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TP53 status for prediction of sensitivity to taxane versus non-taxane neoadjuvant chemotherapy in breast cancer (EORTC 10994/BIG 1-00): a randomised phase 3 trial

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Phase III trial (EORTC 10994/BIG 1-00) assessing the value of p53 using a functional assay to predict sensitivity to a taxane versus non taxane primary chemotherapy in breast cancer: final analysis

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Contributions of authors

HB, MP, JBE, RI participated in the conception and design of the study. HB, MP, JBO, LM, PF, EBR, TP, PR, JJ, EBL, KZ, TC, AL, EL, SA, SL, LDL, VB, DC, JBE, RI participated in the collection and assembly of data. HB, MP, JBO, JJ, SA, SL, VB, DC, JBE, RI participated in the data analysis and interpretation. HB, MP, JBO, LM, PF, JJ, LDL, DC, JBE, RI participated in the manuscript writing. HB, MP, LM, PF, EBR, TP, PR, JJ, EBL, KZ, TC, AL, EL, DC, JBE participated in the provision of study material or patients. All of the authors saw and approved the final version of the report.

Conflicts of interest

The other authors declare that they have no conflicts of interest.

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Abstract

Background—This study tested the hypothesis that docetaxel confers a greater advantage over anthracyclines in p53 mutant compared to p53 wild type breast cancers.

Methods—Patients with locally advanced, inflammatory or large operable breast cancers were randomised to receive neoadjuvant chemotherapy consisting of either a standard anthracycline regimen (FEC 100 or tailored FEC) or a taxane-based regimen (docetaxel for 3 cycles, followed by epirubicin and docetaxel for 3 cycles). In this open label study, randomisation was performed using a minimisation method that stratified by institution and initial tumour stage (large operable versus locally advanced or inflammatory breast cancer). p53 status was assessed with a yeast functional assay on tumour biopsies taken before chemotherapy. The primary endpoint was a comparison of progression-free survival in the two arms according to p53 status and in the entire trial population (by intention to treat). We report the final analysis of the trial. The study is registered in ClinicalTrials.gov, number NCT00017095.

Findings—1856 patients were enrolled and 370 were unassessable for p53 tumour status (the main reason being low tumour cell content in the biopsy). 675 events for the primary endpoint were registered. The hazard ratio (HR) between the two arms for progression-free survival (PFS) was 0.84 (98% CI: 0.63–1.14; p=0.17) in the p53 mutant group and 0.89 (98% CI: 0.68–1.18; p=0.35) in the p53 wild type group. In the entire population, the HR was 0.85 (98% CI: 0.71–1.02; p=0.035) for the use of docetaxel. The most common grade 3 or 4 adverse events were neutropenia in 1598 patients (86.6%), febrile neutropenia in 284 (15.4%), fatigue in 136 (7.4%), infection in 121 (6.6%) and nausea or vomiting in 89 (4.8%). Two patients died of toxicity during or within 30 days of chemotherapy completion and without disease relapse (one in each arm).

Interpretation—Although p53 status is prognostic for overall survival, it was not predictive of preferential sensitivity to taxanes. p53 status tested by yeast assay in this population can not be used to select patients for FEC versus taxane-based chemotherapy.

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Introduction

One consequence of the revolution in genomics is that matching treatments to genotypes will play an increasing role in modern drug development. Most classic cytotoxic drugs were developed before the causal oncogenic mutations in tumours were known. Since the proteins these drugs target are not mutant in tumours, it is difficult to predict individual chemosensitivity to particular drugs. To circumvent this problem, many groups are now trying to derive genomic or transcriptomic signatures that predict the response of tumours to classic chemotherapy. The p53 gene is a prime candidate for inclusion in these signatures. It mediates checkpoint or stress responses to a multitude of insults and suppresses tumour formation through multiple mechanisms including apoptosis, senescence and autophagy.[1]

In the late 1990s, preclinical models suggested that breast tumours with mutant p53 would be resistant to anthracyclines [2, 3] but show similar [4] or increased sensitivity to taxanes.[5] To test the working hypothesis that patients with p53-mutant breast tumours would benefit preferentially from treatment with taxanes, we initiated a clinical trial in patients with large operable, locally advanced or inflammatory breast cancer. When designing the trial we took two important methodological decisions. First, we used a "marker strategy design" so that, if positive, the result would qualify as biomarker level I evidence, as defined by the American Society of Clinical Oncology Tumor Marker Guidelines Committee at that time. [6] Second, to assess p53 status we used a functional assay in yeast (figure 1). [7, 8] This assay detects biologically important mutations that inactivate p53 as a transcription factor but not silent mutations or polymorphisms. In practice, immunohistochemistry lacks sensitivity and specificity, and the functional assay is more sensitive than conventional techniques such as sequencing, mainly because the assay is insensitive to contamination of samples with normal tissue. [9, 10] Tumours were biopsied for p53 assessment and patients then received neoadjuvant therapy with standard anthracycline-based regimen, or with a regimen containing docetaxel. We report here the final analysis of this clinical trial.

Methods

Study design

This intergroup multicentre phase III trial was performed in 42 centres across nine countries. The trial was approved by national ethics committees and local ethics committees in all participating centers. The study is registered in ClinicalTrials.gov, number NCT00017095. The full protocol can be assessed at http://www.cancer.gov/clinicaltrials/EORTC-10994. Before registration, all patients gave signed informed consent for the clinical trial and for research on tumour samples taken before randomisation. A frozen tumour sample was mandatory before inclusion in the trial (either one incisional biopsy or two trucut biopsies taken with a 14G needle). Patients eligible for the trial were women aged less than 71 years with histologically proven invasive carcinoma of the breast suitable for neoadjuvant chemotherapy. Eligible patients had large operable or locally advanced or inflammatory breast cancers, defined as T4a,b,c,d any N; or, any clinical T, N2 or N3; or, large T2 or T3; all were M0 and WHO performance status 0–1. Exclusion criteria are listed in the supplementary information.

Randomisation and masking

In this open label study, randomisation was performed by an inhouse software at the EORTC Headquarters, using a minimization method [11] that stratified by institution and initial tumour stage (large operable versus locally advanced or inflammatory breast cancer). Patients were randomised in a 1:1 ratio to a standard anthracycline regimen (arm A: 5-fluorouracil, epirubicin and cyclophosphamide) or a taxane-based regimen (arm B: three cycles of docetaxel followed by three cycles of docetaxel plus epirubicin). The randomised arm was only calculated and released to the investigator at the time all eligibility criteria were confirmed.

Treatment

In arm A, the nine centers from the Swedish group used tailored FEC; the other centers used FEC100 (the list of centers is given in the Web Appendix). In arm B, we chose to give docetaxel as a single agent upfront for three cycles followed by epirubicin and docetaxel (ET) in combination for three cycles. This approach allowed us to test optimally the hypothesis that p53-mutant tumours are sensitive to taxanes and resistant to anthracyclines, without putting patients at risk of undertreatment. For more details of the chemotherapy regimens see figure 2 and the supplementary methods online. [12–14] At completion of chemotherapy, in the absence of progression, locoregional treatment was planned according to the guidelines described in the protocol. Adjuvant endocrine therapy for 5 years after the end of neoadjuvant chemotherapy was mandatory for women with estrogen receptor (ER) positive or progesterone receptor (PgR) positive tumours. Aromatase inhibitors were used in accordance with local, regional or network policy (this information was registered in a follow-up form). Patients with human epidermal growth factor receptor-2 (HER2) positive tumours were allowed to enter adjuvant clinical trials assessing trastuzumab or to receive this treatment in the adjuvant setting when it became standard practice.

p53 assessment

p53 status was tested at the Swiss Institute for Experimental Cancer Research (ISREC) by yeast functional assay (figure 1) on biopsies that contained ≥20% tumour cells, as previously described, [7, 8, 15] and in the supplementary file online (appendix section 3). To confirm that red colonies contained clonal mutations, plasmids were rescued from red yeast colonies from the first 50 cases scored as mutant. At least four different yeast colonies (clones) were tested for each case. To estimate the positive and negative predictive value, the distribution of % red colonies was decomposed into three peaks by fitting a mixture of normal distributions with the mix function in the mixdist library in the R programming language. This function is based on maximum likelihood estimation using a combination of a Newton-type algorithm and the EM algorithm (for details see the Mix Home page: http://www.math.mcmaster.ca/peter/mix/mix.html).

Statistics

The primary endpoint of the study was progression-free survival, defined as time from randomisation to progression on neoadjuvant chemotherapy, locoregional relapse (invasive cancer), first distant metastasis, death from any cause, or invasive contralateral breast cancer. We did not consider as a primary event: second primary invasive cancer (non breast), or ipsilateral or contralateral ductal or lobular carcinoma in situ. Patients without an event were censored at their last follow-up date. Secondary end-points were: distant metastasis-free survival (time from randomisation to distant progression on neoadjuvant chemotherapy, first distant metastasis, death from any cause), overall survival (time from randomisation to death from any cause), clinical response (according to RECIST criteria),[16] pathological response (complete pathological response was defined as complete disappearance of any invasive cancer in the primary tumour with the exception of very few scattered tumour cells left), and toxicity according to National Cancer Institute Common Toxicity Criteria version 2.0.[17]

The primary objective was to evaluate whether the benefit from the taxane-based arm was essentially restricted to the p53 mutated group. Three co-primary comparisons of progression-free survival between treatment arms were planned: in the patients with p53 wild type tumours; in the patients with p53-mutated tumours; and in all randomised patients. Each primary test was a two-sided log rank test stratified for corrected stage of disease (locally advanced vs. large operable) at 2% alpha error, ensuring an overall 5% alpha error for these correlated co-primary tests. The final test in the p53 mutated group was at a nominal 1.4% alpha error, ensuring experiment-wise 2% alpha level after a per protocol interim analysis at 66% of the required events in this group. Because of the correction for multiplicity, the confidence intervals, although calculated at 98%, are to be interpreted as 95% confidence intervals (experimentwise). The P-values for the 3 primary tests need to be compared to the critical level of 0.02.

The trial was designed to have 80% power to detect hazard ratios of respectively 0.72, 0.67 and 0.80 in the p53 wild type group, the p53 mutated group, and the full sample. This was assuming an overall accrual of 1850 patients followed for an additional 2.5 years after end of accrual, a 15% non assessibility rate for p53, and a 2:1 ratio between wild type and mutated in those assessable.

For further details of the sample size calculation and planned statistical analyses, see the supplementary Methods online. This analysis is based on the final database lock performed on 24 February 2010. All of the analyses were performed on an intention-to-treat basis. Up to May 2009 we observed that there were fewer events than anticipated and that the proportion of p53 wild type tumours was lower than anticipated. As a result, the power of the comparison in the p53 wild type group was compromised. To resolve this problem we sought the advice of an external independent committee. They recommended to carry out the final analysis once the planned 270 events were confirmed in the p53 mutated group. This would ensure that the main goal of the study was met with the planned power (80% power to detect a progression-free survival difference between the arms when p53 was mutant). They recommended to carry out the other planned analyses (overall, p53 wt group and interaction test) at the same time, while acknowledging that these comparisons would be slightly underpowered. Following this recommendation, the clinical cut-off date used was 1 December 2009 after the threshold value of 270 primary endpoint events in the p53 mutated group was met.

Unless otherwise mentioned, all analyses were run in SAS version 9.2. The clinical database resides at EORTC.

Role of the funding source

The sponsor of the trial (EORTC) designed and coordinated the trial. The funding sources of the study had no role in the design of the study; collection, analysis, or interpretation of the data; or in the writing of this report. JB had full access to the raw data. The corresponding author had the final responsibility to submit for publication.

Results

From 25 April 2001 to 20 November 2006, 1856 patients were included; 928 were randomly assigned to the FEC regimen and 928 to the T-ET regimen. The median length of follow-up at the time of database lock was 57 months (quartiles 44 and 70 months, IQR 26 months).

Baseline characteristics and treatment

In the entire series, 1429 patients (77%) presented with large operable tumours, 1194 were ER positive (64%), and 451 were HER2 positive (28%) (table 1). Surgery was performed after neoadjuvant treatment in 1809 patients (97.5%) of whom 764 (42.2%) underwent conservative treatment. Of 1241 patients with ER or PgR positive tumours 1192 (96%) received adjuvant endocrine therapy. Among 451 patients with HER2 positive tumours, only 142 (32%) received adjuvant trastuzumab because this treatment was not yet standard during most of the recruitment period. The compliance with allocated treatment and details on treatment discontinuation are given on the CONSORT diagram (figure 2). Of the 1856 patients enrolled, 22 patients (1.1%) were ineligible and 370 (20%) were unassessable for p53 tumour status (including 6 for both reasons) (figure 3). The main reason was samples with less than 20% tumour cells. These samples were excluded because the normal cells in the sample would have generated a false negative result; they were not technical failures of the yeast assay as such. The characteristics of this group of patients are very similar to the 2 other groups (p53 wild type and mutated) (supplementary table 1).

p53 functional assay

The p53 test was performed on tumour biopsies from 1486 patients (80%) and failed in 17 patients (1.1%) (figure 3). Taking into account all technical reasons, the p53 test was either not performed (370) or failed (17) in 20.9% of cases (figure 3). Tumours from 825 patients were classified as wild type (56.2%) and from 644 patients as mutated (43.8%). The p53 functional assay tests whether p53 is able to activate transcription of a reporter gene in yeast (figure 1). The percentage of red colonies in the yeast assay reflects the relative abundance of wild type and mutant p53 mRNA in the sample. Figure 4 shows the observed distribution of the % red colonies for the 1469 patients for whom the assay was successful. The positive predictive value (PPV) and negative predictive value (NPV) of the assay can be estimated by decomposing the distribution into its three constituent peaks as shown in figure 4: false positives are in the tail of the wild type distribution above the 20% cut-off; false negatives are in the tail of the “heterozygous” distribution below the 20% cut-off. Analysed in this way, the PPV is estimated as 99% and the NPV as 92%.

Progression-free survival

By the time of analysis, 675 events for the primary endpoint had occurred (table 2). Of these 522 (77%) were distant recurrences. The three co-primary comparisons of progression-free survival between the FEC and T-ET arms were performed in the p53 mutant group, the p53 wild type group, and the whole population. No significant difference between the 2 arms was found at the predefined p-values (respectively, 0.014, 0.02 and 0.02; figure 5a). In the p53 mutant group, the HR was 0.84 in favour of T-ET (98.6% CI: 0.63–1.14; log-rank test stratified for stage: $p = 0.17$). The 5-year progression-free survival rates were 59.5% (95%

CI: 53.4–65.1) in the T-ET arm and 55.3% (95% CI: 49.2–60.9) in the FEC arm. In the p53 type wild group, the HR was 0.89 in favour of T-ET (98% CI: 0.68–1.18; log-rank test stratified for stage: p = 0.35). The 5-year progression-free survival rates were 66.8% (95% CI: 61.4–71.6) in the T-ET arm and 64.7% (95% CI: 59.6–69.4) in the FEC arm. In the whole population, the HR in favour of T-ET was 0.85 (98% CI: 0.71–1.02; log-rank test stratified for stage: p = 0.035). The 5-year progression-free survival rates were 65.1% (95% CI: 61.6–68.3) in the T-ET arm and 60.8% (95% CI: 57.3–64.2) in the FEC arm. There was no evidence of an interaction between p53 status and treatment arm (p = 0.68).

Overall survival and distant metastasis-free survival

By the time of analysis, 351 patients (19%) had died. None of the three comparisons for overall survival was significant at the predefined significance level (p = 0.02). Distant metastasis-free survival was similar to progression-free survival because three quarters of the first progression-free survival events were distant metastases. The overall survival and distant metastasis-free survival curves, with their respective HRs, confidence intervals and p-values, are shown in figures 5b and supplementary figure 1.

Complete clinical response and complete pathological response

For both complete clinical response and complete pathological response, we compared treatment arms for all randomised patients, for patients with p53 wild type tumours, and for patients with p53 mutant tumours. For clinical complete response and complete pathological response none of the comparisons reached significance at the 0.02 level (table 3). The pathological complete response rates were respectively 23.5% in the FEC arm and 26.5% in the taxane arm. There was no evidence for an interaction between p53 status, chemotherapy regimen and response to treatment (p = 0.75).

Multivariate analyses

In the first model for progression-free survival and overall survival we used clinical and pathological variables. For progression-free survival, most of the covariates were significant in the univariate models, but only five were retained after backward selection in the multivariate analysis: grade, clinical tumour size (cT), clinical nodal status (cN), endocrine treatment and performance status (table 4). Many factors selected in the univariate model are known to be highly correlated and for this reason were rejected from the multivariate model. For overall survival, the vast majority of the variables were also significant in the univariate analysis but only five were retained in the multivariate analysis : p53 status, cT, cN, endocrine treatment and performance status (table 4). The PFS and OS of patients unassessable for p53 tumour status appear similar to the p53 wild type group (in the univariate analysis HR 1.04 and 1.10 respectively) (table 4).

In the second multivariate model we added to the factors used in the first analysis three additional groups, named “simplified subtypes”, based on hormone receptor and HER2 status: triple negative tumours (ER, PgR and HER2 negative), HER2 positive tumours (and ER and PgR known) and other (all other cases with the three factors known). To explore the role of p53 in these subtypes, we performed univariate and multivariate analyses in a subset of 1422 patients for whom full data were available (table 5). In the analysis for progression-

free survival, "subtype" was not kept as a covariate and thus does not add anything to the first analysis. In the multivariate analysis for overall survival, four variables were retained: p53, cT, cN, and subtype.

Comparison within subgroups for progression-free survival

The treatment effect was broadly similar among all subgroups defined by common clinical characteristics (stage, age, menopausal status post chemotherapy), choice of control regimen (FEC100 or tailored FEC), biological markers (p53 status, endocrine sensitivity, HER2 status) and "simplified subtypes", with the possible exception of triple negatives (figure 6 and supplementary figure 2). The outcome of tailored FEC and FEC100 was similar (the HRs in favor of T-ET were 0.91 and 0.83 respectively).

Toxicity

As expected from the literature on similar regimens, the toxicity profile of the 2 arms were dissimilar, but not different from what has been previously published. We observed a higher frequency of febrile neutropenia and grade 3/4 infection with T-ET arm and a higher frequency of grade 3/4 vomiting in the FEC arm (table 6). Two patients died of toxicity during or within 30 days of chemotherapy completion and without disease relapse: 1 in each arm (table 2).

Discussion

To the best of our knowledge this is the first large prospective clinical trial explicitly designed to assess the ability of a biological marker to predict the response of tumours to different chemotherapy regimens. The interest in identifying predictive markers for classic chemotherapy derives from the hypothesis that only a subgroup of tumours is genetically programmed to respond well to particular drugs. The main conclusion of this trial is that tumour p53 status, defined by a yeast functional assay, can not be used to identify a subgroup of patients more likely to benefit from chemotherapy regimens containing taxanes.

There are several possible explanations for the negative result of the trial. One is that the trial may have been underpowered to reach the particularly stringent significance limits (α = 0.02) we were forced to adopt to accommodate the three primary endpoints. This explains why the overall difference was deemed not to be statistically significant despite the estimated proportional risk reduction being similar to that seen in PACS01, a Franco-Belgian adjuvant trial with similar regimens (15% at p = 0.035 in EORTC 10994 versus 17% in PACS01).[12] The trial may also have been underpowered if the benefit of taxanes in the p53 mutant group was smaller than expected under our hypothesis. In daily practice, only a large effect would be clinically relevant, so it would be difficult to justify performing a much larger trial to confirm the weak trend observed in this study.

The second possibility is that the yeast functional assay did not properly assess p53 status. It might be argued that this study would have been positive if we had used another technique, such as DNA sequencing, to identify p53 mutations. We consider this unlikely because the high p53 mutation rate in this study (44%) is consistent with other reports in breast cancer that used the yeast assay,[18–20] and it is higher than most reports based on DNA

sequencing.[21–24] Sequencing the entire p53 locus from samples microdissected to remove normal tissue could be considered a gold standard for detecting p53 mutations, but it is too technically demanding for use in a routine setting. In our experience the yeast assay is consistently the most sensitive simple technique for detecting p53 mutations.[9, 10] Given this high sensitivity, other methods to assess p53 status have to be considered even less promising in predicting taxane response. There are multiple reasons for the high sensitivity of the yeast assay[25], but the most important is probably the ability to detect mutations in the presence of normal tissue. Because of this we were able to accept samples into the study with only 20% tumour cells. To confirm that the assay was detecting genuine p53 mutations, rather than technical artefacts such as PCR splicing or vector self-ligation,[8] we sequenced p53 from red yeast colonies for the first 50 cases scored as mutant. We identified clonal mutations in every case (supplementary table 2). In addition to false negatives intrinsic to all cDNA-based approaches, such as deletions that remove the promoter or the entire locus, there are several additional potential sources of error. Firstly, p53 uses alternative promoters, alternative splicing and alternative translation start sites, resulting in the expression of a large array of different isoforms, some of which are not detected in the yeast assay.[26–28] Secondly, the yeast assay does not distinguish pure loss-of-function mutants from mutants with simultaneous gain and loss of function, so we might have missed a gain-of-function effect.[14, 29, 30] To explore this point we have embarked on a DNA sequencing study. Finally, the yeast assay does not detect mutation or silencing of other genes in the p53 pathway, such as MDM2, ARF and miR-34a.[1] Taken together, these arguments all point to the p53 pathway being inactivated in a much higher proportion of breast tumours than is generally assumed.

A third possibility is that our hypothesis that p53-mutant tumours would be resistant to anthracyclines but sensitive to taxanes was wrong. The control regimen, FEC, contained three drugs known to activate wild type p53 strongly[3] and was considered at that time to be one of the best standard regimens. The study design thus assumed that the experimental arm (T-ET) would be independent of p53 status (because the taxane would still be active in the p53-mutant group),[4, 5] whereas the control arm (FEC) would show reduced activity in the p53-mutant group. Ironically, over the period the study was recruiting, de Thé and colleagues published a series of papers that question our assumption that FEC would be a good control regimen.[18–20] Specifically, their results suggest that p53-mutant tumours may be more sensitive than wild type tumours to high dose anthracycline-cyclophosphamide.[18, 19] They attribute this in particular to the high dose cyclophosphamide.[20] We think this is not relevant to our study because the dose of cyclophosphamide we used is definitely below that postulated by de Thé to target p53 mutant tumours. The only exception is the Swedish cohort, who received a FEC variant called tailored FEC that contains a higher dose of cyclophosphamide. We analysed the response of p53-mutant tumours in this subgroup (supplementary table 3) and saw no significant difference in progression-free survival (the HR was 0.72 in favour of T-ET). Notwithstanding the trend in the wrong direction, we can not formally exclude the possibility that p53-mutant tumours might be hypersensitive to cyclophosphamide because the small number of p53-mutant tumours in the Swedish cohort (34 treated with FEC, 45 treated with T-ET) means the 95% confidence interval for the hazard ratio includes HR=1.

Several gene signatures have been published that predict p53 status in breast cancer.[19, 31] The genes in these signatures are closely linked to proliferation and ER status. Our study supports these observations: among patients with data available, 18% of grade I tumours were p53 mutant versus 62% of grade III tumours; and 32% of ER+ tumours were p53 mutant versus 70% of ER- tumours (supplementary table 1).

Beside p53, there is considerable interest in using hormone receptors and HER2 to predict the response to taxanes. In the CALGB9344 trial, taxanes were less effective in ER positive tumours,[32] a result not supported by some other studies.[33–35] In our trial there was a 20% reduction in the risk of recurrence in the ER or PgR positive subgroup treated with taxane (95% CI: 0.66–0.98, figure 6). In addition, when taking in consideration HER2 status, no benefit of taxanes was found in the ER positive/HER2 negative subgroup in the CALGB9344 trial,[36] whereas in our trial there was a 16% reduction in the risk of recurrence, albeit with a wide confidence interval (95% CI: 0.64–1.1, supplementary figure 3). A likely explanation for these differences is heterogeneity of the ER positive populations in the different trials.

Our study shows that predicting the response to conventional chemotherapy is a great deal more difficult than predicting the response to targeted therapies. This is presumably because the greatest tumour specificity lies in the oncogenic mutations driving tumour growth, like bcr-abl translocation or HER2 amplification. Viewed from this perspective, p53 status will have the greatest predictive value when it is used to select treatments that act on mutant p53 itself or on its immediate partners to relieve blocks to p53 activation. There is now a massive effort among academic laboratories and the pharmaceutical industry to develop drugs with exactly these properties, such as drugs that stabilize mutants in the wild type conformation and drugs that interfere with p53 degradation.[37] Since patients with p53 mutations have a poor prognosis, there is a clear need for these therapies targeted against mutant or inactivated forms of p53.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Panel: research in context

Systematic review

In the late 1990's when designing this study we did not perform a systematic review. At that time, p53 had been shown in the NCI60 screen [3] to play a pivotal role in the response to a large panel of anti-cancer drugs, with the notable exception of anti-microtubule drugs [3] including taxanes. [4,5] These data suggested that breast tumours with mutant p53 would be resistant to anthracyclines but sensitive to taxanes. Although this was the dominant view at the time, it was controversial because some studies reported conflicting results for individual drugs, in particular between mouse and human tumours, or between different cell lines. The clinical literature could not resolve the issue because it included only retrospective studies that were unable to distinguish the prognostic value of p53 from its ability to predict the response to chemotherapy. In addition these studies used mainly immunohistochemistry, which lacks sensitivity and specificity, or various DNA sequencing techniques, most of which lack sensitivity. We concluded that the only way to solve these problems would be to perform a prospective clinical trial with a more sensitive and specific test to detect p53 mutations.

Interpretation

This is the first large prospective clinical trial explicitly designed to assess the ability of p53 to predict the response of tumours to different chemotherapy regimens. We used a highly sensitive functional yeast assay. Although p53 status is prognostic for overall survival, it does not identify patients more likely to benefit from chemotherapy regimens containing taxanes. Further prospective studies should test the "de Thé hypothesis" [20], or drugs that act directly on p53 and its partners (for example, nutlin, RITA and PRIMA) [37].

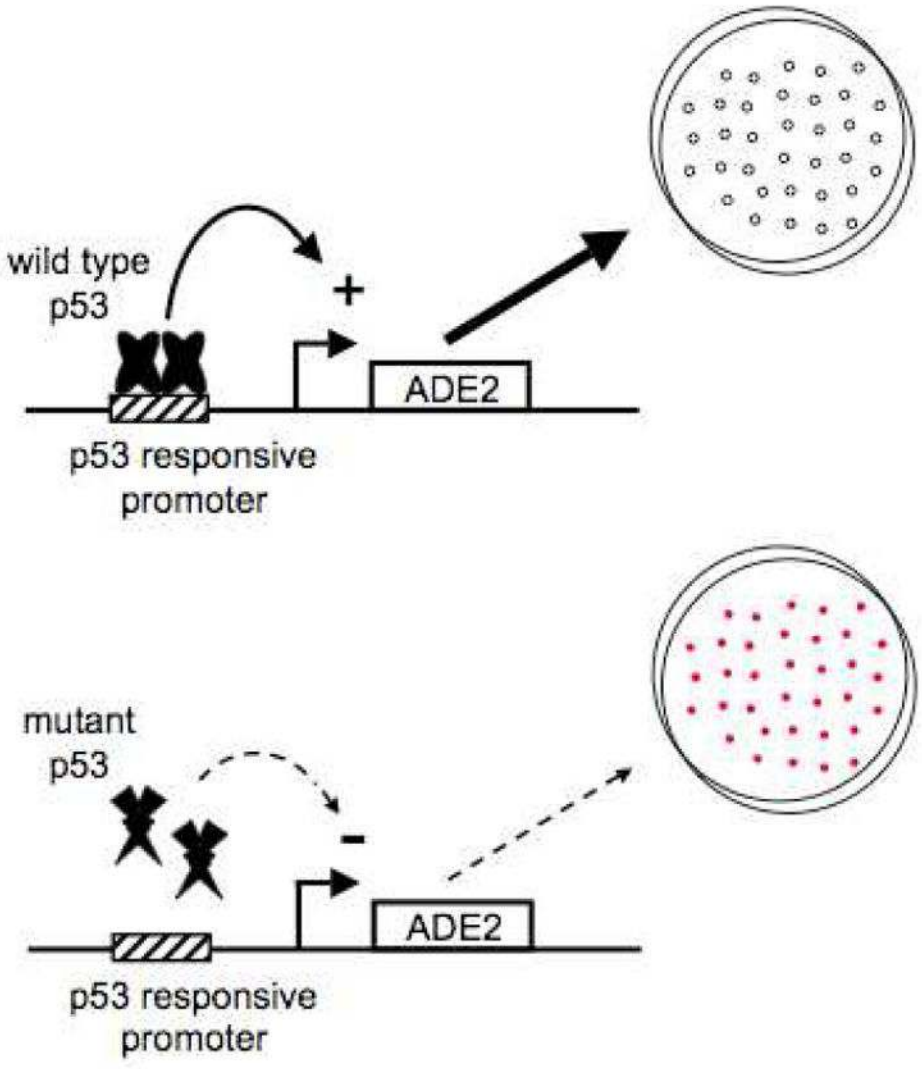


Figure 1.

Outline of the assay

p53 mRNA is extracted from the tumour biopsies, converted to cDNA, and amplified by PCR. The p53 PCR product is transfected into yeast, where it is cloned into a yeast expression vector by homologous recombination. Every yeast colony contains the progeny of a single p53 mRNA. P53 is a transcription factor. When wild type p53 protein is expressed within the yeast cell, it binds to a p53 binding site in a p53 responsive promoter in an ADE2 reporter gene and activates expression of Ade2 protein. Mutant p53 is unable to bind to DNA and fails to induce Ade2 expression. Yeast containing Ade2 protein form white colonies. Yeast lacking Ade2 form red colonies.

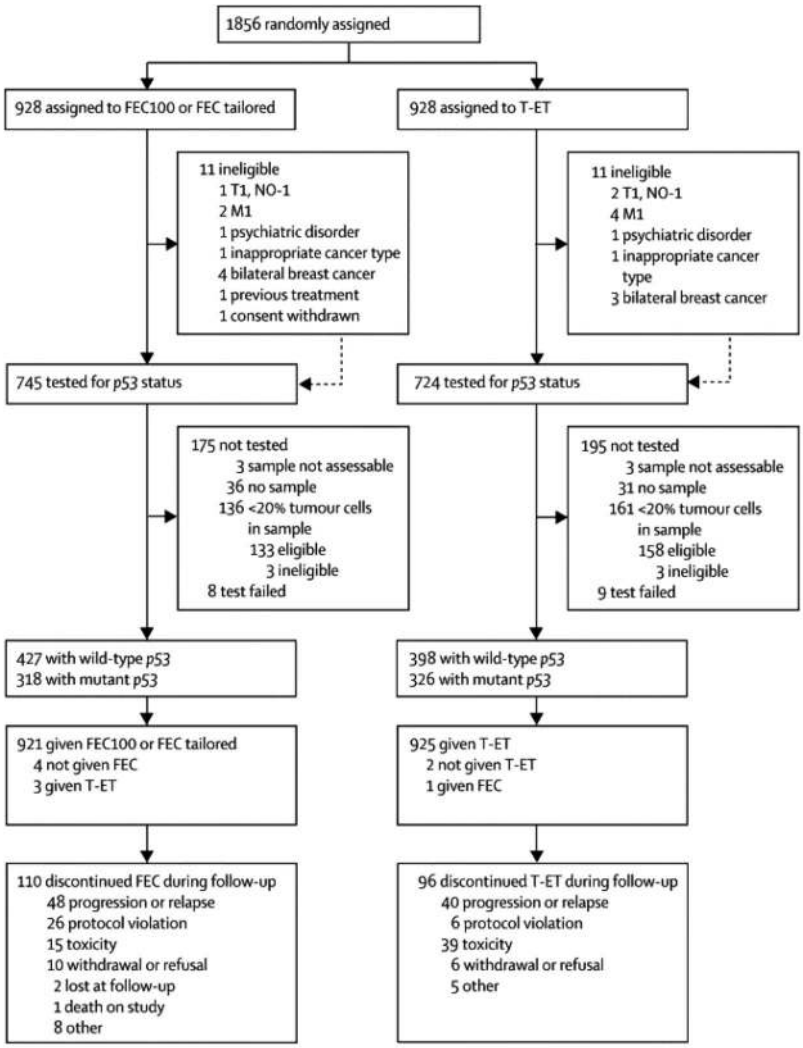


Figure 2.

CONSORT diagram

Abbreviations: FEC100: 5-fluorouracil 500mg/m^2, epirubicin 100mg/m^2, cyclophosphamide 500mg/m^2 all intravenously every 21 days for 6 cycles; Tailored FEC: first cycle on day 1 with 5-fluorouracil 600 mg/m2, epirubicin 75 mg/m2, cyclophosphamide 900 mg/m2, all intravenously every 21 days, with granulocyte colony-stimulating factor (G-CSF) 5 ug/kg on days 5–12 and ciprofloxacin orally 500 mg twice daily on days 2–15, subsequent cycles were modified for each individual as previously described for 6 cycles (maximal epirubicin dose 120 mg/m^2/cycle and cyclophosphamide dose 1200 mg/m^2/cycle); T-ET: docetaxel 100mg/m^2 every 21 days for 3 cycles followed by epirubicin 90mg/m^2 and docetaxel 75mg/m^2 every 21 days without G-CSF for 3 cycles; pts: patients; CT: chemotherapy.

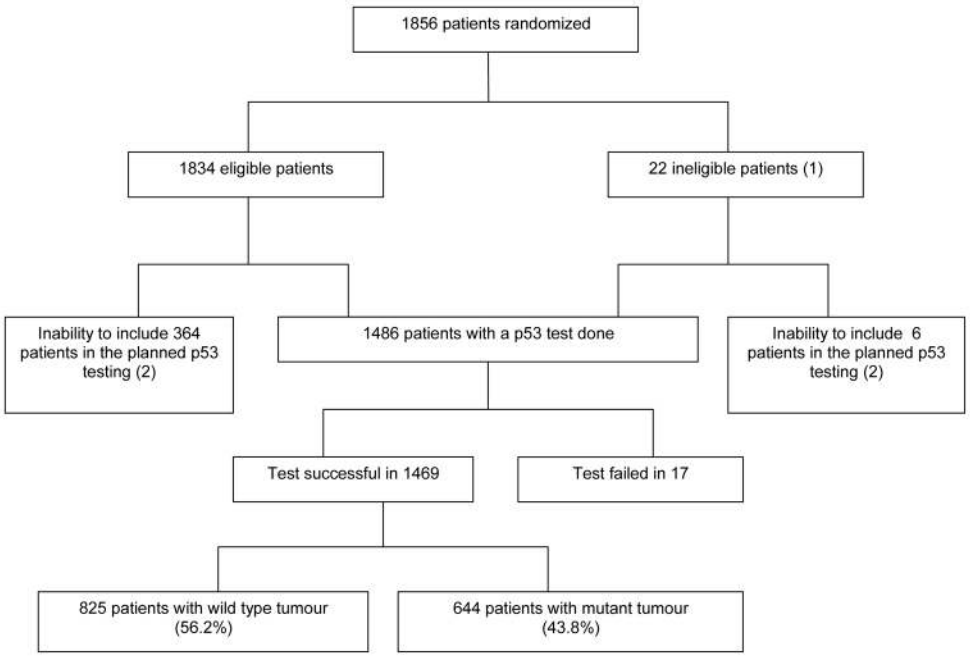


Figure 3.

Trial profile

Abbreviations:

(1) Most important reasons for ineligibility (total=22): Clinical : T1, N0–1 = 3; M1 = 6 ; Psychiatric disorder = 2 ; Inappropriate type of cancer = 2 ; Bilateral BC = 7 ; Prior treatment not allowed = 1 ; Withdrawn consent = 1

(2) Reasons for inability to include patients in the planned testing (total=370): No sample = 67; Not assessable = 6; Samples that could not be tested because <20% tumour cells = 291 eligible patients and 6 ineligible patients

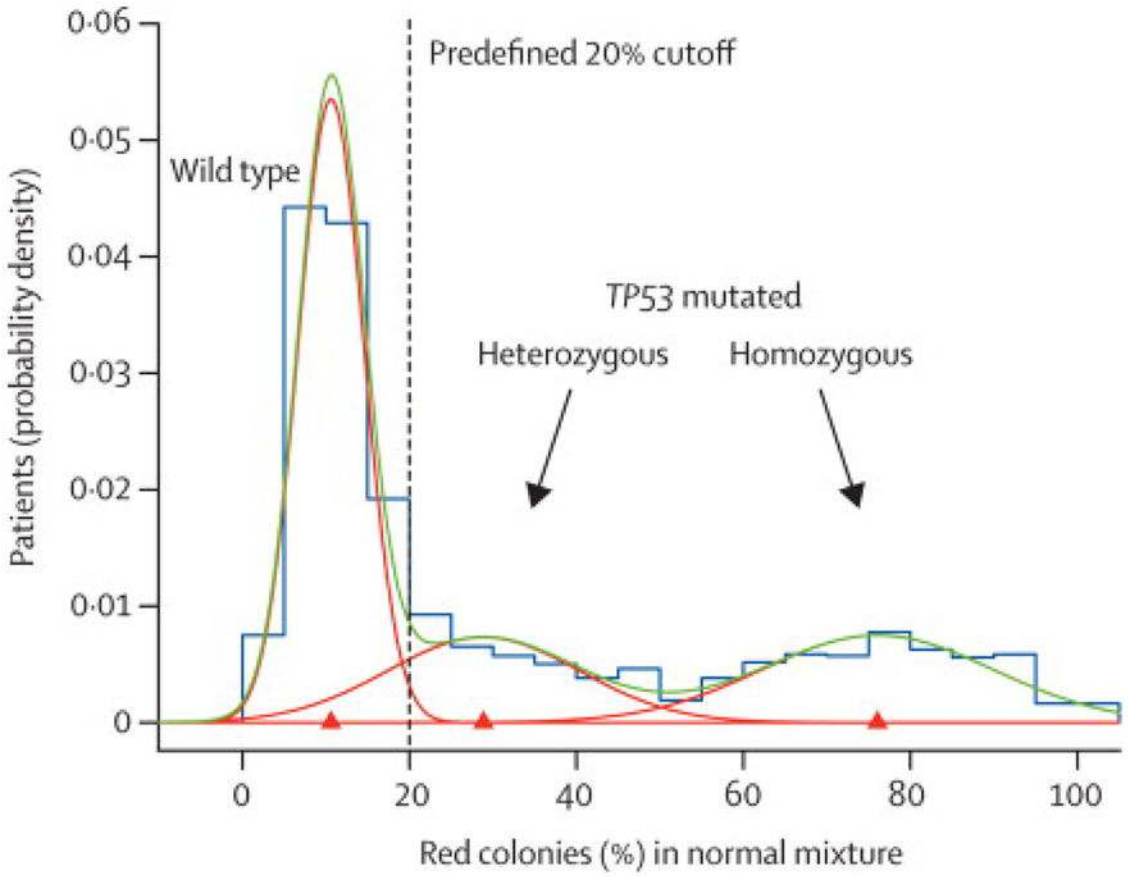
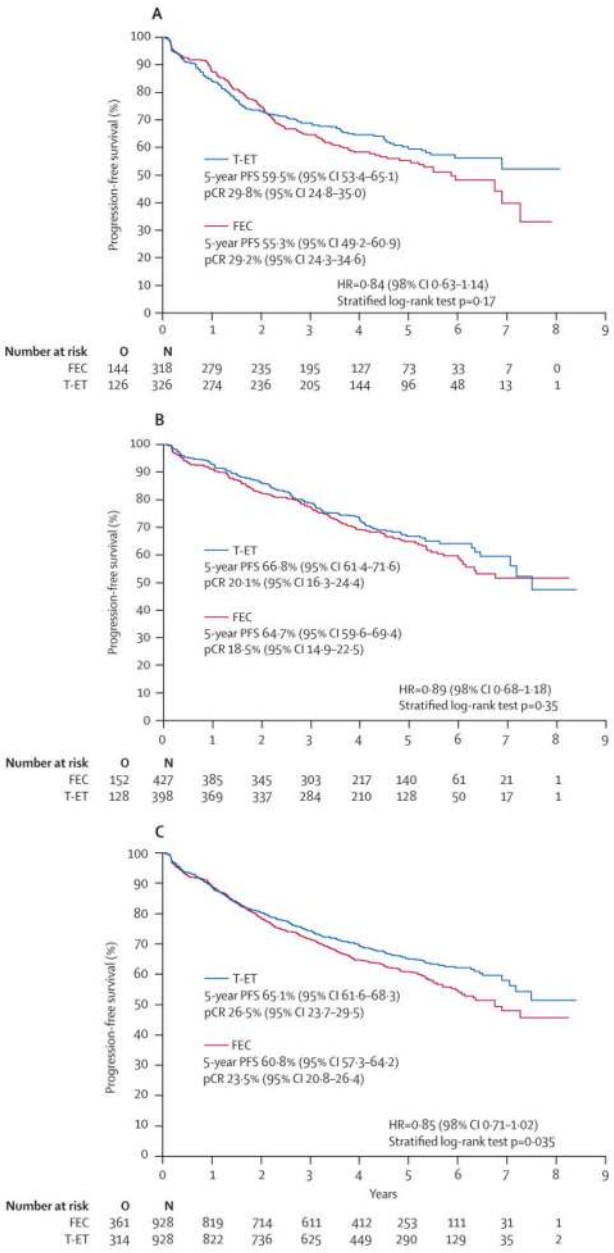


Figure 4.

Distribution of the percentage (%) of red colonies

The percentage of red colonies depends on the amount of mutant mRNA in the tumour cells in the biopsy, the amount of wild type mRNA in the normal cells in the biopsy, and the background. The distribution is modeled as three peaks. The peak on the left corresponds to samples containing only wild type p53 (the background in the assay is thus 11% ± 4%; the main cause is PCR mutations.[14] The peak on the right corresponds to tumours with homozygous p53 mutations (76% ± 14%). The mean wild type p53 mRNA content in the homozygous group is thus ~27% (24% plus the background). Between the wild type and homozygous peaks there is a third peak that is best explained by nonsense p53 mutations or heterozygous (particularly dominant negative) missense p53 mutations, but could potentially be explained by intra-tumoural heterogeneity, an intense inflammatory infiltrate or a florid stromal reaction.



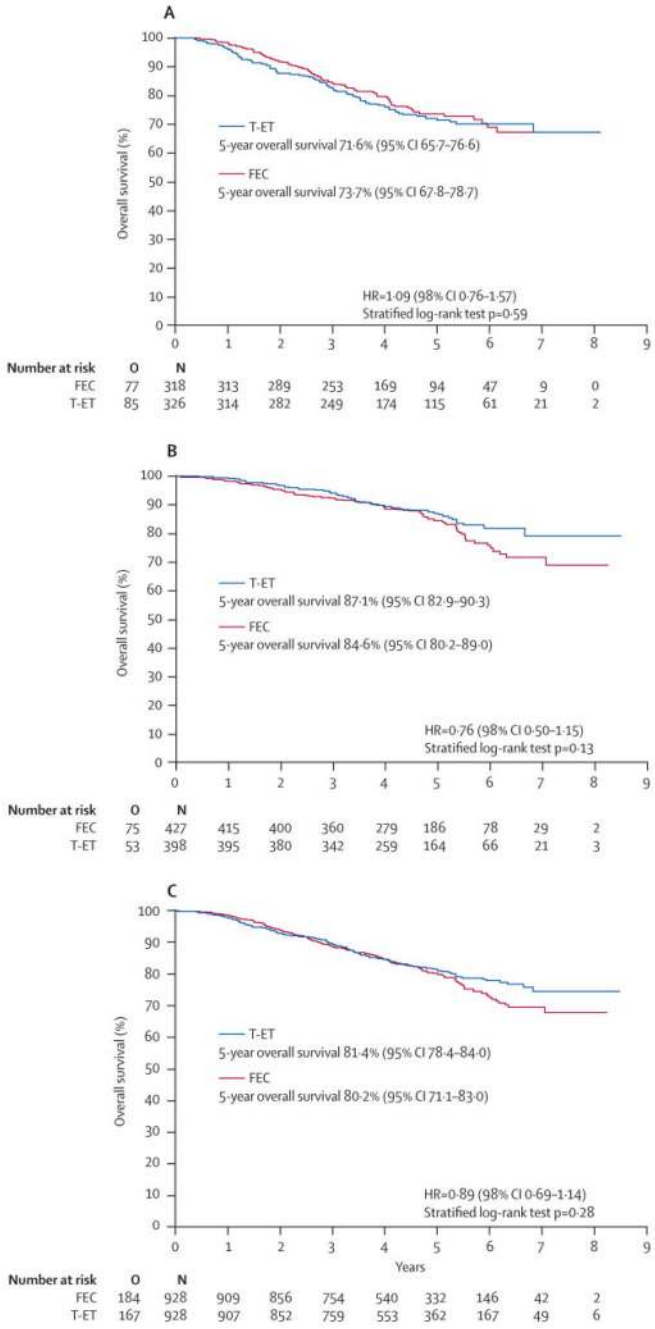


Figure 5.

(a) Progression-free survival and (b) Overall survival in the 3 groups by treatment arm: p53 mutated group, p53 wild type group and all patients

Abbreviations

HR: hazard ratio; 5 yr PFS: 5 year progression-free survival; 5yr OS: 5 year overall survival; CI: confidence interval; FEC: 5-fluorouracil, epirubicin, and cyclophosphamide; T-ET: docetaxel followed by epirubicin and docetaxel.

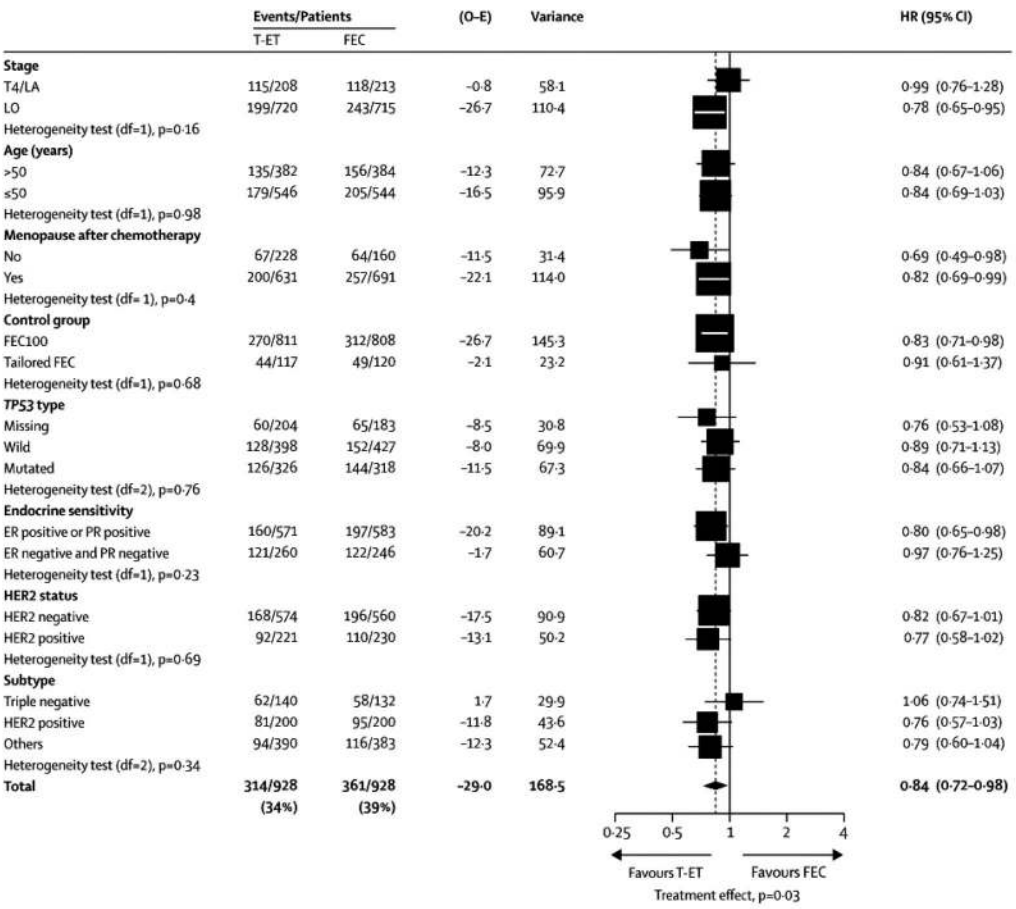


Figure 6.

Hazard ratios for progression-free survival by clinical characteristics, choice of regimen, biological markers, and simplified subtypes

Abbreviations

LA: locally advanced; LO: large operable; Menop. > CT: menopausal status post chemotherapy; Miss: missing; ER: estrogen receptor; PgR: progesterone receptor; HER2: human epidermal growth factor receptor 2; +: positive; −: negative; trip. Neg: triple negative; T-ET: docetaxel followed by epirubicin and docetaxel; FEC: 5-fluorouracil, epirubicin, and cyclophosphamide; HR: hazard ratio; CI: confidence interval.

Table 1

Baseline characteristics, surgery and adjuvant treatment by randomised treatment group

All patients		**FEC arm N=928 N (%)**	**T-ET arm N=928 N (%)**	**Total N=1856 N (%)**
Age	Median	48.9	48.6	48.8
	Range	22.2 – 70.6	24.0 – 70.9	22.2 – 70.9
	≤40	183 (19.7)	192 (20.7)	375 (20.2)
	41–50	361 (38.9)	354 (38.1)	715 (38.5)
	51–70	384 (41.4)	382 (41.2)	766 (41.3)
Menopausal status	Premenoapausal	396 (42.7)	424 (45.7)	820 (44.2)
	Postmenopausal	311 (33.5)	290 (31.3)	601 (32.4)
	Unknown	221 (23.8)	214 (23.0)	435 (23.4)
Category	Ineligible	3 (0.3)	3 (0.3)	6 (0.3)
	LocallyAdv./Inflammatory	213 (23.0)	208 (22.4)	421 (22.7)
	Large operable	712 (76.7)	717 (77.3)	1 429(77.0)
T stage	T1	8 (0.9)	5 (0.5)	13 (0.7)
	T2	442 (47.6)	475 (51.2)	917 (49.4)
	T3	297 (32.0)	276 (29.7)	573 (30.9)
	T4	181 (19.5)	171 (18.4)	352 (19.0)
	Tx	0 (0.0)	1 (0.1)	1 (0.1)
N stage	N0	399 (43.0)	393 (42.3)	792 (42.7)
	N1	455 (49.0)	469 (50.5)	924 (49.8)
	N2	61 (6.6)	55 (5.9)	116 (6.3)
	N3	11 (1.2)	9 (1.0)	20 (1.1)
	Nx	2 (0.2)	2 (0.2)	4 (0.2)
M stage				
	M0	921 (99.2)	922 (99.4)	1 843(99.3)
	M1(*)	7 (0.8)	6 (0.6)	13 (0.7)
Histologic type				
	Ductal	780 (84.1)	766 (82.5)	1 546 (83.3)
	Lobular	96 (10.3)	104 (11.2)	200 (10.8)
	Other	47 (5.1)	47 (5.1)	94 (5.1)
	Unknown	5 (0.5)	11 (1.2)	16 (0.9)
Grade	1	65 (7.0)	56 (6.0)	121 (6.5)
	2	434 (46.8)	425 (45.8)	859 (46.3)
	3	298 (32.1)	317 (34.2)	615 (33.1)
	Unknown or NA	131 (14.1)	130 (14.0)	261 (14.0)
ER status	Negative	283 (30.5)	298 (32.1)	581 (31.3)
	Positive	602 (64.9)	592 (63.8)	1 194 (64.3)
	Unknown	43 (4.6)	38 (4.1)	81 (4.4)
PgR status	Negative	399 (43.0)	379 (40.8)	778 (41.9)
	Positive	431 (46.4)	453 (48.8)	884 (47.6)

All patients		**FEC arm N=928 N (%)**	**T-ET arm N=928 N (%)**	**Total N=1856 N (%)**
HER2(†)	Unknown	98 (10.6)	96 (10.3)	194 (10.5)
	Positive	230 (29)	221 (27.8)	451 (28.3)
	Negative	560 (70.6)	574 (72.1)	1 134(71.4)
	Inconclusive	3 (0.4)	1 (0.1)	4 (0.3)
p53 status	Not done (‡) or failure	183 (19.7)	204 (22.0)	387 (20.9)
	Wild type	427 (46.0)	398 (42.9)	825 (44.5)
	Mutant	318 (34.3)	326 (35.1)	644 (34.7)
Surgery performed after neoadjuvant chemotherapy	No	15 (1.6)	11 (1.2)	26 (1.4)
	Yes	902 (97.2)	907 (97.7)	1809 (97.5)
	Missing	11 (1.2)	10 (1.1)	21 (1.1)
If yes, type of surgery	Lumpectomy/quadrantectomy	378 (41.9)	386 (42.6)	764 (42.2)
	Mastectomy	524 (58.1)	521 (57.4)	1045 (57.8)
Patients with ER and/or PgR positive tumours		**N=628 N (%)**	**N=613 N (%)**	**N=1241 N (%)**
Any adjuvant endocrine treatment	No	21 (3.3)	7 (1.1)	28 (2.3)
	Yes	595 (94.7)	597 (97.4)	1 192 (96.1)
	Unknown	12 (1.9)	9 (1.5)	21 (1.7)
Adjuvant aromatase inhibitor	No	261 (41.6)	267 (43.6)	528 (42.5)
	Yes	350 (55.7)	331 (54.0)	681 (54.9)
	Unknown	17 (2.7)	15 (2.5)	32 (2.6)
HER2-positive patients		**N=230 N (%)**	**N=221 N (%)**	**N=451 N (%)**
Adjuvant trastuzumab	No	147 (63.9)	154 (69.7)	301 (66.7)
	Yes	78 (33.9)	64 (29.0)	142 (31.5)
	Unknown	5 (2.2)	3 (1.4)	8 (1.8)

Abbreviations and legend: ER: estrogen receptor; PgR: progesterone receptor; HER2: human epidermal growth factor receptor 2; FEC: 5-fluorouracil, epirubicin, and cyclophosphamide; T-ET: docetaxel followed by epirubicin and docetaxel;

(*) In 7/13 patients ipsilateral supraclavicular nodes were the only metastatic site

(†) HER2 status was not collected systematically at baseline, but as a result of an amended case report form. This information was obtained for 1589 patients (85.6%), 793 in arm A and 796 in arm B.

(‡) p53 test was not done in 370 cases. The main reason was samples with less than 20% tumour cells. These samples were excluded because the normal cells in the sample would have generated a false negative result; they were not technical failures as such (figure 3).

Table 2

First events contributing to progression-free survival

	FEC arm (N=361)* N (%)	T-ET arm (N=314)* N (%)
Progression while on neoadjuvant chemotherapy	30 (8.3)	29 (9.2)
Distant recurrence	279 (77.3)	243 (77.4)
Invasive loco-regional recurrence	27 (7.5)	21 (6.7)
Invasive contralateral cancer	12 (3.3)	12 (3.8)
Death without prior report of progression	13 (3.6)	9 (2.9)
Progression	2	0
Treatment toxicity‡	1	1
Cancer (non-breast)	5	2
Cardiovascular	1	3
Other	0	2
Unknown	4	1

Abbreviations and legend:

FEC: fluorouracil, epirubicin, cyclophosphamide; T-ET: docetaxel followed by epirubicin and docetaxel;

* Respectively 361 and 314 had a progression-free survival qualifying event

‡ Deaths occuring during chemotherapy or within 30 days of chemotherapy completion and without disease relapse.

Table 3

Clinical and pathological complete responses

	All patients		p53 wild type		p53 mutated	
	FEC arm N=928	**T-ET arm N=928**	**FEC arm N=427**	**T-ET arm N=398**	**FEC arm N=318**	**T-ET arm N=326**
Complete clinical response (cCR)	121	155	51	65	55	56
cCR% (95% CI) *	13.0 (10.9–15.5)	16.7 (14.4–19.3)	11.9 (9.0–15.4)	16.3 (12.8–20.3)	17.3 (13.3–21.9)	17.2 (13.2–21.7)
Odds ratio (98% CI)*	1.34 (0.99–1.82)		1.44 (0.90–2.30)		0.99 (0.61–1.61)	
p-value	0.031		0.072		1.00	
Complete pathological response (pCR)	218	246	79	80	93	97
pCR% (95% CI) *	23.5 (20.8–26.4)	26.5 (23.7–29.5)	18.5 (14.9–22.5)	20.1 (16.3–24.4)	29.2 (24.3–34.6)	29.8 (24.8–35.0)
Odds ratio (98% CI)*	1.17 (0.92–1.51)		1.11 (0.73–1.67)		1.02 (0.69–1.53)	
p-value	0.148		0.60		0.93	

Abbreviations

cCR: complete clinical response

pCR: pathological complete response

* 95% confidence intervals of observed rates (by arm) and estimates, and 98% confidence intervals of odds ratio (between arms)

Table 4

Univariate and multivariate analysis (first model)

			PFS							OS						
			Univ			Multiv				Univ			Multiv			
Factor		**N**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**% selection**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**% selection**
p53	wt	825	1		0.0005	–		–	26.4	1		<0.0001	1		0.036	62.0
	mut	644	1.37	1.16–1.62						1.80	1.43–2.27		1.32	1.02–1.73		
	missing	387	1.04	0.84–1.28						1.10	0.81–1.50		0.93	0.67–1.30		
Stage	LO	1429	1		<0.0001	–		–	14.1	1		<0.0001	–		–	7.9
	LABC/T4d	421	2.18	1.86–2.55						2.48	2.00–3.07					
cT	T1–2	930	1		<0.0001	1		<0.0001	100	1		<0.0001	1		<0.0001	99.9
	T3	573	1.89	1.58–2.27		1.97	1.63–2.39			1.96	1.51–2.55		1.97	1.49–2.60		
	T4	352	3.03	2.50–3.66		2.78	2.25–3.43			3.42	2.63–4.44		2.99	2.24–4.00		
cN	N0	792	1		<0.0001	1		<0.0001	100	1		<0.0001	1		<0.0001	100
	N1	924	1.73	1.46–2,04		1.46	1.22–1.75			2.23	1.74–2.87		1.88	1.43–2.47		
	N2	116	2.44	1.84–3.25		1.67	1.22–2.28			3.55	2.44–5.17		2.34	1.55–3.53		
	N3	20	7.43	4.46–12.39		5.47	3.19–9.38			11.72	6.23–22.04		6.49	3.29–12.79		
PS	0	1748	1		<0.0001	1		0.0008	90.7	1		0.0008	1		0.007	71.6
	1 (2 pts with 2)	108	1.80	1.38–2.35		1.65	1.23–2.21			1.81	1.27–2.56		1.68	1.15–2.46		
Age	50 or more	766	1		0.33	–		–	23.3	1		0.12	–		–	7.7
	<50	1090	0.93	0.80–1.08						0.85	0.69–1.05					
Menopausal status	Post	752	1		0.14				10.7	1		0.08				8.7
	Pre	1099	0.89	0.77–1.04						0.83	0.67–1.03					
Endocrine receptors	ER+ and/or PgR+	1154	1		<0.0001	–	–	–	13.7	1		<0.0001				16.3
	ER− and PgR−	506	1.94	1.65–2.28						2.39	1.92–2.98					
Histological type	Lobular	200	1		0.089				11.2	1		0.16				

			PFS							OS						
			Univ			Multiv				Univ			Multiv			
Factor		**N**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**% selection**	**HR**	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**	**% selection**
	Ductal	1546	1.27	0.98–1.65						1.38	0.94–2.02					
	Other	94	1.52	1.03–2.27						1.65	0.95–2.87					
Grade	1	121	1		<0.0001	1		0.018	78.2	1		<0.0001	–		–	28.7
	2	859	2.10	1.36–3.24		1.55	0.99–2.43			2.65	1.30–5.40					
	3	615	3.15	2.04–4.87		1.74	1.10–2.75			4.34	2.13–8.84					
	Not assessed	253	2.31	1.45–3.68		1.22	0.74–2.01			3.32	1.58–6.99					
Treatment arm (stratified, 98.3% CI)	FEC	928	1		0.035	–		–	44.7	1		0.28	–		–	29.9
	T-ET	928	0.85	0.71–1.02						0.89	0.69–1.14					
Endoc Ttt	Tam/AI	1290	1		<0.0001	1		<0.0001	98.6	1		<0.0001	1		<0.0001	93.0
	None	566	2.11	1.81–2.46		1.95	1.62–2.33			2.59	2.10–3.19		2.27	1.78–2.89		

Abbreviations: cT: clinical tumour size; cN: clinical nodal status; PS: performance status; Endoc Ttt: endocrine treatment; wt: wild type; mut: mutated; LO: large operable; LABC: locally advanced breast cancer; ER: estrogen receptor; PgR: progesterone receptor; FEC: 5-fluorouracil, epirubicin, and cyclophosphamide; T-ET: docetaxel followed by epirubicin and docetaxel; Tam: tamoxifen; AI: aromatase inhibitor; PFS: progression-free survival; OS: overall survival; Univ: univariate; Multiv: multivariate; HR: hazard ratio; CI: confidence interval; % selection: % of times the covariate remained (by backward selection) in the final model in bootstrapped (resampled) models.

Table 5

Multivariate analysis for overall survival (second model with addition of subgroups defined by hormone receptor and HER2 status)

		HR	CI	p
p53	wt	1		0.012
	mut	1.49	1.11–2.01	
	missing	1.00	0.68–1.46	
cT	T1-2	1		<0.0001
	T3	2.12	1.55–2.89	
	T4	3.30	2.36–4.62	
cN	N	1		<0.0001
	N1	1.93	1.42–2.61	
	N2	2.28	1.42–3.67	
	N3	5.98	2.66–13.46	
Subtype	Other	1		<0.0001
	HER2 positive	1.22	0.89–1.66	
	Triple negative	2.10	1.51–2.92	

Abbreviations cT: clinical tumour size; cN: clinical nodal status; wt: wild type; mut: mutated; HER2: human epidermal growth factor receptor.

Table 6

Worst grade 3 or 4 adverse events during chemotherapy in 1846 patients who started treatment according to randomisation

	Sites using FEC100		Sites using Tailored FEC		Total
	FEC100 (N=803) N (%)	T-ET (N=809) N (%)	Tailored FEC (N=118) N (%)	T-ET (N=116) N (%)	(N=1846) N (%)
Haematological (during chemotherapy)					
Anaemia	17 (2.1)	4 (0.5)	4 (3.4)	0 (0.0)	25 (1.4)
Febrile neutropenia	75 (9.3)	173 (21.4)	10 (8.5)	26 (22.5)	284 (15.4)
Leucopenia	476 (59.3)	544 (67.2)	106 (89.8)	102 (88.0)	1228 (66.6)
Neutropenia	653 (81.3)	730 (90.2)	100 (84.7)	115 (99.1)	1598 (86.6)
Thrombocytopenia	19 (2.4)	1 (0.1)	38 (32.2)	0 (0.0)	58 (3.1)
Non haematological					
Allergic reaction	0	12 (1.5)	0	0	12 (0.7)
Cardiac left ventricular function	1 (0.1)	0 (0.0)	0	0	1 (0.1)
Cardiovascular/general-other	3 (0.4)	11 (1.4)	1 (0.8)	0	15 (0.8)
Constitutional symptoms-other	2 (0.2)	6 (0.7)	0	1 (0.9)	9 (0.5)
Dermatology – other	3 (0.4)	3 (0.4)	0	3 (2.6)	9 (0.5)
Diarrhoea	2 (0.2)	11 (1.4)	3 (2.5)	3 (2.6)	19 (1.0)
Edema	0	3 (0.4)	0	0	3 (0.2)
Fatigue	46 (5.7)	68 (8.4)	11 (9.3)	11 (9.5)	136 (7.4)
Gastrointestinal-Other	2 (0.2)	12 (1.5)	2 (1.7)	5 (4.3)	21 (1.1)
Hand-foot skin reaction	0	4 (0.5)	0	0	4 (0.2)
Hypotension	0	3 (0.4)	0	0	3 (0.2)
Infection	31 (3.9)	54 (6.7)	10 (8.5)	26 (22.4)	121 (6.6)
Nausea	21 (2.6)	15 (1.9)	4 (3.4)	2 (1.7)	42 (2.3)
Other toxicities	32 (4.0)	58 (7.2)	17 (14.4)	14 (12.1)	121 (6.6)
Rash desquamation	0	3 (0.4)	0	1 (0.9)	4 (0.2)
Stomatitis/Pharyngitis	15 (1.9)	17 (2.1)	1 (0.8)	2 (1.7)	35 (1.9)
Thrombosis/Embolism	7 (0.9)	6 (0.7)	4 (3.4)	2 (1.7)	19 (1.0)
Vomiting	32 (4.0)	6 (0.7)	8 (6.8)	1 (0.9)	47 (2.5)