A randomised phase III trial of carboplatin compared with docetaxel in *BRCA1/2* mutated and pre-specified triple negative breast cancer "BRCAness" subgroups: the TNT Trial

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Abstract

BRCA1/2 germline mutations predispose to breast cancer (gBRCA-BC) by impairing homologous recombination (HR) causing genomic instability. HR also repairs DNA lesions caused by platinums and PARP inhibitors. Triple Negative Breast Cancers (TNBC) harbour sub-populations with *BRCA1/2* mutations, hypothesised to be especially platinum sensitive. Putative "BRCAness" subgroups may also be especially platinum sensitive. We assessed carboplatin and mechanistically distinct docetaxel in a phase-III trial in unselected advanced TNBC. A pre-specified programme enabled biomarker-treatment interaction analyses in gBRCA-BC and "BRCAness" subgroups: tumour *BRCA1* methylation; *BRCA1* mRNA-low; HR deficiency mutational signatures and basal phenotypes. Primary endpoint was objective response rate (ORR) . In the unselected population (376 patients; 188 carboplatin, 188 docetaxel) carboplatin was not more active than docetaxel (ORR: 31.4v34.0; p=0.66). In contrast in patients with gBRCA-BC carboplatin had double the ORR compared to docetaxel (68%v33%), test for biomarker-treatment interaction (p=0.01). No treatment interaction was observed for *BRCA1* methylation, *BRCA1* mRNA-low status or a Myriad-HRD mutation signature assay. Significant treatment interaction with basal-like subtype was driven by high docetaxel response in the non-basal subgroup. Patients with advanced TNBC benefit from *BRCA1/2* mutation characterization, but not *BRCA1* methylation or Myriad-HRD analysis, informing platinum choices. Basal-like gene expression analysis may also influence treatment choices.

"Triple negative" breast cancer (TNBC) describes the 10-20% of tumours which are estrogen receptor (ER), progesterone receptor (PgR) and HER2 negative. A single TNBC entity is however a fallacy masking considerable histological and biological heterogeneity, understanding of which is needed to optimise therapy selection. Outcome for patients with recurrent/advanced TNBC is especially poor[1]. Chemotherapy is the only approved systemic therapy and, while considered biologically unselective, can have distinct mechanisms of action that target specific biological mechanisms aberrant in cancer. When accompanied by mechanism relevant biomarkers, use of a specific chemotherapeutic in defined populations might be considered a "targeted" therapy.

Whilst genomic classifiers suggest the majority of TNBCs are of basal intrinsic subtype[2,3], recent analyses suggest that TNBC can be sub-classified[4-6]. An immunohistochemical (IHC) approximation of the basal 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* or *BRCA2* mutation associated signatures[8]. Abnormalities also exist in *BRCA1* mRNA expression, largely driven through methylation of the *BRCA1* promoter [9,10] as observed in ovarian cancer[11,12]. This, and the overlap in mutational signatures[8], suggest functional deficiency of homologous recombination (HR) DNA repair genes as a shared characteristic between *BRCA1* familial breast cancers and a substantial, but incompletely defined, subgroup of TNBC. BRCA1 and BRCA2 proteins have important roles in DNA replication fork stabilisation and HR[13] and are components of the Fanconi anaemia protein network[14,15]. The hallmark of deficiency in this network is sensitivity to DNA crosslinks induced by platinums and mitomycin C[16,17]. Historically platinum chemotherapies have only shown modest activity in advanced breast cancer excepting those with chemotherapy naïve disease[18,19].

No trial had directly studied platinum therapy responses 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]. TNT was designed to compare the activity of the standard of care microtubule agent docetaxel with the DNA cross-linking agent carboplatin. We hypothesised greater activity for carboplatin in DNA damage response deficient subgroups. As strong mechanistic evidence existed for the efficacy of platinum DNA salts on cells with *BRCA1* or *BRCA2* 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) germline mutation carriers and putative "BRCAness"[21] TNBC subgroups with ii) *BRCA1* promoter DNA methylation and/or mRNA-low and basal forms of the TNBC defined by iii) gene or iv) protein expression.

Results

Between 25 April 2008 and 18 March 2014 376 patients (188 allocated to carboplatin and 188 to docetaxel) entered the trial, all patients were included in the analysis of the primary endpoint (Figure 1); the trial population largely comprised patients with TNBC and no known *BRCA1/2* mutation (338/376) and baseline characteristics typical of patients with first line relapse of TNBC (Table S2/S3). There were 43 patients with germline *BRCA1/2* mutation (31 *BRCA1* and 12 *BRCA2* Table S2). Of the 31 *BRCA1* mutation carriers 4 had ER+ve disease and of the 12 *BRCA2* mutation carriers 7 had ER+ve disease. Compliance with allocated treatment was good; disease progression and toxicity were the principal reasons for early discontinuation. Median relative dose intensity was 94·0% (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 carboplatin and docetaxel in objective response rate in the overall population (ORR: 59/188 (31·4%) vs. 64/188 (34·0%), absolute difference -2·6%, (95%CI: -12·1 to 6·9), p=0·66; Figure 2A). Following central review of locally classified responses, response rates were 48/188 (25·5%) carboplatin vs. 55/188 (29·3%) docetaxel, absolute difference (C-D) = -3·8 (95%CI: -12·8, 5·2); exact p=0·49, consistent with findings from the main analysis. Similarly, no evidence of a difference was observed for crossover treatments (Figure S1A) or when analysis was limited to those centrally confirmed as having triple negative tumours (see supplementary appendix).

372 (98·9%) patients have had PFS events reported. Median PFS in patients allocated carboplatin was 3·1 months (95%CI: 2·4, 4·2) and 4·4 months (95%CI: 4·1, 5·1) for those allocated docetaxel. No difference in restricted mean PFS was found (difference -0·30 months, p=0·40; Figure 3A).

347 patients are reported to have died. Median 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 and docetaxel respectively. Consistent with the PFS result,

no evidence of a difference was found between treatment groups (difference -0·03 months, p=0·96; Figure S2A).

BRCA subgroup analyses

Protocol pre-specified subgroup analyses by *BRCA1/2* mutation were conducted at the time of the main analysis. Patients with a deleterious *BRCA1/2* germline mutation had a significantly better response to carboplatin than docetaxel (ORR: 17/25 (68·0%) vs. 6/18 (33·3%), absolute difference 34·7%, p=0·03), with no evidence of differential treatment activity in patients with no germline mutation (ORR: 36/128 (28·1%) vs. 50/145 (34·5%), absolute difference -6·4%, p=0·30), resulting in a statistically significant interaction (p=0·01, Figure 2B). This result remained significant (p=0·01) after adjustment for known prognostic factors (see supplementary appendix for details). PFS also favoured carboplatin for patients with a *BRCA1/2* germline mutation (median PFS 6·8 months vs. 4·4 months, difference in restricted mean PFS 2·6 months, interaction p=0·002; Figure 3B) but no difference was found in overall survival (Figure S2B), with interpretation confounded by the pre-planned crossover at progression (Figure S1B). Given the small numbers of *BRCA2* versus *BRCA1* germline mutation carriers randomised, comparative analyses of treatment effect for each gene and in the very small number of ER +ve tumours compared to those that were TNBC were neither significant nor meaningful.

Patients with tumour available for sequencing and a *BRCA1/2* mutation detected in their tumour sample (see Table S4 for overlap of tumour detected mutation with germline *BRCA1/2* mutation status) appeared to have better response to carboplatin than docetaxel (ORR: 12/18 (66·7%) vs. 5/14 (35·7%), absolute difference 31·0%, p=0·15) whilst a treatment effect favouring docetaxel was suggested in patients with wildtype genotype in the tumour (ORR: 23/90 (25·6%) vs. 32/90 (35·6%), absolute difference -10·0%, p=0·20). Given very small patient numbers with tumour mutation data neither of these subgroup analyses attained statistical significance; however, given the effects were in opposite directions, the interaction was significant (p=0·03) (Figure 2C). This however did not hold for PFS or OS (p=0·12, p=0·70 respectively) (Figures 3C and S2C). Eight patients had a wildtype germline genotype but a *BRCA* mutation in their tumour which was therefore classed as a somatic mutation (Table S4); 2/4 had responses with carboplatin and 2/4 with docetaxel, but small numbers limit conclusive interpretation of these data.

Counter to our pre-specified hypothesis, patients with *BRCA1* methylation did not have better response to carboplatin than docetaxel (ORR: 3/14 (21·4%) vs. 8/19 (42·1%), absolute difference -20·7%, p=0·28) with no

evidence of an interaction observed (p=0·35, Figures 2D, 3D, S2D); with similar conclusions when germline *BRCA1/2* mutated patients were excluded.

Concordant with *BRCA1* methylation status, tumours we defined as *BRCA1* mRNA-low, with which methylation was partially associated (Supplementary Figure S3 and Table S5), did not have a better response to carboplatin than docetaxel (ORR: 4/14 (28·6%) vs. 11/17 (64·7%), absolute difference -36·1%, p=0·07) and evidence of an interaction was lacking (p=0·07, Figures 2E, 3E, S2E), again conclusions were not different when 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 mRNA1-low did not suggest any significant signal that supported our *a priori* hypotheses that they would be associated with greater response to carboplatin than 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 homologous recombination could provide 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[8,22-25]. Here we show the result using the combined Myriad HRD assay[26] performed on treatment naïve primary tissue. We find that the great majority of patients with either germline *BRCA1/2* mutation or *BRCA1* methylation have an high Dichotomized "HRD Score" (Figure S4A, S4B) but "HRD Score" high patients, unlike germline *BRCA1/2* mutation carriers, did not have better response to carboplatin than docetaxel (ORR: 13/34 (38.2%) vs. 19/47 (40.4%), absolute difference -2.2%, p=1.0) with no evidence of an interaction observed (p=0·75, Figure 4A). Similar results were found when "HR Deficient" patients, a definition that grouped all *BRCA1/2* mutated patients with those *BRCA1/2* wild-type patients with high HRD score, were examined (Figure 4B). In addition no evidence of treatment specific predictive effect for PFS was found using either HRD definition (Figure S5A,B). Patients with High HRD score had a numerically greater response to both chemotherapy agents than those with low scores but this does not appear statistically significant.

Basal subgroup analyses

Given 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 share a BRCA1 loss of function phenotype with those with mutation by analysing a platinum treatment interaction in this broader basal-like TNBC group. We found no evidence that Prosigna® – PAM50 basal tumours showed greater response to carboplatin compared with docetaxel (ORR: 27/83 (32·5%) vs. 27/87 (31·0%), absolute difference 1·5%, p=0·87). However, in patients with non-basal-like tumours response to docetaxel was significantly better than to carboplatin (ORR: 13/18 (72·2%) vs. 3/18 (16·7%), absolute difference -55·5%, p=0·002), leading to a significant interaction test (p=0·003, Figure 5A) and a similar trend in crossover treatment response (Figure S6). The interaction between treatment and PAM50 subgroups remained significant after adjusting for gBRCA status in the multivariable logistic regression model (p=0·002) (Table S6) and when other known prognostic factors were subsequently included in the model. The interaction was also significant for PFS (p=0·04) (Figure 6A) but not OS (p=0·17) (Figure S7A).

There was no evidence that "core basal" tumours defined by IHC had improved response to carboplatin compared with docetaxel (ORR: 23/67 (34·3%) vs. 19/65 (29·2%), absolute difference 5·1%, p=0·58). While there was a higher response rate to docetaxel compared with carboplatin in patients with non-basal 5 marker negative (5NP) tumours (ORR: 13/31 (41·9%) vs 5/26 (19·2%), absolute difference -22·7%, p=0·09), the difference did not reach statistical significance and the interaction test was non-significant p=0·06 (Figures 5B, 6B, S7B).

Safety

Both carboplatin and docetaxel demonstrated toxicity consistent with their known safety profiles and Grade 3 and 4 adverse events (AEs) were as anticipated for these well-known chemotherapy drugs (Tables S7 and S8). There were more grade 3/4 AEs with docetaxel than with carboplatin. 276 Serious Adverse Events (SAEs) were reported throughout the trial (102 carboplatin; 174 docetaxel). The spectrum of SAEs was as anticipated. Two SAEs were considered to be Suspected Unexpected Serious Adverse Reactions (1 carboplatin; 1 docetaxel). These were i) nausea, vomiting and headaches; ii) low magnesium. One death was considered possibly related to carboplatin treatment; this patient died from pulmonary embolism. As an haplo-insuffiency or dominant negative effect of heterozygous mutation might affect toxicity from HR targeting therapies such as platinum in mutation carriers we sought evidence of excess haematological toxicity as a signal but found none (Table S9).

Although there was a small numerical difference in non-haematological toxicity this was not significant and small numbers preclude firm conclusions from these analyses.

Discussion

This phase III trial utilised two mechanistically distinct single agent chemotherapeutics in unselected advanced TNBC and in *a priori* specified biomarker defined sub-populations thought likely to have targetable defects in HR DNA repair. In the unselected TNBC patients 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 delivered at the full licensed dose. This trial demonstrates significant activity for both agents and the level of response seen for docetaxel is consistent with that seen previously in breast cancer[27] and for carboplatin with that seen in uncontrolled trials of single agent platinums[28,29] or combinations of carboplatin with gemcitabine in unselected TNBC[30]. The only other randomised trial conducted synchronous with our trial and designed to specifically investigate platinum in comparison with a standard of care in advanced TNBC included the substitution of cisplatin for paclitaxel given in a doublet with gemcitabine. In this study treatment was continued until disease progression, as is common practice with paclitaxel, and showed modestly greater activity for cisplatin[31]. A criticism of our study could be that patients did not receive treatment to progression but for 6 cycles (and at investigator discretion maximum of 8 cycles), as was consistent with UK practice with docetaxel at the full licensed 100mg/m^2 dose, as this is rarely tolerated for more than 6-8 cycles. This may explain shorter PFS compared to the study of Hu et al despite similar overall survival[31], and may have underestimated 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 pre-specified 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 cancer[32] and sporadic basal-like breast cancer subtypes show high degrees of chromosomal genomic instability[3]. We hypothesised 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 docetaxel with the drugs showing similar activity. A significant treatment interaction was detected with the Prosigna PAM50 identified subtypes; driven by significantly increased response to docetaxel relative to poor platinum response in

non-basal forms of TNBC. This suggests absence of targetable BRCAness in non-basal TNBC and no evidence to change the standard of care from taxane to a platinum, which our data suggests is inferior in these subtypes. In contrast platinum is a reasonable option in those with basal TNBC particularly in those who fail to tolerate or have previously received a taxane. As the response rate is much less than that of *BRCA1/2* mutation associated breast cancer, 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 yet to be identified subpopulation of this subtype.

BRCA1/2 mutation testing is a clinically validated and widely available biomarker that predicted both greater response and PFS in favour of carboplatin over docetaxel demonstrating clinical utility for treatment selection in this setting. There was no evidence that mutation was associated with reduced activity of docetaxel compared to wildtype; docetaxel remains a valid and active, but inferior, treatment option in this setting. We did not find evidence of an overall survival advantage for carboplatin in *BRCA1/2* mutation carriers, but interpretation is confounded by the crossover design as 56% 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 trial[33], supporting the notion that carboplatin monotherapy is highly active in this patient group. We found approximately one third of *BRCA1/2* carriers did not respond to platinum. Potential resistance mechanisms will be further explored in integrated whole genome and whole transcriptome sequencing analyses in primary tumour material but lack of extensive metastatic tumour from patients immediately prior to platinum treatment will limit sensitivity and ability to draw firm conclusions.

In parallel we tested the hypothesis that epigenetic silencing of *BRCA1* by DNA methylation would show a similar treatment interaction. Despite similar numbers in genetic and epigenetic BRCAness subgroups, patients with *BRCA1* methylation or mRNA low had a higher response to docetaxel than carboplatin. Exploratory analyses seeking optimisation of cut-points and analysis of these epigenetic biomarkers as continuous variables failed to find any signal. In stark contrast to the interaction between *BRCA1/2* mutation and carboplatin treatment effect we find no evidence to support a similar impact of epigenetic BRCAness with no interaction found between either *BRCA1* methylation or *BRCA1* mRNA low status and carboplatin treatment effect. This suggests important differences in the effects of genetic and epigenetic changes at the *BRCA1* locus, at least in

predicting therapy response in metastatic breast cancer exposed to prior adjuvant chemotherapy. These results are consistent with previous results from the non-randomised TBCRC 009 trial in metastatic TNBC[28] where the few tumours with *BRCA1* methylation showed no response to platinum despite evidence of chromosomal instability signatures. The majority of our patients had received adjuvant chemotherapies that cause DNA lesions that engage HR for repair. We measured *BRCA1* methylation and mRNA in archived primary tumour specimens, whereas treatment effect was assessed in metastases. We speculate that in mutation carriers, a higher proportion retain an HR defect in metastatic disease than those with *BRCA1* methylated tumours (Supplementary Figure S9). We suggest mutation creates a more resilient "hard" BRCAness whereas *BRCA1* methylation associated epigenetic BRCAness is more "soft" and plastic[20]. 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 and not selectively platinum sensitive metastases. Our hypothesis is supported by data from both pre-clinical patient derived xenografts and primary breast tumours exposed to neo-adjuvant chemotherapy[34]. In ovarian cancers *BRCA1* mutation but not methylation is associated with improved prognosis after platinum[35,36] and examination of pre- and post-platinum treatment biopsy pairs shows reversion of *BRCA1* methylation in 31% with continued presence of methylation being associated with PARP inhibitor response[37]. While defects in HR are known to be revertable mutational signatures would not be expected to disappear, as they are a permanent "scar" of prior, even if no longer active, HR defects. While our finding that the Myriad HRD assay did not have specific platinum response predictive performance in the advanced TNBC disease setting contrasts to reported association with platinum response in the neoadjuvant setting in TNBC[26] these neoadjuvant studies do not have a comparator arm to allow a test of interaction between biomarker status and any specific treatment effect of platinum chemo as opposed to association with a relatively greater general chemotherapy responsiveness than HRD low status . Where this was examined in the randomised neoadjuvant context the Myriad HRD assay did not show specific predictive performance for platinum response in unplanned retrospective analyses with limited power[38]. Metastatic disease , exposed to prior adjuvant therapy is also a very different biological context. We hypothesise that adjuvant therapy drives reversal of the *BRCA1* methylation "soft' BRCAness[34] HR defect, that we show like *BRCA1* mutation leaves a high HRD score in the primary tumour (Figure S4), erodes the positive predictive value of the HRD score for therapy response in metastasis while a low HRD Score will likely retain negative predictive value by excluding many tumours that have never had an HR defect whether "soft" or "hard". Since our analysis, a novel HR deficiency mutational signature

whole genome sequence analysis methodology called "HRDetect" has been described with preliminary evidence of potential application to FFPE clinical materials[8]. 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 require integration with additional biomarkers of a tumour's current HR status. Analyses of 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 randomised studies have not examined treatment effect in *a priori* defined subpopulations within advanced TNBC[31]. TNT highlights the heterogeneity in TNBC and 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, finding evidence of increased pathological tumour 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 arm backbone[41]. A non-significant trend was noted when a standard cyclophosphamide "backbone" control was used in the CALGB 40603 study[42]. The dose intense carboplatin regimen used in GeparSixto was recently compared with a sequential anthracycline and taxane and high dose cyclophosphamide-containing regimen with no differences found in the primary pathological response measures[44]. It would seem that the use of alkylating agents in early TNBC is important, especially for those that have higher stage disease with associated risk of recurrence requiring 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 more study before platinums are 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 early breast cancer setting.

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 trial [45]

recently reported comparison between the potent PARP inhibitor olaparib, known to trap PARP1 on DNA, in comparison to physicians choice of non-platinum standard of care chemotherapies in anthracycline and taxane exposed advanced gBRCA-BC. Other trials of potent PARP inhibitors are ongoing[46]. 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 platinums in this setting but the TNT trial provides evidence that a widely available affordable off-patent biomarker has utility to select a population, enriched in the TNBCs prevalent in many developing countries[47], who could benefit during this period from the biologically targeted use of highly active and inexpensive platinum chemotherapy agent rather than the current licensed breast cancer standard of care chemotherapies.

Methods

Study design

Conducted in 74 hospitals throughout the UK TNT was a phase III, parallel group, open label randomised controlled trial with pre-planned biomarker subgroup analyses. Trial sponsorship, governance, randomisation procedures and balancing factors are described in the supplementary appendix.

Patients

Eligible patients had to be considered fit to receive either study drug and have measurable, confirmed advanced breast cancer unsuitable for local therapy with histologically confirmed ER, PgR, and HER2 negative primary invasive breast cancer with Allred/quick score <3 or H score <10 or locally determined ER and PgR negative, if other cut-offs used (e.g., 1%, 5% or 10%). HER2 negative was defined as immunohistochemistry scoring 0 or 1+ for HER2, or 2+ and non-amplified for HER2 gene by FISH or CISH. Patients could be ER and HER2 negative and, PgR negative/unknown, or any ER, PgR and HER2 status if known to have *BRCA1* or *BRCA2* germline mutation and otherwise eligible (full eligibility criteria in supplementary appendix). Although patients with TNBC hypothesised to have BRCAness phenotypes were the primary interest, patients with unselected TNBC as well as those with *BRCA1* or *BRCA2* germline mutations were recruited to allow interaction testing of biomarker positive and negative populations in relation to response to each of these mechanistically distinct agents. Patients provided written informed consent.

Procedures

Patients were allocated (1:1 ratio) between six cycles of carboplatin (AUC 6), day 1 3-weekly, and six cycles of docetaxel (100mg/m^2), day 1 3-weekly (see supplementary appendix section 3.1 for details of allocation procedures including minimisation balancing factors used). For patients responding to and tolerating treatment well, a further two cycles could be given subject to local policy. Further details of chemotherapy and supportive medicines are described in the supplementary appendix. Patients were offered six cycles of the alternative ("crossover") treatment upon progression or where allocated treatment was discontinued due to toxicity ("pre-progression crossover"). Subsequent management was at clinician discretion.

Tumour assessment by CT scan was performed after three and six cycles (or at treatment discontinuation if earlier) and three-monthly thereafter until disease progression. Response was assessed as best response by RECIST.

Sample analyses

For consenting patients, one blood sample and archival primary invasive carcinoma, lymph nodes and any recurrent tumour 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 KCL (further details in supplementary appendix).

Germline *BRCA1* and *BRCA2* mutation analysis was conducted and status for subgroup analysis was centrally determined at The Institute of Cancer Research. Genomic DNA from blood white cell preparations was analysed for *BRCA1* and *BRCA2* for intragenic mutations and exon deletions and duplications throughout the coding sequence, and intron-exon boundaries was completed in all cases. This was either performed by Sanger sequencing together with multiplex ligation-dependent probe amplification (MLPA) or by next-generation sequencing using the Illumina TruSight Cancer Panel v1. All intragenic mutations were confirmed by separate bi-directional Sanger sequencing. All exon deletions or duplications were confirmed by 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 from archival primary carcinoma (see supplementary appendix Figure S3 and Supplementary Table S5).

The Myriad HRD test includes three DNA-based measures of homologous recombination deficiency including: whole genome tumour 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 patients with TNBC trials without standard of care control arms[26]. The HRD score is calculated as the sum of the three individual scores, and a previously validated threshold of 42 was utilized in these analyses [26]. As part of the HRD assay, the sequencing data are used to call BRCA1/2 mutations in the tumour, either germline or somatic. The supplementary appendix includes description of HRD assay on *TNT* trial samples.

Primary cancers were classified into basal-like subtypes by several classifiers including an IHC panel[7], and Prosigna[48](further details in supplementary appendix). 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 protocol pre-specified as *a priori* sub-groups analyses are incomplete and will be reported elsewhere.

Outcomes

The primary endpoint was objective tumour response rate (complete or partial). The version of RECIST reporting criteria used for tumour assessment was documented and, where possible, cases assessed using RECIST version 1.0 were subsequently reassessed locally according to RECIST version 1.1. An independent Response Evaluation Committee at study completion reviewed reported responses centrally (local assessment was used for primary analysis).

Secondary endpoints included progression free survival (PFS), overall survival (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 ECOG 2100[49] 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 α=0·05 (two-sided), a sample size of at least 370 patients was required. The protocol recognised *a priori* that equivalence of response, accompanied by reduced toxicity with carboplatin, would also impact clinical practice.

Response rates were compared using 2-sided Fisher's exact tests and logistic regression (see supplementary appendix section 4.10 for further details regarding analysis of subgroups). Survival endpoints were displayed using Kaplan Meier plots and survival analysis modelling utilised restricted mean survival methodology[50] given that the proportionality of hazards assumption required for Cox survival analysis did not hold.

Principal efficacy endpoints were analysed according to intention to treat (ITT) including all 376 patients randomised and according to pre-planned biomarker subgroups (Table S1); additional analysis groups and associated analysis methods are detailed in the supplementary appendix. Analyses are based on a database snapshot taken on 7 March 2016 and performed using STATA 13.

Life Sciences Reporting Summary

Further information on experimental design is available in the Life Sciences Reporting Summary.

Data availability

Gene expression profiling data of the 50 genes used for Prosigna algorithm is available at:

https://doi.org/10.5281/zenodo.1172633.

Other dichotomised biological data used for subgroup analyses is available in supplementary dataset 1.

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Competing Financial Interests

AT, HT, MC, SK, LK, PG, JO, RB, MD, LF, AG, PP, VS, CG, NR, SEP and JMB report grants to their institutional departments from Breast Cancer Now and/or Cancer Research UK, and other research support for costs or consumables in the study from Myriad Genetics, Inc. and NanoString Technologies, Inc. during the conduct of the study. In addition, AT has a patent PCT/EP2015/078987 pending on behalf of King's College London.

MC has a patent "Gene expression profiles to predict relapse of breast cancer" filed in USA and elsewhere with royalties paid.

MD reports personal fees from Myriad outside the submitted work.

AGu reports salary compensation, and stock/options from Myriad Genetics Inc. during conduct of the study, and patent rights assigned to Myriad Genetics.

CMP reports personal fees from Bioclassifier LLC, other from Nanostring Technologies outside the submitted work. In addition, CMP has a patent U.S. Patent No. 9,631,239 with royalties paid.

KMT reports 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, KT 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.

JSL reports salary compensation, and stock/options from Myriad Genetics Inc. during conduct of the study.

The other authors declare no competing interests.

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Figure 1. Consort diagram

Flow of participants in the trial.

Figure 2. Response rates (overall and BRCA subgroups)

Absolute differences between treatment groups within biomarker subgroups are presented; p-values for the differences are calculated using a 2-sided Fisher's exact test. P-values for interactions are based on a logistic regression model of response with terms for biomarker status, treatment group and interaction.

Figure 3. Progression-free survival (overall and BRCA subgroups)

Data presented is the difference in PFS restricted mean (95% CI). A negative value indicates a better response to docetaxel, positive values indicate better response to carboplatin. P-values are calculated using a 2-sided t-test comparing the mean survival between treatments (within biomarker groups as appropriate). C=Carboplatin; D=Docetaxel.

Figure 4. Response rates (HRD subgroups)

Absolute differences between treatment groups within HRD subgroups are presented; p-values for the differences are calculated using a 2-sided Fisher's exact test. P-values for interactions are based on a logistic regression model of response with terms for biomarker status, treatment group and interaction.

Figure 5. Response rates (basal-like subgroups)

Absolute differences between treatment groups within basal subgroups are presented; p-values for the differences are calculated using a 2-sided Fisher's exact test. P-values for interactions are based on a logistic regression model of response with terms for biomarker status, treatment group and interaction.

Figure 6. PFS (basal-like subgroups)

Data presented is the difference in PFS restricted mean within subgroups (95% CI). A negative value indicates a better response to docetaxel, positive values indicate better response to carboplatin. P-values are calculated using a 2-sided t-test comparing the mean survival between treatments within biomarker groups. C=Carboplatin; D=Docetaxel.