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DNA Damage and Repair

Molecular Cancer Research

Biomarker Assessment of HR Deficiency, Tumor *BRCA1/2* Mutations, and *CCNE1* Copy Number in Ovarian Cancer: Associations with Clinical Outcome Following Platinum Monotherapy

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Abstract

The current study evaluated three biomarkers [homologous recombination deficiency (HRD), tumor BRCA1/2 (tBRCA) mutations, and CCNE1 copy-number variation (CNV)] in ovarian tumors from patients enrolled on the SCOTROC4 clinical trial for associations with outcome following carboplatin monotherapy. Ovarian tumors ($n = 250$), with high-grade serous (HGSOC) subgroup analysis ($n = 179$) were classified as HRD positive (HRD score ≥42 or tBRCA mutation) and as CCNE1 amplification positive (CCNE1 CNV score >2.4). Seventy-four (30%) tumors were HRD positive, including 34 (14%) with tBRCA mutations. Forty-seven (19%) were CCNE1 amplification positive, all of which were tBRCA wild-type. HRD and tBRCA, but not CCNE1 amplification, were significantly associated with CA125 complete response in the entire cohort (HRD, $P = 0.00015$; tBRCA $P = 0.0096$), and the HGSOC subgroup (HRD, $P = 0.0016$; tBRCA $P = 0.032$). HRD and lack of CCNE1 amplification were associated with improved progression-free survival (PFS) and overall survival (OS) in the full cohort and HGSOC subgroup (HRD, $P = 0.00021$; CCNE1 status $P = 0.038$). HRD remained significant for OS and PFS after adjusting for clinical factors, while CCNE1 status only remained significant for PFS. Patients with HRD-positive tumors had greater PFS and OS benefit from platinum dose intensification than HRD-negative tumors ($P = 0.049$ and $P = 0.035$, respectively). An alternative exploratory HRD score threshold (≥33 or tBRCA mutation) was also significantly associated with both PFS and OS in the HGSOC subset.

Implications: HRD, tumor BRCA1/2 mutations, and absence of CCNE1 amplification are associated with improved survival of ovarian cancer patients treated with platinum monotherapy and HRD-positive patients may benefit from platinum dose intensification. *Mol Cancer Res; 16(7); 1103–11. ©2018 AACR.*

Introduction

Defects in the homologous recombination (HR) pathway are associated with increased sensitivity to DNA-damaging agents and targeted agents, such as PARP inhibitors, across many cancer types. The most well-studied markers of HR pathway defects are mutations in *BRCA1* or *BRCA2* (*BRCA1/2*). For example, previous studies have shown that triple-negative breast cancer (TNBC) tumors and ovarian cancer tumors with *BRCA1/2* mutations show improved sensitivity to platinum-based chemotherapy relative to *BRCA1/2* wild-type tumors (1, 2). Similarly, ovarian cancer tumors with mutations in *BRCA1/2* have shown improved sensitivity to PARP inhibitors (3–5). However, defects in the HR pathway are not confined to mutations in *BRCA1/2* in ovarian cancer. Studies report HR pathway defects in as many as 50% of epithelial ovarian cancers, a third of which may be caused by something other than a mutation in *BRCA1* or *BRCA2* (6).

In order to improve the identification of tumors with HR pathway defects that are likely to respond to DNA-damaging agents, a three-biomarker measure of homologous recombination deficiency (HRD) has been developed. The HRD assay quantitates genomic instability in a tumor genome (7) based on three independent measures of genomic instability: loss of heterozygosity (LOH; ref. 8), telomeric allelic imbalance (TAI; ref. 9), and large-scale state transition (LST; ref. 10). Each individual measure has been shown to be associated with response to platinum-based therapy in either TNBC or ovarian cancer (9–11), and the combined score has been shown to be a better predictor of HRD than any of the individual scores (12).

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An HRD score threshold of 42 was recently developed in a cohort of breast and ovarian chemotherapy-naïve tumor samples with known *BRCA1/2* deficiency status (13). This threshold is used in combination with tumor *BRCA1/2* mutation status to differentiate tumors with HR deficiency (HRD positive; HRD score $\geq$42 or a tumor *BRCA1/2* mutation) from HR nondeficient tumors (HRD negative; HRD score < 42 and wild-type *BRCA1/2*). In an independent cohort, HRD positive was significantly associated with response to platinum-based treatment in TNBC (13).

Copy-number amplification of the cell-cycle regulator cyclin E1 (*CCNE1*) is observed only in tumors with wild-type *BRCA1/2* and has been associated with early primary treatment failure and reduced patient survival in ovarian cancer (14, 15). In a recent study, Etemadmoghadam and colleagues demonstrated that *CCNE1*-amplified ovarian tumors require the presence of functional BRCA1 protein and may be responsive to the proteasome inhibitor bortezomib (16). In addition, *CCNE1*-amplified ovarian xenograft models were observed to be sensitive to a combination of a CDK2 inhibitor and an AKT1 inhibitor in a high-throughput screen (17).

Here, we evaluated using a predefined analysis plan the association of three molecular biomarkers (HRD status using an HRD score of $\geq$42 or tBRCA mutation, *BRCA1/2* mutations, and *CCNE1* copy-number amplification) with clinical outcomes following monotherapy with the DNA-damaging agent carboplatin at primary presentation. This was done in a cohort of tumors from patients enrolled in the SCOTROC4 phase III trial of stage IC to IV epithelial ovarian carcinoma, primary fallopian tube carcinoma, or ovarian-type peritoneal carcinomatosis treated with platinum monotherapy, with or without dose intensification (18). Available clinical endpoints in this study included CA125 response, progression-free survival (PFS), and overall survival (OS). All three biomarkers were assessed for their ability to predict response to platinum monotherapy and for their association with patient survival outcomes.

Recently, the predictive power of the HRD threshold of $\geq$42 (5th percentile of HRD scores observed in *BRCA1/2*-mutant tumors) was evaluated for the prediction of PFS benefit due to the PARP inhibitor niraparib in second-line platinum-sensitive germline *BRCA1/2*-negative HGSOC (4). While the HRD $\geq$42 threshold was associated with significant niraparib PFS benefit, the patient group falling below this threshold also received significant, albeit reduced, benefit. These data suggest that a revision of the threshold might better define the responding patient group. To explore this concept in this study, we tested an HRD threshold of $\geq$33 (1st percentile of HRD scores observed in *BRCA1/2*-mutant tumors) against CA125 response, PFS, and OS in the HGSOC patient set.

The SCOTROC4 trial was a randomized trial of flat dosing versus intrapatient dose escalation of single-agent carboplatin as first-line chemotherapy for advanced ovarian cancer (18). Although the trial showed that intrapatient dose escalation of carboplatin based on nadir blood counts is feasible and safe, it provided no improvement in PFS or OS compared with flat dosing. However, we hypothesized that HRD-positive tumors might gain additional benefit from dose intensification and have explored potential differences depending on HRD status between patients in the dose escalation and flat dosing arms of the SCOTROC4 trial.

Materials and Methods

Patients and treatment

SCOTROC4 was a phase III randomized trial that enrolled patients with stage IC to IV epithelial ovarian carcinoma, primary fallopian tube carcinoma, or ovarian-type peritoneal carcinomatosis (18). Patients were randomized into treatment arms and received 6 cycles of 3 weekly carboplatin either at a flat dose or with an intrapatient dose escalation. The flow of patients and samples through the study is described in Supplementary Fig. S1. Tumor collection for this study was approved by local Ethics Committee and informed written consent was obtained from patient. Among patients from SCOTROC4 with epithelial ovarian carcinoma, 250 were included in this study based on patient consent and tumor sample availability. This includes 120 patients in the arm without dose intensification and 130 patients in the dose intensification arm. Based on pathologic review of tumor slides from all samples and *TP53* mutation status, 179 samples were classified as HGSOC. Of 179 patients with HGSOC tumors, 115 were in the flat dose arm and 64 were in the dose escalation arm.

Clinical assessments and endpoints

Response to therapy was monitored by CA125 response (19). CA125 measurements were carried out at baseline, before each cycle of treatment, and then twice monthly. Patients were followed up for 2 years every 2 months and then every 3 months. PFS was determined according to RECIST version 1.0 (20). CT scans were carried out at baseline and after 6 cycles of treatment and also carried out if CA125 rose or clinical progression was suspected. PFS was the time from randomization until PD or death from any cause (whichever occurred first).

Molecular analysis

DNA from patient samples was extracted from three to five 10-μm formalin-fixed paraffin-embedded (FFPE) tissue sections from each available tumor sample after scraping areas with the highest tumor cell density (Promega Maxwell 16 LEV FFPE Plus kit AS1290, Promega). FFPE tissue was incubated overnight in 20 μL Proteinase K and 180 μL incubation buffer at 70°C in a shaking heat block. An additional 20 μL Proteinase K was then added, followed by 3 hours digestion at 70°C. RNase A (A1973, 10 μL; Promega) was added followed by RNA digestion at 37°C for 20 minutes. Lysis buffer (420 μL) was then added, and the samples were loaded into Maxwell cartridges. gDNA was eluted in 110 μL of water.

The DNA analysis approach used here has been previously described (13). Genome-wide SNP data were generated using a custom hybridization enrichment panel, which targets 54,091 SNPs distributed across the human genome. *TP53*, *BRCA1*, and *BRCA2* mutation data were also evaluated in the context of this study. Details of the methods used for identification of *BRCA1*- and *BRCA2*-deficient tumors are provided in Timms and colleagues (7). Deleterious and suspected deleterious mutations were included in the analysis (21, 22).

Allelic imbalance profiles were generated to determine the scores for each individual biomarker component (TAI, LST, and LOH), and the combined HRD score is the sum of the individual biomarker scores (7, 13). An HRD score threshold of 42 (5th percentile of HRD scores observed in *BRCA1/2*-deficient tumors) has been previously developed to identify HR-deficient tumors (13). Tumors are considered HR deficient (HRD positive) if they

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have a high HRD score (≥42) or a tumor *BRCA1* or *BRCA2* (tBRCA) mutation and HR nondeficient (HRD negative) if they have a low HRD score (<42) and wild-type *BRCA1/2* (13). In this study, we explored whether lowering the threshold from the 5th percentile level of HRD scores observed in *BRCA1/2*-deficient tumors (HRD score ≥42) to the 1st percentile (HRD score ≥33) might better define the responding patient group. In these analyses, HRD-positive status was defined as an HRD score either greater than or equal to the exploratory threshold of 33 or a *BRCA1/2* mutant with any HRD score. This exploratory threshold was evaluated in the HGSOC subgroup only.

To identify tumors with *CCNE1* copy-number amplification, the copy number was averaged for the 3 SNPs on the HRD SNP assay which surround the *CCNE1* locus. The average copy number was then adjusted by the average copy number across all SNPs of the sample to produce a relative amplification score (Supplementary Fig. S2). CCNE1 amplification values of between 0.5 and 2 were considered to be within the accepted range for tumor sample variability and therefore did not represent CCNE1 amplification. Assuming these nonamplified samples to be log-normally distributed, the derived mean and standard deviation yielded at 99th percentile gave an amplification value of 2.4. Samples that exceed a CCNE1 amplification score of 2.4 were designated as *CCNE1* amplification positive.

Statistical analysis

Clinical and molecular variables were evaluated as predictors of CA125 response in terms of odds ratios (OR) and Wald confidence intervals (CI) from logistic regression models. Associations with PFS and OS were assessed with hazard ratios (HR) from Cox proportional hazards (PH) models; categorical variables were also evaluated with Kaplan–Meier (KM) curves and Mantel–Cox log-rank tests. *P* values from logistic regression and Cox PH models were based on likelihood ratio tests. *P* values are reported as two-sided unless otherwise noted.

Results

Study cohort

Patient demographic and clinical data are shown in Supplementary Table S1. CA125 response was available for 139 patients, while PFS and OS were available for all patients ($N = 250$). Overall, 74 (30%) of tumors were HRD positive (≥42), including 34 (14%) with tBRCA mutations, and 47 (19%) were identified as having amplification of *CCNE1* (Supplementary Table S1). *CCNE1* amplification was observed only in tumors without *BRCA1/2* mutations, which is consistent with previous reports (14, 15). *CCNE1* amplification was observed more frequently in HRD-negative tumors in this cohort (logistic $P = 1.6 \times 10^{-4}$; OR, 5.50; 95% CI, 1.89–16.0) compared with HRD positive (≥42). The HGSOC subset included 64 (36%) HRD positive (≥42) tumors, 29 (16%) of which had tBRCA mutations, and 39 (22%) tumors with *CCNE1* amplification.

Association with response to platinum monotherapy

CA125 response and molecular results were available for 139 tumors from the entire cohort and 113 HGSOC tumors. The distribution of HRD scores stratified by CA125 response category is shown in Fig. 1. HRD (≥42) and tBRCA mutation status were both significantly associated with CA125 complete response (CR) in the entire cohort ($P = 0.00015$ and $P = 0.0096$, respectively),

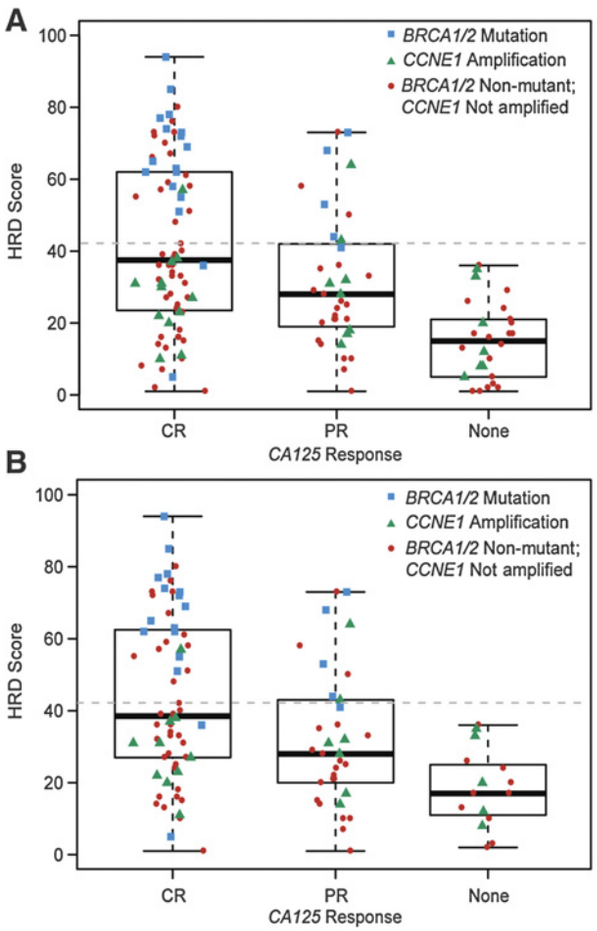

Figure 1.

Biomarker status and CA125 response. HRD status, tBRCA mutation status, and *CCNE1* amplification as predictors of CA125 response in (**A**) the overall cohort ($n = 137$) and (**B**) the HGSOC subgroup. One *BRCA1* mutation carrier is not shown due to failed HRD score. CR, complete response; PR, partial response; none, no response.

and in the subgroup of HGSOC patients ($P = 0.0016$ and $P = 0.032$, respectively; Supplementary Table S2). In the HGSOC subgroup, the HRD-positive rate increases from 37% to 52% when the HRD threshold is reduced from ≥42 to ≥33. HRD status defined as ≥33 or *BRCA1/2* mutant remains statistically significantly associated with CA125 complete response ($P = 5.0 \times 10^{-4}$) (Supplementary Table S2). A receiver-operating curve (ROC) was used to compare sensitivity and specificity of different thresholds as predictors of CA125 response (Supplementary Fig. S2). *CCNE1* amplification was not significantly associated with CA125 response in either the overall cohort or the HGSOC subgroup (Supplementary Table S2).

In a multivariate logistic regression analysis of CA125 complete response adjusted for clinical variables (age at surgery, histology, grade, stage, bulk of residual disease after surgery, and performance status), HRD status remained significantly associated with response in the overall cohort ($P = 3.6 \times 10^{-4}$; Supplementary Table S3). Similarly, HRD status (≥42) retained statistical significance in the HGSOC subset after adjusting for clinical

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Table 1. Univariate Cox PH analysis of PFS and OS for HRD and tBRCA

		Overall cohort		HGSOC subset	
Variable	**Levels**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
PFS					
HRD status (≥42)	HRD positive	0.65 (0.46–0.93)	0.014	0.50 (0.34–0.73)	0.00021
	HRD negative	Ref		Ref	
HRD status (≥33)	HRD positive	ND	ND	0.51 (0.36–0.72)	0.00014
	HRD negative	ND		Ref	
tBRCA mutation status	Mutant	0.61 (0.38–0.99)	0.034	0.48 (0.29–0.79)	0.0017
	Wild-type	Ref		Ref	
CCNE1 amplification status[a]	Amplified	1.91 (1.32–2.75)	0.0011	1.56 (1.04–2.34)	0.038
	Not amplified	Ref		Ref	
OS					
HRD status (≥42)	HRD positive	0.57 (0.36–0.92)	0.016	0.45 (0.27–0.74)	0.0011
	HRD negative	Ref		Ref	
HRD status (≥33)	HRD positive	ND	ND	0.43 (0.27–0.69)	0.00033
	HRD negative	ND		Ref	
tBRCA mutation status	Mutant	0.64 (0.35–1.17)	0.12	0.50 (0.26–0.95)	0.022
	Wild-type	Ref		Ref	
CCNE1 amplification status[a]	Amplified	1.82 (1.15–2.88)	0.015	1.72 (1.04–2.85)	0.043
	Not amplified	Ref		Ref	

[a]CCNE1 amplification status was determined for 248 of 250 patients in the full cohort, and 178 of 179 patients in the HGSOC subcohort.

variables ($P = 0.0050$). In these multivariable analyses of the overall cohort and HGSOC subset (Supplementary Table S3), HRD status was the only variable that was significantly associated with CA125 response. HRD status as defined using the exploratory threshold of ≥33 also retained statistical significance after adjusting for clinical factors ($P = 9.4 \times 10^{-4}$; Supplementary Table S4). tBRCA was significantly associated with CA125 response in the full cohort ($P = 0.049$), but not the HGSOC subset after adjusting for clinical factors (Supplementary Table S5).

Association of HRD, tBRCA, and CCNE1 with PFS or OS

HRD status (≥42) was significantly associated with both improved PFS and OS in the overall cohort ($P = 0.014$ and $P = 0.016$, respectively) and in the HGSOC subgroup ($P = 2.1 \times 10^{-4}$ and $P = 0.0011$, respectively; Table 1). The HRD-positive rate in the HGSOC subgroup increases from 35.8% to 48.6% for PFS and OS when the threshold is reduced from ≥42 to ≥33. HRD status remains significantly associated with both improved PFS and OS in the HGSOC subgroup when the threshold is reduced to ≥33 in both univariate ($P = 1.4 \times 10^{-4}$ and $P = 3.3 \times 10^{-4}$, respectively; Table 1) and multivariate ($P = 3.0 \times 10^{-6}$and $P = 3.1 \times 10^{-4}$, respectively) Cox proportional hazards models (Supplementary Table S6). Improvements in median PFS and OS were similar to those observed for the prespecified threshold (Supplementary Fig. S3).

tBRCA mutation status was significantly associated with only PFS in the entire cohort ($P = 0.034$), and with both PFS and OS in the HGSOC subgroup ($P = 0.0017$ and $P = 0.022$, respectively; Table 1). *CCNE1* amplification was significantly associated with both PFS and OS in the overall cohort (0.0011 and 0.015, respectively) and in the HGSOC subgroup ($P = 0.038$ and 0.043, respectively; Table 1).

In the overall cohort, significant improvements in median survival were observed for all three biomarkers (Fig. 2). HRD status was associated with a 7-month improvement in PFS (18.9 months for HR deficient vs. 11.6 months for nondeficient) and a 20-month improvement in OS (48.5 months for HR deficient vs. 28.1 months for nondeficient; Supplementary Table S7). Similarly, tBRCA mutations were associated with an 8-month improvement in PFS and 18-month improvement in OS. *CCNE1* amplification was associated with a 6-month reduction in PFS and a 27-month reduction in OS. Similar associations were observed in the HGSOC subset (Fig. 3; Supplementary Table S8).

In multivariate Cox PH analyses including all patients, HRD status remained significantly associated with both PFS ($P = 2.1 \times 10^{-5}$) and OS ($P = 0.0012$; Table 2). Clinical variables, which were also significantly associated with outcome, were grade ($P = 0.013$ and 0.0064), stage (PFS only, $P = 0.00014$), and bulk of residual disease after surgery (PFS only, $P = 0.0049$; Table 2). Age at surgery, histology, and performance status were not significantly associated with either PFS or OS in this analysis. When multivariate analysis was restricted to HGSOC, HRD status remained significant for PFS and OS ($P = 2.2 \times 10^{-4}$ and $P = 0.0048$, respectively). Stage and bulk of residual disease also remained significant in the HGSOC subset for only PFS ($P = 0.019$ and $P = 0.0055$, respectively; Table 2). Age at surgery and performance status were not significantly associated with outcome in this analysis. In multivariable models restricted to the subset of tBRCA nonmutant patients, HRD status was significantly associated with PFS ($P = 0.0023$, HR, 0.50; 95% CI, 0.31–0.80) and OS ($P = 0.015$; HR, 0.47; 95% CI, 0.25–0.91) in the entire cohort ($N = 216$), and in HGSOC patients ($N = 150$; PFS $P = 0.017$, HR, 0.55; 95% CI, 0.33–0.92; OS $P = 0.037$, HR, 0.49; 95% CI, 0.24–0.99).

CCNE1 amplification was associated with PFS ($P = 1.8 \times 10^{-4}$) in the overall cohort after adjusting for clinical factors (Table 3). When multivariate analysis was restricted to HGSOC, *CCNE1* amplification remained significant for PFS ($P = 0.0033$; Table 3). tBRCA was associated with PFS in the overall cohort ($P = 0.0015$) and the HGSOC subcohort (0.0019) after adjusting for clinical factors (Supplementary Table S9).

In Cox PH analyses of the full cohort adjusted for clinical factors, HRD and *CCNE1* amplification, HRD was associated with both PFS and OS ($P = 7.3 \times 10^{-4}$ and $P = 0.0052$ respectively) while *CCNE1* amplification was associated with PFS only ($P = 0.0087$). When the same models were examined in the HGSOC subset, HRD maintained significant associations with both PFS and OS ($P = 0.0027$ and $P = 0.019$, respectively; Supplementary Table S10).

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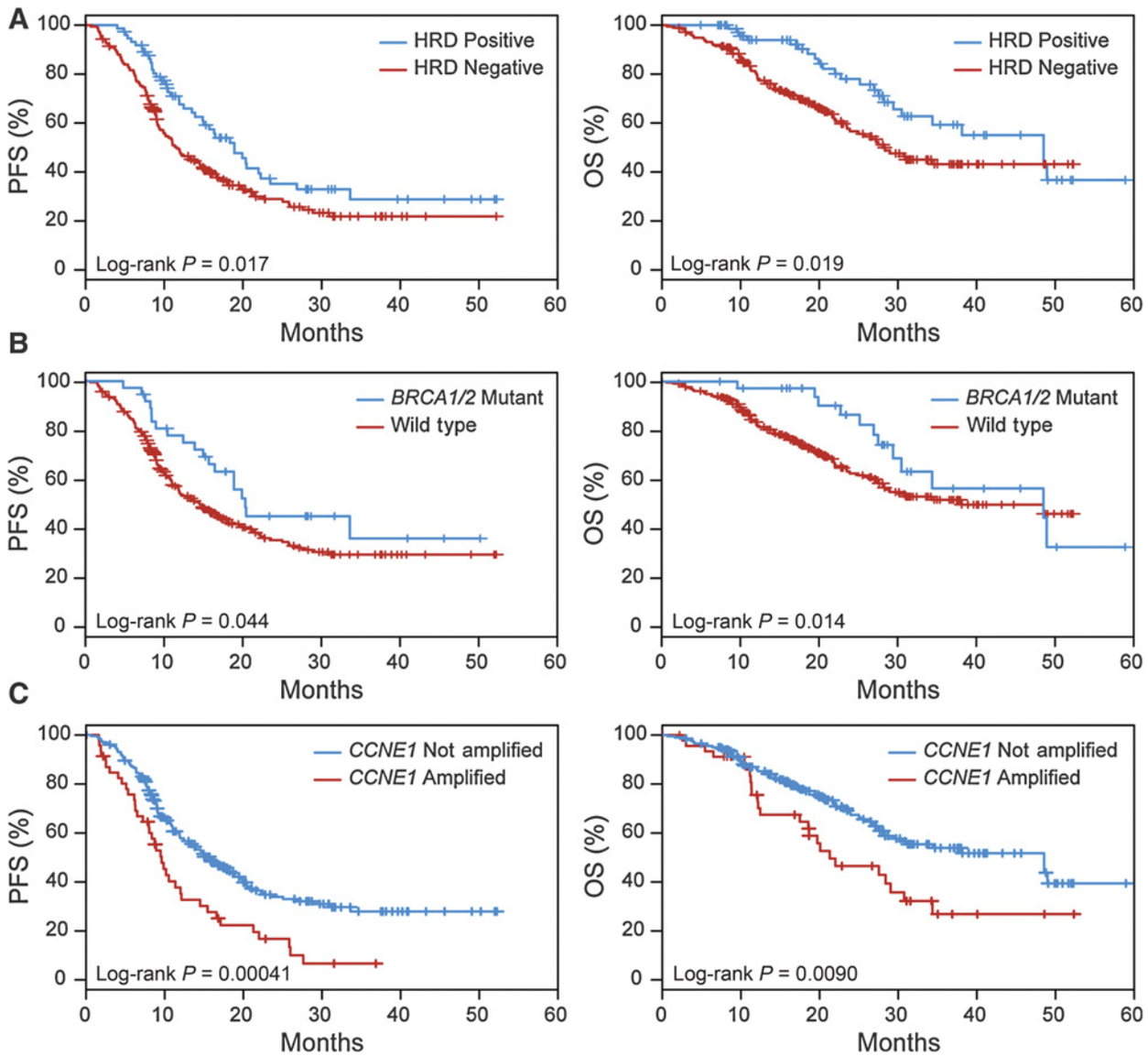

Figure 2.

Biomarker status and survival in the overall SCOTROC4 cohort. Kaplan–Meier survival curves for the overall cohort ($N = 250$) according to (**A**) HRD status, (**B**) tBRCA mutation status, and (**C**) *CCNE1* amplification. Details of numbers of events and median survival with 95% CI are shown in Supplementary Table S7.

Association of HRD with dose intensification

We hypothesized that the improved outcomes observed for HRD-positive tumors were due to increased platinum sensitivity, and that these tumors might gain additional benefit from dose intensification. One hundred thirty patients were in the dose-intensified arm (42 HRD positive) and 120 patients (32 HRD positive) were in the arm without dose intensification. In subset analyses of both arms combined, there were no significant differences in PFS rates due to dose intensification in either the HRD-negative (HR, 1.13; 95% CI, 0.79–1.62) or HRD-positive (HR, 0.62; 95% CI, 0.33–1.14) groups. However, Cox PH analysis of the full cohort stratified by treatment arm suggested that the effect on PFS of platinum dose intensification was greater in the HRD-positive group (one-sided interaction $P = 0.049$). Similarly, for OS there were no significant differences in OS rates in the HRD-negative (HR 1.54; 95% CI, 0.96–2.45) or HRD-positive (HR, 0.61; 95% CI, 0.25–1.48) groups, but the effect of dose intensification on OS was significantly greater for HRD-positive tumors (one-sided interaction $P = 0.035$). These data support the hypothesis that patients with HR-deficient tumors may benefit from dose intensification by intrapatient carboplatin dose escalation.

Discussion

The HR deficiency score based on measures of genomic instability and *BRCA1/2* mutations are markers of HR pathway defects, and previous studies have demonstrated that these molecular markers predict response to DNA-damaging agents in some cancer

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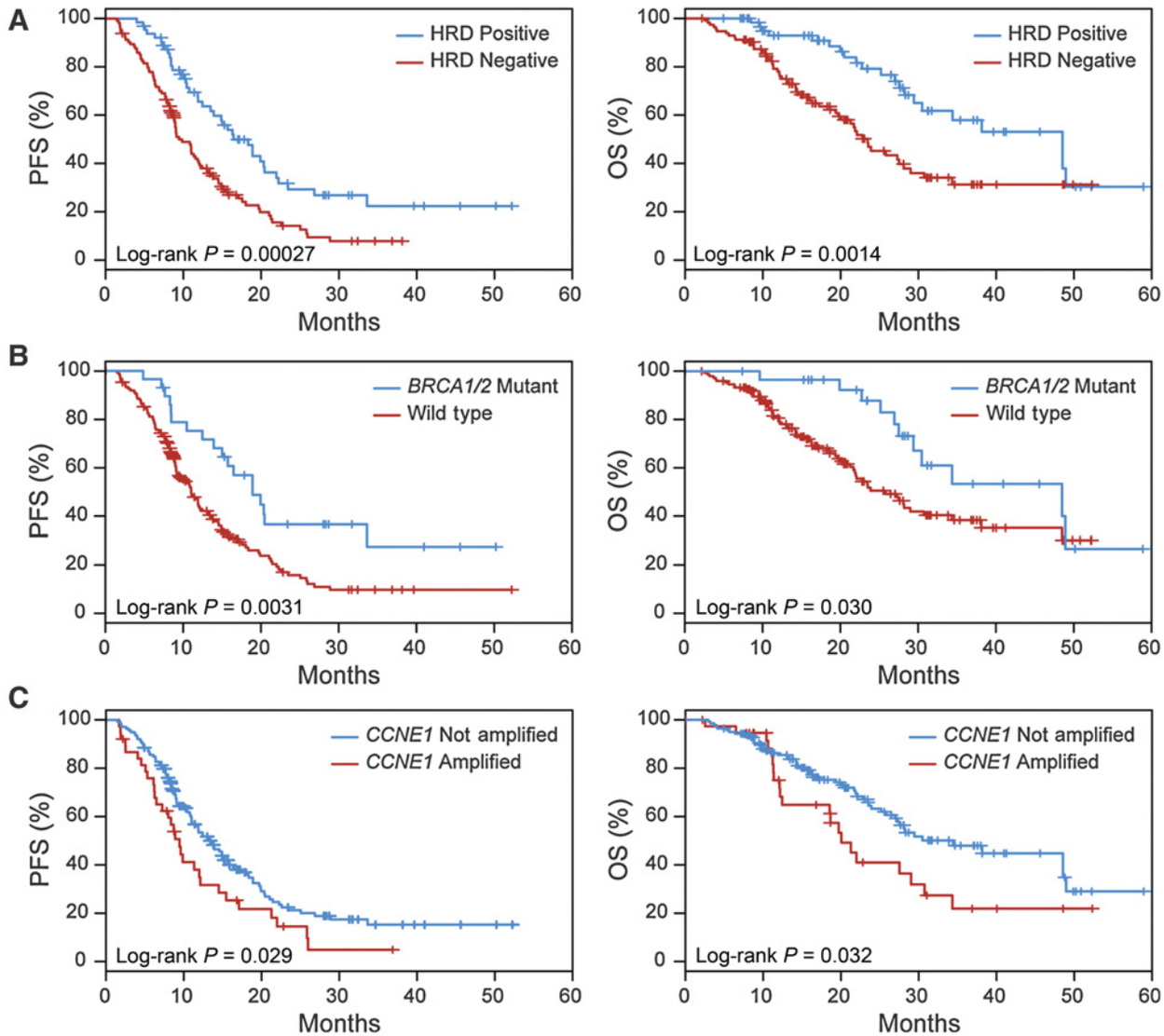

Figure 3.

Biomarker status and survival in the HGSOC SCOTROC4 cohort. Kaplan–Meier survival curves for the HGSOC subgroup ($N = 179$) according to (**A**) HRD status, (**B**) tBRCA mutation status, and (**C**) *CCNE1* amplification. Details of numbers of events and median survival with 95% CI are shown in Supplementary Table S8.

types (1–5, 13–15, 23). In addition, *CCNE1* amplification has been associated with chemotherapy resistance and poor prognosis in HGSOC (14, 15). Standard of care for first-line treatment of advanced ovarian cancer is carboplatin/paclitaxel combination therapy. However, the SCOTROC4 study provided an opportunity to investigate the ability of these three molecular markers to predict treatment response and outcomes following platinum monotherapy in a cohort of women with ovarian cancer and in the subset with HGSOC, thus avoiding potential confounding effects of paclitaxel. HGSOC histotype was based on pathological review of tumor slides by two gynecologic pathologists and *TP53* mutation status. While we recognize the importance of defining histotype in this heterogeneous disease, some non–high-grade serous tumors (endometrioid and mucinous) can have defective homologous repair as determined by the HRD score (see Supplementary Table S1). Because the SCOTROC4 trial was all epithelial ovarian cancer, we had a predetermined analysis plan that would analyze all available tumors and then a high-grade serous subgroup analysis.

A positive relationship was observed between the HRD score and *BRCA1/2* mutation status, which is consistent with previously published data (7, 13, 22). In addition, *CCNE1* amplification was observed only in tumors without *BRCA1/2* mutations, as previously reported (14, 15). A similar relationship was observed between low HRD score (<42) and *CCNE1* amplification here, suggesting that *CCNE1*-amplified tumors may require functional homologous recombination repair or represent alternative tumor development pathways.

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Table 2. Multivariate Cox PH analysis of HRD as a predictor of PFS and OS

			PFS		OS	
Variable	**Levels**	**Patients *N* (%)**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
All patients						
HRD status	HRD positive	71 (31)	0.44 (0.30–0.65)	2.1×10^{-5}	0.45 (0.27–0.74)	0.0012
	HRD negative	155 (69)	Ref		Ref	
Age at surgery	Years	226 (100)	1.01 (0.99–1.02)	0.55	1.00 (0.98–1.03)	0.68
Histology	Serous[a]/clear cell	189 (84)	1.34 (0.72–2.49)	0.34	1.18 (0.53–2.63)	0.68
	Other	37 (16)	Ref		Ref	
Grade	Low	20 (9)	Ref	0.013	Ref	0.0064
	High	206 (91)	2.59 (1.11–6.05)		4.70 (1.13–19.51)	
Stage	IC–II	56 (25)	Ref	0.00014	Ref	0.12
	III	144 (64)	3.33 (1.80–6.16)		1.84 (0.84–4.05)	
	IV	26 (12)	2.37 (1.12–4.98)		1.13 (0.42–3.05)	
Bulk of residual disease	None/microscopic	85 (38)	Ref	0.0049	Ref	0.091
	Macroscopic <2 cm	54 (24)	1.35 (0.80–2.30)		1.41 (0.69–2.86)	
	Macroscopic >2 cm	87 (38)	2.04 (1.28–3.24)		1.92 (1.03–3.61)	
Performance status	0	69 (31)	Ref	0.19	Ref	0.17
	1	122 (54)	1.17 (0.75–1.84)		1.02 (0.57–1.83)	
	2	35 (15)	1.66 (0.94–2.92)		1.73 (0.85–3.56)	
HGSOC						
HRD status	HRD positive	63 (36)	0.46 (0.30–0.70)	2.2×10^{-4}	0.47 (0.28–0.81)	0.0048
	HRD negative	110 (64)	Ref		Ref	
Age at surgery	Years	173 (100)	1.01 (0.99–1.03)	0.39	1.02 (0.99–1.04)	0.19
Stage	IC–II	31 (18)	Ref	0.019	Ref	0.12
	III	120 (69)	2.12 (1.07–4.20)		1.59 (0.63–4.00)	
	IV	22 (13)	1.28 (0.56–2.90)		0.78 (0.25–2.49)	
Bulk of residual disease	None/microscopic	49 (28)	Ref	0.0055	Ref	0.32
	Macroscopic <2 cm	48 (28)	1.37 (0.77–2.44)		1.11 (0.52–2.36)	
	Macroscopic >2 cm	76 (44)	2.15 (1.28–3.60)		1.54 (0.78–3.03)	
Performance status	0	41 (24)	Ref	0.083	Ref	0.18
	1	100 (58)	1.27 (0.75–2.15)		1.13 (0.56–2.30)	
	2	32 (18)	1.98 (1.05–3.75)		1.91 (0.83–4.38)	

[a]One patient with serous or endometrioid histology was categorized as serous for this analysis.

Table 3. Multivariate Cox PH analysis of CCNE1 as a predictor of PFS and OS

		PFS		OS	
Variable	**Levels**	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Overall cohort (*N* = 225)					
CCNE1 status	Amplified	2.19 (1.49–3.22)	1.8×10^{-4}	1.63 (1.01–2.63)	0.052
	Not amplified	Ref		Ref	
Age at surgery	Years	1.02 (1.00–1.03)	0.051	1.02 (0.99–1.04)	0.15
Histology	Serous[a]/clear cell	1.31 (0.71–2.43)	0.37	1.18 (0.53–2.62)	0.68
	Other	Ref		Ref	
Grade	Low	Ref	0.072	Ref	0.020
	High	2.03 (0.87–4.73)		3.89 (0.94–16.1)	
Stage	IC–II	Ref	7.9×10^{-5}	Ref	0.17
	III	3.35 (1.86–6.03)		1.77 (0.83–3.80)	
	IV	2.57 (1.25–5.29)		1.17 (0.44–3.08)	
Bulk of residual disease	None/microscopic	Ref	0.0036	Ref	0.099
	Macroscopic ≤2 cm	1.10 (0.66–1.83)		1.19 (0.59–2.38)	
	Macroscopic >2 cm	1.91 (1.21–3.04)		1.81 (0.96–3.38)	
Performance status	0	Ref	0.48	Ref	0.28
	1	1.06 (0.68–1.65)		0.92 (0.51–1.65)	
	2	1.37 (0.78–2.42)		1.45 (0.71–2.99)	
HGSOC subset (*N* = 172)					
CCNE1 status	Amplified	1.95 (1.28–2.99)	0.0033	1.69 (1.01–2.84)	0.056
	Not amplified	Ref		Ref	
Age at surgery	Years	1.02 (1.00–1.04)	0.019	1.03 (1.00–1.06)	0.018
Stage	IC–II	Ref	0.010	Ref	0.13
	III	2.45 (1.26–4.75)		1.71 (0.70–4.20)	
	IV	1.61 (0.73–3.57)		0.89 (0.29–2.75)	
Bulk of residual disease	None/microscopic	Ref	0.0031	Ref	0.25
	Macroscopic ≤2 cm	1.07 (0.61–1.87)		0.94 (0.45–1.97)	
	Macroscopic >2 cm	1.99 (1.19–3.30)		1.45 (0.74–2.85)	
Performance status	0	Ref	0.29	Ref	0.36
	1	1.12 (0.67–1.88)		0.99 (0.49–2.01)	
	2	1.58 (0.84–2.97)		1.52 (0.66–3.51)	

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CA125 response data showed significant association with both HRD status and *BRCA1/2* mutation status, but not with *CCNE1* amplification. In multivariate analysis, only HRD status retained statistical significance. This result is consistent with previously published observations in both TNBC and ovarian cancer (3–5, 13, 23) and supports the hypothesis that HRD status (as defined by HRD score in combination with *BRCA1/2* mutation screening) predicts sensitivity to DNA-damaging agents.

An exploratory analysis of an alternate HRD score threshold at the first percentile (≥33) of HRD scores in *BRCA1/2*-deficient tumors showed that HRD status remained significantly associated with CA125 response, while the fraction of biomarker positive to biomarker negative patients increased with the reduction in the HRD threshold. In a companion diagnostic context, such a threshold adjustment would enable more patients to receive drug benefit, although it will also increase the number of patients receiving treatment with limited benefit.

HRD and *BRCA1/2* mutation status was also significantly associated with improved patient survival in this study, in both the overall cohort and in the HGSOC subgroup. *CCNE1* amplification was also significantly associated with reduced survival in the overall study cohort, consistent with previous reports (14, 15). Both HRD status and *CCNE1* amplification remained significantly associated with outcome in multivariate analysis.

Based on the positive association between HRD status and both response and outcome in this cohort, it was hypothesized that HRD-positive tumors would show more benefit from platinum dose intensification than HRD-negative tumors. The effect of dose intensification on PFS and OS was significantly greater in the HRD-positive group, suggesting that patients whose tumors are defective in HR may benefit from dose escalation based on intrapatient measures of toxicity as in the dose escalation arm of SCOTROC4 (18).

HRD status as defined by a three-biomarker HRD score in combination with *BRCA1/2* mutation screening provided significant improvement over clinical variables in identifying patients with ovarian cancer who had improved response to platinum monotherapy, and was prognostic in this setting. HRD-positive tumors were observed predominantly in HGSOC tumors. In the clinical setting, the HRD test could be used to identify patients with increased likelihood of response to DNA-damaging agents, or other agents that target the DNA-damage repair pathways. *CCNE1* amplification is also prognostic with patients whose tumors have amplification of this locus having significantly worse outcomes. Therapies that target this defect may provide an opportunity to improve outcomes for patients with *CCNE1*-amplified ovarian tumors.

Disclosure of Potential Conflicts of Interest

K.M. Timms is employed with Myriad Genetics, Inc. and has ownership interest (including patents) in the same. E. Hughes and K. Brown are employed with Myriad Genetics, Inc. A. Gutin is SVP of Bioinformatics at Myriad Genetics, Inc. and is manager of automation engineering for the same. H. Gabra is vice president at AstraZeneca. J.S. Lanchbury is CSO at Myriad Genetics Inc. and has ownership interest (including patents) in the same. R. Brown reports receiving a commercial research grant from Myriad Genetics Inc. No potential conflicts of interest were disclosed by the other authors.

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Biomarker Assessment of HR Deficiency, Tumor *BRCA1/2* Mutations, and *CCNE1* Copy Number in Ovarian Cancer: Associations with Clinical Outcome Following Platinum Monotherapy

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