Survival and contralateral breast cancer in CHEK2 1100delC breast cancer patients: impact of adjuvant chemotherapy

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Background: We assessed the sensitivity to adjuvant chemotherapy in cell cycle checkpoint kinase 2 (CHEK2) vs non-CHEK2 breast cancer patients by comparing the contralateral breast cancer incidence and distant disease-free and breast cancer-specific survival between both groups, stratified for adjuvant chemotherapy.

Methods: One Dutch hereditary non-BRCA1/2 breast cancer patient cohort (n = 1220) and two Dutch cohorts unselected for family history (n = 1014 and n = 2488, respectively) were genotyped for CHEK2 1100delC. Hazard ratios for contralateral breast cancer, distant disease-free and breast cancer-specific death for mutation carriers vs noncarriers were calculated using the Cox proportional hazard method, stratified for adjuvant chemotherapy.

Results: The CHEK2 mutation carriers (n = 193) had an increased incidence of contralateral breast cancer (multivariate hazard ratio 3.97, 95% confidence interval 2.59–6.07). Distant disease-free and breast cancer-specific death were similar in the first 6 years in mutation carriers compared with noncarriers, but diverted as of 6 years after breast cancer diagnosis (multivariate hazard ratios and 95% confidence intervals 2.65 (1.79–3.93) and 2.05 (1.41–2.99), respectively). No significant interaction between CHEK2 and adjuvant chemotherapy was observed.

Conclusions: The CHEK2 1100delC-associated breast cancer is associated with a higher contralateral breast cancer rate as well as worse survival measures beyond 6 years after diagnosis. No differential sensitivity to adjuvant chemotherapy was observed in CHEK2 patients.
Three different cohorts of women with invasive early breast cancer were included in this study. Overall inclusion criteria were: age at diagnosis of first breast cancer < 80 years, diagnosis of first breast cancer after 1970, no distant metastases at or within 3 months from primary diagnosis, no proven BRCA1 or BRCA2 mutation and follow-up data available.

The first cohort (n = 1220) consisted of breast cancer patients ascertained through the Rotterdam Family Cancer Clinic. All selected patients were tested negative for BRCA1 and BRCA2 mutations. We refer to this cohort as the ‘non-BRCA1/2’ cohort.

The second cohort (n = 1014, after exclusion of 64 patients already included in the non-BRCA1/2 cohort) consisted of patients enrolled in the ORIGO study, designed to investigate the percentage of BRCA1/2 mutations in an unselected breast cancer population. This study concerned a consecutive series of breast cancer patients. Data on contralateral breast cancer rate and survival for CHEK2 mutation carriers vs non-carriers have been previously described (de Bock et al, 2004). We refer to this cohort as ‘ORIGO’.

The third cohort was drawn from the Rotterdam Medical Oncology Tumour bank (RMOT) database (n = 2488, after exclusion of 114 patients already included in the non-BRCA1/2 cohort and 1 patient already included in the ORIGO cohort). The RMOT database included breast cancer patients mainly diagnosed in the South Western region of the Netherlands from whom fresh frozen breast tumour specimens were submitted to the Erasmus Medical Centre for routine ER and progesterone receptor (PgR) measurement between 1978 and 2004. We refer to this cohort as ‘RMOT’.

Part of the non-BRCA1/2 cohort (57%) and the entire ORIGO cohort were also included in the study of Weischer et al (2012), investigating survival and contralateral breast cancer risk in CHEK2 1100delC mutation carriers. For the current study, follow-up of the patients in both cohorts has been updated to July 2010.

For all eligible patients of the three cohorts, data on patient and tumour characteristics, treatment and follow-up were collected from the medical records. The study was approved by the Institutional Review Board.
treatment factors, including age at and year of diagnosis, cohort (non-BRCA1/2, ORIGO, RMOT), tumour size (T1, T2, T3 or T4, unknown), nodal status (negative, positive, unknown), hormone receptor status (negative, positive, unknown), differentiation grade (1, 2, 3, unknown), type of surgery (lumpectomy, mastectomy, none or unknown), adjuvant chemotherapy (yes, no, unknown), adjuvant hormonal therapy (yes, no, unknown) and radiotherapy (yes, no, unknown), and the models for distant disease-free and breast cancer-specific survival also for contralateral breast cancer as time-dependent variable. Tumour ER and PgR analyses were performed by ligand binding assay or enzyme-linked immunoassay. The cutoff for ER and PgR positivity was 10 fmol mg$^{-1}$ cytosolic protein or $>10\%$ immunopositive cells. A negative hormone receptor status was defined as negativity for both ER and PgR, and a positive hormone receptor status as positivity for ER and/or PgR. Hormone receptor status was considered as unknown if information on both ER and PgR status was lacking, or in case of negative ER and unknown PgR status.

The proportional hazard assumption was assessed by using an extended Cox model with time-depending covariables. A likelihood

| Table 2. Patient and tumour characteristics of cell cycle checkpoint kinase 2 (CHEK2) 1100delC mutation carriers and noncarriers |
|---------------------------------------------------------------|
| **Carrier** | **Noncarrier** |
| n | % | n | % | P-value |
| Number of patients | 193 | 4529 | 6.8 (0.6–31.9) | 7.2 (0.1–38.9) |
| Median follow-up in years | | | |
| Age at breast cancer diagnosis | | | |
| Median (years) | 50.0 | 51.4 | | 0.003 |
| Mean (years) | 50.1 | 52.8 | | |
| Range (years) | 22.5–78.0 | 18.7–79.9 | | 0.14 |
| <40 | 37 | 19.2 | 714 | 15.8 |
| 40–50 | 59 | 30.6 | 1326 | 29.3 |
| ≥ 50 | 97 | 50.3 | 2489 | 55.0 |
| Year of diagnosis | | | |
| <1990 | 58 | 30.1 | 1590 | 35.1 |
| 1990–2000 | 83 | 43.0 | 1996 | 44.1 |
| > 2000 | 52 | 26.9 | 943 | 20.8 |
| Synchronous contralateral breast cancer | | | |
| No | 189 | 97.9 | 4443 | 98.1 |
| Yes | 4 | 2.1 | 86 | 1.9 |
| T stage | | | |
| T1 | 95 | 51.9 | 2074 | 47.2 |
| T2 | 72 | 39.3 | 1886 | 42.9 |
| T3 | 11 | 6.0 | 264 | 6.0 |
| T4 | 5 | 2.7 | 168 | 3.8 |
| Unknown | 10 (5.2) | 137 | (3.0) | |
| N stage | | | |
| Negative | 105 | 57.1 | 2388 | 53.9 |
| 1–3 | 35 | 19.0 | 1096 | 24.7 |
| 4+ | 44 | 23.9 | 949 | 21.4 |
| Unknown | 9 (4.7) | 96 | (2.1) | |
| Differentiation grade | | | |
| 1 | 21 | 14.7 | 370 | 10.5 |
| 2 | 52 | 36.4 | 1155 | 32.6 |
| 3 | 70 | 49.0 | 2014 | 56.9 |
| Unknown | 50 (25.9) | 990 | (21.9) | |
| Hormone receptor status | | | |
| Negative | 9 | 5.5 | 759 | 19.3 |
| Positive | 157 | 94.5 | 3173 | 80.7 |
| Unknown | 30 (15.5) | 597 | (13.2) | |
| HER2/neu | | | |
| Negative | 73 | 76.0 | 1886 | 80.7 |
| Positive | 23 | 24.0 | 452 | 19.3 |
| Unknown | 97 (50.2) | 2191 | (48.3) | |
ratio test was used to test whether an extended model was significantly different compared with the proportional hazard model. The extended models for CHEK2 and tumour size were significant for both distant metastases ($P = 0.003$ and $P = 0.002$, respectively) and breast cancer-specific death ($P = 0.02$ and $P = 0.04$), indicating that the hazards for distant metastases and breast cancer-specific death by CHEK2 1100delC and tumour size were not proportional over time. Because the survival curves for distant disease-free and breast cancer-specific survival for CHEK2 mutation carriers and noncarriers were similar over the first 6 years, but started to diverge after 6 years (Figure 2A and D), we chose for an extended Cox model with tumour size as time-dependent variable and that contains a heaviside function (Kleinbaum and Klein, 2005), together with the CHEK2 1100delC variable, with a cutoff point of 6 years. The corresponding model then provided two hazard ratios for CHEK2 1100delC, one for the first 6 years and one for beyond 6 years after breast cancer diagnosis.

Analyses for contralateral breast cancer distant disease-free and breast cancer-specific survival were stratified for adjuvant treatment with chemotherapy (yes/no). Analyses for distant disease-free survival were also stratified for type of adjuvant chemotherapy (anthracycline-based/nonanthracycline-based) and adjuvant hormonal therapy (yes/no).

Interaction terms between CHEK2 1100delC mutation status and administration of adjuvant chemotherapy (yes/no) for the different end points were calculated. In addition, the prognostic impact of age at diagnosis, tumour size, nodal status, ER status, chemotherapy and hormonal therapy were analysed separately for CHEK2 1100delC and non-CHEK2 patients.

All analyses were performed using SPSS (version 20.0; SPSS, Inc., Chicago, IL, USA) and Stata (version 12; Stat Corporation, College Station, TX, USA). A two-sided $P<0.05$ was considered as statistically significant.

**RESULTS**

In total, 193 (4.1%) of the 4722 included female breast cancer patients tested positive for the CHEK2 1100delC mutation (Table 1). The percentage of CHEK2 1100delC mutations was highest in the non-BRCA1/2 cohort (6.3%, $n = 77$) vs 2.9% ($n = 29$) in the ORIGO and 3.5% ($n = 87$) in the RMOT cohort, respectively.

Patient and tumour characteristics of the 193 CHEK2 1100delC mutation carriers and the 4529 non-carriers are shown in Table 2. The median follow-up time was 6.8 years for mutation carriers and 7.2 years for noncarriers. The CHEK2 1100delC-associated breast cancer patients were significantly younger at diagnosis than the noncarriers (median 50.0 vs 51.4 years). In addition, mutation carriers were more often diagnosed with breast cancer after 2000 (26.9% vs 20.8%), probably reflecting the higher percentage of CHEK2 1100delC mutation carriers in the non-BRCA1/2 cohort, as patients in this cohort were more often diagnosed after 2000 (51%) compared with patients from the RMOT cohort (<1%) and ORIGO cohort (37%; data not shown in tables). Breast cancers in mutation carriers were more often hormone receptor positive compared with cancers in noncarriers (94.5% vs 80.7%). No significant differences in tumour size, nodal status, differentiation grade and her2 expression were observed between CHEK2 1100delC mutation carriers and noncarriers.

Types of treatment are shown in Table 3. Significantly less CHEK2-associated breast cancer patients received radiotherapy compared with the noncarriers (62.0% vs 71.3%). This observation could not be explained by differences in surgical therapy (lumpectomy vs mastectomy), TN stage or age at diagnosis, but might be partly explained by differences in the year of diagnosis between the two groups (see Table 2). Adjuvant chemotherapy was administered to 31% of the patients, mainly consisting of anthracycline-containing regimens, and 24% of the patients received adjuvant endocrine therapy, mainly consisting of tamoxifen. This was not significant different between CHEK2 mutation carriers and noncarriers.

**Contralateral breast cancer risk.** The incidence of metachronous contralateral breast cancer was higher in CHEK2 1100delC mutation carriers than in noncarriers, with a 10-year risk of 28.9% vs 8.5%, respectively (Table 4 and Figure 1). In a multivariate analysis, the risk of contralateral breast cancer remained higher in mutation carriers compared with noncarriers (HR 3.97, 95% CI 2.59–6.07).

If stratified for adjuvant chemotherapy, the increased contralateral breast cancer rate for CHEK2 1100delC-associated breast cancer patients was observed in both the group treated with and without chemotherapy (HR multivariate 6.95, 95% CI 3.21–15.00 and 3.15, 95% CI 1.86–5.35, respectively). Although the HR was higher for...

| Table 3. Types of treatment in CHEK2 1100delC mutation carriers and noncarriers |
|-----------------|--------------|--------------|--------------|---|
| **Surgical therapy** | | | | |
| None | 0 | 0 | 10 | 0.2 | 0.80 |
| Lumpectomy | 88 | 47.1 | 2087 | 46.4 |
| Mastectomy | 99 | 52.9 | 2405 | 53.4 |
| Unknown | 6 | 27 | | |

| **Adjuvant systemic therapy** | | | | |
| None | 117 | 63.2 | 2646 | 59.3 | 0.42 |
| Only chemotherapy | 32 | 17.3 | 826 | 18.5 |
| Only hormonal therapy | 12 | 6.5 | 441 | 9.9 |
| Chemo- and hormonal therapy | 24 | 13.0 | 547 | 12.3 |
| Unknown | 8 | 69 | | |

| **(Neo)-adjuvant chemotherapy** | | | | |
| None | 129 | 69.4 | 3089 | 69.0 | 0.41 |
| Anthracyclines | 38 | 20.4 | 838 | 18.7 |
| Anthracyclines/taxanes | 0 | 0 | 68 | 1.5 |
| Other | 16 | 8.6 | 435 | 9.7 |
| Unknown type | 3 | 1.6 | 45 | 1.0 |
| Unknown if treated | 7 | 54 | | |

| **Adjuvant hormonal therapy** | | | | |
| None | 143 | 77.3 | 3392 | 76.0 | 0.46 |
| LHRH/ other | 6 | 3.2 | 84 | 1.9 |
| Tamoxifen | 33 | 17.8 | 812 | 18.2 |
| aromatase inhibitor | 1 | 0.5 | 29 | 0.6 |
| Sequential tamoxifen/aromatase inhibitor | 2 | 1.1 | 120 | 2.7 |
| Unknown type | 0 | 0 | 27 | 0.6 |
| Unknown if treated | 8 | 65 | | |

| **Radiotherapy** | | | | |
| No | 73 | 38.0 | 1298 | 28.7 | 0.006 |
| Yes | 119 | 62.0 | 3220 | 71.3 |
| Unknown | 1 | 11 | | |

Abbreviations: CHEK2 = cell cycle checkpoint kinase 2, LHRH = luteinising-hormone-releasing hormone.
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*Four patients received taxanes.
**Four patients were treated with radiotherapy of the ovaries or oophorectomy.
patients treated with adjuvant chemotherapy, no significant interaction between the CHEK2 1100delC mutation and adjuvant chemotherapy was observed (HR for interaction 2.15; \( P = 0.10 \); not shown in Tables). Stratifying for adjuvant endocrine therapy was not possible because only 19 CHEK2 1100delC-associated breast cancer patients were treated with hormonal therapy. No contralateral breast cancers were observed in these 19 patients.

**Survival.** The data regarding distant disease-free survival are shown in Table 5 and Figure 2A–C and regarding breast cancer-specific survival in Table 5 and Figure 2D–F. The Kaplan–Meier curves showed a nonsignificant decreased distant disease-free (\( P = 0.06 \)) and breast cancer-specific survival (\( P = 0.14 \)) for CHEK2-associated patients compared with non-CHEK2 patients (Figure 2A and D).

As can be observed in the Kaplan–Meier curves, the distant disease-free (Figure 2A) and breast cancer-specific survival (Figure 2D) were similar over the first 6 years after breast cancer diagnosis for CHEK2 1100delC mutation carriers and noncarriers and started to diverge after 6 years. In the first 6 years after breast cancer diagnosis, the multivariate hazard ratios for CHEK2 vs non-CHEK2 breast cancer patients were not significant for distant disease-free (1.08; 95% CI 0.83–1.42) and breast cancer-specific survival (1.00, 95% CI 0.71–1.41). Survival outcomes beyond 6 years after breast cancer diagnosis, however, were significantly worse for CHEK2 mutation carriers compared with noncarriers. Multivariate HRs for the period beyond 6 years after breast cancer diagnosis were 2.65 (95% CI 1.79–3.93) for distant disease-free and 2.05 (95% CI 1.41–2.99) for breast cancer-specific survival, respectively.

If we stratified our analyses for adjuvant chemotherapy, a significantly worse distant disease-free (\( P = 0.002 \)) and a trend for a worse breast cancer-specific survival (\( P = 0.05 \)) were observed for CHEK2-associated compared with non-CHEK2-associated breast cancer patients in the chemotherapy group (Figure 2C and F) that was not observed in the group not treated with chemotherapy (\( P = 0.52 \) and \( P = 0.40 \), respectively, Figure 2B and E). However, in both treatment groups, the group treated with and the group treated without adjuvant chemotherapy, we observed in the first 6 years after breast cancer diagnosis no significant differences in distant disease-free survival between CHEK2 mutation carriers and noncarriers (HR multivariate 1.41, 95% CI 0.91–2.16 and HR multivariate 0.98, 95% CI 0.69–1.38, respectively; Table 5), whereas beyond 6 years after breast cancer diagnosis, a significantly worse distant disease-free survival in CHEK2 mutation carriers compared with noncarriers was observed for both the adjuvant chemotherapy group (HR 4.54, 95% CI 2.41–8.65) and the group without adjuvant chemotherapy (2.31, 95% CI 1.39–3.83). Although the HRs were higher in the adjuvant chemotherapy group compared with the group without adjuvant chemotherapy, no significant interaction was observed between CHEK2 1100delC and adjuvant chemotherapy (HR interaction 1.51; \( P = 0.08 \); data not shown in tables).

If analysing patients treated with anthracycline-based chemotherapy and nonanthracyline-based chemotherapy separately, we observed in both groups a significantly worse distant disease-free survival beyond 6 years after breast cancer diagnosis for CHEK2 mutation carriers compared with noncarriers (HR multivariate 4.16, 95% CI 1.69–10.22 and 4.73 95% CI 1.85–12.09, respectively; Table 5).

In addition, stratifying for treatment with hormonal therapy (yes/no) had no major impact on the hazard ratio for disease-free survival. The CHEK2-associated breast cancer patients had a significantly worse distant disease-free survival than noncarriers beyond 6 years after diagnosis, independently of treatment with adjuvant hormonal therapy (HR 3.24, 95% CI 1.16–9.03) or without adjuvant hormonal therapy (HR 2.88, 95% CI 1.88–4.41; Table 5).

For breast cancer-specific survival a similar phenomenon was observed; only beyond 6 years after breast cancer diagnosis a significantly worse breast cancer-specific survival was observed for CHEK2 mutation carriers vs noncarriers in both adjuvant chemotherapy groups (i.e., the group treated with and without adjuvant chemotherapy). In the first 6 years after breast cancer diagnosis, we observed no significant difference in breast cancer-specific survival between CHEK2 mutation carriers and noncarriers (Figure 2D–F and Table 5).

In Table 6, multivariate HRs with respect to breast cancer-specific survival are presented for several prognostic factors, separately for CHEK2 and non-CHEK2 patients. Age at diagnosis, tumour size and nodal status appeared to be prognostic factors in both groups, although the prognostic value of age at diagnosis was not significant in the CHEK2 group. Interestingly, ER status appeared only to be a prognostic factor in the non-CHEK2 group.

### Table 4. Rate of metachronous contralateral breast cancera

| Carrier | Noncarrier | HR univariate | HR multivariate |
|---------|------------|---------------|-----------------|
| % (95% CI) | % (95% CI) | % (95% CI) | % (95% CI) |
| **All patients** | | | |
| 5 Years | 18.3 (11.7–28.0) | 3.8 (3.1–4.5) | <0.001 | 4.07 (2.66–6.21) | 3.97 (2.59–6.07b) |
| 10 Years | 28.9 (19.1–42.1) | 8.5 (7.2–9.7) | 3.18 (1.87–5.40) | 3.15 (1.86–5.35b) |
| **No adjuvant chemotherapy** | | | |
| 5 Years | 17.7 (10.3–29.3) | 4.2 (2.3–5.0) | <0.001 | 7.35 (3.42–15.08) | 6.95 (3.21–15.00b) |
| 10 Years | 24.1 (14.1–39.2) | 8.8 (7.3–10.2) | 4.16, 95% CI 1.69–10.22 and 4.73 95% CI 1.85–12.09, respectively; Table 5. |
| **Adjuvant chemotherapy** | | | |
| 5 Years | 21.9 (9.1–42.2) | 2.8 (1.7–4.2) | <0.001 | 7.35 (3.42–15.08) | 6.95 (3.21–15.00b) |
| 10 Years | 42.1 (22.6–77.4) | 7.4 (5.3–10.6) | 4.16, 95% CI 1.69–10.22 and 4.73 95% CI 1.85–12.09, respectively; Table 5. |

**Abbreviations:** CHEK2 = cell cycle checkpoint kinase 2; CI = confidence interval; HR = hazard ratio.

*a*For analyses regarding contralateral breast cancer (CBC), the non-BRCA1/2 cohort was excluded, because this cohort was probably enriched with patients with CBC.

*b*Adjusted for year of diagnosis, cohort and type of surgery. Other covariates, that is, age at diagnosis, tumour size, nodal status, hormone receptor status, differentiation grade, type of surgery, type of chemotherapy, hormonal therapy and radiotherapy, had an impact of <10% on the HR.
In this study, we observed a worse distant disease-free and breast cancer-specific survival for CHEK2 1100delC-associated compared with noncarrier patients, becoming apparent beyond 6 years after breast cancer diagnosis. Furthermore, we confirmed the higher incidence of contralateral breast cancer in breast cancer patients carrying the CHEK2 1100delC mutation, as observed in earlier studies (de Bock et al, 2004; Schmidt et al, 2007; Weischer et al, 2012). Both observations were not influenced by chemotherapy, suggesting no differential efficacy of chemotherapy in CHEK2 1100delC mutation carriers.

Interestingly, the worse distant disease-free and breast cancer-specific survival in CHEK2 1100delC mutation carriers was only observed beyond 6 years after breast cancer diagnosis, rather than immediately after breast cancer diagnosis. Although earlier studies also observed or suggested a worse (distant disease-free) survival in CHEK2 1100delC mutation carriers (de Bock et al, 2004; Schmidt et al, 2007; Weischer et al, 2012), none of these studies showed the phenomenon that we observed. In view of these discordant results, we further performed analyses in the three patient cohorts, and found that the trend for a worse distant disease-free and breast cancer-specific survival beyond 6 years was only observed in the RMOT cohort, whereas in the other two cohorts the survival became worse as of diagnosis (significant for the ORIGO cohort and nonsignificant for the non-BRCA1/2 cohort; data not shown). A reason for this different observation in the RMOT compared with the non-BRCA1/2 and ORIGO cohorts might be different patient characteristics in the RMOT cohort, such as the higher age at diagnosis (median 55 years compared with 44 and 52 years, respectively) and earlier diagnose years, resulting in a lower percentage of patients treated with adjuvant chemotherapy (19% vs 52% and 37%, respectively) and adjuvant hormonal therapy (14% vs 36% and 35%, respectively) and other types of treatment (more often CMF and less often anthracycline-based or taxane-based regimens). As adjuvant hormonal therapy is generally given for a period of 5 years, it can be hypothesised that the worse survival beyond 6 years in CHEK2-associated breast cancer patients might be an effect of the hormonal therapy. However, evidence for this hypothesis is not found in this study as we observed in both groups (i.e., the group treated with and the group treated without adjuvant hormonal therapy) a worse survival in CHEK2 mutation carriers compared with noncarriers more than 6 years after breast cancer diagnosis. It can also be hypothesised that the worse distant disease-free and breast cancer-specific survival for CHEK2 mutation carriers after 6 years is caused by the higher incidence of contralateral breast cancer in this group. However, this worse distant disease-free and breast cancer-specific survival remained if we censored at time of metachronous contralateral breast cancer and/or local recurrences (data not shown) and if we included contralateral breast cancer as time-dependent covariable in the extended Cox models, indicating that the observed worse survival measures are not only due to the higher incidence of contralateral breast cancer and/or local recurrences in the CHEK2 group. As the

Figure 1. Contralateral breast cancer rate for CHEK2-positive (green line) and CHEK2-negative patients (blue line) in (A) all patients, (B) patients not treated with systemic therapy and (C) patients treated with systemic therapy. *Patients with synchronous contralateral breast cancer and patients from the non-BRCA1/2 cohort were excluded for this analysis regarding metachronous contralateral breast cancer. #In (B and C), stratified for adjuvant chemotherapy, one additional carrier and four noncarriers for whom treatment with adjuvant chemotherapy was unknown were excluded. The full colour version of this figure is available at British Journal of Cancer online.
worse distant disease-free and breast cancer-specific survival cannot be explained by a reduced sensitivity to systemic therapy and by the higher incidence of contralateral breast cancer, we suppose that a different tumour biology might play a role. More research is needed into tumour biology of CHEK2 breast cancers.

The CHEK2 kinase acts as a tumour suppressor and plays a critical role in DNA damage repair. In response to double-strand DNA breaks, CHEK2 is activated by ataxia telangiectasia mutated (ATM) and involved in cell cycle control, DNA repair and apoptosis. The CHEK2 kinase phosphorylates TP53 and BRCA1, which upon BRCA1 represses the nonhomologous end-joining pathway and activates the homologous recombination repair pathway (Nevanlinna and Bartek, 2006; Tung and Silver, 2011; 2012). Based on the role of CHEK2 kinase in activating the repair mechanism of double-strand DNA breaks and the role of CHEK2 in the coordination of the mitotic spindle assembly, we hypothesised that CHEK2 1100delC mutation carriers might be more sensitive to chemotherapy. For BRCA1-associated breast cancer patients, there is growing evidence from in vitro studies on BRCA1-deficient cells and retrospective clinical studies on BRCA1 mutation carriers suggesting a higher sensitivity to chemotherapeutic agents causing double-strand DNA breaks, such as platinum or anthracyclines (Kriege et al, 2009; Bayraktar and Gluck, 2012). However, the results found in the current study do not support the hypothesis that CHEK2 mutation carriers have an increased sensitivity to chemotherapy, neither for anthracycline-based regimens nor for nonanthracycline-based regimens. In contrast, the observations on the worse distant disease-free and breast cancer-specific survival and the higher contralateral breast cancer rate in CHEK2 mutation carriers being most pronounced in the group treated with chemotherapy suggest that CHEK2 mutation carriers are rather less than more sensitive to chemotherapy compared with noncarrier breast cancer patients. However, as the interaction between CHEK2 and adjuvant chemotherapy is not significant, we cannot prove a decreased sensitivity to adjuvant chemotherapy in CHEK2 breast cancer patients. It might be that differences in patient and tumour characteristics between the groups treated with and without adjuvant chemotherapy may have influenced the results of the analyses stratified for adjuvant chemotherapy. Patients treated with adjuvant chemotherapy were younger at diagnosis, were more often diagnosed after 2000 (anthracycline-based chemotherapy being common), had a larger tumour size, had more often node positive and hormone receptor negative tumours and were more often treated with mastectomy and hormonal therapy than patients not treated with adjuvant chemotherapy. It might be that especially younger or node-positive CHEK2-associated breast cancer patients have a worse prognosis compared with noncarriers.

The increased risk of contralateral breast cancer in CHEK2 1100delC mutation carriers was observed in patients treated with adjuvant chemotherapy as well as in patients not receiving adjuvant chemotherapy. A factor to take into account hereby is radiotherapy, as it was suggested that CHEK2 mutation carriers might be more sensitive to ionising radiation (Broeks et al, 2004, 2007; Mellemkjaer et al, 2008). However, in multivariate analyses we found an increased risk of contralateral breast cancer in both patients treated with (HR 4.12 95% CI 2.49–6.83) and without radiotherapy (HR 3.17 95% CI 1.36–7.35; data not shown).

The strengths of our study include the relatively large sample size, the known tumour and treatment data and the homogeneous population from one country. To our knowledge, this is in fact the largest study on survival in CHEK2 1100delC breast cancer patients with available treatment data enabling to investigate the effect of adjuvant chemotherapy in this data set.

We are however aware of some limitations. First, this is a retrospective study and consequently treatment was provided on indication, according to the Dutch guidelines existing for the respective treatment years. Although treatment guidelines have changed over time, in general, patients with the worst prognosis (based on age at diagnosis and tumour stage/characteristics) received adjuvant chemotherapy. This probably is the reason why patients receiving adjuvant chemotherapy had a worse distant disease-free survival (median 6.5 and 15.0 years for CHEK2 mutation carriers and noncarriers, respectively; Figure 2C) compared with patients not receiving adjuvant chemotherapy (11.0 and 16.8 years for mutation carriers and noncarriers, respectively; Figure 2B).

Another possible limitation is longevity bias in the non-BRCA1/2 group, developed by selection of preferably long-living patients.

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### Table 5. Distant disease-free and breast cancer-specific survival

|                      | Univariate HR (95% CI) | Multivariate HR (95% CI) | Univariate HR (95% CI) | Multivariate HR (95% CI) |
|----------------------|------------------------|--------------------------|------------------------|--------------------------|
| **Distant disease-free survival** |                        |                          |                        |                          |
| All patients         | 1.02 (0.78–1.33)       | 1.08 (0.83–1.42)         | 2.15 (1.45–3.18)       | 2.65 (1.79–3.93)         |
| No adjuvant chemotherapy | 0.90 (0.64–1.27)    | 0.98 (0.69–1.38)         | 1.98 (1.19–3.28)       | 2.31 (1.39–3.83)         |
| Adjuvant chemotherapy | 1.38 (0.90–2.13)       | 1.41 (0.91–2.16)         | 3.64 (1.94–6.81)       | 4.54 (2.41–8.56)         |
| Anthracycline-based chemotherapy | 1.58 (0.92–2.72) | 1.59 (0.92–2.75)         | 3.64 (1.55–8.55)       | 4.16 (1.69–10.22)        |
| Nonanthracycline-based chemotherapy | 1.23 (0.61–2.50) | 1.16 (0.57–2.35)         | 3.76 (1.49–9.47)       | 4.73 (1.85–12.09)        |
| No adjuvant hormonal therapy | 1.01 (0.75–1.36) | 1.08 (0.80–1.45)         | 2.43 (1.59–3.71)       | 2.88 (1.88–4.41)         |
| Adjuvant hormonal therapy | 1.22 (0.66–2.23) | 1.36 (0.74–2.51)         | 2.79 (1.01–7.73)       | 3.24 (1.16–9.03)         |

| **Breast cancer-specific survival** |                        |                          |                        |                          |
| All patients         | 0.96 (0.68–1.35)       | 1.00 (0.71–1.41)         | 1.75 (1.20–2.54)       | 2.05 (1.41–2.99)         |
| No adjuvant chemotherapy | 0.92 (0.60–1.41) | 1.00 (0.65–1.53)         | 1.68 (1.03–2.73)       | 2.03 (1.25–3.32)         |
| Adjuvant chemotherapy | 1.10 (0.61–1.97)       | 1.05 (0.59–1.88)         | 2.33 (1.29–4.21)       | 2.55 (1.40–4.64)         |

Abbreviations: CI = confidence interval; HR = hazard ratio.

*Adjusted for tumour size and chemotherapy, and other covariates had an impact of < 10% on the HR.

*Adjusted for tumour size, and other covariates had an impact of < 10% on the HR.

*Adjusted for tumour size and hormonal therapy, and other covariates had an impact of < 10% on the HR.
Patients at risk
Carriers 193 109 40 10
Non-carriers 4529 2660 1168 319
Cumulative number of events
Carriers 0 52 75 82
Non-carriers 0 1167 1515 1603
Cumulative number of contralateral breast cancers
Carriers 4 29 35 37
Non-carriers 86 249 338 379

Patients at risk
Carriers 129* 78 30 5
Non-carriers 3089* 1921 1362 372
Cumulative number of events
Carriers 0 30 44 50
Non-carriers 0 808 1061 1603
Cumulative number of contralateral breast cancers
Carriers 4 21 24 25
Non-carriers 44 166 233 271

Patients at risk
Carriers 57* 27 7 2
Non-carriers 1386* 838 341 88
Cumulative number of events
Carriers 0 10 22 23
Non-carriers 0 203 344 373

Patients at risk
Carriers 129* 88 34 8
Non-carriers 3089* 2199 997 268
Cumulative number of events
Carriers 0 18 34 37
Non-carriers 0 495 772 859

Figure 2. Distant disease-free survival for CHEK2-positive (green line) and CHEK2-negative patients (blue line) in (A) all patients, (B) patients not treated with adjuvant chemotherapy and (C) patients treated with adjuvant chemotherapy, and breast cancer-specific survival for CHEK2-positive (green line) and CHEK2-negative patients (blue line) in (D) all patients, (E) patients not treated with adjuvant chemotherapy and (F) patients treated with adjuvant chemotherapy. *In (B, C, E and F) stratified for adjuvant chemotherapy, an additional 7 carriers and 54 noncarriers for whom treatment with adjuvant chemotherapy was unknown were excluded.
who were tested for BRCA1/2 mutations (long) after their breast cancer diagnosis. After stratifying for cohort, we indeed observed the longest survival within the non-BRCA1/2 cohort. However, we also performed left-truncated analyses, and the results were similar as those from the initial analyses, with a worse distant disease-free and breast cancer-specific survival beyond 6 years after diagnosis for CHEK2 mutation carriers compared with noncarriers (data not shown).

Finally, genetic testing for CHEK2 1100delC within the RMOT cohort was performed on DNA isolated from tumour tissue and not from peripheral blood. It is possible that some germline CHEK2 1100delC mutations were missed because the mutant CHEK2 allele was lost in the tumour instead of the wild-type allele (Sodha et al., 2002; Kilpivaara et al., 2003). Moreover, a detected CHEK2 1100delC mutation in the tumour may have been acquired somatically, being different from a germline mutation. Importantly, there were no discrepancies in DNA analyses test results of CHEK2 status observed when comparing blood and tumour sample test of 115 patients.

Currently, genotyping for CHEK2 is not yet performed at genetic testing for breast cancer as it is not considered to have clinical consequences. Moreover, implementation of CHEK2 genotyping (and especially presymptomatic testing of relatives) is complicated by the observation that in breast cancer families the breast cancer risk does not segregate completely with the presence or absence of the 1100delC mutation (The CHEK2 Breast Cancer Case-Control Consortium, 2004). However, the high incidence of contralateral breast cancer in CHEK2 1100delC mutation carriers found in the current study (10-year risk 24.1%) and in earlier studies is comparable to the incidence of contralateral breast cancer that we observed in BRCA1 and BRCA2 mutation carriers in an earlier study (10-year risk 25% and 20%, respectively) (Brekelmans et al., 2006) and in line with observations of other groups (10-year risk ranging between 13% and 37%) (Haffty et al., 2002; Metcalfe et al., 2004; Robson et al., 2005; Pierce et al., 2006; Graeser et al., 2009; van der Kolk et al., 2010). Based on this increased contralateral breast cancer rate, affected BRCA1/2 mutation carriers have a more intensive follow-up scheme for contralateral breast cancer detection including MRI or can opt for a risk-reducing contralateral mastectomy, even though it is unknown whether such a risk-reducing contralateral mastectomy can improve survival (Tung, 2011). In our opinion, this follow-up regimen should also be offered to CHEK2 1100delC mutation carriers after breast cancer diagnosis. In addition, as the population frequency of the CHEK2 1100delC mutation in the Netherlands is high, it may be relevant to discuss the implementation of genotyping for CHEK2 in breast cancer patients. Moreover, genetic testing for CHEK2 1100delC and may be other variants may also be considered in other populations where the mutation frequency is relevantly high such as Finland, Sweden, United Kingdom and North America (Hollestelle et al., 2010).

In conclusion, CHEK2 1100delC mutation carriers had a significantly higher incidence of contralateral breast cancer compared with noncarriers that may have consequences for follow-up strategies in clinical practice. Distant disease-free and breast cancer-specific survival were similar over the first 6 years after diagnosis, but became worse beyond 6 years after diagnosis for CHEK2 1100delC mutation carriers compared with noncarrier BC patients. No significant interaction between adjuvant chemotherapy and a CHEK2 1100delC mutation was observed regarding contralateral breast cancer or distant disease-free survival.

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**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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Chemotherapy and survival in CHEK2 breast cancer patients

BRITISH JOURNAL OF CANCER

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