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Predictive Factors for Response to Chemotherapy in Advanced Breast Cancer

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Most breast cancer patients receive chemotherapy at some phase of their illness but only about half of them benefit from it. Identifying the factors predicting response to chemotherapy would also assist the clinician in selection of appropriate patients for chemotherapy, thus saving others from unnecessary exposure to toxic agents. At the present time, there is no tumour biological factor available for clinical use in the prediction of chemotherapy response in advanced breast cancer apart from oestrogen receptor status, which predicts response to hormonal therapy, or the HER2 receptor, which predicts response to trastuzumab. Interestingly, they both are also targets for those therapies. Several groups have tried to find such predictive factors for chemotherapy in advanced breast cancer but the results are so far disappointing. This review collects the rapidly expanding data published so far on the predictive value of tumour biological factors for chemotherapy response in advanced breast cancer. In conclusion, none of them is yet good enough for clinical use in advanced breast cancer.

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Breast cancer is the most common cancer in females in the western world. Most breast cancer patients receive chemotherapy at some phase of their illness but only about half of them benefit from it. Since the inception of cytotoxic treatment for cancer the question of why some patients respond to therapy while others do not has been asked. It would be of considerable clinical value to know in advance which patients respond to chemotherapy, thus saving others from unnecessary exposure to toxic agents. Identifying and understanding the factors predicting response to chemotherapy might also help in elucidation of the basic mechanism behind response to cytotoxic agents in malignant disease.

In vitro, the breast cancer cell lines most likely to respond to chemotherapy are those with a short doubling time and no oestrogen receptor (1, 2). In vivo, the outcome is much more complicated and depends on several known and unknown patient and tumour biological factors that may also interact with each other. Since the 1970s many studies have been undertaken to discover which factors might predict response to chemotherapy in breast cancer.

A factor has predictive value only if its presence or absence is associated with resistance or sensitivity to a particular therapy; such factors may or may not have associated prognostic value. Table 1 summarizes the most commonly investigated tumour biological factors tested as predictive factors for response to chemotherapy in advanced breast cancer.

The purpose of the present review was to collect the data published so far on the predictive value of tumour biologic factors for chemotherapy response in advanced breast cancer. Only the studies where chemotherapy (i.e. not hormonal therapy or both) was used to treat advanced breast cancer (i.e. locally advanced or metastatic; not adjuvant treatment) were reviewed. The literature was searched from the medline database. The phrases used in the search were advanced/metastatic breast cancer, chemotherapy, predictive factor, tumour biological factor (i.e. ER, p53, etc). In addition, the references cited in the papers were reviewed. Congress abstracts were not included.

Oestrogen receptor and progesterone receptor status

In the earliest studies seeking to relate tumour biological characteristics to chemotherapy response investigated the correlation between oestrogen receptor (ER) and chemotherapy responsiveness. The first report suggested that ER negativity correlated positively with response to chemotherapy (3). This finding was not confirmed by subsequent studies (4–9) and a summary of these early studies indicates that there is no obvious correlation between chemotherapy responsiveness and oestrogen recep-

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Table 1

Tumour biological factors tested in prediction of response to chemotherapy in advanced breast cancer

Tumour biological factor
Oestrogen receptor
Progesterone receptor
Histological grade
Ploidy
Proliferation activity (TLI, S-phase fraction, Ki-67, mib-1)
Drug resistance related factors (MDR, GST)
Oncogenes (c-erbB-2)
Apoptosis related genes (p53, bcl-2, bax)
Miscellaneous (elastosis, topoisomerase IIalpha)

tor contents of the tumour (10). In some of the more recently published studies the results are also conflicting (Table 2). However, most of these reports have utilized ER measurements taken some time before chemotherapy and changes in ER status may occur, especially after hormone therapy (11). Stewart et al. prospectively studied whether receptor status immediately before commencement of chemotherapy influenced response rate but also in that study no relationship was found between ER status and response to cytotoxic chemotherapy (12).

There is a paucity of early literature on progesterone receptor (PgR) as a predictive factor for chemotherapy. In recent studies the results are contradictory (Table 2). Interestingly, in some studies ER and PgR predict response to chemotherapy in different ways (13–15). In conclusion, hormone receptors have not shown any usefulness as predictive indicators for response to chemotherapy in advanced breast cancer.

Tumour differentiation

The relationship between the histological grade of the primary tumour and response to chemotherapy in advanced breast cancer has been investigated in many studies (Table 3). Most studies state that the response to chemotherapy is not dependent on histological grade (15–21). However, in two studies high-grade tumours were more resistant either to neoadjuvant treatment with weekly

Table 2

Studies on the predictive value of hormone receptors for chemotherapy response in locally advanced or metastatic breast cancer

Investigator and year	n	Chemotherapy	Factor	Method	Cut point	RR% negative	RR% positive	Association with RR	p-value	Ref.
Lippman et al. 1978	70	Various (no P)	ER	Quantitative	10	76%	12%	High worse	<0.0001	(3)
Kiang et al. 1980	40	CAF, CMF ± V, P	ER	Quantitative	3–4	43%	89%	High better	<0.01	(6)
Hilf et al. 1980	44	NA	ER	Quantitative	10	68%	50%	Not predictive	NS	(4)
Jonat et al. 1980	53	CM ± F, V, P; A	ER	Quantitative	20	33%	61%	Not predictive	NS	(5)
Rosenbaum et al. 1980	30	CMF ± P; AVCR; mitovinbl	ER	Quantitative	10	43%	75%	High better	<0.05	(7)
Rubens et al. 1980	47	CMF ± vinbl; A ± V; C	ER	Quantitative	5	57%	62%	Not predictive	NS	(8)
Samal et al. 1980	81	A; VCMFP; AF ± C, M; AC	ER	Quantitative	3–4	62%	78%	Not predictive	NS	(9)
Remvikos et al. 1989	60	Neoadjuvant CAF	ER	EIA	250	83%	70%	Not predictive	NS	(16)
Chevillard et al. 1996	63	Neoadjuvant CAF or CTF	ER, PgR	ICC	250	NA	NA	Not predictive	NS	(18)
MacGrogan et al. 1996	128	Neoadjuvant EVM, MTV	ER	IHC	10	NA	NA	High worse	0.001	(15)
			PgR	IHC	10	NA	NA	Not predictive	NS	
Colleoni et al. 1999	73	Neoadjuvant FUFAvino	ER	IHC	10	63%	57%	Not predictive	NS	(13)
			PgR	IHC	10	73%	38%	High worse	0.05	
Zambetti et al. 1999	67	Neoadjuvant A/E	ER	IHC	NA	68%	74%	Not predictive NS	(21)	
			PgR	IHC	NA	67%	75%	Not predictive	NS	
Daidone et al. 1999		Neoadjuvant A, CMF, FAC, FEC, FmxC	ER	DCC	10	73%	78%	Not predictive	NS	(14)
			PgR	DCC	25	68%	86%	High bette	0.03	
Järvinen et al. 1998	55	First line E weekly	ER	IHC	20	26%	67%	High better	0.002	(20)
			PgR	IHC	20	43%	63%	High better	0.04	

Abbreviations, ER = oestrogen receptor; PgR = progesterone receptor; IHC = immunohistochemistry; EIA = enzyme immuno assay; DCC = dextran-coated charcoal; NA = not available; NS = not significant; RR = response rate.

Table 3

Studies on the predictive value of histological grade for chemotherapy response in locally advanced or metastatic breast cancer

Investigator and year	n	Chemotherapy	RR% with histological grades I–III	Association with RR	p-value	Ref.
Remvikos et al. 1989	60	Neoadjuvant FAC	55% gr I vs 83% gr II–III	Not predictive	NS	(16)
Belembaogo et al. 1992	58	Neoadjuvant AVCF ± M	NA	Not predictive	NS	(17)
Aas et al. 1996	63	Neoadjuvant A weekly	NA	High grade resistant	0.018	(22)
Chevillard et al. 1996	63	Neoadjuvant FAC or CTF	NA	Not predictive	NA	(18)
MacGrogan et al. 1996	128	Neoadjuvant EVM, MTV	NA	Not predictive	NS	(15)
Rozan et al. 1998	167	Neoadjuvant FAC	12% CR gr I vs 21% gr II vs 37% gr III	High grade sensitive	0.04	(24)
Zambetti et al. 1999	70	Neoadjuvant A/E	NA	Not predictive	NS	(21)
Masters et al. 1987	125	First line A ± V	NA	Not predictive	NS	(19)
Niskanen et al. 1997	103	First line FEC	59% gr I vs 30% gr II vs 24% gr III	Low grade sensitive	<0.001	(23)
Järvinen et al. 1998	55	First line E weekly	43% gr I–II vs 64% gr III	Not predictive	NS	(20)

Abbreviations: NA = not available; RR = response rate; NS = not significant.

doxorubicin (22) or first-line treatment with FEC (5-fluorouracil, epirubicin, cyclophosphamide) given either weekly or monthly (23), whereas in one study high-grade tumours were more sensitive to neoadjuvant treatment with FAC (5-fluorouracil, adriamycin, cyclophosphamide) (24).

Elastosis is considered as a marker of well-differentiated tumours. In their study, Masters et al. found that tumours with abundant elastosis were more likely to respond to chemotherapy than those with little or no elastosis, but histological grade as such was not associated with response to chemotherapy (19). In summary, tumour differentiation does not offer any help in selecting patients for chemotherapy in advanced breast cancer.

Ploidy

Because DNA is one of the main targets of cytotoxic drugs, there has been interest in testing the DNA content (ploidy) of the primary tumour as a predictive factor for chemotherapy. A summary of the published studies on chemotherapy response and ploidy is presented in Table 4. In the neoadjuvant setting aneuploidy predicts either a better response to chemotherapy (25, 26) or no difference in treatment outcome between diploid and aneuploid tumours (14, 16–18, 27). In the metastatic setting, ploidy did not predict response to chemotherapy (19, 20, 28). Taken together, these studies do not indicate any obvious relationship between DNA content and chemotherapy sensitivity in breast cancer.

Tumour proliferation

The potential of proliferative markers in predicting response to chemotherapy in breast cancer has been assessed in a number of clinical studies (Table 5). In the first of these studies tumour proliferation was measured by tumour cell uptake of tritiated thymidine, which detects cells

Table 4

Studies on the predictive value of tumour ploidy for chemotherapy in locally advanced or metastatic breast cancer

Investigator and year	n	Chemotherapy	RR% Diploid	RR% Aneuploid	Association with RR	p-value	Ref.
Spyratos et al. 1992	35	Neoadjuvant AVCMF	10%	60%	Aneuploidy better	0.008	(26)
Remvikos et al. 1989	60	Neoadjuvant FAC	63%	82%	Not predictive	0.15	(16)
Bonadonna et al. 1990	92	Neoadjuvant CMF/FAC/FEC	76%	73%	Not predictive	NS	(27)
Belembaogo et al. 1992	126	Neoadjuvant AVCF ± M	NA	NA	Not predictive	NS	(17)
O'Reilly et al. 1992	16	Neoadjuvant FAC/FEC	50%	100%	Aneuploidy better	0.06	(25)
Chevillard et al. 1996	71	Neoadjuvant CAF or CFT	NA	NA	Not predictive	NS	(18)
Daidone et al. 1999	136	Neoadjuvant A, CMF, FAC, FEC, FmxC	89%	79%	Not predictive	NS	(14)
Masters et al. 1987	76	First line A ± V	69%	45%	Not predictive	NS	(19)
Hietanen et al. 1995	72	First line FEC	44%	25%	Not predictive	NS	(28)
Järvinen et al. 1998	55	First line E weekly	55%	50%	Not predictive	NS	(20)

Table 5

Studies on the predictive value of tumour proliferation for chemotherapy response in locally advanced or metastatic breast cancer

Investigator and year	n	Chemotherapy	Method	Cut point	RR% Low	RR% High	Association with RR	p-value	Ref.
Sulkes et al. 1979	25	First line CAV	TLI	9	0	69	High better	0.001	(30)
Silvestrini et al. 1987	52	Neoadjuvant	TLI	3.6	50%	50%	Not predictive	NS	(33)
Bonadonna et al. 1990	115	Neoadjuvant CMF/FAC/FEC	TLI	NA	79%	70%	Not predictive	NS	(27)
Daidone et al. 1991	26	Neoadjuvant CM/F	TLI	3.6	NA	NA	Not predictive	NS	(32)
Gardin et al. 1994	36	Neoadjuvant FAC	TLI	1.4	56%	83%	High better	NS $p = 0.06$	(31)
Collecchi et al. 1998	70	Neoadjuvant FEC	TLI	3.1	62%	88%	High better	0.014	(29)
Daidone et al. 1999	164	Neoadjuvant A, CMF, FAC, FEC, FmxC	TLI	3	82%	75%	Not predictive	NS	(14)
Remvikos et al. 1989	60	Neoadjuvant FAC	SPF	5–10	46%	84% 100%	High better	0.0017	(16)
Remvikos et al. 1993	184	Neoadjuvant FAC	SPF	5	57%	86%	High better	<0.003	(36)
Remvikos et al. 1993	71	Neoadjuvant CAF/CTF	SPF	5	56%	89%	High better	<0.002	(35)
			BrdU	3.3	56%	89%	High better	<0.002	
Spyratos et al. 1992	35	Neoadjuvant AVCMF	SPF	7.5	26	83	High better	0.004	(26)
O'Reilly et al. 1992	22	Neoadjuvant FAC/FEC	SPF	10.2	60	100	High better	<0.05	(25)
Belembaogo et al. 1992	91	Neoadjuvant AVCF+M	SPF	10	NA	NA	Not predictive, High better	NS	(17)
Chevillard et al. 1992	87	Neoadjuvant CAF/CTF	SPF	5	NA	NA	High better	0.01	(18)
MacGrogan et al. 1996	128	Neoadjuvant EVM, MTV	MIB-1	40	NA	NA	High better	0.009	(15)
Rozan et al. 1998	167	Neoadjuvant FAC	Ki-67	10	21% CR	30% CR	Not predictive	NS	(24)
			SPF	3	14% CR	39% CR	High better	0.01	
Willsher et al. 1998	50	Neoadjuvant MMM	mib-1	NA	NA	NA	Not predictive	NS	(40)
Colleoni et al. 1999	73	Neoadjuvant AC/5-FUFA-vino	mib-1	20	42%	76%	High better	0.05	(13)
Zambetti et al. 1999	68	Neoadjuvant A/E	mib-1	10	62%	75%	High better	NS	(21)
Amadori et al. 1997	76	Miscellaneous metastatic	TLI	3.1	15%	47%	Higher better	0.01	(34)
Masters et al. 1987	69	First line A ± V	SPF	7.0	57%	53%	Not predictive	NS	(19)
Hietanen et al. 1995	72	First line FEC	SPF	4.2/12.5	18%	45%	High better	0.008	(28)
Järvinen et al. 1998	55	First line E weekly	SPF	8	55%	50%	Not predictive	NS	(20)
Bonnetti et al. 1998	55	CMF, FAC, FEC, metastatic	Ki-67	25	24%	44%	Not predictive	NS (0.19)	(41)
Sjöström et al. 2000	72	Docetaxel metastatic	mib-1	25	56%	51%	Not predictive	NS	(42)
	62	MF metastatic	mib-1	25	54%	16%	Low better	0.01	(42)

Abbreviations: RR = response rate; TLI = thymidine labelling index; SPF = S-phase fraction; BrdU = bromodeoxyuridine; NA = not available; CR = complete response; NS = not significant.

performing DNA synthesis. Two out of seven studies demonstrated that highly proliferative tumours as measured by uptake of tritiated thymidine had a better response to neoadjuvant chemotherapy (29, 30), while another study showed a trend towards better response (31) and the rest no difference (14, 27, 32, 33). The one study on metastatic disease demonstrated a better response to highly proliferative tumours and, interestingly, the difference was significant only in previously untreated patients (34). This suggests that previously administered treatment, i.e. hormone therapy or chemotherapy, might alter the proliferation-related chemosensitivity as measured from the primary tumour.

Bromodeoxyuridine (BrdU), a halogenated analog of thymidine, has also been used as a measure of tumour proliferation in one study (35). Measuring the uptake of tritiated thymidine or BrdU requires fresh tumour material. Consequently, more feasible methods have been developed, and determination of S-phase fraction (SPF) by flow cytometry as a reliable and simple measurement of tumour proliferation activity replaced the thymidine labelling index. In all studies in the neoadjuvant setting a positive association was found between high tumour proliferation as measured by SPF and response to various neoadjuvant chemotherapy regimens (Table 5) (16–18, 24–26, 35, 36). However, in the metastatic setting, SPF of the primary tumour was not associated with response to chemotherapy in two studies (19, 20) while in one study a high SPF predicted a better response to chemotherapy (28).

Since flow cytometry requires large tumour volumes, immunohistochemical determination of cell proliferation antigens was applied to measure tumour proliferation. Ki-67 is a proliferation-related antigen expressed in late G1, S, G2 and M phases of the cell cycle (37). Ki-67 monoclonal antibody reacts with an epitope that is denatured with tissue fixation. Mib-1 monoclonal antibody was

produced using a 66-base pair recombinant part of the Ki-67 antigen as an immunogen. Its selectivity is similar to Ki-67 but it recognizes the target in fixed embedded tissues (38, 39). Later, a polyclonal Ki-67 antigen, which is also able to function in fixed embedded tissues, was introduced. The results with either Ki-67 or mib-1 antibody in both the neoadjuvant and metastatic settings are inconsistent with some studies indicating a better response (13, 15), others showing no difference (21, 24, 40, 41) and in one study an even poorer response (42) in highly proliferating tumours.

Of the different methods measuring tumour proliferation from primary tumours, SPF seems to be the one most often and uniformly associated with response to preoperative chemotherapy. However, the clinical utility of this knowledge is poor, since many patients with slowly proliferating tumours also benefit from chemotherapy.

Drug-resistance factors

The mechanisms of drug resistance are still poorly understood. Multi-drug resistance (MDR) may play a role in some tumours but it seems evident that there are many different pathways by which drug resistance may arise (43). Multi-drug resistance is caused by overexpression of P-glycoprotein, a membrane protein that pumps amphophilic organic substances out of the cell (44). Several cytotoxic agents including anthracyclines, vinca-alkaloids and etoposide are substrates for P-glycoprotein. In a small series of breast tumours, P-glycoprotein expression was detected in a semiquantitative manner taking into consideration both the number of positive cells and the intensity of staining. A high degree of staining correlated well with poor response to chemotherapy (45) (Table 6). In another small study, a large proportion of cells expressing P-glycoprotein were observed significantly more often in tumours showing less than partial response to preoperative chemotherapy for locally advanced disease (46). Finally, in a fairly large study, mdr-1 gene expression as assessed by reverse transcriptase PCR (polymerase chain reaction) but not P-glycoprotein expression as assessed by immunohistochemistry (IHC) predicted resistance to neoadjuvant chemotherapy (18).

Glutathione S-transferases (SGT) is a family of detoxifying enzymes that may be involved in drug resistance (47). The only clinical studies published found no correlation between SGT expression of the primary tumour and response to chemotherapy in locally advanced or metastatic breast cancer (15, 48).

So far, only a few small studies on single, specific, drug-resistance factors have been published and they seem to be of little use in the clinic. This is not surprising since there are so many different mechanisms for drug resistance in tumours.

C-erbB-2 and topoisomerase IIalpha

One of the most extensively studied tumour biologic factors in breast cancer is the HER-2/neu oncogene, also referred to as c-erbB-2, which encodes a transmembrane tyrosine kinase receptor that belongs to a family of epithelial growth factor receptors structurally related to the Epidermal Growth Factor Receptor (erbB-1) (49). The activation of c-erbB-2 leads to increased transcription of nuclear proteins and cellular growth. C-erbB-2 is overexpressed in 20–25% of breast cancers, and these tumours seem to be clinically aggressive (50).

In a few of the early breast cancer studies, it has been suggested that patients with c-erbB-2 overexpressing tumours are less responsive to CMF-containing adjuvant chemotherapy (51, 52) but benefit from anthracyclines (53, 54). In the neoadjuvant and metastatic treatment of breast cancer, the information on cerbB-2 and response to chemotherapy is more conflicting (Table 7). The only published studies indicating that tumours overexpressing c-erbB-2 respond better to chemotherapy have been those on patients receiving either the latest vinca-alkaloid vinorelbine or taxanes (13, 55). In the remaining studies, where patients have been treated with either an anthracy-

Table 6

Studies on the predictive value of drug resistance factors for chemotherapy in locally advanced or metastatic breast cancer

Investigator and year	n	Chemotherapy	Factor	Method	Cut point	RR% Low	RR% High	Association with RR	p-value	Ref.
Ro et al. 1991	40	Neoadjuvant ACVP	Pgp	IHC	0	NA	NA	Positive resistant	0.005	(46)
Verrelle et al. 1991	20	Neoadjuvant + metastatic AVCF ± M	Pgp	IHC	75	NA	NA	Positive resistant	<0.02	(45)
Chevillard et al. 1996	73	Neoadjuvant CAF or	MDR1	RT-PCR		NA	NA	Positive resistant	0.07 (<0.02)	(18)
	63	CTF	Pgp	IHC	1	NA	NA	Not predictive	NS	
MacGrogan et al. 1996	128	Neoadjuvant EVM, MTV	GST	IHC	1	NA	NA	Not predictive	NS	(15)
Wright et al. 1992	68	First line mitoxantrone	GST	IHC	0	NA	NA	Not predictive	NS	(48)

Abbreviations: Pgp = P-glycoprotein; IHC = immunohistochemistry; NA = not available; MDRI = multi-drug resistance gene I; GST = glutathione S transferase; RR = response rate.

Table 7

Studies on the predictive value of c-erbB-2 for response to chemotherapy in locally advanced or metastatic breast cancer

Investigator and year	n	Chemotherapy	Antibody	Cut point	Positive	RR% Low	RR% High	Association with RR	p-value	Ref.
MacGrogan et al. 1996	128	Neoadjuvant EVM, MTV	Rabbit polyclonal Dako	1	1	NA	NA	Not predictive	NS	(15)
Rozan et al. 1998	167	Neoadjuvant FAC	NCL-CB11 Novocastra	No/ weak/ strong		CR score 0 20%; score 1 24%	CR score 2 31%	Not predictive CR	NS	(24)
Willsher et al. 1998	50	Neoadjuvant MMM	Rabbit polyclonal Dako	5		73%	30%	High worse	0.0025	(40)
Colleoni et al. 1999	73	Neoadjuvant FUFAvino	TAB250 Triton	10	10%	56%	86%	High better	0.03	(13)
Wright et al. 1992	68	First line mitoxantrone	NCL-CB11 Novocastra	50	23%	40%	31%	Not predictive	NS	(48)
Niskanen et al. 1997	103	First line FEC	NCL-CB11 Novocastra	50	14%	Score 34%; score 1 22%	Score 2 50%	Not predictive	NS	(23)
Baselga et al. 1997	122	Taxane metastatic	Mouse monoclonal 4D5	10	38%	36%	36%	High better	0.002	(55)
Järvinen et al. 1998	55	First line E weekly	TAB250 Triton	NA	35%	64%	64%	High worse	0.006	(20)
Jukkola et al. 2001	72	Anthracyclines metastatic	NCL-CB11 Novocastra	NA	32%	35%	35%	High worse	NA	(56)
	52	Non-anthracyclines metastatic	NCL-CB11 Novocastra	NA	29%	27%	27%	High worse	NA	
Sjöström et al. 2002	66	Docetaxel metastatic	Rabbit polyclonal Dako	10	45%	53%	53%	Not predictive	0.50	(57)
	62	MF metastatic	Rabbit polyclonal Dako	10	39%	19%	19%	Not predictive	0.60	

Abbreviations: RR = response rate; NA = not available; CR = complete response; NS = not significant.

cline-based or some other chemotherapy regimen, a positive c-erbB-2 status was indicative of a poorer (20, 40, 56) or equal response (15, 23, 24, 48, 57) compared with c-erB-2-negative tumours.

Topoisomerase IIalpha, the molecular target for anthracyclines and other topoisomerase II inhibitors, might theoretically be an even better predictive factor for anthracyclines. Some recent preclinical data support this hypothesis. The topoisomerase IIalpha gene is namely located close to the c-erbB-2 oncogene and c-erbB-2 amplification is often associated with either amplification or deletion of topoisomerase IIalpha (58). In breast cancer cell lines with c-erbB-2 gene amplification, Järvinen et al. have shown that topoisomerase IIalpha gene amplification is associated with increased protein expression and sensitivity to doxorubicin, whereas topoisomerase IIalpha gene deletion is associated with decreased protein expression and resistance to doxorubicin (59). These interesting findings may explain why patients with c-erbB-2-positive tumours seem to benefit more from adjuvant chemotherapy with anthracyclines or more intensive schedules of anthracyclines (53, 54) and topoisomerase IIalpha may prove to be an even better indicator of sensitivity to anthracyclines than c-erbB-2. However, in metastatic breast cancer, topoisomerase IIalpha, as assessed by IHC, did not predict response to first-line chemotherapy with epirubicin while c-erbB-2 overexpression was associated with a poorer response (20). Results from other clinical studies are eagerly awaited.

The controversial results with c-erbB-2 in trials with an advanced disease setting may partly result from some methodological problems. Immunohistochemical assays lack a uniformly approved scoring system and different antibodies have been used. Assessment of c-erbB-2 gene amplification by fluorescence in situ hybridization (FISH) is considered to be a better method for detecting c-erbB-2 status than assessment of protein overexpression by IHC. However, there are not yet any clinical FISH data on the predictive value of c-erbB-2 for chemotherapy in advanced breast cancer. Taken together, it is apparent that c-erB-2 overexpression cannot yet be readily applied as a predictive factor for response to chemotherapy in advanced breast cancer. C-erbB-2 amplification and also topoisomerase IIalpha data are still pending.

Factors relating to cell cycle checkpoint processes or apoptosis

It has become apparent that, regardless of distinct mechanisms of action, most anticancer agents ultimately kill

cancer cells by inducing apoptosis. p53 and bcl-2 family proteins take part in the regulation of apoptosis triggered by cytotoxic drugs. In preclinical models, mutations in apoptotic programs can produce treatment-resistant tumours (60).

p53

The p53 protein is a transcription factor that participates in cell-cycle checkpoint processes and apoptosis. Under normal physiological conditions, p53 acts to limit proliferation following damage to genomic DNA through two alternative mechanisms: by G1 (or G2) arrest or by apoptosis (61). The relationship between chemosensitivity and p53 can be considered from two mutually exclusive points of view: it increases chemosensitivity due to apoptosis or decreases chemosensitivity due to growth arrest and DNA repair. The apoptosis or arrest decision depends on tissue type, anticancer agent, dose and mutational status of the tumour.

The p53 gene is mutated in at least 50% of human cancers (62). The clinical reports on the value of p53 status in predicting response to chemotherapy in breast cancer are discordant (Table 8). Half of the studies reported thus far have found no difference in response to various chemotherapy regimens according to p53 status as assessed by IHC (15, 20, 41, 63, 64). In one study, normal wild-type (wt) p53 was associated with a better response to neoadjuvant chemotherapy (14), whereas in another study poorer responses were seen in tumours with wt p53 (13). Notably, also the studies that used sequencing as the method of assessing p53 status reported conflicting results, i.e. either resistance (65, 66) or response (67) to anthracyclines in tumours with mutated p53. Interestingly, in the study by Kandioler-Eckersberger et al. the tumours with wt p53 either by IHC, sequencing or both were highly sensitive to anthracycline-based chemotherapy but more resistant to paclitaxel (66).

These clinical data have been disappointing, since mutant p53 has been correlated with a poorer response to cytotoxic treatment in vitro (68) and in mice (60). Methodological differences may explain some of the discordance between preclinical and clinical studies (69). Using IHC methods, nearly all missense mutations of p53 in the core domain can be detected, but their sensitivity is lower for mutations that produce protein truncation or for mutations that lie outside the core domain as well as null mutations. Moreover, for several reasons, positive immunohistochemical staining for p53 may be present without mutations. Namely, detectable levels may exist as a result of normal cell-cycle fluctuation, response to DNA damage, stabilization caused by interaction with other cellular proteins such as mdm-2, or failure of feedback loops or degradation pathways. Sequencing gives the most complete picture of the mutational p53 status but does not necessarily give the whole picture on the functional status of p53 and defects elsewhere in the p53 pathway (70). Recently, recommendations for analysing p53 status were given (71). In addition to methodological problems, an important notion is that the in vitro and preclinical in vivo results cannot be applied to clinical conditions where tumours are not experimentally induced and where probably many other biological factors also determine tumour regression, e.g. angiogenesis, growth factors, etc (72). Finally, heterogeneous clinical data may also result from the notion that drugs not causing DNA damage, for instance taxanes and vinca-alkaloids, may induce apoptosis via p53 independent pathways.

p21/WAF1

The wt p53-activated fragment 1 (WAF1, also known as p21) functions as an inhibitor of cyclin-dependent kinases (cdks) (73). In response to DNA damaging agents, p21 is an important downstream effector in the p53-specific growth arrest pathway (74, 75) but not in the p53-dependent apoptotic pathway (76).

To date, the utility of p21 in predicting response to chemotherapy has been assessed in only two studies, where no significant relationship between p21 and clinical response to chemotherapy was found (Table 9) (42, 77).

Bcl-2 family proteins

Bcl-2, the oncogene responsible for the development of follicular lymphoma (78), inhibits apoptosis. Over 20 genes whose encoded proteins share amino acid sequence homology with bcl-2 have been identified and are called bcl-2 family genes (79). Some of these function as blockers of cell death, whereas others promote apoptosis. The bax gene encodes a protein that belongs to this family and inhibits the action of bcl-2 and thus promotes apoptosis (80).

Because drugs with distinct primary targets end up in apoptotic cell death, mutations in apoptotic pathways could cause multi-drug resistance. In preclinical models, overexpression of bcl-2 promotes chemoresistance, whereas induction of bax increases chemosensitivity (81). Clinically, it is unclear whether monitoring expression of apoptosis-related proteins provides additional information on how patients will respond to treatment. The data on the predictive value of these proteins for chemotherapy in advanced breast cancer are presented in Table 10. The majority of studies have shown no relationship between bcl-2 expression and response to chemotherapy (13, 14, 63, 82–84), although in one study bcl-2 overexpression predicted a poorer response (41).

Interestingly, the first clinical study on the predictive value of bax demonstrated that patients having primary tumours with reduced levels of bax showed poorer response rates to chemotherapy, especially in the subgroup of patients treated with a lower dose intensity regimen of chemotherapy (83). This notion is in accordance with

Table 8

Studies on the predictive value of p53 for response to chemotherapy in locally advanced or metastatic breast cancer

Investigator and year	n	Chemotherapy	Factor	Method	Cut point	RR% p53 negative #	RR% p53 positive #	Association with RR	p-value	Ref.
Mathieu et al. 1995	86	Neoadjuvant AVCMF	p53	IHC	5	57%	58%	Not predictive	NS	(64)
Aas et al. 1996	63	Neoadjuvant (+first line) A weekly	p53	CDGE or sequencing		47%; 8% PD	39%; 69% PD	Not predictive for response; positive resistant	0.57	(65)
Chevillard et al. 1997	53	Neoadjuvant CAF or CTF	p53	Sequencing		Wt 79% resistant	50% responsive	Negative resistant	0.03	(67)
MacGrogan et al. 1996	128	Neoadjuvant EVM, MTV	p53	IHC	1	NA	NA	Not predictive	NS	(15)
Rozan et al. 1998	167	Neoadjuvant FAC	p53	IHC	0/5	CR 20%/26%	CR 26%	Not predictive for CR	NS	(24)
Colleoni et al. 1999	73	Neoadjuvant FUFAvino	p53	IHC	10	53%	86%	Negative worse	0.04	(13)
Daidone et al. 1999	136	Neoadjuvant A, CMF, FAC, FEC, FmxC	p53	IHC	5	94%	72%	Negative better	0.004	(14)
Bottini et al. 2000	143	Neoadjuvant CMF, E	p53	IHC	Various	NA	NA	Not predictive	NS	(63)
				IHC	10	27% CR	9.4% CR	Negative better	<0.04	
Niskanen et al. 1997	103	First line FEC	p53	IHC	10	37%	24%	Not predictive	NS	(23)
Bonetti et al. 1998	55	CMF, FAC. FEC metastatic	p53	IHC	NA	NA	NA	Not predictive	NS	(41)
Järvinen et al. 1998	55	First line E weekly	p53	IHC	20	57%	44%	Not predictive	NS	(20)
Kandioler-Eckersberger et al. 2000	35	FEC metastatic	p53	Sequencing		64%	0%	Negative better	<0.0001	(66)
				IHC	10	85%	7%	Negative better	0.003	
				Both		94%	6%	Negative better	<0.0001	
	32	Paclitaxel metastatic	p53	Sequencing		38%	86%	Negative worse	0.08	(66)
				IHC	10	35%	67%	Not predictive	NS	
				Both		24%	73%	Negative worse	0.01	
Sjöström et al. 2000	72	Docetaxel metastatic	p53	IHC	10	54%	50%	Not predictive	NS	(42)
	62	MF metastatic	p53	IHC	10	26%	22%	Not predictive	NS	(42)

p53 negative means immunohistochemical staining negative/low or no mutation detected by sequencing.
p53 positive means immunohistochemical staining positive/high or mutation detected by sequencing.
Abbrevations: RR = response rate; IHC = immunohistochemistry; CDGE = constant denaturant gel electrophoresis; NA = not available; CR = complete response; NS = not significant.

previous in vitro studies where for most anticancer drugs, elevations in the anti-apoptotic/pro-apoptotic protein ratios do not prevent drug-induced apoptosis but rather shift the dose-response curve to the right so that higher concentrations of drugs are required to achieve an equivalent percentage of tumour cell kill (85). These findings suggest that bax may be a useful marker in selecting patients for more intensive treatment schedules instead of the weekly treatment schedules, although the findings should be confirmed in other studies using various dose intensities.

Emerging clinical evidence thus far has not demonstrated any clear connection between bcl-2 family proteins and response to chemotherapy in breast cancer. This may be related to the high number of bcl-2 proteins normally seen in breast epithelium (79) so measuring only one or two bcl-2 family proteins may not give enough indication of the functional state of this apoptotic pathway. It is probable that the whole apoptotic pathways instead of single genes or proteins should be investigated and the recent applications of DNA microarrays and proteomics may help in this respect. Moreover, because of the multiplicity of apoptotic pathways, defects at one site might lead to the use of another pathway. Finally, much work is still needed to increase our understanding of when

Table 9

Studies on the predictive value of p21 for response to chemotherapy in locally advanced or metastatic breast cancer

Investigator and year	n	Chemotherapy	Method	Cutpoint	RR% Low	RR% High	Association with RR	p-value	Ref.
Ellis et al. 1997	56	Neoadjuvant A weekly	IHC	0	NA	NA	Not predictive	NS	(77)
Sjöström et al. 2000	72	Docetaxel metastatic	IHC	10	59%	46%	Not predictive	NS	(42)
	62	MF metastatic	IHC	10	21%	28%	Not predictive	NS	(42)

Abbreviations: IHC = immunohistochemistry; RR = response rate; NS = not significant.

Table 10

Studies on the predictive value of bcl-2 family protiens for response to chemotherapy in locally advanced or metastatic breast cancer

Investigator and Year	n	Chemotherapy	Factor	Method	Cut point	RR% Low	RR% High	Association with RR	p-value	Ref.
Ellis et al. 1998	36	Neoadjuvant ECF	bcl-2	IHC	10	NA	NA	Not predictive	0.8	(82)
Colleoni et al. 1999	73	Neoadjuvant FUFAvino	bcl-2	IHC	10	52%	63%	Not predictive	NS	(13)
Daidone et al. 1999	113	Neoadjuvant A, CMF, FAC, FEC, FmxC	bcl-2	IHC	30	74%	85%	Not predictive	NS	(14)
	82		bax	IHC	10	80%	79%	Not predictive	NS	
Bottini et al. 2000	143	Neoadjuvant CMF, E	bcl-2	IHC	various	NA	NA	Not predictive	NS	(63)
Krajewski et al. 1995	104	FEC first line	bcl-2	IHC	10	NA	NA	Not predictive	NS	(83)
			Bax	IHC	10	21%	43%	High better	0.02	(83)
	49	FEC weekly	bax	IHC	10	6%	42%	High better	0.01	(83)
	55	FEC monthly	bax	IHC	10	30%	44%	Not predictive	NS	(83)
Bonetti et al. 1998	55	CMF, FAC, FEC metastatic	bcl-2	IHC	NA	44%	13%	High worse	0.019	(41)
Sjöström et al. 2002	64	Docetaxel metastatic	bcl-2	IHC	5	54%	52%	Not predictive	NS	
	57		bax	IHC	5	54%	52%	Not predictive	NS	
	59	MF metastatic	bcl-2	IHC	5	14%	32%	Not predictive	0.07	(84)
	55		bax	IHC	5	22%	28%	Not predictive	NS	

Abbreviations: RR = response rate; IHC = immunohistochemistry; NA = not available; NS = not significant.

and how these multiple apoptotic pathways contribute to chemosensitivity.

Sequential assessment of tumour biological factors

Attempts have been made to analyse early changes in cytokinetics and other tumour biological factors during neoadjuvant chemotherapy to correlate possible changes with treatment outcome. Remvikos et al. found that modifications in histograms, namely a decrease in S-phase fraction and an increase in the G2M phase of the cell cycle, were associated with a response to chemotherapy ($p < 0.001$) (35). In another study sequential assessment of MDR phenotype identified three groups of tumours with distinct outcomes: positive constant expression of MDR1 predicted resistance to anthracyclines, constant lack of expression of MDR1 predicted response to chemotherapy, and early acquired or increased MDR1 gene expression predicted resistance to various chemotherapy regimens (18). An increasing level of PCNA (proliferating cell nuclear antigen) and erbB-1 (epidermal growth factor receptor) was associated with resistance to neoadjuvant chemotherapy and a decrease in ER with response (86). Interestingly, the apoptotic index and Ki-67 decreased while bcl-2 expression increased significantly in residual tumour specimens as compared to pretreatment specimens after neoadjuvant chemotherapy in the study by Ellis et al. (82). Although the findings were preliminary, the investigators suggested that the phenotype of reduced apoptosis and cell proliferation combined with increased bcl-2 might be associated with breast cancer cells resistant to chemotherapy.

Further studies are needed to find out whether sequential measurements of tumour biologic factors during chemotherapy add something to the more conventional assessment of response to treatment using radiologic imaging techniques.

DISCUSSION

Several groups have tried to find predictive factors for chemotherapy response but the results with any factor have thus far been conflicting (87). Of various tumour biological factors, only high SPF seems to be uniformly associated with response to preoperative chemotherapy. However, the clinical utility of this knowledge is poor since many patients with slowly proliferating tumours also benefit from chemotherapy.

Some methodological aspects should be taken into account when these disappointing results of predictive factors for chemotherapy response in advanced breast cancer are evaluated. First, it is not optimal to assess predictive factors for chemotherapy in metastatic disease from primary tumours. Metastases do not always show clonal relationship with the corresponding primary tumour (88). Previous therapies may also have an effect on clonal selection or chemosensitivity (11, 34). However, in practice it is difficult and sometimes perhaps also unethical to take histological samples from metastases. Thus, the most appropriate way to investigate predictive tumour biological factors would be in the neoadjuvant setting. Nevertheless, the findings of neoadjuvant studies do not necessarily apply to metastatic disease.

Another source of inaccuracy in immunohistochemical studies is that, when heterogeneously expressed, they give only estimates of positive staining of different proteins. Hence, although a panel of distinct factors is investigated, no firm conclusions on the possible interactions can be

made. In that respect, a double immunohistochemical technique might be better. Additionally, the sensitivity and specificity of antibodies used in IHC may vary (89). The conflict in the results of different studies may partly be due to differences in the type of chemotherapy. This raises the possibility of biological markers being used to assist in the selection of the type of chemotherapy regimen administered to particular tumour biological phenotypes. Finally, it is too simplistic to expect that any individual biomarker will be sufficiently predictive to be clinically useful alone. Increasing knowledge about tumour biology has made it clear that no single variable regulates chemosensitivity. Whole-cell biological pathways instead of single genes or proteins should be assessed.

Most clinical studies on prediction of chemotherapy response are fairly small, with less than 100 patients. However, to be a useful marker not only statistically but also clinically, the difference in chemotherapy response according to the investigated tumour biologic factor should be strong enough to be seen preliminarily in these smaller studies (90). Of course, the clinical utility of putative markers needs to be established in larger prospective studies.

Future aspects

To date, no tumour biological factor is available for clinical use in the prediction of chemotherapy response in advanced breast cancer other than oestrogen receptor status, which predicts response to hormonal therapy, or c-erbB-2 status, which predicts response to trastuzumab — a humanized monoclonal antibody directed against the extracellular domain of the HER2 receptor (87). Interestingly, they are both also the targets for those therapies.

Cancer is a disease marked by multiple primary alterations in DNA and there are multiple different pathways for malignant transformation to occur. Patients with breast cancer may have rather different diseases at the molecular level. Moreover, within a tumour mass, there is a large amount of heterogeneity (91). Consequently, distinct subpopulations may require distinct types of therapy and thus have distinct predictive factors according to which therapy should be tailored. In the future, it is hoped that biochip diagnostics will make it possible to identify the specific genetic lesions of each tumour and then individual treatments can be tailored accordingly.

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