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Review

ERBB2 Oncogene in Human Breast Cancer and its Clinical Significance

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We reviewed the relationships between *ERBB2* amplification and/or overexpression in human breast cancer and the clinicopathological parameters described in the literature (97 studies involving 22 616 patients) in order to draw conclusions regarding its clinical interest. The mean of *ERBB2* positivity (26%, ranging from 5 to 55%) is not dependent on the method used to evaluate *ERBB2* amplification or overexpression. Despite the discrepancies observed between the different studies, several associations between *ERBB2* positivity and the classical clinicopathological parameters were noted. There are clear relationships between *ERBB2* positivity and the lack of steroid receptors, the histological subtypes of mammary tumours (ductal invasive and *in situ*), worse histological and nuclear grades, aneuploidy and high rate of proliferation. In univariate analyses, *ERBB2* is strongly associated with poor prognosis. All these data indicate that *ERBB2* is a marker of aggressiveness of the tumour. However, *ERBB2* does not retain a clinical prognostic significance in multivariate analyses, since it is associated with several strong prognostic parameters. When considering the prognostic value of *ERBB2* in relation to treatment, a significantly worse survival of the treated patients is noted in *ERBB2* positive patients. This suggest that *ERBB2* could be a marker of reduced response to chemotherapy and hormonal treatment. With respect to the tumour response to treatment, the results, provided as yet by pilot studies, remain controversial and further investigations are necessary to evaluate the predictive value of *ERBB2*. Finally, new therapeutic approaches targeting the cells overexpressing *ERBB2* have been developed. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: *ERBB2*, breast cancer, prognostic value, response to treatment
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INTRODUCTION

THE *ERBB2* oncogene [1, 2], also called *HER2/neu* [3], is the human homologue of the *neu* oncogene identified in DNA from rat neuroglioblastomas induced by ethyl-nitrosourea [4]. Located on chromosome 17q, the gene encodes a trans-membrane glycoprotein (p185) with tyrosine kinase activity, which is closely related to epidermal growth factor receptor (EGFR) [5]. These proteins, together with those encoded by *ERBB3* (*HER3*) [6, 7] and *ERBB4* (*HER4*) [8], constitute the type I growth factor receptor gene family [9, 10].

All four members of the family are expressed in breast cancer cells *in vitro*. For primary breast cancer, since increased levels of EGFR [11] and *ERBB2* [12] were first reported, several thousand cases have been studied and the clinical significance of EGFR has been extensively examined [13]. Elevated expression of *ERBB3* has been observed [14–16], but until now this has been poorly documented. Although isolated from breast cancer cells, *ERBB4* expression has not yet been assayed in breast cancers.

Despite the numerous studies, the prognostic significance and the value of *ERBB2* in predicting the response to treatment remain somewhat unclear. Here we review the biological and clinical data on *ERBB2* in breast cancer and discuss the clinical usefulness of this parameter.

ANALYTICAL REVIEW METHOD

For this paper, the review of Klijn and associates [13] on EGFR in breast cancer, was used as a model. We selected the

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papers reporting relevant data on one or more of the clinical aspects of *ERBB2* in breast cancer. The source of the articles was the Cancerlit database. When the same group published several papers with increasing numbers of patients, we used the most recent paper. We found 97 different studies involving 22 616 patients. The relationship between *ERBB2* and prognosis was described in 34 different studies. Given the great differences in their procedural and statistical methods, cut-off values for *ERBB2* positivity and patient characteristics, we decided to summarise these results in descriptive terms. We gave priority to the conclusions based on the larger number of patients, but when studies with numerous patients (> 150) provided a conclusion different from the general conclusion, this was specified.

ERBB2 ACTIVATION IN HUMAN BREAST CANCER

In human breast cancer, activation of the *ERBB2* proto-oncogene by translocation has not yet been described, and rearrangements of *ERBB2* have rarely been observed [12]. Activating *trans*-membrane point mutations, observed in the rat homologue *neu* oncogene [17], have not been found in human breast cancer [18]. The main mechanism of *ERBB2*

Table 1. ERBB2 *amplification in primary breast cancer measured by Southern blot (SB) and polymerase chain reaction (PCR)*

First author [Ref.]	Year	*n*	Method used	Amplification range	Positive tumours *n* (%)
Cline [24]	1987	53	SB	3–10-fold	8 (15)
Slamon* [12]	1987	189	SB	2–> 20-fold	53 (28)
Varley [25]	1987	41	SB	5–40-fold	7 (17)
Venter [26]	1987	36	SB	2–> 100-fold	12 (33)
Zhou* [27]	1987	86	SB	3–30-fold	15 (17)
Berger [28]	1988	51	SB	2–15-fold	13 (25)
Fontaine [29]	1988	15	SB	2–14-fold	7 (46)
Guérin* [20]	1988	116	SB	2–> 30-fold	23 (20)
Adnane [30]	1989	292	SB	2–30-fold	61 (21)
Guérin* [31]	1989	221	SB	3–30-fold	60 (27)
Gutman [32]	1989	36	SB	8 and 12-fold	2 (5)
Ro [33]	1989	66	SB	2–> 8-fold	13 (20)
Roux-Dosseto [34]	1989	170	SB	2–> 20-fold	53 (31)
Seshadri [35]	1989	73	SB	2–> 5	17 (23)
Slamon [21]	1989	526	SB	2–> 20	146 (27)
Zeillinger [36]	1989	291	SB	2–> 20	52 (17)
Zhou [37]	1989	157	SB		17 (11)
Borg* [38]	1990	310	Slot blot	2–< 20	52 (18)
Brouillet [39]	1990	140	SB	≥ 2	32 (23)
Heintz [40]	1990	50	SB	2–> 50	17 (34)
Kury* [41]	1990	77	SB	2–> 5	24 (31)
Meyers [42]	1990	99	SB	2–> 30	9 (9)
Borg* [43]	1991	539	SB	2–> 30	102 (19)
Clark* [44]	1991	362	SB	2–> 20	119 (33)
Dati [22]	1991	77	SB	2–> 15	19 (25)
Olsson [45]	1991	72	SB	3–12	22 (31)
Tommasi [46]	1991	107	SB	≥ 2	34 (32)
Berns [47]	1992	1052	SB	3–43	197 (19)
Ciocca [48]	1992	1103	SB		231 (21)
Tiwari [49]	1992	61	SB	2–7	17 (28)
Todd [50]	1992	1532	Slot blot		217 (14)
Descotes [51]	1993	149	SB		28 (19)
Gaffey [52]	1993	50	SB	2–10	13 (26)
Henry [53]	1993	103	SB	2–49	28 (27)
Keith [54]	1993	50	SB	5–7	6 (12)
Knyazev [55]	1993	60	SB	3–> 15	15 (25)
Kreipe [56]	1993	60	SB	1.8–19	16 (27)
Smith [57]	1993	117	SB	2.3–58	25 (21)
Borg [58]	1994	783	SB	2–30	147 (19)
Odagiri [59]	1994	41	SB		11 (27)
Prost [60]	1994	178	SB	3–30	30 (17)
Ito [61]	1994	494	SB		103 (20)
Lönn [62]	1992	39	PCR	2–> 10	7 (19)
Liu [63]	1992	122	PCR	2–> 8	26 (21)
Hubbard [64]	1994	287	PCR		158 (55)
Sestini [65]	1994	22	PCR		7 (32)
An [66]	1995	195	PCR	2–9	52 (26)

*Studies not included in Table 3 as there is evidence that the patients are the same as in another referenced study.

Table 2. ERBB2 *overexpression in primary breast cancer measured by Northern blot (NB), fluorescence* in situ *hybridisation (FISH), Western blot (WB), flow cytometry (FC) and immunohistochemistry (IHC; P, paraffin embedded; F, frozen sections; MAb, monoclonal antibody; pAb, polyclonal antibody)*

First author [Ref.]	Year	*n*	Method used	Antibody used	Positive tumours *m* (%)
Rio [67]	1987	114	NB		23 (20)
Guérin* [20]	1988	116	NB		42 (36)
Guérin* [31]	1989	126	NB		49 (39)
Slamon* [21]	1989	187	NB		69 (37)
May [68]	1990	89	NB		20 (23)
Delvenne [69]	1992	47	NB		16 (34)
Kallioniemi [70]	1992	44	FISH		10 (23)
Tandon [71]	1989	728	WB		118 (16)
Borg* [38]	1990	360	WB		68 (19)
Giai [72]	1994	157	WB		65 (41)
Wiltschke [73]	1994	105	WB		22 (21)
Wildenhain [74]	1990	56	WB		23 (41)
Dati* [22]	1991	132	WB		51 (39)
Barnes* [75]	1988	195	IHC-P	pAb 21N	58 (30)
Berger [28]	1988	47	IHC-P	pAb 20N	14 (30)
Gusterson* [76]	1988	103	IHC-P	pAb 21N	14 (14)
Gusterson* [77]	1988	137	IHC-P	pAb 21N	22 (16)
Van de Vijver [78]	1988	189	IHC-P	MAb 3B5	27 (14)
Harris [79]	1989	184	IHC-P	pAb 21N	31 (17)
Spandidos [80]	1989	100	IHC-P	pAb1	35 (35)
Thor [81]	1989	313	IHC-P	MAb TA1	47 (15)
Walker [82]	1989	85	IHC-P	pAb 21N	20 (23.5)
Wright* [81]	1989	185	IHC-P	pAb 21N	31 (17)
Bacus [84]	1990	45	IHC-F	Slamon	22 (50)
De Potter [85]	1990	71	IHC-P	MAb 3B5	27 (38)
Kommos [86]	1990	50	IHC-P	pAb1	11 (22)
			IHC-F		5 (10)
Mizukami [87]	1990	82	IHC-P	MAb 3B5	40 (49)
Richner [88]	1990	79	IHC-P	pAb 21N	20 (25)
Dykins [89]	1991	187	IHC-P	MAb NCL-CB11	41 (22)
Gullick [90]	1991	483	IHC-P	pAb 21N	103 (21)
Kallioniemi [91]	1991	319	IHC-P	MAb1	72 (23)
Lovekin [92]	1991	497	IHC-P	pAb 21N	74 (15)
McCann [93]	1991	314	IHC-P	pAb 21N	52 (11)
Munzel [94]	1991	30	IHC-F		7 (23)
O'Reilly [95]	1991	172	IHC-P	pAb 21N	39 (23)
Poller [96]	1991	85	IHC-P	pAb 21N	18 (21)
Rilke [97]	1991	1210	IHC-P	Slamon	278 (23)
Gasparini [98]	1992	165	IHC-P	pAb 21N	45 (27)
Gusterson [99]	1992	1506	IHC-P	MAb ICR 12	258 (17)
Isola [100]	1992	289	IHC-P	MAb TAb250	45 (16)
Lee [101]	1992	83	IHC-F	MAb1	24 (29)
Liu* [63]	1992	122	IHC-P	pAb OA-11-854	45 (37)
Pavelic [102]	1992	56	IHC-P	MAb E21A7	23 (41)
Poller [103]	1992	146	IHC-F	pAb 21N	60 (40)
Schroeter [104]	1992	232	IHC-P	pAb 21N	35 (15)
Treurniet [105]	1992	296	IHC-P	MAb 3B5	53 (18)
Wright* [106]	1992	65	IHC-P	pAb 21N	14 (22)
Bianchi [107]	1993	230	IHC-P	MAb1	48 (21)
Kynast [108]	1993	37	IHC-P	MAb 9G6	9 (24)
Ji [109]	1993	193	IHC-P	MAb1	60 (31)
Nicholson* [110]	1993	103	IHC-F	pAb 21N	27 (26)
Press [111]	1993	210	IHC-P	pAb R60	62 (29)
Soomro [112]	1993	81	IHC-P	pAb 21N MAb 4D5	8 (10)
Thomas [113]	1993	120	IHC-P	pAb 21N	36 (30)
Delarue [114]	1994	73	IHC-P	Home made	10 (14)
Gasparini* [15]	1994	212	IHC-P	pAb 21N	50 (24)
Gasparini [115]	1994	254	IHC-P	pAb 21N	54 (21)
Hartmann [116]	1994	354	IHC-P	pAb (Triton biosciences)	72 (20)
Hubbard [64]	1994	282	IHC-P	pAb 21N	31 (11)
Jacquemier [117]	1994	81	IHC-P	MAb 3B5	19 (23)

(continued)

Table 2—contd

Muss [118]	1994	397	IHC-P	pAb OA-11-854	115 (29)
Pechoux [119]	1994	31	IHC-P	MAb NCL-CB11	11 (35)
Schneider [120]	1994	31	IHC-P	MAb NCL-CB11	9 (29)
Tetu [121]	1994	888	IHC-P	pAb (Triton biosciences)	143 (16)
Archer [122]	1995	92	IHC-P	pAb1	24 (26)
Goussia [123]	1995	40	IHC-P	OM 11 952	16 (40)
Keshgesian [124]	1995	320	IHC-P	MAb NCL-CB11	75 (23)
Quenel [125]	1995	942	IHC-P	pAb (Dako)	229 (24)
Resnick [126]	1995	40	IHC-P	MAb 3B5	10 (25)
Stal [127]	1995	152	FC	MAb 9G6	33 (22)
Soubeyran [128]	1996	74	IHC-P	pAb (Dako)	19 (26)

*Studies not included in Table 3 as there is evidence that the patients are the same as in another referenced study.

activation involves amplification, which has always been associated with overexpression, as revealed by increased levels of p185 and its mRNA [12, 19]. However, it should be noted that several breast cancer specimens have shown overexpression of *ERBB2*, despite the lack of gene amplification [20–22]. A recent study suggests that there are two separate tumour populations with, respectively, low and high levels of *ERBB2* overexpression, the gene amplification being restricted to the latter group [23].

ERBB2 POSITIVITY IN HUMAN BREAST CANCER

Amplification of the *ERBB2* gene was evaluated in most of the studies by Southern blot (34 studies, Table 1) and in recent reports by the polymerase chain reaction (PCR) method (five studies; Table 1). Overexpression has been determined by various methods (Table 2), including Northern blot (six studies), fluorescent *in situ* hybridisation (one study), Western blot (six studies), flow cytometry (one study) and immunohistochemistry (57 studies). The immunohistochemical staining, using at least 17 different monoclonal and polyclonal antibodies, was performed on paraffin embedded material in nearly all the studies, apart from six, which used frozen sections.

The variations in *ERBB2* positivity in human breast cancer, with respect to the method used for evaluation, are presented in Table 3. When the size of the series is taken into account (Table 3), the percentage of *ERBB2* positivity appears to be very similar with the various methods (20–23.6%), apart from the PCR method which provided higher values (37.6%). The overall rate of positivity in the 22 616 cases examined was 21.4%.

When considering the individual studies, Western blot and PCR methods give the highest values (29.7 and 30.6%). The mean *ERBB2* positivity was 26% (range 5–55%).

ASSOCIATION WITH CLINICOPATHOLOGICAL PARAMETERS

Hormonal receptor status

The mean *ERBB2* positivity is 2.4 times higher in oestrogen receptor (ER) negative than in ER positive tumours (35.5% versus 13.3%), and it is twice as high in progesterone receptor (PR) negative than in PR positive tumours (32.2% versus 14.8%) (Table 4). Accordingly, Heintz and colleagues [40] reported that 79 and 61% of *ERBB2* negative tumours were, respectively, ER and PR positive, whilst only 53 and 24% of *ERBB2* positive tumours were ER and PR positive. Todd and associates [50] observed that ER negative tumours were twice as likely to be *ERBB2* positive than ER positive tumours (23.7% versus 9.7%) and that 10.1% of PR positive tumours were *ERBB2* positive, while 19.2% of PR negative tumours exhibited *ERBB2* positivity.

These results clearly demonstrate that *ERBB2* positivity is strongly and inversely related to ERs and PRs.

Age at diagnosis and menopausal status

Numerous reports found no significant relationship between the age of the patient at diagnosis and *ERBB2* positivity (4685/5785 patients; Table 5). This result was surprising, as *ERBB2* is inversely related to ER and PR, these parameters being correlated with age. However, several studies reported that *ERBB2* positivity was more frequent in younger patients [34, 43, 91]. Accordingly, the frequency

Table 3. Mean ERBB2 *positivity by method used for evaluation*

		Patients*		*ERBB2* + (%)†	
Method	No. of studies	*n*	*ERBB2* + (%)	Mean	Range
SB	35	8374	1678 (20.0)	22.7	(5–46)
PCR	5	665	250 (37.6)	30.6	(19–55)
NB	3	250	59 (23.6)	25.6	(20–34)
FISH	1	44	10 (23)	23.0	
WB	3	1046	228 (21.8)	29.7	(16–41)
IHC	50	12237	2614 (21.4)	24.6	(10–50)
Total	97	22616	4839 (21.4)	26.0	(5–55)

SB, Southern blot; PCR, polymerase chain reaction; NB, Northern blot; FISH, fluorescent *in situ* hybridisation; WB, Western blot; IHC, immunohistochemistry. *Mean *ERBB2* positivity based on all individual patients included. †Mean and range of *ERBB2* positivity based on the reported percentages for each separate series.

Table 4. Relationship between steroid receptors (oestrogen (ER) and progesterone receptors (PR)) and ERBB2 *positivity*

First author [Ref.]	Method used for *ERBB2*	Relationship with ER	% *ERBB2* positivity ER− versus ER+	ER threshold (fmol/mg)	% ER positivity	Relationship with PR	% *ERBB2* positivity PR− versus PR+	PR threshold (fmol/mg)	% PR positivity
Guérin [20]	SB	Neg	42 versus 4	10		Neg	25 versus 10	10	
Slamon [12]	SB	NS	18 versus 18	3	63	NS	17 versus 19	5	50
Adnane [30]	SB	Neg	35 versus 16	10	71	Neg	34 versus 15	10	70
Ro [33]	SB	NS	29 versus 19	20	65				
Roux-Dosseto [34]	SB	Neg	43 versus 24	10	65	Neg	44 versus 22	10	58
Zeillinger [36]	SB	Neg	28 versus 14	10	72	Neg	22 versus 16	10	59
Borg [38]	SB	Neg	34 versus 6	10	69	Neg	30 versus 5	10	59
Borg [43]	SB	Neg	37 versus 16	10	65	Neg	32 versus 18	10	59
Clark [44]	SB	NS	29 versus 35		74	NS	33 versus 34		55
Dati [22]	SB	NS	22 versus 26	10	88	NS	41 versus 21	10	77
Berns [47]	SB	Neg	31 versus 14	10	78	Neg	27 versus 13	10	68
Ciocca [48]	SB	Neg	29 versus 18	3	80	Neg	25 versus 18	5	63
Henry [53]	SB	Neg	36 versus 16	> 10 units ER mRNA					
Ito [61]	SB	Neg	30 versus 13	5	56	Neg	31 versus 16	5	69
Rio [67]	NB	NS			72				
May [68]	NB	Neg	43 versus 16	10	76				
Barnes [75]	IHC	NS	45 versus 30		76	NS	35 versus 38		59
De Potter [85]	IHC	Neg	71 versus 19	10	64	Neg	59 versus 15	10	51
Kommos [86]	IHC	Neg	43 versus 7	10	58	NS		10	
Mizukami [87]	IHC	NS	40 versus 43	5	62	NS	38 versus 41	5	58
Kalloniemi [91]	IHC	Neg	39 versus 11	10	57	Neg	27 versus 10	10	24
Lovekin [92]	IHC	Neg	35 versus 12	5	55				
McCann [93]	IHC	NS	19 versus 13		40				
O'Reilly [95]	IHC	Neg	33 versus 12	10	79	Neg	24 versus 10	10	57
Pavelic [102]	IHC	NS	50 versus 43	5	45	NS	40 versus 50	5	45
Schroeter [104]	IHC	NS	23 versus 13	10	61				
Hartmann [116]	IHC	Neg	13 versus 5		66				
Tetu [121]	IHC	Neg (N-)	29 versus 11		71	Neg	24 versus 10		57
Dati [22]	WB	NS	43 versus 39	10	83	Neg	54 versus 34	10	72
Wiltschke [73]	WB	Neg	33 versus 12	10	57	Neg	32 versus 12	10	55
Tandon [71]	WB	Neg	25 versus 14	3		Neg	25 versus 11	5	
Quenel [125]	IHC	Neg	34 versus 21	10	73	Neg	33 versus 17	15	56
Stal [127]		Neg	39 versus 11	0.1 fmol/μg DNA	62				

Neg, negative relationship; NS, not significant; SB, Southern blot, NB, Northern blot; WB, Western blot; IHC, immunohistochemistry.

of *ERBB2* positivity was found to be significantly lower in post-menopausal women (858 patients) [43, 91]. Moreover, Borg and associates [38] found that menopausal status was not associated with amplification of *ERBB2*, but that overexpression marginally increased among premenopausal women. Conversely, Gusterson and colleagues [99], reported that postmenopausal status was associated with *ERBB2* positivity, but exclusively in node-positive patients.

Tumour size

Most of the studies found no relationship between *ERBB2* positivity and tumour size (5897/8143 patients; Table 5). However, *ERBB2* was associated with larger tumours in a few studies [43, 91, 97]. In node negative patients, Gusterson and colleagues [99] reported a relationship between increased tumour size (> 2 cm) and *ERBB2*.

Lymph node status and recurrences

The relationship between lymph node status and *ERBB2* was analysed according to either the presence or the number of involved lymph nodes. Most studies found no association between *ERBB2* and the presence (5450/6418 patients) or the number (4051/7207 patients) of lymph node metastases (Table 5). Borg and associates [43] found a statistically significant relationship between the presence of positive nodes and *ERBB2*. Several reports described that *ERBB2* was associated with the number of involved lymph nodes [12, 31, 43, 60, 71, 91, 116, 121].

The relationship between recurrence and *ERBB2* positivity was also examined. Firstly, good correlation was observed between *ERBB2* status in primary tumours and in recurrences. McCann and associates [93] reported that 95% of *ERBB2* positive patients had comparable staining patterns of *ERBB2* expression between the primary and recurrent lesions. Similarly, Niehans and colleagues [129] reported that *ERBB2* immunoreactivity of primary tumour and later metastases was congruent and that the staining pattern at different metastatic sites was rarely heterogenous. Barnes and associates [75] found that *ERBB2* immunoreactivity in metastatic lesions was similar to that seen in the respective primary breast carcinomas.

ERBB2 positivity has also been significantly related to the site of first metastases. Kallioniemi and colleagues [91]

Table 5. *Relationship between* ERBB2 *positivity and the classical pathological parameters*

First author [Ref.]	*n*	Age at diagnosis	Menopausal status	Tumour size	Presence of involved nodes	Number of involved nodes	Tumour grade	Ploidy	Proliferation rate
Slamon [12]	189			NS		Pos			
Guérin [20]	116	NS					NS		
Dati [22]	77			NS		NS			
Cline [24]	53			NS	Pos				
Berger [28]	51					Pos			
Adnane [30]	292	NS				NS	NS		
Guérin [31]	221					Pos			
Ro [33]	66	NS		NS				NS	
Roux-Dosseto [34]	170	Neg	NS	NS	NS	NS	NS		
Zhou [37]	157				NS				
Heintz [40]	50	NS			NS				Pos (mitotic index)
Borg [43]	539	Neg	Neg	Pos	Pos	Pos		Neg	Pos (S phase)
Clark [44]	362	NS		Pos*		NS			
Olsson [45]	72	Neg							
Tommasi [46]	107		NS	NS	NS		NS		
Ciocca [48]	1103	NS		NS		NS		Neg	Pos (S phase)
Tiwari [49]	61				Pos				
Gaffey [52]	50	NS		NS	Pos				Pos (mitotic index)
Henry [53]	103			NS	NS		Pos (histological)		
Kreipe [56]	60	NS		NS	NS		NS		NS
Prost [60]	178					Pos			
May [68]	89	NS			Pos		NS		
Delvenne [69]	47			NS	Pos				
Tandon [71]	728	NS		NS		Pos			
Wiltschke [73]	105			Pos	NS				
Barnes [75]	195						Pos (histological)		
Bacus [84]	45							Neg	
De Potter [85]	71				NS				
Kommos [86]	50								NS
Gullick [90]	483	NS		NS	NS		Pos (histological)		
Kallioniemi [91]	319	Neg	Neg	Pos		Pos	Pos (histological)	Neg	Pos (mitotic index)
Lovekin [92]	497						Pos (histological)		
McCann [93]	314	NS	NS	NS		NS	Pos (histological)		
Münzel [94]	30								Pos (Ki-67)
O'Reilly [95]	172			NS	NS	NS	NS	Neg	NS
Poller [96]	85								Pos (S phase)
Rilke [97]	1210			Pos	NS		Pos (histological)		
Gusterson [99]	1506		Pos (N^+)	Pos (N^-)	NS	NS			
Lee [101]	83			NS	NS			Neg	Pos (S phase, Ki67)
Pavelic [102]	56				Pos				
Poller [103]	146						Pos (histological)		
Schroeter [104]	232	NS		Pos*	NS				
Bianchi [107]	230	NS		NS			NS		
Ji [109]	193							Neg	
Nicholson [110]	103								NS
Delarue [114]	73			Pos	Pos				
Hartmann [116]	354			NS		Pos	Pos (nuclear)	NS	NS
Tetu [121]	888	NS		NS		Pos	Pos (histological) Pos (nuclear)	Neg	NS
Quenel [125]	942			NS	NS				
Stal [127]	152							Neg	Pos (S phase)

*Slight trend found. Pos, positive relationship; neg, negative relationship; NS, not significant; N^+, node-positive; N^-, node-negative.

observed that more lung, liver and brain metastases and fewer bone metastases occurred in *ERBB2* positive cases as compared with *ERBB2* negative cases. Moreover, Schroeter and associates [104] reported a significant relationship between *ERBB2* positivity and liver metastases as a first site of relapse.

An increased risk of recurrence has been associated with *ERBB2* positivity [27, 60, 85, 111].

Tumour type

As shown in Table 6, several relationships have been observed between *ERBB2* positivity and the histological type of breast cancer. In invasive carcinomas, *ERBB2* positivity has a higher incidence in ductal (22% overall positivity) than in lobular (7% overall positivity) carcinomas. For Guérin and colleagues [20] and Dati and coworkers [22], this association

Table 6. Relationship between ERBB2 *positivity and the histological subtypes of benign and malignant mammary tumours*

Type	Guérin [20]	Dati [22]	Varley [25]	Gusterson [77]	Van de Vijver [78]	McCann [93]	Poller [96, 130]	Liu [63]	Soomro [112, 131]	Sommerville [132]	Heatley [133]	Ramachandra [134]	Barnes [135]	Barnes [136]	De Potter [137]	Porter [138]	Overall
Ductal *in situ*				33/74 (44%)		7/11 (64%)	60/116 (52%)	13/27 (48%)	17/80 (21%)	22/48 (46%)							152/356 (43%)
Comedo							13/16 (81%)					18/29 (62%)	15/20 (75%)	8/11 (73%)	23/55 (42%)		102/176 (58%)
Solid					19/29 (66%)		2/2					2/4 (50%)	2/10 (20%)				
Cribriform							0/11					1/6 (17%)	0/19				
Papillary				0/3	0/16		0/1					0/3		2/30 (7%)	1/20 (5%)		4/109 (3%)
Lobular *in situ*				0/16					0/1	0/10			1/9 (11%)			1/57 (1.7%)	2/93 (2%)
Ductal invasive	35/95 (37%)	42/104 (40%)	7/37 (19%)	22/137 (16%)		30/208 (14%)		23/122 (21%)	10/64 (16%)	10/50 (20%)	12/64 (19%)						191/881 (22%)
Lobular invasive	1/5 (20%)	6/14 (43%)		0/12		2/43 (5%)			0/27	1/24 (4%)	0/7					0/15	10/147 (7%)
Mucoid/ colloid						1/6 (17%)											1/6 (17%)
Medullary	0/1			1/12 (8%)					0/13	1/7							2/33 (6%)
Tubular		0/5	0/1	1/9 (11%)		0/3			0/10								1/28 (3%)
Papillary			0/2						0/4								0/6 (0%)
Fibro sarcoma	0/3		0/6														0/9 (0%)
Fibro adenoma			0/1								0/20						0/21 (0%)
Mucinous			0/1						1/23 (4%)		0/3						1/27 (4%)
Paget's disease				5/6 (83%)													5/6 (83%)

was limited to *ERBB2* amplification (they found no significant difference between ductal and lobular carcinomas with respect to *ERBB2* overexpression). In ductal carcinomas, *ERBB2* positivity was found to be twice as high in *in situ* carcinomas (43% overall positivity) than in invasive carcinomas (22% overall positivity). In ductal carcinomas *in situ* (DCIS), the incidence of *ERBB2* positivity was very high in DCIS of large cell type (58% overall positivity in comedo and solid subtypes), while DCIS of small cell type were very rarely *ERBB2* positive (3% overall positivity in cribriform and papillary subtypes). In Paget's disease, Gusterson and colleagues [77], Lammie and associates [139] and Wolber and co-workers [140] reported that, respectively, 83, 91 and 79% of the cases studied were *ERBB2* positive. *ERBB2* positivity is associated with the inflammatory character of breast cancer [31, 60, 68, 109].

Tumour grade

Several studies (involving 4509/5745 cases) have reported an association between *ERBB2* positivity and a higher histological or nuclear grade [141–143] (Table 5). Four reports failed to find this relationship. On the whole, these results suggest that *ERBB2* positivity is related to a worse grade.

Tumour ploidy

A higher frequency of *ERBB2* positivity in aneuploid tumours (mean 29%, range 20–40%) than in diploid tumours (mean 13%, range 5–24%) (3429/4021 patients) was reported in several studies (Table 5). For Bacus and associates [84], all tumours overexpressing *ERBB2* had tetraploid or near-tetraploid DNA content. In rare cases, no relationship was found between *ERBB2* and tumour ploidy [116].

Cellular proliferation parameters

The cellular proliferation parameters analysed included S phase fraction, mitotic index, thymidine labelling index (TLI) and Ki-67 positive fraction. Several studies found a close association between *ERBB2* positivity and a higher rate of proliferation (2583/3938 patients, Table 5). Conversely, O'Reilly and colleagues [95] failed to find this association.

Oncogenes, suppressor genes and proteases

Henry and associates [53] found no relationship between *ERBB2* and c-*myc* amplifications, while Adnane and colleagues [30] found a close association. *Int-2* amplification was not related to *ERBB2* positivity [30, 53]. Dati and associates [22] showed a strong correlation between p21 ras and p185 levels, while Archer and colleagues [122] did not.

As regards suppressor genes, several studies reported a close correlation between *ERBB2* expression and positive tumour *p53* status [73, 103, 109, 144–146], while others found no relationship [122, 147].

With respect to proteases, two studies reported an association between *ERBB2* amplification or overexpression and high levels of cathepsin D [121, 148] (1469 patients). Two other studies reported that high cytosolic cathepsin D concentrations were not correlated with *ERBB2* positivity [39, 149] (comprising 202 patients).

Other type I growth factor receptor genes

As already extensively reported by Klijn and colleagues [13], there is no agreement on the relationship between EGFR and *ERBB2*. Some studies described an association [150, 151], while others found either an inverse relationship or no relationship between these parameters [36, 66, 86, 110]. Although they observed no significant associations between either EGFR status or increasing levels of EGFR expression and *ERBB2* immunostaining, Nicholson and associates [110] reported that subdivision of the EGFR data according to *ERBB2* measurements revealed a relationship between *ERBB2* immunostaining and worsened patient outlook in moderately EGFR positive tumours.

With regard to *ERBB3* overexpression, two studies reported no significant association with *ERBB2* overexpression in primary breast cancer [14–16]. Conversely, Gasparini and colleagues [15] observed that *ERBB3* overexpression in node negative tumours was statistically significantly associated with *ERBB2* positivity.

*Serum p*185 *protein level*

The extracellular domain of the p185 oncoprotein has been shown to be released from the surface of human breast cancer cells overexpressing *ERBB2* [152]. This approximately 105 kDa soluble p185 fragment has been also detected in sera from breast cancer patients [153]. Several studies were performed to evaluate the relationship between serum p185 levels and tissue expression assayed immunohistochemically. No statistically significant association between serum level and tissue expression of ERBB2 was found by Breuer and colleagues [154] and Kandl and associates [155]. Conversely, in patients with distant metastases, Narita and co-workers [156] observed a close association between expression of erbB2 protein in the primary tumour and serum p185 levels, while no correlation was noted in stage I/II patients. Since high levels of serum p185 have been found to be associated with metastatic disease, it is considered useful as a tumour marker in postoperative follow-up of breast cancer patients with *ERBB2* overexpression of the primary tumour [108, 156, 157]. Moreover, Kandl and associates [155] reported that the presence of serum soluble erbB2 had a significant impact on the survival of patients with advanced disease while it had no influence on response to either initial or salvage treatment for stage IV disease.

PROGNOSTIC VALUE OF *ERBB2*

The relationship between *ERBB2* positivity and clinical outcome in primary breast cancer was examined, taking into consideration both the whole population of patients (lymph node-positive and negative patients, Table 7) and the two subpopulations of patients separately (lymph node positive patients, Table 8; lymph node negative patients, Table 9). In all the studies, the well accepted prognostic factors, such as the number of involved lymph nodes, the steroid receptors, histopathological grading and tumour size, maintained their prognostic value.

Relationship between ERBB2 *positivity and relapse-free survival (RFS)*

Using univariate analysis, 11 reports (involving 3005 patients) showed that *ERBB2* positivity was significantly related to a worse RFS in the whole population of breast cancer patients (Table 7). In other studies, this relationship was shown to be restricted to subgroups of patients: PR positive [22], p53 positive [73] and patients with T3 and T4 lesions [81]. In contrast, two additional studies (644 patients) found no association between *ERBB2* status and RFS

Table 7. Relationship between ERBB2 *positivity and prognosis in the whole population of breast cancer, comprising lymph node positive (N^+) and negative (N^-) patients*

First author [Ref.]	Population	*ERBB2* positivity *n* (%)	Median follow-up (months)	Systemic treatment	Classical prognostic parameters	Significance of *ERBB2* on			
						RFS		OS	
						UV	MV	UV	MV
Walker [82]	$27N^-$, $58N^+$	20/85 (23.5%)	24	?			< 0.0002 RR = 3.85 (1.86–7.97)		< 0.009 RR = 2.97 (1.29–6.84)
Thor [81]	$141N^-$, $120N^+$	47/313 (15%)	102	N^+	Node, ER	NS 0.0018 in T3, T4 ($n = 49$)	NS	NS 0.0002 in T3, T4 ($n = 49$)	NS
Wright [83]	$44N^-$, $62N^+$ 79uk	31/185 (16.7%)	24	None	Nodes, ER	< 0.005	0.025	< 0.001	0.04
May [68]	$35N^-$, $41N^+$ 13 IBC	20/89 (23%)	30	N^+ IBC	ER	< 0.0001	< 0.02 RR = 4.9	ND	ND
Kury [41]	?	24/77 (31%)	40.5	$n = 47$	Node, size, grade	ND	ND	NS	NS
Clark [44]	$177N^-$, $185N^+$	119/362 (33%)	75	78%N^+, 20%N^-	Node, PR, size	NS	NS	NS	NS
Dati [22]	$56N^-$, $76N^+$	51/132 (39%)	31	N^+	Node > 3, size, PR	NS 0.01 in PR^+ ($n = 91$)	ND	NS 0.047 in PR^+ ($n = 91$)	ND
Gullick [90]	$192N^-$, $212N^+$ 79uk	103/483 (21%)			Node, ER grade, size	0.001	0.007	0.0007	0.02
McCann [93]	$127N^-$, $111N^+$ 76uk	52/314 (17%)	48	$n = 121$	Node, grade	< 0.001	NS	< 0.001	0.01
Rilke [97]	$520N^-$, $660N^+$ 30uk	279/1210 (23%)		None	Node, grade size	ND	ND	0.00002	NS
Kallioniemi [91]	$174N^-$, $145N^+$	72/319 (22.5%)	117.6	(Stage III, $n = 14$)	Node, size, ER, PR	0.01	NS	0.0004	0.03 RR = 1.5
Berns [158]	$95N^-$, $187N^+$	42/282 (23%)	74	$n = 71$	Size, node, grade, ER, PR	NS	NS	0.035	NS
Schroeter [104]	$102N^-$, $110N^+$	33/212 (15%)		$n = 125$	Size	18 months: 0.008 24 months: 0.01	NS	36 months: 0.05 42 months: 0.02	NS
Gasparini [98]	$83N^-$, $82N^+$	45/165 (27%)	42	N^+	Node, DNA ploidy	0.004	NS	NS	ND
Henry [53]	$30N^-$, $51N^+$ 22uk	28/103 (27%)	24	Yes (%?)		< 0.0001	ND	< 0.0001	ND
Thomas [113]	$66N^-$, $54N^+$	25/120 (21%)		All	Stage, size, nodes	< 0.01	NS	< 0.01	NS
Wiltschke [73]	$30N^-$, $75N^+$	22/105 (21%)	71.3	All N^+ N^- (%?)		NS 0.037 in $p53^+$ ($n = 16$)	ND	ND	ND
Delarue [114]	$33N^-$, $40N^+$	10/73 (14%)	78	All N^+ none N^-		0.001 RR = 4.5	0.001		
Quenel [125]	$398N^-$, $544N^+$	229/942 (24%)	83.5	85% N^+ 17% N^-	Size, node, grade, ER, PR	< 0.0001	NS	0.0001	NS

uk, unknown; IBC, inflammatory breast cancer; RFS, relapse-free survival; OS, overall survival; UV, univariate analysis; MV, multivariate analysis performed using the Cox regression model; NS not significant; ND, not described; RR, relative risk.

Table 8. *Relationship between* ERBB2 *positivity and prognosis in lymph node positive (N^+) patients*

First author [Ref.]	*ERBB2* positivity *n* (%)	Median follow-up (months)	Systemic treatment	Classical prognostic parameters	Significance of *ERBB2* on RFS UV	RFS MV	OS UV	OS MV
Slamon [21]	101/345 (27)	57		Node, PR, size	0.01	0.006	0.041	0.045
Thor [81]	16/120 (13)	102	Yes (%NP)	Node, ER	NS		NS	NS
Tandon [71]	60/350 (17)	50		Node, PR	0.0014	0.029	< 0.0001	0.0022
Borg [38]	22/120 (18)	41	44%	Node, PR, size	NS (overexpressed) 0.035 (amplified)	NS	ND	NS
Lovekin [92]	40/229 (17)				ND	ND	0.003	NS
O'Reilly [95]	17/85 (20)		50	Node	0.016	0.02	0.04	NS
Rilke [97]	158/660 (24)		None	Node, size, age	ND	ND	0.00003	NS
Kallioniemi [91]	43/145 (29.6)	117.6	Stage III ($n = 14$)	Node, size, ER, PR	ND	ND	5 years = 0.0005	ND
Anbazhagan [159]	29/211 (13.7)	108	All	Node, postmenopause	0.0002	0.036	< 0.0001	NS
Gusterson [99]	140/746 (19)	72	All	NP		0.0003 HR = 1.54		< 0.0001 HR = 2.15
Tetu [121]	143/888 (16)		73.9%		< 0.0001	HR = 2.055	< 0.0001	HR = 1.800
Hartmann [116]	27/354 (7.6)		91%	Node, ER, size, grade	0.01	NS	0.03	NS
Quenel [125]	143/544 (24.6)	83.5	85%	Size, node, grade, ER, PR	0.008	NS	0.0002	NS

RFS, relapse-free survival; OS, overall survival; PR, progesterone receptors; ER, oestrogen receptors; NP, not precise; UV, univariate analysis; MV, multivariate analysis; HR, hazard ratio; NS, not significant; ND, not described.

Table 9. *Relationship between* ERBB2 *positivity and prognosis in lymph node negative (N^-) patients*

First author [Ref.]	*ERBB2* positivity *n* (%)	Median follow-up (months)	Systemic treatment	Classical prognostic parameters	Significance of *ERBB2* on RFS UV	RFS MV	OS UV	OS MV
Slamon [21]	45/181 (25)	59		Node, PR, size	NS	NS	NS	NS
Ro [33]	13/66 (20)	85	None	ND	NS	ND	0.021	ND
Thor [81]	20/141 (14)	102	None	Node, ER	NS	NS	NS	NS
Tandon [71]	60/378 (16)	57		Node, PR	NS	NS	NS	NS
Richner [88]	10/79 (13)	54	62%	ER	NS	ND	NS	ND
Lovekin [92]	31/250 (12.4)				ND	ND	NS	ND
O'Reilly [95]	11/87 (13)		$n = 50$	Node	NS	ND	NS	ND
Rilke [97]	110/520 (21.3)		None	Node, size, age	ND	ND	NS	ND
Kallioniemi [91]	29/174 (16.7)	117.6	None	Node size, ER, PR	ND	ND	0.02	0.03
Gusterson [99]	118/760 (16)	72	66%	NP	NS	ND	0.01	ND
Isola [100]	45/289 (15.6)	96					0.01	NS
Allred [160]	97/677 (14.3)	60			NS	ND	NS	NS
Press [111]	25/210 (12)	108	None	ER, size		0.0012 OR = 3.0 (1.5–6.1)		ND
Bianchi [107]	20/230 (8.7)		None		5 years: 0.03	NS	5 years: 0.0007	NS
Gasparini [115]	54/254 (21)	62			NS	NS	NS	NS
Quenel [125]	95/398 (24)	83.5		Size, node, grade, ER, PR	0.0004	0.04 RR = 1.7	NS	NS

PR, progesterone receptor; ND, not described; ER, oestrogen receptor; NP, not precise; RFS, relapse-free survival; UV, univariate analysis; MV, multivariate analysis; OS, overall survival; NS, not significant; OR, odds ratio; RR, relative risk.

[44, 158]. By multivariate analysis, five reports (915 patients) confirmed the prognostic value of *ERBB2* on RFS [68, 82, 83, 90, 114], while six others (918 patients) did not [91, 93, 98, 104, 113, 125].

In lymph node positive patients (Table 8), seven studies (2,777 patients) demonstrated a significant relationship between *ERBB2* status and RFS, by univariate analysis. For Borg and colleagues [38] the relationship was restricted to *ERBB2* amplification (not valid for overexpression). Using multivariate analysis, six studies (2,625 patients) confirmed the prognostic value of *ERBB2* [21, 71, 95, 99, 121, 159]. For Anbazhagan and colleagues [159], the significance of *ERBB2* was only maintained for the subpopulation of premenopausal patients. Finally, three reports (1,018 patients) failed to confirm the association between *ERBB2* and RFS [38, 116, 125].

In lymph node negative patients, two reports (628 patients) found a prognostic value of *ERBB2* on RFS [107, 125] (Table 9). For Allred and colleagues [160], *ERBB2* positivity was associated with reduced RFS in a subset of patients with small, ER positive, predominantly invasive tumours. Most studies found no relationship between *ERBB2* and RFS. Using multivariate analysis, Quenel and associates [125] and Press and colleagues [111] confirmed that *ERBB2* was an independent prognostic factor for RFS.

Relationship between ERBB2 *positivity and overall survival (OS)*

Concerning the population of lymph node-positive and negative patients (Table 7), 10 reports (4,170 patients) found that *ERBB2* was a prognostic factor for OS by univariate analysis. Five studies (1,386 patients) found that *ERBB2* positivity was an independent prognostic indicator [82, 83, 90, 91, 93], while others (2,869 patients) did not [53, 97, 104, 113, 125, 158].

In the population of lymph node positive patients (Table 8), univariate analyses revealed that *ERBB2* status was significantly related to OS (10 studies, 3,811 patients). Multivariate analysis confirmed the prognostic value of *ERBB2* on OS in four studies involving 2,329 patients [21, 71, 99, 121]. However, in six other reports (comprising 2,083 patients), the significance was not maintained in multivariate analysis [91, 95, 97, 116, 125, 159].

In the population of lymph node negative patients (Table 9), 10 studies (2,703 patients) did not find an association between *ERBB2* and OS, while five other reports did (1,520 patients). Rilke and colleagues [97] reported that this relationship was restricted to breast carcinoma without lymphoplasmacytic infiltration (LPI). For Allred and colleagues [160], the prognostic value of *ERBB2* on OS was limited to the small (< 3 cm), ER positive, invasive tumours. By multivariate analysis, Kallioniemi and associates [91] confirmed the significant association between *ERBB2* and OS.

PROGNOSTIC VALUE OF *ERBB2* IN RELATION TO TREATMENT

Hormonotherapy

When considering node-positive, steroid positive tumour patients, Borg and colleagues [58] noted a favourable effect of adjuvant tamoxifen on survival for patients with *ERBB2* negative tumours, while patients with *ERBB2* positive tumours did not benefit from tamoxifen administration. Giai and associates [72] observed that node positive breast cancer patients co-expressing *ERBB2* and *RAS* had a worse outcome than patients not co-expressing these oncogenes, when treated with tamoxifen. Recently, Soubeyran and colleagues [128] reported that *ERBB2* showed no significant variation under tamoxifen, increasing in only 3 cases and decreasing in 13 cases among the 74 cases studied. No relationship was found between these variations and the efficiency of hormonotherapy.

Chemotherapy

Allred and colleagues [160] evaluated the relationship between overexpression of *ERBB2* and the response to adjuvant chemotherapy (CMFP regimen) in a subgroup of high risk node-negative breast cancer patients defined as having either ER negative or large ER positive tumors. The patients were randomised to be either observed or to receive adjuvant chemotherapy after surgery. While patients with *ERBB2* negative tumours showed significantly longer disease-free survival (DFS) than their untreated counterparts, patients with *ERBB2* positive tumours showed a similar DFS with or without treatment, demonstrating no benefit from adjuvant therapy in this subgroup. Similarly, Stal and associates [127] demonstrated that patients with highly proliferative tumours that did not overexpress *ERBB2* benefitted most, in terms of survival, from a CMF regimen. Moreover, Gusterson and colleagues [99] observed that in node positive patients, the effect of prolonged duration chemotherapy (CMF regimen) on DFS was greater in patients without *ERBB2* overexpression than in those overexpressing *ERBB2*. In node-negative patients, these authors observed a greater effect of peri-operative chemotherapy on DFS in *ERBB2* negative than in *ERBB2* positive patients. Concurrently, in women with node-positive early breast cancer, Muss and associates [118] reported that patients receiving a high dose regimen of adjuvant chemotherapy (cyclophosphamide, doxorubicin and fluorouracil) had significantly longer DFS and OS if their tumours were *ERBB2* positive but not if their tumours were *ERBB2* negative. Interestingly, *in vitro* [57] and *in vivo* [54, 57, 161] studies reported co-amplifications of *ERBB2* and topoisomerase II_{α}, a target enzyme for doxorubicin, in 12–50% of the cases examined and the cell line most sensitive to topoisomerase II_{α} (m-AMSA and mitoxantrone) had amplified *ERBB2* and topoisomerase II_{α} genes [57]. For Bitran and colleagues [162], patients with high risk stage II and IIIA breast cancer who have overexpression of *HER2/neu*, appear to have a high risk of relapse, even when treated with high dose chemotherapy and autologous haematopoietic progenitor cell support.

In contrast, Jacquemier and co-workers [117] did not find a significant correlation between response to adjuvant chemotherapy and *ERBB2* positivity. More recently, no beneficial effect from high-doses of chemotherapy including mitoxantrone, was observed on the course of patients with a high serum level of p185 [163].

ERBB2 AND TUMOUR RESPONSE TO TREATMENT

Hormonotherapy

Wright and colleagues [106] reported that among patients receiving endocrine therapy as first-line treatment for relapse, 7% of *ERBB2* positive patients responded to tamoxifen treatment compared with 37% of *ERBB2* negative patients ($P < 0.05$). Moreover, *ERBB2* and EGFR appeared to have

an additive effect in reducing the likelihood of response, since 0 of 8 patients with EGFR and *ERBB2* positive tumours benefitted from endocrine therapy [106]. Similarly, Nicholson and colleagues [110] observed *ERBB2* expression most frequently in patients failing to respond to endocrine measures ($P < 0.05$). These authors reported that *ERBB2* expression did not influence the proportion of patients responding to endocrine therapy in the EGFR negative group, while a significantly further loss of hormone sensitivity was observed in the moderately EGFR positive group. Moreover, the response rate to second-line endocrine therapy was 41% in patients with low serum ERBB2 levels and only 21% in patients with elevated serum ERBB2 [164]. In contrast, no statistically significant relationship between *ERBB2* positivity and response to treatment was reported by Archer and colleagues [122] in patients with either locally advanced or metastatic breast cancer, treated with primary endocrine therapy.

Chemotherapy

In advanced breast cancers, Wright and associates [165] reported that tumours overexpressing *ERBB2* showed a lower response rate and shorter duration of response to treatment with mitoxantrone compared with *ERBB2* negative tumours. These associations were not statistically significant, but survival following the start of treatment was significantly shorter in the *ERBB2* positive group. Conversely, Resnick and colleagues [126] reported that in locally advanced breast carcinoma, *ERBB2* positive patients showed a greater response to pre-operative chemotherapy, including doxorubicin, than *ERBB2* negative patients: 60% of *ERBB2* positive cases had a near-complete to complete response, whereas only 30% of *ERBB2* negative cases were similarly chemosensitive ($P = 0.052$). Finally, MacGrogan and associates [166] found no predictive value of *ERBB2* on the tumour response to primary chemotherapy in patients with invasive breast cancers. In keeping with this result, we observed that plasma *ERBB2* positivity had no predictive value for metastatic breast cancer patients treated by chemotherapy [167].

THERAPEUTIC PERSPECTIVES

As several lines of evidence have suggested that breast cancer patients with *ERBB2* overexpression exhibit a reduced response to conventional treatments, new therapeutic approaches, targeting the cells overexpressing *ERBB2* and based on monoclonal antibodies (MAbs) or on antisense technology, have been developed.

In the first approach, murine MAbs directed against the extracellular domain of p185 have been shown to inhibit the growth, in cell lines and in xenograft models, of human breast cancer cells overexpressing *ERBB2* (see [168] for a review). However, to overcome the problem of immunogenicity which compromises the therapeutic efficacy of these antibodies, Carter and associates [169] constructed humanised anti-p185 MAbs. Recently, a phase II study demonstrated that the recombinant humanised anti-p185 MAb is well tolerated and clinically active in patients with metastatic breast cancer overexpressing *ERBB2* who had received extensive prior therapy [170]. In addition, the antiproliferative effects of cytokine or drugs, including tumour necrosis factor α (TNF_α) cisplatin, doxorubicin, paclitaxel and tamoxifen, are potentiated by anti-p185 antibodies, both *in vitro* and in xenografts [171–175].

Based on anti-p185 MAbs, other strategies have been developed in order to convert their cytostatic effect into a cytotoxic effect. In the first method, anti-p185 MAbs were conjugated either to toxins [176–178], or to enzymes which then convert a non-toxic prodrug to a cytotoxic drug, minimising systemic toxicity and maximising drug concentrations in the tumour [179]. The second method involved the construction of antibodies with dual specificity for *ERBB2* and for triggering molecules on cytolytic effector cells [180–183].

Besides the MAb based therapies, other promising new potential agents for the treatment of breast cancer patients overexpressing *ERBB2* include antisense oligonucleotides. Several studies have already reported that *ERBB2* antisense oligonucleotides downregulated *ERBB2* expression in breast cancer cell lines [184–186]. In addition, the growth and DNA synthesis of breast cancer cell lines overexpressing *ERBB2* were specifically inhibited by these ERBB2 antisense oligonucleotides [185]. These results suggest that ERBB2 antisense oligonucleotides are suitable candidates for treatment of breast cancer patients that overexpress *ERBB2*.

CONCLUSION

The variations in *ERBB2* positivity in human breast cancer were analysed taking into account the different methods used for evaluation. The range of *ERBB2* positivity described in the different papers was very wide in every method. In immunohistochemical studies, a probable explanation for the variable overexpression rates reported in the literature was provided by Press and colleagues [187], who demonstrated the highly variable ability of the *ERBB2* antibodies to detect overexpression in archival tissue samples. Nevertheless, apart from the PCR method, which gave higher rates of positivity but which was used in a minority of studies, no clear differences were observed between the mean rates of *ERBB2* positivity obtained with the various methods.

The relationships between *ERBB2* positivity and the classical clinicopathological parameters were analysed. Despite some discrepancies observed between the different studies (due to the number of patients included, the tumour characteristics, the method used to evaluate *ERBB2* positivity and the method of tumour scoring, and the cut-off level of *ERBB2* positivity), several associations have been noted. Nearly all the studies report a strong inverse relationship between *ERBB2* positivity and the steroid receptors. There is a clear association between *ERBB2* positivity and the histological type of the tumour, worse nuclear and histological grades, tumour aneuploidy and a high rate of proliferation. Conversely, almost all the papers report that age at diagnosis, tumour size, and nodal status are not related to *ERBB2*, with just a few studies reporting an association. The relationship between *ERBB2* positivity and either menopausal status or the number of involved lymph nodes remains unclear. Finally, there is no agreement regarding a positive association between *ERBB2* and the other type I growth factor receptors.

The associations described between *ERBB2* positivity and prognosis in human breast cancer are somewhat controversial. In lymph node-positive patients, most studies indicate that *ERBB2* is associated with decreased RFS and/or OS in univariate analyses. This finding indicates that *ERBB2* is a biological marker of a more aggressive form of breast cancer. However, in multivariate analyses, *ERBB2* is an independent prognostic factor in several studies, although not

in others. As previously reported [102], one possible explanation for this could be the differing duration of follow-up in the various studies. Globally, it seems that the independent prognostic value of *ERBB2* is observed in studies which carried out investigation in the years immediately following diagnosis. On the contrary, in a long follow-up period, no difference in prognosis is found between *ERBB2* positive and *ERBB2* negative patients. Moreover, since most node-positive patients received systemic adjuvant therapy, it cannot be excluded that treatment could affect clinical correlations, although in these studies, treatment decisions were not dependant on *ERBB2* status.

In the case of lymph node-negative patients, most studies did not find *ERBB2* to be a prognostic indicator. Several reasons could explain this finding. First, the statistical power to demonstrate an effect on survival is directly dependant on the number of events (relapses or deaths) in the study. Since the risk of recurrences in the node-negative disease is relatively low, many cases would be required to obtain sufficient events to draw statistically significant conclusions. Consequently, it is possible that the number of patients included is too small and the follow-up duration is too short to show statistical significance. Another possibility concerns the influence of systemic adjuvant therapy on the prognostic significance of *ERBB2*. The apparent difference in the prognostic value of *ERBB2* between node-positive and node-negative patients might be, in part, related to an involvement of this oncogene in drug resistance, since most node-positive patients received systemic adjuvant treatments [97, 119, 156].

When considering the prognostic value of *ERBB2* in relation to treatment, it seems that the favourable effect of both hormonotherapy and chemotherapy on survival was restricted to the *ERBB2* negative patients. A significantly worse survival of the treated patients was noted in *ERBB2* positive patients [58, 99, 160]. This suggests that *ERBB2* could be involved in a drug resistance mechanism or could possibly at least be a marker for drug resistance through an unknown mechanism. This relative resistance to chemotherapy in *ERBB2* positive tumours seems to be associated with CMF regimens. In a pilot study, patients receiving high-dose regimens including doxorubicin had significantly longer survival if their tumour was *ERBB2* positive.

With respect to the tumour response to treatment, it remains difficult to draw general conclusions, since there are few reports and all of them are pilot studies. Two studies have indicated that *ERBB2* positivity is associated with a reduced response to hormonotherapy, while another failed to find this association. The loss of hormone sensitivity seems to increase in the subgroup of moderately EGFR positive patients. In patients receiving chemotherapy, the results are also controversial. On the whole, these results indicate that further investigations performed on larger populations of patients are necessary to evaluate the predictive value of *ERBB2* on tumour response to treatment. It should be noted that studies performed in patients receiving neoadjuvant chemotherapy could be very useful, since the results can be obtained rapidly (the patients generally receiving six courses of chemotherapy).

New therapeutic approaches targeting the cells overexpressing *ERBB2* have been developed and are currently being evaluated. The preliminary results show that these treatments can cause regression of human breast cancer. This promising finding justifies further evaluation of *ERBB2*.

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