O-1. Trends in prognosis of patients with primary metastatic breast cancer between 1975 and 2002

van Gestel CMJ, Voogd AC, Roukema JA, van de Poll-Franse LV, Vreugdenhil G, Ernst MF. *Tilburg, Netherlands*

Approximately 5% of all patients diagnosed with breast cancer have metastases at time of diagnosis. This percentage has slightly decreased during the last years. In the same period there was a great change in treatment of metastatic breast cancer. We studied the prognosis of all patients with primary metastatic breast cancer who were treated in one hospital in Southeast Netherlands between 1975 and 2002. During this period 21,522 patients with breast cancer were diagnosed; in 5% (1081 patients) metastatic disease was present. Follow-up was complete until the 1st of July 2003.

An univariate analysis showed no improvement in prognosis between 1975 and 2002. The median survival time (the period in which 50% of patients deceases) from 1975 to 1984, 1985 to 1994 and 1995 to 2002 was 18, 17.2 and 19.5 months respectively. There were no significant differences in patients in all age-groups. However, patients <50 years of age showed a small improvement in prognosis after two year survival. In multivariate analysis the period of diagnosis turned out to be no independent prognostic factor. Older age, visceral metastases and number of metastases showed to be unfavourable prognostic factors.

In conclusion there seems to be no significant improvement in prognosis of patients with primary metastatic breast cancer in the period 1975–2002. The improvement in the younger population in the period 1995–2002 is probably due to the introduction of the taxanes.

O-2. Microarray profiling and real time PCR validation of genes associated with Tamoxifen resistance

Speirs V, Scott DJ, Parkes AT, Limer JL, Poola I. *University of Leeds & Harvard University USA*

The onset of tamoxifen (TAM) resistance in breast cancer is a serious clinical dilemma. Despite intense research, there is still no satisfactory explanation for the resistant phenotype. To help understand the underlying mechanisms, we established an *in vitro* model of TAM-resistance by continuous culture of the breast cancer cell line, MCF-7, in 100nM 4-hydroxytamoxifen (4-HT) for 12 months. 4-HT-treated cells, termed MCF-7^{MMU1}, became refractory to the inhibitory effects of 4-HT after approximately 3 months in culture. These cells also exhibited a greatly reduced response to 17β-estradiol but retained expression of estrogen receptor (ER)-α and -β protein at similar levels to wild-type (wt) MCF-7 cells. This phenotype was stable. We then determined gene expression profiles of MCF-7^{MMU1} and wtMCF-7 in order to identify genes associated with the resistant phenotype. Of approximately 22000 oligonucleotides analysed using Affymetix GeneChip microarrays, 131 genes were up-regulated and 156 were down-regulated in MCF-7^{MMU1} cells relative to controls by at least 3-fold. In general, genes associated with cell cycle, cell adhesion or extracellular matrix, were up-regulated while those associated with apoptosis or growth factors/hormones were down-regulated. Real time RT-PCR analysis of a subset of these genes positively validated the microarray data. Genes that were consistently up-regulated included *A1B1*, *CAV1*, *HMGCS2*, *SGP28*, *CEACAM6*, *EP4*, *COX-1*, *SOX9*, *TIMP3* and *CLIC3*, while *IGF1R*, *NPY1R*, *EP3* and *GREB1* were down-regulated. Many of these genes have not previously been implicated in breast carcinogenesis. Additionally, the prostaglandin and IGF-1 signalling pathways were differentially regulated at multiple levels. In summary, our data illustrates the complexity of 4-HT-resistance and the capacity of microarray technology to aid identification of novel genes not previously implicated in this phenomenon. These genes have significance as predictors to determine the likelihood of relapse in breast cancer patients receiving TAM.

O-3. Prediction of hormone response in breast cancer by microarray analysis of sequential tumour biopsies from patients receiving neoadjuvant therapy with Letrozole

Miller WR, Renshaw L, Larionov A, Anderson TJ, White S, Hampton G, Walker JR, Ho S, Krause A, Evans DB, Dixon JM. *Western General Hospital, Edinburgh, Novartis Research Foundation, San Diego, USA & Novartis Pharma Basel, Switzerland*

Background: Changes in tumour RNA expression may be monitored in sequential biopsies of individual tumours taken during neoadjuvant therapy and analysed by microarray analysis. In the present study, changes occurring within 10–14 days have been related to clinical response status assessed after 3 months of treatment.

Methods: 58 postmenopausal women with large operable ER-rich breast cancers were treated for 3 months with neoadjuvant letrozole. Clinical response was based on clinical and ultrasound changes. Cancers were sampled at diagnosis, 10–14 days and 3 months; RNA was extracted and hybridized on Affymetrix HG_U133A GeneChips.

Results: 52 cases were assessable for response; 37 (71%) responded (>50% reduction in tumour volume) and 15 were classified as minimal or no response. Changes in expression of 135 gene probes were informative in distinguishing between tumours subsequently displaying clinical response and those not. The gene onthology of the probe sets included protein synthesis/degradation (24%), transcription (23%), signal transduction (22%), cell proliferation/apoptosis (13%). Clustering of these gene changes produced profiles highly predictive of response/resistance to letrozole.

Conclusions: Changes in pattern of gene expression can be detected in biopsies taken before and after 14 days treatment with neoadjuvant letrozole. These may elucidate the mechanisms of tumour response and allow early recognition of response/resistance. Patterns of expression changes can be used to predict subsequent tumour response to treatment.