Additive hormonal treatment (Table I) became available during the 1950s with the commercial production of steroid hormones. Since involution of the embryological breast bud which develops in all early, sexually undifferentiated embryos was known to occur under the influence of androgens it was perhaps not surprising that androgens could cause regression of female breast cancer. More surprising perhaps was the finding that oestrogens, which appeared to be necessary for the maintenance and growth of breast cancer in younger women, could also be beneficial, albeit usually in older, postmenopausal women. Other steroid hormones such as the gestagens and modified androgens such as danazol are also active.

The term 'changing the hormonal milieu' was coined to indicate a hypothetical alteration between growth-stimulatory and as yet (undefined) growth-inhibitory hormonal influences.

During the 1960s much effort went into attempts to define levels of plasma hormones or of total hormone production as measured by urinary excretion of sex steroids; this was intended to provide an index of hormone responsiveness and prognosis in breast cancer. Ultimately none of these tests achieved any real measure of success since these investigations focused on patient determinants rather than the nature of the tumour. However, several clinical features were defined which provided some guidelines for hormone treatment of breast cancer (Table I). In the absence of more specific information, these clinical guidelines remain useful.

Table II. Clinical features and response to hormone treatment in advanced breast cancer

<table>
<thead>
<tr>
<th>Favourable</th>
<th>Unfavourable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt; 60</td>
<td>Age &lt; 45</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>Premenopausal</td>
</tr>
<tr>
<td>Long disease-free interval</td>
<td>Rapid development of metastases</td>
</tr>
<tr>
<td>Bone, skin, node</td>
<td>Visceral metastases</td>
</tr>
</tbody>
</table>

Hormone receptors and the response of breast cancer to hormonal manipulation

Of course breast cancer is not the only tissue responsive to oestrogens. Normal breast and uterine tissue responds to oestrogens while at the same time oestrogenic hormones have no apparent effect on the growth of other organs. Early studies using both animal and human models helped to define oestrogen-responsive tissues and to demonstrate the association of a targeting mechanism with specific accumulation against a concentration gradient of oestrogen in responsive tissues. The first successful methods of measuring potential hormone responsiveness were based on the phenomenon of binding of oestrogen, and involved the use of a specific cellular receptor with high affinity for oestrogen that was able to recognise and competitively bind it. A specific oestrogen receptor (ER) was subsequently found in oestrogen-responsive tissues and in a proportion of breast tumours that were excised and kept fresh enough for the phenomenon to be demonstrated by a number of 'ligand-binding' methods.
These methods were the first to offer a rational explanation for hormonal effects on breast cancer. The early model of steroid hormone action suggested that oestrogen would penetrate the cell membrane, bind to a cytoplasmic receptor and that the complex would then be translated to the cell nucleus where the hormone-receptor complex would interact with the genome to induce changes in cell function and growth. Among the recognizable changes induced by oestrogens is the induction of mRNA followed by synthesis of the progesterone receptor (PR) protein.

Both early and subsequent studies showed that only some breast cancers are ER-positive. There is an inverse relationship between the age of the patient in whom the tumour develops and the likelihood of ER expression. This relationship parallels the previously observed rate of hormonal responsiveness of about 30% for breast cancer in premenopausal women, to some 50 - 60% for breast cancers in postmenopausal women in the sixth and seventh decades of life.

In South Africa, with its varied population and varied distribution of cancer, not only breast cancer incidence but also ER expression have been found to be different in different racial groups. The frequency of breast cancer in black women is about one-quarter of that in whites (Table III), although there is evidence that the frequency of breast cancer in the black population is rising. In addition the pattern of breast cancer, including age distribution, stage at presentation (Table IV), hormonal receptor status and overall prognosis, appears to be different in the different ethnic groups. The lower frequency of receptor-positive tumours in black women with breast cancer cannot, however, be explained by the age distribution alone, since this difference is present even when stage and race are taken into account, breast cancer seen at the Breast Clinic, Johannesburg and Hillbrow hospitals, University of the Writwatersrand.

| Table V. ER status analysed by ethnic group and age (deciles) |
|------------------|---------|---------|
| Decile | ER status | Black | White |
| 20 - 29 | ER+ | 9 | 45 | 3 | 38 |
| | ER- | 11 | 55 | 5 | 62 |
| Total | | 20 | 6 |
| 30 - 39 | ER+ | 44 | 47 | 17 | 41 |
| | ER- | 50 | 53 | 24 | 59 |
| Total | | 94 | 41 |
| 40 - 49 | ER+ | 59 | 51 | 67 | 60 |
| | ER- | 56 | 49 | 44 | 40 |
| Total | | 115 | 111 |
| 50 - 59 | ER+ | 44 | 42 | 85 | 63 |
| | ER- | 48 | 58 | 50 | 37 |
| Total | | 92 | 135 |
| 60 - 69 | ER+ | 47 | 49 | 141 | 71 |
| | ER- | 48 | 51 | 58 | 29 |
| Total | | 95 | 199 |
| 70 - 79 | ER+ | 18 | 69 | 112 | 71 |
| | ER- | 8 | 31 | 45 | 29 |
| Total | | 26 | 157 |
| 80+ | ER+ | 6 | 75 | 29 | 76 |
| | ER- | 2 | 25 | 9 | 24 |
| Total | | 8 | 38 |

Table VI. Multivariate analysis of breast cancer survival including age, ethnic group, T, N and OR

<table>
<thead>
<tr>
<th>Variable</th>
<th>Co-efficient</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>0.4529</td>
<td>0.0958</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>N</td>
<td>0.4448</td>
<td>0.0824</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>ER</td>
<td>0.4582</td>
<td>0.1475</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Race</td>
<td>0.3708</td>
<td>0.1664</td>
<td>= 0.007</td>
</tr>
<tr>
<td>Age</td>
<td>-0.0098</td>
<td>0.0056</td>
<td>= 0.78</td>
</tr>
</tbody>
</table>

Analysis based on 1 139 patients with breast cancer seen at the Breast Clinic, Johannesburg and Hillbrow hospitals, University of the Witswatersrand.

**Oestrogen-receptor blocking agents and hormone treatment of breast cancer**

The introduction of tamoxifen, the first of the nonsteroidal, OR blocking agents, into the therapeutic armamentarium against breast cancer had some major therapeutic advantages, not because tamoxifen works more frequently than other hormone manipulations but because of the relative lack of side-effects. The initial theory was that tamoxifen exerted its effects through a specific block of the hormone/receptor interaction within the cytoplasm of the tumour cell.

There is a clear relationship between response to tamoxifen therapy and the hormone receptor status of the tumour, and between the level of receptor expression and both response rate (Fig. 1) and response duration. King and Greene were the first to demonstrate, by using immunocytochemical techniques with a monoclonal antibody directed against the isolated and purified ER, that specific ER localised to the nucleus rather than the...
cytoplasm of the malignant cell. One spin-off of this work was that ER can now be determined either by measuring the protein itself by immunoassay or by demonstrating its presence in the malignant cells by immuno-histochemical methods rather than measurement of the oestrogen (ligand-) binding capacity of cytosol preparations which may contain variable mixtures of tumour and non-malignant stromal cells. Antibody-based methods of ER estimation have generally begun to replace ligand-binding methods.

The change of localisation of the receptor can be accommodated reasonably easily in the outline of hormone action referred to earlier. What had previously been measured in the cytoplasm was nuclear receptor leaking out as cells were disrupted during the process of preparation for analysis.

Hormone growth factor interactions

Based on studies with the MCF 7 breast cancer cell line, a cell line which displays hormone receptors and which is responsive both to growth stimulation by oestrogens and to growth inhibition by tamoxifen, Lippman and co-workers first showed a relationship between hormones and growth factors in breast cancer. Growth factors are small peptides of between 10 000 and 20 000 daltons produced by a range of cells and tissues which serve to regulate growth and differentiation. A number of these factors (Table VII) have been isolated and purified; more recently, genes coding for these factors have been cloned. Aberrant expression of growth factors or insertion of genes for growth factors into cells in an unregulated fashion was, from the inception of this field of research, considered to be a possible mechanism for stimulation of tumour growth.

Growth factors were also shown to have specific cellular receptors. The interaction of growth factor and receptor serves as a signal for cellular responses, most prominent among these being cell growth. Aberrant receptor expression or receptor-like function could also conceivably be a cause of tumour growth.

Indeed, several growth factors or their receptors have been identified as products of oncogenes — those genes thought initially to be specifically involved in the genesis of cancer, but now increasingly recognised as important cellular control genes in normal cells.

Not just stimulation of growth of pre-existing tumours but indeed tumour-like behaviour in normal or at least otherwise fairly normal cells can be demonstrated as a result of the action of certain growth factors. This property leads to the definition of so-called transforming growth factors. Growth factors (including transforming growth factors) may also exert negative effects and inhibit growth of specific types of cell. The functional expression of growth factor effects is influenced both by the type of receptor and the cell or tissue under consideration.

The model proposed by Lippman and co-workers was that hormonal effects on growth of MCF 7 cells were indirect, i.e. they entailed stimulating the elaboration and release of either growth-stimulatory or growth-inhibitory factors. In the MCF 7 cell line, TGFα (induced by oestrogens) acts as a positive growth signal while TGFβ (upregulated by tamoxifen) appears to act as a negative growth factor (Fig. 2). This model is an example of the wider field of autoregulatory phenomena and autocrine growth stimulation.

![Fig. 1. Relationship between level of tumour ER and response to therapy with tamoxifen. The response at all metastatic sites is related to tumour ER level.](image)

![Fig. 2. Hormone/growth factor interactions in breast cancer based on MCF 7 cell line model. Oestrogenic stimulation results in production of a positive growth factor TGFα, while tamoxifen administration results in elaboration of an inhibitory growth factor TGFβ.](image)
however, not always so. Oestrogen receptor may still be present when hormone treatment has failed, while some tumours may still respond to hormone manipulation with another type of hormone treatment. In a previous study from this institution it was demonstrated that response to a second course of hormone treatment is significantly related to tumour cell ploidy (a measure of the quantity of DNA per cell), with diploid tumours tending to respond to a second round of hormone treatment while aneuploid tumours usually fail to respond. These studies suggest that loss of hormone responsiveness is a phenomenon which occurs in tumour cell populations that have undergone considerable mutation.

Another issue arises from receptor expression and may be related to the concept of continuing cell mutation. This is the problem of the hormone receptor-positive tumour not responsive to hormonal treatment. The predictive value of positive ER is less than absolute. In fact, the overall probability of response to hormone treatment even in patients with ER-positive tumours is about 50%. Are the receptors mutated in these instances? Different domains or regions of the receptor molecule, including both a DNA-binding domain and a hormone-binding domain, can be defined. Receptor measurement by methods dependent on demonstration of the hormone-binding site, either its capacity to bind oestrogen or by means of an antibody directed specifically against the portion of the receptor that binds oestrogen, may fail to give information on the entire receptor molecule and its functional capacity. The portion that binds to DNA may be mutated or abnormal and either cause or fail to induce gene activation, irrespective of whether hormone binding occurs. Receptor mutations have indeed been identified but the frequency of such mutations and the definition of specific types of mutations require further investigation.

The involvement of growth factors other than TGFs in clinical breast cancer

How representative are the results with the MCF 7 cell line, and are the two growth factors which seem to be of prime importance for stimulating or suppressing growth of these cells the only ones of major importance in clinical breast cancer? Evidence shows the in vivo situation to be more complex. Other growth-promoting factors may also be involved in clinical breast cancer. One such factor of current interest is the cERB2-HER2/neu oncogene product. This cellular gene, which was first described in a carcinogen-induced rat neuroglioblastoma and which is homologous with a gene (v-ERB) isolated from cells infected with the avian erythroblastosis virus, has been the subject of extensive investigation.

Overexpression of the cERB2 gene as determined by molecular biological techniques involving quantitative mRNA analysis using PCR appears to be an adverse prognostic factor in breast cancer. The protein encoded by the cERB2-HER2/neu gene has recently been identified, and structurally appears to belong to the group of growth factor receptors.

cERB2 expression can now be demonstrated by immunochemical techniques, using antibodies directed against the gene product. The cERB2 protein on the cell membrane may, when overexpressed by cancer cells, be stimulated by other growth factors such as epidermal growth factor, which can cross-react with this receptor. There may also be a specific ligand for this receptor produced by the tumour cells themselves. This ligand (itself a membrane protein) appears to require proteolytic cleavage from the cell membrane before it can activate the receptor and act as a growth factor.

While cERB2 mRNA overexpression appears to be an adverse prognostic factor in breast cancer, the relationship between cERB2 protein expression and progression of breast cancer is more complex. An apparently paradoxical finding is that membrane staining for cERB2 protein appears to be most intense in intraduct carcinomas which have not as yet penetrated the basement membrane and which are thought to represent the earliest and least aggressive forms of breast cancer. The development of micro-invasion is usually associated with a decrease in the intensity of cERB2 staining.

Soluble cERB2 protein, possibly derived from the cell membrane by proteolytic cleavage or produced by the cells as a truncated receptor molecule, has also been found in the serum of approximately 14 - 30% of patients with breast carcinoma. A recent study undertaken at the University of the Witwatersrand showed that there was no correlation between tissue expression of cERB2 protein (as detected by immunostaining) and the presence of soluble cERB2 protein in serum. Furthermore, while tissue expression of cERB2 protein had no influence on prognosis of patients with advanced breast cancer, patients who had soluble cERB2 protein in the serum had a significantly poorer prognosis (Fig. 3). It is possible that soluble cERB2 protein acts as a binding protein for other growth factors, resulting in preferential delivery of as yet unknown growth-stimulatory factors to the tumour cells. In this regard, the function of soluble cERB2 protein may be similar to that of the soluble interleukin-6 (IL-6) receptor which appears to be necessary for IL6 binding to the myeloma cells for which it is a growth factor.

Fig. 3. Influence of the presence (o) or absence (■) of soluble cERB2 on survival in advanced breast cancer. The difference was statistically significant — P = 0.002 (log rank), P = 0.01 (Wilcoxon).
Another growth factor which may be involved in progression of breast cancer is platelet-derived growth factor (PDGF). We have found elevated levels thereof in the serum of patients with more advanced and more bulky disease. Immunochemical studies have also demonstrated PDGF expression in cytological material from breast tumours as well as a correlation between PDGF levels and expression and prognosis in advanced breast cancer (Fig. 4). While these findings suggest involvement of PDGF in breast cancer progression, the demonstration of receptors for PDGF, particularly in breast cancer cell lines and breast tumours, has been more difficult and appears to be complicated by the presence of apparently activated intracellular receptors and possible internal autocrine pathways. We are currently investigating whether clinical breast tumour specimens express the PDGF receptor.

Another avenue of research is just beginning to open up — interaction of tumour cells and stroma. It must always be remembered that cancer occurs against a background of non-cancerous stromal cells. The two components can, and evidently do, interact.

Recent studies have shown that in patients with clinical breast cancer who are given tamoxifen, it may be the stromal cells rather than the cancer cells that respond by showing increased immunostaining for TGFβ. This finding may offer an explanation for the puzzling finding in clinical studies that in the adjuvant treatment setting, tamoxifen appears to be effective, at least to a degree, even in patients who have ER-negative tumours.

Tamoxifen thus appears to be able to influence the function of stromal cells such as fibroblasts. Stromal cell production of growth-suppressive factors may be sufficient to suppress the growth of a subclinical tumour burden.

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Another area of the hormone/growth factor axis requires further investigation. It is believed that oestrogens are not only involved in the progression of cancer but are of importance in the genesis of breast cancer. Are we to believe that oestrogenic hormones are only involved in the growth of hormone receptor-positive tumours? After all, women who develop ER-negative breast cancers are still women and still have oestrogens. Various lines of evidence suggest that the relationship involves not only the tumour cell ER. Breast tumours accumulate as well as synthesise oestrogens from oestrogenic precursors, irrespective of the hormone receptor status of the tumour. Miller and O'Neill have shown that the residual, histologically normal tissue in the quadrant of the breast in which the tumour arises generally has a higher content of the enzyme called aromatase, responsible for converting androgenic precursors to oestrogens. Is such oestrogen accumulation merely an epiphenomenon of the malignant phenotype? We have demonstrated a relationship between the oestrogen content of the tumour-bearing area and prognosis of breast cancer, a relationship independent of tumour hormone receptor content (Fig. 6).

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Furthermore, in the course of a study based on the premise that if tumour cells can be induced to proliferate temporarily by switching them on with a growth signal (in this case oestrogen) they may be more sensitive to the effects of chemotherapy, we found that ER-positive as well as ER-negative tumours showed an increase of the proliferative index. These findings suggest some non-receptor-mediated effect of oestrogen on breast cancer cells themselves or on the surrounding non-malignant stromal cells, including non-malignant breast epithelial cells, which might be induced to produce positive growth signals for ER-negative but growth-factor-responsive tumours.

Future studies will look to identify the entire complex of tumour-plus-stroma and to recognize that clinical breast cancer is merely a part of a more widespread field abnormality which forms the background for competing hormonal and growth-factor stimuli. The eventual emergence and progression of a particular cell that gives rise to clinical breast cancer is probably determined by the interaction of these effects and remains influenced by them.

REFERENCES