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# p53 Status Identifies Two Subgroups of Triple-negative Breast Cancers with Distinct Biological Features

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**Objective:** Despite the clinical similarities triple-negative and basal-like breast cancer are not synonymous. Indeed, not all basal-like cancers are negative for estrogen receptor, progesterone receptor and HER2 expression while triple-negative also encompasses other cancer types. P53 protein appears heterogeneously expressed in triple-negative breast cancers, suggesting that it may be associated with specific biological subgroups with a different outcome.
**Methods:** We comparatively analyzed p53 expression in triple-negative tumors from two independent breast cancer case series (633 cases from the University of Ferrara and 1076 cases from the University of Nottingham).
**Results:** In both case series, p53 protein expression was able to subdivide the triple-negative cases into two distinct subsets consistent with a different outcome. In fact, triple-negative patients with a p53 expressing tumor showed worse overall and event-free survival.
**Conclusions:** The immunohistochemical evaluation of p53 expression may help in taming the currently stormy relationship between pathological (triple-negative tumors) and biological (basal breast cancers) classifications and in selecting patient subgroups with different biological features providing a potentially powerful prognostic contribution in triple-negative breast cancers.

*Key words: breast cancer – triple-negative – prognosis – biological marker*

## INTRODUCTION

Clinically, breast cancer patients fall into three main groups: those with estrogen (ER) and progesterone (PR) receptor-positive tumors, who are generally managed with anti-hormone treatments with/without chemotherapy; those with HER2-positive tumors, who can receive a HER2-targeted therapy; those with ER, PR and HER2-negative tumors, for whom the lack of tailored therapies makes chemotherapy the only available modality of systemic care (1).

Genome-wide DNA microarray analysis was used to classify breast cancers into five main expression profile groups,

two of them ER-positive (luminal A and B) and three ER-negative [normal breast-like, ERBB2 (also known as HER2) and basal-like] (2–4). Consistent grouping were also obtained resorting either to routine or experimental immunohistochemical markers (5,6). In addition, single nucleotide polymorphism association studies indicated that different genetic risk factors can be associated to ER-positive or ER-negative tumors, and that they may also vary according to the expression of HER2 or basal cancer markers (7). The basal-like cancer group includes tumors that lack both steroid hormone receptors and HER2 expression, the so-called triple-negative cancers (3,8–10). However, despite the clinical similarities between basal-like and triple-negative tumors, including higher incidence in younger patients (11,12), higher histologic grade (9–11), aggressive clinical behavior and poor prognosis (13–15), triple-negative and basal-like breast cancers are not synonymous. Indeed, not all basal-like cancers are negative for ER, PR and HER2 expression (8) and the triple-negative group encompasses also non-basal-like tumors, namely normal breast-like cancers (10). Notably, although normal breast-like tumors have a somewhat better prognosis than basal-like cancers (3,4,16), they do not respond to neoadjuvant chemotherapy as well as basal-like cancers do (17,18).

Salient features of triple-negative breast cancers include overexpression of epidermal growth factor receptor (EGFR) and c-KIT, a high proliferative rate, frequent genomic alterations, phenotypic similarity to *BRCA1*-associated cancers and frequent mutations of the *TP53* gene with the corresponding protein heterogeneously expressed (10,19,20). Hence, we comparatively analyzed p53 expression in triple-negative breast cancers from two independent breast tumor case series to assess whether it was associated with specific subgroups with different biological features.

## PATIENTS AND METHODS

### Ferrara Case Series

A series of 633 patients who underwent surgery for primary infiltrating breast cancer between 1983 and 1992 at the University of Ferrara was analyzed (5). Informed written consent was obtained from all patients and the study was approved by the University of Ferrara Research Ethics Committee. ER, PR, Ki-67/MIB-1 proliferation index (Ki-67), HER2 and p53 levels were assessed by immunohistochemistry (IHC) as elsewhere described (5). Briefly, the H222 (ER-ICA Abbott, Abbott Laboratories, Chicago, IL, USA) and 6F11 antibodies (NeoMarkers, Fremont, CA, USA) were used to reveal the ER with equivalent results. The KD68 (PR-ICA Abbott) and PR-1A6 (NeoMarkers) antibodies were used to reveal the PR. Staining for ER and PR was done on cryostat sections (ER-ICA or PR-ICA kits, following the manufacturer's instructions) or on paraffin-embedded sections using the streptavidin-biotin-peroxidase method (Biogenex, San Ramon, CA, USA). Ki-67 was determined on cryostat sections with Ki-67 (Dako, Glostrup, Denmark) and on permanent section with MIB1 (Biomeda, Foster City, CA, USA). HER2/NEU was revealed with Ab-1 (Zymed Lab, Inc., San Francisco, CA, USA) and p53 was revealed with DO7 (NeoMarkers). Immunohistochemical procedures were done with an automatic immunostaining device (Ventana Medical System, Tucson, AZ, USA) and Ventana Kits (Strasbourg, France). Immunostaining for ER, PR, Ki-67 and p53 was quantified with a Computerized Image Analysis System (CAS 200, Becton Dickinson, San Jose, CA, USA), as previously described (5). Only cancer cells with distinct nuclear immunostaining for ER, PR, Ki-67 and p53 were recorded as positive and the percentage of stained nuclei was calculated as the proportion of the stained area versus the total nuclear area. Cancer cells were considered positive for HER2/NEU when they showed distinct plasma membrane immunoreactivity. As percent expression values of ER, PR and HER2 tended to distribute around discrete values (0, 10, 25, 50, 75 and 100% of tumor cells), they were categorized accordingly, whereas percentages of Ki-67 and p53 expressing cells were analyzed without discretization. Triple-negative cases ($n = 33$) were defined as 0% for the ER, PR and HER2 markers.

### Nottingham Case Series

The distribution of p53 expression values within triple-negative cases was also investigated on an independent data set of 1076 cases from the Nottingham Tenovus Primary Breast Carcinoma Series (21). In the original study, aimed at immunohistochemically profiling breast cancer, tumor samples were evaluated for 25 different markers: hormone receptors (ER, PR and androgen receptor), EGFR family members (EGFR, c-ErbB2, c-ErbB3 and c-ErbB4), tumor suppressor genes (BRCA1, FHIT and p53), cell adhesion molecules (E-cadherin and P-cadherin), mucins (NCL-MUC1, MUC1-core and NCL-MUC2) and markers associated with luminal (CK7/8, CK18 and CK19) and basal (CK5/6, CK14, SMA and p63) phenotype, or with apocrine (GCDFP-15) or neuroendocrine (chromogranin A and synaptophysin) differentiation. 1D5, PgR636 and HER2 antibodies (DakoCytomation, Dako, Glostrp, Denmark) were used to reveal ER, PR and HER2, respectively, whereas p53 was revealed with DO7 (Novocastra Laboratories, Newcastle upon Tyne, UK) antibody. Levels of IHC reactivity were categorized using a modified *H* score so that it includes a semiquantitative assessment of both the intensity of staining and the percentage of positive cells. For the intensity, a score of 0–3, corresponding to negative, weak, moderate and strong positivity, was recorded. In addition, the percentage of positive cells at each intensity was estimated. The *H* score is calculated as $0 \times$ negative $\% + 1 \times$ weak $\% + 2 \times$ moderate $\% + 3 \times$ strongly stained %. The range of possible scores is thus 0–300 (21). Triple-negative cases ($n = 185$) were defined as having a zero *H* score for ER, PR and HER2. An *H* score equal to zero was also

applied to define p53 status. Despite the different method of IHC and the not strictly equivalent scoring system applied to assess ER, PR and HER2 status, according to the conversion table proposed by Shousha (22), triple-negative tumors were defined consistently between the two case series. Table 1 summarizes the main clinicopathological features of the triple-negative subgroup from the Ferrara and Nottingham case series.

### Statistical Analysis

According to the expression of ER, PR, HER2, Ki-67 and p53, in the original Ferrara study, Ambrogi et al. (5) defined four breast tumor clusters, essentially corresponding to previously identified breast cancer phenotypes (2,16). Considering the expression of 25 biomarkers in the Nottingham case series, the original hierarchical classification in six groups proposed by Abd El-Rehim et al. (21) has been subsequently refined using the *k*-means algorithm and additional approaches, comparatively reported (6). In the present study, we focused on the clusters related to the basal phenotype owing to its relationship with the molecular triple-negative status (8,11).

To show how triple-negative tumors grouped according to p53 expression, a Sammon Mapping was adopted as multivariate visualization technique with suitable properties for preserving, on a bidimensional plot, similarity relationships between tumors in different clusters. The purpose of Sammon non-linear mapping is to provide a bidimensional representation of the tumors, characterized by the considered biological markers, such that any distortion of the representation is minimized (23). Therefore, similar tumors were likely plotted near to each other in the graph without axis scales because not informative as only relative distances between points are meaningful indicating how triple-negative patients are closer to each other than non-triple-negative patients do. However, to help in interpreting the plot, the Spearman correlations of each axis with the biological markers were considered.

Ever since the availability of full follow-up information on the Ferrara case series, the outcome analysis of p53 status in triple-negative tumors has been based on overall and event-free survival, defined as the time elapsed from surgery to the first occurrence of an adverse event including other primaries and death without recurrence. Kaplan–Meier survival curves with hazard ratio interval estimates and log-rank tests were performed. No additional multivariable models were considered because we assumed TP53 gene alteration and consequently p53 protein dysfunction as the upstream step in the chain of events leading to a more aggressive phenotype that reflects in some patho-biological features. According to this assumption, the adjustment for features downstream causally related to the activity of p53 as the transcription factor, would only mask its primary association with the outcome (24). Owing to the very limited size of the triple-negative group, the additional role of therapy on the outcome could not be investigated. However, according to the standard treatments applied in the institutions and reported in the original references (5,25), throughout the period these samples were collected, a modulation of the response to therapy according to p53 status was not expected.

**Table 1.** Clinicopathological features of Ferrara and Nottingham triple-negative case series

| | Ferrara *n* (%) | Nottingham *n* (%) |
|---|---|---|
| Age (years) | | |
| ≤40 | 3 (9) | 43 (23) |
| 41–50 | 8 (24) | 59 (32) |
| 51–55 | 3 (9) | 19 (10) |
| 56–70 | 15 (46) | 64 (35) |
| >70 | 4 (12) | |
| Histologic type | | |
| Ductal | 30 (91) | 154 (83) |
| Lobular | 2 (6) | 3 (2) |
| Medullary | 1 (3) | 21 (11) |
| Other[a] | | 7 (4) |
| Pathologic stage | | |
| I | 20 (61) | 88 (47) |
| II | 13 (39) | 92 (50) |
| III | | 5 (3) |
| No. of metastatic lymph nodes | | |
| 0 | 20 (61) | 118 (64) |
| 1–3 | 7 (21) | 53 (29) |
| >3 | 6 (18) | 14 (7) |

[a]This class includes tubular, mucinous, papillary and cribriform histotype.

## RESULTS

In the present study, p53 expression was able to subdivide all the triple-negative Ferrara tumors into two distinct subsets (Fig. 1a), which were included in the previously defined Clusters 2 and 3 (5). Strikingly, p53 expression was only seen associated with the original Cluster 3 previously defined as 'basal-like', whereas low-to-nil p53 expression was observed only in the original Cluster 2, which had a less homogeneous definition. Concerning the Nottingham case series (Fig. 1b), the results indicated that triple-negative cases were mainly grouped (164 out of 185, 89%) in the two clusters with basal features (6), according to the cytokeratines, but distinguished by the presence of p53. The other triple-negative cases were spread across the other clusters, being mostly (17 out of 21, 81%) negative for p53 expression.

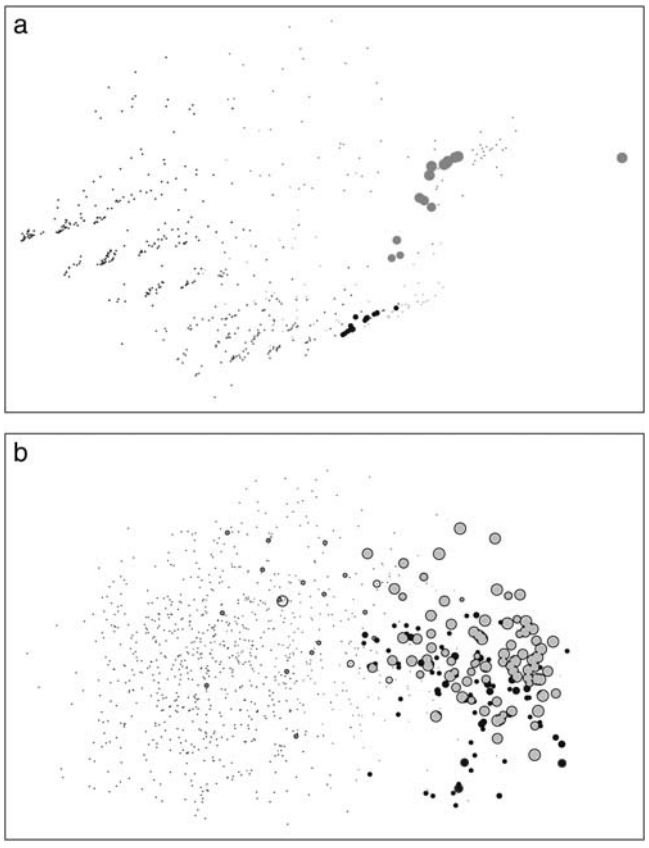


**Figure 1.** Sammon non-linear mapping. Triple-negative tumors are represented by bullets proportional to their p53 level. Axis scales are not reported because not informative as only relative distances between points are meaningful indicating how triple-negative patients are closer to each other than non-triple-negative patients do. (a) Ferrara case series. The points represent 633 tumors (5). The first axis is negatively correlated with ER and PR, while the second axis is mainly positively correlated with p53. Triple-negative tumors with a p53-negative expression, represented by the dark small bullets, are included in the original Cluster 2. Triple-negative tumors with a p53-positive expression, represented by gray large bullets are included in the original Cluster 3. (b) Nottingham case series. The points represent 1076 tumors. The first axis is negatively correlated with the markers associated with a luminal phenotype, while the second axis appears mainly correlated with EGFR family members. P53 is positively correlated with the first axis and, moderately, with the second axis. Black bullets represent triple-negative tumors included in the basal cluster with low p53. Gray bullets represent triple-negative tumors included in the basal cluster with high p53. Circles not filled represent triple-negative tumors not clustered in basal-like groups.

As expected, the triple-negative cases of the independent data set from the Nottingham series (21) showed a decreased expression of luminal phenotype-associated markers (MUC1, CK7/8, CK18 and CK19) and an increased expression of basal-like phenotype markers (EGFR, CK5/6 and CK14) (data not shown). In consistence with Ferrara data, the two clusters with basal features were characterized by a different p53 expression with a substantial lack of modulation of the other markers (Fig. 2a and b). Of interest, and notwithstanding the different immunohistochemical methodologies applied to evaluate p53 expression, in the two overall case series, p53 scores distributed continuously around low/intermediate values (Fig. 3, left panels). On the other hand, in both triple-negative subsets the distribution of p53 was sharply dichotomic, i.e. high versus low-to-nil values) (Fig. 3, central panels), thus supporting the hypothesis of two biologically distinct phenotypes associated to the clinical definition of triple negativity, according to p53 expression.

Overall and event-free survival curves are reported in Fig. 4. Notably, no difference in the survival of p53 negative versus positive cases was observed in the overall case series, as well as in non-triple-negative breast cancers. Conversely, p53 expression subdivided triple-negative cases in two biological distinct subgroups that, in addition to different aggressiveness features, were associated with worse (p53-positive) or better (p53-negative) prognosis.

## DISCUSSION

The clinical definition of triple-negative breast cancer was claimed to not fully encompass the biological definition of basal-like phenotype as emerging from several studies based on genomic analysis and protein expression panels, like those considered in the present study. In particular, this work provides evidence that a biological refinement of the triple-negative condition, mainly associated with basal-like features, can be achieved by splitting basal-like cancers in two distinct subgroups according to p53 expression. Indeed, in the present study, p53 protein expression was shown to be able to subdivide triple-negative tumors in two subgroups, supporting the hypothesis that triple-negative cancers *de facto* include two different biological entities: basal-like (p53-positive) and normal breast-like (p53-negative) tumors. Owing to this dichotomy within triple-negative cancers, p53 expression, routinely evaluated by IHC as a surrogate marker for mutation status, may help in identifying, on a biological basis, patient subgroups with different aggressiveness features and prognosis. However, more sophisticated statistical analysis for investigating causal dependencies among p53 clinicopathological features and outcome are needed instead of simple adjustment in a multivariable model. This issue was addressed in a paper recently published by our group (26). In fact, missense *TP53* gene mutations often lead to a high stability of p53 proteins that become detectable by IHC (27,28). Such mutant p53 proteins can functionally become dominant-negative with gain-of-function properties with respect to truncated p53 proteins that are largely unstable and cannot be revealed by IHC analysis, similar to wild-type p53. As a consequence, missense *TP53* mutations are predominantly IHC positive (92.9%), whereas truncating *TP53* mutations are predominantly IHC negative (88.5%) (25). Breast cancer patients carrying missense *TP53* mutations show worse disease-free survival than those with wild-type *TP53* or with truncating *TP53* mutations (27).

In consistence with the above, Langerod et al. (29) demonstrated that, among the five breast cancer subgroups

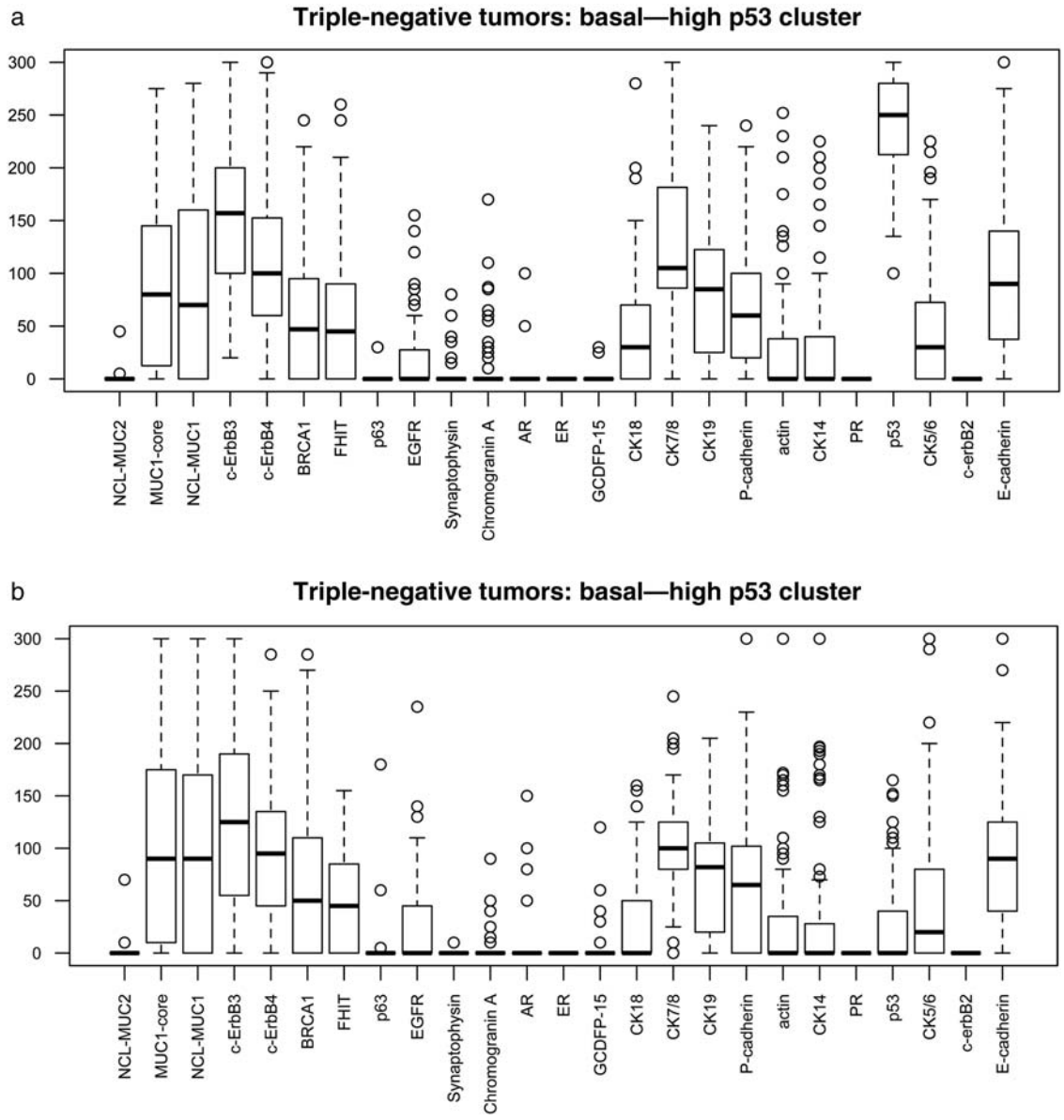


**Figure 2.** Distribution of the 25 different biomarkers evaluated in the Nottingham case series and expressed as *H* score only for the 164 triple-negative patients included in the two clusters with basal features, as defined according to cytokeratin expression, but distinguished by p53 values (see (6) for details). Namely, the box plots at the top show the distributions of the 25 different biomarkers evaluated in the original study (21) for the triple-negative cases in the clusters with basal features and characterized by a low p53 expression; at the bottom, the box plots show the distribution for the triple-negative cases in the clusters with basal features with a high p53 expression.

proposed by Perou et al. (2), the basal-like and ERBB2+ cases show the highest *TP53* mRNA expression while the normal-like phenotype has the lowest *TP53* mRNA levels. These findings were confirmed at the protein level. As IHC detection of the p53 protein largely identifies missense *TP53* mutations, p53 positivity may represent a useful biological marker to discriminate more aggressive triple-negative basal-like tumors from triple-negative tumors with a normal-like phenotype. Our findings consistently support the evidence that p53 expression subdivides triple-negative cases in two subgroups with different prognosis and, in agreement with a very recent independent paper reporting p53 as strongly predictive for relapse-free survival and overall survival in triple-negative patients (30), support the use of p53 expression as a prognostic marker in triple-negative breast cancers.

In addition, as *TP53* gene mutations are predictive of response to taxanes in reconstituted model systems (31) and in patients (32,33), knowledge of p53 status may also provide powerful information for selecting, among the triple-negative tumors, those more likely to benefit from taxane versus anthracycline/alkylating agent-based chemotherapy (17,18,34).

Taken together, our findings suggest that the immunohistochemical evaluation of p53 expression may help in

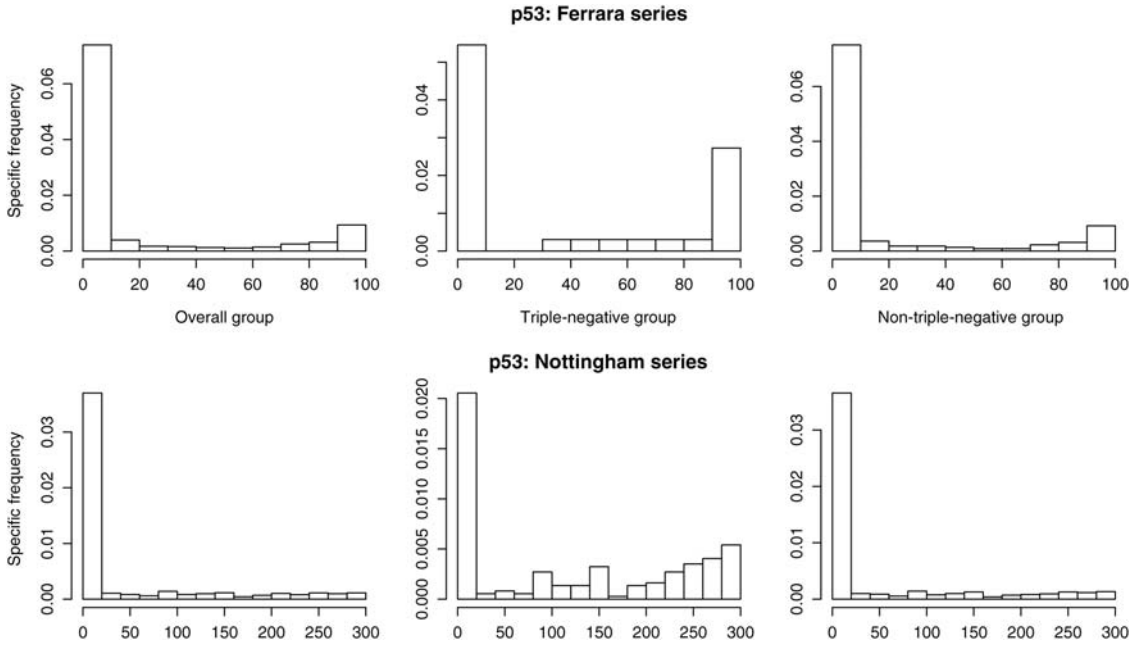


**Figure 3.** Distribution of p53 expression in the two case series evaluated as specific frequency (i.e. the frequency of patients in each histogram class divided by the class length: respectively, 10 in Ferrara and 20 in Nottingham case series). Left panels show the distribution of p53 values for overall case series. The central panels show the distribution of p53 values for the triple-negative subgroup. The right panels show the distribution of p53 in non-triple-negative cases. For the Ferrara case series, the proportion of the stained area versus the total nuclear area is reported. For the Nottingham case series, the *H* score combining the percentage of positive cells and intensity is reported. In the two overall case series, p53 distributed continuously around low/intermediate values, whereas in both triple-negative subsets the distribution of p53 was sharply dichotomic, i.e. high versus low-to-nil values.

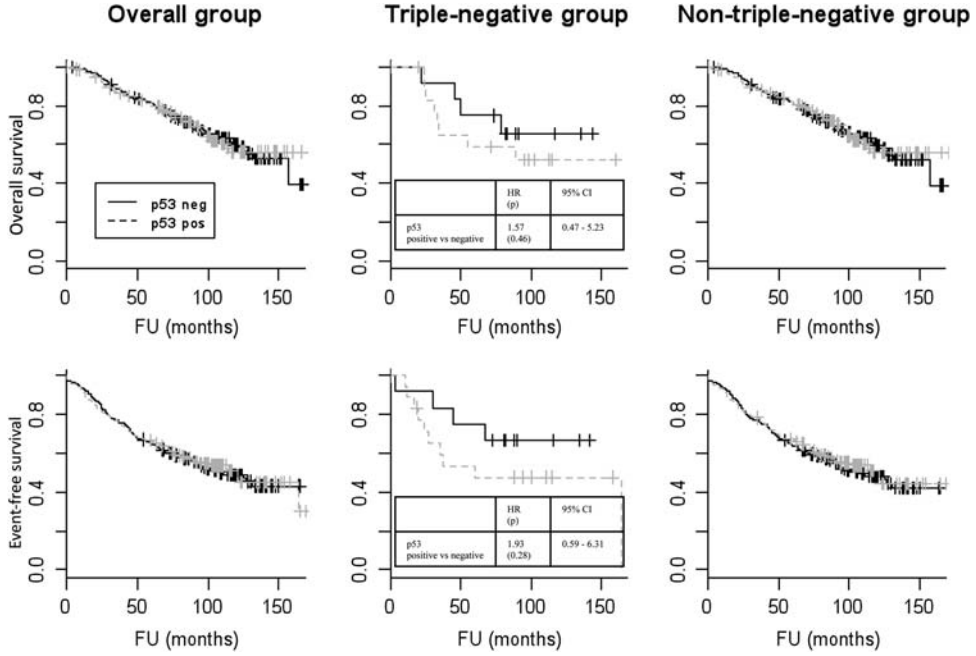


**Figure 4.** Kaplan–Meier survival curves in the overall, triple-negative and non-triple-negative groups. The upper panels show the overall survival and the lower panels the event-free survival. In the box, hazard ratio interval estimates and the log-rank test are provided. FU, follow up.

selecting patient subgroups with different biological histories among triple-negative breast cancers, providing a potentially powerful prognostic contribution in such a peculiar group of breast cancers. In fact, as recently pointed out, the identification as a basal-like cancer cannot simply be substituted by evidence for a triple-negative status (35). Our findings support this notion, showing that triple-negative breast cancer is not a unique biological entity and may also help in

taming the currently stormy relationship between pathological (triple-negative tumors) and biological (basal-like breast cancers) classifications even though conclusive studies, based on other/prospective large case series, are needed to confirm such evidence.

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## Conflict of interest statement

None declared.

## References

1. Widakowich C, de Azabuja E, Gil T. Molecular targeted therapies in breast cancer: where are we now? *Int J Biochem Cell Biol* 2007;39:1375–87.
2. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. *Nature* 2000;406:747–52.
3. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci* 2001;98:10869–74.
4. Sørlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc Natl Acad Sci* 2003;100:8418–23.
5. Ambrogi F, Biganzoli E, Querzoli P, Ferretti S, Boracchi P, Alberti S, et al. Molecular subtyping of breast cancer from traditional tumor marker profiles using parallel clustering methods. *Clin Cancer Res* 2006;12:781–90.
6. Soria D, Garibaldi JM, Ambrogi F, Green AR, Powe D, Rakha E, et al. A methodology to identify consensus classes from clustering algorithms applied to immunohistochemical data from breast cancer patients. *Comput Biol Med* 2010;doi:10.1016/j.compbiomed.2010.01.003.
7. Garcia-Closas M, Chanock S. Genetic susceptibility loci for breast cancer by estrogen receptor status. *Clin Cancer Res* 2008;14:8000–9.
8. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. *Clin Cancer Res* 2004;10:5367–74.
9. van de Rijn M, Perou CM, Tibshirani R, van de Rijn M, Perou CM, Tibshirani R, et al. Expression of cytokeratins 17 and 5 identifies a group of breast carcinomas with poor clinical outcome. *Am J Pathol* 2002;161:1991–6.
10. Cleator S, Heller W, Coombes RC. Triple-negative breast cancer: therapeutic options. *Lancet Oncol* 2007;8:235–44.
11. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. *Clin Cancer Res* 2007;13:4429–34.
12. Tischkowitz M, Brunet J-S, Bégin LR, Huntsman DG, Cheang MC, Akslen LA, et al. Use of immunohistochemical markers can refine prognosis in triple-negative breast cancer. *BMC Cancer* 2007;7:134.
13. Rakha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. *Cancer* 2007;109:25–32.
14. Brenton JD, Carey LA, Ahmed AA, Caldas C. Molecular classification and molecular forecasting of breast cancer: ready for clinical application? *J Clin Oncol* 2005;23:7350–60.
15. Fulford LG, Reis-Filho JS, Ryder K, Jones C, Gillett CE, Hanby A, et al. Basal-like grade III invasive ductal carcinoma of the breast: patterns of metastasis and long-term survival. *Breast Cancer Res* 2007;9:R4.
16. Sotiriou C, Neo S-Y, McShane LM, Korn EL, Long PM, Jazaeri A, et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proc Natl Acad Sci* 2003;100:10393–8.
17. Rouzier R, Perou CM, Symmans WF, Ibrahim N, Cristofanilli M, Anderson K, et al. Breast cancer molecular subtypes respond differently to preoperative chemotherapy. *Clin Cancer Res* 2005;11:5678–85.
18. Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, et al. The triple-negative paradox: primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res* 2007;13:2329–34.
19. Lakhani SR, van de Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L, et al. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. *J Clin Oncol* 2002;20:2310–8.
20. Pusztai L, Ayers M, Stec J, Clark E, Hess K, Stivers D, et al. Gene expression profiles obtained from fine-needle aspirations of breast cancer reliably identify routine prognostic markers and reveal large-scale molecular differences between estrogen-negative and estrogen-positive tumors. *Clin Cancer Res* 2003;9:2406–15.
21. Abd El-Rehim DM, Ball G, Pinder SE, Rakha E, Paish C, Robertson JF, et al. High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. *Int J Cancer* 2005;116:340–50.
22. Shousha S. Oestrogen receptor status of breast carcinoma: Allred/H score coversion table. *Histopathology* 2008;53:346–7.
23. Venables WN, Ripley BD. *Modern Applied Statistics with S*, 4th edn. New York: Springer 2003.
24. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. *Epidemiology* 2004;15:615–25.
25. Rocha EA, El-Sayed ME, Green AR, Lee AH, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. *Cancer* 2007;109:25–32.
26. Stefanini F, Coradini D, Biganzoli E. Conditional independence relations among biological markers may improve clinical decision as in the case of triple negative breast cancers. *BMC Bioinformatics* 2009;10:S13.
27. Tsuda H, Hirohashi S. Association among p53 gene mutation, nuclear accumulation of the p53 protein and aggressive phenotypes in breast cancer. *Int J Cancer* 1994;57:498–503.
28. Ozcelik H, Pinnaduwage D, Bull SB, Andrulis IL. Type of *TP53* mutation and ERBB2 amplification affects survival in node-negative breast cancer. *Breast Cancer Res Treat* 2007;105:255–65.
29. Langerød A, Zhao H, Borgan Ø, Nesland JM, Bukholm IR, Ikdahl T, et al. *TP53* mutation status and gene expression profiles are powerful prognostic markers of breast cancer. *Breast Cancer Res* 2007;9:R30.
30. Chae BJ, Bae JS, Lee A, Park WC, Seo YJ, Song BJ, et al. P53 as a specific prognostic factor in triple-negative breast cancer. *Jpn J Clin Oncol* 2009;39:217–24.
31. Cimoli G, Malacarne D, Ponassi R, Valenti M, Alberti S, Parodi S. Meta analysis of the role of p53 status in isogenic systems tested for sensitivity to cytotoxic anti-neoplastic drugs. *Biochem Biophys Acta* 2004;1705:103–20.
32. Harris LN, Broadwater G, Liu NU. Molecular subtypes of breast cancer in relation to paclitaxel response and outcomes in women with metastatic disease: results from CALGB 9342. *Breast Cancer Res* 2006;8:R66.
33. Wahl AF, Donaldson KL, Fairchild C. Loss of normal p53 function confers sensitization to Taxol by increasing G2/M arrest and apoptosis. *Nature Med* 1996;2:72–9.
34. Di Leo A, Tanner M, Desmedt C, Paesmans M, Cardoso F, Durbecq V, et al. P53 gene mutations as a predictive marker in a population of advanced breast cancer patients randomly treated with doxorubicin or docetaxel in the context of a phase III clinical trial. *Ann Oncol* 2007;18:997–1003.
35. Sorlie T. Introducing molecular subtyping of breast cancer into the clinic? *J Clin Oncol* 2009;27:1153–4.

## Appendix

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