322

## ASPECTS OF ALTERED IMMUNEFUNCTIONS IN BREAST CANCER PATIENTS:

Krainer M., Wiltschke C., Stragans M., Budinsky A., Michl I., and Zielinski CC. 1st. Dep. of Internal Med. and 1st. Dep. of Gynaecol. and Obst., University of Vienna, Austria.

Phytohemagglutinin (PHA)- stimulation of peripheral blood mononuclear cells (PMNC) was studied in patients at various stages of breast cancer and after various adjuvant therapies. As reported previously, tumor-bearing patients had a significantly decreased mitogenic stimulation as compared to healthy age matched control individuals. In this study we are able to show that this immunedefect is not only detectable in untreated breast cancer patients but is persistent also in patients after therapy. No significant difference of mitogenstimulation between patients after mitogenstimulation could be detected also in 5 patients with complete remissions up to 6 years after diagnosis and initial treatment. This reduced mitogenstimulation could not be overcome by addition of IL-2 to the cultures, which implicates that this immunedefect in breast cancer patients is not due to a reduced IL-2 availibility.

323

## ASSOCIATION OF LYTIC EFFECTOR CELL FUNCTION WITH HIGH EXPRESSION OF HER-2 EXPRESSION IN BREAST CANCER PATIENTS.

Wiltschke C., Krainer M., Budinski A., Tyl E., Speiser P., Zeillinger R., Kubista E. and Zielinski CC. 1st Dep. of Internal Med. and 1st. Dep. of Gynaecol. and Obstetrics, University of Vienna, Austria.

Increased expression of the HER-2/neu oncogen in breast cancer correlates with decreased estrogen receptor concentration and seems to be an important prognostic factor. In the present study, we have investigated whether there is a connection between HER-2 expression and immunologic parameters directed against tumor defense.

In patients with breast cancer, NK-activity was significantly higher, as compared to patients with benign tumors ($p<0.002$) or healthy control individuals ($p<0.01$). Moreover, 25% of patients with breast cancer showed an overexpression of HER-2 protein. Within this group of patients, NK cell activity was significantly lower (45.6 ± 16.1%), as compared to the group with no HER-2 overexpression ( 57.3 ± 11.0%, $p< 00.6$). NK activity was never increased in patients with HER-2 overexpression. Thus, there was a statisitcally significant correlation of cytolytic effector cell function with HER-2 expression of the tumor ($p<0.006$), and HER-2 overexpression correlated with a a negative hormone receptor status ($p<0.004$). In conclusion, these data add further evidence to previous observations from this laboratory that certain tumor characteristics may be associated with reactions of the host in breast cancer.

324

## THE PROGNOSTIC ROLE OF DNA PLOIDY IN PRIMARY TUMORS OF METASTATIC BREAST CANCER TREATED WITH COMBINATION CHEMOTHERAPY (CEF)

P. Hietanen, C. Blomqvist, V-M. Wasenius, E. Niskanen, K. Franssila.
Division of Radiobiology and Radiopathology, Deparment of Radiotherapy and Oncology. University Central Hospital of Helsinki, Finland

In a previous study (Proc. ASCO vol 11, 74 1992) it was shown that in patients with metastatic breast cancer the outcome of cytotoxic treatment was dependent on scheduling of drug administration. Two randomized groups of patients received the same monthly dose of CEF (cyclophosphamide, epirubicin, 5-Fu) either on a once a week or once a month bases. 158 patients were evaluable for response. Response and survival were significantly better in the group treated once a month.

DNA ploidy was assesable in 107 (84%) of the primary tumors studied using paraffin-embedded tissue derivates from 128 patients. No significant difference was observed when comparing DNA ploidy and response to treatment either in the patient material as a whole or in the two treatment groups separately. Time to progression was also similar among diploid and aneuploid tumors. In Cox's multivariate analysis of overall survival four factors emerged as significant: treatment group, Er receptor status, disease limited to soft tissue and ploidy. Surprisingly aneuploid tumors were assosiated with a better survival than diploid.

The results indicate that DNA-ploidy is not an useful factor to predict response to CEF-regimen. DNA aneuploidy as a predictor of longer survival after recurrence remains an obscure finding which needs further study.

325

## MEDULLARARY CARCINOMA OF THE BREAST, PROGNOSTIC IMPORTANCE OF CHARACTERISTIC FEATURES EVALUATED IN A MULTIVARIATE COX ANALYSIS.

L Pedersen, S Holck, T Schioedt, K Zedeler, H T Mouridsen.

Dept. of Oncology and Pathology, Herlev Hospital and Rigshospitalet, Copenhagen, Denmark.

In the first part of this study, dealing with 136 breast cancers with medullary features registered in the Danish Breast Cancer Group (DBCG) from 1982-87, we confirmed the prognostic importance of a new definition of MC, recently proposed by us, and deduced in a former study on a corresponding tumour material (DBCG 77-82). Looking at the three histological diagnostic criteria, however, only one of these (syncytial growth pattern) bore significant prognostic impact. Prognostic trends were, however, the same for all histological characteristics in the two tumour populations, and we therefore combined the two consequitive populations to perform a multivariate Cox regression analysis, including histopathological parameters and diagnostic subgrouping (MCnew def and Ridolfi). Four histological parameters retained positive prognostic importance in the final Cox model, beeing 1) predominantly syncytial growth pattern, 2) diffuse stromal infiltration with mononuclear cells, 3) no tubular component and 4) sparse necrosis. Beeing deduced from a multivariate analysis on a rather comprehensive tumour material we expect these histological parameters to be of valid prognostic importance in MC, and we propose that these four parameters are stressed in the histological diagnosis of medullary carcinoma of the breast.

326

## TPS- A PROGNOSTIC MARKER IN BREAST CANCER

**V.Barak, B.Nisman, B.Burde, I.Roisman, O.Drize T.Peretz**
Immunol. Lab for Tumor Diagnosis, Oncology Dep., Hadassah Medical Center, Jerusalem, Israel.

The TPS assay measures the M3 specific epitope of tissue polypeptide antigen in serum. It indicates the tumor proliferative rate, rather then the tumor burden and thus may prove to be a prognostic factor. In this retrospective study, we analyzed the usefulness of TPS evaluations in breast cancer patients (pts) in clinical follow up and response to treatment.

TPS, as well as other Tumor Marker (CEA, CA 15-3) were evaluated. The study population consisted of 146 pts. Pts were divided according to active (clinically or radiologically demonstrated) disease (57 pts) versus non active disease - NED (89 pts). In active disease sensitivity of TPS (cut-off 80 u/l), CA 15-3 (cut-off 30 u/ml) and CEA (cut-off 3 ng/ml) was 72%, 60% and 56% respectively. In this group a three marker combination gave sensitivity of 91%. TPS levels were elevated in 31 out of 89 patients with NED. There was a good correlation of TPS with response to treatment . We conclude that the TPS proliferation marker provides additive information in breast cancer pts and may serve for prognosis.

**Keywords:** TPS, prognostic marker, breast cancer

327

## FLOW CYTOMETRIC ANALYSIS OF FRESH HUMAN TUMOR TISSUE: A NEW SEPARATION SYSTEM FOR IDENTIFICATION OF SINGLE TUMOR CELLS AND APPLICATION TO ANALYSIS OF TUMOR-ASSSOCIATED PROTEASES.

Harbeck N., Yamamoto N., Schüren E., Schmitt M., *Höfler H., Graeff H. Frauenklinik und *Pathologisches Institut, Technische Universität, Munich, Germany.

Flow cytometric single cell analysis from fresh tumor specimens is complicated by the heterogeneity of the tumor tissue and its surrounding stroma. We developed a technique for separating native tumor tissue into single tumor cell suspensions without destroying or labelling surface antigens or receptors. There are no specific antibodies against tumor cells. Therefore they were isolated by "negative selection" through a magnetic separation system (Fa. Miltenyi, Bergisch-Gladbach, Germany): All "unwanted" cells like granulocytes, monocytes, T-/B-cells, macrophages or fibroblasts were labelled with specific monoclonal antibodies and then bound via anti-isotype magnetic microbeads to a steel-wool matrix in a magnetic field. Our technique enables fast and simple separation of single tumor cells from fresh tumor specimens. These still unlabelled cells can then be analyzed by flow cytometry or confocal laser scan microscopy for proteases like the urokinase-type plasminogen activator (u-PA), its inhibitor PAI-1, cathepsins or the u-PA receptor. Multiparameter analysis such as simultaneous S-phase and ploidy determination is also possible.