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The Clinical Significance of Epidermal Growth Factor Receptor (EGF-R) in Human Breast Cancer: A Review on 5232 Patients*

J. G. M. KLIJN, P. M. J. J. BERNS, P. I. M. SCHMITZ, AND J. A. FOEKENS

Department of Medical Oncology (J.G.M.K., P.M.J.J.B., J.A.F.), Division of Endocrine Oncology, and Department of Statistics (P.I.M.S.), The Dr. Daniel den Hoed Cancer Center, P.O. Box 5201, 3008 AE Rotterdam, The Netherlands.

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I. Introduction

EPIDERMAL growth factor (EGF) is a 53-amino-acid polypeptide (mol wt 6.045 K) that can influence proliferation and differentiation of a wide variety of cells (1–6). EGF as well as transforming growth factor-α (TGF-α), both of which can activate EGF receptor (EGF-R), are probably produced locally in many tissues as local growth factors rather than as systemic hormones. There is evidence that EGF plays a role in carcinogenesis and that the EGF-stimulated growth regulatory system (apart from that of benign cells) is also involved in proliferation of malignant cells (3). Cellular events are induced by EGF via its cell membrane receptor (EGF-R). The EGF-R is a 170 K glycoprotein that can be divided into an extracellular domain binding EGF or TGF-α, a short transmembrane domain, and an intracellular domain carrying tyrosine kinase activity (7). This intracellular domain shows close sequence homology with the c-erbB-2 and with *neu* (8), the rat homolog of c-erbB-2 oncogene. Increased expression of the EGF-R gene has been found in a variety of tumors, generally indicating a more aggressive behavior of cancers compared to those with low or normal expression (9–10) although this association is not invariant (11). EGF-R has been identified by several methods including radioligand binding assays, autoradiography, immunocytohistochemistry, immunoenzymatic assays, and measurement of EGF-R transcripts.

EGF can stimulate the growth of normal mammary epithelium and human breast cancer cells *in vitro* (12, 13). Receptors for EGF have been demonstrated on several breast cancer cell lines, especially on estrogen receptor (ER)-negative tumor cells, and in human primary tumors and metastases (4, 13, 14). At present there is no agreement on the clinical relationships and prognostic value of EGF-R in human breast cancer. Therefore, the objective of this review is to examine the clinical associations reported with EGF-R and breast cancer in a large series of publications including those from our group.

II. Analytical Review Method

The source of the articles for this review was the computerized Medline/EBSCO data-base. By such ex-

* Supported by Grant RRTI 88-9 from the Dutch Cancer Society.
Address requests for reprints and all correspondence to: Dr. J. G. M. Klijn, M.D., Ph.D., Division Endocrine Oncology, Dr. Daniel den Hoed Cancer Center, Groene Hilledijk 301, 3075 EA Rotterdam, The Netherlands.

tensive review of the literature we selected all papers reporting relevant data on one or more clinical aspects of EGF-R in breast cancer. Most studies on prognostic factors such as EGF-R concern pilot studies (<150 patients), sometimes followed by more definitive studies by the same investigators (15). Several groups published more than one paper on this subject. Comparing the results of different groups of investigators, for each separate parameter, we have used the most representative series of patients reported in the most recent paper from each group containing the largest series of patients. Ultimately we found data on EGF-R status in tumors of 5232 patients reported by 40 different groups of investigators. With respect to some clinical relationships we combined the results of relevant studies, giving more weight to larger studies, in order to confirm significant relationships suspected in smaller individual studies. With respect to the relationship between EGF-R levels and prognosis as published by nine different groups of investigators, we have chosen to summarize these results in descriptive terms in view of the great differences in their procedural and statistical methods, chosen cut-off values for EGF-R positivity, and patient characteristics.

III. Results

A. EGF-R measurement and positivity

Several methods for identifying EGF-R in human breast cancer have been applied including ligand binding assays (14, 16–54), autoradiography (55–59), immunocytohistochemistry/immunoenzymatic assays (22, 25, 39, 43, 60–70), measurement of EGF-R transcripts (71–74), or EGF-R associated phosphotyrosine kinase activity (75) (Table 1). At present, there is no agreement on how best to define EGF-R positivity. Apart from different techniques, different cut-off levels have been used resulting in a wide variety of reported EGF-R positivity incidence ranging from 14–91% (Table 1). With Scatchard analysis the reported dissociation constant (K_d) values varied from 0.2–4.6 nM. Some authors (25, 51, 54) report a good correlation between Scatchard analysis and single saturation dose ("two-point") assays. With respect to tumor EGF-R content, median values were reported between 1.1 and 40 fmol/mg membrane protein, while levels of individual tumors ranged from 0–3600 fmol/mg protein (Table 1). In fact, while significant specific binding of EGF (41) and phosphotyrosine kinase activity (75) was shown in 91% of tumors, a high level of EGF-R determined by Scatchard analysis was present in only about a third of all tumors (41). EGF-R gene amplification is rare, occurring in 0–14% of breast cancers (71, 76, 77); however, EGF-R transcripts are demonstrable in 46–51% of primary tumors (71–74).

In 2500 (48%) of 5232 tumors investigated by 40 different study groups, EGF-R status was assessed as positive (Table 2). This percentage of 48% does not clearly differ from the mean (45%) of the EGF-R positivities reported by these individual study groups (Table 2). When comparing the results of different techniques it is striking to note that the average EGF-R positivity showed less difference between the respective techniques used, varying from 42% for immunological methods to 45%, 46%, and 48% for the radioligand binding assay, autoradiography, and measurement of EGF-R transcripts, respectively (Table 2). Only the single study detecting EGF-R associated kinase activity showed 91% positivity. There appears to be a close correlation between biochemical and immunological methods of detection of EGF-R (22, 25, 36, 43, 64). Grimaux *et al.* (64) and Koenders *et al.* (53) demonstrated no difference in results after either short- or long-term storage of tumors or membranes, respectively.

B. Relationship with other prognostic factors

1. Relationship with steroid receptor status. In contrast to two initial studies (14, 16) showing only a tendency to a negative relationship and one immunohistochemical study (66) of EGF-R, at least 28 different groups have reported a negative relationship between EGF-R and ER levels irrespective of the measuring technique used (Table 3). Interestingly, this was true despite great differences in reported ER-positive rates ranging from 34–82%. In general, EGF-R positivity was observed in 29–91% (mean: 59%) of ER-negative and in 4–51% (mean: 29%) of ER-positive tumors reported by 21 different laboratories (14, 21, 26, 35, 37–39, 41, 43, 44, 47, 48, 50, 52–54, 61, 64, 66, 67, 71).

With respect to the progesterone receptor (PR), most groups (12 of 19) have reported a negative relationship between EGF-R and PR levels (Table 3). In PR-negative tumors EGF-R positivity ranged from 23–91% (mean 51%) and in PR-positive tumors from 6–55% (mean 32%) (14, 35, 37–39, 41, 50, 53, 64, 66, 67, 71). In summary, mean EGF-R positivity is twice as high in ER or PR-negative tumors (59–51%) as compared to ER or PR-positive tumors (29–32%).

With respect to the ER-regulated proteins ER-D5 and P24, Horne *et al.* (78) failed to demonstrate any significant relationship between these proteins and either EGF-R or ER status.

Finally, although an association between EGF-R and total protein kinase C determined by phorbol-ester binding was not observed, Wyss *et al.* (31) demonstrated a negative relationship between total protein kinase C levels and steroid receptor levels in human primary breast cancer.

2. Relationship with age or menopausal status. Nine

groups of investigators have studied the relationship between age and tumor EGF-R levels. Seven groups (14, 33, 35, 39, 41, 44, 54, 64) found no correlation between age and EGF-R, while two groups (31, 47) reported a negative relationship. Grimaux *et al.* (39, 64) made similar observations for both the biochemical and immunological EGF-R assays. With respect to menopausal status, Sainsbury *et al.* (24) reported 45% and 30% EGF-R positivity in premenopausal and postmenopausal patients, respectively. Six other groups found no relationship between menopausal status and EGF-R levels (20, 35, 39, 41, 44, 66).

TABLE 1. EGF-R positivity in primary breast cancers measured by radioligand binding assay (RBA), autoradiography (AUT), immunocytochemistry or immunohistochemistry (IH), immunoenzymetric assay (IEMA), EGF-R transcripts (mRNA), and EGF-R associated phosphotyrosine kinase activity (PKA)

First author	Ref.	Year	n	Method	K_d (nM)	EGF-R content (fmol/mg)[e]			Cut-off (fmol/mg)	Positive (%)
						Mean	Median	Range tumors		
A										
1. Fitzpatrick	(14)	1984	137	RBA	2.0	8.4	3.2	0–121	1	48
2. Peyrat	(16)	1984	65	RBA	1.0				S.B > 1%[d]	48
3. a. Perez	(17)	1984	95	RBA	1.84	17.3	11.0	0–64	1	42
b. Skoog	(18)	1986	37	RBA	2.0			0–80[a]	10[a]	27
c. Macias	(20)	1987	72	RBA				0–120[a]	10[a]	27
d. Rios	(21)	1988	225	RBA					1	43
4. a. Sainsbury	(22)	1985	104	RBA	0.7–2.3			0–43	4	32
b. Sainsbury	(23)	1985	108	RBA				0–47	4 or S.B/ T>75%	42
c. Sainsbury	(24)	1987	135	RBA					10	35
d. Harris	(25)	1988	228	RBA	0.17–2.9			0–187	10	35
e. Nicholson	(26)	1988	246	RBA	2.0				10	32
f. Sainsbury	(27)	1988	264	RBA					10	33
g. Nicholson	(30)	1990	231	RBA					10	35
5. Wyss	(31)	1987	238	RBA			5.5	0–173	1.4	54
6. Pekonen	(33)	1988	171	RBA	1–3				S.B>2%	±15 (7–26)
7. a. Delarue	(34)	1988	100	RBA	3.9		4.0	0–210	5–10	22
b. Spyratos	(35)	1990	109	RBA	4.6	47			10	34
8. a. Battaglia	(36)	1988	55	RBA	2.0		1.1	0–55	1.5	42
b. Battaglia	(37)	1988	89	RBA			3.7	0–25	1.5	57
9. Cappelletti	(38)	1988	136	RBA	0.55	47	27	0–275	30	46
									45	32
10. Grimaux	(39)	1989	68	RBA		6	4	0–33	5	37
11. a. Foekens	(40)	1989	44	RBA	0.2		13	0–215	Signif S.B.	(70)
b. Foekens	(41)	1989	214	RBA	0.32		1.3	0–317	Signif S.B.	91
12. Fekete	(42)	1989	335	RBA	0.3	74			1	67
13. a. Toi	(43)	1989	50	RBA	0.67	8.3		0–35	1	36
b. Toi	(44)	1990	91	RBA					1	43
14. Bauknegt	(45)	1989	59	RBA					1	33
15. Zeillinger	(46)	1989	50	RBA					10	16
16. Costa	(47)	1989	68	RBA					10	16
17. Barker	(48)	1989	44	RBA				0–57	3	34
18. Llorens	(49)	1989	67	RBA				0–50	3	36
19. Bolla	(50)	1990	177	RBA	1.3				5	25
20. Etienne	(51)	1990	120	RBA	0.6		10	0–1448		79
21. Lefebvre	(52)	1990	55	RBA					"Non-occupied"	47
									"Total" EGF-R	76
22. Koenders	(53)	1991	531	RBA	0.5		40	3–3600	3	57
23. Bolufer	(54)	1990	220	RBA	2.5				0.5[e]	42
B										
24. a. Spitzer	(55)	1987	24	AUT			8.9	0–21[b]	1.2[b]	66
b. Spitzer	(56)	1988	50	AUT					1.0[b]	64
25. a. Reubi	(57)	1988	35	AUT						29
b. Reubi	(58)	1989	36	AUT						28

continued

TABLE 1. *Continued*

First author	Ref.	Year	n	Method	MoAb	Mean	Median	Range	Cut-off (fmol/mg)	Positive tumors (%)
						(fmol/mg[e] or score of staining)				
C										
26. a. Sainsbury	(22)	1985		I.H.	EGF-R1			0–3+		Correlated with RBA
b. Harris	(25)	1988	48	I.H.	EGF-R1			0–3+		48
27. Walker	(60)	1986	48	I.H.	EGF-R1			0–3+	≥2+	42
28. Wrba	(61)	1988	88	I.H.	EGF-R1			0–3+	≥1+	60
29. Betta	(62)	1989	54	I.H.	EGF-R1			0–3+		65
30. Toi	(43)	1989	50	I.H.	EGF-R1			0–2+	≥1+	34
31. Möller	(63)	1989	197	I.H.	EGF-R1			0–2+	≥1+	34
32. Grimaux	(64)	1990	220–280	IEMA	EGF-R1	18	13	0–100	≥20 fmol/mg	33 correlated with RBA
					EGF-R528					
33. a. Gasparini	(65)	1990	86	I.H.	EGF-R1					60
b. Bevilacqua	(66)	1990	134	I.H.	EGF-R1			0–2+	≥1+	51
34. Lewis	(67)	1990	90	I.H.	EGF-R1			0–3+	≥2+	14
35. Tsutsumi	(68)	1990	36	I.H.						50
36. Kommoss	(69)	1990	55	I.H.						60
37. Hainsworth	(70)	1991	71	I.H.	EGF-R1			0–4+		17
D										
38. Guerin	(71)	1989	221	EGF-R mRNA						46
39. Coombes	(73)	1990	107	EGF-R-mRNA						51
E										
40. Baugnet-Mahieu	(75)	1990	134	EGF-R PKA						91

[a] Atomoles/μg DNA.
[b] Femtomoles/mg tissue protein.
[c] Femtomoles/mg homogenate.
[d] S.B., Specific binding.
[e] Femtomoles/mg membrane protein.

3. Relationship with tumor size. Fourteen groups (20–21, 33, 35, 39, 41, 44, 47, 50, 54, 61, 62, 66, 67, 70) have found no significant correlation between tumor size and EGF-R levels. On the other hand, Sainsbury and associates (24) and Harris and Nicholson (25) reported a significant positive correlation between EGF-R and increasing tumor size. In addition, Spitzer *et al.* (56) found higher EGF-R levels in larger tumors (>4 cm). In contrast, Cappelletti *et al.* (38) observed that tumors larger than 3 cm were mostly EGF-R-negative, while smaller tumors (<3 cm) were almost equally distributed among the EGF-R-positive and -negative subsets. Bolla *et al.* (50) and our group (41) found a tendency to higher EGF-R levels in T1 tumors when compared to T2 and T3 tumors, respectively. Thus only two (25, 56) of 17 study groups found a positive correlation between tumor size and EGF-R levels, while three others (38, 41, 50) found a trend toward a negative relationship.

4. Relationship with lymph node status and recurrences. Reports on the relationship between EGF-R and lymph node status are contradictory. EGF-R relationship to nodal status can be expressed in different ways, *i.e.* 1) EGF-R positivity or levels in primary tumors correlated with number of lymph nodes involved by metastases, or 2) EGF-R positivity or levels measured in lymph node metastases compared with those measured in the primary tumors.

Sainsbury *et al.* (24) observed that EGF-R positivity in primary tumors is higher in patients with nodal involvement (N+) as compared to node-negative (N−) patients (55% *vs.* 30%). Battaglia *et al.* (37) also reported a higher incidence in N+ patients (77.7%) than in N− patients (25.7%). In addition, Bolufer *et al.* (54) found that nodal involvement correlated significantly with EGF-R status only in the ER+ tumor subgroup, but not in all tumors. In noninflammatory breast cancer Guérin *et al.* (71) found that the presence of EGF-R transcripts increased linearly with the number of positive lymph nodes. Hainsworth *et al.* (70) also found that EGF-R expression correlated with the number of involved lymph nodes. However, 15 groups found no significant correlation between EGF-R positivity in the primary tumor and the presence or number of axillary lymph node metas-

TABLE 2. Mean EGF-R positivity by method in 40 different series of patients

Method	No. of series (n)	A. Patients		B. EGF-R positivity (%)	
		n	EGF-R+	Mean	Range
RBA	23	3533	1723 (49%)	45	(15–91)
Autoradiography	2	86	42 (49%)	46	(28–64)
Immunologically	12	1151	451 (39%)	42	(14–65)
EGF-R transcripts	2	328	162 (49%)	48.5	(46–51)
EGF-R associated kinase activity	1	134	122 (91%)	91	
Total	40	5232	2500 (48%)	45%	(14–91)

Part A, Mean EGF-R positivity based on all individual patients included, giving more importance to larger series of patients than to smaller series.

Part B, Mean and range of EGF-R positivity based on the reported percentages for each separate series (irrespective the size of the series).

tases (16, 21, 33, 35, 39, 41, 44, 47, 50, 54, 56, 61, 62, 66, 67). Grimaux *et al.* (39), however, reported that EGF-R was often elevated in the tumors of patients with less than four involved nodes, while Toi *et al.* (43) observed a positive correlation between EGF-R status and lymphatic vessel invasion. Spitzer *et al.* (56) observed higher EGF-R levels in the primary tumors of patients with lymph node metastases than in those primary tumors that lacked lymph node metastases (8.1 *vs.* 3.4 fmol/mg, respectively), but this difference was not significant ($P < 0.1$).

With respect to EGF-R positivity and EGF-R levels in lymph node metastases or recurrences *vs.* those in the primary tumors, Sainsbury *et al.* (22) reported 71% positivity in the lymph node metastases *vs.* 32% in the primary tumors. Macias *et al.* (19) also reported a significantly higher incidence of EGF-R positivity in lymph node metastases (52%) or recurrences (47%) than in primary tumors (25–7%). Battaglia *et al.* (37) observed a median increase in EGF-R levels of 157% (range 27–590%) in lymph node metastases; overall, EGF-R levels were significantly ($P < 0.05$) higher in metastases than in primary tumors (median: 3.7 *vs.* 2.2 fmol/mg protein). Grimaux *et al.* (39) also found higher mean EGF-R levels in tumor-bearing nodes as compared to primary tumors (10.37 *vs.* 6.03 fmol/mg protein), but this difference did not appear to be statistically significant. These authors did observe a significant correlation between EGF-R levels in the nodes and primary tumors of 10 patients investigated.

In conclusion, nine (19, 24, 37, 39, 43, 54, 56, 70, 71) of 20 studies showed a positive relationship between EGF-R and lymph node status, but in only five (19, 24, 36, 70, 71) of these was this relationship statistically significant.

5. Relationship with histology. Fitzpatrick *et al.* (14) reported that [^{125}I]EGF binding approached a significant correlation with the percentage of malignant breast cells. Using immunohistochemistry Möller *et al.* (63) showed EGF-R expression in ductal, lobular, and myoepithelial cells, but only occasionally in stroma cells. In addition, Battaglia *et al.* (37) reported that the stroma was completely unreactive to monoclonal antibodies against EGF-R, while only malignant cells stained positive. Using autoradiography Spitzer *et al.* (55) also observed that fat and connective tissues were almost devoid of any EGF binding. On the other hand, Tsutsumi *et al.* (68) found that in a few carcinomas EGF-R positivity localized only to stromal cells. EGF-R levels may be decreased, increased, or unchanged in cancer cells as compared with their normal counterpart (3). Pekonen *et al.* (33) reported no difference in EGF-R binding in normal tissue from that found in cancer tissue, and the only normal tissue with high EGF binding was adjacent to a carcinoma.

Generally speaking, there seems to be no clear relationship between EGF-R and histological type of breast cancer (Table 4). A higher EGF-R positivity was found in ductal carcinomas as compared to lobular carcinomas by Skoog *et al.* (18) (36% *vs.* 0%), Sainsbury *et al.* (27) (34% *vs.* 21%), and Costa *et al.* (47) (23% *vs.* 6%), while Wrba *et al.* (61) (58% *vs.* 57%), Toi *et al.* (44) (43% *vs.* 33%), and Grimaux *et al.* (64) (31% *vs.* 42%) found no significant difference in EGF-R positivity between ductal and lobular carcinomas, respectively. Spitzer *et al.* (55) also found no significant difference in EGF-R levels between ductal and lobular carcinomas (9.7 *versus* 7.4 fmol/mg). EGF-R has been demonstrated in most subtypes of primary breast cancer (18, 27, 61, 64), although positivity of EGF-R seems somewhat lower in mucoid and tubular tumors (20%), which appears in agreement with the reported better long-term survival attributed to these well differentiated tumor types (79). As summarized in Table 4, EGF-R-positivity was frequently found in medullary carcinomas (88%), but Möller *et al.* (63), using immunohistochemistry, reported finding EGF-R in only one of seven patients with medullary breast tumors. EGF binding correlated with number of mitoses (55, 56) especially in node-positive patients (56), and EGF-R transcripts were more frequently detected in inflammatory breast cancer (58%) than in noninflammatory breast cancer (39%) (71). It is interesting to note that Reubi and Torhorst (57) reported high EGF-R density in necrotic tumor areas.

6. Relationship with tumor differentiation and grade. Seventeen groups studied the relationship between EGF-R and tumor differentiation and grade. Ten different groups (23–25, 33, 34, 39, 44, 50, 54, 56, 63, 64, 70)

TABLE 3. Relationship between steroid receptors (ER, PR) and EGF-R

First author	Ref.	Method for EGF-R	Relation with ER	% of EGFR+ ER-*vs* ER+ (%)	Cut-off for ER (fmol/mg)	ER+ Tumors (%)	Relation with PR	% of EGFR+ PR-*vs* PR+ (%)	Cut-off for PR (fmol/mg)	PR+ tumors
1. Fitzpatrick	(14)	RBA	NS	41 *vs.* 40	50		NS	43 *vs.* 38	50	
2. Peyrat	(16)	RBA	NS		10		NS		10	
3. a. Perez	(17)	RBA	Neg	51 *vs.* 33	10	51				
b. Skoog	(18)	RBA	Neg		0.4[a]	50				
c. Macias	(20)	RBA	Neg	39 *vs.* 22	0.1–0.3[a]	68				
d. Rios	(21)	RBA	Neg	54 *vs.* 29	10	45				
4. a. Sainsbury	(22)	RBA	Neg	61 *vs.* 8	5	51				
b. Sainsbury	(23)	RBA	Neg	71 *vs.* 6	5					
c. Sainsbury	(24)	RBA	Neg	61 *vs.* 6	5					
d. Harris	(25)	RBA	Neg	52 *vs.* 12	5	43				
e. Nicholson	(26)	RBA	Neg	53 *vs.* 13	5	46				
5. Wyss	(31)	RBA	Neg		10	76	Neg		10	65
6. Pekonen	(33)	RBA	Neg				Neg			
7. a. Delarue	(34)	RBA	Neg	50 *vs.* 13	10	76	Neg	37 *vs.* 11	10	61
b. Spyratos	(35)	RBA	Neg	52 *vs.* 22	10	61	Neg	43 *vs.* 19	10	39
8. a. Battaglia	(36)	RBA	NS	58 *vs.* 33	10	65	Neg	61 *vs.* 22	20	49
b. Battaglia	(37)	RBA	Neg	68 *vs.* 42	10	40	Neg	68 *vs.* 24	10	24
9. Cappelletti	(38)	RBA	Neg	73 *vs.* 35	10	70	Neg	65 *vs.* 37	25	63
				58 *vs.* 20[b]				49 *vs.* 23		
10. Grimaux	(39)	RBA	Neg	59 *vs.* 26	10	68	NS	41 *vs.* 32	10	50
11. a. Foekens	(40)	RBA	Neg	91 *vs.* 47	10	50	Neg	91 *vs.* 47	10	50
b. Foekens	(41)	RBA	Neg		10	78	Neg		10	56
12. Fekete	(42)	RBA	Neg		10	82	Neg		10	68
13. a. Toi	(43)	RBA	Neg	63 *vs.* 24	5	67				
b. Toi	(44)	RBA	Neg	72 *vs.* 24	5	60				
14. Costa	(47)	RBA	Neg	29 *vs.* 5	20	54				
15. Barker	(48)	RBA	Neg	57 *vs.* 17	5	52	NS		10	
16. Llorens	(49)	RBA	Neg			51	Neg			
17. Bolla	(50)	RBA	Neg	45 *vs.* 19	10	77	Neg	34 *vs.* 19	10	62
18. Etienne	(51)	RBA	Neg							
19. Lefebvre	(52)	RBA	Neg	65 *vs.* 37	10					
			NS	75 *vs.* 77[c]	10					
20. Koenders	(53)	RBA	Neg	85 *vs.* 46	10	72	Neg	72 *vs.* 49	10	65
21. Bolufer	(54)	RBA	Neg	48 *vs.* 31		34				
22. Wrba	(61)	IH	Neg	77 *vs.* 51	Erica 1-3[b]	65				
23. Betta	(62)	IH	Neg							
24. Toi	(43)	IH	Neg	69 *vs.* 18	Erica > 10%[b]	68				
25. Grimaux	(64)	IH	Neg	55 *vs.* 25	10	80	Neg	41 *vs.* 27	10	73
26. Bevilacqua	(66)	IH	NS	50 *vs.* 51	Erica > 5%	73	NS	46 *vs.* 55	Prica > 5%[b]	51
27. Lewis	(67)	IH	Neg	32 *vs.* 4	10	64	NS	23 *vs.* 6	10	54
28. Hainsworth	(70)	IH	Neg				Neg			
29. Guerin	(71)	mRNA	Neg	61 *vs.* 33		62	NS	50 *vs.* 35		46
30. Coombes	(74)	mRNA	Neg							
31. Baugnet-Mahieu	(75)	PKA	Neg							

See Table 1 for patient number, EGF-R cut-off levels and EGF-R positivity.

RBA, Radioligand binding assay; IH, immunocytohistochemistry; mRNA, EGF-R transcripts; PKA, EGF-R associated phosphotyrosine kinase activity; NS, not significant.

[a] Atomoles/μg DNA.

[b] Other cut-off levels for EGF-R (see Table 1).

[c] "Total" EGF-R.

demonstrated a positive relationship between EGF-R and grade, poorly differentiated tumors showing a higher percentage of EGF-R positivity and higher EGF-R levels. In contrast, eight groups (16, 35, 41, 60–62, 66, 67) did not find a significant correlation between these two parameters. Bolufer *et al.* (54) found a positive relationship within the ER-negative, but not the ER-positive subgroup.

7. Relationship with tumor ploidy. Seven groups (20, 35, 39, 47, 60, 64, 65) studied the relationship between EGF-R and tumor ploidy, also known as the tumor DNA-index or content as determined by flow cytometry. Only

TABLE 4. The EGF-R in different histological subtypes of mammary tumors

Type	Skoog (18)	Sainsbury (27)	Costa (47)	Wrba (61)	Toi (44)	Grimaux (64)	Overall
Ductal invasive	8/22 (36%)	81/239 (34%)	10/44 (23%)	31/53 (58%)	34/80 (43%)	69/220 (31%)	233/658 (35%)
Ductal *in situ*				2/3	2/4	7/17 (41%)	11/24 (46%)
comedo-type				3/4			3/4
Lobular invasive	0/9 (0%)	3/14 (21%)	1/18 (6%)	8/14 (57%)	2/6 (33%)	10/24 (42%)	24/85 (28%)
Lobular *in situ*					1/1	1/2	2/3
Mucoid/Colloid	0/4	1/3		2/3		2/10 (20%)	5/20 (20%)
Apocrine						1/2	1/2
Clear cell		0/1					0/1
Medullary	2/2			4/5		1/1	7/8 (88%)
Tubular		0/3		2/4		0/3	2/10 (20%)
Papillary						1/1	1/1
Carcinoid		0/1					0/1
Cystosarcoma		1/2				1/1	2/3
Fibrosarcoma		0/1		0/1			0/2
Fibroadenomas	0/3					2/2	2/5
Total:							293/831 (35%)

N.B. Spitzer *et al.* (55): mean EGF-R ductal ca (n = 16) = 9.7 fmol/mg
mean EGF-R lobular ca (n = 18) = 7.4 fmol/mg > NS

Walker and Camplejohn (60) found a significant correlation between these two parameters while six groups did not find any significant relationship (Table 5). All series showed a higher incidence of EGF-R positivity in aneuploid tumors (mean 36%; range 21–50%) than in diploid tumors (mean 13%; range 0–29%). Taking the absolute numbers reported in five studies together (Table 5), we calculated the presence of EGF-R positivity in 88 of 253 aneuploid tumors (35%) *vs.* only 17 of 114 diploid tumors (15%) [$P < 0.0001$, method of Der Simonian and Laird (79a), 1986]. Thus, most investigators did not find any significant correlation between EGF-R and DNA-index probably due to the small number of patients in their series of patients, but the overall incidence of EGF-R may be 2 to 3 times higher in aneuploid than in diploid tumors.

8. Relationship with parameters for cellular proliferation. Nine groups studied the relationship of EGF-R with thymidine labeling index (n = 1), S-phase fraction (n = 3), Ki-67 index (n = 4), or mitotic activity (n = 1). Macias *et al.* (20) found EGF-R positivity in 33% of 39 tumors with a high (>5%) labeling index *vs.* 13% of 16 tumors with a low (<5%) index, but this difference was not statistically significant. Walker and Camplejohn (60) demonstrated a close correlation ($P < 0.01$) between the presence of EGF-R and high (>14%) S-phase content in 48 tumors investigated, but Costa *et al.* (47) and Grimaux *et al.* (64) did not find a significant correlation in series of 28 and 60 tumors, respectively. With respect to Ki-67 immunoreactivity, Toi *et al.* (44) found that in 27 tumors the average proportion of Ki-67-stained cells in EGF-R-positive tumors was 25.4% in contrast to 8.6% in EGF-R-negative ones ($P < 0.01$). Three other groups (61, 62, 66) were not able to demonstrate a significant correlation between these two parameters in series of 88, 54, and 134 patients, respectively. Finally, Spitzer *et al.* (55, 56) reported a significant positive correlation between the number of mitoses counted under light microscopy and EGF binding, but this association was mainly in N+ patients (56). In sum, only three (44, 55, 60) of the nine studies reported any correlation between EGF-R and parameters relating to high cellular proliferation, but these series were all relatively small.

9. Relationship with oncogenes. Although EGF-R is structurally homologous to the c-erbB-2/*neu* oncogene (8) and both appear to be negatively correlated with steroid receptor status, there is no agreement on a (potential) positive association between EGF-R and c-erbB-2/HER-2/*neu*. Hainsworth *et al.* (70) and Marx *et al.* (80) did find a positive correlation between expression of EGF-R and c-erbB-2, but other reports (30, 46, 81, 82) indicate the opposite or no relationship. Tsutsumi *et al.* (68) observed a single case of breast cancer that overexpressed both EGF-R and *neu* in reciprocal immunohistochemical staining patterns, indicating that EGF-R positive cells were also not overexpressing the *neu* protein. Zeillinger *et al.* (46) investigated the relationship between HER-2 amplification, steroid receptors, and EGF-R in 291 primary breast cancers. HER-2 gene amplification was demonstrated in 18% of the tumors, in 14% of ER-positive and in 28% of ER-negative tumors. In this large series no association between HER-2 amplification and either EGF-R or androgen receptor was observed.

Hainsworth *et al.* (70) showed that EGF-R membrane

TABLE 5. Relationship between EGF-R status and DNA pattern

Authors (Ref.)	Absolute numbers EGF-R+	% EGF-R+	
	Aneuploid *vs.* diploid	Aneuploid *vs.* diploid	*P*
1. Macias (20)	15/46 *vs.* 3/24	33 *vs.* 13	NS
2. Walker (60)	19/38 *vs.* 1/10	50 *vs.* 10	$P < 0.05$
3. Spyratos (35)	29/78 *vs.* 9/31	37 *vs.* 29	NS
4. Grimaux (RBA) (39)	14/38 *vs.* 0/16	37 *vs.* 0	NS
5. Grimaux (IEMA) (64)	11/53 *vs.* 4/33	21 *vs.* 12	NS
6. Costa (47)			NS
7. Gasparini (65)			$P = 0.08$
Total	88/253 (35%) *vs.* 17/114 (15%)	$\bar{x}$ = 36% *vs.* 13%	$P < 0.0001$[a]

[a] Method of Der Simonian and Laird (79a).

staining was a much stronger prognostic indicator than c-erbB-2 product or *ras* p21 staining; in contrast, Wright *et al.* (82), applying multivariate analysis, demonstrated that both lymph node status and c-erbB-2 oncoprotein staining were more important prognosticators than EGF-R as measured by ligand binding assay.

Nicholson *et al.* (30) reported that patient prognosis based on *neu* was independent of EGF-R status and that the combination of *neu* positivity with EGF-R increased the prognostic power, showing an apparently additive effect in predicting a more aggressive course of disease. This is in agreement with recent experimental data showing that combinations of moderate levels of expression of EGF receptors and c-erbB-2, which are not individually capable of cell transformation, can together produce fully transformed cells (83, 84).

EGF-R was barely detectable in *ras* transfected cells, while *myc* transfected cells were much more sensitive to the growth-stimulatory effects of EGF without an increase in EGF-R levels as compared to nontransfected cells (6, 25).

10. Relationship with other growth factor or peptide hormone receptors. In agreement with the results of Pekonen *et al.* (33) we did not find an association between EGF-R and insulin-like growth factor I receptor levels (41). In addition, Peyrat *et al.* (16) and Fekete *et al.* (42) demonstrated no relationship with tumor PRL-R concentrations and [D-Trp^6]-LHRH binding sites, respectively.

Reubi *et al.* (59) discussed an inverse relationship between the presence of somatostatin receptors (SS-R) and EGF-R measured by autoradiography. They demonstrated the presence of EGF-R in only 18 of 71 (25%) SS-R-positive primary breast cancers (57, 58). The tissue location of SS-R did not coincide with that of EGF-R (59). Whereas SS-R were located on tumor tissue, EGF-R were often seen on adjacent normal lobules and ducts. On the other hand, using a biochemical assay for EGF-R, we did not observe a significant correlation between EGF-R and SS-R (41). Also, Fekete *et al.* (42) found no relationship between EGF-R and SS-R both measured by Scatchard analysis.

11. Relationship with membrane-bound tissue plasminogen activator. There is a large amount of evidence that plasminogen activators (PA) are involved in tumor invasion and metastasis. Harris and Nicholson (25) divided 43 breast cancers into ER+/EGF-R−, ER-/EGF-R+, and ER-/EGF-R− subgroups. While there was no significant difference in total PA between these groups, tissue PA (tPA) was significantly lower in ER-/EGF-R+ tumors. The lack of PA appeared to be associated with an aggressive group of tumors, and it was suggested that EGF-R might have a role in suppressing tissue PA secretion (25).

C. Relationship with prognosis and survival

1. Relationship with relapse-free (RFS) and overall survival (OS). Thus far nine different groups (20–21, 24, 30, 32, 35, 39, 41, 44, 67, 72–74) have reported on the relationship between EGF-R and survival. The size of the series of patients investigated ranged from 55–376 with EGF-R positivity in 14–55% (Table 6). The median (12–66 months) and maximal (30–96 months) follow-up showed great differences. In the majority of the studies systemic adjuvant therapy was given in a number of patients, mostly in the node-positive group. Using univariate analysis, five groups (21, 24, 30, 32, 39, 67) found a significant relationship between EGF-R status and RFS or OS at a certain time point of follow-up (Table 6). This finding was confirmed in two of three studies using multivariate analysis.

Sainsbury *et al.* (24) indicated that by multivariate analysis EGF-R status was the most important variable in predicting RFS and OS in lymph node-negative patients and the second most important variable in lymph node-positive patients. RFS and OS were significantly worse for patients with EGF-R-positive tumors as compared to patients with EGF-R-negative tumors. The best

TABLE 6. Relationship between EGF-R and relapse free (RFS) and overall survival (OS)

First author	Year	Ref.	n	Systemic adjuvant therapy	EGF-R+ (%)	Follow-up (months)		Significance of EGF-R				Subgroups			
						Median	Maximal	Univariate		Multivariate		N−	N+	ER−	ER+
								RFS	OS	RFS	OS				
1. a. Sainsbury	1987	24	135	None	35	33	42	$P < 0.001$ (39 *vs.* 76%)°	$P < 0.001$	$P < 0.001$	$P < 0.025$	$P < 0.01$	NS	$P < 0.025$	
b. Nicholson	1990	30	231	None	35	45	60	$P < 0.001$	$P < 0.001$			$P < 0.01$		$P < 0.1$ (RFS) $P < 0.05$ (OS)	$P < 0.05$
2. a. Macias	1987	20	77	N+, T > 3 cm	27		72	NS (50 *vs.* 60%)°				NS			
b. Rios	1988	21	179		43	±15	30	$P < 0.05$						NS	$P < 0.05$
3. Costa	1988	32	376	N+ (n = 180)	±50	12		$P < 0.01$				$P < 0.08$		$P < 0.09$	
4. Foekens	1989	41	203	N+ (n = 52)	>0.5ˣ = 67 >2.0ˣ = 34	42	70	$P = 0.09$		$P = 0.08$		NS	$P < 0.1$	NS	$P < 0.1$
5. Grimaux	1989	39	55	N+ (=all)	37	66	92	NS	$P = 0.05$*					$P = 0.07$* (RFS) $P = 0.12$ (OS)	
6. Spyratos	1990	35	109 75N−	N+ (n = 34) None	34	60	96	NS ($P = 0.29$)				$P = 0.14$ $P = 0.05$	NS		
7. Lewis	1990	67	90	None	14	±18	36	$P < 0.003$		$P = 0.04$				$P = 0.02$	
8. Coombes	1990	73, 74	64–107		51–55	42	72	NS ($P = 0.79$)	NS ($P = 0.86$)						
9. Toi	1990	44	91	N+	43	25	40	?(77 *vs.* 94%)°							

None, no systemic adjuvant therapy; N−, node-negative patients; N+, node-positive patients.
* = only at 40 months, not at 90 months
° = RFS for EGF-R+ vs EGF-R − patients
ˣ = fmol/mg

discriminative effect was found in subgroups with lymph node-negative or ER-negative tumors. In contrast, we found only a tendency (P 0.09) for a negative relationship between EGF-R and RFS (41). Macias *et al.* (20) initially reported a 6-yr relapse rate in 10 of 20 (50%) patients with EGF-R positive tumors *vs.* 22 of 52 (40%) patients with EGF-R negative tumors, but this difference was not statistically significant. They later observed a significant relationship in a larger series of patients with shorter follow-up interval (21). Grimaux *et al.* (39) reported a nearly significant prognostic value (P 0.051) when overall survival curves were analyzed at 40 months follow-up [which is comparable to the follow-up duration in the study of Sainsbury (24)], but EGF-R failed to predict long-term outcome. Spyratos *et al.* (35) also found that EGF-R had no predictive value for long-term outcome. Two other studies, by Costa *et al.* (32) and Lewis *et al.* (67), reported a significant prognostic relationship, but their patient follow-up period was very short. Toi *et al.* (44) observed a relapse in 23% of EGF-R-positive patients and only 6% in EGF-R-negative patients, but the statistical significance in this was not indicated. Coombes *et al.* (74), measuring transcripts for EGF-R, found no relationship with relapse-free survival.

In contrast to Sainsbury *et al.* (24) we found the best discriminative effects of EGF-R to be in lymph node-positive or ER-positive patient subsets (41). Lewis *et al.* (67), in agreement with Sainsbury's results (24), found a significant discriminative effect of EGF-R in the ER-negative subgroup of patients, but the other groups of investigators (20, 21, 32, 35, 39, 41) did not make such an observation, although a tendency to such relationship was present in two (32, 39) of these studies (Table 6). Rios *et al.* (21) found only a significant discriminative effect of EGF-R in ER-positive patients. EGF-R+/ER− patients showed the poorest and EGF-R-/ER+ patients the best survival probability (21, 39) due, no doubt, to the better discriminative effect of EGF-R in ER+ patients (21). Lymph node status and steroid receptor content appeared to be better prognosticators than EGF-R (20, 41), but in a subgroup of 55 node-positive patients Grimaux *et al.* (39) reported that EGF-R was the only biological parameter to reach statistical significance in predicting for early death. Macias *et al.* (20) also reported that within node- positive patients, EGF-R had prognostic value that was not present within the node-negative patient subgroup. Furthermore, Spyratos *et al.* (35) and Costa *et al.* (32) found no significant prognostic effect in node-negative patients. However, when, in the former study (35), patients receiving prior adjuvant therapy were excluded, EGF-R appeared as the only significant prognostic variable (P 0.05). For those studies (24, 30, 67) in which systemic adjuvant therapy had not been given, the best discriminative effect of EGF-R was observed.

In summary, five (21, 24, 32, 39, 67) of nine study groups have shown significant prognostic value for EGF-R and survival after short-term (1–4 yr) follow-up, while three (20, 39, 41) of five groups (20, 35, 39, 41, 74), having a maximal follow-up interval of at least 6 yr, found only a tendency ($0.10 > P > 0.05$) associating EGF-R with survival. It appears that EGF-R-positive tumors are more prone to first relapses at visceral sites (24, 28, 44, 54), while EGF-R-negative tumors more often recur in the bone.

2. Relationship with response to endocrine and chemotherapy. Nicholson *et al.* (28, 30) reported that expression of EGF-R is associated with lack of response to endocrine therapy in recurrent breast cancer. Only 8% of EGF-R-positive tumors showed an objective response to first-line treatment with tamoxifen in metastatic breast cancer while 30% of EGF-R-negative tumors responded ($P < 0.05$). Only one of 28 EGF-R +/ER− tumors achieved an objective response. Patients with EGF-R-positive primary tumors showed more rapid disease progression after start of first-line endocrine therapy than those with EGF-R-negative tumors. In one preliminary study, Harris (29) observed that in 25 patients, who received first-line single chemotherapy with mitoxantrone, there was no correlation between EGF-R status and response to therapy, time to tumor progression, or survival.

IV. Summary

EGF-R positivity was shown to be present in 2500 (48%) of 5232 breast tumors in 40 different series of patients. The mean of the percentages of EGF-R positivity in the individual series reported by these 40 different groups of investigators is 45% (range 14–91%). Overall there are generally no clear differences between results obtained by radioligand binding assays, immunological methods, autoradiography, and measurement of EGF-R transcripts although the mean percentage of EGF-R-positive tumors determined by immunological methods tends to be somewhat lower. Nearly all studies indicate a negative relationship between EGF-R and steroid receptor status (28 of 31 studies for ER, 12/19 for PR) showing that EGF-R positivity is twice as high in ER or PR- negative tumors compared to ER or PR- positive tumors (~50–60% *vs.* 30%). With regard to other prognostic factors the majority of investigators (10/18) also reported a significant (positive) correlation with tumor grade, but only a minority found a significant relationship between EGF-R status and patient age (2/9), menopausal status (1/7), histological type (3/7), tumor size (2/17), nodal status (5–9/20), ploidy (1/7), or proliferation indices (3/9). No relationship was observed with tumor insulin-like growth factor I receptor, PRL receptor (PRL-R), and LHRH receptor (LHRH-R) status, but an

inverse relationship between EGF-R and somatostatin receptor may be present. However, it has to be stressed that the series in which the relationship between EGF-R status and other prognostic factors were investigated, contained relatively few patients (mostly <100). Therefore, when larger groups of patients are investigated, more significant relationships may be observed, especially with respect to nodal status, tumor ploidy, and proliferation indices. In fact, we calculated the presence of EGF-R positivity overall in 35% of 253 aneuploid tumors *vs.* in only 15% of 114 diploid tumors ($P < 0.0001$). In addition most studies observed a trend, if no significant correlation, between higher EGF-R levels in tumors with the highest percentages of S-phase or Ki-67 expression. With regard to relapse-free and overall survival, five of nine different groups of investigators showed significant prognostic value of EGF-R after short-term (1- to 4-yr) follow-up, indicating that patients with EGF-R-positive tumors have a poor prognosis. However, three of five groups with a maximal follow-up of at least 6 yr found only a tendency for any relationship between EGF-R status and long-term outcome.

V. Discussion and Conclusions

The efficacy and cost effectiveness of adjuvant systemic therapy in women with primary breast cancer are important subjects of current debate (85, 86). More selective use of chemotherapy to maximize the benefit to individual patients may be possible with refinements in risk stratification and better assessment of the patients' risk preferences. There are numerous prognostic factors reported for primary breast cancer (see review in Ref. 87). EGF-R is a more recent tumor marker whose prognostic value has been well studied but remains controversial. This may be due to differences in techniques used for EGF-R measurements or cut-off levels chosen for EGF-R positivity. However, we could find no clear differences between the results obtained by radioligand binding assay, immunological methods, autoradiography, or measurement of EGF-R transcripts. The slighty lower EGF-R positivity using immunological methods might by explained by a lower assay sensitivity than for ligand binding assays, accounting for EGF-R-positive tumors by ligand binding assay that are negative by immunohistochemical analysis (25). In some cases EGF-R was undetectable by ligand binding or immunochemistry but could be detected by enhancement of autophosphorylation with EGF (25).

Despite the great variation in cut-off levels for EGF-R positivity, nearly all investigators report a negative relationship between tumor EGF-R status and steroid receptor levels. Although ER levels are positively correlated with age (87, 88), there is an absence of such a relation between EGF-R and age or menopausal status, as observed by nearly all investigators. We also found a negative relationship between cytosolic EGF/TGF-α-like activities [growth factors that can influence the number of detectable EGF-R (89, 90)] and steroid receptor levels in breast cancer (40). Macias *et al.* (91) showed higher TGFα contents in metastatic than in primary breast cancer, but, in contrast to our findings, did not find a correlation between tumor TGFα content and ER levels. Measuring gene transcripts of EGF, ER, and PR, Dotzlaw *et al.* (92) recently showed that EGF was more frequently detectable in ER or PR-positive tumors than in ER or PR-negative breast tumors. They also demonstrated a positive association between EGF messenger RNA and steroid receptor levels. However, Barrett-Lee *et al.* (72) and Coombes and co-workers (73, 74) observed no association between TGFα mRNA and survival.

The results correlating EGF-R status and RFS are very controversial: some groups have found a very close correlation, while others have found only a tendency or no significant relationship between these parameters. Comparing EGF-R status with previously validated prognostic indices, Hainsworth *et al.* (70) observed a significant association, but Lewis *et al.* (67) did not. Sainsbury *et al.* (24) found by multivariate analysis that EGF-R status was the most important variable in predicting RFS and OS in lymph node-negative patients and the second most important variable in lymph node-positive patients. Spyratos *et al.* (35) noted that although EGF-R status had overall no significant prognostic value, it was the only significant prognosticator in node-negative patients, while Lewis *et al.* (67) stated that EGF-R was the third most important prognosticator after nodal status and grade. However, with respect to subgroup analysis by nodal or ER status, there is no agreement on the best prognosticator and in which subgroup EGF-R status offers the most predictive power. The differences in results and conclusions might partly be due to differences in patient number, cut-off levels for both EGF-R and ER, application of systemic adjuvant therapy, and duration of patient follow-up—generally concerns affecting the evaluation of other prognostic factors (15). In view of the prominent predictability value of EGF-R status in those series in which patients were not treated with adjuvant therapy, and the observed association between tumor EGF-R positivity and lack of response to endocrine therapy (but not to chemotherapy) in patients with metastatic disease, the overall influence of adjuvant systemic treatment on the prognostic power of EGF-R status has yet to be established (especially for long-term outcome).

Furthermore, it has to be stressed that the size of all reported series are generally small, ranging from 20 or 30 to a few hundred patients. Only three studies (30, 32,

41) included more than 200 patients. Furthermore, apart from assessment by *P* values in univariate and multivariate analysis in relation to RFS and OS, the potential impact of a prognostic factor should preferably also be expressed by positive and negative predictive values and their differences. For example Nicholson *et al.* (30) report a 5 yr RFS of approximately 20% (derived from the figure) for patients with EGF-R+ tumors (positive predictive value at 5 yr) and a 5 yr RFS of 38% for patients with EGF-R-tumors (negative predictive value at 5 yr of follow-up). The difference of these values (18%) is a measure of the impact of the prognostic value of EGF-R. Similarly, Foekens *et al.* (41) have shown a 5 yr RFS of approximately 43% and 67% for patients with low or high and intermediate EGF-R tumor values, respectively, thus a difference of 24%. Unfortunately, in the other studies indicated in Table 6, it appears difficult to evaluate the prognostic value of EGF-R other than in terms of significant *P* values. Therefore, to make definite conclusions about the relationships and predictive value of EGF-R status, larger confirmatory studies with long-term follow-up periods are warranted.

It would also be essential to have a standardized EGF-R assay. In view of the lack of a uniform method for EGF-R measurement and uniform criterion for positivity (cut-off point), the authors propose standardization of EGF-R assays. In this respect it is noteworthy to mention that the EORTC Receptor Study Group has recently declared the hydroxylapatite method as the method of choice to separate bound from free ligand (93). After minor modification, this HAP-assay adopts a membrane protein threshold of 0.2 mg/ml in order to avoid false-negative results due to too low protein content (53). A clinically valuable significant cut-off level for positivity of EGF-R using this assay has yet to be determined by taking length of RFS and OS as endpoints. For smaller tissue samples or cytological samples, autoradiography or immunoassays may be used. Apart from the consensus reached by the EORTC Receptor Study Group to use HAP adsorption of EGF-R as a tool to separate bound from free ligand by low-speed centrifugation procedures, more work has to be done before the EGF-R assay will be completely standardized. Major aspects that need standardization include the membrane preparation to be used and the reference parameter on which to express EGF-R (*e.g.* protein, DNA, wet weight, or a membrane marker such as 5′-nucleotidase).

In view of the close similarity between EGF-R (c-erbB-1) and HER2/*neu* (c-erbB-2) oncogene protein, it is interesting to compare the clinical relationships and prognostic value of these two membrane markers. For the EGF-R gene a great difference has been found between the incidence of gene amplification (0–14%) (71, 76, 77) relative to protein (over)expression (about half of the patients), in contrast to HER2/*neu* in which there is a strong correlation between amplification and overexpression. Amplification and/or overexpression of the HER2/*neu* gene has been described in approximately 20% of breast tumors (range 8–64%) (94–100). In our most recent update (100) of the literature, data encompassing a total of 11,408 breast tumors, we calculated a mean HER2/*neu* positive incidence of 20% without finding any significant difference between the incidence of amplification and overexpression. This means that EGF-R-positive tumors relate to a greater proportion (45%) of breast cancer patients than do HER2/*neu* positive tumors (20%), which might suggest an advantage for the measurement of EGF-R as prognostic parameter. The prognostic value of HER2/*neu* amplification in breast cancer has been studied extensively since the initial report by Slamon *et al.* (101), showing that HER2/*neu* amplification is an independent prognostic factor in node-positive patients. Recent reviews (94–99) suggest that there is no association between elevated HER2/*neu* and patient age, only a tentative relationship with tumor grade, size, or nodal involvement, and an inverse association with steroid hormone receptors. There is no consensus on the independent prognostic power of HER2/*neu* to predict either RFS or OS. Most interestingly, these relationships and conclusions are similar and comparable to those described for EGF-R in this review. Few authors (70, 82) have compared the prognostic power of EGF-R and HER2/*neu* in the same study, and these show conflicting results (see *Section B.9*).

Conclusions

Based on 40 separate studies comprising 5232 patients, the mean percentage of EGF-R positivity reported in breast cancer is 45% (range 14–91%).

Overall, there is no clear difference in results between radioligand binding assays, immunological methods, autoradiography, and measurement of EGF-R transcripts (mean EGF-R positivity for respective techniques ranging between 42–48%), although EGF-R positivity by immunological methods tends to be lower.

In view of the lack of an uniform method for EGF-R measurement and uniform criterion for positivity (cut-off point), the authors propose standardization of EGF-R assays as established by the EORTC in order to employ such standard methods for clinical trials involving EGF-R.

Correlations with other prognostic factors and with prognosis in lymph node-negative and positive disease can be made based on the current literature but will likely be more clear if such standard methodology is accepted, as occurred for ER and PR.

There is a negative correlation between EGF-R and

steroid receptor (ER, PR) levels showing that EGF-R-positivity is twice as high in ER- or PR-negative tumors than in ER- or PR-positive tumors (~50–60% *vs.* 30%).

There is no relationship between EGF-R status and age or menopausal status.

There is no clear association between EGF-R and tumor size.

Some authors suggest that there is a higher incidence of EGF-R positivity for primary tumors in patients with nodal involvement or higher EGF-R levels in lymph node metastases/recurrences as compared to primary tumors, but other studies disagree.

There may be higher EGF-R positivity in ductal carcinomas than in lobular carcinomas, but this is not a uniform observation.

There is a likely association between high EGF-R levels and poor tumor differentiation and grade.

When the results of several studies are combined there appears to be an association between a higher (2- to 3-fold) incidence of EGF-R positivity and aneuploid tumors as compared to diploid tumors.

A few studies indicate a positive correlation between EGF-R and higher rates of breast cancer proliferation.

There is no relationship between EGF-R and IGF-1-R, PRL-R, or LHRH-R, but possibly an inverse relationship with SS-R.

There is little agreement on the prognostic value of EGF-R, with most studies indicating a tendency or weak association between EGF-R and RFS or OS. There is also no agreement on the subgroups of patients in which EGF-R may have a discriminative prognostic effect. The discriminatory effect of EGF-R status with respect to prognosis seems to decrease with long-term follow-up compared to short follow-up as previously shown and discussed by some authors (88, 102) for the ER status.

Patients with advanced disease and EGF-R-positive tumors respond less well to first-line endocrine treatment as compared to EGF-R-negative tumors, but the significance of this independence of ER status is unknown. There is no apparent relationship between EGF-R status and response to first-line chemotherapy, but this has not been well studied.

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