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1 **Carboplatin in *BRCA1/2*-Mutated and Triple Negative Breast Cancer BRCAness subgroups:**

2 **The TNT Trial**

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56 **Abstract**

57 *BRCA1/2* germline mutations predispose to breast cancer (gBRCA-BC) by impairing homologous
58 recombination (HR) causing genomic instability. HR also repairs DNA lesions caused by platinum and PARP
59 inhibitors. Triple Negative Breast Cancers (TNBC) harbour sub-populations with *BRCA1/2* mutations,
60 hypothesised to be especially platinum sensitive. Putative “BRCAness” subgroups may also be especially
61 platinum sensitive. We assessed carboplatin and mechanistically distinct docetaxel in a phase-III trial in
62 unselected advanced TNBC. A pre-specified programme enabled biomarker-treatment interaction analyses in
63 gBRCA-BC and “BRCAness” subgroups: tumour *BRCA1* methylation; *BRCA1* mRNA-low; HR deficiency
64 mutational signatures and basal phenotypes. Primary endpoint was objective response rate (ORR) . In the
65 unselected population (376 patients; 188 carboplatin, 188 docetaxel) carboplatin was not more active than
66 docetaxel (ORR: 31.4% v 34.0%; p=0.66). In contrast in patients with gBRCA-BC carboplatin had double the ORR
67 compared to docetaxel (68% v 33%), test for biomarker-treatment interaction (p=0.01). No treatment interaction
68 was observed for *BRCA1* methylation, *BRCA1* mRNA-low status or a Myriad-HRD mutation signature assay.
69 Significant treatment interaction with basal-like subtype was driven by high docetaxel response in the non-basal
70 subgroup. Patients with advanced TNBC benefit from *BRCA1/2* mutation characterization, but not *BRCA1*
71 methylation or Myriad-HRD analysis, informing platinum choices. Basal-like gene expression analysis may also
72 influence treatment choices.

73 **Introduction**

74 “Triple negative” breast cancer (TNBC) describes the 10-20% of tumours which are estrogen receptor (ER),
75 progesterone receptor (PgR) and HER2 negative. A single TNBC entity is however a fallacy masking
76 considerable histological and biological heterogeneity, understanding of which is needed to optimise therapy
77 selection. Outcome for patients with recurrent/advanced TNBC is especially poor¹. Chemotherapy is the only
78 approved systemic therapy and, while considered biologically unselective, can have distinct mechanisms of
79 action that target specific biological mechanisms aberrant in cancer. When accompanied by mechanism relevant
80 biomarkers, use of a specific chemotherapeutic in defined populations might be considered a “targeted” therapy.

81

82 Whilst genomic classifiers suggest the majority of TNBCs are of basal intrinsic subtype^{2,3}, recent analyses
83 suggest that TNBC can be sub-classified⁴⁻⁶. An immunohistochemical (IHC) approximation of the basal intrinsic
84 subtype has been termed “core basal”⁷. A common feature of sporadic basal TNBC is genomic instability with
85 mutational and rearrangement signatures indicative of abnormalities in DNA repair and replication stress that
86 overlap *BRCA1* or *BRCA2* mutation associated signatures⁸. Abnormalities also exist in *BRCA1* mRNA
87 expression, largely driven through methylation of the *BRCA1* promoter^{9,10} as observed in ovarian cancer^{11,12}.
88 This, and the overlap in mutational signatures⁸, suggest functional deficiency of homologous recombination
89 (HR) DNA repair genes as a shared characteristic between *BRCA1* familial breast cancers and a substantial, but
90 incompletely defined, subgroup of TNBC. BRCA1 and BRCA2 proteins have important roles in DNA
91 replication fork stabilisation and HR¹³ and are components of the Fanconi anaemia protein network^{14,15}. The
92 hallmark of deficiency in this network is sensitivity to DNA crosslinks induced by platinum and mitomycin
93 C^{16,17}. Historically platinum chemotherapies have only shown modest activity in advanced breast cancer
94 excepting those with chemotherapy naïve disease^{18,19}.

95

96 No trial had directly studied platinum therapy responses in comparison to standard of care in advanced
97 unselected TNBC, its majority basal subtype or subgroups of TNBC with features of aberrant BRCA1/2
98 associated function or “BRCAness”²⁰. TNT was designed to compare the activity of the standard of care
99 microtubule agent docetaxel with the DNA cross-linking agent carboplatin. We hypothesised greater activity for
100 carboplatin in DNA damage response deficient subgroups. As strong mechanistic evidence existed for the
101 efficacy of platinum DNA salts on cells with *BRCA1* or *BRCA2* mutations, accrual of patients known to have
102 these germline mutations was allowed, irrespective of ER, PgR and HER2 status. We pre-specified analyses of

103 i) germline mutation carriers and putative “BRCAness”²¹ TNBC subgroups with ii) *BRCA1* promoter DNA
104 methylation and/or mRNA-low and basal forms of the TNBC defined by iii) gene or iv) protein expression.

105

106

107 **Results**

108 Between 25 April 2008 and 18 March 2014 376 patients (188 allocated to carboplatin and 188 to docetaxel)
109 entered the trial, all patients were included in the analysis of the primary endpoint (Figure 1); the trial population
110 largely comprised patients with TNBC and no known *BRCA1/2* mutation (338/376) and baseline characteristics
111 typical of patients with first line relapse of TNBC (Table S2/S3). There were 43 patients with germline
112 *BRCA1/2* mutation (31 *BRCA1* and 12 *BRCA2* Table S2). Of the 31 *BRCA1* mutation carriers 4 had ER+ve
113 disease and of the 12 *BRCA2* mutation carriers 7 had ER+ve disease. Compliance with allocated treatment was
114 good; disease progression and toxicity were the principal reasons for early discontinuation. Median relative dose
115 intensity was 94.0% (IQR 84.2, 99.8) for carboplatin and 94.8% (IQR: 84.8, 100.0) for docetaxel.

116

117 **Overall results**

118 There was no evidence of a difference between carboplatin and docetaxel in objective response rate in the
119 overall population (ORR: 59/188 (31.4%) vs. 64/188 (34.0%), absolute difference -2.6%, (95%CI: -12.1 to
120 6.9), $p=0.66$; Figure 2A). Following central review of locally classified responses, response rates were 48/188
121 (25.5%) carboplatin vs. 55/188 (29.3%) docetaxel, absolute difference (C-D) = -3.8 (95%CI: -12.8, 5.2); exact
122 $p=0.49$, consistent with findings from the main analysis. Similarly, no evidence of a difference was observed for
123 crossover treatments (Figure S1A) or when analysis was limited to those centrally confirmed as having triple
124 negative tumours (see supplementary appendix).

125

126 372 (98.9%) patients have had PFS events reported. Median PFS in patients allocated carboplatin was 3.1
127 months (95%CI: 2.4, 4.2) and 4.4 months (95%CI: 4.1, 5.1) for those allocated docetaxel. No difference in
128 restricted mean PFS was found (difference -0.30 months, $p=0.40$; Figure 3A).

129

130 347 patients are reported to have died. Median OS was 12.8 months (95%CI: 10.6, 15.3) and 12.0 months
131 (95%CI: 10.2, 13.0) for those allocated carboplatin and docetaxel respectively. Consistent with the PFS result,

132 no evidence of a difference was found between treatment groups (difference -0.03 months, $p=0.96$; Figure
133 S2A).

134

135 ***BRCA subgroup analyses***

136 Protocol pre-specified subgroup analyses by *BRCA1/2* mutation were conducted at the time of the main analysis.

137 Patients with a deleterious *BRCA1/2* germline mutation had a significantly better response to carboplatin than

138 docetaxel (ORR: 17/25 (68.0%) vs. 6/18 (33.3%), absolute difference 34.7%, $p=0.03$), with no evidence of

139 differential treatment activity in patients with no germline mutation (ORR: 36/128 (28.1%) vs. 50/145 (34.5%),

140 absolute difference -6.4%, $p=0.30$), resulting in a statistically significant interaction ($p=0.01$, Figure 2B). This

141 result remained significant ($p=0.01$) after adjustment for known prognostic factors (see supplementary appendix

142 for details). PFS also favoured carboplatin for patients with a *BRCA1/2* germline mutation (median PFS 6.8

143 months vs. 4.4 months, difference in restricted mean PFS 2.6 months, interaction $p=0.002$; Figure 3B) but no

144 difference was found in overall survival (Figure S2B), with interpretation confounded by the pre-planned

145 crossover at progression (Figure S1B). Given the small numbers of *BRCA2* versus *BRCA1* germline mutation

146 carriers randomised, comparative analyses of treatment effect for each gene and in the very small number of ER

147 +ve tumours compared to those that were TNBC were neither significant nor meaningful.

148 Patients with tumour available for sequencing and a *BRCA1/2* mutation detected in their tumour sample (see

149 Table S4 for overlap of tumour detected mutation with germline *BRCA1/2* mutation status) appeared to have

150 better response to carboplatin than docetaxel (ORR: 12/18 (66.7%) vs. 5/14 (35.7%), absolute difference

151 31.0%, $p=0.15$) whilst a treatment effect favouring docetaxel was suggested in patients with wildtype genotype

152 in the tumour (ORR: 23/90 (25.6%) vs. 32/90 (35.6%), absolute difference -10.0%, $p=0.20$). Given very small

153 patient numbers with tumour mutation data neither of these subgroup analyses attained statistical significance;

154 however, given the effects were in opposite directions, the interaction was significant ($p=0.03$) (Figure 2C).

155 This however did not hold for PFS or OS ($p=0.12$, $p=0.70$ respectively) (Figures 3C and S2C). Eight patients

156 had a wildtype germline genotype but a *BRCA* mutation in their tumour which was therefore classed as a

157 somatic mutation (Table S4); 2/4 had responses with carboplatin and 2/4 with docetaxel, but small numbers

158 limit conclusive interpretation of these data.

159

160 Counter to our pre-specified hypothesis, patients with *BRCA1* methylation did not have better response to

161 carboplatin than docetaxel (ORR: 3/14 (21.4%) vs. 8/19 (42.1%), absolute difference -20.7%, $p=0.28$) with no

162 evidence of an interaction observed ($p=0.35$, Figures 2D, 3D, S2D); with similar conclusions when germline
163 *BRCA1/2* mutated patients were excluded.

164

165 Concordant with *BRCA1* methylation status, tumours we defined as *BRCA1* mRNA-low, with which
166 methylation was partially associated (Supplementary Figure S3 and Table S5), did not have a better response to
167 carboplatin than docetaxel (ORR: 4/14 (28.6%) vs. 11/17 (64.7%), absolute difference -36.1%, $p=0.07$) and
168 evidence of an interaction was lacking ($p=0.07$, Figures 2E, 3E, S2E), again conclusions were not different
169 when germline BRCA mutations were excluded. Furthermore, exploratory analyses examining any relationship
170 between high response to carboplatin and the cut-point for *BRCA1* methylation or BRCA1 mRNA1-low did not
171 suggest any significant signal that supported our *a priori* hypotheses that they would be associated with greater
172 response to carboplatin than a taxane (data not presented).

173

174 ***Homologous Recombination Deficiency subgroup analyses***

175 In the initial trial design and first protocol we hypothesized that changes in the genome landscape which may
176 arise as a consequence of defects in homologous recombination could provide an indicator of platinum salt
177 sensitivity and should be examined for interaction with treatment effect in both treatment arms. A number of
178 these assays have been reported^{8,22-25}. Here we show the result using the combined Myriad HRD assay²⁶
179 performed on treatment naïve primary tissue. We find that the great majority of patients with either germline
180 *BRCA1/2* mutation or *BRCA1* methylation have an high Dichotomized “HRD Score” (Figure S4A, S4B) but
181 “HRD Score” high patients, unlike germline *BRCA1/2* mutation carriers, did not have better response to
182 carboplatin than docetaxel (ORR: 13/34 (38.2%) vs. 19/47 (40.4%), absolute difference -2.2%, $p=1.0$) with no
183 evidence of an interaction observed ($p=0.75$, Figure 4A). Similar results were found when “HR Deficient”
184 patients, a definition that grouped all *BRCA1/2* mutated patients with those *BRCA1/2* wild-type patients with
185 high HRD score, were examined (Figure 4B). In addition no evidence of treatment specific predictive effect for
186 PFS was found using either HRD definition (Figure S5A,B). Patients with High HRD score had a numerically
187 greater response to both chemotherapy agents than those with low scores but this does not appear statistically
188 significant.

189

190 ***Basal subgroup analyses***

191 Given association between germline *BRCA1* mutation and the development of basal-like breast cancers we
192 sought to formally test the premise that all basal-like cancers share a BRCA1 loss of function phenotype with
193 those with mutation by analysing a platinum treatment interaction in this broader basal-like TNBC group. We
194 found no evidence that Prosigna® – PAM50 basal tumours showed greater response to carboplatin compared
195 with docetaxel (ORR: 27/83 (32.5%) vs. 27/87 (31.0%), absolute difference 1.5%, $p=0.87$). However, in
196 patients with non-basal-like tumours response to docetaxel was significantly better than to carboplatin (ORR:
197 13/18 (72.2%) vs. 3/18 (16.7%), absolute difference -55.5%, $p=0.002$), leading to a significant interaction test
198 ($p=0.003$, Figure 5A) and a similar trend in crossover treatment response (Figure S6). The interaction between
199 treatment and PAM50 subgroups remained significant after adjusting for gBRCA status in the multivariable
200 logistic regression model ($p=0.002$) (Table S6) and when other known prognostic factors were subsequently
201 included in the model. The interaction was also significant for PFS ($p=0.04$) (Figure 6A) but not OS ($p=0.17$)
202 (Figure S7A).

203

204 There was no evidence that “core basal” tumours defined by IHC had improved response to carboplatin
205 compared with docetaxel (ORR: 23/67 (34.3%) vs. 19/65 (29.2%), absolute difference 5.1%, $p=0.58$). While
206 there was a higher response rate to docetaxel compared with carboplatin in patients with non-basal 5 marker
207 negative (5NP) tumours (ORR: 13/31 (41.9%) vs 5/26 (19.2%), absolute difference -22.7%, $p=0.09$), the
208 difference did not reach statistical significance and the interaction test was non-significant $p=0.06$ (Figures 5B,
209 6B, S7B).

210

211 *Safety*

212 Both carboplatin and docetaxel demonstrated toxicity consistent with their known safety profiles and Grade 3
213 and 4 adverse events (AEs) were as anticipated for these well-known chemotherapy drugs (Tables S7 and S8).

214 There were more grade 3/4 AEs with docetaxel than with carboplatin. 276 Serious Adverse Events (SAEs) were
215 reported throughout the trial (102 carboplatin; 174 docetaxel). The spectrum of SAEs was as anticipated. Two
216 SAEs were considered to be Suspected Unexpected Serious Adverse Reactions (1 carboplatin; 1 docetaxel).

217 These were i) nausea, vomiting and headaches; ii) low magnesium. One death was considered possibly related
218 to carboplatin treatment; this patient died from pulmonary embolism. As an haplo-insufficiency or dominant
219 negative effect of heterozygous mutation might affect toxicity from HR targeting therapies such as platinum in
220 mutation carriers we sought evidence of excess haematological toxicity as a signal but found none (Table S9).

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

223

224 **Discussion**

225 This phase III trial utilised two mechanistically distinct single agent chemotherapeutics in unselected advanced
226 TNBC and in *a priori* specified biomarker defined sub-populations thought likely to have targetable defects in
227 HR DNA repair. In the unselected TNBC patients no evidence of a superior response to carboplatin was
228 observed when compared with a standard of care taxane, docetaxel. Carboplatin was better tolerated than
229 docetaxel delivered at the full licensed dose. This trial demonstrates significant activity for both agents and the
230 level of response seen for docetaxel is consistent with that seen previously in breast cancer²⁷ and for carboplatin
231 with that seen in uncontrolled trials of single agent platinum^{28,29} or combinations of carboplatin with
232 gemcitabine in unselected TNBC³⁰. The only other randomised trial conducted synchronous with our trial and
233 designed to specifically investigate platinum in comparison with a standard of care in advanced TNBC included
234 the substitution of cisplatin for paclitaxel given in a doublet with gemcitabine. In this study treatment was
235 continued until disease progression, as is common practice with paclitaxel, and showed modestly greater activity
236 for cisplatin³¹. A criticism of our study could be that patients did not receive treatment to progression but for 6
237 cycles (and at investigator discretion maximum of 8 cycles), as was consistent with UK practice with docetaxel
238 at the full licensed 100mg/m² dose, as this is rarely tolerated for more than 6-8 cycles. This may explain shorter
239 PFS compared to the study of Hu et al despite similar overall survival³¹, and may have underestimated the effect
240 of carboplatin in those without a progression event during treatment and who might have continued event free
241 for longer had treatment continued.

242

243 In contrast to the unselected population, the pre-specified analyses of treatment effect in subgroups found
244 evidence of clinically and statistically significant biomarker-treatment interactions. There is a strong association
245 between *BRCA1* mutation and basal-like cancer³² and sporadic basal-like breast cancer subtypes show high
246 degrees of chromosomal genomic instability³. We hypothesised that if, as has been widely speculated, there was
247 a shared profound BRCAness phenotype sporadic basal-like cancers might have very high platinum sensitivity.
248 We found no evidence that basal-like biomarkers predicted higher response to platinum than docetaxel with the
249 drugs showing similar activity. A significant treatment interaction was detected with the Prosigna PAM50
250 identified subtypes; driven by significantly increased response to docetaxel relative to poor platinum response in

251 non-basal forms of TNBC. This suggests absence of targetable BRCAness in non-basal TNBC and no evidence
252 to change the standard of care from taxane to a platinum, which our data suggests is inferior in these subtypes.
253 In contrast platinum is a reasonable option in those with basal TNBC particularly in those who fail to tolerate or
254 have previously received a taxane. As the response rate is much less than that of *BRCA1/2* mutation associated
255 breast cancer, if there is a profound BRCAness phenotype that remains prevalent in metastatic basal-like breast
256 cancer, beyond the context of *BRCA1* or *BRCA2* mutation, it appears to lie within a yet to be identified
257 subpopulation of this subtype.

258

259 *BRCA1/2* mutation testing is a clinically validated and widely available biomarker that predicted both greater
260 response and PFS in favour of carboplatin over docetaxel demonstrating clinical utility for treatment selection in
261 this setting. There was no evidence that mutation was associated with reduced activity of docetaxel compared to
262 wildtype; docetaxel remains a valid and active, but inferior, treatment option in this setting. We did not find
263 evidence of an overall survival advantage for carboplatin in *BRCA1/2* mutation carriers, but interpretation is
264 confounded by the crossover design as 56% received carboplatin at progression. The high levels of response
265 seen for carboplatin were similar to those reported for the combination of carboplatin and paclitaxel in an
266 essentially similar population in the reference comparator arm in the phase II BROCADE trial³³, supporting the
267 notion that carboplatin monotherapy is highly active in this patient group. We found approximately one third of
268 *BRCA1/2* carriers did not respond to platinum. Potential resistance mechanisms will be further explored in
269 integrated whole genome and whole transcriptome sequencing analyses in primary tumour material but lack of
270 extensive metastatic tumour from patients immediately prior to platinum treatment will limit sensitivity and
271 ability to draw firm conclusions.

272

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

281 predicting therapy response in metastatic breast cancer exposed to prior adjuvant chemotherapy. These results
282 are consistent with previous results from the non-randomised TBCRC 009 trial in metastatic TNBC²⁸ where the
283 few tumours with *BRCA1* methylation showed no response to platinum despite evidence of chromosomal
284 instability signatures. The majority of our patients had received adjuvant chemotherapies that cause DNA
285 lesions that engage HR for repair. We measured *BRCA1* methylation and mRNA in archived primary tumour
286 specimens, whereas treatment effect was assessed in metastases. We speculate that in mutation carriers, a higher
287 proportion retain an HR defect in metastatic disease than those with *BRCA1* methylated tumours
288 (Supplementary Figure S9). We suggest mutation creates a more resilient “hard” BRCAness whereas *BRCA1*
289 methylation associated epigenetic BRCAness is more “soft” and plastic²⁰. The methylation of *BRCA1* may be
290 both more heterogeneous and/or more revertible in subclinical metastases that, when subjected to selection
291 pressure by DNA damaging adjuvant therapy, lose their HR defect and survive subsequently developing as HR
292 proficient and not selectively platinum sensitive metastases. Our hypothesis is supported by data from both pre-
293 clinical patient derived xenografts and primary breast tumours exposed to neo-adjuvant chemotherapy³⁴. In
294 ovarian cancers *BRCA1* mutation but not methylation is associated with improved prognosis after platinum^{35,36}
295 and examination of pre- and post-platinum treatment biopsy pairs shows reversion of *BRCA1* methylation in
296 31% with continued presence of methylation being associated with PARP inhibitor response³⁷. While defects in
297 HR are known to be revertable mutational signatures would not be expected to disappear, as they are a
298 permanent “scar” of prior, even if no longer active, HR defects. While our finding that the Myriad HRD assay
299 did not have specific platinum response predictive performance in the advanced TNBC disease setting contrasts
300 to reported association with platinum response in the neoadjuvant setting in TNBC²⁶ these neoadjuvant studies
301 do not have a comparator arm to allow a test of interaction between biomarker status and any specific
302 treatment effect of platinum chemo as opposed to association with a relatively greater general chemotherapy
303 responsiveness than HRD low status. Where this was examined in the randomised neoadjuvant context the
304 Myriad HRD assay did not show specific predictive performance for platinum response in unplanned
305 retrospective analyses with limited power³⁸. Metastatic disease, exposed to prior adjuvant therapy is also a very
306 different biological context. We hypothesise that adjuvant therapy drives reversal of the *BRCA1* methylation
307 “soft” BRCAness³⁴ HR defect, that we show like *BRCA1* mutation leaves a high HRD score in the primary
308 tumour (Figure S4), erodes the positive predictive value of the HRD score for therapy response in metastasis
309 while a low HRD Score will likely retain negative predictive value by excluding many tumours that have never
310 had an HR defect whether “soft” or “hard”. Since our analysis, a novel HR deficiency mutational signature

311 whole genome sequence analysis methodology called “HRDetect” has been described with preliminary evidence
312 of potential application to FFPE clinical materials⁸. As HRDetect is also a cumulative historical measure of
313 lifetime HR deficiency the positive predictive value of this method may also be eroded by the effects of reversal
314 of epigenetic HR defects in treatment exposed metastatic disease and require integration with additional
315 biomarkers of a tumour’s current HR status. Analyses of HRDetect and multiple additional mutational
316 signatures, and their integration with transcriptional signatures of BRCAness and treatment response^{8,23,26,39,40}
317 are planned but require whole genome sequencing currently being piloted in TNT Trial FFPE material . These
318 future analyses are beyond the scope of this manuscript.

319

320 Previous randomised studies have not examined treatment effect in *a priori* defined subpopulations within
321 advanced TNBC³¹. TNT highlights the heterogeneity in TNBC and need to investigate therapeutic effects with
322 planned analyses of biological subgroups. We provide the first evidence of the clinical utility of *BRCA1/2*
323 genotyping to inform therapy choice in metastatic familial breast cancer and TNBC. In early TNBC three recent
324 trials have tested the role of the addition of platinum to anthracycline and taxane based neoadjuvant schedules,
325 finding evidence of increased pathological tumour response⁴¹⁻⁴³. These studies are underpowered for survival
326 endpoints, but where reported, significant effects on disease free survival were only seen when the alkylating
327 agent cyclophosphamide was omitted from the control arm backbone⁴¹. A non-significant trend was noted when
328 a standard cyclophosphamide “backbone” control was used in the CALGB 40603 study⁴². The dose intense
329 carboplatin regimen used in GeparSixto was recently compared with a sequential anthracycline and taxane and
330 high dose cyclophosphamide-containing regimen with no differences found in the primary pathological response
331 measures⁴⁴. It would seem that the use of alkylating agents in early TNBC is important, especially for those that
332 have higher stage disease with associated risk of recurrence requiring a maximally effective therapy, to reduce
333 this risk and achieve optimal surgery. The balance of additional toxicity and paucity of appropriately powered
334 survival analyses testing interaction with potential predictive biomarkers for platinum response suggest the need
335 for more study before platinum are used routinely across all stages and biological subtypes of early TNBC.
336 Data from our trial although conducted in advanced TNBC inform this landscape and raise important hypotheses
337 for further testing in the early breast cancer setting.

338

339 Many countries now perform inexpensive local *BRCA1/2* germline testing. Our results support *BRCA1/2*
340 germline testing to select patients for platinum chemotherapy for advanced disease. The OlympiAD trial ⁴⁵

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

351

352 **Methods**

353

354 *Study design*

355 Conducted in 74 hospitals throughout the UK TNT (NCT00532727) was a phase III, parallel group, open label
356 randomised controlled trial with pre-planned biomarker subgroup analyses. Trial sponsorship, governance,
357 randomisation procedures and balancing factors are described in the supplementary appendix.

358

359 *Patients*

360 Eligible patients had to be considered fit to receive either study drug and have measurable, confirmed advanced
361 breast cancer unsuitable for local therapy with histologically confirmed ER, PgR, and HER2 negative primary
362 invasive breast cancer with Allred/quick score <3 or H score <10 or locally determined ER and PgR negative, if
363 other cut-offs used (e.g., 1%, 5% or 10%). HER2 negative was defined as immunohistochemistry scoring 0 or
364 1+ for HER2, or 2+ and non-amplified for HER2 gene by FISH or CISH. Patients could be ER and HER2
365 negative and, PgR negative/unknown, or any ER, PgR and HER2 status if known to have *BRCA1* or *BRCA2*
366 germline mutation and otherwise eligible (full eligibility criteria in supplementary appendix). Although patients
367 with TNBC hypothesised to have BRCAness phenotypes were the primary interest, patients with unselected
368 TNBC as well as those with *BRCA1* or *BRCA2* germline mutations were recruited to allow interaction testing of
369 biomarker positive and negative populations in relation to response to each of these mechanistically distinct
370 agents. Patients provided written informed consent.

371

372

373 ***Procedures***

374 Patients were allocated (1:1 ratio) between six cycles of carboplatin (AUC 6), day 1 3-weekly, and six cycles of
375 docetaxel (100mg/m²), day 1 3-weekly (see supplementary appendix section 3.1 for details of allocation
376 procedures including minimisation balancing factors used). For patients responding to and tolerating treatment
377 well, a further two cycles could be given subject to local policy. Further details of chemotherapy and supportive
378 medicines are described in the supplementary appendix. Patients were offered six cycles of the alternative
379 (“crossover”) treatment upon progression or where allocated treatment was discontinued due to toxicity (“pre-
380 progression crossover”). Subsequent management was at clinician discretion.

381

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

385

386 ***Sample analyses***

387 For consenting patients, one blood sample and archival primary invasive carcinoma, lymph nodes and any
388 recurrent tumour specimens, or a research biopsy from a metastatic site, were collected. There was no
389 requirement for a recurrent specimen to be provided. DNA was extracted using standard methodology. Central
390 review of ER, PgR and HER status was performed at KCL (further details in supplementary appendix).

391

392 Germline *BRCA1* and *BRCA2* mutation analysis was conducted and status for subgroup analysis was centrally
393 determined at The Institute of Cancer Research. Genomic DNA from blood white cell preparations was analysed
394 for *BRCA1* and *BRCA2* for intragenic mutations and exon deletions and duplications throughout the coding
395 sequence, and intron-exon boundaries was completed in all cases. This was either performed by Sanger
396 sequencing together with multiplex ligation-dependent probe amplification (MLPA) or by next-generation
397 sequencing using the Illumina TruSight Cancer Panel v1. All intragenic mutations were confirmed by separate
398 bi-directional Sanger sequencing. All exon deletions or duplications were confirmed by MLPA. The mutation
399 nomenclature was in accordance with clinical convention with numbering starting at the first A of the ATG
400 initiation site, using BRCA1 LRG_292_t1 and BRCA2 LRG_293_t1.

401

402 The DNA methylation status of the regulatory region of *BRCA1* was determined using bisulfite sequencing and
403 *BRCA1* mRNA expression level from total-RNA-sequencing from archival primary carcinoma (see
404 supplementary appendix Figure S3 and Supplementary Table S5).

405

406 The Myriad HRD test includes three DNA-based measures of homologous recombination deficiency including:
407 whole genome tumour loss of heterozygosity profiles (LOH), telomeric allelic imbalance (TAI) and large-scale
408 state transitions (LST)²²⁻²⁴. All three scores are highly correlated with defects in *BRCA1/2* and predict response
409 to platinum-containing neoadjuvant chemotherapy in patients with TNBC trials without standard of care control
410 arms²⁶. The HRD score is calculated as the sum of the three individual scores, and a previously validated
411 threshold of 42 was utilized in these analyses²⁶. As part of the HRD assay, the sequencing data are used to call
412 *BRCA1/2* mutations in the tumour, either germline or somatic. The supplementary appendix includes
413 description of HRD assay on *TNT* trial samples.

414

415 Primary cancers were classified into basal-like subtypes by several classifiers including an IHC panel⁷, and
416 Prosigna⁴⁸(further details in supplementary appendix). Integration of transcriptional and whole genome
417 chromosomal instability, rearrangement and mutational signatures that have been associated with *BRCA1* or
418 *BRCA2* mutation and *BRCA1* methylation and may specifically interact with carboplatin response^{8,22-26,39,40}
419 were protocol pre-specified as *a priori* sub-groups analyses are incomplete and will be reported elsewhere.

420

421 ***Outcomes***

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

427

428 Secondary endpoints included progression free survival (PFS), overall survival (OS), response to crossover
429 treatment (as per primary endpoint), tolerability and safety.

430

431 Adverse events were assessed throughout treatment; graded according to National Cancer Institute Common
432 Toxicity Criteria (version 3·0) and coded according to the Medical Dictionary for Regulatory Activities
433 (MedDRA version 14·0) with central clinical review (by the Chief Investigator) at study completion.

434

435 ***Statistical analyses***

436 Evidence to inform sample size calculations was scarce; however ECOG 2100⁴⁹ suggested a 20-30% response
437 rate for single agent taxane. TNT was designed on the premise of demonstrating superiority of carboplatin with
438 a 15% improvement in response rates designated as clinically important. Assuming 90% power and type I error
439 $\alpha=0\cdot05$ (two-sided), a sample size of at least 370 patients was required. The protocol recognised *a priori* that
440 equivalence of response, accompanied by reduced toxicity with carboplatin, would also impact clinical practice.

441

442 Response rates were compared using 2-sided Fisher's exact tests and logistic regression (see supplementary
443 appendix section 4.10 for further details regarding analysis of subgroups). Survival endpoints were displayed
444 using Kaplan Meier plots and survival analysis modelling utilised restricted mean survival methodology⁵⁰ given
445 that the proportionality of hazards assumption required for Cox survival analysis did not hold.

446

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

451

452 ***Life Sciences Reporting Summary***

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

454

455

456

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470

471 **Author contributions statement**

472 AT - Chief Investigator, trial design, protocol development, participant recruitment, data collection, data
473 interpretation, writing, Trial Management Group member; HT - statistical analysis, data interpretation, writing,
474 Trial Management Group member; MCUC - translational substudy lead, biological data analysis, data
475 interpretation, writing, Biological Sub-committee Trial Management Group member; SK - trial management,
476 data collection, data management, Trial Management Group member; LK - trial design, protocol development,
477 statistical analysis, data interpretation, writing, Trial Management Group member; PG - biological analyses; JO
478 - biological analyses; JA - participant recruitment, data collection; SB - participant recruitment, data collection;
479 PB-L - participant recruitment, data collection, Trial Management Group member; RB - biological analyses,
480 writing, Biological Sub-committee Trial Management Group member; SC - participant recruitment, data
481 collection; MD - biological analyses; JMF - biological analyses, writing; LF - trial management, data collection,
482 Trial Management Group member; AG - biological analyses, Biological Sub-committee Trial Management
483 Group member; AGu - biological analyses; CH-W - participant recruitment, data collection, Trial Management
484 Group member; MQH - participant recruitment, data collection; KAH - biological analyses; JP - Response
485 Evaluation Committee member, independent radiology review; PP - Trial Management Group member; CMP -
486 biological analyses, Biological Sub-committee Trial Management Group member; RR - participant recruitment,
487 data collection, Trial Management Group member; VS - biological analyses; AS - germline genetics advisor for
488 biological analyses and data interpretation, protocol development, writing, Trial Management Group member;
489 IES - participant recruitment, data collection, Trial Management Group member; KMT - biological analyses;
490 AMW - participant recruitment, data collection, Trial Management Group member; GW - participant

491 recruitment, data collection; CG - TNT tissue bank lead, biological analyses, Trial Management Group member;
492 JSL - biological analyses; AA - Trial Management Group member; NR - germline genetics advisor for
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494 MH - trial design, protocol development, participant recruitment, data collection, Trial Management Group
495 member; PE - trial design, protocol development, participant recruitment, data collection, Trial Management
496 Group member; SEP - study lead pathologist, biological analyses, Trial Management Group member; JMB -
497 trial design, protocol development, study conduct oversight, statistical analysis, data interpretation, writing,
498 Trial Management Group member. All authors reviewed the manuscript prior to submission.

499

500 **Competing Financial Interests**

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504 conduct of the study. In addition, AT has a patent PCT/EP2015/078987 pending on behalf of King's College
505 London.

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

508 MD reports personal fees from Myriad outside the submitted work.

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

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

513 KMT reports personal fees from Myriad Genetics, Inc. during the conduct of the study, and personal fees from
514 Myriad Genetics, Inc. outside the submitted work. In addition, KT has the following patents pending:

515 13/164,499; 14/554,715; 15/010,721; 15/192,497; 14/245,576; 62/000,000; 62/311,231; 62/332,526;

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517 201280070358.0; 12860530.0; 2014-548965; 2014248007; 2,908,745; 14779403.6; 2016-506657; 712,663;

518 PCT/US15/045561; PCT/US15/064473; and the following patents issued to Myriad Genetics, Inc.: 9,279,156;
519 9,388,427 and 625468.

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

521 The other authors declare no competing interests.

522

523

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670

671

672 **Figure 1. Consort diagram**

673 Flow of participants in the trial.

674

675 **Figure 2. Response rates (overall and BRCA subgroups)**

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

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

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

684 **Figure 4. Response rates (HRD subgroups)**

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

688 **Figure 5. Response rates (basal-like subgroups)**

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

692 **Figure 6. PFS (basal-like subgroups)**

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

697

698 *Data availability*

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

700 <https://doi.org/10.5281/zenodo.1172633>.

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

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