

Clinical studies of p53 in treatment and benefit of breast cancer patients

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Abstract

This article describes p53 as a prognostic and predictive factor, together with some information on how best to determine the p53 status. By December 1998, 13 000 articles on p53 were identified on Pub Med, the National Library of Medicine. Within one week a further 62 articles were recorded making it difficult to give the complete p53 story. This review article will focus on discussing p53 in relation to its predictive potential. So far, no firm conclusions can be made based on the articles studied. This may be, in part, because many studies have used less than optimal techniques for determination of the p53 status, together with the fact that the studies lacked power to detect a potential difference in outcome from specific therapy in relation to p53 status.

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Background

The tumour suppressor gene p53 and its protein control critical cellular functions involved in apoptosis and in the control of the cell cycle (Lane 1992) (reviewed in Harris 1996). The encoded protein consists of 393 amino acids giving a molecular mass of 53 000 Daltons. The N-terminal part of the protein is involved in transcription control, the middle portion is responsible for the DNA binding and the carboxy-terminal third of the protein facilitates the tetramerisation of the protein, which is claimed to be required for its function (reviewed in Kirsch & Kastan 1998). The p53 gene can be activated via the ataxia telangiectasia gene (ATM) by carcinogens, cytostatics, radiation, ultraviolet light, hypoxia or by an oncogene (Graeber *et al.* 1994, Serrano *et al.* 1997) (reviewed in Harris 1996, Kirsch & Kastan 1998, Bergh 1998a). When cells acquire irreparable damage, the apoptotic machinery is activated, while in the case of reparable damage the cell cycle is retarded via p53-initiated downstream activation of the cyclin-dependent kinases. This results in inhibition of the cyclins, together with interaction with the retinoblastoma gene product aiming at controlling the cell cycle at specific check points.

The normal p53 function can be inactivated by somatic and germ line mutations, binding to the oncogene murine double minute (MDM2) and binding to different viral oncoproteins (human papilloma virus protein E6, SV40 large T antigen, hepatitis B viral X protein, adenovirus protein E1A) (reviewed in Harris 1996). Somatic mutation

of the p53 gene is, so far, the most common genetic abnormality described in human cancer.

In pre-clinical model systems it was demonstrated that tumours with wild-type p53 status responded better to certain oncological therapeutic modalities than tumours with an altered p53 status (Clarke *et al.* 1993, Lowe *et al.* 1993, 1994, O'Connor *et al.* 1993, Fan *et al.* 1994, Lim *et al.* 1994). Despite these straightforward findings it is also obvious that radiation and certain cytostatics may also induce apoptosis via a p53-independent pathway (Clarke *et al.* 1993) (reviewed in Beck & Dalton 1997). However, the issue is complex, since mutant p53 has been claimed to interfere 'with the p53-independent pathways of apoptosis' (Li *et al.* 1998). p40, p51 and p73 have been demonstrated to have more or less sequence homology with p53 (Jost *et al.* 1997, Kaghad *et al.* 1997, Osada *et al.* 1998, Trink *et al.* 1998).

These genes are now grouped together in the p53 family. How they are activated and may replace and function when p53 is mutated is not known. These aspects may be important both for p53-dependent and -independent apoptosis.

How to determine the p53 status

A variety of largely molecular biological techniques have been used in preclinical studies, while immunohistochemistry and other protein based methods have frequently been used in clinical studies (reviewed in Bergh 1998a). A principal comment is that measurement of the p53 protein is sound so long as it represents the functional

Table 1 IMH p53 status in relation to mutation status and type of mutation detected. Figures within parentheses represent total number detected for each type of mutation.

IMH status	Mutation status	Mutation type
64 positive IMH →	19 wild-type 1 unknown 44 mutations	→ 40 point mutations (45)** 2 deletions (13) 2 insertions (4) 0 stop codons (7)
248 negative IMH →	222 wild-type 3 unknown 23 mutations	→ 5 point mutations (45)** 11 deletions (13) 1 insertion (4) 6 stop codons (7)

Modified from Sjögren *et al.* 1996 and Sjögren 1997 with permission. IMH, immunohistochemistry.

aspect of p53. In comparative studies, the use of immunohistochemistry and another protein measurement will result in false negative and false positive results (Sjögren *et al.* 1996, Børresen-Dale 1997, Norberg *et al.* 1998, Williams *et al.* 1998), e.g. a 30% false positive and a 9% false negative frequency using immunohistochemistry with Pab 1801 compared with cDNA-based sequencing (Table 1) (Sjögren *et al.* 1996, Sjögren 1997).

The protein based methods, mainly immunohistochemistry, miss stop codons and deletions in a high frequency, but detect point mutations (Table 1) (Sjögren *et al.* 1996, Børresen-Dale 1997, Norberg *et al.* 1998, Williams *et al.* 1998).

Prognostic versus predictive factors

Prognostic factors should give information on the prognosis for different subgroups of patients, optimally describing the natural cause and outcome, and be unrelated to different therapeutic interventions. So far, we still use the classical prognostic factors of tumour size and lymph node status, and, with increasing frequency, different histological grading systems. The list of potential prognostic factors for breast cancer is almost exhaustingly long. The need for further prognostic factors is limited, while the need for adequate predictive factors indicating the response to a certain therapy modality is substantial.

A predictive factor should give information on the outcome in relation to a certain oncological therapeutic modality, e.g. oestrogen and progesterone receptor status in relation to hormonal therapy: 60-70% versus 5-10% chance to respond in receptor-positive versus receptor-negative tumours respectively (Roodi *et al.* 1995).

Many prognostic and predictive factors will be analysed on one biopsy from the primary breast cancer, despite the study being focused on the metastatic stage. Solid tumours including breast cancer will most likely demonstrate a marked tumour cell heterogeneity with reference to different markers. Furthermore, it has not been studied in detail whether the primary breast cancer has the same geno- and phenotypic characteristics as the micro- and macrometastases. These considerations highlight the need to analyse the degree of heterogeneity both in the primary tumour and in the metastases, and to be able to obtain representative tissue samples based on these prerequisites. Ideally, in the future the selection of oncological therapeutic modalities should be tailor-made, based on analyses of the tumour's biological profile both in the primary tumour and in the metastases.

p53 as a prognostic factor

Mutations in the p53 gene or increased expression of the p53 protein as an indirect marker of a mutation have been described as prognostic factors for a long list of human malignant tumours, including breast cancer, being associated with worse prognosis (Cattoretti *et al.* 1988, Callahan 1992, Allred *et al.* 1993, Andersen *et al.* 1993, Barnes *et al.* 1993, Elledge *et al.* 1993, Silvestrini *et al.* 1993, Thor & Yandell 1993, Bergh *et al.* 1995, Gasparini *et al.* 1998).

A large study on 1400 node-negative breast cancer patients demonstrated a survival disadvantage for those patients with an increased immunohistochemical expression of p53 (Silvestrini *et al.* 1996); this was also demonstrated in other studies on node-negative patients using immunohistochemistry (Allred *et al.* 1993,

Table 2 Clinical studies on p53 and tamoxifen

Number of patients	Technique and antibody for p53 demonstration	Negative correlation between p53 and response to therapy and survival		Reference
		Response	Survival	
92	IMH, CMI	No	No	Archer <i>et al.</i> (1995)
48	Seq	—*	Yes	Bergh <i>et al.</i> (1995)
205	IMH, 1801	No	Yes	Elledge <i>et al.</i> (1997)
401	LIA	Yes	Yes	Berns <i>et al.</i> (1998)

—, no information; *, not relevant, adjuvant therapy; IMH, immunohistochemistry; Seq, sequencing; LIA, p53 lumino-metric immunoassay.

Gasparini *et al.* 1998). However, sequence based analyses of the p53 status on a smaller cohort of 205 node-negative patients could not verify the potential prognostic information for the node-negative group (Bergh *et al.* 1995).

Molecular biological techniques add a further dimension while certain mutation sites, the zinc binding domains L2 and L3 and the evolutionarily conserved regions II and V respectively, have been described as being associated with particularly poor survival (Bergh *et al.* 1995, Børresen *et al.* 1995).

p53 as a predictive factor

Cytostatics, tamoxifen and radiation can induce apoptosis via either p53-dependent or p53-independent pathways. In the following paragraphs the effects of tamoxifen, cytostatics and radiation will be described in more detail, with focus on the p53-dependent apoptotic pathway.

p53 and tamoxifen

The oestrogen and progesterone receptor-positive cell line MCF-7 was transfected with mutant p53 (Elledge *et al.* 1995). The mutation was localised to the amino acid position 179, resulting in an amino acid shift from histidine to glutamine (Elledge *et al.* 1995). Five transfected clones were studied with reference to the response to tamoxifen. In soft agar experiments four out of five mutants retained their sensitivity to tamoxifen. Based on these transfection experiments the authors concluded that the introduction of a mutation in amino acid position 179 did not result in oestrogen-independent growth or tamoxifen resistance (Elledge *et al.* 1995). However, these authors correctly considered that different p53 mutation sites may be associated with different functional implications (Halevy *et al.* 1990, Milne *et al.* 1992, Dittmer *et al.* 1993, Mukhopadhyay & Roth 1993, Bergh *et al.* 1995, Børresen *et al.* 1995).

Ninety-two patients with metastatic or locally advanced breast cancer received primary hormonal therapy (Table 2) (Archer *et al.* 1995). The expression of

p53, c-erbB-2 and ras p21 was analysed with immunohistochemistry, for p53 using the polyclonal antibody CMI. No statistically significant relationships were demonstrated between these parameters and the response to therapy, time to treatment failure or total survival (Archer *et al.* 1995). In another immunohistochemistry-based study, the response to tamoxifen was tested in 205 oestrogen receptor-positive and metastatic breast cancer patients in relation to the protein expression of bcl-2 and p53 (Table 2) (Elledge *et al.* 1997). Patients with high immunohistochemical levels of p53 either in the primary breast cancer or in the metastatic tumour, demonstrated a statistically significant shorter median survival compared with those with a lower p53 expression, 20 months versus 36 months ($P=0.008$) respectively (Elledge *et al.* 1997). Patients with a tumour containing a higher immunohistochemical value demonstrated no significant difference in response (58% versus 50%, $P=0.36$) compared with the tumours with a lower immunohistochemical expression (Elledge *et al.* 1997). Taken together, these data indicate that p53 is a prognostic indicator for this set of patients, but not a predictor for response to endocrine therapy (Elledge *et al.* 1997). Contrary to these findings, we have previously demonstrated a significantly worse overall survival for lymph node-positive patients receiving adjuvant tamoxifen together with loco-regional radiotherapy, if the tumours had a mutant p53 status compared with those with wild-type p53 status, using cDNA-based sequencing (Table 2) (Bergh *et al.* 1995). Furthermore, in the metastatic setting, 401 tamoxifen-treated patients with relapse of breast cancer were studied in relation to the p53 protein levels in the primary breast cancers (Table 2) (Berns *et al.* 1998). The p53 protein levels were reported not to be related to the oestrogen and progesterone levels (Berns *et al.* 1998). These authors demonstrated an association between the p53 protein levels and response to tamoxifen therapy. Patients with higher p53 protein levels demonstrated a 42% response while those with lower p53 protein levels demonstrated a 56% response, (Berns *et al.* 1998). This observation re-

Table 3 Clinical studies on p53 and chemotherapy

Number of patients	Chemotherapy type	Technique and antibody for p53 demonstration		Negative correlation between p53 and response and relapse/DFS/survival		Reference
		Technique	Antibody	Response	Relapse/DFS/survival	
40	CMF	ELISA	Not given	Yes	–	Koehler <i>et al.</i> (1994)
57	MMT	IMH	1801, 240	No	–	Makris <i>et al.</i> (1995)
86	AVCMF	IMH	DO7	No	–	Mathieu <i>et al.</i> (1995)
86	Weekly doxorubicin	CDGE	Seq	–	Yes	Aas <i>et al.</i> (1996)
128	EVM, MTV	IMH	DO7	No	No	MacGrogan <i>et al.</i> (1996)
277	CMF	IMH	DO1	–*	No	Dublin <i>et al.</i> (1997)
35	5-FU + RT	IMH	DO7	Yes	–	Formenti <i>et al.</i> (1997)
40 (70)	AC, HD-pacl	IMH	DO7	No	Yes**	Linn <i>et al.</i> (1997)
90	MM(M)T	IMH	1801,240	No	–	Makris <i>et al.</i> (1997)
127	FEC vs 1/4 FEC Perioperative	IMH	DO7	–	No	NoNiskanen <i>et al.</i> (1997)
441	FAC	IMH	DO7	–*	Yes	Clahsen <i>et al.</i> (1998)
282	CMF	IMH	DO7	–*	No	Degeorges <i>et al.</i> (1998)
329	FAC or RT	IMH	DO7	No	No	Rozaan <i>et al.</i> (1998)
994	FAC	IMH	1801	–*	Yes***	Thor <i>et al.</i> (1998)
126	CMF	Seq	Not relevant	–*	Yes	Larsson <i>et al.</i> (submitted)

Seq, sequencing; CDGE, constant denaturant gel electrophoresis; ELISA, enzyme-linked immunoassay; IMH, immunohistochemistry; DFS, disease-free survival; –, no information; *, not relevant, adjuvant therapy; **, worse survival for patient with p53 positive and P-gp positive tumours; ***, interaction with c-erbB-2, significant for survival; AC, doxorubicin, cyclophosphamide; AVCMF, doxorubicin, vincristine, cyclophosphamide, methotrexate, 5-fluorouracil; CMF, cyclophosphamide, methotrexate, 5-fluorouracil; EVM, epirubicin, vincristine, methotrexate; FAC, 5-fluorouracil, doxorubicin, cyclophosphamide; FEC, 5-fluorouracil, epirubicin, cyclophosphamide; HD-pacl, high dose paclitaxel; MM(M)T, mitoxantrone, methotrexate, (+/- mitomycin C), tamoxifen; MTV, mitomycin C, thiotepa, vindesine; RT, radiotherapy.

tained significance in the multivariate model (odds ratio 0.48, confidence interval 0.31 to 0.74; $P < 0.001$).

p53 and cytostatics

p53 is involved in different aspects of cellular resistance. As an example, mutant p53 has been reported to stimulate the MDR1 promoter (Chin *et al.* 1992, Zastawny *et al.* 1993). There is a wide spectrum of cellular resistance mechanisms to cytostatics, including p53 related effects, not to mention the pharmacokinetic factors (Bergh 1998b). In different pre-clinical experiments using mouse embryo fibroblasts and human foreskin fibroblasts, the authors demonstrated that carboplatin, cisplatin and paclitaxel may have increased activity on cells with inactivated p53 function (Hawkins *et al.* 1996, Wahl *et al.* 1996). However, contradictory results have also been published (Wu & El-Deiry 1996, Houldsworth *et al.* 1998). The reason for this discrepancy may, in part, be related to some of the model systems and one may wonder whether inactivation of the p53 protein with viruses will result in similar functional alterations as a p53 mutation. The topoisomerase I inhibitors, 10-hydroxycamptothecin and camptothecin, have been investigated on human breast

cancer cell lines with reference to their apoptotic capacity (Liu & Zhang 1998). In the cell lines used, these drugs were able to induce apoptosis via both p53-dependent and -independent pathways (Liu & Zhang 1998).

Based on the results in the preclinical models on p53 with reference to the effect of chemotherapy, these observations have been expanded to retrospective clinical studies on breast cancer patients and to other tumour types not included in this review. The use in many clinical studies of different polychemotherapy regimens makes it potentially difficult to interpret the results. Different cytostatics included in one combination may work via p53-dependent and -independent pathways respectively, which may dilute the p53-related effects.

In a neo-adjuvant chemotherapy study on 86 patients treated with AVCMF therapy (doxorubicin, vincristine, cyclophosphamide, methotrexate, 5-fluorouracil), no correlation was demonstrated between p53 protein expression using the DO7 monoclonal antibody and tumour response (Table 3) (Mathieu *et al.* 1995). One further neoadjuvant study on 57 patients was also negative: immunohistochemistry of p53-negative patients had a response rate of 81%, 29 of 31 patients responded. (Table 3) (Makris *et al.* 1995). The corresponding figure

for those with positive immunohistochemistry using Pab 240 was 16 of 21 treated patients. The chemotherapy in this study consisted of mitoxantrone, methotrexate combined with tamoxifen. In a follow-up study on 90 patients with primary operable breast carcinoma, p53 was analysed together with Ki-67, bcl-2 and c-erbB-2 together with the receptors and no correlation was detected between the immunohistochemical expression for p53 and the response to the mitoxantrone-based polychemotherapy regimen in combination with tamoxifen (Table 3) (Makris *et al.* 1997). In another immunohistochemical study on 130 patients receiving CMF (cyclophosphamide, methotrexate, 5-fluorouracil) or no adjuvant chemotherapy, the prognostic value for p53 was demonstrated (Table 3) (Dublin *et al.* 1997). The effects of adjuvant CMF in both the p53-positive and -negative tumours were very similar. The relative survival risk was 2.3 (95%, confidence interval 1.2-4.3) and 2.1 (95%, confidence interval 1.4-3.0) for the immunohistochemically p53-positive and -negative tumours respectively (Dublin *et al.* 1997). In another study on 282 patients receiving CMF, no significant correlation between positive immunohistochemistry using the DO7 monoclonal antibody and survival could be demonstrated (Table 3) (Degeorges *et al.* 1998). The effect of neo-adjuvant 5-fluorouracil, doxorubicin and cyclophosphamide or radiotherapy before surgery was evaluated on 329 patients in relation to the immunohistochemical expression of c-erbB-2 and p53 (Table 3) (Rozaan *et al.* 1998). No correlation could be demonstrated between the p53 expression and response to therapy (Rozaan *et al.* 1998).

In 127 patients with metastatic breast cancer p53, c-erbB-2 and cathepsin-D were analysed and none of these factors predicted the outcome after polychemotherapy with 5-fluorouracil, epirubicin and cyclophosphamide (Table 3) (Niskanen *et al.* 1997).

Similarly, p53 analysed with immunohistochemical techniques did not predict the response to chemotherapy in a study of 40 patients with locally advanced breast cancer and other breast cancer subgroups (Linn *et al.* 1997), or in another study on 128 breast carcinomas (Table 3) (MacGrogan *et al.* 1996).

On the other hand, positive clinical studies have also been published. No obvious patterns identify the negative and positive studies respectively, but the technical aspects of determination of the p53 status and the techniques used, as well as the representativeness of the patient populations may have influenced the results (Elledge 1996, Silvestrini 1996, Sjögren *et al.* 1996, Bergh 1998a).

Four hundred and forty-one premenopausal and node-negative patients were investigated for the immunohistochemical expression of p53, c-erbB-2, Ki-67, receptors and angiogenesis (Table 3) (Clahsen *et al.* 1998). Patients with negative immunohistochemistry for p53

using DO7 had a statistically significant ($P<0.01$) benefit in 4-year disease-free survival from preoperative 5-fluorouracil, doxorubicin and cyclophosphamide therapy compared with the patients not receiving perioperative chemotherapy (Clahsen *et al.* 1998). No benefit ($P=0.8$) could be demonstrated for the p53-positive group. In another retrospective study, p53 and c-erbB-2 were analysed in a group of 992 lymph node-negative breast cancer patients receiving different dose levels of doxorubicin combined with cyclophosphamide and 5-FU (Table 3) (Thor *et al.* 1998). This study demonstrated that patients with c-erbB-2-expressing tumours needed to have high or moderate doxorubicin doses and an interaction was described between p53 immunohistochemical expression and the chemotherapy dose (Thor *et al.* 1998).

In a study of only 40 patients, the authors claimed a positive correlation between p53 protein levels, measured with an enzyme-linked immunoassay, and response to CMF therapy (Table 3) (Koechli *et al.* 1994).

In a study of 86 patients with locally advanced or metastatic breast cancer who were treated with weekly doses of 20 mg doxorubicin (Table 3) (Aas *et al.* 1996), only 3 out of 40 (8%) breast cancers progressed during therapy if p53 was of the wild-type, while the corresponding figure for tumour progression or relapse was 11 of 16 (69%) patients when p53 was mutant ($P<0.05$) (Aas *et al.* 1996). Furthermore, in patients with tumours containing p53 mutations within the zinc finger binding regions L2 or L3, 8 out of 10 ($P=0.01$) patients had tumours demonstrating relapse or progression during follow-up (Aas *et al.* 1996). In another study on 126 patients, those with breast cancers with mutant p53 had a statistically worse outcome compared with those with wild-type p53 when receiving adjuvant CMF (Table 3) (Larsson *et al.* submitted).

Thirty-five women with locally advanced breast cancer received a preoperative continuous infusion of 5-fluorouracil and loco-regional radiotherapy (Table 3) (Formenti *et al.* 1997). Lack of p53 overexpression was statistically significantly ($P=0.01$) correlated with a histopathological response to the therapy (Formenti *et al.* 1997).

A Finnish group has performed a multivariate analyses on 103 patients with inclusion of p53, c-erbB-2, bcl-2, bax, S-phase fraction, ploidy, receptors and histological grade with reference to time to progression and survival, once more with a negative result for p53 using immunohistochemistry (Sjöström *et al.* 1998).

Recently, 226 patients with stage I or early stage II breast cancer were analysed for p53 and c-erbB-2 using an artificial neural networks system (Burke *et al.* 1998). The authors reported that the TNM-staging system was no better at predicting response for stage I and early stage II patients than flipping a coin. p53 and c-erbB-2 and the

number of positive nodes were, however, useful predictors for response to systemic adjuvant therapy, chemotherapy or tamoxifen, or postoperative radiotherapy (Burke *et al.* 1998).

p53 and radiotherapy

Cells with mutant p53 status were initially described as being less sensitive to radiotherapy (Lowe *et al.* 1994). It has also been demonstrated that heterozygous cell lines (mutant/wild-type) are less sensitive to radiation compared with wild-type cells (Delia *et al.* 1996). In studies of human lymphoblastoid cell lines, it was demonstrated that radiation was able to induce apoptosis to the same extent as in the line with wild-type p53 status, but with a time delay for the mutant cell line (Xia *et al.* 1995).

Clinical studies on node-negative breast cancer patients have demonstrated that those with mutant p53 or increased p53 protein levels have a statistically significantly decreased risk for relapse if they received postoperative radiotherapy compared with the corresponding patients who did not receive postoperative radiotherapy (Jansson *et al.* 1995, Silvestrini *et al.* 1997). On the other hand, patients with wild-type p53 tended to gain much less benefit from postoperative radiotherapy (Jansson *et al.* 1995).

From these data, one may extrapolate that certain p53 mutation sites may be associated with a partly retained apoptotic capacity, possibly due to the prolonged half-life of mutant p53 (Bergh 1997). Furthermore, another less likely explanation for the results would be that there is retention of a second and normal p53 allele with intact functional properties. We have demonstrated in a study of 100 breast cancer patients with 26 somatic p53 mutations that in 21 of the tumours we had loss of heterozygosity, in one tumour we could not find loss of heterozygosity and in four tumours the data were not informative (Williams *et al.* 1998).

Conclusion

p53 has critical functions for the control of the cell cycle and apoptosis. Cytostatics, tamoxifen and radiation may induce apoptosis via p53-dependent and -independent pathways. The clinical data with reference to the potential predictive value of p53 are still conflicting, which is due partly to suboptimal methods for determination of the p53 status, and partly to too small studies combined with selected patient materials. The data so far indicate that CMF-based regimens and tamoxifen may be suboptimal for patients with mutant p53 and postoperative radiotherapy may be extra beneficial for breast cancers with mutant p53. For the future, larger studies on population-based cohorts using optimal methods for p53

measurements are warranted, and ideally the studies should have a prospective design based on randomised patient populations.

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