Table 1. Combined effect of diabetes mellitus and obesity on postmenopausal breast cancer risk, Italy 1991–1994

	Diabetes mellitus				
	No		Yes		All
Severe obesity*	Cases:controls	OR (95% CI)†	Cases:controls	OR (95% CI)†	OR (95% CI)†
No	1117:1287	1‡	62:49	1.6 (1.1–2.4)	1‡
Yes	348:362	1.2 (1.0–1.4)	44:39	1.6 (1.0–2.5)	1.2 (1.0–1.4)
All	1465:1649	1‡	106:88	1.5 (1.1–2.0)	

* Women who belonged to the 5th highest quintile of body mass index ($kg/m^2 > 28.8$). † Odds ratios (OR) and 95% confidence intervals (CI) from unconditional multiple logistic regression equations including terms for study area, age, education, parity, menopausal status, plus diabetes and severe obesity, when required. ‡ Reference category.

(e.g. increase of physical activity [5]), as well as treatment (e.g. pharmacological inhibitors of IGF action [6]).

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p21^{WAF1} and p53 Immunohistochemical Expression in Breast Carcinoma may Predict Therapeutic Response to Adjuvant Treatment

M. Barbareschi,[1] C. Doglioni,[2] S. Veronese,[3] M. Bonzanini,[1] P. Dalla Palma,[1] A.L. Harris[4] and O. Caffo[5]

[1]Departments of Histopathology and [5]Medical Oncology; S. Chiara Hospital, Medical Oncology Department, 38100 Trento; [2]Department of Histopathology, Hospital of Belluno, 32100, Belluno; [3]Department of Histopathology, Niguarda-Ca'Granda Hospital, Piazza Ospedale Maggiore 1, 20100 Milano, Italy; [4]Imperial Cancer Research Fund, University of Oxford, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, U.K.

P53 GENE alterations may play a major role in determining cellular chemosensitivity [1, 2]. However, *in vivo* studies using p53 immunohistochemical overexpression as a marker of *P53* mutation provided conflicting results [3–5]. One problem with immunohistochemical studies is that p53 overexpression does not always reflects *P53* mutation and loss of function. A way to investigate the functional status of *P53* is to evaluate the expression of some of its downstream effectors, such as p21^{WAF1}, which acts by blocking cyclin-dependent kinases.

We investigated the immunohistochemical expression of p53 and p21 in 170 invasive breast carcinomas, treated with adjuvant systemic therapy. 140 patients were node-positive (N1/2) and 30 were node-negative (N0); median follow-up

Correspondence to O. Caffo.
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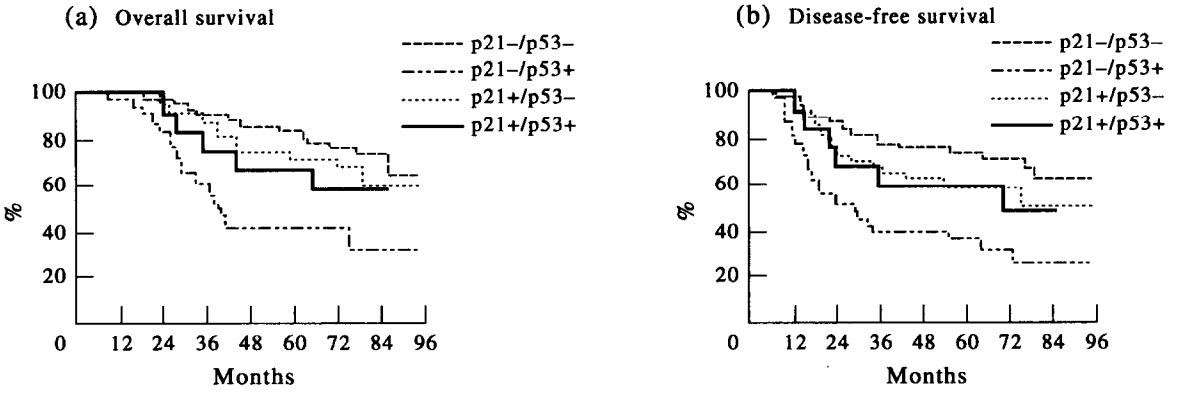

Figure 1. DFS and OS curves for the 170 patients stratified on the basis of the p53/p21 phenotype.

was 66 months (range 9–96). 86 patients (72 N1/2 and 14 N0) received adjuvant chemotherapy (cyclophosphamide, methotrexate, 5-fluorouracil), 75 patients (59 N1/2 and 16 N0) received adjuvant hormonotherapy (tamoxifen 20 mg daily), and 9 node-positive patients were treated with both hormono- and chemotherapy. p53 and p21 immunostaining were carried out on paraffin sections using the DO-7 (Dako, Glostrup, Denmark) and EA10 (Oncogene Science, Cambridge, Massachusetts, U.S.A.) monoclonal antibodies, respectively. p53 overexpression (p53 +, defined as >15% of reacting cells) was seen in 48 (28%) cases. p21 overexpression (p21 +, defined as staining of >10% of cells) was seen in 52 (31%) cases.

p53 positivity was related to short disease-free survival (DFS) and breast cancer-related overall survival (OS) ($P = 0.0002$ and $P = 0.0001$, respectively; Kaplan–Meier method, log-rank test). p21 positivity was not related to prognosis. Bivariate analysis of the combined p53/p21 phenotype showed that p53 + /p21− tumours had the worst prognosis (5 year DFS 36%, OS 41%), p53 − /p21+ and p53 + /p21+ tumours were associated with intermediate prognosis (5 year DFS 60% and 58%, respectively; 5 year OS 72% and 67%, respectively), while p53 − /p21− tumours had the best prognosis (5 year DFS 72%, OS 83%) (Figure 1). Multivariate analysis (Cox proportional hazard method) showed that the only variables independently associated with DFS and OS were tumour size and the p53 + /p21− phenotype ($P = 0.0022$ and 0.0148 for DFS respectively, and $P = 0.0001$ and $P = 0.0004$ for OS respectively); nodal status and oestrogen receptor status were independently associated only with DFS ($P = 0.0001$ and $P = 0.0017$, respectively).

The different p53/p21 phenotypes suggest the presence of at least four different situations: (1) p53 + /p21− cases should be those without any *P53* function, since p53 is overexpressed, and hence probably mutated, and its effector is lacking; (2) p53 + /p21+ cases could bear either a wild-type p53 protein which induces p21, or a mutated p53 protein which is still able to induced p21; (3) p53 − /p21+ cases should have wild-type p53, and p21 expression could be due to p53-independent mechanisms; (4) p21 − /p53− cases could be those with wild-type p53 and without p21 activation. The p53 + /p21− phenotype was associated with the worst DFS and OS, which, in this group of treated patients, may be considered as an index of a high degree of treatment failures; conversely the p53 + /p21+ phenotype was associated with a relatively good prognosis, similar to that of p53 negative tumours. We hypothesise that the p53 + /p21− phenotype could correspond to cases with impaired G1 checkpoint, which may not be able to activate the apoptotic cascade in response to DNA-damaging drugs. Conversely, p53 + /p21+ cases may bear an intact *P53* function, and hence may be able to activate apoptosis in response to adjuvant treatment.

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