

Carboplatin in *BRCA1/2*-mutated and triple-negative breast cancer BRCAness subgroups: the TNT Trial

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Germline mutations in *BRCA1/2* predispose individuals to breast cancer (termed germline-mutated *BRCA1/2* breast cancer, gBRCA-BC) by impairing homologous recombination (HR) and causing genomic instability. HR also repairs DNA lesions caused by platinum agents and PARP inhibitors. Triple-negative breast cancers (TNBCs) harbor subpopulations with *BRCA1/2* mutations, hypothesized to be especially platinum-sensitive. Cancers in putative 'BRCAness' subgroups—tumors with *BRCA1* methylation; low levels of *BRCA1* mRNA (*BRCA1* mRNA-low); or mutational signatures for HR deficiency and those with basal phenotypes—may also be sensitive to platinum. We assessed the efficacy of carboplatin and another mechanistically distinct therapy, docetaxel, in a phase 3 trial in subjects with unselected advanced TNBC. A prespecified protocol enabled biomarker-treatment interaction analyses in gBRCA-BC and BRCAness subgroups. The primary endpoint was objective response rate (ORR). In the unselected population (376 subjects; 188 carboplatin, 188 docetaxel), carboplatin was not more active than docetaxel (ORR, 31.4% versus 34.0%, respectively; $P = 0.66$). In contrast, in subjects with gBRCA-BC, carboplatin had double the ORR of docetaxel (68% versus 33%, respectively; biomarker, treatment interaction $P = 0.01$). Such benefit was not observed for subjects with *BRCA1* methylation, *BRCA1* mRNA-low tumors or a high score in a Myriad HRD assay. Significant interaction between treatment and the basal-like subtype was driven by high docetaxel response in the nonbasal subgroup. We conclude that patients with advanced TNBC benefit from characterization of *BRCA1/2* mutations, but not *BRCA1* methylation or Myriad HRD analyses, to inform choices on platinum-based chemotherapy. Additionally, gene expression analysis of basal-like cancers may also influence treatment selection.

TNBC describes the 10–20% of tumors that are estrogen receptor (ER)-, progesterone receptor (PgR)- and HER2-negative. However, assuming that TNBC is a single entity is a fallacy

masking considerable histological and biological heterogeneity among tumors, which must be understood to optimize therapy selection. Outcome for a patient with recurrent and/or advanced

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TNBC is especially poor¹. Chemotherapy is the only approved systemic therapy and, although considered biologically unselective, can have distinct mechanisms of action that target specific biological mechanisms that are aberrant in cancer. When accompanied by mechanism-relevant biomarkers, the use of a specific chemotherapeutic in defined populations might be considered a ‘targeted’ therapy.

Although genomic classifiers suggest that the majority of TNBCs are of the basal-like intrinsic subtype^{2,3}, recent analyses suggest that TNBC can be subclassified^{4–6}. An immunohistochemical (IHC) approximation of the basal-like intrinsic subtype has been termed ‘core basal’⁷. A common feature of sporadic basal TNBC is genomic instability with mutational and rearrangement signatures indicative of abnormalities in DNA repair and replication stress that overlap *BRCA1/2*-mutation-associated signatures⁸. Abnormalities also exist in *BRCA1* mRNA expression that are largely driven through methylation of the *BRCA1* promoter^{9,10}, as observed in ovarian cancer^{11,12}. This and the overlap in mutational signatures⁸ suggest functional deficiency of genes with known roles in DNA repair by HR as a shared characteristic between *BRCA1* hereditary breast cancers and a substantial, but incompletely defined, subgroup of TNBC. *BRCA1* and *BRCA2* proteins have important roles in DNA replication fork stabilization and HR¹³ and are components of the Fanconi anemia protein network^{14,15}. The hallmark of deficiency in this network is sensitivity to DNA crosslinks induced by platinum and mitomycin C^{16,17}. Historically, platinum chemotherapies have only shown modest activity in advanced breast cancer except for chemotherapy-naïve disease^{18,19}.

No trial has directly studied responses to platinum therapy in comparison to standard of care in advanced unselected TNBC, its majority basal subtype or subgroups of TNBC with features of aberrant *BRCA1/2*-associated function or ‘BRCAness’²⁰. The TNT Trial was designed to compare the activity of the standard of care microtubule-disrupting agent docetaxel with the DNA-crosslinking agent carboplatin. We hypothesized that carboplatin would have greater activity than docetaxel in subgroups deficient in DNA damage response. Considering that strong mechanistic evidence exists for the efficacy of carboplatin and cisplatin in cells with *BRCA1/2* mutations, accrual of patients known to have these germline mutations was allowed irrespective of ER, PgR and HER2 status. We pre-specified analyses of (i) *BRCA1/2* germline mutation carriers and of members of putative BRCAness²¹ TNBC subgroups with (ii) DNA methylation at the *BRCA1* promoter and/or low *BRCA1* mRNA expression and the basal-like phenotype as defined by (iii) gene or (iv) protein expression.

Results

Between 25 April 2008 and 18 March 2014, 376 subjects (188 allocated to carboplatin and 188 to docetaxel) entered the trial, and all subjects were included in the analysis of the primary endpoint (Fig. 1); the trial population largely comprised patients with TNBC and no known *BRCA1/2* mutation (338 of 376) and with baseline characteristics typical of patients with relapse of TNBC following first-line therapy (Supplementary Tables 2 and 3). There were 43 subjects with germline *BRCA1/2* mutation (31 *BRCA1* and 12 *BRCA2*; Supplementary Table 2). Of the 31 carriers of a *BRCA1* mutation, 4 had ER-positive disease, and 7 of the 12 carriers of a *BRCA2* mutation had ER-positive disease. Compliance with allocated treatment was good; disease progression and toxicity were the principal reasons for early discontinuation. The median relative dose intensity was 94.0% (interquartile range (IQR), 84.2–99.8%) for carboplatin and 94.8% (IQR, 84.8–100.0%) for docetaxel.

Overall results. There was no evidence of a difference between the ORR to carboplatin and to docetaxel in the overall population (ORR, 31.4% (59 of 188 carboplatin-treated subjects) versus

34.0% (64 of 188 docetaxel-treated subjects); absolute difference (calculated as carboplatin ORR – docetaxel ORR), –2.6%; 95% CI, –12.1 to 6.9; $P=0.66$; Fig. 2a). Following central review of locally classified responses, response rates were determined to be 25.5% (48 of 188 subjects) for carboplatin versus 29.3% (55 of 188 subjects) for docetaxel (absolute difference, –3.8%; 95% CI: –12.8 to 5.2; $P=0.49$), consistent with findings from the main analysis. Similarly, no difference was observed based on responses to crossover treatment with the alternative drug (crossover treatment; Supplementary Fig. 1a) or when analysis was limited to those centrally confirmed as having TNBC tumors (Supplementary Note).

Of the 376 subjects in the trial, 372 (98.9%) had progression-free survival (PFS) events reported. Median PFS in subjects allocated carboplatin was 3.1 months (95% CI, 2.4–4.2), and median PFS was 4.4 months (95% CI, 4.1–5.1) for those allocated docetaxel. No difference in restricted mean PFS was found (difference in restricted mean PFS (calculated as carboplatin restricted mean PFS – docetaxel restricted mean PFS), –0.30 months; $P=0.40$; Fig. 3a).

There were 347 subjects who were reported to have died. Median overall survival (OS) was 12.8 months (95% CI, 10.6–15.3) and 12.0 months (95% CI, 10.2–13.0) for those allocated carboplatin or docetaxel, respectively. Consistent with the PFS result, no evidence of a difference in OS was found between treatment groups (difference in OS (calculated as carboplatin restricted mean OS – docetaxel restricted mean OS), –0.03 months; $P=0.96$; Supplementary Fig. 2a).

BRCA subgroup analyses. Analyses of *BRCA1/2* mutation subgroups, which were prespecified in study protocols, were conducted at the time of the main analysis. Subjects with a deleterious *BRCA1/2* germline mutation had a significantly better response to carboplatin than to docetaxel (ORR, 68% (17 of 25 carboplatin-treated subjects) versus 33.3% (6 of 18 docetaxel-treated subjects); absolute difference, 34.7%; $P=0.03$), and there was no evidence of differential treatment activity in subjects with no germline mutation (ORR, 28.1% (36 of 128 carboplatin-treated subjects) versus 34.5% (50 of 145 docetaxel-treated subjects); absolute difference, –6.4%; $P=0.30$), resulting in a statistically significant interaction between therapy and *BRCA1/2* mutation status ($P=0.01$; Fig. 2b). This result remained significant ($P=0.01$) after adjustment for known prognostic factors (Supplementary Note). PFS was also longer in subjects with a *BRCA1/2* germline mutation who were treated with carboplatin (median PFS, 6.8 months (carboplatin) versus 4.4 months (docetaxel); difference in restricted mean PFS, 2.6 months; interaction $P=0.002$; Fig. 3b), but no difference was found between groups in OS (Supplementary Fig. 2b), with interpretation confounded by the preplanned crossover at progression (Supplementary Fig. 1b). Considering the small number of *BRCA2* versus *BRCA1* germline mutation carriers who were randomized, comparative analyses of interaction between treatment effect and each mutated gene were neither significant nor meaningful; this also held true for assessment of treatment interaction with ER status, as there was a very small number of ER-positive tumors.

Subjects with tumor tissue available for sequencing and a *BRCA1/2* mutation detected in their tumor sample (see Supplementary Table 4 for overlap of mutation detected in tumor with germline *BRCA1/2* mutation status) appeared to have a better response to carboplatin than to docetaxel (ORR, 66.7% (12 of 18 carboplatin-treated subjects) versus 35.7% (5 of 14 subjects); absolute difference, 31.0%; $P=0.15$); however, docetaxel had a more positive treatment effect in subjects with wild-type *BRCA1/2* in their tumor sample (ORR, 25.6% (23 of 90 carboplatin-treated subjects) versus 35.6% (32 of 90 docetaxel-treated subjects); absolute difference, –10.0%; $P=0.20$). Given the very small number of subjects with tumor mutation data, neither of these subgroup analyses attained statistical significance; however, considering that the effects were in opposite directions, the interaction between treatment effect and *BRCA1/2* status in

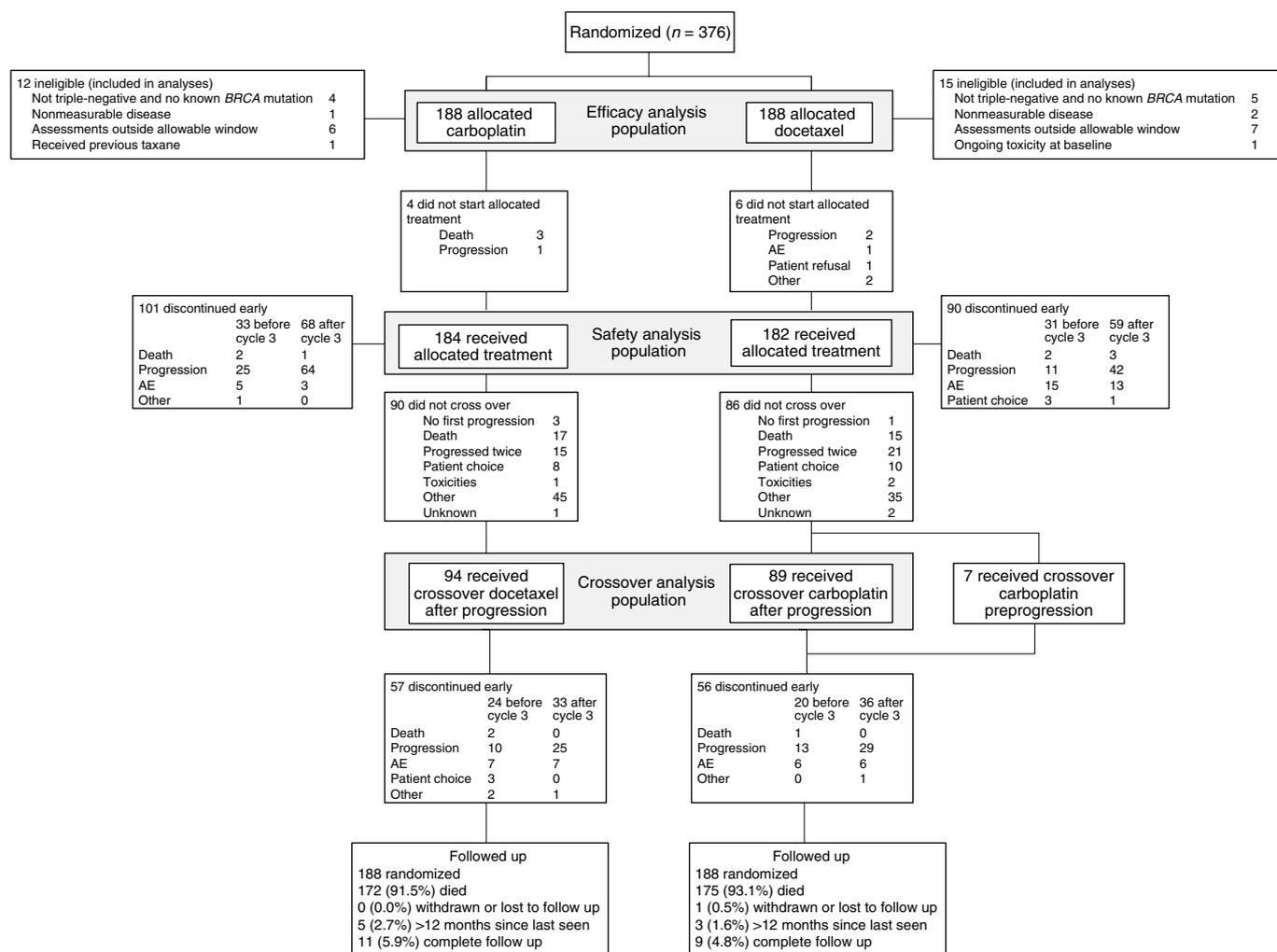


Fig. 1 | Diagram of study. Flowchart for allocation of participants enrolled in the trial into treatment groups

tumor was significant ($P=0.03$; Fig. 2c). This, however, did not hold for PFS or OS ($P=0.12$ and $P=0.70$, respectively; Fig. 3c and Supplementary Fig. 2c). Eight subjects had a germline wild-type *BRCA* genotype but a *BRCA* mutation in their tumor, which was classed as a somatic mutation (Supplementary Table 4); two of four carboplatin-treated subjects with these mutations responded to therapy, as did two of four docetaxel-treated subjects, but small numbers of subjects limit a conclusive interpretation of these data.

Counter to our prespecified hypothesis, subjects with *BRCA1* methylation did not have a better response to carboplatin than to docetaxel (ORR, 21.4% (3 of 14 carboplatin-treated subjects) versus 42.1% (8 of 19 docetaxel-treated subjects); absolute difference, -20.7% ; $P=0.28$) with no evidence of an interaction observed ($P=0.35$; Figs. 2d and 3d and Supplementary Fig. 2d); we arrived at similar conclusions when subjects with germline *BRCA1/2* mutations were excluded.

Concordant with *BRCA1* methylation status, tumors we defined as *BRCA1* mRNA-low, which methylation was partially associated with (Supplementary Fig. 3 and Supplementary Table 5), did not have a better response to carboplatin than to docetaxel (ORR, 28.6% (4 of 14 carboplatin-treated subjects) versus 64.7% (11 of 17 docetaxel-treated subjects); absolute difference, -36.1% ; $P=0.07$), and evidence of an interaction was lacking ($P=0.07$; Figs. 2e and 3e and Supplementary Fig. 2e). Again, conclusions were not different when subjects with germline *BRCA* mutations were excluded. Furthermore, exploratory analyses examining any relationship

between high response to carboplatin and the cut-point for *BRCA1* methylation or *BRCA1* mRNA-low did not reveal any notable signal that supported our a priori hypotheses that these factors would be associated with a better response to carboplatin than to a taxane (data not presented).

Homologous Recombination Deficiency subgroup analyses.

In the initial trial design and first protocol, we hypothesized that changes in the genome landscape, which may arise as a consequence of defects in HR, could be an indicator of platinum salt sensitivity and should be examined for interaction with treatment effect in both treatment arms. A number of these assays have been reported^{18,22–25}. Here we show the result using the combined Myriad HRD assay²⁶ performed on treatment-naïve primary tissue. We found that the great majority of subjects with either germline *BRCA1/2* mutation or *BRCA1* methylation have a high dichotomized ‘HRD Score’ (Supplementary Fig. 4a,b), but subjects with a high HRD Score, unlike carriers of a germline *BRCA1/2* mutation, did not have a better response to carboplatin than to docetaxel (ORR, 38.2% (13 of 34 carboplatin-treated subjects) versus 40.4% (19 of 47 docetaxel-treated subjects); absolute difference -2.2% ; $P=1.0$), with no evidence of an interaction observed ($P=0.75$; Fig. 4a). Similar results were found when ‘HR Deficient’ subjects, a definition that grouped all subjects with a *BRCA1/2* mutation and subjects with wild-type *BRCA1/2* with a high HRD score, were examined (Fig. 4b). In addition, no evidence of treatment-specific predictive effects on PFS

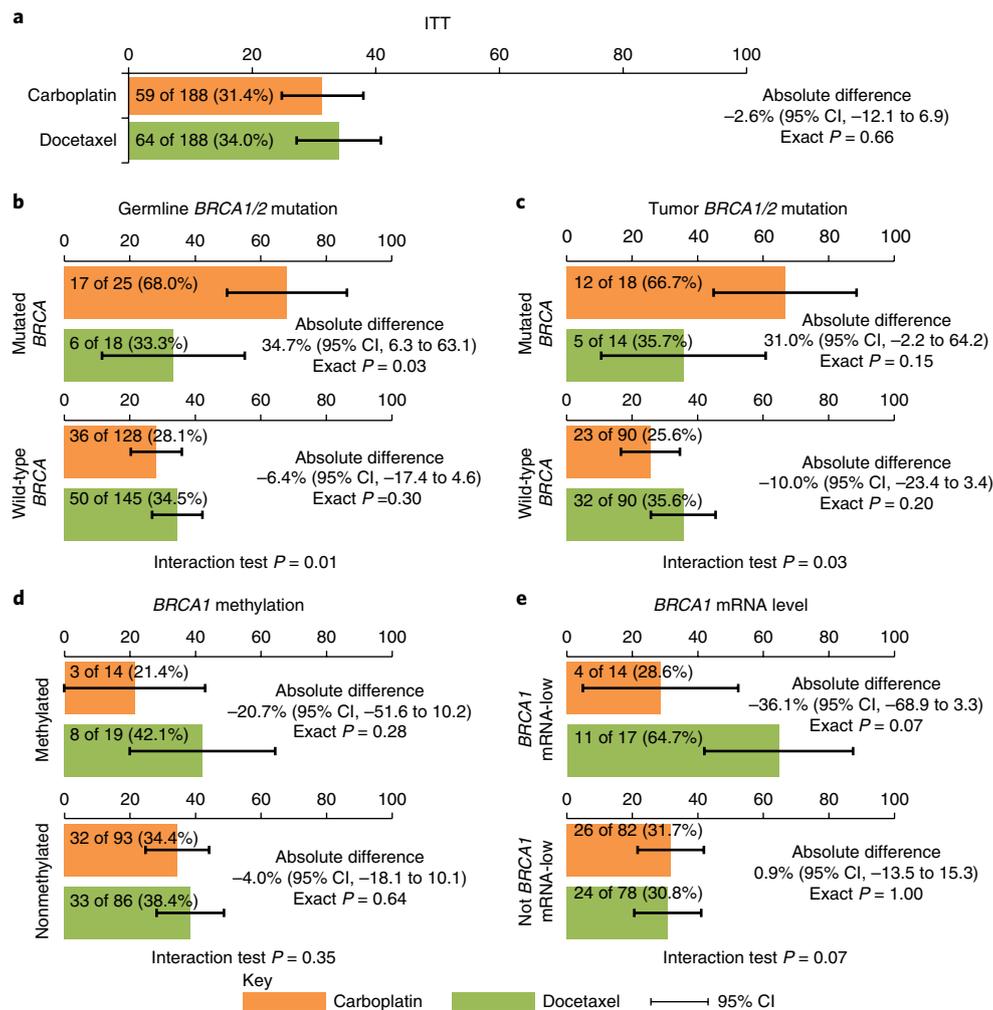


Fig. 2 | Response rates documented in the overall population and within *BRCA* subgroups. **a**, The absolute difference in the percentage of subjects who responded to treatment (carboplatin responders (%) – docetaxel responders (%)) between treatment groups within the overall patient population. ITT, intention to treat. **b–e**, The absolute differences in the percentage of subjects who responded to treatment between treatment groups with subjects stratified according to germline *BRCA1/2* mutation status (**b**), tumor *BRCA1/2* mutation status (**c**), *BRCA1* methylation status (**d**) and *BRCA1* mRNA-low status (**e**). P values for absolute differences were calculated using a two-sided Fisher’s exact test. P values for the interaction tests are based on the Wald statistic from a logistic regression model of response, with terms for biomarker status, treatment group and interaction. The number of subjects who responded to treatment out of all subjects in the group is shown on each bar. Axes scales show the percentage of subjects with a response.

was found using either HRD definition (Supplementary Fig. 5a,b). Subjects with a high HRD score had a greater response to both chemotherapy agents than those with low scores, but this was not statistically significant.

Basal subgroup analyses. Given the association between germline *BRCA1* mutation and the development of basal-like breast cancers, we sought to formally test the premise that all basal-like cancers and *BRCA1*-mutated tumors share a loss-of-function phenotype of *BRCA1* through analyzing interaction between basal-like status and platinum treatment in this broader basal-like TNBC group. We found no evidence that Prosigna–PAM50 basal tumors had a better response to carboplatin compared to docetaxel (ORR, 32.5% (27 of 83 carboplatin-treated subjects) versus 31.0% (27 of 87 docetaxel-treated subjects); absolute difference, 1.5%; $P=0.87$). However, in patients with non-basal-like tumors, response to docetaxel was significantly better than to carboplatin (ORR, 72.2% (13 of 18 docetaxel-treated subjects) versus 16.7% (3 of 18 carboplatin-treated subjects); absolute difference, -55.5% ; $P=0.002$), leading to a significant interaction test ($P=0.003$; Fig. 5a) and a similar trend

in crossover treatment response (Supplementary Fig. 6). The interaction between treatment and PAM50 subgroups remained significant after adjusting for g-*BRCA*-BC status in the multivariable logistic regression model ($P=0.002$; Supplementary Table 6) and when other known prognostic factors were subsequently included in the model. The interaction was also significant for PFS ($P=0.04$; Fig. 6a) but not OS ($P=0.17$; Supplementary Fig. 7a).

There was no evidence that ‘core basal’ tumors defined by IHC analysis had improved response to carboplatin compared with docetaxel (ORR, 34.3% (23 of 67 carboplatin-treated subjects) versus 29.2% (19 of 65 docetaxel-treated subjects); absolute difference, 5.1%; $P=0.58$). Although there was a higher response rate to docetaxel compared with carboplatin in subjects with tumors that were negative for expression of ER, PgR, HER2, EGFR and Ck5/6 and were nonbasal by IHC (ORR, 41.9% (13 of 31 docetaxel-treated subjects) versus 19.2% (5 of 26 carboplatin-treated subjects); absolute difference, -22.7% ; $P=0.09$), the difference did not reach statistical significance, and the interaction test was nonsignificant ($P=0.06$; Figs. 5b and 6b and Supplementary Fig. 7b).

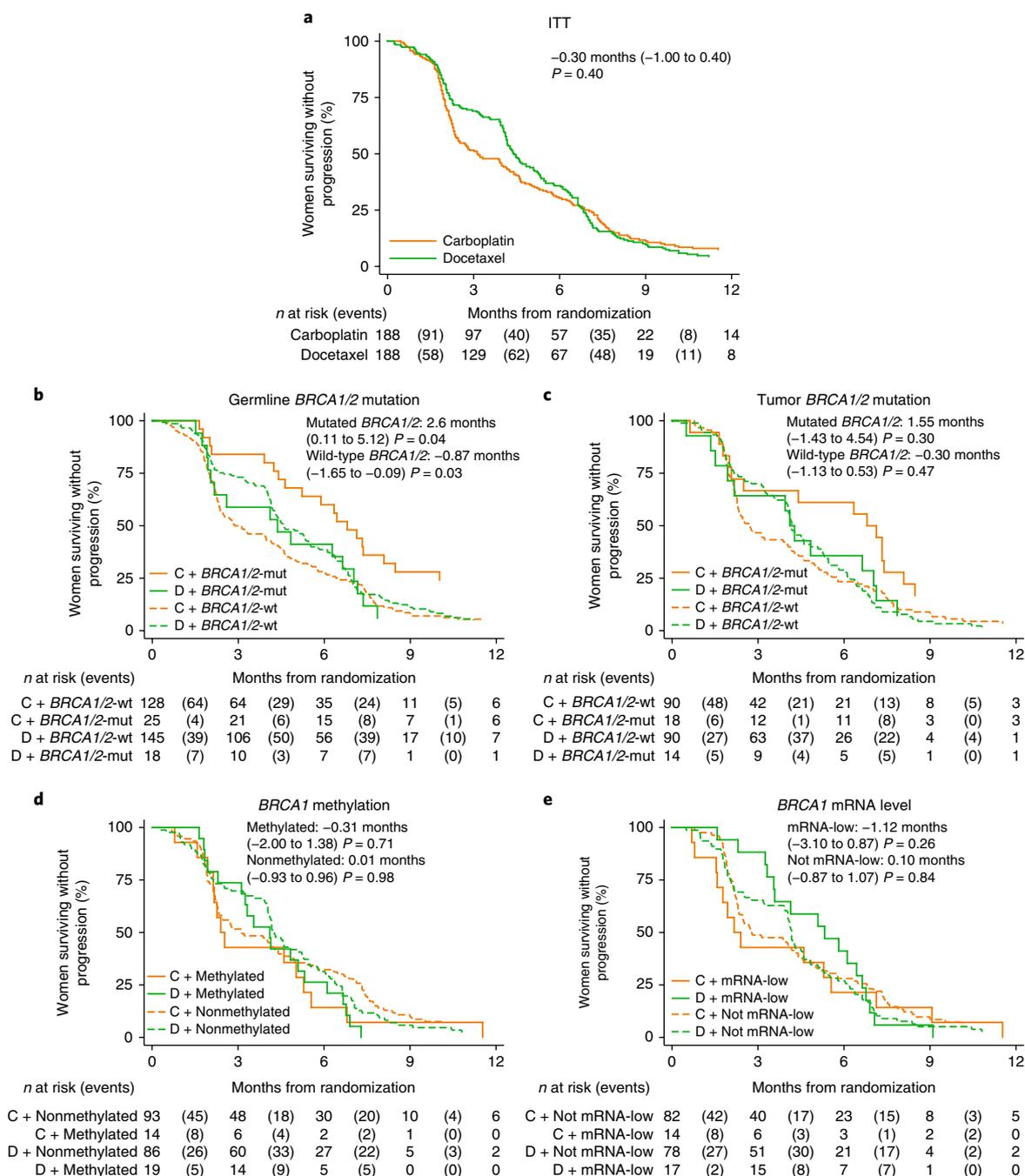


Fig. 3 | PFS in the entire population and in subgroups stratified according to BRCA status. **a**, PFS for the overall ITT population of subjects treated with carboplatin or docetaxel. The difference in restricted mean and 95% CI are shown on the figure. **b–e**, PFS for treatment groups with subjects stratified according to germline *BRCA1/2* mutation status (**b**), tumor *BRCA1/2* mutation status (**c**), *BRCA1* methylation status (**d**) and *BRCA1* mRNA-low status (**e**). The difference in restricted means with 95% CIs for stratified groups are shown on the figures. A negative mean indicates a better response to docetaxel, and a positive mean indicates a better response to carboplatin. P values were calculated using a two-sided Student’s t -test comparing the restricted mean PFS between treatments (within biomarker groups as indicated). C, carboplatin; D, docetaxel. n at risk (events) shows the number of subjects who remain in the analysis set at a given time point and the number of PFS events reported between time points.

Safety. Both carboplatin and docetaxel demonstrated toxicity consistent with their known safety profiles, and grade 3 and 4 adverse events (AEs) occurred as anticipated for these well-known chemotherapy drugs (Supplementary Tables 7 and 8). There were more grade 3 and 4 AEs following treatment with docetaxel than with carboplatin. Throughout the trial, 276 serious adverse events (SAEs) were reported (102 and 174 in carboplatin- and docetaxel-treated subjects, respectively). The spectrum of SAEs was as anticipated.

Two SAEs were considered to be suspected unexpected serious adverse reactions (1 in each treatment group); these were (i) nausea, vomiting and headaches and (ii) low magnesium. One death was considered possibly related to carboplatin treatment; this subject died from pulmonary embolism. As a haploinsufficiency or dominant-negative effect of heterozygous mutation might affect toxicity from HR-targeting therapies, such as platinum, in carriers of a *BRCA* mutation, we sought evidence of excess hematological

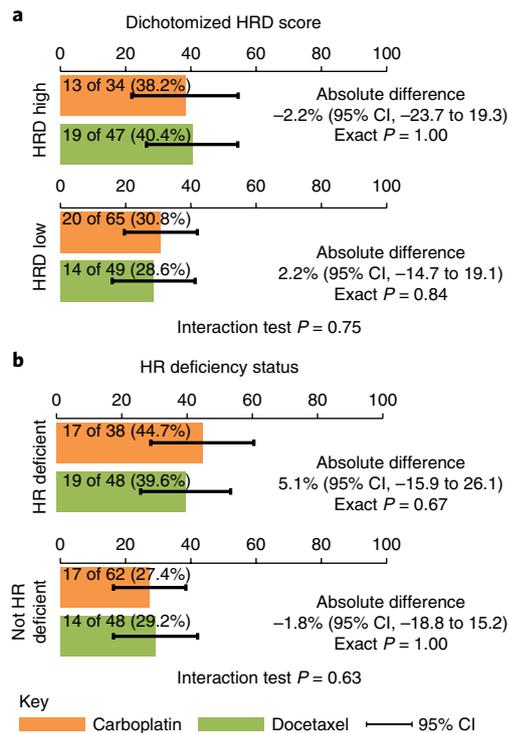


Fig. 4 | Response rates in groups stratified according to HRD status. **a, b**, Absolute differences in the percentage of subjects who responded to treatment between treatment groups within subgroups stratified by dichotomized HRD score (**a**) and HR deficiency status (**b**). P values for the differences were calculated using a two-sided Fisher's exact test. P values for interaction tests are based on a logistic regression model of response, with terms for biomarker status, treatment group and interaction. The number of subjects who responded to treatment out of all subjects in the group is shown on each bar. Axes scales show the percentage of subjects with a response.

toxicity as a signal but found none (Supplementary Table 9). Although there was a small difference in nonhematological toxicity, this was not significant, and small numbers preclude firm conclusions from these analyses.

Discussion

This phase 3 trial utilized two mechanistically distinct single-agent chemotherapeutics in unselected advanced TNBC and in biomarker-defined subpopulations that were specified a priori and were thought likely to have targetable defects in HR DNA repair. In the unselected subjects with TNBC, no evidence of a superior response to carboplatin was observed when compared with a standard of care taxane, docetaxel. Carboplatin was better tolerated than docetaxel when delivered at the full licensed dose. This trial demonstrates clinically important activity for both agents. The level of response seen for docetaxel in this trial is consistent with that seen previously in breast cancer²⁷, and the level of response for carboplatin was comparable to that seen in uncontrolled trials of single-agent platinum^{28,29} or combinations of carboplatin with gemcitabine in unselected TNBC³⁰. The only other randomized trial that was conducted synchronously with our trial and was designed to specifically investigate platinum therapy in comparison with a standard of care in advanced TNBC included the substitution of the platinum therapy cisplatin for paclitaxel that was administered in a doublet with gemcitabine. In this study, treatment was continued until disease progression, as is common practice with paclitaxel, and the cisplatin doublet showed modestly increased activity compared

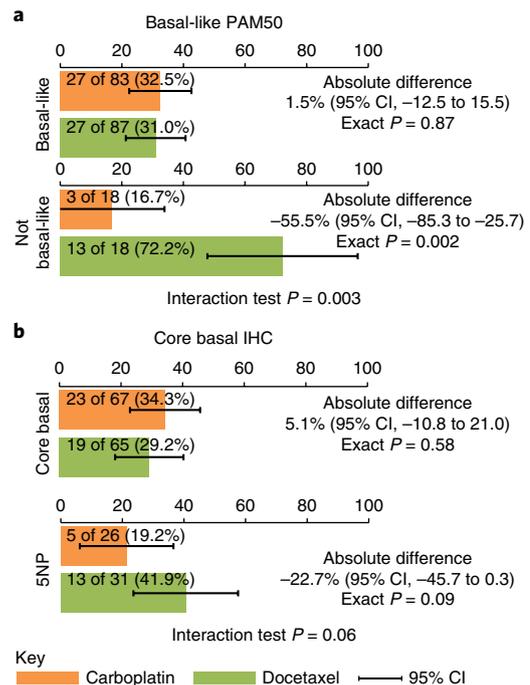


Fig. 5 | Response rates in groups stratified according to basal-like status. **a, b**, Absolute differences in the percentage of subjects who responded to treatment between treatment groups within subgroups stratified by basal-like status as defined by PAM50 signatures (**a**) and core basal status as determined by IHC analysis (**b**). P values for the differences were calculated using a two-sided Fisher's exact test. P values for interactions were based on a logistic regression model of response, with terms for biomarker status, treatment group and interaction. 5NP, tumors that were negative for expression of ER, PgR, HER2, EGFR and Ck5/6 and were nonbasal by IHC. The number of subjects who responded to treatment out of all subjects in the group is shown on each bar. Axes scales show the percentage of subjects with a response.

to the paclitaxel doublet standard of care therapy³¹. A criticism of our study could be that subjects did not receive treatment to progression but only for six cycles (and, at investigator discretion, to a maximum of eight cycles). This was consistent with UK practice with docetaxel at the full licensed 100 mg/m² dose, as this dose is rarely tolerated for more than six to eight cycles. This limited duration may explain the shorter PFS in our study compared to that in Hu et al.³¹ despite similar OS between studies and may have caused us to underestimate the effect of carboplatin in those without a progression event during treatment and who might have continued event-free for longer had treatment continued.

In contrast to the unselected population, the prespecified analyses of treatment effect in subgroups found evidence of clinically and statistically significant biomarker–treatment interactions. There is a strong association between *BRCA1* mutation and basal-like cancer³², and sporadic basal-like breast cancer subtypes show high degrees of chromosomal genomic instability³. We hypothesized that if, as has been widely speculated, there was a shared, profound BRCAness phenotype, sporadic basal-like cancers might have very high platinum sensitivity. We found no evidence that basal-like biomarkers predicted higher response to platinum than to docetaxel, with the drugs showing similar activity. A significant interaction was detected between treatment and the Prosigna–PAM50-identified subtypes that was driven by significantly increased response to docetaxel relative to poor platinum response in nonbasal forms of TNBC. This suggests an absence of targetable BRCAness in nonbasal TNBC and provides no evidence to change the standard of

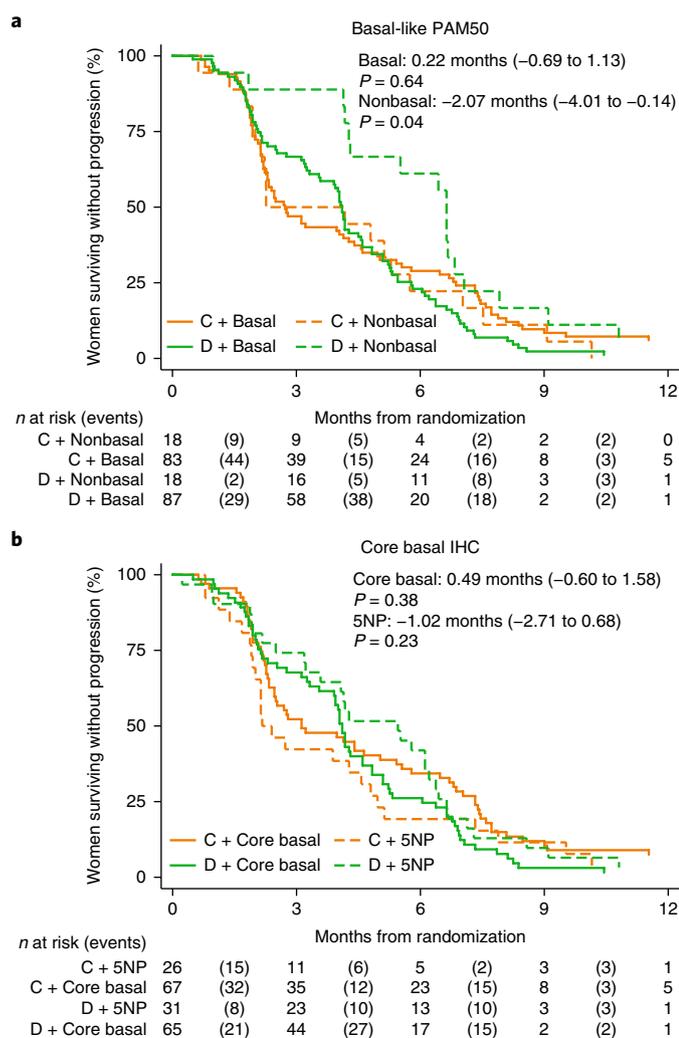


Fig. 6 | PFS for groups stratified according to basal-like status. a, b, PFS for treatment groups with subjects stratified according to basal-like status as defined by PAM50 (**a**) and core basal status as determined using IHC analysis (**b**). A negative value indicates a better response to docetaxel, and a positive value indicates a better response to carboplatin. The difference between restricted means with 95% CIs for stratified groups are shown on the figures. P values were calculated using a two-sided Student's t -test comparing the restricted mean survival between treatments within biomarker groups. n at risk (events) shows the number of patients who remain in the analysis set at a given time point and the number of PFS events reported between time points.

care from a taxane to a platinum, which our data suggest is inferior in these subtypes. In contrast, platinum is a reasonable option in individuals with basal-like TNBC, particularly in those who fail to tolerate or have previously received a taxane. As the response rate in these cancers is much lower than that of breast cancer associated with a *BRCA1/2* mutation, if there is a profound BRCAness phenotype that remains prevalent in metastatic basal-like breast cancer beyond the context of *BRCA1* or *BRCA2* mutation, it appears to lie within a subpopulation of this subtype that is yet to be identified.

BRCA1/2 mutation is a clinically validated and widely available biomarker that has predicted both a greater response and longer PFS in subjects treated with carboplatin than in those treated with docetaxel, demonstrating clinical utility for treatment selection in this setting. There was no evidence that *BRCA1/2* mutation was associated with reduced activity of docetaxel compared to the wild

type; docetaxel remains a valid and active, but inferior, treatment option in this setting. We did not find evidence of an OS advantage conferred by carboplatin therapy in carriers of a *BRCA1/2* mutation, but interpretation is confounded by the crossover design, as 56% of these subjects received carboplatin at progression. The high levels of response seen for carboplatin were similar to those reported for the combination of carboplatin and paclitaxel in an essentially similar population in the reference comparator arm in the phase II BROCADE trial³³, supporting the notion that carboplatin monotherapy is highly active in this subject group. We found approximately one-third of subjects with a *BRCA1/2* mutation did not respond to platinum therapy. Potential mechanisms underlying resistance will be further explored in integrated whole-genome and whole-transcriptome sequencing analyses in primary tumor material, but the lack of extensive metastatic tumor samples from subjects immediately before platinum treatment will limit both the sensitivity of the analyses and the ability to draw firm conclusions.

In parallel, we tested the hypothesis that epigenetic silencing of *BRCA1* through DNA methylation would show a similar treatment interaction. Despite a similar number of subjects in genetic and epigenetic BRCAness subgroups, subjects with *BRCA1* methylation or mRNA-low tumors had a higher response to docetaxel than to carboplatin. Exploratory analyses with the aim of optimizing cut-points for these biomarkers and analyses of these epigenetic biomarkers as continuous variables failed to find any signal of potential carboplatin treatment interaction. In stark contrast to the interaction between *BRCA1/2* mutation and carboplatin treatment effect, no evidence was found that supported a similar impact of epigenetic BRCAness; there was no interaction found between either *BRCA1* methylation or *BRCA1* mRNA-low status and carboplatin treatment effect. This suggests that genetic and epigenetic changes at the *BRCA1* locus have important differences, at least in the context of predicting therapy response in metastatic breast cancer exposed to prior adjuvant chemotherapy. These results are consistent with previous results from the nonrandomized TBCRC 009 trial in metastatic TNBC²⁸, in which the few tumors with *BRCA1* methylation showed no response to platinum therapy despite evidence of chromosomal instability signatures. The majority of subjects in our study had received adjuvant chemotherapies that cause DNA lesions that engage HR in their repair. We measured *BRCA1* methylation and mRNA level in archived primary tumor specimens, whereas treatment effect was assessed in metastases. We speculate that a higher proportion of *BRCA1/2*-mutation carriers retain an HR defect in metastatic disease than those with *BRCA1* methylated tumors (Supplementary Fig. 9). We suggest that mutation creates a more resilient ‘hard’ BRCAness, whereas epigenetic BRCAness associated with *BRCA1* methylation is more ‘soft’ and plastic²⁰. The methylation of *BRCA1* may be both more heterogeneous and/or more revertible in subclinical metastases that, when subjected to selection pressure by DNA-damaging adjuvant therapy, lose their HR defect and survive, subsequently developing as HR-proficient clinical metastases that are not selectively platinum-sensitive. Our hypothesis is supported by data from both preclinical patient-derived xenografts and primary breast tumors exposed to neoadjuvant chemotherapy³⁴. In ovarian cancers, *BRCA1* mutation, but not methylation, is associated with improved prognosis after platinum therapy^{35,36}, and examination of biopsy pairs obtained pre- and postplatinum treatment shows reversion of *BRCA1* methylation in 31% of tumors, with continued presence of methylation being associated with a PARP inhibitor response³⁷. Although defects in HR are known to be revertible, mutational signatures would not be expected to disappear, as they are a permanent ‘scar’ of prior, even if no longer active, HR defects. Although our finding that the Myriad HRD assay did not specifically predict response to platinum as opposed to docetaxel in the setting of advanced TNBC disease contrasts to the reported association of a high Myriad HRD score

with platinum response in the neoadjuvant setting in TNBC²⁶, these neoadjuvant studies do not have a comparator arm to allow testing for interaction between biomarker status and any specific treatment effect of platinum chemotherapy as opposed to a relatively greater general chemotherapy responsiveness in HRD-high tumors than in HRD-low tumors. Where this specific effect of platinum therapy was examined in the randomized neoadjuvant context, the Myriad HRD assay did not show specific predictive performance for platinum response in unplanned retrospective analyses with limited power³⁸. Metastatic disease exposed to prior adjuvant therapy is also a very different biological context than therapy in a treatment-naïve neoadjuvant or adjuvant context. We hypothesize that adjuvant or neoadjuvant therapy drives reversal of the *BRCA1*-methylation-driven soft BRCAness³⁴ HR defect, which we show, like *BRCA1* mutation, produces a high HRD score in the primary tumor (Supplementary Fig. 4). This high score erodes the positive predictive value of the HRD score for therapy response in metastases, whereas a low HRD score will likely retain negative predictive value by excluding many tumors that have never had an HR defect, whether soft or hard. Since our analysis, a new HR deficiency mutational signature whole-genome sequence analysis methodology called 'HRDetect' has been described, with preliminary evidence of potential application to formalin-fixed paraffin-embedded (FFPE) clinical materials⁸. As HRDetect is also a cumulative historical measure of lifetime HR deficiency, the positive predictive value of this method may also be eroded by the effects of reversal of epigenetic HR defects in treatment-exposed metastatic disease and may require integration with additional biomarkers revealing a tumor's current HR status. Analyses using HRDetect and multiple additional mutational signatures and their integration with transcriptional signatures of BRCAness and treatment response^{8,23,26,39,40} are planned but require whole-genome sequencing currently being piloted in TNT Trial FFPE material. These future analyses are beyond the scope of this manuscript.

Previous randomized studies have not examined treatment effect in a priori-defined subpopulations within advanced TNBC³¹. TNT highlights the heterogeneity in TNBC and the need to investigate therapeutic effects with planned analyses of biological subgroups. We provide the first evidence of the clinical utility of *BRCA1/2* genotyping to inform therapy choice in metastatic familial breast cancer and TNBC. In early TNBC, three recent trials have tested the role of the addition of platinum to anthracycline- and taxane-based neoadjuvant schedules and found evidence of increased pathological tumor response^{41–43}. These studies are underpowered for survival endpoints, but where reported, significant effects on disease-free survival were only seen when the alkylating agent cyclophosphamide was omitted from the control arm⁴¹. A nonsignificant trend was noted when a standard cyclophosphamide 'backbone' control was used in the CALGB 40603 study⁴². The dose-intense carboplatin regimen used in GeparSixto was recently compared with a sequential anthracycline and taxanes regimen and a high-dose cyclophosphamide-containing regimen, with no differences found in the primary pathological response measures⁴⁴. It would seem that the use of alkylating agents in early TNBC is important, especially for individuals who have higher-stage disease with associated risk of recurrence and require a maximally effective therapy, to reduce this risk and achieve optimal surgery. The balance of additional toxicity and paucity of appropriately powered survival analyses testing interaction with potential predictive biomarkers for platinum response suggest the need for further studies before platinum is used routinely across all stages and biological subtypes of early TNBC. Data from our trial, although conducted in advanced TNBC, inform this landscape and raise important hypotheses for further testing in the setting of early breast cancer.

Many countries now perform inexpensive local *BRCA1/2* germline testing. Our results support *BRCA1/2* germline testing to

select patients for platinum chemotherapy for advanced disease. The OlympiAD trial⁴⁵ recently reported a comparison between the potent PARP inhibitor olaparib, known to trap PARP1 on DNA, and the physician choice of nonplatinum standard of care chemotherapies in anthracycline- and taxane-exposed advanced gBRCA-BC. Other trials of potent PARP inhibitors are ongoing⁴⁶. The PARP inhibitor olaparib is now approved in advanced gBRCA-BC, but this treatment may remain unaffordable to many health care systems and patients for many years. It remains unknown how potent PARP1-trapping inhibitors would compare with platinum in this setting, but the TNT Trial provides evidence that a widely available, affordable off-patent biomarker, which is enriched in the TNBCs prevalent in many developing countries⁴⁷, has utility in selecting a population that could benefit during this period from the biologically targeted use of a highly active and inexpensive platinum chemotherapy agent rather than the current licensed standard-of-care chemotherapies for breast cancer.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at <https://doi.org/10.1038/s41591-018-0009-7>.

Received: 24 November 2017; Accepted: 2 March 2018;

Published online: 30 April 2018

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Acknowledgements

The authors would like to thank all subjects and the families of those who took part in the trial and all involved staff at the participating centers. In addition, we acknowledge R. Buus and B. Haynes for laboratory support for NanoString assays, S. Ferree of NanoString for provision of Prosigna reagents and manuscript review and R. Seitz of Insight Genetics for assistance in TNBC type analysis and manuscript review. The authors also acknowledge past and present colleagues on the TNT Trial Management Group, the Independent Data Monitoring Committee and Trial Steering Committee who oversaw the trial, the Response Evaluation Committee who conducted the independent radiology review and Cancer Research UK and Breast Cancer Now (and their legacy charity Breakthrough Breast Cancer) who funded the study (Cancer Research UK grant number CRUK/07/012) as well as the National Institute for Health Research Cancer Research Networks in England and their equivalent NHS research and development (R&D)-funded networks in Scotland, Wales, and Northern Ireland for 'in-kind' support. Funding was provided from Myriad Genetics, Inc., to cover costs of nucleic extraction from tumor blocks appropriate for next-generation sequencing, and Prosigna reagent kits were provided by NanoString Technologies, Inc.

Author contributions

A.T., H.T., S.K., L.K., P.B.-L., L.F., C.H.-W., P.P., R.R., I.E.S., A.M.W., C.G., A.A., N.R., M.H., P.E., S.E.P. and J.M.B. are members of the Trial Management Group (TMG), and M.C.U.C., A.Gr., C.M.P., A.S. and R.B. are in the Biological Subcommittee of the TMG. J.P. is a Response Evaluation Committee member. A.T. was the Chief Investigator and chair of the Biological Subcommittee of the TMG and performed trial design and protocol development, including writing of the translational subsection of the protocol at trial outset to test the BRCAness hypotheses. A.T. also performed participant recruitment, data collection, data interpretation and writing of the manuscript. H.T. performed statistical analysis, data interpretation and writing of the manuscript. M.C.U.C. was the lead biostatistician for the translational substudies and performed data analysis of biological data generated from the biomarker assays, including basal-like subtype by NanoString (Prosigna) and IHC, *BRCA1* methylation, *BRCA1/2* mutation status, HRD score and total RNA-seq, data interpretation and writing of the manuscript. S.K. performed trial management, data collection and data management. L.K. performed trial design, protocol development, statistical analysis, data interpretation and writing of the manuscript. P.G., J.O. and V.S. performed TNT tissues resource preparation. J.A., S.B., P.B.-L., S.C., C.H.-W., M.Q.H., R.R., I.E.S., A.M.W. and G.W. performed participant recruitment and data collection. R.B. and J.M.F. performed data analysis of *BRCA1* methylation and writing of the manuscript. M.D. performed the NanoString (Prosigna) experiment. L.F. performed trial management and data collection. A.Gr. and A.Gu. performed HRD analysis. K.A.H. and C.M.P. performed total RNA-seq from which *BRCA1* mRNA was derived. J.P. performed independent radiology review. A.S. served as a germline genetics advisor for biological analyses and data interpretation, supported the germline *BRCA1/2* mutation analysis and performed protocol development and writing of the manuscript. K.M.T. and J.S.L. performed tumor *BRCA1/2* mutation analysis, *BRCA1* methylation analysis and HRD analysis. C.G. served as the TNT tissue bank lead and performed TNT tissues resource preparation, ER/PgR and HER2 central testing, basal breast cancer subtyping by IHC and gene expression analysis. N.R. served as a germline genetics advisor for biological analyses and data interpretation, led the germline *BRCA1/2* mutation analysis and performed protocol development and writing of the manuscript. M.H. and P.E. performed trial design, protocol development, participant recruitment and data collection. S.E.P. served as the study's lead pathologist and performed ER/PgR, HER2 central testing, and basal breast cancer subtyping by IHC. J.M.B. performed trial design, protocol development, study conduct oversight, statistical analysis, data interpretation and writing of the manuscript. All authors reviewed the manuscript prior to submission.

Competing interests

A.T., H.T., M.C.U.C., S.K., L.K., P.G., J.O., R.B., M.D., L.F., A.G., P.P., V.S., C.G., N.R., S.E.P. and J.M.B. report that their institutional departments have received grants from Breast Cancer Now and/or Cancer Research UK and other support for costs or consumables in this research from Myriad Genetics, Inc. and NanoString Technologies, Inc. during the conduct of the study. M.C.U.C. has a patent: US Patent No. 9,631,239 with royalties paid. M.D. reports receiving personal fees from Myriad Genetics, Inc. outside of the submitted work. A.Gu. reports receiving salary compensation and stock/options from Myriad Genetics, Inc. during conduct of the study and has patent

rights assigned to Myriad Genetics. C.M.P. reports receiving personal fees from Bioclassifier LLC, consulting fees from Nanostring Technologies outside the submitted work. In addition, C.M.P. has a patent: U.S. Patent No. 9,631,239 with royalties paid. K.M.T. reports receiving personal fees from Myriad Genetics, Inc. during the conduct of the study and personal fees from Myriad Genetics, Inc. outside the submitted work. In addition, K.T. has the following patents pending: 13/164,499; 14/554,715; 15/010,721; 15/192,497; 14/245,576; 62/000,000; 62/311,231; 62/332,526; 14/962,588; 2802882; 11796544.2; 15189527.3; 2,839,210; 12801070.9; 2014-516031; 2012358244; 2,860,312; 201280070358.0; 12860530.0; 2014-548965; 2014248007; 2,908,745; 14779403.6; 2016-506657; 712,663; PCT/US15/045561; PCT/US15/064473; and the following patents issued to Myriad Genetics, Inc.: 9,279,156; 9,388,427 and 625468. J.S.L. reports salary compensation and stock/options from Myriad

Genetics Inc. received during conduct of the study. The other authors declare no competing interests.

Additional information

Supplementary information is available for this paper at <https://doi.org/10.1038/s41591-018-0009-7>.

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Methods

Study design. Conducted in 74 hospitals throughout the United Kingdom, TNT (NCT00532727) was a phase 3, parallel group, open-label randomized controlled trial with preplanned biomarker subgroup analyses. The trial was cosponsored by The Institute of Cancer Research and King's College London. It was approved by the East London and The City Main Research Ethics Committee. Trial sponsorship, governance, randomization procedures and balancing factors are described in the Supplementary Note.

Subjects. Eligible patients had to be considered fit to receive either study drug (carboplatin or docetaxel) and had to have measurable, confirmed advanced breast cancer unsuitable for local therapy that was histologically confirmed as ER-, PgR- and HER2-negative primary invasive breast cancer that either had an Allred/quick score of <3 or a histo score (H-score) of <10 or that were locally determined to be ER- and PgR-negative if other cut-offs were used (for example, 1%, 5% or 10%). The HER2-negative phenotype was defined as an IHC score of 0 or 1+ for HER2 or a fluorescence in situ hybridization (FISH) or chromogenic in situ hybridization (CISH) score of 2+ and no amplification for the *HER2* gene. An included subject could be ER- and HER2-negative, PgR-negative/unknown or any ER, PgR and HER2 status if the subject was known to have a *BRCA1* or *BRCA2* germline mutation and was otherwise eligible to participate (full eligibility criteria are listed in the Supplementary Note). Although subjects with TNBC who were hypothesized to have BRCAness phenotypes were the primary interest, subjects with unselected TNBC as well as those with *BRCA1* or *BRCA2* germline mutations were recruited to allow interaction testing of biomarker-positive and biomarker-negative populations in relation to response to each of these mechanistically distinct agents. Subjects provided written informed consent.

Procedures. Subjects were allocated (in a 1:1 ratio) to groups that received six cycles of carboplatin (area under the curve (AUC) 6), day 1 of a 3-weekly cycle, or six cycles of docetaxel (100 mg/m²), day 1 of a 3-weekly cycle (see Supplementary Note for details of allocation procedures, including the minimization-balancing factors used). For subjects responding to and tolerating treatment well, a further two cycles of treatment could be given, subject to local policy. Further details of chemotherapy and supportive medicines are described in the Supplementary Note. Subjects were offered six cycles of the alternative (crossover) treatment upon progression or where allocated treatment was discontinued due to toxicity (preprogression crossover). Subsequent management was at discretion of the clinician.

Tumor assessment through computed tomography (CT) scan was performed after three and six cycles (or at treatment discontinuation if earlier) and once every 3 months thereafter until disease progression. Response was assessed as best response according to response evaluation criteria in solid tumors (RECIST).

Sample analyses. For consenting patients, one blood sample and archival primary invasive carcinoma, lymph node and any recurrent tumor specimens or a research biopsy from a metastatic site were collected. There was no requirement for a recurrent specimen to be provided. DNA was extracted using standard methodology. Central review of ER, PgR and HER status was performed at King's College London (further details in Supplementary Note).

The Institute of Cancer Research is where germline *BRCA1* and *BRCA2* mutation analysis was conducted and where status for subgroup analysis was centrally determined. Genomic DNA from white blood cell preparations was analyzed for intragenic mutations in *BRCA1* and *BRCA2* and for exon deletions and duplications throughout the coding sequence, and intron–exon boundaries was completed in all cases. This was either performed using Sanger sequencing together with multiplex ligation-dependent probe amplification (MLPA) or via next-generation sequencing using the Illumina TruSight Cancer Panel version 1. All intragenic mutations were confirmed through separate bidirectional Sanger sequencing. All exon deletions or duplications were confirmed through MLPA. The mutation nomenclature was in accordance with clinical convention, with numbering starting at the first A of the ATG initiation site, using *BRCA1* LRG_292_t1 and *BRCA2* LRG_293_t1.

The DNA methylation status of the regulatory region of *BRCA1* was determined using bisulfite sequencing and *BRCA1* mRNA expression level from total RNA sequencing data from archival primary carcinoma (Supplementary Note, Supplementary Fig. 3 and Supplementary Table 5).

The Myriad HRD test includes three DNA-based measures of homologous recombination deficiency: whole-genome tumor loss of heterozygosity profiles

(LOH), telomeric allelic imbalance (TAI) and large-scale state transitions (LST)^{22–24}. All three scores are highly correlated with defects in *BRCA1/2* and predict response to platinum-containing neoadjuvant chemotherapy in subjects with TNBC in trials without standard of care control arms²⁶. The HRD score is calculated as the sum of the three individual scores, and a previously validated threshold of 42 was used in these analyses²⁶. As part of the HRD assay, the sequencing data were used to call *BRCA1/2* mutations, either germline or somatic, in the tumor. The Supplementary Note includes a description of HRD assay on TNT Trial samples.

Primary cancers were classified into basal-like subtypes using several classifiers, including an IHC panel⁷ and Prosigna⁴⁸ (further details in Supplementary Note). Integration of transcriptional and whole-genome chromosomal instability, rearrangement and mutational signatures that have been associated with *BRCA1* or *BRCA2* mutation and *BRCA1* methylation and may specifically interact with carboplatin response^{8,22–26,39,40} were prespecified in the protocols as a priori subgroups analyses, but these integrated analyses are incomplete and will be reported elsewhere.

Outcomes. The primary endpoint was ORR (complete or partial). The version of RECIST used for tumor assessment was documented and, where possible, cases assessed using RECIST version 1.0 were subsequently reassessed locally according to RECIST version 1.1. At study completion, an independent Response Evaluation Committee reviewed reported responses centrally (local assessment was used for primary analysis).

Secondary endpoints included PFS, OS, response to crossover treatment (as per primary endpoint), tolerability and safety.

Adverse events were assessed throughout treatment, graded according to National Cancer Institute Common Toxicity Criteria (version 3.0) and coded according to the Medical Dictionary for Regulatory Activities (MedDRA version 14.0) with central clinical review (by the Chief Investigator) at study completion.

Statistical analyses. Evidence to inform sample size calculations was scarce; however, the ECOG 2100 trial⁴⁹ suggested a 20–30% response rate for single-agent taxane. TNT was designed on the premise of demonstrating superiority of carboplatin, with a 15% improvement in response rates designated as clinically important. Assuming 90% power and type I error $\alpha = 0.05$ (two-sided), a sample size of at least 370 patients was required. The protocol recognized a priori that equivalence of response, accompanied by reduced toxicity with carboplatin, would also impact clinical practice.

Response rates were compared using two-sided Fisher's exact tests and logistic regression (see Supplementary Note for details regarding analysis of subgroups). Survival endpoints were displayed using Kaplan–Meier plots, and for survival analysis modeling, we utilized restricted mean survival methodology⁵⁰ considering that the proportional hazards assumption required for Cox survival analysis did not hold.

Principal efficacy endpoints were analyzed according to the ITT population, including all 376 subjects randomized, and according to pre-planned biomarker subgroups (Supplementary Table 1); additional analysis groups and associated analysis methods are detailed in the Supplementary Note. Analyses are based on a database snapshot taken on 7 March 2016 and performed using STATA 13.

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary.

Data availability. Gene expression profiling data for the 50 genes used for Prosigna algorithm are available at: <https://doi.org/10.5281/zenodo.1172633>.

Other dichotomized biological data used for subgroup analyses are available in Supplementary Dataset 1.

References

- Wallden, B. et al. Development and verification of the PAM50-based Prosigna breast cancer gene signature assay. *BMC Med. Genomics* **8**, 54 (2015).
- Miller, K. et al. Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. *N. Engl. J. Med.* **357**, 2666–2676 (2007).
- Royston, P. & Parmar, M. K. Restricted mean survival time: an alternative to the hazard ratio for the design and analysis of randomized trials with a time-to-event outcome. *BMC Med. Res. Methodol.* **13**, 152 (2013).

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. [For final submission](#): please carefully check your responses for accuracy; you will not be able to make changes later.

▶ Experimental design

1. Sample size

Describe how sample size was determined.

Response rate to docetaxel was expected to be 20-30%. The trial was powered to detect superiority of carboplatin over docetaxel with an improvement in response rates of 15%. With 90% power and 2-sided alpha of 0.05, at least 370 patients were required.

2. Data exclusions

Describe any data exclusions.

No patients were excluded from the analyses. For a priori specified biomarker subgroup analyses only those with known biomarker status were included.

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

No experimental replication was attempted - N/A in a phase III randomised controlled clinical trial

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

ICR-CTSU allocated patients to carboplatin or docetaxel (1:1 ratio) utilising a computerised minimisation algorithm with a random element. Balancing factors were centre, previous adjuvant taxane chemotherapy, presence of liver or lung metastasis, performance status (0/1 vs 2) and recurrent locally advanced vs metastatic carcinoma.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Treatment allocation was not blinded; this would have been impractical due to different side effect profiles and administration of the drugs. However those analysing samples to generate biomarker data for a priori planned biomarker subgroup analyses were blinded to treatment and response.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

- n/a Confirmed
- The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
 - A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
 - A statement indicating how many times each experiment was replicated
 - The statistical test(s) used and whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
 - A description of any assumptions or corrections, such as an adjustment for multiple comparisons
 - Test values indicating whether an effect is present
Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.
 - A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
 - Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on [statistics for biologists](#) for further resources and guidance.

► Software

Policy information about [availability of computer code](#)

7. Software

Describe the software used to analyze the data in this study.

Statistical analysis was carried out in Stata v13.
Other software used for biological analyses were:
CASAVA 1.8 <http://www.illumina.com/>
MapSplice v12_07 <http://www.netlab.uky.edu/p/bioinfo/MapSplice>
Picard Tools v1.64 - <http://picard.sourceforge.net/>
UBU v1.0 <https://github.com/mozack/ubu>
RSEM v1.1.13 - <https://deweylab.github.io/RSEM/>
TCGA GAF - <https://api.gdc.cancer.gov/v0/data/a0bb9765-3f03-485b-839d-7dce4a9bcfeb>

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* [guidance for providing algorithms and software for publication](#) provides further information on this topic.

► Materials and reagents

Policy information about [availability of materials](#)

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

The trial associated tissue biological resource is accessible through application to the TNT Trial Management Group Biological sub-committee where proposed research is covered by protocol and the TNT trial ethical permission and patient consent.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

ER: anti-Estrogen Receptor (ER) (SP1) Rabbit Monoclonal Ventana 790-4324/4325 antibody
PgR: anti-Progesterone Receptor (PR) (1E2) Rabbit Monoclonal Ventana 790-2223/4296 Ab
HER2: anti-HER-2/neu (4B5) Rabbit Monoclonal Ventana 790-2991 (05278368001) antibody
Ck5: Ck5 (XM26) Mouse monoclonal Novocastra NCL-CK5 antibody
EGFR: EGFR.25) Mouse monoclonal Novocastra NCL-L-EGFR-384 antibody

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used - NA

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used - NA

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used - NA

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by [ICLAC](#), provide a scientific rationale for their use.

No eukaryotic cell lines were used - NA

► Animals and human research participants

Policy information about [studies involving animals](#); when reporting animal research, follow the [ARRIVE guidelines](#)

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

No animals were used.

Policy information about [studies involving human research participants](#)

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

All patients were female, aged between 26 and 81. Patients had triple negative metastatic or recurrent locally advanced breast cancer or patients had a known germline BRCA1/2 mutation and had metastatic or recurrent locally advanced breast cancer.