

ORIGINAL ARTICLE



Assessment of structural chromosomal instability phenotypes as biomarkers of carboplatin response in triple negative breast cancer: the TNT trial

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Background: In the TNT trial of triple negative breast cancer (NCT00532727), germline *BRCA1/2* mutations were present in 28% of carboplatin responders. We assessed quantitative measures of structural chromosomal instability (CIN) to identify a wider patient subgroup within TNT with preferential benefit from carboplatin over docetaxel. **Patients and methods:** Copy number aberrations (CNAs) were established from 135 formalin-fixed paraffin-embedded primary carcinomas using Illumina OmniExpress SNP-arrays. Seven published [allelic imbalanced CNA (AiCNA); allelic balanced CNA (AbCNA); copy number neutral loss of heterozygosity (CnLOH); number of telomeric allelic imbalances (NtAI); *BRCA1*-like status; percentage of genome altered (PGA); homologous recombination deficiency (HRD) scores] and two novel [Shannon diversity index (SI); high-level amplifications (HLAMP)] CIN-measurements were derived. HLAMP was defined based on the presence of at least one of the top 5% amplified cytobands located on 1q, 8q and 10p. Continuous CIN-measurements were divided into tertiles. All nine CIN-measurements were used to analyse objective response rate (ORR) and progression-free survival (PFS).

Results: Patients with tumours without HLAMP had a numerically higher ORR and significantly longer PFS in the carboplatin (C) than in the docetaxel (D) arm [56% (C) versus 29% (D), $P_{HLAMP,quiet} = 0.085$; PFS 6.1 months (C) versus 4.1 months (D), $P_{interaction/HLAMP} = 0.047$]. In the carboplatin arm, patients with tumours showing intermediate telomeric NtAI and AiCNA had higher ORR [54% (C) versus 20% (D), $P_{NtAI,intermediate} = 0.03$; 62% (C) versus 33% (D), $P_{AiCNA,intermediate} = 0.076$]. Patients with high AiCNA and PGA had shorter PFS in the carboplatin arm [3.4 months (high) versus 5.7 months (low/intermediate); and 3.8 months (high) versus 5.6 months (low/ intermediate), respectively; $P_{interaction/AiCNA} = 0.027$, $P_{adj.interaction/AiCNA} = 0.125$ and $P_{interaction/PGA} = 0.053$, $P_{adj.interaction/PGA} = 0.176$], whilst no difference was observed in the docetaxel arm.

Conclusions: Patients with tumours lacking HLAMP and demonstrating intermediate CIN-measurements formed a subgroup benefitting from carboplatin relative to docetaxel treatment within the TNT trial. This suggests a complex and paradoxical relationship between the extent of genomic instability in primary tumours and treatment response in the metastatic setting.

Key words: metastatic triple negative breast cancer, carboplatin, genomic instability, allelic imbalance

INTRODUCTION

The TNT trial (NCT00532727), a phase III, open label, randomised clinical trial compared carboplatin (C) with docetaxel (D) in patients with recurrent locally advanced or metastatic triple negative breast cancer (TNBC) or with

recurrent locally advanced or metastatic disease in germline *BRCA1/2* mutation carriers, irrespective of Estrogen receptor/Progesterone receptor/HER2 status. TNT trial patients with germline *BRCA1/2* mutations had a significantly better objective response rate (ORR) to carboplatin and showed improved progression-free survival (PFS) with this agent.¹ As some TNBC patients without known germline defects of *BRCA1/2* benefit from platinum-based chemotherapy, biomarkers that better predict treatment response for this subgroup of patients are urgently required.^{2,3}

Most TNBCs display highly aberrant genomes as a consequence of defects in DNA damage response (DDR)

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pathways. In \sim 35% of TNBCs, this increased genomic instability can be explained by functional inactivation of BRCA1/2,³ leading to homologous recombination deficiency (HRD).⁴ Using a range of platforms, including array comparative genomic hybridisation (aCGH),⁵ SNP-arrays,⁶⁻⁹ targeted sequencing panels¹⁰⁻¹² and whole genome sequencing,^{3,10,12,13} measures of unique patterns of chromosomal instability (CIN) have been developed to identify 'BRCAness'¹⁴ and HRD, which potentially identify sensitivity to DDR-targeting drugs compared with other standards of care. Such measures are sometimes referred to as 'genomic scars' and include mutational and rearrangement signatures. In the neoadjuvant setting, these 'genomic scars' have been shown to carry clinically relevant information for platinum-based chemotherapy response in TNBC patients.^{2,9,11} However, their value for patients with advanced disease is still debatable. High levels of HRD were associated with platinum response in the single-agent platinum TBCR009 study,¹⁵ whilst the Myriad HRD score² was not specifically associated with improved carboplatin ORR or PFS compared to docetaxel in the TNT trial.¹

Here, we have quantitatively assessed a suite of nine CINmeasurements based on genome profiles of primary tumours from the TNT trial to identify a wider patient subgroup benefitting from carboplatin over docetaxel. We compared their prevalence to the patient's pathogenic germline and somatic *BRCA1/2* mutation and *BRCA1* promoter methylation status. Then, we asked whether a primary tumour's degree of genomic instability has predictive value with regards to treatment response of the metastatic disease and whether prediction was selective of carboplatin response. As a result, biomarker defined subgroups of patients for whom platinum-based treatment may be selectively beneficial in the metastatic setting were deciphered.

PATIENTS AND METHODS

We analysed genome-wide allele-specific copy number profiles from 135 TNT trial patients (NCT00532727)¹ using Illumina HumanOmniExpress 24 SNP-arrays. The cohort included 131 (97%) TNBC cases and 4 ER+ *BRCA1/2* mutation carriers. Cases were categorised as: (i) germline or somatic *BRCA1/2* mutation carriers without *BRCA1* promoter methylation (n = 20); (ii) *BRCA1* methylated cases without *BRCA1/2* mutations (n = 19); (iii) *BRCA1/2* wild-type cases (n = 75). Germline and somatic *BRCA1/2* mutated cases were grouped together, as no statistically significant different chromosomal instability patterns were observed. Samples with ambiguous *BRCA1/2* deficiency status (n = 21) were excluded when the associations of CIN-measurements with *BRCA1/2* mutation and *BRCA1*

The majority of the analysed *BRCA1/2* mutated and *BRCA1* promoter methylated cases were associated with loss of heterozygosity (LOH) 19/20 (95%) of the *BRCA1/2* mutated cases, 17/19 (89.5%) of the *BRCA1* methylated cases. Three cases without LOH associated had moderate to low tumour purity (60%, 49% and 27%), however, the



Figure 1. CONSORT diagram showing the number of evaluable primary tumour samples. Samples with ambiguous *BRCA1/2* deficiency status (n = 21) were excluded when the associations of CIN-measurements with *BRCA1/2* mutation and *BRCA1* methylation status were examined.



Figure 2. Overview of the characterisation of the CIN-measurements among the primary tumour samples (n = 135).

(A) Frequency of copy number gains and losses across the whole genome in the TNT copy number subset (n = 135). CNAs were determined based on ASCAT copy number estimates of the primary tumour samples. (B) Summary of the CIN-measurements of the primary tumour samples. The samples are ordered according to the PGA score within the HLAMP groups. The bar plot shows the PGA values for each sample. The coloured bars display the association with *BRCA1/2* mutation and *BRCA1* methylation status: burgundy - BRCA1/2 mutation, dark blue - BRCA1 methylation, green - both *BRCA1/2* mutation and *BRCA1* methylation. The displayed covariates include HLAMP group, Shannon diversity index group, *BRCA1*-like status and HRD status. NtAI, AiCNA, AbCNA, CnLOH values are displayed as z-scores. Tumour purity (estimated by ASCAT algorithm) is shown as quartiles (0% representing the lowest quartile with samples of lowest tumour purity). *BRCA1/2* mutational status and *BRCA1* methylation status are displayed together with loss of heterozygosity status for the patients with mutation or methylation of *BRCA12* mutation. (C) Comparison of the distribution of the CIN-measurements among the *BRCA1/2* deficiency subgroups: AiCNA, AbCNA, CnLOH, NtAI, PGA are displayed on boxplots; HLAMP,

Myriad scores were >42, thus indicating HR deficiency. Two cases with *BRCA1* methylation without LOH exhibited only a moderate *BRCA1* methylation level, yet above the 10% threshold.¹

The clinical baseline characteristics of the whole TNT trial (n = 376) was comparable with the subset of patients with primary tumours (n = 196), and the study cohort (n = 135) (Figure 1, supplementary Table S1, available at https://doi.org/10.1016/j.annonc.2020.10.475) (for details see supplementary Materials, available at https://doi.org/10.1016/j.annonc.2020.10.475).

The copy number aberrations (CNAs) identified were used to derive the assessed quantitative measurements of CIN. Allelic imbalanced CNA (AiCNA), allelic balanced CNA (AbCNA), copy number neutral loss of heterozygosity (CnLOH) and number of telomeric allelic imbalances (NtAI) were calculated as previously described.⁹ Percentage of genome altered (PGA) and Shannon diversity index¹⁶ (SI) were quantified based on the copy number (CN) profiles. Based on the observed unimodal distributions of the continuous CIN-measurements, equally-sized tertiles (low, intermediate, high) were established. The BRCA1-like classifier¹⁷ was used to identify tumours with similar CN profiles to BRCA1 mutation carriers. We composed a novel score termed high-level amplifications (HLAMP), which was defined based on the presence of at least one of the top 5% of recurrently amplified genomic regions (cytobands) in this cohort. These cytobands were located on 1g, 8g and 10p chromosomal arms (including 1q21.1-24.1, 1q42.2-44, 8q11.21-24.3 and 10p15.3-14). The cohort was divided into three HLAMP groups: (i) samples lacking these amplifications were referred to as quiet; (ii) those with <50%amplified cytobands as low; (iii) >50% amplified cytobands as high HLAMP, which was chosen based on the observed distribution of the HLAMP score. Cut-off points for all continuous CIN-measurements and the HLAMP score were determined blinded to the patient outcome. The Myriad HRD score was used to divide the cohort into HR deficient and HR proficient subgroups, as defined in the previous report of the TNT trial.¹

Illumina TruSight Cancer v2 targeted sequencing panel¹⁸ was used to identify pathogenic germline variants of 97 genes associated with predisposition to cancer.

The association of CIN-measurements with ORR and PFS was assessed using logistic regression and restricted mean survival analysis, respectively. Detailed procedures are provided in the supplementary Material, available at https://doi.org/10.1016/j.annonc.2020.10.475. In the reporting process, the REMARK guidelines were followed where applicable (supplementary Table S2, available at https://doi.org/10.1016/j.annonc.2020.10.475).

RESULTS

Association between CIN features, BRCA1/2 mutation and BRCA1 promoter methylation

Of 376 patients randomised in the TNT trial, genome profiles of primary tumours from 135 patients were suitable for chromosomal instability assessment (see CONSORT diagram in Figure 1). Many of these tumours displayed highly aberrant genomes (Figure 2A), comparable with those in previously published series of TNBCs, such as the Guy's Hospital King's College London⁹ and METABRIC¹⁹ cohorts, when considering only those patients who, as in the TNT trial, developed metastases (supplementary Material, supplementary Figure S1, available at https://doi.org/10. 1016/j.annonc.2020.10.475). As the majority of the samples were TNBCs, characteristic CNAs including gains on 1q, 3q, 8q, 10p or 12p and losses on 4q, 5q or 8p chromosomal arms were seen²⁰ (Figure 2A).

We first established nine different CIN-measurements to capture the consequences of diverse defects in DDR mechanisms that could lead to excessive genomic instability in TNBCs (Figure 2B). These included our three previously published 'scores of chromosomal instability scarring' (SCINS) measures, namely AiCNA, AbCNA and CnLOH.⁹ We also quantified the PGA measure,²¹ a general proxy for the total amount of CNA across the whole genome; NtAI,⁶ that was shown to be indicative of DDR deficiency and platinum sensitivity in TNBC patients; and the aCGH-based BRCA1like classifier (BRCA1-like),⁵ that was shown to predict benefit from high-dose platinum-based chemotherapy. To measure the heterogeneity of the aberrant CN states, we introduced the SI.¹⁶ In addition, a novel score termed HLAMP was derived from the observed amplifications in the CN profiles within the TNT cohort. The distribution of the novel HLAMP score was confirmed in the SCAN-B,³ a TNBC cohort, and the TNBC subset of the METABRIC¹⁹ dataset. For both independent studies, tumours were selected when patients who, as necessary for TNT trial eligibility, developed relapse or distant metastasis (supplementary Material, supplementary Figure S2, available at https://doi.org/10. 1016/j.annonc.2020.10.475). To complete this compendium of CIN-measurements, the Myriad HRD score, as reported in the TNT study,¹ was also included.

Then, we ensured that the characteristics of the CINmeasurements of the ER+ *BRCA1/2* mutation carriers were consistent with the rest of the TNT study cohort (supplementary Figure S3, supplementary Table S3, available at https://doi.org/10.1016/j.annonc.2020.10.475).

Next, the extent of each of the nine CIN-measurements was compared between those TNT trial cases with pathogenic germline or somatic *BRCA1/2* mutations, *BRCA1*

Shannon index (SI), *BRCA1*-like and HRD status are displayed on stacked bar plots. The somatic *BRCA1* mutated cases are coloured in burgundy on the boxplots for the continuous variables, and the number of somatic *BRCA1* mutated cases in each subgroup of the categorical CIN-measurements are displayed next to the bar plots. *P* values of Kruskal–Wallis rank sum tests for AiCNA, AbCNA, CnLOH, NtAI and PGA and Fisher's exact tests for HLAMP, SI, *BRCA1*-like and HRD are shown. The *P* values are corrected for multiple comparisons by the Benjamini–Hochberg method.

AbCNA, allelic balanced CNA; AiCNA, allelic imbalanced CNA; CnLOH, copy number neutral loss of heterozygosity; HLAMP, high-level amplifications; HRD, homologous recombination deficiency; MET, *BRCA1* promoter methylated; MUT, *BRCA1/2* mutated; NtAI, number of telomeric allelic imbalances; PGA, percentage of genome altered; WT, *BRCA1/2* wild-type.



Figure 3. Objective response rate (ORR) in the carboplatin and docetaxel treatment arms across (A) NtAI, (B) AiCNA and (C) HLAMP subgroups (low, intermediate, high, as defined by tertiles for NtAI and AiCNA; and quiet, low, high for HLAMP). Ninety-five per cent confidence intervals, Fisher's exact test *P* values and percentage of subjects who responded to treatment in the group are shown on the bar plots. Kaplan—Meier survival plots showing the progression-free survival (PFS) in the carboplatin and docetaxel arms between the (D) HLAMP, (E) AiCNA and (F) PGA subgroups. *P* values of likelihood ratio tests for interaction between the CIN-measurements and treatment group are shown with and without adjustment for clinical covariates. *N* at risk (events) shows the number of subjects who remain in the analysis set at a given time point and the number of PFS events reported between time points.

methylated and BRCA1/2 wild-type cancers. Continuous CIN-measurements, such as NtAI, AiCNA, AbCNA, CnLOH and PGA scores displayed similar distributions across all three subgroups (Figure 2C). In alignment with our previous study,¹ HR deficient cases were clearly associated with the presence of BRCA1/2 mutation and BRCA1 promoter methylation (Kruskal–Wallis rank sum test P = 1.61e-17) (Figure 2C, supplementary Table S4, available at https://doi. org/10.1016/j.annonc.2020.10.475). The majority of tumours (76%, 103/135) were classified as BRCA1-like,¹⁷ including 80% (16/20) of BRCA1/2 mutated and 73% (14/ 19) of BRCA1 methylated cases. In 55% (11/20) of germline and somatic BRCA1/2 mutation carriers, tumours were categorised as quiet HLAMP, whilst 35% (7/20) and 10% (2/ 20) were grouped into the low and high HLAMP groups, respectively. Conversely, tumours with BRCA1 promoter methylation were most prominent in the low HLAMP subgroup (68%, 13/19), and were present at a significantly lesser extent in the quiet (3/19) and high (4/19) HLAMP categories (Fisher's exact $P_{adi} = 0.029$, Figure 2C, supplementary Table S4, available at https://doi.org/10. 1016/j.annonc.2020.10.475).

Association of germline variants in additional DDR-related cancer predisposition genes with CIN features

Pathogenic germline variants in DDR genes¹⁸ increase the risk of developing cancer and were identified in peripheral blood leukocyte DNA in 8/135 patients, not including *BRCA1/2* (supplementary Table S5, available at https://doi.org/10.1016/j.annonc.2020.10.475). The majority (62.5%, 5/8) of these cases were part of the low HLAMP group and were completely absent in the high group (Figure 2B). Moreover, tumours of patients with germline variants in DDR genes had high Shannon diversity score (62.5%, 5/8) and were more often classified as being *BRCA1*-like (75%, 6/8) or HR deficient (62.5%, 5/8), but small numbers limit conclusive interpretation of these data (Figure 2B).

CIN measures as biomarkers for chemotherapy response

Next we asked whether any of the nine established CINmeasurements carried prognostic or predictive value within the TNT trial.

Subgroup analyses indicated that patients with tumours of the intermediate NtAI subgroup had a significantly better response to carboplatin than docetaxel (ORR: 13/24 (54%) versus 4/20 (20%) $P_{NtAI,intermediate} = 0.03$) (Figure 3A, supplementary Table S6, available at https://doi.org/10. 1016/j.annonc.2020.10.475), and patients with tumours of the intermediate AiCNA subgroup also appeared to have better response to carboplatin than docetaxel (ORR: 13/21 (62%) versus 8/24 (33%) $P_{AiCNA,intermediate} = 0.076$) (Figure 3B, supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2020.10.475). For both, a trend for interaction between treatment group and AiCNA ($P_{interaction/AiCNA} = 0.060$) and NtAI ($P_{interaction/NtAI} = 0.083$)

was observed, which remained evident after adjustment for clinical covariates (for details see supplementary Material, available at https://doi.org/10.1016/j.annonc.2020.10.475), including *BRCA1/2* mutation status ($P_{adj.interaction/AiCNA} = 0.024$, $P_{adj.interaction/NtAI} = 0.016$). Whilst no significant interactions were found between treatment and any of the other tested CIN-measurements, a numerically higher ORR was observed in the carboplatin arm in the intermediate CnLOH group [ORR: 12/25 (48%) (C) versus 3/20 (15%) (D), $P_{CnLOH,medium} = 0.027$] and in the quiet HLAMP group [ORR: 14/25 (56%) (C) versus 7/24 (29%) (D), $P_{HLAMP,quiet} = 0.085$] (Figure 3C, supplementary Table S6, available at https://doi.org/10.1016/j.annonc.2020.10.475).

Patients with carcinomas in the quiet HLAMP group had an improved PFS with carboplatin versus docetaxel; and this association remained significant following adjustment for clinical variables including BRCA1/2 mutation [restricted mean PFS 6.1 months (C) versus 4.1 months (D), $P_{interaction/HLAMP} = 0.047$ and $P_{adj.interaction/HLAMP} = 0.033$; Figure 3D]. Trends for interaction of treatment with AiCNA ($P_{interaction/AiCNA} = 0.027$) and with PGA ($P_{interaction/PGA} =$ 0.053) were observed, showing the shortest PFS in cases with the highest PGA scores and in the high AiCNA subgroup in the carboplatin arm. However, these interactions were lost after adjustment for clinical covariates $(P_{adj.interaction/AiCNA} = 0.125, P_{adj.interaction/PGA} = 0.176)$ (Figures 3E and 3F). Sixty-seven of 135 primary tumours showed low to intermediate CIN burden based on AiCNA and PGA scores. Within this subgroup carboplatin responders were more prevalent (64%, 18/28) in comparison with docetaxel responders (39%, 9/23) (supplementary Figure S4, available at https://doi.org/10.1016/j.annonc.2 020.10.475).

Lastly, we excluded the four ER+ *BRCA1/2* mutation carriers from the outcome analyses, which showed that the results and derived conclusions remained essentially unaffected, supporting the plausibility of the inclusion the ER+ cases (supplementary Table S7, available at https://doi.org/10.1016/j.annonc.2020.10.475).

DISCUSSION

The FDA approved olaparib and talazoparib in 2018 for patients with confirmed germline *BRCA1/2* mutation,^{22,23} including those with TNBC, provided one of the first targeted therapy options for a subset of TNBC patients. However, the majority of TNBC patients lack germline *BRCA1/2* mutations and are treated with either standard-of-care chemotherapy or, in some circumstances, with immunotherapy.²⁴ By exploring the highly aberrant genomes of TNBC, several 'genomic scars' caused by disruptions of DDR mechanisms have been developed and carry some predictive value for treatment responses to chemotherapy in the neoadjuvant setting. However, the specificity of the prediction of platinum response, which is distinct from more generic chemotherapy response, is unclear in this setting.^{2,5-7,9,25}

The randomised, controlled TNT trial provided the opportunity to dissect genomic features and differentiate response to mechanistically highly distinct carboplatin and docetaxel treatments in metastatic or locally advanced TNBC. Indeed, we identified intermediate levels of allelic imbalanced CNAs, as measured by AiCNA, that focuses on genomic segments larger than 8 Megabase pair,⁹ and telomeric NtAl⁶ as being differentially associated with improved ORR in the carboplatin arm. Moreover, we noticed that in the TBCRC009 trial,¹⁵ in which metastatic TNBC patients were treated with platinum monotherapy, the highest levels of tumour response were observed in cases with medium levels of the 'genomic scar' assays developed by Myriad that measure large LOH events (HRD-LOH)⁷ and large-scale state transition events (HRD-LST),⁸ both of which have been associated with HR deficiency. Our analyses of the TNT trial allow the testing of the specific interaction of these measures with platinum, as opposed to mechanistically distinct docetaxel chemotherapy, and suggest that an intermediate CIN phenotype may represent a selective biomarker for platinum-based treatment response (as opposed to taxanes) in TNBC. Furthermore, AiCNA, and PGA, as well as the HLAMP scores, were associated with differential carboplatin effect as defined by PFS. As HLAMP was developed by analysis within this dataset, this result must be regarded as hypothesis-generating.

Response to carboplatin, a DNA cross-linking agent, is related to the cell's failure to successfully repair and survive the induced DNA damage. This prompted us to examine the utility of CIN-measurements as predictors of carboplatin response, as they can provide genomic evidence of disruption of DDR mechanisms reflected in acquired genome damage. In contrast, the cytotoxic effect of docetaxel is mediated by the stabilisation of normally dynamic microtubule assembly during mitotic cell division, leading to cell death. In agreement with our observations it was, therefore, not anticipated that 'genomic scars' of DNA repair deficiency should be selectively associated with docetaxel response.

Limitations of this study include the low resolution of the SNP-array platform that was used and the potential confounding factor of selecting a certain biological subset of TNBC. Although the ideal tissue resource for a predictive biomarker study of patients with metastatic/advanced breast carcinoma would be a set of metastatic biopsies, these were not regularly collected at the time of conduct of the TNT trial. There may, therefore, have been selection of DDR-related resistance by DNA damage inducing adjuvant therapy between primary diagnosis and trial entry with advanced disease. Hence, the biology of these recurrent tumours may not be adequately represented by archival primary invasive cancer tissues. Nevertheless, the copy number landscape of these archival primary tumours in the TNT trial did display distinctive CNAs, including known amplifications and losses that are characteristic of TNBCs occurring in patients who develop metastatic disease.

In summary, the somatic genome profiles of these series of TNT trial cases provide an opportunity to explore the

molecular features of TNBC and their association with treatment response of metastases to two single-agent chemotherapies with highly distinct mechanisms of action. The finding that intermediate levels of allelic imbalanced CNAs determined by AiCNA and NtAI are selectively predictive of carboplatin responses offers a potential approach to find specific associations to platinum response. Moreover, we found a signal that requires validation in other TNBC cohorts that patients with tumours displaying intermediate CIN scores, as well as those with tumours lacking HLAMP, have differential prediction of response. If our findings are substantiated they may potentially facilitate the prediction of a wider subgroup of TNBC patients who might be selected for platinum-based chemotherapy and support the potential integration of 'genomic scars' as a decision tool in clinical practice.

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DISCLOSURE

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