Eur J Cancer Clin Oncol, Vol. 24, No. 1, pp. 21–28, 1988.
Printed in Great Britain

0277–5379/88$3.00+0.00
Pergamon Journals Ltd.

Cellular Aspects of Breast Cancer: Workshop Report

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INTRODUCTION

THE AIM of this review is to summarize the posters and the workshop session on 'Cellular Aspects' of cancer of the breast. Since this was a working conference most of the posters were orientated towards the practical question of how laboratory investigations can help in the management of patients. Some presentations were concerned with how we can predict tumour behaviour by measurements performed either on human tumour tissue or on samples of peripheral blood and some were directed towards attempts to understand the mechanisms of disease behaviour such as metastatic potential or endocrine responsiveness. It is through this type of information that we may be able to derive new approaches to treatment.

There are considerable problems concerning the interpretation of the value of markers of tumour behaviour measured in tissue extracts or histological sections of tumours. The heterogeneous nature of most tumours may lead to considerable sampling errors. Furthermore, measurements of the end points of relapse free survival (RFS), overall survival (OS) and response to therapy are affected by several variables. For example, the time to relapse is influenced by local radiotherapy and whether the patient population was treated with adjuvant systemic therapy. The estimation of the value of a marker of response, for example to endocrine therapy, may depend on how the 'no change' category is viewed by particular investigators. Estimations of the relative importance of markers of metastatic potential and endocrine responsiveness should preferably be performed on large populations and employ multivariate methods. In addition in the clinical field we must apply other more pragmatic criteria; not only asking 'is it new?', but also 'is it clinically useful?' and if so 'is it of sufficient value to be incorporated into clinical practice?'

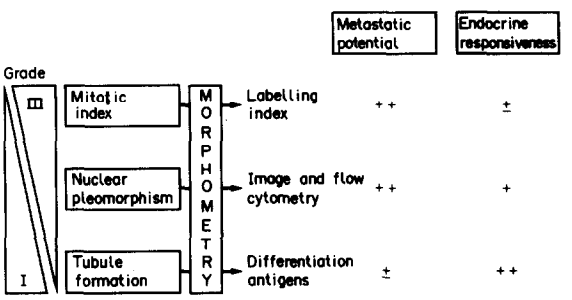

Fig. 1. Schematic representation of a hypothesis relating tumour-derived data to metastatic potential and endocrine responsiveness.

MARKERS OF METASTATIC POTENTIAL

The most important prognostic marker is axillary lymph node status. Patients with node involvement relapse sooner and die earlier than those without involved nodes. Interpretation is not confounded by other major indicators of prognosis. The proportion of patients responding to endocrine and chemotherapy is similar regardless of whether the axillary nodes are involved or not. This is unusual since most other prognostic markers, to a variable extent, also indicate responsiveness to systemic treatment. Thus the interpretation of their value is dependent upon whether therapy has been given either as an adjuvant or for advanced disease and whether or not there was a response. For example, tumour grade is a marker of prognosis because it indicates both metastatic potential and endocrine responsiveness. Grade I tumours have a good prognosis, not only because they are relatively unlikely to metastasize, but if they do they are more likely to respond to endocrine therapy. Figure 1 attempts to ascribe to the components of grade their relative importance as predictors of metastatic potential as

well as predictors of endocrine responsiveness.

Several papers were presented concerning new methods of measuring the three features most frequently used in grading systems; mitotic activity, nuclear pleomorphism and tubule formation. Morphometric techniques can be used in the measurement of all three components. To some degree mitotic activity is also related to thymidine labelling, nuclear pleomorphism to image and flow cytometry and tubule formation to expression of differentiation antigens. Clearly these are not exact correlations but, as indicated in Fig. 1, this is another way of looking at the problem.

1. *Morphometry*

Although individual dedicated pathologists can reliably estimate tumour grade it is well recognized that there is great inter-observer variation. Several groups have attempted to define the individual features of grade more precisely by morphometric techniques using imaging systems linked to computers to estimate nuclear area and diameter, the number of mitotic figures and the degree of tubule formation [1–3]. A more precise estimate of prognosis has been found using such morphometric measurements than by conventional histological estimation of grade [2]. It was pointed out, however, that at present these techniques are time consuming, but that the future may lie with automation. Two groups indicated during the workshop that they considered the number of mitoses gave the greatest prognostic information and that a careful estimate of the mitotic index could possibly be used in place of all the other components [2, 4]. The patient population in these reports, and in several others mentioned below, was often small and highly heterogeneous. There is a great need for pathologists to combine their data in an international collaborative effort to find the 'best buy' for prognostic factors based on morphology. Perhaps overview methods, similar to those used to evaluate adjuvant therapy, could be employed.

2. *Image and flow cytometry*

Some years ago Auer showed that tumour cell ploidy as determined by static cytometry was related to prognosis. At the conference data comparing image and flow cytometry demonstrated that the two techniques gave equivalent results [5, 6]. In addition Auer's group [5] compared results of ploidy estimates for each tumour quadrant and showed that they were similar.

Thus flow cytometry may be used instead of the more laborious technique of static cytometry as tumour heterogeneity does not appear to be a major problem. It may also be possible to get accurate information concerning ploidy from fine needle aspirates [7].

There does appear to be a problem, however, in the relatively poor prognostic distinctions given by estimates of ploidy in breast cancer. In a group of 566 tumours van de Velde *et al.* [8] showed that patients with aneuploid tumours had a poorer prognosis than those with diploid tumours. In addition, using Cox analysis, ploidy was shown to be an independent prognostic factor. However, in agreement with other studies reported at the conference [9] and elsewhere, the prognostic separation between diploid and aneuploid tumours was relatively minor and it is clear that ploidy does not provide meaningful separation into prognostic sub groups. No data were presented on the prognostic significance of ploidy after relapse.

3. *Labelling index (LI)*

Thymidine LI should give a more precise indicator of metastatic potential than mitotic index since the labelling value is approximately an order of magnitude greater, because S phase is much longer than the duration of mitosis. Highly significant inverse correlations between LI, RFS and OS of patients with node negative tumours was shown by two groups [10, 11]. This has important implications for selecting poor prognostic subgroups of node negative patients for adjuvant therapy. Daidone *et al.* [10] also presented LI data on node positive patients, all of whom were treated with adjuvant CMF. The prognostic significance for RFS and OS of LI was lost, presumably because chemotherapy differentially improved the outlook of patients with a high LI. The relationship of LI to survival after relapse is not clear. The Milan group [10], however, did present survival data on patients with locally advanced tumours treated by chemotherapy. Those with a high LI had a significantly poorer prognosis suggesting that chemotherapy did not improve the prognosis of the high LI group compared to that of the low LI group. More data are required concerning the relationship of LI to endocrine responsiveness.

4. *Immunostaining of proliferating cells*

KI-67 is a commercially available monoclonal antibody which reacts with nuclear antigens associated with cell proliferation. Data were presented showing that it is possible to estimate the proportion of cells which express the antigen in smears from aspiration biopsies of tumours [12]. Importantly there was a clear difference between benign and malignant tumours. This supports data already available on solid tumour specimens. We need more data on the prognostic significance of expression of this antigen and others of a similar type.

5. *Differentiation antigens*

Inasmuch as antigens found in the human milk fat globule are most highly expressed during pregnancy and lactation they may be regarded as differentiation antigens. These antigens are also expressed in breast carcinomas. Data were presented [13] showing that the antigens recognized by antibodies HMFG1 and HMFG2 tend to be expressed in oestrogen receptor (ER) and progesterone receptor (PR) positive tumours and were considered by this group to be as good indicators of endocrine responsiveness as are receptor measurements. As with the receptors, however, their expression is poorly related to metastatic potential. Co-expression of receptors and differentiation antigens suggests that receptors may be regarded as features of differentiation. This contention is supported by a report of increased expression of ER during clonal growth of mammary tumour cells in agar [14].

The antibodies HMFG1 and HMFG2 recognize oligosaccharide moieties on mucin-like molecules. The core protein of this mucin shows less expression in normal tissues than it does in carcinomas. This may be because there is defective glycosylation in the tumours. This finding may be important, both with regard to diagnosis and immune-directed therapy [15].

The prognostic significance of PR remains controversial; one group found it to be unimportant for predicting either RFS or OS [16] and another showed that it was a highly important indicator of both time periods [17]. Marked differences such as these are possibly related to the variable use of adjuvant therapy and of therapy after relapse. It may be hypothesized that both ER and PR are prognostic indicators because they predict response to endocrine therapy; if adjuvant endocrine therapy is given relapse will be delayed in receptor positive tumours but, because receptors are largely unrelated to metastatic potential, no relationship between receptors and RFS will be seen in the absence of treatment. This proposal was supported for ER by one presentation at the meeting [18]. Thorpe *et al.* [19], however, showed that premenopausal node negative patients could be divided into a good and a poor subgroup on the basis of presence or absence of PR.

6. *Oncogenes*

The place of oncogenes in the prediction of metastatic potential is becoming clearer. In his review lecture Dr Cardiff predicted from data derived from rodent tumours that both tumour morphology and prognosis will prove to be related to the numbers and types of oncogenes activated. A report by van de Vijver *et al.* [20] confirmed that the neu (c-erbB-2) oncogene is amplified in human mammary tumours and is often accompanied by amplification of the linked c-erbA oncogene. This group has produced monoclonal antibodies against amino acid sequences from oligonucleotide fragments of the gene which can be used to detect oncogene expression in formalin-fixed archival tumours. This will enable the relationship between c-erbB-2 and prognosis to be further evaluated. The expression of the p62 protein product of the c-myc oncogene, measured in suspensions of nuclei from paraffin embedded tumours, was shown to be related to survival [21]. In addition the p21 protein of the c-Ha-ras was found to be over-expressed in 60% of human tumours, mainly in ER-positive ones [22]. More studies to define the relationship of these and other oncogenes to metastatic potential are clearly required. It represents a new and exciting field of investigation.

Another useful approach, which may ultimately be used to define risk, is to look for restriction fragment length polymorphisms near oncogenes in an attempt to define altered alleles with predictive value. The observation that a number of haplotypes of the 3′ flanking region of the c-Ha-ras gene were expressed in patients but not controls provides a prospect for defining a nucleotide sequence as a marker for breast cancer [23, 24].

7. *Markers in tissues*

Anti-epithelial membrane antigen (EMA) monoclonal antibodies may be used to detect the presence of small metastases in tissue sections. Metastases were detected in 19% of 929 lymph nodes using conventional histology but were found in 23% using immunohistochemical staining with an antibody (CT-M-1) against EMA [25]. This antibody is also useful for detecting malignant cells in effusions; six of 15 cytologically negative effusions were found to be positive using CT-M-1 [26]. The value of anti-EMA antibodies for detecting micrometastases in aspirates of bone marrow at the time of primary surgery was confirmed [27]. Eight of 15 patients with EMA positive marrows subsequently developed metastases compared with only two of 30 patients with negative marrows.

MARKERS OF TUMOUR RESPONSE

This section included studies of steroid hormone receptors and other proteins which have a potential ability to predict endocrine responsiveness, observations on the mechanisms of endocrine response and the measurement of markers of tumour burden in serum.

1. *Steroid hormone receptors*

Monoclonal antibodies to ER have undoubtedly revolutionized measurement of receptors in biopsies and, in particular, in aspirates of tumours [28]. Several groups have shown that the immunoradio-

metric (ER-EIA), and the immunocytochemical assay (ER-ICA) using the Abbott antibodies are equivalent to ER detected by dextran coated charcoal assays [29–33]. It was also shown that it is possible to estimate ER on formalin-fixed sections [34, 35]. However, it was pointed out in the workshop that we need to calibrate the new assays in relation to endocrine responsiveness rather than to the old methods of assay. During the workshop discussion it was stated that if a single cell was positive it represented an endocrine responsive tumour. Clearly this needs to be confirmed. There is also a need for response data in relation to evaluation of PR using the new monoclonal antibodies [32, 36]. Androgen receptors were shown to be related to both grade and ER and to give similar information to the more conventionally measured receptors [37]. Hormone receptors appear to be more frequently expressed in small non-palpable tumours [38]. However, the expression of the oestrogen receptor associated antigen (D5) was found to be higher in infiltrating carcinomas than in either benign or pre-malignant lesions [39]. These data, taken together, suggest that increased receptor expression occurs with early invasive growth and that receptors are then gradually lost with tumour progression.

Another study demonstrated that there may be translocation mutants of ER similar to those seen for glucocorticoids in lymphoma cells. Translocation defective tumours were unresponsive to endocrine therapy [40].

Several groups have demonstrated that many tumours may synthesize potential autocrine mediators of growth such as alpha TGF and the IGFs. In an intriguing study Mittra [41] showed, both by immunostaining with specific antibodies and by *in situ* hybridization, that the lactogenic hormones (prolactin, placental lactogen and growth hormone) were present in 80% of human tumours. There was a significant inverse correlation with ER. Mittra suggested that ER negative tumours constitutively synthesize lactogens which act directly within the cell at the nuclear level. Clearly this finding needs confirmation and further studies on the specificity of lactogenic hormone binding to DNA would be of value. A number of groups have suggested that there may be serum inhibitors of mammary tumour cell growth and data were presented showing that oestrogens may act by overcoming this inhibition [42]. Inhibitors in foetal bovine serum may be bovine prolactin which is known to bind to receptors but not to activate them. However, the inhibitors in serum from humans may be specific molecules and it is important for their nature and specificity to be determined.

The presence of gonadatrophin releasing hormone receptors was reported in 45% of tumours [43] and this again raises the question of a direct action of LHRH agonists on the breast; however, the concentrations required for half maximal binding make this unlikely. Other actions of tamoxifen (inhibition of calmodulin and stimulation of microtubule disassembly) were confirmed [44]; but their importance *in vivo* is not clear. An association of these mechanisms with endocrine responsiveness has not been demonstrated. The possible importance of local production of oestrogens from precursors in the breast was supported by a study which demonstrated that significant aromatase activity may be found in the breast and that the highest activity was in the quadrant where the tumour was found [45]. In addition the same group measured 17β-hydroxysteroid dehydrogenase, an important enzyme for the interconversion of androgens and oestrogens, and demonstrated that higher levels were found in breasts with tumours which failed to respond to systemic therapy [46].

2. *Other indicators of response*

Cyclic AMP protein (cAMP-B) levels in rat mammary tumours change markedly during endocrine induced tumour growth and inhibition . Miller *et al.* [47] measured the binding protein in human mammary tumours and related levels to subsequent response to endocrine therapy. There was no relationship between the cAMP-B levels and response; however, when (as has been demonstrated with rat tumours) the data were expressed as a ratio of ER/cAMP-B a distinction between responders and non-responders was found. The assay for cAMP-B is not simple, but the introduction of monoclonal antibodies against this protein may lead to a more readily available approach to prediction of response. Other putative markers of endocrine responsiveness were HMFG [13], breast cyst protein [48], creatine kinase-BB [49] and a dysplastic mammographic pattern [50]. One group found that β and kappa casein were not detectable in tumours and thus were of no value in determining responsiveness [51].

Nomura *et al.* [52] showed that 195 of 357 (55%) mammary tumours produced colonies in agar and that the proportion of those responding to tamoxifen, oestradiol and medroxyprogesterone acetate were similar to that expected *in vivo*. Growth in agar is not a technique which can be used routinely but is of value in determining the regulation of mammary tumour cell growth.

3. *Markers in serum*

Several groups presented data from studies which show the value of the Ca 15.3 assay and how it compares with CEA and other tumour marker measurements [53–58]. Like CEA the newer assay

is of little value in determining the presence of metastatic disease at presentation or for usefully increasing the lead time before relapse. However, it is a good marker of tumour burden and useful for following response to therapy. It should be regarded as complementary to CEA since there are tumours which secrete one marker but not the other. The serological detection of a 90 K proteolipid released from human tumour cells *in vitro* [59] and of the normal breast antigen 3E1-2 [60] may also prove to be useful as markers.

OTHER STUDIES

Two groups presented data relating to the observation that the monocytes of most patients with carcinoma of the breast fuse to form giant cells when cultured *in vivo* for 1–3 weeks [61–63]. This phenomenon was seen in only 10% of controls but in a high proportion of patients with benign breast disease [62]. Al-Sumidaie *et al.* [61] also demonstrated that fusion could be inhibited by tamoxifen and was associated with the release from fused cells of the enzyme reverse transcriptase. This raises again the possibility of the association of breast cancer with a retrovirus.

SUMMARY

It has not been possible in this review to cover all the submitted posters nor indeed all the points discussed during the workshop session. It is hoped, however, that major points of interest have been presented and that areas where there is consensus and areas where controversy remains concerning the clinical value of particular investigations have been highlighted. It is also hoped that some new potential growth areas for investigation of tumour biology have been defined.

REFERENCES

1. Chleq C, Lesty C, Nonnemmacher L *et al.* Mathematical morphology applied to breast carcinoma. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D1.5.
2. Ellis IO, Elston CW, Todd JH *et al.* Development of an objective morphometric histological grading system in primary breast cancer and comparison with subjective histological grade. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D1.4.
3. Schnurch H-G, Bender HG, Jesdinsky HJ. Towards a more precise description of breast cancer: prognostic impact of morphological features of the primary tumor. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D1.3.
4. Uyterlinde AM, Schipper NW, Baak JPA. Additional prognosic value of cellular DNA content to classical and morphometric parameters in invasive ductal breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D1.6.
5. Von Rosen A, Askensten U, Auer G. Does nuclear DNA content vary within the same tumour? A combined image and flow cytometric study of 21 primary mammary adenocarcinomas. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D4.2.
6. Arnerlov C, Roos G, Emdin SO. DNA content as a prognostic indicator in breast carcinoma—a comparison between static and flow cytometry of archival material. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D4.3.
7. Miller WR, Mullen P, Levack PA, Anderson TJ. DNA analysis of breast tumour fine needle aspirates using flow cytometry. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D4.5.
8. Van de Velde CJH, Cornelisse CJ, Caspers RJC, Van Driel OJR. DNA ploidy and survival in breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D4.6.
9. Blijham GH, Schutte B, Reynders M, Schouten L. The importance of DNA flow cytometry for adjuvant studies; in particular in node negative patients. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D4.1.
10. Daidone MG, Silvestrini R, Canova S, Orefice S, Bonadonna G. Cell kinetics and prognosis in human breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D4.9.
11. Hery M, Namer M, Gioanni J, Courdi A, Monticelli J. The labelling index: a prognostic factor in node negative breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D4.8.
12. Kuenen-Boumeester V, Blonk DI, Van der Kwast TH. Immunocytochemical determination of the proliferation fraction in fine needle aspiration smears of breast tumours. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D4.10.
13. Howell A, Baildam AD, Barnes DM, Sellwood RA. The clinical significance of heterogeneous expression of milk fat globule antigen (HMFG1) in human mammary tumours. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.11.
14. Werner A, Bellmann O, Vogel J. Estrogen receptor expression and lectin histochemistry in *in vitro* cultured endocrine-related tumors. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.17.
15. Girling A, Burchell J, Gendler S, Gillett C, Taylor-Papadimitriou J, Millis RR. SM3—a monoclonal antibody to the core protein of the mucin found in human milk. 4th EORTC

Breast Cancer Working Conference, 1987, Abstract No. D3.12.
16. Hawkins RA, White G, Bundred NJ, Dixon JMJ, Miller WR, Stewart H, Forest APM. The prognostic significance of oestrogen and progestogen receptor activities in human breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.11.
17. Klijn JGM, Alexieva-Figusch J, Van Putten WLJ, Blankenstein MA, Blonk-van der Wijst J. The prognostic value of estrogen and progestin receptors in primary human breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.23.
18. Passalacqua R, Bozzetti C, Bisagni G *et al.* Proposal of a new method for selecting the cut-off value of estrogen receptor (ER) in primary breast carcinoma (pbc). Prognostic correlations. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.10.
19. Thorpe SM, Rose C. Biology and clinical significance of steroid hormone receptors. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.12.
20. Van de Vijver M, Van de Berselaar R, Devilee P, Cornelisse C, Peterse J, Nusse R. Amplification of the neu (c-erb-B-2) oncogene in human mammary tumours is relatively frequent and is often accompanied by amplification of the linked c-erb-A oncogene. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.3.
21. Blamey RW, Locker A, Dowle CS *et al.* The relationship of p62c-myc oncogene levels in operable breast cancer to patients survival and tumour prognostic factors. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.5.
22. Sismondi P, Giai M, Piffanelli A, Bottero A, Selvatici R, De Bortoli M. p21ras expression defines different tumor populations in human breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.4.
23. Sismondi P, Camaschella C, Giai M *et al.* Distribution of haplotypes of the human Ha-RAS proto-oncogene in breast cancer patients and in a control population. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.1.
24. Sismondi P, Giai M, Saglio G *et al.* Differences in Ha-RAS haplotypes between peripheral blood leucocytes and tumour tissue in human breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.2.
25. Abarca J, Van Eeckhout P, Van Bogaert L-J *et al.* Improved detection of metastases in axillary lymph nodes in breast carcinomas. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.14.
26. Abarca J, Bussolati G, Van Bogaert L-J, Trouet A. Improved detection of metastatic carcinoma cells in pleural effusions from patients with breast carcinoma. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.16.
27. Untch M, Harbeck N, Eiermann W, Hepp H. Immunocytochemical detection of tumor cells in bone marrow in breast cancer patients at time of primary therapy. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.15.
28. Dicato M, Schroell B, Dondelinger RF, Kurdziel J-C, Ries F. Hormone receptor measurement in metastatic breast cancer by monoclonal antibodies in minimal tissue samples obtained by interventional radiological procedures. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.9.
29. Piccart M, Leclercq G, Domange AM, Lenglet G, Paridaens R. Estrogen receptors (ER) in breast carcinomas: a comparative study of enzyme immunoantibody (Abbot-EIA) and dextran-coated charcoal binding cytosol (DCC) assays. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.1.
30. Faverly D, Devleeschouwer N, Lenglet G, Paridaens R. Estrogen receptor assays on small samples of human breast cancer obtained by tru-cut biopsy and fine needle aspiration. Correlations between immunoenzymatical, immunocytochemical and biochemical methods. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.8.
31. Eiermann W, Matzner G, Schenk U, Untch M, Jawny J. Comparative investigations for the detection of steroid receptors in breast cancer with mABs. 4th EORTC Breast Cancer Working Conference 1987, Abstract No. D2.4.
32. Delarue JC, Contesso G, Mouriesse H *et al.* A comparison between the biochemical method of measuring estrogen (ER) and progesterone receptors (PR) and an immunohistochemical method (ER-ICA) in human breast tumors. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No.D2.3.
33. Toi M, Hamada Y, Seto Y, Tanimoto M, Niimoto M, Hattori T. Clinical application of estrogen receptor immunocytochemical assay (ER-ICA) in breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.6.
34. Andersen J, Orntoft T, Rasmussen BB, Thorpe S, Rose C, Poulsen HS. Immunohistochemical analysis of estrogen receptors (ER) using formalin-fixed paraffin embedded tissue—relationship to steroid binding assay and the prognostic and predictive value in patients with breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.7.
35. Skovbon H, Poulsen HS. Double immunocytochemical staining for estrogen receptors (ER) and estrogen-induced 24-K protein in formalin-fixed paraffin sections of human breast cancer tissue—relationship to prognostic and predictive value in patients with breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.18.
36. Foekens JA, Van Driel J, Portengen H, Henkelman MS, Haije WG, Klijn JGM. Comparison of progesterone receptor enzyme immunoassay (Abbot PR-EIA) and radioligand binding

assay (PR-RBA) in stored human breast cancer tissue samples. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.5.
37. Reiner G, Jakesz R, Kolb R *et al.* Androgen receptors and prognostic factors in human breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.21.
38. Tinnemans JGM, Raemaekers JMM, Wobbes T, Beex LVAM, De Boer HHM. High incidence of oestrogen (ER) and progesterone (PgR) receptor activity in nonpalpable breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.14.
39. Girling A, Caleffi M, King RJB, Millis RR. An immunohistochemical study of oestrogen receptor related protein in normal breast tissue and benign breast disease. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.16.
40. Raam S, Tamaura H, Teixeira T *et al. In vitro* translocation abnormalities of estrogen receptors (ER) predict hormone therapy failures in breast cancers. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.13.
41. Mittra I. Intracellular synthesis and nuclear accumulation of lactogenic hormones in autocrine regulation of human breast cancer: a new proposal for hormone responsiveness. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.6.
42. Lykkesfeldt AE, Rose C, Thorpe S, Lykkesfeldt G, Laursen I, Briand P. The mechanism for estradiol stimulation of human breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.9.
43. Kaufmann M, Kiesel L, Klinga K, Runnebaum B, Kubli F. Gonadotropin-releasing hormone receptors in human breast carcinoma. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.22.
44. Gulino A, Barrera G, Vacca A *et al.* Growth inhibition of breast cancer cells and calmodulin antagonism in the activation of cAMP-phosphodiesterase and microtubule disassembly by triphenylethyline antiestrogens. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.10.
45. O'Neill JS, Miller WR. Oestrogen biosynthesis in breast adipose tissue. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.14.
46. O'Neill JS, Miller WR. Levels of 17β-hydroxysteroid dehydrogenase in breast adipose tissue from breast cancer patients. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.15.
47. Miller WR, Watson DMA, Hawkins RA, Stewart HJ, Bundred NJ. Tumour cyclic AMP binding proteins and endocrine responsiveness of breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.21.
48. Bundred NJ, Stewart HJ, Sturgeon C *et al. In vitro* production of CEA and breast cyst protein by breast carcinomas: relationship to receptor status and response to hormonal therapy. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.8.
49. Scambia G, Santeusanio G, Panici PB, Baiocchi G, Iacobelli S, Mancuso S. Immunohistochemical localization of creatine kinase-BB in primary breast cancer: correlation with estrogen receptor content. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.19.
50. Frigerio A, Burke P, Fasciano F, Temporelli A. Correlation of hormone receptor levels with mammographic patterns in breast cancer: a report on 154 patients. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D2.15.
51. Earl HM, McIlhinney RAJ, Wilson P, Gusterson BA, Coombes RC. Immunohistochemical studies of human beta and kappa caseins in human breast, and breast carcinomas using monoclonal antibodies. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.17.
52. Nomura Y, Tashiro H, Takaeko F, Hisamatsu K. Detection of hormone dependency of human breast cancer in the *in vitro* clonogenic assay. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.7.
53. Filipovic S, Filipovic B. First clinical experiences with tumor marker CA 15-3 in the evaluation of breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.1.
54. Fumoleau P, Cuilliere JC, Ricolleau G *et al.* CA 15-3 tumor markers in the follow up of 381 breast cancer patients. A prospective study, comparison with CEA. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.2.
55. Diegarten K, Stieber P, Fateh-Moghadam A, Eiermann W. CA 15-3: clinical evaluation and comparison with CEA and TPA in breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.3.
56. Rogers K, Duncan JL, Milford-Ward R. Detection of serum antigens by monoclonal antibody CA 15-3 in patients with breast carcinoma. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.4.
57. Bonfrer JMG, Nooyen W, Hilkens J. CA 15-3, a marker in breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.5.
58. Namer M, Anicet-Pons D, Krebs BP, Hery M, Khater R, Frenay M. Comparison of CEA and CA 15-3 as tumor markers for non metastatic breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.7.
59. Iacobelli S, Scambia G, Arno E *et al.* Detection of antigens defined by a novel monoclonal antibody in the sera of patients with breast cancer and their modulation by interferon. 4th

EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.18.
60. Stewart J, Burton R, Smart C. Elevated serum levels of 3EI breast cancer antigen in patients with advanced breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D3.6.
61. Al-Sumidaie AM, Leinster SJ. Tamoxifen effect on giant cell formation in patients with breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.11.
62. Carragher A, Odling-Smee W. Multinucleate giant cell formation in breast cancer. 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.12.
63. Al-Sumidaie AM, Leinster SJ, Hart CA, Green CD, McCarthy K. Is human breast cancer caused by retrovirus? 4th EORTC Breast Cancer Working Conference, 1987, Abstract No. D5.13.