Extreme chromosomal instability forecasts improved outcome in ER-negative breast cancer: a prospective validation cohort study from the TACT trial


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Background: Chromosomal instability (CIN) has been shown to be associated with drug resistance and poor clinical outcome in several cancer types. However, in oestrogen receptor (ER)-negative breast cancer we have previously demonstrated that extreme CIN is associated with improved clinical outcome, consistent with a negative impact of CIN on tumour fitness and growth. The aim of this current study was to validate this finding using previously defined CIN thresholds in a much larger prospective cohort from a randomised, controlled, clinical trial.

Patients and methods: As a surrogate measurement of CIN, dual centromeric fluorescence in situ hybridisation was performed for both chromosomes 2 and 15 on 1173 tumours from the breast cancer TACT trial (CRUK01/001). Each tumour was scored manually and the mean percentage of cells deviating from the modal centromere number was used to define four CIN groups (MCD1–4), where tumours in the MCD4 group were defined as having extreme CIN.

Results: In a multivariate analysis of disease-free survival, with a median follow-up of 91 months, increasing CIN was associated with improved outcome in patients with ER-negative cancer (P trend = 0.03). A similar pattern was seen in ER-negative/HER2-negative cancers (P trend = 0.007).

Conclusions: This prospective validation cohort study further substantiated the association between extreme CIN and improved outcome in ER-negative breast cancers. Identifying such patients with extreme CIN may help distinguish good from poor prognostic groups, and therefore support treatment and risk stratification in this aggressive breast cancer subtype.

Key words: chromosomal instability, intratumour heterogeneity, breast cancer, modal centromere deviation, prognostic biomarker

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Introduction

Chromosomal instability (CIN) is characterised by an increased rate of gain or loss of whole or fractions of chromosomes, and is
a known driver of intercellular and intratumour heterogeneity [1, 2]. CIN can be classified into structural and/or numeral CIN. Structural CIN, such as duplications, deletions and gene amplifications, may be precipitated by telomeric dysfunction [3–5] or DNA replication stress [3, 6–8]. Numerical CIN, a gain or loss of whole chromosomes, may be precipitated by defects in the mitotic checkpoint [9, 10], the attachment of chromosomes to the mitotic spindle [11], centromere amplification [12], aberrant sister chromatid cohesion [13–15] and cytokinetic failure [16]. Increasingly it is recognised that structural CIN can precipitate numerical CIN and vice versa [17, 18]. An association between chromosomally unstable tumours and poor clinical outcome has been demonstrated in several solid tumour types [19–22]. This may be a consequence of increased intratumour heterogeneity and cellular diversity allowing tumours to adapt to various microenvironmental selection pressures [23]. This increased adaptive ability may also lead to drug resistance [24–26], and therefore disease progression [27, 28]. In addition, chromosomally unstable tumours can provide a substrate for the selection of copy number aberrations of genes associated with tumour proliferation, conferring a selective advantage, and therefore increased cellular fitness [29]. However, evidence is emerging that CIN may also have a negative impact on tumour fitness and growth. In yeast and mouse experimental studies, aneuploidy has been shown to result in decreased proliferation and cellular fitness [30, 31]. This may be related to the continuous gross numerical and structural chromosomal changes seen in CIN tumours resulting in the accumulation of deleterious genomic events affecting cancer cell survival [32]. It therefore follows that a threshold of CIN may exist, such that up to a certain level of instability, tumour growth, adaptation and progression may be enhanced, but that beyond this level cancer cell survival may be disadvantageous for tumour growth [23, 33].

To optimise breast cancer treatment and management, there is a need to identify and develop improved methods to predict prognosis, treatment benefit and risk of early relapse. Prognostic gene expression signatures, such as MammaPrint and Oncotype DX, have been shown to reflect tumour CIN status [21], and whilst these signatures are of prognostic value in oestrogen receptor (ER)-positive breast cancer, their value is limited in ER-negative breast cancer [34]. This may be related to the paradoxical non-monotonic relationship between extreme CIN and clinical outcome in this subgroup of breast cancer, which has thus far only been demonstrated in small retrospective studies. We have previously applied the CIN70 expression signature [19] to the gene expression profile of 265 patients with ER-negative breast cancer, and demonstrated that tumours with extreme CIN (upper quartile of CIN70 expression) were associated with improved prognosis relative to the other three quartiles [35]. This paradoxical relationship between increasing CIN and improved prognosis was also seen in gastric, ovarian and non-small-cell lung cancers [35]. We further validated these findings in a small discovery cohort study of 246 patients with primary breast cancer using a dual centromeric fluorescence in situ hybridisation (FISH) assay as a surrogate measurement of CIN [36]. In patients with ER-negative breast cancer, extreme CIN was associated with improved prognosis. Using this established assay with pre-defined CIN thresholds [36], we present here the largest prospective validation cohort study of patients with primary breast cancer in whom CIN has been measured and correlated with the clinical outcome [36]. This cohort of patients was chosen from within the TACT trial (CRUK01/001) where detailed clinical and follow-up data were available.

patients and methods

study population

The TACT trial (CRUK01/001) was a multi-centre, open-label, phase III clinical study. TMAs were created containing cores, 0.6 mm in diameter and 4 mm in thickness, selected from representative tumour areas as determined by a consultant breast histopathologist from haematoxylin and eosin-stained sections. For more details, see supplementary Appendix, available at Annals of Oncology online.

FISH analysis

Using previously established methods [36], dual-colour FISH was carried out using centromeric probes CEP2 and CEP15 labelled with spectrum red and green, respectively (Abbott Laboratories). Forty nuclei per core showing clear and discrete hybridisation signals for both chromosomes were scored manually.

statistical analysis

Using previously established methods [36], a CIN score was derived based on centromeric signals and by counting the numbers of centromeres in a minimum of 40 nuclei per core. The mean (chromosomes 2 and 15) percentage of cells deviating from the modal centromere number was used to define four CIN score groups (Modal Centromere Deviation groups 1–4: MCD1, 0%–15%; MCD2, 15%–30%; MCD3 30%–45%; and MCD4, >45%). To confirm the validity of this approach in identifying tumours with the most extreme CIN, the MCD score was compared with the Shannon Diversity Index (SDI) [37], using a previously established formula [37].

The primary clinical endpoint was invasive disease-free survival (DFS) as previously reported [38]. Correlation between patient characteristics and biomarkers was examined using Spearman’s rank correlation coefficient. For survival-related endpoints, Kaplan–Meier product limit curves were plotted and prognostic and predictive effects examined by the use of Cox proportional hazards regression models. Time-to-event analyses were stratified by the control regimen and included all patients with available biomarker data on an intention-to-treat basis.

For more details, see supplementary Appendix, available at Annals of Oncology online.

results

TMA cohort

Thirty TMAs were assessed for this current study, with each TMA containing an average of 100 tumours. All TMA slides were hybridised using centromeric probes for chromosomes 2 and 15 (CEP2 and CEP15). For some TMA slides, hybridisation was not successful and, in some cases, tumour cores were no longer present. CEP2 hybridisation was successful in 1994 cores for CEP2, and 1721 cores for CEP15. One thousand, three hundred and seventy-four hybridisation was successful in 1994 cores for CEP2, and 1721 cores for CEP15. For some TMA slides, hybridisation was not successful and, in some cases, tumour cores were no longer present. CEP2 hybridisation was successful in 1994 cores for CEP2, and 1721 cores for CEP15. One thousand, three hundred and seventy-four
population, with no statistically significant differences between the two (supplementary Table S1, available at *Annals of Oncology* online). Central ER, progesterone receptor (PR), and HER2 status testing was performed according to UK guidelines.

**patient characteristics and histopathological variables across MCD groups**

The distribution of the percentage of nuclei deviating from the modal centromere signals and allocation of MCD1 to MCD4 groups. (C) Representative FISH images of MCD1 to MCD4 tumours with centromeric probes for chromosomes 2 and 15 labelled in the pink and green, respectively, and DNA stained with DAPI, 40,6-diamidino-2-phenylindole.

**clinical outcome and MCD group in ER-negative breast cancer**

In our previous discovery cohort, patients with ER-negative cancer and extreme CIN had an improved prognosis [36]. In a univariate analysis of DFS, with a median follow-up of 91 months, patients with ER-negative cancer in MCD4 group had an improved outcome compared with the other MCD groups, but this was not statistically significant (HR = 0.61, 95% CI 0.29–1.26; P = 0.18) (Figure 3A). In contrast, patients with ER-positive cancer in the MCD4 group had worse outcome, although this was also not statistically significant (HR = 1.28, 95% CI 0.74–2.21; P = 0.38). An improved outcome in the MCD4 group was also seen in ER-negative/HER2-negative cancers in the MCD4 group (0.41, 0.14–1.17; P = 0.1) (Figure 3B). Using breast cancer-specific death as the outcome measure, a similar pattern was seen in ER-negative cancers (0.67, 0.29–1.54, P = 0.35) (Figure 3C). In a multivariate analysis in ER-negative patients, nodal status and increasing CIN were weakly positively correlated with increasing SDI, but this was not statistically significant on univariate analysis (data not shown). There was no association or trend found in ER-positive cancers.
Using $P$ trend as a statistical measure of significance (see statistical analysis section), there was evidence of an improved outcome with increasing MCD group in ER-negative ($P$ trend = 0.03) and ER-negative/HER2-negative cancers ($P$ trend = 0.007) (supplementary Table S2 and S3, available at Annals of Oncology online).

In determining whether MCD group had any influence on response to taxane therapy, and therefore improved DFS, MCD1/MCD2 and MCD3/MCD4 groups were combined (due to small patient numbers), and patients treated with the anthracycline/taxane-based regimen were compared with patients treated with the anthracycline-based regimen. In ER-negative cancers, the HR was 1.05 (0.72–1.53; $P$ = 0.80) in MCD1/MCD2 versus 0.89 (0.51–1.53; $P$ = 0.66) in MCD3/MCD4 group, demonstrating no difference between the combined MCD groups (supplementary Figure S2A, available at Annals of Oncology online). Similarly, there was no significant correlation found in the ER- and HER2-negative cancers (supplementary Figure S2B, available at Annals of Oncology online). Overall there was no evidence that DFS in the anthracycline/taxane treated group was dependent on the MCD group, but it is important to note that with such small patient numbers this analysis was insufficiently powered to detect an interaction and therefore a difference in the outcome between the two treatment arms.

**Discussion**

Increased CIN has been shown to be associated with poor prognosis in several tumour types [19–22, 40–42], suggesting that greater genomic instability may promote cancer cell growth and therefore tumour progression. However, both aneuploidy and CIN have also been shown to be disadvantageous for cancer cell survival and fitness [12, 43–47], such that extreme levels of genomic instability are associated with an improved clinical outcome [35, 36, 48]. The mechanisms behind the transition from advantageous to disadvantageous effects of CIN, in terms of tumour growth and progression, are unknown, but they may involve overwhelming genomic instability and mutational burden coupled with impaired DNA repair ability [48]. Both aneuploidy and CIN are features of malignant tumours, which may represent potential targetable phenotypes for anti-cancer therapy [49–51], and identifying such tumour properties may help guide therapeutic intervention.

In our initial discovery cohort study of 246 patients, extreme CIN was associated with improved long-term survival in ER-negative cancers (HR = 0.0827, 95% CI 0.0097–0.7066, $P$ = 0.0228) [36]. In this prospective validation cohort study, representing 1173 patients, we further substantiate this finding by identifying

| Table 1. Clinicopathological characteristics and the distribution of histopathological variable across MCD groups |
|-----------------|--------|--------|--------|--------|--------|--------|--------|--------|
|                  | Study cohort | MCD1 | MCD2 | MCD3 | MCD4 | Rs | P-value |
| N (%) | N (%) | N (%) | N (%) | N (%) |
| Number of patients | 1173 | 354 (30.2) | 447 (38.1) | 283 (24.1) | 89 (7.7) | 0.03 | 0.34 |
| Median age (range) | 49 (24–88) | 48 (25–88) | 49 (28–67) | 49 (25–70) | 49 (27–75) | 0.03 | 0.38 |
| <40 years | 216 | 66 (30.6) | 77 (35.7) | 58 (26.9) | 15 (6.9) |
| ≥40 years | 957 | 288 (30.1) | 370 (38.7) | 225 (23.5) | 74 (7.7) | 0 | 0.80 |
| Involved lymph nodes | 0 | 242 | 61 (25.2) | 105 (43.4) | 59 (24.4) | 17 (7) |
| 1–3 | 524 | 181 (34.5) | 191 (36.5) | 114 (21.8) | 38 (7.3) |
| 4+ | 407 | 112 (27.5) | 151 (37.1) | 110 (27) | 34 (8.4) | 0.03 | 0.38 |
| Tumour size | 0–2 cm | 311 | 103 (33.1) | 124 (39.9) | 63 (20.3) | 21 (6.8) |
| 2–5 cm | 771 | 224 (29.1) | 294 (38.1) | 195 (25.3) | 58 (7.5) |
| >5 cm | 91 | 27 (29.7) | 29 (31.9) | 25 (27.5) | 10 (11) | 0.05 | 0.07 |
| Grade | I | 53 | 28 (52.8) | 16 (30.2) | 4 (7.6) | 5 (9.4) |
| II | 378 | 137 (36.2) | 144 (38.1) | 76 (20.1) | 21 (5.6) |
| III | 742 | 189 (25.5) | 287 (38.7) | 203 (27.4) | 63 (8.5) | 0.15 | <0.001 |
| ER status | Negative | 429 | 93 (21.7) | 179 (41.7) | 121 (28.2) | 36 (8.4) |
| Positive | 744 | 261 (35.1) | 268 (36) | 162 (21.8) | 53 (7.1) | −0.09 | 0.003 |
| PR-status | Negative | 579 | 134 (23.1) | 232 (40.1) | 160 (27.6) | 53 (9.2) |
| Positive | 594 | 220 (37) | 215 (36.2) | 123 (20.7) | 36 (6.1) | −0.16 | <0.001 |
| HER2 status | Negative | 886 | 305 (34.4) | 326 (36.8) | 193 (21.8) | 62 (7) |
| Positive | 287 | 49 (17.1) | 121 (42.2) | 90 (31.4) | 27 (9.4) | 0.16 | <0.001 |
| Treatment | Control | 588 | 169 (28.7) | 241 (41) | 136 (23.1) | 42 (7.1) |
| FEC-T | 585 | 185 (31.6) | 206 (35.2) | 147 (25.1) | 47 (8) | N/A |
a statistically significant trend in improved prognosis with increasing CIN, as measured by the MCD group, using identical thresholds and methodology to define the MCD groups. However, our study is not without limitations. Hybridisation for chromosomes 2 and 15 was performed on the same slide for the majority of tumours but in some cases, for example, if hybridisation for one of the chromosomes failed, a different slide from the same tumour block was used. Given the existence of intratumour and intercellular heterogeneity, different tumour nuclei may have been scored for the same tumour. In addition, whilst CIN is a genome-wide phenomenon, our MCD FISH-based assay relied upon the signal from only two centromeres, limiting our ability to fully characterise CIN in each tumour. Nevertheless, this was sufficient enough to validate our hypothesis in the discovery cohort, which assessed the same two centromeres.

The development of gene expression-based signatures has aided the assessment of prognosis and risk of disease relapse in certain breast cancer subtypes, in particular ER-positive tumours [52, 53]. However, there remains a need to supplement existing, and identify new, prognostic signatures in ER-negative breast tumours [36]. In view of the potential prognostic value of tumour CIN status, the development of reproducible and efficient techniques to assess the CIN status in tumour samples may be of significant benefit. Using defined thresholds for CIN, such as MCD group, may help distinguish between good and bad...
prognostic groups, supporting both treatment and risk stratification in breast cancer patients. In addition, stratification of ER-negative breast cancer patients by the MCD group may help identify a cohort of patients with an improved outcome, which may be important to take into account when assessing therapeutic response and clinical outcome, especially in the context of clinical trials.

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disclosure

The authors have no disclosures to declare.

references

Inhibition of the phosphoinositide 3-kinase pathway for the treatment of patients with metastatic \emph{metaplastic} breast cancer

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\textbf{Background:} Mesenchymal/metaplastic breast cancers (MpBCs) are often triple-negative (TNBC), and chemo-refractory, and can harbor phosphoinositide 3-kinase (PI3kinase) alterations; thus, therapy with mTor inhibitors may demonstrate activity.

\textbf{Patients and methods:} Patients with mesenchymal/MpBC treated with temsirolimus-based regimens were evaluated. Mutational analyses [polymerase chain reaction (PCR)-based DNA sequencing method, mass spectrometric detection (Sequenom MassARRAY), or next-generation sequencing] as well as loss of phosphatase and tensin homolog (PTEN) (immunohistochemistry) were performed (archived tissue when available).

\textbf{Results:} Twenty-three patients (one of whom was on two separate trials) were treated using temsirolimus-containing regimens: temsirolimus alone ($n = 1$ patient) or combined with the following: liposomal doxorubicin and bevacizumab.