eprints@whiterose.ac.uk
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Abstract

BRCA1/2 germline mutations predispose to breast cancer by impairing homologous recombination (HR) causing genomic instability. HR also repairs DNA lesions caused by platinums and PARP inhibitors. Unselected Triple Negative Breast Cancers (TNBC) harbour a sub-population with BRCA1/2 mutations, hypothesised to be especially platinum sensitive. Additional putative "BRCAness" subgroups may also be especially platinum sensitive. We assessed carboplatin and mechanistically distinct docetaxel in a phase III randomised trial in unselected advanced TNBC. A pre-specified correlative biology programme enabled biomarker-treatment interaction analyses in BRCA1/2 mutation associated breast cancer (gBRCA-BC) and putative BRCAness biomarker 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 randomised; 188 carboplatin, 188 docetaxel) carboplatin was not more active than docetaxel (ORR: 31.4 v 34.0; p=0.66). In contrast in patients with gBRCA-BC carboplatin was highly active with double the ORR compared to docetaxel (68% v 33%), 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. We conclude TNBC patients benefit from BRCA1/2 mutation characterization, but not BRCA1 methylation or Myriad HRD analysis. Basal-like gene expression analysis may also influence treatment choices.

73

"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

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   **Methods**

107

108   **Study design**

109   Conducted in 74 hospitals throughout the UK TNT was a phase III, parallel group, open label randomised

110   controlled trial with pre-planned biomarker subgroup analyses. Trial sponsorship, governance, randomisation

111   procedures and balancing factors are described in the supplementary appendix.

112

113   **Patients**

114   Eligible patients had to be considered fit to receive either study drug and have measurable, confirmed advanced

115   breast cancer unsuitable for local therapy with histologically confirmed ER, PgR, and HER2 negative primary

116   invasive breast cancer with Allred/quick score <3 or H score <10 or locally determined ER and PgR negative, if

117   other cut-offs used (e.g., 1%, 5% or 10%). HER2 negative was defined as immunohistochemistry scoring 0 or

118   1+ for HER2, or 2+ and non-amplified for HER2 gene by FISH or CISH. Patients could be ER and HER2

119   negative and, PgR negative/unknown, or any ER, PgR and HER2 status if known to have BRCA1 or BRCA2

120   germline mutation and otherwise eligible (full eligibility criteria in supplementary appendix). Although patients

121   with TNBC hypothesised to have BRCAness phenotypes were the primary interest, patients with unselected

122   TNBC as well as those with BRCA1 or BRCA2 germline mutations were recruited to allow interaction testing of

123   biomarker positive and negative populations in relation to response to each of these mechanistically distinct

124   agents. Patients provided written informed consent.

125

126   **Procedures**

127   Patients were allocated (1:1 ratio) between six cycles of carboplatin (AUC 6), day 1 3-weekly, and six cycles of

128   docetaxel (100mg/m^2), day 1 3-weekly. For patients responding to and tolerating treatment well, a further two

129   cycles could be given subject to local policy. Further details of chemotherapy and supportive medicines are

130   described in the supplementary appendix. Patients were offered six cycles of the alternative ("crossover")

131   treatment upon progression or where allocated treatment was discontinued due to toxicity ("pre-progression

132   crossover"). Subsequent management was at clinician discretion.

133

134   Tumour assessment by CT scan was performed after three and six cycles (or at treatment discontinuation if

135   earlier) and three-monthly thereafter until disease progression. Response was assessed as best response by

136   RECIST.

137

138   **Sample analyses**

139   For consenting patients, one blood sample and archival primary invasive carcinoma, lymph nodes and any

140   recurrent tumour specimens, or a research biopsy from a metastatic site, were collected. There was no

141   requirement for a recurrent specimen to be provided. DNA was extracted using standard methodology. Central

142   review of ER, PgR and HER status was performed at KCL (further details in supplementary appendix).

143

144   Germline BRCA1 and BRCA2 mutation analysis was conducted and status for subgroup analysis was centrally

145   determined at The Institute of Cancer Research. Genomic DNA from blood white cell preparations was analysed

146   for BRCA1 and BRCA2 for intragenic mutations and exon deletions and duplications throughout the coding

147   sequence, and intron-exon boundaries was completed in all cases. This was either performed by Sanger

148   sequencing together with multiplex ligation-dependent probe amplification (MLPA) or by next-generation

149   sequencing using the Illumina TruSight Cancer Panel v1. All intragenic mutations were confirmed by separate

150   bi-directional Sanger sequencing. All exon deletions or duplications were confirmed by MLPA. The mutation

151   nomenclature was in accordance with clinical convention with numbering starting at the first A of the ATG

152   initiation site, using BRCA1 LRG_292_t1 and BRCA2 LRG_293_t1.

153

154   The DNA methylation status of the regulatory region of BRCA1 was determined using bisulfite sequencing and

155   BRCA1 mRNA expression level from total-RNA-sequencing from archival primary carcinoma (see

156   supplementary appendix Figure S3 and Supplementary Table S5).

157

158   The Myriad HRD test includes three DNA-based measures of homologous recombination deficiency including:

159   whole genome tumour loss of heterozygosity profiles (LOH), telomeric allelic imbalance (TAI) and large-scale

160   state transitions (LST)[22-24]. All three scores are highly correlated with defects in BRCA1/2 and hypothesized to

161   be associated with sensitivity to platinum agents. The HRD score is calculated as the sum of the three individual

162   scores [25]. As part of the HRD assay, the sequencing data are used to call BRCA1/2 mutations in the tumour,

163 either germline or somatic. The supplementary appendix includes description of HRD assay on TNT trial

164 samples.

166 Primary cancers were classified into basal-like subtypes by several classifiers including an IHC panel[7], and

167 Prosigna[26] (further details in supplementary appendix). Integration of transcriptional and whole genome

168 chromosomal instability, rearrangement and mutational signatures that have been associated with BRCA1 or

169 BRCA2 mutation and BRCA1 methylation and may specifically interact with carboplatin response [8,22-25,27-29]

170 were protocol pre-specified as a priori sub-groups analyses are incomplete and will be reported elsewhere.

172 All genomics data reported in this manuscript will be available for public access.

174 **Outcomes**

175 The primary endpoint was objective tumour response rate (complete or partial). The version of RECIST

176 reporting criteria used for tumour assessment was documented and, where possible, cases assessed using

177 RECIST version 1.0 were subsequently reassessed locally according to RECIST version 1.1. An independent

178 Response Evaluation Committee at study completion reviewed reported responses centrally (local assessment

179 was used for primary analysis).

181 Secondary endpoints included progression free survival (PFS), overall survival (OS), response to crossover

182 treatment (as per primary endpoint), tolerability and safety.

184 Adverse events were assessed throughout treatment; graded according to National Cancer Institute Common

185 Toxicity Criteria (version 3·0) and coded according to the Medical Dictionary for Regulatory Activities

186 (MedDRA version 14·0) with central clinical review (by the Chief Investigator) at study completion.

188 **Statistical analyses**

189 Evidence to inform sample size calculations was scarce; however ECOG 2100[30] suggested a 20-30% response

190 rate for single agent taxane. TNT was designed on the premise of demonstrating superiority of carboplatin with

191 a 15% improvement in response rates designated as clinically important. Assuming 90% power and type I error

192 α=0·05 (two-sided), a sample size of at least 370 patients was required. The protocol recognised a priori that

193 equivalence of response, accompanied by reduced toxicity with carboplatin, would also impact clinical practice.

194

195 Response rates were compared using Fisher's exact tests and logistic regression (see supplementary appendix

196 section 4.11 for further details regarding analysis of subgroups). Survival endpoints were displayed using

197 Kaplan Meier plots and survival analysis modelling utilised restricted mean survival methodology[31] given that

198 the proportionality of hazards assumption required for Cox survival analysis did not hold.

199

200 Principal efficacy endpoints were analysed according to intention to treat (ITT) including all 376 patients

201 randomised and according to pre-planned biomarker subgroups (Table S1); additional analysis groups and

202 associated analysis methods are detailed in the supplementary appendix. Analyses are based on a database

203 snapshot taken on 7 March 2016 and performed using STATA 13.

204

205 **Results**

206 Between 25 April 2008 and 18 March 2014 376 patients (188 allocated to carboplatin and 188 to docetaxel)

207 entered the trial, all patients were included in the analysis of the primary endpoint (Figure 1); the trial population

208 largely comprised patients with TNBC and no known BRCA1/2 mutation (338/376) and baseline characteristics

209 typical of patients with first line relapse of TNBC (Table S2/S3). There were 43 patients with germline

210 BRCA1/2 mutation (31 BRCA1 and 12 BRCA2 Table S2). Of the 31 BRCA1 mutation carriers XX had ER+ve

211 disease and of the 12 BRCA2 mutation carriers XX had ER+ve disease. Compliance with allocated treatment

212 was good; disease progression and toxicity were the principal reasons for early discontinuation. Median relative

213 dose intensity was 94·0% (IQR 84·2, 99·8) for carboplatin and 94·8% (IQR: 84·8, 100·0) for docetaxel.

214

215 **Overall results**

216 There was no evidence of a difference between carboplatin and docetaxel in objective response rate in the

217 overall population (ORR: 59/188 (31·4%) vs. 64/188 (34·0%), absolute difference -2·6%, (95%CI: -12·1 to

218 6·9), p=0·66; Figure 2A). Following central review of locally classified responses, response rates were 48/188

219 (25·5%) carboplatin vs. 55/188 (29·3%) docetaxel, absolute difference (C-D) = -3·8 (95%CI: -12·8, 5·2); exact

220 p=0·49, consistent with findings from the main analysis. Similarly, no evidence of a difference was observed for

221 crossover treatments (Figure S1A) or when analysis was limited to those centrally confirmed as having triple

222 negative tumours (see supplementary appendix).

223

224 372 (98·9%) patients have had PFS events reported. Median PFS in patients allocated carboplatin was 3·1

225 months (95%CI: 2·4, 4·2) and 4·4 months (95%CI: 4·1, 5·1) for those allocated docetaxel. No difference in

226 restricted mean PFS was found (difference -0·30 months, p=0·40; Figure 3A).

227

228 347 patients are reported to have died. Median OS was 12·8 months (95%CI: 10·6, 15·3) and 12·0 months

229 (95%CI: 10·2, 13·0) for those allocated carboplatin and docetaxel respectively. Consistent with the PFS result,

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

231 S2A).

232

233 **BRCA subgroup analyses**

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

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

236 docetaxel (ORR: 17/25 (68·0%) vs. 6/18 (33·3%), absolute difference 34·7%, p=0·03), with no evidence of

237 differential treatment activity in patients with no germline mutation (ORR: 36/128 (28·1%) vs. 50/145 (34·5%),

238 absolute difference -6·4%, p=0·30), resulting in a statistically significant interaction (p=0·01, Figure 2B). This

239 result remained significant (p=0·01) after adjustment for known prognostic factors (see supplementary appendix

240 for details). PFS also favoured carboplatin for patients with a BRCA1/2 germline mutation (median PFS 6·8

241 months vs. 4·4 months, difference in restricted mean PFS 2·6 months, interaction p=0·002; Figure 3B) but no

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

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

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

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

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

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

248 better response to carboplatin than docetaxel (ORR: 12/18 (66·7%) vs. 5/14 (35·7%), absolute difference

249 31·0%, p=0·15) whilst a treatment effect favouring docetaxel was suggested in patients with wildtype genotype

250 in the tumour (ORR: 23/90 (25·6%) vs. 32/90 (35·6%), absolute difference -10·0%, p=0·20). Given very small

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

252 however, given the effects were in opposite directions, the interaction was significant (p=0·03) (Figure 2C).

253 This however did not hold for PFS or OS (p=0·12, p=0·70 respectively) (Figures 3C and S2C). Eight patients

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

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

256 limit conclusive interpretation of these data.

257

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

259 carboplatin than docetaxel (ORR: 3/14 (21·4%) vs. 8/19 (42·1%), absolute difference -20·7%, p=0·28) with no

260 evidence of an interaction observed (p=0·35, Figures 2D, 3D, S2D); with similar conclusions when germline

261 BRCA1/2 mutated patients were excluded.

262

263 Concordant with BRCA1 methylation status, tumours we defined as BRCA1 mRNA-low, with which

264 methylation was partially associated (Supplemntary Figure S3 and Table S5), did not have a better response to

265 carboplatin than docetaxel (ORR: 4/14 (28·6%) vs. 11/17 (64·7%), absolute difference -36·1%, p=0·07) and

266 evidence of an interaction was lacking (p=0·07, Figures 2E, 3E, S2E), again conclusions were not different

267 when germline BRCA mutations were excluded. Furthermore, exploratory analyses examining any relationship

268 between high response to carboplatin and the cut-point for BRCA1 methylation or BRCA1 mRNA1-low did not

269 suggest any significant signal that supported our a priori hypotheses that they would be associated with greater

270 response to carboplatin than a taxane (data not presented).

271

272 **Homologous Recombination Deficiency subgroup analyses**

273 In the initial trial design and first protocol we hypothesized that changes in the genome landscape which may

274 arise as a consequence of defects in homologous recombination could provide an indicator of platinum salt

275 sensitivity and should be examined for interaction with treatment effect in both treatment arms. A number of

276 these assays have been reported[8,22-24,27]. Here we show the result using the combined Myriad HRD assay[25]

277 performed on treatment naïve primary tissue. We find that the great majority of patients with either germline

278 BRCA1/2 mutation or BRCA1 methylation have an high Dichotomized "HRD Score" (Figure S4A, S4B) but

279 "HRD Score" high patients, unlike germline BRCA1/2 mutation carriers, did not have better response to

280 carboplatin than docetaxel (ORR: 13/34 (38.2%) vs. 19/47 (40.4%), absolute difference -2.2%, p=1.0) with no

281   evidence of an interaction observed (p=0·75, Figure 4A). Similar results were found when "HRD Deficient"

282   patients, a definition that grouped all BRCA1/2 mutated patients with those BRCA1/2 wild-type patients with

283   high HRD score, were examined (Figure 4B). In addition no evidence of treatment specific predictive effect for

284   PFS was found using either HRD definition (Figure S5A,B). Patients with High HRD score had a numerically

285   greater response to both chemotherapy agents than those with low scores but this does not appear statistically

286   significant.

287

288   **Basal subgroup analyses**

289   Given association between germline BRCA1 mutation and the development of basal-like breast cancers we

290   sought to formally test the premise that all basal-like cancers share a BRCA1 loss of function phenotype with

291   those with mutation by analysing a platinum treatment interaction in this broader basal-like TNBC group. We

292   found no evidence that Prosigna® – PAM50 basal tumours showed greater response to carboplatin compared

293   with docetaxel (ORR: 27/83 (32·5%) vs. 27/87 (31·0%), absolute difference 1·5%, p=0·87). However, in

294   patients with non-basal-like tumours response to docetaxel was significantly better than to carboplatin (ORR:

295   13/18 (72·2%) vs. 3/18 (16·7%), absolute difference -55·5%, p=0·002), leading to a significant interaction test

296   (p=0·003, Figure 5A) and a similar trend in crossover treatment response (Figure S6). The interaction between

297   treatment and PAM50 subgroups remained significant after adjusting for gBRCA status in the multivariable

298   logistic regression model (p=0·002) (Table S6) and when other known prognostic factors were subsequently

299   included in the model. The interaction was also significant for PFS (p=0·04) (Figure 6A) but not OS (p=0·17)

300   (Figure S7A).

301   There was no evidence that "core basal" tumours defined by IHC had improved response to carboplatin

302   compared with docetaxel (ORR: 23/67 (34·3%) vs. 19/65 (29·2%), absolute difference 5·1%, p=0·58). While

303   there was a higher response rate to docetaxel compared with carboplatin in patients with non-basal 5 marker

304   negative (5NP) tumours (ORR: 13/31 (41·9%) vs 5/26 (19·2%)., absolute difference -22·7%, p=0·09), the

305   difference did not reach statistical significance and the interaction test was non-significant p=0·06 (Figures 5B,

306   6B, S7B).

307

308   **Safety**

309   Both carboplatin and docetaxel demonstrated toxicity consistent with their known safety profiles and Grade 3

310   and 4 adverse events (AEs) were as anticipated for these well-known chemotherapy drugs (Tables S7 and S8).

311 There were more grade 3/4 AEs with docetaxel than with carboplatin. 276 Serious Adverse Events (SAEs) were

312 reported throughout the trial (102 carboplatin; 174 docetaxel). The spectrum of SAEs was as anticipated. Two

313 SAEs were considered to be Suspected Unexpected Serious Adverse Reactions (1 carboplatin; 1 docetaxel).

314 These were i) nausea, vomiting and headaches; ii) low magnesium. One death was considered possibly related

315 to carboplatin treatment; this patient died from pulmonary embolism. As an haplo-insuffiency or dominant

316 negative effect of heterozygous mutation might affect toxicity from HR targeting therapies such as platinum in

317 mutation carriers we sought evidence of excess haematological toxicity as a signal but found none (Table S9).

318 Although there was a small numerical difference in non-haematological toxicity this was not significant and

319 small numbers preclude firm conclusions from these analyses.

320

321 **Discussion**

322 This phase III trial utilised two mechanistically distinct single agent chemotherapeutics in unselected advanced

323 TNBC and in a priori specified biomarker defined sub-populations thought likely to have targetable defects in

324 HR DNA repair. In the unselected TNBC patients no evidence of a superior response to carboplatin was

325 observed when compared with a standard of care taxane, docetaxel. Carboplatin was better tolerated than

326 docetaxel delivered at the full licensed dose. This trial demonstrates significant activity for both agents and the

327 level of response seen for docetaxel is consistent with that seen previously in breast cancer[32] and for carboplatin

328 with that seen in uncontrolled trials of single agent platinums[33,34] or combinations of carboplatin with

329 gemcitabine in unselected TNBC[35]. The only other randomised trial conducted synchronous with our trial and

330 designed to specifically investigate platinum in comparison with a standard of care in advanced TNBC included

331 the substitution of cisplatin for paclitaxel given in a doublet with gemcitabine. In this study treatment was

332 continued until disease progression, as is common practice with paclitaxel, and showed modestly greater activity

333 for cisplatin[36]. A criticism of our study could be that patients did not receive treatment to progression but for 6

334 cycles (and at investigator discretion maximum of 8 cycles), as was consistent with UK practice with docetaxel

335 at the full licensed 100mg/m^2 dose, as this is rarely tolerated for more than 6-8 cycles. This may explain shorter

336 PFS compared to the study of Hu et al despite similar overall survival[36], and may have underestimated the effect

337 of carboplatin in those without a progression event during treatment and who might have continued event free

338 for longer had treatment continued.

339

340   In contrast to the unselected population, the pre-specified analyses of treatment effect in subgroups found

341   evidence of clinically and statistically significant biomarker-treatment interactions. There is a strong association

342   between BRCA1 mutation and basal-like cancer[37] and sporadic basal-like breast cancer subtypes show high

343   degrees of chromosomal genomic instability[3]. We hypothesised that if, as has been widely speculated, there was

344   a shared profound BRCAness phenotype sporadic basal-like cancers might have very high platinum sensitivity.

345   We found no evidence that basal-like biomarkers predicted higher response to platinum than docetaxel with the

346   drugs showing similar activity. A significant treatment interaction was detected with the Prosigna PAM50

347   identified subtypes; driven by significantly increased response to docetaxel relative to poor platinum response in

348   non-basal forms of TNBC. This suggests absence of targetable BRCAness in non-basal TNBC and no evidence

349   to change the standard of care from taxane to a platinum, which our data suggests is inferior in these subtypes.

350   In contrast platinum is a reasonable option in those with basal TNBC particularly in those who fail to tolerate or

351   have previously received a taxane. As the response rate is much less than that of BRCA1/2 mutation associated

352   breast cancer, if there is a profound BRCAness phenotype that remains prevalent in metastatic basal-like breast

353   cancer, beyond the context of BRCA1 or BRCA2 mutation, it appears to lie within a yet to be identified

354   subpopulation of this subtype.

355

356   BRCA1/2 mutation testing is a clinically validated and widely available biomarker that predicted both greater

357   response and PFS in favour of carboplatin over docetaxel demonstrating clinical utility for treatment selection in

358   this setting. There was no evidence that mutation was associated with reduced activity of docetaxel compared to

359   wildtype; docetaxel remains a valid and active, but inferior, treatment option in this setting. We did not find

360   evidence of an overall survival advantage for carboplatin in BRCA1/2 mutation carriers, but interpretation is

361   confounded by the crossover design as 56% received carboplatin at progression. The high levels of response

362   seen for carboplatin were similar to those reported for the combination of carboplatin and paclitaxel in an

363   essentially similar population in the reference comparator arm in the phase II BROCADE trial[38], supporting the

364   notion that carboplatin monotherapy is highly active in this patient group. We found approximately one third of

365   BRCA1/2 carriers did not respond to platinum. Potential resistance mechanisms will be further explored in

366   integrated whole genome and whole transcriptome sequencing analyses in primary tumour material but lack of

367   extensive metastatic tumour from patients immediately prior to platinum treatment will limit sensitivity and

368   ability to draw firm conclusions.

369

370   In parallel we tested the hypothesis that epigenetic silencing of BRCA1 by DNA methylation would show a

371   similar treatment interaction. Despite similar numbers in genetic and epigenetic BRCAness subgroups, patients

372   with BRCA1 methylation or mRNA low had a higher response to docetaxel than carboplatin. Exploratory

373   analyses seeking optimisation of cut-points and analysis of these epigenetic biomarkers as continuous variables

374   failed to find any signal. In stark contrast to the interaction between BRCA1/2 mutation and carboplatin

375   treatment effect we find no evidence to support a similar impact of epigenetic BRCAness with no interaction

376   found between either BRCA1 methylation or BRCA1 mRNA low status and carboplatin treatment effect. This

377   suggests important differences in the effects of genetic and epigenetic changes at the BRCA1 locus, at least in

378   predicting therapy response in metastatic breast cancer exposed to prior adjuvant chemotherapy. These results

379   are consistent with previous results from the non-randomised TBCRC 009 trial in metastatic TNBC[33] where the

380   few tumours with BRCA1 methylation showed no response to platinum despite evidence of chromosomal

381   instability signatures. The majority of our patients had received adjuvant chemotherapies that cause DNA

382   lesions that engage HR for repair. We measured BRCA1 methylation and mRNA in archived primary tumour

383   specimens, whereas treatment effect was assessed in metastases. We speculate that in mutation carriers, a higher

384   proportion retain an HR defect in metastatic disease than those with BRCA1 methylated tumours

385   (Supplementary Figure S9). We suggest mutation creates a more resilient "hard" BRCAness whereas BRCA1

386   methylation associated epigenetic BRCAness is more "soft" and plastic[20]. The methylation of BRCA1 may be

387   both more heterogeneous and/or more revertible in subclinical metastases that, when subjected to selection

388   pressure by DNA damaging adjuvant therapy, lose their HR defect and survive subsequently developing as HR

389   proficient and not selectively platinum sensitive metastases. Our hypothesis is supported by data from both pre-

390   clinical patient derived xenografts and primary breast tumours exposed to neo-adjuvant chemotherapy[40]. In

391   ovarian cancers BRCA1 mutation but not methylation is associated with improved prognosis after platinum[41,42]

392   and examination of pre- and post-platinum treatment biopsy pairs shows reversion of BRCA1 methylation in

393   31% with continued presence of methylation being associated with PARP inhibitor response[43]. While defects in

394   HR are known to be revertable mutational signatures would not be expected to disappear, as they are a

395   permanent "scar" of prior, even if no longer active, HR defects. While our finding that the Myriad HRD assay

396   did not have specific platinum response predictive performance in the advanced TNBC disease setting contrasts

397   to reported association with platinum response in the neoadjuvant setting in TNBC[25] these neoadjuvant studies

398   do not have a comparator arm to allow a test of interaction between biomarker status and any specific

399   treatment effect of platinum chemo as opposed to association with a relatively greater general chemotherapy

400 responsiveness than HRD low status . Where this was examined in the randomised neoadjuvant context the

401 Myriad HRD assay has not shown specific predictive performance for platinum response[44]. Metastatic disease ,

402 exposed to prior adjuvant therapy is also a very different biological context. We hypothesise that adjuvant

403 therapy drives reversal of the BRCAl methylation "soft' BRCAness[40] HR defect, that we show like BRCAl

404 mutation leaves a high HRD score in the primary tumour (Figure S4), erodes the positive predictive value of the

405 HRD score for therapy response in metastasis while a low HRD Score will likely retain negative predictive

406 value by excluding many tumours that have never had an HR defect whether "soft" or "hard". Since our

407 analysis, a novel HR deficiency mutational signature whole genome sequence analysis methodology called

408 "HRDetect" has been described with preliminary evidence of potential application to FFPE clinical materials[8].

409 As HRDetect is also a cumulative historical measure of lifetime HR deficiency the positive predictive value of

410 this method may also be eroded by the effects of reveral of epigenetic HR defects in treatment exposed

411 metatstatic disease and require integration with additional biomarkers of a tumours current HR status. Analyses

412 of HRDetect and multiple additional mutational signatures, and their integration with transcriptional signatures

413 of BRCAness and treatment response[8,23,25,28,29] are planned but require whole genome sequencing currently

414 being piloted in TNT Trial FFPE material . These future analyses are beyond the scope of this manuscript.

415

416 Previous randomised studies have not examined treatment effect in a priori defined subpopulations within

417 advanced TNBC[36]. TNT highlights the heterogeneity in TNBC and need to investigate therapeutic effects with

418 planned analyses of biological subgroups. We provide the first evidence of the clinical utility of BRCAl/2

419 genotyping to inform therapy choice in metastatic familial breast cancer and TNBC. In early TNBC three recent

420 trials have tested the role of the addition of platinum to anthracycline and taxane based neoadjuvant schedules,

421 finding evidence of increased pathological tumour response[45-47]. These studies are underpowered for survival

422 endpoints, but where reported, significant effects on disease free survival were only seen when the alkylating

423 agent cyclophosphamide was omitted from the control arm backbone[45]. A non-significant trend was noted when

424 a standard cyclosphosphamide "backbone" control was used in the CALGB 40603 study[46]. The dose intense

425 carboplatin regimen used in GeparSixto was recently compared with a sequential anthracycline and taxane and

426 high dose cyclophosphamide-containing regimen with no differences found in the primary pathological response

427 measures[48]. It would seem that the use of alkylating agents in early TNBC is important, especially for those that

428 have higher stage disease with associated risk of recurrence requiring a maximally effective therapy to reduce

429 this risk and achieve optimal surgery. The balance of additional toxicity and paucity of appropriately powered

430   survival analyses testing interaction with potential predictive biomarkers for platinum response suggest the need

431   for more study before platinums are used routinely across all stages and biological subtypes of early TNBC.

432   Data from our trial although conducted in advanced TNBC inform this landscape and raise important hypotheses

433   for further testing in the early breast cancer setting.

434

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

436   select patients for platinum chemotherapy for advanced disease. No PARP inhibitor is yet licensed in breast

437   cancer. The OlympiAD trial [49] recently reported comparison between the potent PARP inhibitor olaparib,

438   known to trap PARP1 on DNA, in comparison to physicians choice of non-platinum standard of care

439   chemotherapies in anthracycline and taxane exposed advanced gBRCA-BC. Other trials of potent PARP

440   inhibitors are ongoing[50]. The PARP inhibitor olaparib is now approved in advanced gBRCA-BC but this

441   treatment may remain unaffordable to many health care systems and patients for many years. It remains

442   unknown how potent PARP1-trapping inhibitors would compare with platinums in this setting but the TNT trial

443   provides evidence that a widely available affordable off-patent biomarker has utility to select a population,

444   enriched in the TNBCs prevalent in many developing countries[51], who could benefit during this period from the

445   biologically targeted use of highly active and inexpensive platinum chemotherapy agent rather than the current

446   licensed breast cancer standard of care chemotherapies.

447

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460   Seitz of Insight Genetics for assistance in TNBCtype analysis and manuscript review.

461

462   **Author Contributons**

463   AT - Chief Investigator, trial design, protocol development, participant recruitment, data collection, data

464   interpretation, writing, Trial Management Group member; HT - statistical analysis, data interpretation, writing,

465   Trial Management Group member; MC - translational substudy lead, biological data analysis, data

466   interpretation, writing, Biological Sub-committee Trial Management Group member; SK - trial management,

467   data collection, data management, Trial Management Group member; LK - trial design, protocol development,

468   statistical analysis, data interpretation, writing, Trial Management Group member; PG - biological analyses; JO

469   - biological analyses; JA - participant recruitment, data collection; SB - participant recruitment, data collection;

470   PB-L - participant recruitment, data collection, Trial Management Group member; RB - biological analyses,

471   writing, Biological Sub-committee Trial Management Group member; SC - participant recruitment, data

472   collection; MD - biological analyses; LF - trial management, data collection, Trial Management Group member;

473   JF - biological analyses, writing; AG - biological analyses, Biological Sub-committee Trial Management Group

474   member; CH-W - participant recruitment, data collection, Trial Management Group member; MQH - participant

475   recruitment, data collection; KAH - biological analyses; JP - Response Evaluation Committee member,

476   independent radiology review; PP - Trial Management Group member; CMP - biological analyses, Biological

477   Sub-committee Trial Management Group member; RR - participant recruitment, data collection, Trial

478   Management Group member; VS - biological analyses; AS - germline genetics advisor for biological analyses

479   and data interpretation, protocol development, writing, Trial Management Group member; IS - participant

480   recruitment, data collection, Trial Management Group member; KMT - biological analyses; AMW - participant

481   recruitment, data collection; GW - participant recruitment, data collection; CG - TNT tissue bank lead,

482   biological analyses, Trial Management Group member; JSL - biological analyses; AA - Trial Management

483   Group member; NR - germline genetics advisor for biological analyses and data interpretation, protocol

484   development, writing, Trial Management Group member; MH - trial design, protocol development, participant

485   recruitment, data collection, Trial Management Group member; PE - trial design, protocol development,

486   participant recruitment, data collection, Trial Management Group member; SEP - study lead pathologist,

487   biological analyses, Trial Management Group member; JMB - trial design, protocol development, study conduct

488   oversight, statistical analysis, data interpretation, writing, Trial Management Group member. All authors

489   reviewed the manuscript prior to submission.

490

491   **Competing Financial Interests**

492   AT, HT, MC, SK, LK, PG, JO, RB, MD, LF, AG, PP, VS, CG, NR, SEP and JMB report grants to their

493   institutional departments from Breast Cancer Now and/or Cancer Research UK, and other research support for

494   costs or consumables in the study from Myriad Genetics, Inc. and NanoString Technologies, Inc. during the

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

496   London.

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

498   royalties paid.

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

500   CMP reports personal fees from Bioclassifier LLC, other from Nanostring Technologies outside the submitted

501   work. In addition, CMP has a patent U.S. Patent No. 9,631,239 with royalties paid.

502   KMT reports personal fees from Myriad Genetics, Inc. during the conduct of the study, and personal fees from

503   Myriad Genetics, Inc. outside the submitted work. In addition, KT has the following patents pending:

504   13/164,499; 14/554,715;   15/010,721; 15/192,497; 14/245,576; 62/000,000; 62/311,231; 62/332,526;

505   14/962,588; 2802882; 11796544.2; 15189527.3; 2,839,210; 12801070.9; 2014-516031; 2012358244; 2,860,312;

506   201280070358.0; 12860530.0; 2014-548965; 2014248007; 2,908,745; 14779403.6; 2016-506657; 712,663;

507   PCT/US15/045561; PCT/US15/064473; and the following patents issued to Myriad Genetics, Inc.: 9,279,156;

508   9,388,427 and 625468.

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

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

511   patent rights assigned to Myriad Genetics.

512   The other authors declare no competing interests.

513

514

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657

659   **Figure 1. Consort diagram**

Randomised (N=376)

12 ineligible (included in analyses)
Not triple negative and no known BRCA mutation	4
Non measurable disease	1
Assessments outside allowable window	6
Received previous taxane	1

188 allocated carboplatin

Efficacy analysis population

188 allocated docetaxel

15 ineligible (included in analyses)
Not triple negative and no known BRCA mutation	5
Non measureable disease	2
Assessments outside allowable window	7
Ongoing toxicity at baseline	1

4 did not start allocated treatment
Death	3
Progression	1

6 did not start allocated treatment
Progression	2
AE	1
Patient refusal	1
Other	2

101 discontinued early
	33 before cycle 3	68 after cycle 3
Death	2	1
Progression	25	64
AE	5	3
Other	1	0

184 received allocated treatment

Safety analysis population

182 received allocated treatment

90 discontinued early
	31 before cycle 3	59 after cycle 3
Death	2	3
Progression	11	42
AE	15	13
Patient choice	3	1

90 did not cross over
No first progression	3
Death	17
Progressed twice	15
Patient choice	8
Toxicities	1
Other	45
Unknown	1

86 did not cross over
No first progression	1
Death	15
Progressed twice	21
Patient choice	10
Toxicities	2
Other	35
Unknown	2

94 received cross-over docetaxel after progression

Crossover analysis population

89 received cross-over carboplatin after progression

7 received crossover carboplatin pre-progression

57 discontinued early
	24 before cycle 3	33 after cycle 3
Death	2	0
Progression	10	25
AE	7	7
Patient choice	3	0
Other	2	1

56 discontinued early
	20 before cycle 3	36 after cycle 3
Death	1	0
Progression	13	29
AE	6	6
Other	0	1

Followed-up
188 randomised
172 (91.5%) died
0 (0.0%) withdrawn/lost to follow up
5 (2.7%) >12 months since last seen
11 (5.9%) complete follow up

Followed-up
188 randomised
175 (93.1%) died
1 (0.5%) withdrawn/lost to follow up
3 (1.6%) >12 months since last seen
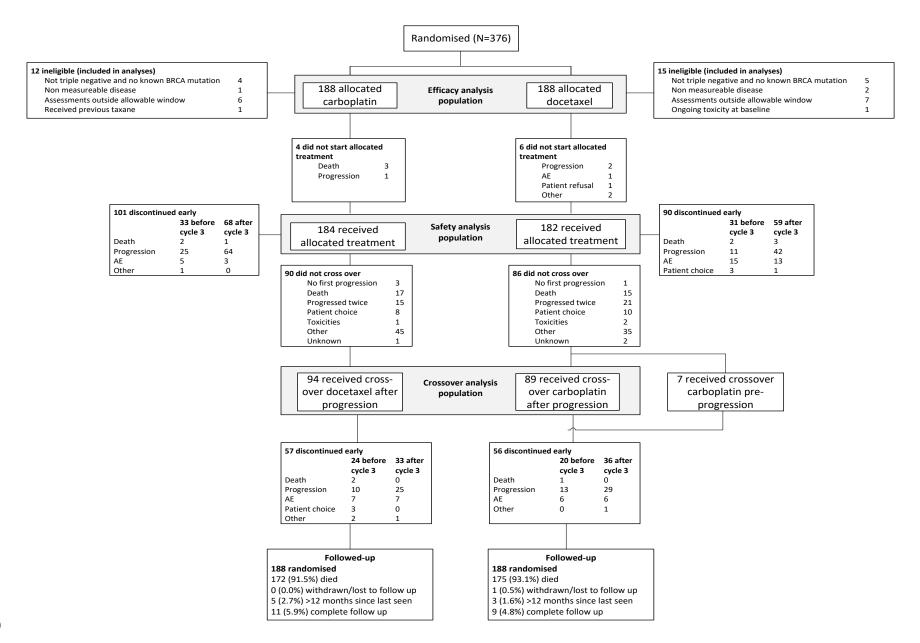9 (4.8%) complete follow up

660

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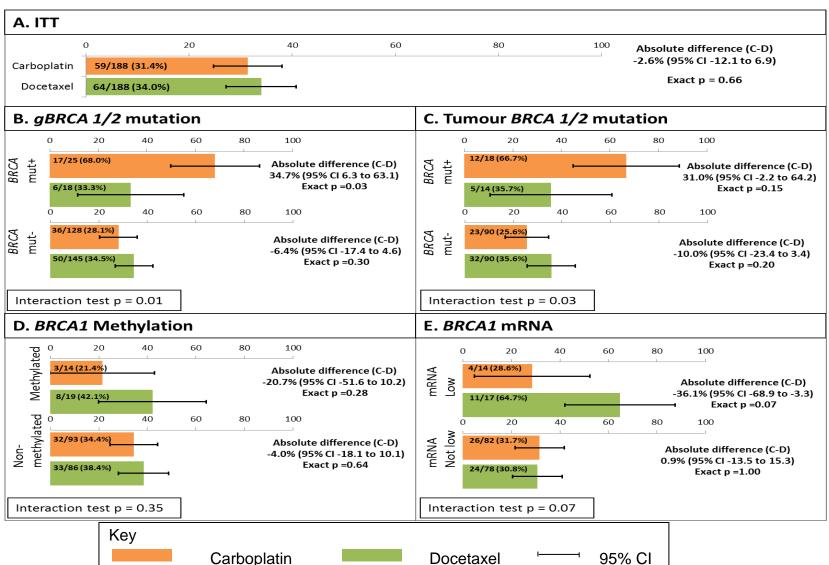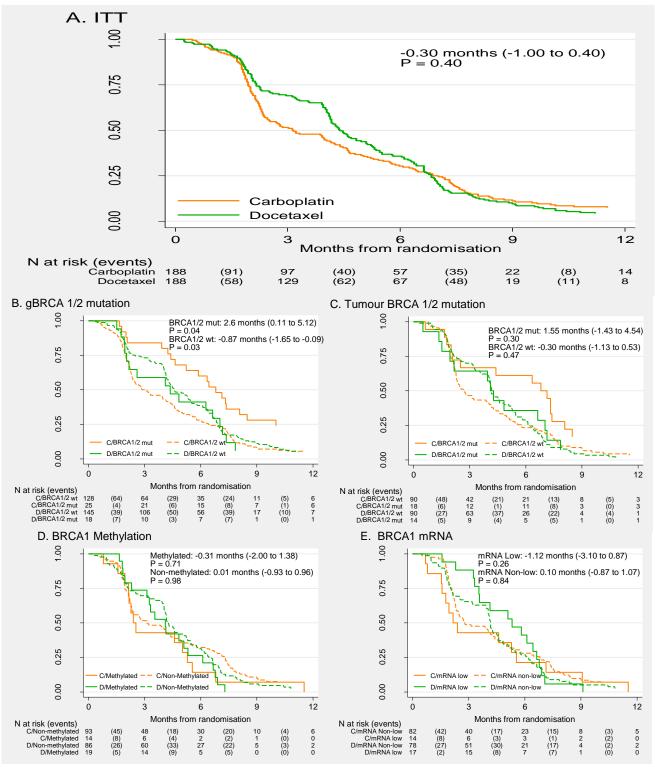
Figure 3. Progression-free survival (overall and BRCA subgroups)

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)

A. Dichotomized HRD score

HRD High
- 13/34 (38.2%) — Carboplatin
- 19/47 (40.4%) — Docetaxel

Absolute difference (C-D)
-2.2% (95%CI -23.7 to 19.3)
Exact p = 1.00

HRD Low
- 20/65 (30.8%) — Carboplatin
- 14/49 (28.6%) — Docetaxel

Absolute difference (C-D)
2.2% (95%CI -14.7 to 19.1)
Exact p = 0.84

Interaction test p = 0.75

B. HR Deficiency status

HR Deficient
- 17/38 (44.7%) — Carboplatin
- 19/48 (39.6%) — Docetaxel

Absolute difference (C-D)
5.1% (95%CI -15.9 to 26.1)
Exact p = 0.67

HR Non-deficient
- 17/62 (27.4%) — Carboplatin
- 14/48 (29.2%) — Docetaxel

Absolute difference (C-D)
-1.8% (95%CI -18.8 to 15.2)
Exact p = 1.00

Interaction test p = 0.63

Key
Carboplatin   Docetaxel   ⊢───┤ 95% CI

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.
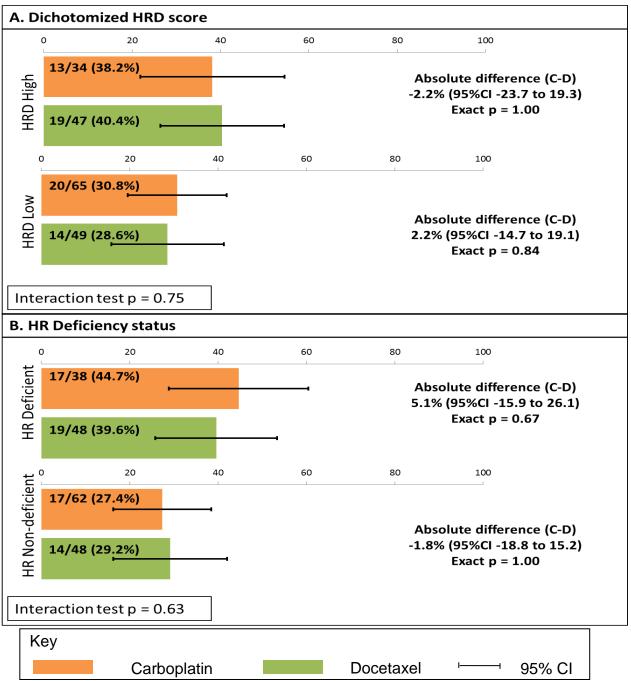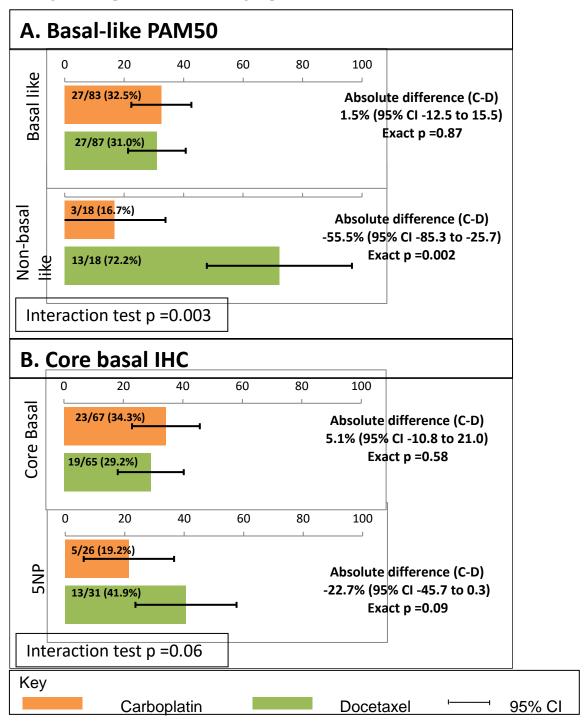
Figure 5. Response rates (basal-like groups)

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 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.

1.13)

.01 to -0.14)

12

(2)	0
(3)	5
(3)	1
(2)	1

.60 to 1.58)

0.68)

12

(3)	1
(3)	5
(3)	1
(2)	1

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 t-test comparing the mean survival between treatments (within biomarker groups as appropriate). C=Carboplatin; D=Docetaxel.