The Breast xxx (2015) 1–4

Contents lists available at ScienceDirect

The Breast

journal homepage: www.elsevier.com/brst

p53 status identifies triple-negative breast cancer patients who do not respond to adjuvant chemotherapy

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ARTICLE INFO

Article history:
Received 9 May 2014
Received in revised form
8 November 2014
Accepted 28 January 2015
Available online xxx

Keywords:
Breast cancer
Triple negative
p53 status
Adjuvant chemotherapy

ABSTRACT

Genomic analysis and protein expression assimilate triple-negative breast cancers (TNBC) with basal-like breast tumors. TNBCs, however, have proved to encompass also tumors with normal-like phenotype and known to have favorable prognosis and to respond to chemotherapy. In a recent paper, we have provided evidence that p53 status is able to subdivide TNBCs into two distinct subgroups with different outcome, and consistent with basal- and normal-like phenotypes. Based on this finding, we explored the contribution of p53 status in predicting the response to adjuvant CMF or CMF followed doxorubicin chemotherapy of a group of TNBC patients. Results indicated that TNBC patients with a p53-positive tumor had a shorter relapse-free and overall survival than patients carrying a p53-negative TNBC, corroborating our hypothesis about the relationship between TNBC phenotype (basal-like *versus* normal-like) and p53 status as predictor of response to antracycline/CMF-based chemotherapy.

Triple-negative breast cancers (TNBC)s are characterized by an estrogen (ER), progesterone receptor (PR) and HER2-negative status. This clinical class represents a relevant issue because of its high incidence in young patients, high aggressiveness and lack of target therapies. Because of their triple negativity, TNBCs do not benefit neither from anti-hormone or HER2-targeted therapy [1,2] and conventional chemotherapy remains the only available modality of systemic care [3,4].

Genomic analysis and protein expression have assimilated TNBCs with basal-like breast tumors although immunohistochemical studies have clearly demonstrated that not all basal-like cancers are TNBCs and suggested that the clinical category "triple-negative" also includes tumors with a normal-like phenotype [5,6].

In agreement with the suggested biological heterogeneity of the TNBC class, in a recent study [7], we found that p53 protein expression was able to subdivide TNBCs into two distinct groups with different clinical outcome. Patients with a p53-positive TNBC showed shorter event-free and overall survival compared with patients with a p53-negative TNBC. Since a p53-positive status is generally associated with a more aggressive phenotype, we suggested the immunohistochemical evaluation of p53 expression as useful to distinguish basal-like TNBCs from tumors with a normal breast-like phenotype, which are recognized to have a better prognosis than basal-like tumors and to respond to neoadjuvant chemotherapy [8–11].

To challenge such a discriminative prognostic capacity, we explored the contribution of p53 status evaluation in predicting the response to adjuvant chemotherapy of a group of node-positive TNBCs enrolled, between November 1981 and July 1990, in a prospective randomized trial carried out at the Istituto Nazionale Tumori in Milan, Italy.

Methods

Patients

Main goal of the trial was to evaluate the response to adjuvant treatment with cyclophosphamide, methotrexate, and fluorouracil

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http://dx.doi.org/10.1016/j.breast.2015.01.007
0960-9776/

(CMF) alone for 12 cycles or CMF for eight cycles followed by doxorubicin (Adriamycin, ADM) for four cycles [12,13] in a group of patients who had a radical mastectomy or a quadrantectomy plus axillary dissection for unilateral breast cancer and who had histological evidence of involvement of one to three axillary nodes. After stratification by menopausal status, patients were randomly assigned to receive either CMF alone or the CMF followed by ADM. No adjuvant endocrine therapy was administered and irradiation was limited to the remaining portions of the breast after quadrantectomy. In the absence of symptoms, physical examination was performed every 3 weeks during chemotherapy treatment, then every 6 months for the first 5 years, and every 12 months thereafter.

Of the original case series, composed of 552 patients (275 treated with CMF alone and 277 treated with CMF followed by ADM), 514 (259 treated with CMF alone and 255 treated with CMF followed by ADM) had a p53 immunohistochemical evaluation. According to baseline characteristics, study population and selected subset were found similar as to indicate no evident bias of selection. Of the 514 patients with p53 evaluation, 57 (10.3%) were found to carry a TNBC (Table 1). As regards adjuvant treatment, 30 received CMF alone and 27 received CMF followed by ADM.

Relapse-free survival (RFS) was estimated considering as treatment failure the first documented evidence of new manifestation of disease in locoregional areas (including homolateral supraclavicular adenopathy), distant sites, contralateral breast, or any combination of these sites. Of 514 patients with p53 evaluation, 250 experienced new manifestation of disease (64 locoregional, 120 distant, 32 distant plus locoregional and 34 contralateral lesions) whereas 24 had a second primary tumor that was not considered as a treatment failure. In these cases, for RFS estimation, follow-up was censored at the date of the last visit with no cancer evidence or at the occurrence of the second primary tumor. The median follow-up time was of 180 months.

Overall survival (OS) was estimated considering death from all causes, ascertained through medical records, death certificates or family doctors. In total, 162 patients died of breast cancer and 25 died of other causes.

Immunohistochemistry

Immunohistochemical evaluation of a panel of markers was performed on paraffin-embedded tissue. In addition to estrogen (clone 1D5, 1:200 diluted, DBA, Segrate, Milan, Italy) and progesterone receptor (MAb 1A6, 1.100 diluted, DBA), HER2 (anti-c-erbB2 CB11, 1:100 diluted, Ylem, Avezzano, Italy) and p53 protein expression (anti-p53 MAb DO7, 1:500 diluted, Novocastra, Newcastle upon Tyne, UK) were immunohistochemically evaluated as elsewhere described [13]. Immunostaining was performed by a sensitive peroxidase-streptavidin method on paraffin-embedded material and using an automated immunostainer (TechMate 1000, Dako, Copenhagen, Denmark). As a negative control, the primary antibody was replaced with a nonimmune serum from the same species in which the primary antibody was produced. Appropriate cases with known reactivity were used as positive controls. Section were scored positive when more than 10% of tumor cells were labeled, except for HER2 which was scored as positive when strong membrane labeling was observed. With this scoring system, CB11+ cases were found to correspond to tumor scoring 3+ with HerceptTest (Dako) as evaluated on the same primary breast carcinomas.

Table 1
Tumor marker status of patients with p53 evaluation.

Variable		TNBC		Non-TNBC	
		No. patients	(%)	No. patients	(%)
p53	Neg.	37	64.9	386	84.5
	Pos.	20	35.1	71	15.5
ER	Neg.	57	100	205	44.9
	Pos.	0	0	252	55.1
PR	Neg.	57	100	219	47.9
	Pos.	0	0	238	52.1
HER2	Neg.	57	100	384	84.1
	Pos.	0	0	73	15.9

Abbreviations: TNBC, triple-negative breast cancer; Neg., negative; Pos., positive; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2. For cutoff values see Methods section.

Statistical analysis

To evaluate the effect of treatment according to p53 status, RFS and OS probability curves were estimated by the Kaplan–Meier method whereas the differential effect of treatment according to p53 status was tested by Cox regression model. Statistical analysis was performed using R: A Language and Environment for Statistical Computing (R Core Team, R Foundation for Statistical Computing, Vienna, Austria, 2013, http://www.R-project.org) and a P-value ≤ 0.05 was considered significant.

Results

The 20 TNBC patients with a p53-positive tumor had a shorter RFS and OS than patients with a p53-negative TNBC (Fig. 1). Because of the small number of events, while RFS reached the statistical evidence ($p = 0.0206$), OS did not ($p = 0.3620$). Furthermore, results showed that the unfavorable outcome observed in p53-positive TNBCs was independent from the adjuvant regimen applied since treatment failure was observed in both CMF alone and CMF followed by ADM (data not shown). Conversely, in non-TNBC group, p53 status did not appear predictive for response to adjuvant chemotherapy.

Conclusions

As derived from a prospective randomized trial, present results provided reliable evidence that, in TNBC patients, p53 status was associated with a short time to recurrence and corroborated our hypothesis on the relationship between p53 status, as predictor of response to adjuvant chemotherapy, and TNBC phenotype (specifically, basal-like *versus* normal-like). In addition, they suggest a probable link between two clinical findings until now not directly connected, *i.e.*, the observation that TNBCs encompass basal-like and normal-like phenotype, and that p53 mutations are more frequently associated with basal-like phenotype than non basal-like [14]. Furthermore, present results provided a possible explanation for the debatable consistency of p53 status as response predictor for different adjuvant or neoadjuvant treatments, including alkylating agents, anthracyclines, platinum salts, and taxanes [15–18], and suggested the use of p53 immunohistochemical evaluation to identify patients who would benefit from adjuvant strategy alternative to conventional chemotherapy. An interesting alternative could be a gene therapy based on interfering RNA approach and aimed to inhibit the synthesis of the mutated p53 protein [19,20]. It should be recalled, in fact, that unlike other tumor-suppressor genes that are downregulated typically by deletion, truncation or silencing, the majority of *TP53* alterations are missense mutations in the DNA-binding domain. Generally, this type of mutation leads to a high constitutive expression of mutant p53 protein with dominant–negative

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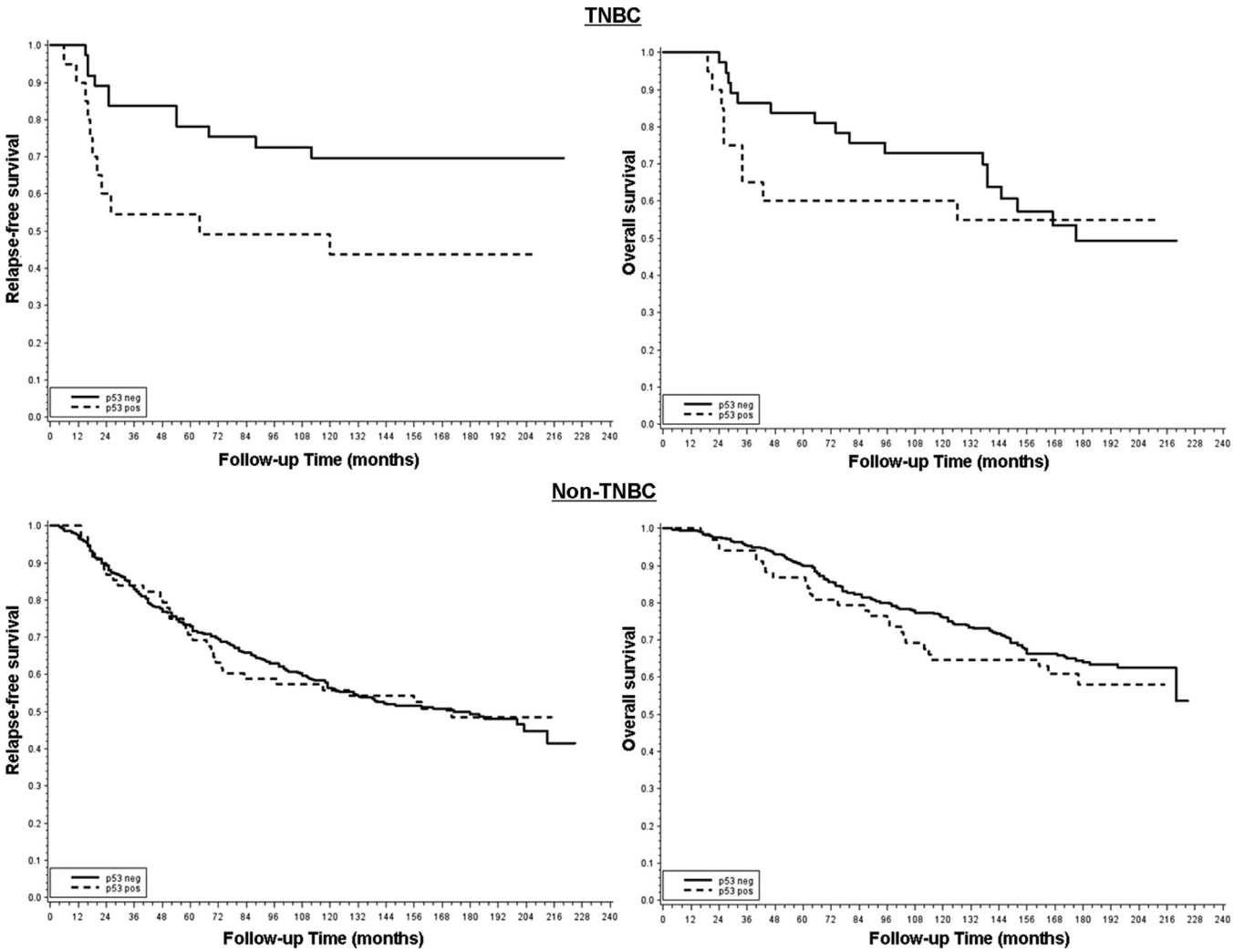

Fig. 1. Relapse-free survival and overall survival according to p53 status.

activity able to interfere with the oncosuppressive function of normal p53 and with the response to chemotherapeutic regimens that include anthracyclines.

Ethical approval

The original prospective randomized trial, of which present investigation is an ancillary study, was approved by the member of the institute's research and ethics committees.

Funding source

Supported in part by the Associazione Italiana Ricerca sul Cancro, Milan, Italy. The study sponsor had no involvement in the study design, in the collection, analysis and interpretation of data, in the writing of the manuscript, and in the decision to submit the manuscript for publication.

Conflict of interest statement

All authors declared any financial and personal conflict of interest.

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