed by the PCR-SSCP method. Specimens showing a differential band were amplified and used for direct DNA sequencing.

Results: Two mutations were detected, Glu1735Lys and Asp1778His. The first mutation was identified in a family with five breast cancer patients in first-degree, distributed between two generations. One patient showed bilateral tumor. The second missense mutation appeared in a 44-year-old patient with a sporadic invasive ductal carcinoma with axillary involvement and negative steroid receptors.

Conclusions: The two new mutations detected may represent a functional change in the transactivation potential of the BRCA1 gene.

547 POSTER

The expression of new protein taking part in cancerogenesis, p65, and its correlation with steroid receptors in ductal carcinoma of female breast

H. Niewiadomska, A. Jeziorski. *Medical University of Lodz, Dept. of Oncology, ul. Paderewskiego 4, 93509 Lodz, Poland*

A 65-KD phosphoprotein (p65) was isolated from human breast carcinoma cell line MCF7. The aminoacid sequence analysis of N-terminal part of the p65 molecule was similar to the steroid receptor protein. This raises the possiblility that the p65 gene may belong to the family of genes which encode nuclear receptors for various hydrophobic ligands of steroid hormones. Paraffin-embeded tissue slides from 89 infiltrating ductal carcinoma specimens were assessed immunohistochemically with the usage of monoclonal antibodies against human p65 antigen. The p65 expression was correlated with oestrogen receptor (OR) and progesterone receptor (PR) levels and grade of malignancy according to Bloom and Richardson scale. It is suggested that the high OR and PR levels are accompanied by the presence of p65 in breast cancer tissue. The funcion of p65 and its ligands is still unknown. However, p65 may be important in the process of development of tumours. It is probable that the conserved cysteine-rich domains found in the human p65 and which are also common to human OR provide an important biological funcion.

548 POSTER

A prospective study on genetic risk factors in an unselected sample of breast cancer patients who receive adjuvant radiotherapy

C.C. Wárlám-Rodenhuis[1], C.H.F. Gimbrère[2], R.P. van Helvoirt[1], K.E. van der Wiele[3], H.F.A. Vasen[4], R.B. van der Luyt[3], M.G.E.M. Ausems[3]. *[1]Dept. of Radiotherapy University Hospital Utrecht (UHU), PO-box 85500, 3508 GA; [2]Comprehensive Cancer Center; [3]Clinical Genetics Center Utrecht; [4]Dept. of Internal Medicin UHU, Utrecht, The Netherlands*

5–8% of all breast cancers (BCs) are due to hereditary factors. Detection of women at high risk for BC and offering genetic counselling (GC) to them might be worthwhile. In high risk women regular mammography (started at younger age) and preventive surgery (in some cases) may result in substantial gains in health and in life expectancy. Women at high risk for BC can be identified by systemetically searching for presumed risk factors. The Radiotherapy Department of the University Hospital (UH) in Utrecht together with the Clinical Genetics Center and the Comprehensive Cancer Center prepared a study to prospectively evaluate the prevalence of risk factors for hereditary BC in 1.000 patients. All BC patients referred for radiotherapy as part of curative treatment for their disease are included (60% of all newly

diagnosed BCs in the region). At first visit a checklist with 8 presumed risk factors is used. To patients who have one or more risk factors the composition of an extensive family tree is proposed. Information concerning the family history of BC (age at diagnosis, bilaterality) and the occurrence of other cancers such as ovarian cancer is taken into account. Guided by specific criteria, referral to the Family Cancer Clinic at the UH is suggested. GC and DNA testing for BRCA1 and BRCA2 mutations is offered to selected patients. In this study insight will be gained in the interest of unselected BC patients and their families for GC and DNA testing. In addition the correlation between several risk factors, the probability of hereditary BC and the detectability of DNA mutations is studied. During the first 8 months 304 patients were registered. 132 patients had 1 or more risk factors: 85 of them agreed with the construction of a family tree, 51 fulfilled the criteria for referral to the Family Cancer Clinic and 35 accepted GC. Preliminary results will be presented.

549 POSTER

CIP-1 Protein expression in node-positive breast cancer patients

P. Hupperets[1], E. Thunnissen[2], J. Peterse[3], H. Schouten[1]. *Departments of [1]Internal Medicine; [2]Pathology, University Hospital Maastricht; [3]Department of Pathology, Netherlands Cancer Institute, Amsterdam, The Netherlands*

CIP-1 is a cyclin dependent kinase inhibitor which negatively controls cell proliferation. Since chemotherapy may affect cell cycle regulation, in this study the hypothesis was tested that increased levels of CIP-1 may be associated with poor response to chemotherapy and with dismal clinical outcome. CIP-1 protein was assessed by immunohistochemistry (IHC) in 26 node-positive breast cancer patients (pts) (≥10 tumor containing axillary nodes or tumor containing infraclavicular node). All 26 pts had been treated with 4 cycles of conventional chemotherapy followed by high-dose chemotherapy supported by bone marrow stem cells. In 1 pt no tumor was left in the paraffin section for IHC. Nuclear staining for CIP-1 was observed in tumor cells in 18/25 of tumors (with usually moderate (+) and sometimes equal intensity (++) compared to internal controls). Nine of the pts with this staining had no evidence of disease (NED) after a median follow-up of 3 yrs, whereas 8 had recurrent disease. Five pts without this staining pattern (intensity 0 or ±) had NED, whereas 2 pts died, one with, and one without disease. Nuclear staining for CIP-1 in an estimated area of >50% of tumor area was observed in 18/25 of tumors. No differences in clinical outcome could be detected: 10 pts with nuclear staining of >50% of tumor area had NED, whereas 8 pts had recurrent disease. Those pts with minimal or absent nuclear staining (≤10% of tumor area) (3 pts) had NED. CIP-1 expression is found in a high percentage of nuclei in breast cancer tumor cells of pts with bad prognosis breast cancer. CIP-1 expression is not associated with clinical outcome in these heavily treated pts, whereas the absence of CIP-1 expression seems to be associated with good prognosis.

550 POSTER

A study of correlation between DNA ploidy pattern and aberration of chromosome 8 detected by fluorescence in situ hybridization in human breast cancer

S. Hara, R.S. Terada, H. Ayabe, Y. Tagawa. *Nagasaki University School of Medicine, First Department of Surgery, 1-7-1 Sakamoto, Nagasaki-city, J-8528501 Nagasaki, Japan*

Purpose: To compare DNA ploidy pattern (DP) by flow cytometry (FCM) with the aberration of chromosome 8 (Chr.8) by fluorescence in situ hybridization (FISH) in human breast cancer and to study the correlation between them with axillary lymphnodes metastasis.

Methods: Fifty cases of breast cancer which had no chemotherapy and radiation therapy before radical operation were studied. Tissues obtained by operation were divided two sections. The one were sliced and DP were analyzed by propidium iodide staning using FCM (FACScan). The stump sections were made from the others and fixed with acetone, and aberration of Chr.8 were analyzed on 200 cancer cells by FISH using D8Z1/biotin (Oncor) probe which detects centromere of chr.8.

Results: Thirty-three (66%) of 50 had DNA aneuploidy, 17 (34%) had DNA diploidy. The aberration rates of Chr.8 widely ranged from 19 to 75%. There was no significant correlation between DP and aberration rates of chr.8. There was significant correlation only between axillary lymphnodes status and aberration rates of chr.8 ($p = 0.023$). There was no correlation between DP and axillary lymphnodes status.

Conclusion: These results show that breast cancer with high aberration rates of chr.8 tend to involve axillary lymphnodes.

551 POSTER

The antioxidant status of breast cancer patients

D. Korman. *Laboratory of Oncology, Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russia*

The different changes of AOS have been established at various pathological processes including cancerogenesis and tumor growth. The extent of unsaturation of the tissues and blood lipids, which can be defined by the number of double bounds (C = C) in lipids (NDB), may be considered as an integral parameter for the AOS of a tissue and whole body characterization.

We determined NDB in lipids extracted from blood plasma and erytrocytes before treatment in 113 breast cancer patients (BCP) and 94 healthy women. NDB was measured using the special device -"double bounds analysator – DBA". It was discovered that NDB was significantly higher in the lipids of BCP than in that of controls and correlated with the extent of tumor. The patients with advanced (stage IV) BC had mean value of NDB $3.5 \div 0.3 \times 10^{18}$/mg of lipids, with operable BC – $1.8 \div 0.3 \times 10^{18}$, with benign breast tumors – $0.2 \div 0.05 \times 10^{18}$, in control – $0.4 \div 0.03 \times 10^{18}$. The higher DB level was corresponded to various unfavorable prognostic clinico-morfological factors.

The changes of NDB in blood lipids are considered as a reflection of the tumor-host interrelations. The assay of this parameter in cancer patients may be useful for define tumor extent, for monitoring of patients status and results of treatment, for determination of indications for treatment with antioxidants.

552 POSTER

Presurgery chemotherapy and DNA ploidy of the adenocarcinoma breast cells

T. Nikolaeva, Ya. Dobrynin. *N. Blokhin Cancer Research Center RAMS, Moscow 115478, Russia*

Purpose: To evaluate effectiveness of adenocarcinoma breast (AB) preoperation chemotherapy (PC).

Methods: We studied by FCM the changes of DNA synthesizing cells fraction (SF) (19 cases) and ploidy (29 cases). The biopsies was taken before and after course of PC during surgical operation.

Results: Discovered reduce SF cells after clinically successful treatment in 13 pts with diploid AB. The mean life time this pts was 4 years, 4 pts survived 6 years. In 6 nonresponders to treatment showed the increase the SF cells in all cases. In this group the recurrence onset was detected at 8–18 mo. and the mean life time was less than 2 years. FCM analysis before PC treatment demonstrated in 17 cases DNA content like diploid (D) and in 12 ones aneuploid (A). The analysis after PC showed the diploidy in 12 cases (1 group) and aneuploidy in 8 ones (IY group). 5 D tumors became A ones (II group), 4 A tumors became D ones (III group). 2 pts (16.7%) of the I group have recurrences through 25 and 11 mo. and died through 36 and 20 mo., 10 pts lived 57–80 mo. 3 (65%) pts of the II group have recurrences after 10–12 mo. and died shortly (12–15 mo.), 2 (33%) pts with D tumors becoming tetraploid after PC. They have not recurrences and lived 52–86 mo. In 2 cases (III group) the modification of A population (hyperdiploid) into D led to rapid progression of the disease (10–12 mo.) and pts died through 15–50 mo. The modification A population in any site (IY group, 7 cases) accompanied the disease progression and reduced survival length (6 pts died through 24–49 mo.).

Conclusion: The conservation D or tetraploidy after PC is prognostic factor of the PC efficacy in AB pts in III stage of the decease.

553 POSTER

p-Glycoprotein (pGP) and p53 expression in primary breast cancer

P.G. Betta[1], G. Bottero[1], M.F. Cosimi[1], G. Spinoglio[1], M. Pavesi[2], M. Pastormerlo[2]. *[1]Department of Oncology, Azienda Ospedaliera, Alessandria; [2]Unit of Pathology, Santo Spirito Hospital, Casale Monferrato, Italy*

Purpose: To assess the putative relationship between the expression of multidrug resistance-associated protein (pGP) and p53 protein accumulation in primary breast cancer surgical specimens (n = 40).