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ORIGINAL REPORT

Polymorphisms in *XRCC1*, *XRCC3*, and *CCND1* and Survival After Treatment for Metastatic Breast Cancer

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ABSTRACT

Purpose
Single nucleotide polymorphisms (SNPs) in DNA repair and cell cycle control genes may alter protein function and therefore the efficacy of DNA damaging chemotherapy. We retrospectively evaluated the association of SNPs in DNA repair genes, XRCC1-01 (Arg399Gln) and XRCC3-01 (Thr241Met), and a cell cycle control gene, CCND1-02 (A870G), with progression-free survival (PFS) and breast cancer specific survival (BCSS) in patients with metastatic breast cancer (MBC).

Patients and Methods
SNPs in 95 patients with MBC enrolled onto one of five prospective clinical trials of high-dose chemotherapy and autologous stem-cell transplantation were evaluated using genotyping assays.

Results
For XRCC1-01, the hazard ratio (HR) for BCSS was 2.8 (95% CI, 1.60 to 5.00) and the HR for PFS was 2.0 (95%CI, 1.12 to 3.43). For XRCC3-01, the HR for BCSS was 2.0 (95%CI, 1.12 to 3.70) and the HR for PFS was 2.0 (95%CI, 1.09 to 3.59). For CCND1-02, the HR for BCSS was 1.8 (95%CI, 1.12 to 2.78) and the HR for PFS was 1.8 (95%CI, 1.15 to 2.85). Patients carrying one variant genotype (HR, 1.7; 95%CI, 1.07 to 2.82) or combinations of any two variant genotypes (HR, 4.7; 95% CI, 2.41 to 8.94) had significantly poorer BCSS compared with patients carrying zero variants. In multivariable analysis, XRCC1-01, presence of liver metastases, and bone metastases independently predicted BCSS. Combinations of any two variant genotypes were stronger independent predictors of BCSS and PFS than the presence of liver or bone metastases.

Conclusion
XRCC1-01, XRCC3-01, and CCND1-01 may be predictive of survival outcome in patients with MBC treated with DNA damaging chemotherapy.

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INTRODUCTION

DNA repair and cell cycle control mechanisms maintain genomic stability. When DNA damage occurs, DNA repair pathways, cell cycle arrest, and apoptosis may be activated. Radiation therapy and treatment with chemotherapeutic drugs, such as alkylating agents (cyclophosphamide) and anthracyclines (adriamycin), can damage DNA directly, through intercalation and also by lipid peroxidation and the formation of by-products, such as reactive oxygen species.[1,2] In vitro and in vivo studies have shown associations between alterations in DNA repair and cell cycle control genes and/or proteins and sensitivity to a broad range of drugs[3-9] and patient survival.[10-12] In addition, single nucleotide polymorphisms (SNPs) in genes involved in DNA repair and cell cycle control can affect repair efficiency,[13,14] increase cancer risk[15,16] and significantly alter patient responses to cancer treatments.[11,12,17,18] These include SNPs in the DNA repair genes X-ray repair cross complementing group 1 (*XRCC1*)[19-23] and x-ray repair cross complementing group 3 (*XRCC3*)[24-26] and in the cell cycle control gene, cyclin D1 (*CCND1*).[27-30]

XRCC1 is a base excision repair and single strand break repair protein that may play an important role in resistance to a variety of DNA damaging agents. In vitro, Chinese hamster ovary and breast cancer cells lacking functional XRCC1 protein are hypersensitive to a broad range of DNA damaging agents[31,32] and XRCC1 transcript levels correlate positively with cisplatin chemoresistance in cancer cell lines.[6]

A SNP in the *XRCC1* gene, consisting of a nucleotide substitution of G to A, designated as XRCC1-01, results in an arginine (Arg) to glutamine (Gln) amino acid change at codon 399. Although the functional consequences of this polymorphism are unknown, it may affect several protein-protein

interactions.[33] In vitro, tumor cell lines homozygous for the XRCC1-01 AA genotype are more resistant to a diverse array of anticancer and cytotoxic drugs compared with the AG or the GG (least resistant) variants.[3] These include alkylating agents such as busulfan, thiotepa, carboplatin, and cisplatin; DNA/RNA antimetabolites such as fluorouracil; and antimitotics such as vinblastine.[3]

XRCC3 is involved in the repair of double-strand breaks and interstrand crosslinks. Increased levels of *XRCC3* in cell lines and clinical samples correlate with increased resistance to DNA interstrand crosslinking agents, such as cisplatin and melphalan.[34] A SNP in the *XRCC3* gene, involving a C to T substitution designated as XRCC3-01 results in a threonine (Thr) to methionine (Met) amino acid change at codon 241. Although the functional consequences of this SNP are presently unknown, hypothetical modeling suggests that the amino acid substitution may remove a phosphorylation site and thus affect repair function.[35]

CCND1 is a key regulatory protein of the G1/S cell cycle checkpoint that monitors for unrepaired DNA damage. In vitro, increased expression of CCND1 correlates with increased resistance to cisplatin in head and neck[36] and colon cancer cells[37] and inhibition of CCND1 is associated with increased sensitivity to fluoropyrimidines and platinum compounds in pancreatic cancer cells.[38] CCND1 may be important in the development and/or progression of many cancers including bladder,[39,40] prostate,[41] breast[42,43], ovarian,[44] colorectal,[45] and lung.[27]

The SNP, CCND1-02 (A870G, exon 4), results in alternate splicing of CCND1 mRNA to generate the cyclin D1b variant.[46] Although the exact function of this variant is unknown, studies suggest a role in cancer development and progression. In vitro, the cyclin D1b variant coded by the A allele is oncogenic, localizing to the nucleus and resulting in a loss of contact inhibition and cellular transformation when compared to cyclin D1a.[46-48] CCND1-02 is associated with risk[29,49,50] and outcome in a number of cancers.[51-53]

Because SNPs in DNA repair and cell cycle control genes are associated with clinical outcome in other cancers, we hypothesized that they may be associated with survival in patients with metastatic breast cancer (MBC). Thus, we retrospectively analyzed SNPs in *XRCC1* (XRCC1-01), *XRCC3* (XRCC3-01), and *CCND1* (CCND1-02) and their association with progression-free survival (PFS) and breast cancer specific survival (BCSS) in 95 patients with MBC who had received high-dose chemotherapy (HDC) with autologous stem-cell transplantation (ASCT).

PATIENTS AND METHODS

Patient Population

Patient characteristics and HDC treatment regimens are presented in Table 1. Patients were selected from 134 patients enrolled onto one of five clinical trials of HDC with ASCT at Sudbury Regional Hospital (SRH; Sudbury, Ontario, Canada) between 1992 and 1997. Within this group, there were 102 stage IV patients who received HDC and ASCT and 95 patients for whom DNA was available for genotyping. Some information such as estrogen and progesterone receptor status was not available for all patients (not done or inconclusive in chart information). Information on tissue human epidermal growth factor receptor 2 (HER-2) status was unavailable.

All patients received two to four cycles of mobilization chemotherapy consisting of combinations of cyclophosphamide, adriamycin or epirubicin, and flurouracil, or fluorouracil, epirubicin, and mitoxantrone. HDC treatment groups and cumulative dosages are described in Table 1. In addition, six patients received a second HDC consisting of cyclophosphamide, mitoxantrone, and either carboplatin (n = 4) or vinblastine (n = 1). The clinical trials and study were approved by the research ethics board, SRH, Laurentian Site, Sudbury, Ontario, and informed signed consent was obtained from all patients.

Table 1. Clinical Characteristics and Treatment Regimens of Metastatic Breast Cancer Patients

Clinical Characteristic	Patients No.	Patients %
Total (stage IV)	95	
Age		
< 40	23	24
40-49	49	52
50-59	23	24
ER (n = 84)		
Negative	35	42
Positive	49	58
PR (n = 80)		
Negative	41	51
Positive	39	49
No. of metastatic sites		
UD, 1	54	57
≥ 2	41	43
Metastatic sites*		
Bone	53	56
Lung	32	34
Lymph node	26	27
Liver	14	14
Other	22	23
HDC regimen		
Mitox, cyclo, vin	27	28
Mitox, cyclo, carbo	21	22
Mitox, cyclo, paclitaxel	42	44
Thiotepa, cyclo, carbo	4	4
Mitox, cyclo	1	1

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; UD, unassessable disease; HDC, high dose chemotherapy; Mitox, mitoxantrone cumulative dose: 70 mg/m^2; Cyclo, cyclophosphamide cumulative dose 6 g/m^2; Vin, vinblastine cumulative dose: 12 mg/m^2; Carbo, carboplatin cumulative dose: 800 mg/m^2; Paclitaxel cumulative dose: 250-400 mg/m^2; thiotepa cumulative dose: 500 mg/m^2.
*Includes metastasis to more than one site.

Analysis of SNPs

DNA was extracted from cryopreserved, apheresis blood product, peripheral blood or bone marrow samples using the DNA Blood MiniKit (Qiagen, Mississauga, Ontario, Canada) following the manufacturer's protocol.

A candidate approach was used to select for nonsynonymous SNPs with moderate frequency in genes previously reported to be associated with chemotherapeutic sensitivity and cancer risk, progression, or survival. In addition, selection was based on studies that have indicated possible interactions between these various DNA repair mechanisms.[26,34,54,55] The National Cancer Institute SNP500 Cancer database[56] was used to obtain information on SNPs including target sequence, frequency estimates and referenced TaqMan assays (Applied Biosystems, Foster City, CA). The TaqMan assay consists of two primers for polymerase chain reaction amplification of the sequence of interest and two allele specific fluorescent probes. SNPs and primer and probe sequences are described in Appendix Table A1 (available online only). Genotyping was conducted using the ABI PRISM 7900HT sequence detection system (Applied Biosystems) according to the manufacturer's protocol. For quality control purposes, random samples were repeated for each SNP (n = 8% of all samples genotyped). Assignment of genotypes was performed independently by two investigators blinded to the survival end points.

Statistical Methods

Deviations from Hardy-Weinberg equilibrium for each SNP genotype were assessed using the Pearson χ^2 test. Survival curves were generated using the Kaplan-Meier product limit estimate of the survivorship function. Patients were observed for 111.7 months. PFS is defined as time (months) from study registration until documented progression of metastatic disease or censorship (had not progressed during follow-up time period). BCSS is defined as time (months) from study registration until death from metastatic disease or censorship (were alive at the end of the follow-up time period). Survival information was collected from hospital medical records, primary care physicians, and/or family.

Equality of survivorship functions was assessed using the log-rank test. The Cox proportional hazards regression model defined hazard ratios (HR) and 95% CIs.

Multivariable analysis using proportional hazards regression models used variables identified as significant in univariable analysis for both BCSS and PFS and that were available for the full data set. The estimates from these models provided HR and 95% CI adjusted for all variables in the model.

Statistical analysis was done using Stata version 8.0 (Stata Corporation, College Station, TX).

RESULTS

Patient Characteristics

The median age was 45 years (range, 19 to 56). During the follow-up period of 111.7 months, disease progression occurred in 91 patients (96%) and 91 patients (96%) died due to MBC. The time at risk for disease progression ranged from 1.6 months to 111.7 months. The time at risk for death ranged from 4.8 months to 111.7 months. For the total group (n = 95), the median PFS was 10.4 months and the median BCSS was 22.4 months.

Patients were assigned to four major groups based on differences in treatment regimens (Table 1). There were no significant differences in PFS and BCSS (Kaplan-Meier survival curves, log-rank analysis of survivorship function) between the major treatment groups, between patients receiving one or two HDC treatments, between clinical trials groups, or for patients treated with or without carboplatin (data not shown). In the reduced groups with known hormone receptor status, there were significant differences for PFS ($\chi^2 = 0.02$) and BCSS ($\chi^2 = 0.005$) by estrogen receptor status, (n = 84) but not by progesterone receptor status (n = 80; PFS, $\chi^2 = 0.27$; BCSS, $\chi^2 = 0.09$). Survival differences were significant for patients with metastatic site(s) that included liver (n = 14; PFS, $\chi^2 < 0.001$; BCSS, $\chi^2 < 0.001$). Patients with site(s) of metastases that included bone had significantly longer survival than any other metastastic sites (n = 53, PFS: $\chi^2 = 0.03$; BCSS: $\chi^2 = 0.02$).

Patients with more than one metastatic site (n = 41) had significantly poorer PFS ($\chi^2 = 0.002$) but not BCSS ($\chi^2 = 0.15$) compared with patients with unassessable disease or one metastatic site.

Genotypic Frequencies of Polymorphisms

The genotypic frequencies for each polymorphism are presented in Table 2. They are not significantly different than what would be expected if the population was in Hardy-Weinberg equilibrium and are similar to the frequencies reported in the National Cancer Institute SNP500 database.

BCSS and PFS

Significant differences in PFS and BCSS were observed for all three polymorphisms. For each SNP, analyses were done for each of the genotypes separately and also for the heterozygous genotype grouped with a homozygous genotype with similar median BCSS (Table 2). Kaplan-Meier survival curves for BCSS are shown in Figure 1A to C. For XRCC1-01, for the combined GG + AG genotypes versus the AA genotype, the HR was 2.8 (95% CI, 1.60 to 5.00) for BCSS and

Table 2. SNPs and Progression-Free Survival and Breast Cancer Specific Survival

Genotype	No.	Median PFS (months)	*P**	Age Adjusted HR	Age Adjusted 95%CI	Median BCSS (months)	*P**	Age Adjusted HR	Age Adjusted 95% CI
XRCC1-01†									
GG	38	11.6		1	reference	25.3		1	reference
AG	40	9.8		1.0	0.77 to 1.95	26.6		0.9	0.58 to 1.44
AA	16	8.8	.04	2.2	1.18 to 3.99	12.1	.001	2.7	1.45 to 5.00
GG + AG	78	10.8		1	reference	25.7		1	reference
AA	16	8.8	.02	2.0	1.12 to 3.43	12.1	.0003	2.8	1.60 to 5.00
XRCC3-01									
CC	37	10.8		1	reference	24.9		1	reference
CT	44	10.6		1.0	0.65 to 1.61	23.3		0.9	0.62 to 1.54
TT	14	8.2	.06	2.0	1.05 to 3.82	14.7	.05	2.0	1.06 to 3.82
CC + CT	81	10.7		1	reference	24.8		1	reference
TT	14	8.2	.02	2.0	1.09 to 3.59	14.7	.01	2.0	1.12 to 3.70
CCND1-02									
AA	25	11.6		1	reference	25.7		1	reference
AG	42	10.9		0.8	0.50 to 1.33	24.8		0.8	0.48 to 1.35
GG	28	8.3	.03	1.6	0.90 to 2.69	14.7	.03	1.5	1.89 to 2.67
AA + AG	67	11.1		1	reference	25.3		1	reference
GG	28	8.3	.01	1.8	1.15 to 2.85	14.7	.01	1.8	1.12 to 2.78

Abbreviations: SNP, single nucleotide polymorphism; BCSS, breast cancer specific survival; PFS, progression-free survival; HR, hazard ratio.
*Log-rank for comparison of Kaplan-Meier survival curves were derived from comparisons among all three genotypes or between two groups in the combined genotype model.
†XRCC1-01 could not be determined for one patient.

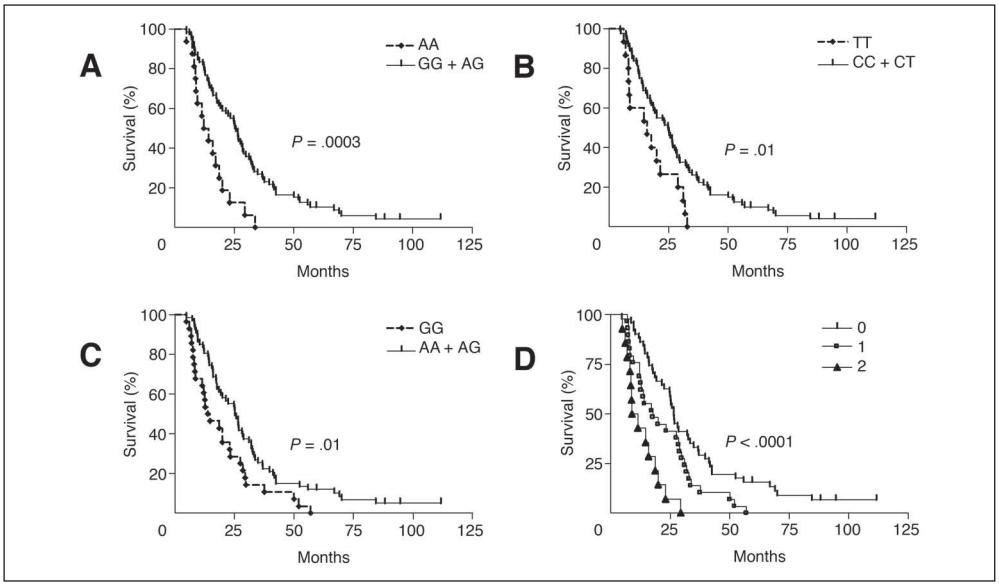

Fig 1. Kaplan-Meier breast cancer specific survival (BCSS) curves of (A) XRCC1-01 GG + AG versus AA genotypes ($P = .0003$); (B) XRCC3-01 CC + CT versus TT genotypes ($P = .01$); (C) CCND1-02 AA + AG versus GG genotypes ($P = .01$); and (D) number of variant genotypes zero (none), one (one of XRCC1-01, XRCC3-01, or CCND1-02), two (two of XRCC1-01, XRCC3-01, or CCND1-02). $P < .0001$ (for difference among groups). All P values are log-rank.

the HR was 2.0 (95% CI, 1.12 to 3.43) for PFS. For XRCC3-01, for the combined CC + CT genotypes versus the TT variant genotype, the HR was 2.0 (95% CI, 1.12 to 3.70) for BCSS and the HR was 2.0 (95% CI, 1.09 to 3.59) for PFS. For CCND1-02 for the combined AA + AG combined genotypes versus the GG genotype, the HR was 1.8 (95% CI, 1.12 to 2.78) for BCSS and the HR was 1.8 (95% CI, 1.15 to 2.85) for PFS.

In a Cox proportional hazards model that included bone and liver metastases (significantly associated with both PFS and BCSS in univariate analysis), XRCC1-01 and XRCC3-01 and the presence of liver metastases were strong independent predictors of BCSS. Although results for XRCC1-01 and CCND1-02 were not significant, they were suggestive of an effect for PFS. XRCC3-01 and the presence of liver metastases remained significant for PFS. Metastatic sites that included bone were protective and independently predicted better BCSS (Table 3).

Combination of Variant Genotypes and Survival

We explored the effect of increasing numbers of variant genotypes (XRCC1-01 AA, XRCC3-01 TT, CCND1-02 GG) on survival (Table 4). Highly significant differences for BCSS were found for patients carrying two variant genotypes ($P = .0001$, log-rank). Median BCSS decreased progressively for patients carrying zero, one, and two variant genotypes. The median PFS decreased significantly for patients with zero and one variant genotype and decreased slightly for patients carrying two of the variant genotypes. The HR for patients with two variant genotypes for BCSS was 4.7 (95% CI, 2.41 to 8.94) and for PFS was 3.6 (95% CI, 1.89 to 6.99). SNP combinations for the 14 patients with two variant genotypes were: six patients with XRCC1-01 (AA) and CCND1-02 (GG), two patients with XRCC1-01 (AA) and XRCC3-01 (TT), and six patients with XRCC3-01 (TT) and CCND1-02 (GG). There were no patients with all three of the variant genotypes. The strong association of increasing number of variant genotypes with BCSS is shown in Figure 1D. In a multivariable model, carrying two variant SNPs was a stronger independent predictor of BCSS than the presence of liver or bone metastases (Table 5).

Table 3. Multivariable Analysis of SNPs and Survival

Variable	PFS		BCSS	
	HR	95% CI	HR	95% CI
Bone metastases	0.68	0.44 to 1.05	0.60	0.39 to 0.93
Liver metastases	2.4	1.27 to 4.34	2.7	1.49 to 4.97
XRCC1-01	1.7	0.94 to 2.96	2.3	1.27 to 4.16
XRCC3-01	1.9	1.06 to 3.58	2.4	1.30 to 4.48
CCND1-02	1.5	0.94 to 2.51	1.6	0.97 to 2.54

Abbreviations: SNP, single nucleotide polymorphism; PFS, progression-free survival; BCSS, breast cancer specific survival; HR, hazard ratio.

DISCUSSION

Population-based studies have shown an association between SNPs involved in DNA repair and cell cycle control and an increased risk of breast,[20,57] prostate,[58,59] bladder,[60] skin,[61] lung,[62] and colorectal cancers.[63] Some of these polymorphisms are also associated with decreased survival after chemotherapy.[12,21,52,64]

In this study, we have shown that SNPs in two DNA repair genes, *XRCC1* and *XRCC3* and in a cell cycle control gene, *CCND1*, either alone or in combination were significantly associated with PFS and BCSS in a group of patients with MBC. Other studies have shown associations of XRCC1-01 and XRCC3-01 with survival and/or risk in non–small-cell lung cancer, colorectal, and laryngeal squamous cell cancer.[21,22,26,53] CCND1-02 was associated with survival differences in the large population based case-control Shanghai Breast Cancer Study in patients with stage III and IV breast cancer.[52]

To our knowledge, this is the first study to show that polymorphisms in *XRCC1* and *XRCC3* are associated with survival outcome of patients with MBC. These patients were treated with a variety of DNA damaging agents in the mobilization and HDC treatment regimens. Treatment regimens included alkylating agents such as carboplatin, cyclophosphamide, and thiotepa, which can form crosslinks between DNA strands, and anthracyclines, such as adriamycin, epirubicin, and

Table 4. Combination of Variant Genotypes and Survival

No. of Variant Genotypes	No. of Patients	PFS				BCSS			
		Duration (months)	Age Adjusted HR	Age Adjusted 95% CI	*P**	Duration (months)	Age Adjusted HR	Age Adjusted 95% CI	*P**
0	51	12.5	1	reference		26.6	1	reference	
1	29	8.3	1.8	1.09 to 2.83	.0001	17.7	1.7	1.07 to 2.82	< .0001
2	14	8.2	3.6	1.89 to 6.99		8.8	4.7	2.41 to 8.94	

Abbreviations: PFS, progression-free survival; BCSS, breast cancer specific survival; HR, hazard ratio.
*Log-rank for comparison of Kaplan-Meier survival curves, number of variant genotypes was modelled using indicator variable.

mitoxantrone, whose mechanism of action includes intercalation into DNA.[65,66] Treatment with fluorouracil during mobilization primarily inhibits thymidylate synthetase but also can damage DNA.[67] Antimitotics, such as vinblastine and paclitaxel, used in the HDC treatment of some of these patients have also been shown to damage DNA or be affected by repair polymorphisms.[3,68] Therefore, the levels and efficiency of DNA repair mechanisms may affect sensitivity to a variety of chemotherapeutic drugs.

High doses of DNA-damaging drugs were used in the treatment of these patients and it is not known whether the association of these SNPs with outcome will translate into the conventional treatment setting. However, the association of these SNPs with survival outcome in the treatment of other cancers at lower drug doses suggests that this is not a dose response effect. The lack of improvement in BCSS found in clinical trials of HDC with ASCT compared with conventional chemotherapy for women with MBC[69] may in part be due to these genetic variations in DNA repair and cell cycle control and their associated drug resistance. Presently, there are few prognostic/predictive factors for patients with MBC and treatment goals are directed toward palliation primarily using DNA damaging chemotherapy.[70,71] Direct association studies of SNPs within candidate genes may provide important clues concerning the role of genetic variation in treatment response and outcome. Future studies examining combinations of polymorphisms in genes involved in multiple DNA repair and drug metabolism pathways are required in order to establish the use of screening for multiple SNPs for cancer risk assessment and the selection of multimodality treatments.[72] In this study, we observed that combinations of two variant genotypes were stronger independent predictors of survival in patients than any single variant genotype or the presence of liver or bone metastases.

In this study, XRCC1-01, XRCC3-01, and CCND1-02 appear to be more strongly associated with BCSS than PFS. In an age-adjusted, multivariable model, which controlled for the presence of bone and liver metastases, both XRCC1-01 and XRCC3-01 remained strong independent predictors of BCSS. The results for CCND1-02 were suggestive of an effect but were not significant for both PFS and BCSS. While PFS is not as accurate an end point as BCSS, the PFS end point may provide information concerning the mechanism(s) by which these SNPs affect outcome and was therefore included in the analysis. In addition, in these patients, progression has already occurred after treatment in the adjuvant setting. As a result, other drug resistance mechanisms or new mutations may be attenuating the affect of these polymorphisms. Some of these SNPs may play a more important role in earlier stages of cancer. In a similar study, survival differences appeared stronger for XRCC1-01 for patients with stage III versus stage IV non–small-cell lung cancer.[21]

Table 5. Multivariable Analysis of Combined SNPs and Survival

Variable	PFS HR	PFS 95% CI	BCSS HR	BCSS 95% CI
Bone metastases	0.67	0.43 to 1.04	0.58	0.37 to 0.90
Liver metastases	2.3	1.25 to 4.11	2.5	1.41 to 4.59
1 variant genotype	1.7	1.07 to 2.81	1.9	1.13 to 3.03
2 variant genotype	2.7	1.39 to 5.42	3.8	1.96 to 7.45

Abbreviations: SNP, single nucleotide polymorphism; PFS, progression-free survival; BCSS, breast cancer specific survival; HR, hazard ratio.

Complete information concerning post-HDC treatment(s) for this group of stage IV patients was not available for this study although there were no planned treatments and any subsequent treatment for progression was palliative. Although survival differences in these patients are unlikely to be related to differences in post-HDC systemic or radiation treatment, the possibility cannot be definitively excluded.

Other studies have suggested an interaction between DNA repair SNPs and treatment outcome using platinum drugs.[21,22,73,74] We explored whether there was a differential effect of XRCC1-01, XRCC3-01, or CCND1-02 and carboplatin treatment by stratifying the data on ever receiving carboplatin treatment. The largest differential effect was seen for XRCC3-01. For risk of death, in the group of patients who had not received carboplatin, the XRCC3-01 TT genotype was associated with an age-adjusted HR 1.9 (95% CI, 0.93 to 3.98) whereas in the patient group who had received carboplatin, the XRCC3-01 TT genotype was associated with a HR of 2.6 (95% CI, 0.88 to 7.88). While suggestive of a possible differential effect, the interaction term in a Cox model that contained XRCC1-01 TT variant group, and ever receiving carboplatin treatment was not significant ($HR_{interaction\ term}$, 1.14; 95% CI, 0.33 to 3.88). However, we acknowledge that this small study had low power to detect an interaction.

As an early marker study, we have followed the reporting guidelines as outlined for tumor marker prognostic studies (Reporting Recommendations for Tumor Marker Prognostic Studies [REMARK]).[75] Limitations to this retrospective study include its small sample size and incomplete information concerning hormone receptor and tissue HER-2 status. Although the results presented in this study suggest that XRCC1-01 and XRCC3-01 may be prognostic markers as defined by REMARK guidelines (since the primary end point was survival), future studies examining the association of these markers in different therapies are required to determine their status as prognostic and/or predictive

markers.[75-77] The SNPs examined in this study may be associated with resistance and sensitivity to HDC with a variety of DNA damaging drugs and have separated patients into good and poor survival outcome groups. Large randomized trials examining these SNPs in patients with MBC treated with conventional doses of these drugs or treated with drugs that do not damage DNA may help classify whether these SNPs are predictive markers for responses to a specific therapy. The development of well-designed, larger prospective studies examining multiple SNPs in the conventional treatment setting for patients with primary and metastatic breast cancer may also further help define their role and value in the clinical setting.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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