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THE SIGNIFICANCE OF HORMONE RECEPTORS TO PREDICT THE ENDOCRINE RESPONSIVENESS OF HUMAN BREAST CANCER

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Abstract

The paper reviews clinical data on the correlation between the response of human breast cancer to endocrine therapy and the tumour cell content of receptors of e.g. oestrogen (OeR), progesterone (PgR), androgens (AR) and the epidermal growth factor (EGFR). In advanced disease there is a well established correlation betweeen OeR content and the rate of objective response to all types of endocrine therapy. However, if selection of first-line salvage therapy based on OeR status will result in prolonged survival or improved quality of life remains controversial. Assays of PgR, AR, and EGFR—in addition to OeR—increase the predictive ability but no study has been able to define an entirely unresponsive subgroup of patients on the basis of receptor status. In the adjuvant setting conflicting relationships have been reported. Some authors have found a benefit with tamoxifen also among OeR negative patients whereas others have concluded that adjuvant tamoxifen is ineffective in such patients. Prospective randomized trials are warranted to further assess the predictive value of hormone receptors, particularly in view of the increased frequency of thrombotic events and endometrial cancer associated with long-term adjuvant tamoxifen.

Key words: Breast cancer, hormone receptors, endocrine therapy, epidermal growth factor.

About one-third of all patients with advanced breast cancer respond to endocrine therapy (1). Several clinical and histological parameters are correlated with endocrine responsiveness, e.g. age, length of recurrence-free interval, dominant side of disease (2–4), and tumour differentiation (5–7). With the discovery of the oestrogen receptor (OeR) and other receptors more accurate methods have become available. This paper reviews the current status of OeR and other receptors for selection of patients who might benefit from endocrine therapy in advanced disease as well as in the adjuvant setting.

Tumour growth control

Oestrogens appear to be the most important steroid hormones involved in growth regulation of hormone-dependent breast cancer (1, 8). Oestrogens interact directly with tumour cells via OeR, resulting in an increased synthesis of several enzymes involved in DNA synthesis, progesterone receptors (PgR), and certain proteins with unknown functions in the cell. The oestrogen-OeR interaction also results in an increased synthesis of growth factors which may stimulate growth of the tumour cell itself (autocrine stimulation) or of surrounding cells (paracrine stimulation) through binding to specific receptors. Conversion of the hormone-dependent to the hormone-independent phenotype may be the result of an increased, non-steroid hormone-regulated synthesis of growth factors.

There is still a lack of information concerning the correlation between endocrine responsiveness and the levels of activity of most of the mentioned oestrogen-induced proteins. Most of the available clinical data concern OeR, PgR, and receptors for the epidermal growth factor (EGFR).

Oestrogens may interact with breast cancers via other mechanisms than OeR (1, 8, 9). They may induce stromal fibroblasts to secrete growth factors resulting in a paracrine stimulation of the cancer cells. It is also possible that androgens and glucocorticoids stimulate tumour growth via specific receptors. However, these alternative mechanisms for hormone-induced tumour growth remain hypothetical and have not been substantiated clinically.

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Evaluation of response

Objective response (complete or partial remission) is the most frequently reported measure of endocrine responsiveness in advanced disease but may be difficult to evaluate in many patients. It is possible that other measures—e.g. response rate including both objective remissions and stable disease or time to disease progression—are clinically more relevant. For instance, it has been reported that the survival of patients with stable disease is similar to that of patients showing complete or partial remission (10).

In the adjuvant setting there is no endpoint equivalent to objective remission. For analysis of treatment interactions most authors have used recurrence-free survival which is related to the endpoint time to progression in advanced disease. Since the endpoints are different, and since the endocrine responsiveness may be different in early and late disease, clinical correlations established in studies of advanced breast cancer may not be relevant in the adjuvant setting and vice versa.

OeR and response

In 1971 Jensen et al. (11) showed that there was a correlation between OeR status and the objective response rate among patients with advanced disease treated with adrenalectomy. Since then several reports have confirmed and extended this observation: the objective response rate is about 50–70% among patients classified as OeR positive and less than 5–10% among OeR negatives (12). The rate among OeR positives has been reported to be the same with most types of endocrine therapy with the possible exception of androgens which appear to yield slightly lower response rates: 30–40% (13, 14). The objective response rate is closely correlated with the total amount of OeR with a rate of up to 80% among those with high levels (Table 1) (15).

OeR content is correlated with many clinical and histological parameters that also predict response to endocrine therapy, e.g. age, length of recurrence-free interval, dominant site of disease, number of metastatic sites, and tumour grade. Comparative studies have demonstrated that OeR has greater predictive ability than the mentioned parameters even though some of them (e.g. age and menopausal status) may have a predictive value that is independent of OeR status (15, 16).

Table 1

Relationship between the objective response rate to endocrine therapy and the tumour cell content of oestrogen receptors. From Lippman et al. (15)

OeR content[a]	Objective response rate (%)
0–9	3/33 (9)
10–19	3/10 (30)
20–49	7/11 (63)
50+	24/31 (77)
Total	37/85 (44)

[a] fmol OeR/mg protein.

Ligand-binding- versus immuno-assays

Most of the early clinical studies were based on ligand-binding receptor assays. With the development of monoclonal antibodies new assays have become commercially available: the immunocytochemical assay (ICA) and the enzyme immuno assay (EIA). Several studies have shown that results with these new methods are significantly correlated with those obtained with the older ligand-binding assays (17–24). It has been suggested that immunoassays—in addition to being less time-consuming—give more reproducible results and are less influenced by e.g. the endogenous levels of steroid hormones and non-specific, low-affinity hormone binding. The response rate among those classified as OeR positive with an immunoassay appears to be slightly higher than would be expected with a ligand-binding assay (Table 2). Conversely, the response rate among OeR negatives appear to be slightly lower than expected. These observations suggest that immuno-assays have a better predictive ability than ligand-binding assays but this hypothesis remains to be established in comparative clinical studies.

The EIA is based on homogenized tumour specimens and gives a quantitative estimate of the mean receptor content of the cells in the specimen. The ICA is done on thin slices of tissue or on fine-needle aspirates and permits evaluation of the receptor content of individual tumour cells. The results can be expressed as the percentage of receptor positive cells or as the average staining intensity i.e. a semiquantitative estimate of the average receptor content. The percentage of positive cells is possibly a more important predictor of response than the staining intensity but the optimal cut-off point is not well known. In a paper

Table 2

Objective response rate according to OeR-ICA results

Study	OeR-ICA positive[a]	OeR-ICA negative
	Objective response rate (%)	
Pertschuk et al. (21)	7/20 (35)	1/23 (4)
Hawkins et al.[b] (22)	6/11 (55)	0/3 (0)
McClelland et al. (23)	21/29 (72)	1/27 (4)
Jonat et al. (24)	6/11 (55)	1/9 (11)
McCarty et al. (46)	13/14 (93)	1/9 (11)
Total	62/87 (71)	5/69 (7)

[a] As defined by the authors.
[b] Patients whose tumours regressed during therapy were classified as responders.

by Gaskell et al. (25) which described results of treatment with tamoxifen for primary breast cancer in elderly patients, the percentage of positive cells significantly predicted the likelihood of response: only 1/12 tumours with less than 25% positive cells responded (8%) compared to 26/31 (84%) of those with more than 25% positive cells. The average staining intensity was not found to be predictive. In 56 patients with advanced disease Coombes (26) reported a response rate of 70% among those with more than 50% positive cells compared to only 4% among those with fewer than 50% positive cells. Many authors have combined the percentage of positive cells and the average staining intensity into a 'staining index' but here is no consensus on how this index should be defined.

OeR and PgR versus response

Irrespective of which assay is used, some OeR positive tumours do not respond to endocrine therapy. Such tumours may have a defect in the oestrogen response pathway distal to the OeR. Progesterone receptors are synthesized by tumour cells that are stimulated by oestrogens. Therefore, it has been hypothesized that PgR-status might help to predict endocrine responsiveness since the presence of PgR may indicate that the OeR pathway is functional.

Several studies have shown that the classification of patients in terms of both OeR and PgR status increases the predictive ability: the objective response rate is 70–80% for those who are positive for both receptors, 30–50% for those who are positive for only one of the receptors, and 5–10% for those classified as negative for both receptors (27–29). When the predictive ability of the two receptors has been compared, OeR has usually been found to be better.

OeR negative/PgR positive tumours have sometimes been described as laboratory artifacts representing patients with a false negative OeR assay. In such a case one would expect a response rate of about 70–80% among such patients. In reality, it appears to be about 40–50% which suggests that these patients have tumours that represent a biologic entity distinct from tumours that are positive for both OeR and PgR.

As mentioned before PgR does not appear to have a better predictive ability than OeR. Several hypotheses have been suggested to explain this observation. The low levels of circulating oestrogens in postmenopausal women may result in an insufficient stimulation of hormone dependent tumour cells to synthesize PgR. In premenopausal women high levels of progestagens may bind to PgR making it undetectable. PgR assays are possibly also more likely to yield false negative results due to technical difficulties. Misclassification of patients in regard to PgR status would tend to obscure the predictive ability of the assay (27–32).

The receptor status of a tumour may be different in different metastatic sites and may change over time. Receptor status is often measured in one site, e.g. the primary tumour or a local recurrence, and the response to endocrine therapy evaluated in another site, e.g. bone or liver. The rate of discordant receptor status in multiple, asynchronous biopsies has been reported to be about 20–25% for patients who initially were classified as OeR positive, OeR negative or PgR negative (31). In contrast, about 50% of initially PgR positive patients were reported to be PgR negative in subsequent biopsies (Table 3). Gross et al. (30) reported that interval endocrine therapy was an important factor contributing to the development of PgR negative status among initially PgR positive patients.

Table 3

Discordance of OeR and PgR status in asynchronous tumour biopsies. From Harland et al. (31)

Status at first biopsy	Status at second biopsy	
	Positive (%)	Negative (%)
OeR positive (n = 54)	43 (80)	11 (20)
OeR negative (n = 34)	9 (26)	25 (74)
PgR positive (n = 37)	19 (51)	18 (49)
PgR negative (n = 50)	8 (16)	42 (84)

Raemaekers et al. (29) found that the predictive value of PgR-status was increased if the assay was done on a tumour specimen excised immediately prior to the initiation of therapy compared to if the assay had been done more than 6 months prior to the start of treatment.

These observations may help to explain why PgR status in most studies have not been shown to have a greater predictive ability than OeR. Another possible explanation is illustrated by in vitro studies that have shown a dissociation between oestrogen-induced tumour growth and synthesis of PgR. For instance, the MDA-MB-134 human breast cancer cell line has a high OeR content and a low PgR content. Oestradiol stimulates the growth of these cells but fails to increase PgR levels (33). This observation accords with the mentioned clinical observations, i.e. that a substantial proportion of PgR negative tumours still may be regulated by oestrogens and may respond to endocrine therapy.

EGFR and response

There is an inverse relationship between OeR positivity and the presence of EGFR: few OeR positive tumours are also positive for EGFR (Table 4) (34, 35). In 1989, Nicholson et al. (36) showed that EGFR positivity predicted failure to respond to endocrine therapy: only 1 of 28 tumours (4%) classified as positive for both OeR and EGFR showed an objective response to endocrine therapy. The greatest response rate was observed among those classified as OeR positive/EGFR negative (Table 5). These

Table 4

Relationship between EGFR- and OeR status, summary of two studies

Study EGFR status	OeR positive (%)	OeR negative (%)	Total (%)
Sainsbury et al. (34)			
EGFR positive	1 (3)	12 (41)	13 (21)
EGFR negative	31 (97)	17 (59)	48 (79)
Total	32 (100)	29 (100)	61 (100)
Macias et al. (35)			
EGFR positive	11 (22)	9 (39)	20 (28)
EGFR negative	37 (78)	14 (61)	52 (72)
Total	49 (100)	23 (100)	72 (100)

Table 5

Objective response rate according to OeR and EGFR status. From Nicholson et al. (36)

EGFR status	OeR negative	OeR positive	Total
	Objective response rate (%)		
EGFR positive	1/28 (4)	2/7 (29)	3/35 (9)
EGFR negative	1/12 (8)	10/25 (40)	11/37 (30)
Total	2/40 (5)	12/32 (38)	14/72 (19)

observations suggest an inverse relationship between tumour growth regulation by steroid hormones and non-steroid hormone-regulated tumour growth achieved by autonomous secretion of growth factors.

Responses in OeR negative tumours

About 5–10% of OeR negative tumours show an objective response during endocrine therapy but the duration of the reported responses is not well known. It is also unclear if endocrine therapy prolongs the time to disease progression in OeR negatives. The clinical significance of objective responses among these patients thus remains unclear.

The mechanism of response among OeR negatives has mainly been studied for treatment with tamoxifen. Several hypotheses have been suggested (37, 38). Specific binding sites for anti-oestrogens (AEBS) have been described that may be present both in tumour cells as well as in surrounding stromal cells. Binding of tamoxifen to AEBS may result in secretion of growth inhibitory substances. High concentrations of anti-oestrogens may have direct cytotoxic effects on tumour cells. Tamoxifen has also been shown to have effects on the immune system, for instance increasing the activity of natural killer cells.

Androgen receptors

About 30–50% of all primary breast cancers exhibit specific androgen receptors (AR). There is a positive correlation between AR levels and both OeR and PgR levels (39). AR positive patients are more likely to respond to endocrine therapy than AR negative patients: Bryan et al. (40) reported that the objective response rate among AR positives was 54% compared to 14% among AR negatives. However, it is not known if AR status has a predicitive ability that is independent of OeR/PgR status. Therefore, the clinical relevance of AR assays remains controversial.

Clinical relevance of receptors in advanced disease

On the basis of receptor status patients with advanced disease can be classified into groups with a low, intermediate, or high probability of response to endocrine therapy (Table 6) but there is no consensus on the clinical relevance of such a classification. Many clinicians favour the use of endocrine therapy as the first-line salvage treatment even when the probability of a response is low. The rationale is that modern endocrine therapy of breast cancer is associated with only few and usually mild adverse side effects. Despite a relatively high objective response rate, chemotherapy of advanced breast cancer has not been associated with any major improvement of the overall survival and it is frequently associated with side effects that might decrease the quality of life. Moreover, responses with chemotherapy are usually shorter than those achieved with endocrine therapy. In view of these circumstances it might be argued that chemotherapy should be restricted to those patients who fail to respond to endocrine therapy.

On the other hand, hormone receptor status is the most important predictor of endocrine responsiveness and response to treatment is an important determinant of the quality of life of cancer patients. Therefore, therapy recommendations based on receptor status can be expected to minimize the proportion of patients whose quality of life is decreased due to an ineffective, first-line endocrine treatment. This argument is, admittedly, speculative and

Table 6

Probability of response to endocrine therapy in relation to receptor status

Low <5–10%	Intermediate 30–40%	High >40–50%
OeR[a]	OeR+	OeR++
OeR – EGFR+	OeR + PgR –	OeR + EGFR –
OeR – PgR –	OeR – PgR +	OeR + PgR +
OeR – AR –	OeR – AR + (?)	OeR + AR +

[a] Oe – EIA or ligand-binding assay: <5–15 fmol/mg protein or <0.05–0.15 fmol/μg DNA, OeR – ICA: <25–50% positive cells or a low 'staining index'.

the clinical relevance of hormone receptors in the management of patients with recurrent breast cancer remains controversial.

The clinical value of receptor status may depend on the routines for follow-up after primary treatment. If the follow-up includes extensive routine examinations for early diagnosis of recurrent disease many patients will be diagnosed with asymptomatic recurrences. In that setting it might be reasonable to use endocrine therapy in all patients, even if the probability of response is low. If the follow-up is by clinical examination alone most patients with recurrent disease will be diagnosed because of tumour-related symptoms. In that setting it appears reasonable to use chemotherapy in receptor negative patients in order to minimize the proportion of non-reponders to first-line salvage therapy.

Clinical relevance of receptors for adjuvant therapy

In many of the large randomized trials of adjuvant tamoxifen versus no adjuvant endocrine therapy, retrospective analysis have been done of the correlation between treatment effect and OeR content. Conflicting relationships have been reported. For instance, in the NATO-trial the effect of tamoxifen was the same irrespective of OeR level (41). In a Scottish trial the effect of tamoxifen was correlated with OeR level: the greatest benefit was observed among those with high OeR levels but there was some benefit also among those with a low receptor content (42). In contrast, in the Stockholm trial there was a significant correlation between OeR level and treatment effect: tamoxifen appeared to be ineffective among those classified as OeR negative (Table 7) (43). The Danish DBCG 77c trial also failed to show a treatment benefit for OeR negative patients. In that study only patients with a high OeR content appeared to benefit (44).

In the Stockholm trial the PgR status appeared to modify the effect of tamoxifen among the OeR positives but the PgR assay did not help to identify an unresponsive subgroup of patients: some effect was observed also among those classified as OeR positive/PgR negative (Table 8) (45).

It has been suggested that misclassification of patients in regard to OeR status may help to explain the results of the British trials. The OeR protein is sensitive and may become undetectable if the tumour specimen is not rapidly

Table 7

The number of treatment failures among 1 184 patients included in the Stockholm Adjuvant Tamoxifen Trial by OeR level and allocated treatment. From Rutqvist et al. (43)

OeR level[a]	Tamoxifen (n = 590)		Control (n = 594)		Rate ratio[b]	p[c] (logrank)
	No. of cases	No. of failures (%)	No. of cases	No. of failures (%)		
OeR−	117	39 (33)	118	34 (29)	1.16	n.s.
OeR+	229	45 (20)	246	74 (30)	0.62	0.01
OeR++	244	41 (17)	230	66 (29)	0.52	<0.01

[a] OeR−: <0.05 fmol/μg DNA, OeR+: 0.05 − 0.88 fmol/μg DNA, OeR++: >0.88 fmol/μg DNA.
[b] Tamoxifen allocated patients versus controls.
[c] n.s. indicates a p-value >0.05, test for trend in rate ratios for all patients: $p < 0.01$.

Table 8

The number of treatment failures among 737 patients included in the Stockholm Adjuvant Tamoxifen Trial by receptor status and allocated treatment. Results for patients classified as OeR negative/PgR positive are not included because of small numbers. From Rutqvist et al. (45)

Receptor status[a]	Tamoxifen (n = 359)		Control (n = 378)		Rate ratio[b]	p[c] (logrank)
	No. of cases	No. of failures (%)	No. of cases	No. of failures (%)		
OeR neg./PgR neg.	68	26 (37)	75	23 (31)	1.45	n.s.
OeR pos./PgR neg.	134	22 (16)	158	39 (25)	0.65	0.08
OeR pos./PgR pos.	157	18 (11)	145	33 (22)	0.48	0.01

[a] OeR/PgR negative: <0.05 fmol/μg DNA.
[b] Tamoxifen allocated patients versus controls.
[c] n.s indicates a p-value >0.05, test for trend in rate ratios for all patients: $p < 0.01$.

Table 9

Features of selected European adjuvant tamoxifen trials

Trial	No. with OeR (% of all patients)	No. of laboratories	Histological verification of specimens	OeR-neg[a] (%)	Effect of tamoxifen among OeR negatives
Stockholm (43)	1 184 (84)	1	Yes	20	no
DBCG 77c (44)	308 (18)	1	Yes	21	no
Scottish (42)	732 (56)	2	?	26	Yes
NATO (41)	525 (46)	7	No	37	Yes

[a] As defined by the authors.

chilled after excision. Moreover, when the assays are done in several laboratories there is a risk of significant inter-laboratory variation. Table 9 summarizes a few important features of the mentioned adjuvant trials. In the British trials the assays were done in several laboratories and the percentage of patients classified as OeR negative was generally higher than in the Scandinavian trials in which all assays were done in one laboratory. The higher percentages of OeR negative patients suggest that some OeR positives may have been classified as OeR negatives which may explain the observation of a treatment effect also in that subgroup.

One should perhaps expect a treatment benefit also among OeR negatives due to the observation that OeR negative patients with advanced disease occasionally may respond to endocrine therapy. It is possible that the Danish and Stockholm trials simply had too few OeR negative patients in order to observe a small benefit with adjuvant tamoxifen. In summary, prospective randomized trials are needed to ascertain the role of adjuvant tamoxifen in OeR negative patients. The acute toxicity of tamoxifen is low and the drug is generally well tolerated but reports of an increased frequency of thrombotic events and endometrial cancer associated with long-term treatment emphasize the need to select only those patients for which tamoxifen potentially might be effective.

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