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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

Euan A. Stronach[1*], James Paul[2*], Kirsten M Timms[3*], Elisha Hughes[3], Krystal Brown[3], Christopher Neff[3], Michael Perry[3], Alexander Gutin[3], Mona El-Bahrawy[4], Jennifer H. Steel[1], Xinxue Liu[1], Liz-Anne Lewsley[2], Nadeem Siddiqui[5], Hani Gabra[1,6], Jerry S Lanchbury[3], Robert Brown[1,7]

[1] Division of Cancer and Ovarian Cancer Action Research Centre, Department of Surgery and Cancer, Imperial College London, London W12 0NN, UK
[2] University of Glasgow (Cancer Research UK Clinical Trials Unit) & SGCTG
[3] Myriad Genetics Inc, 320 Wakara Way, Salt Lake City, UT 84108, USA
[4] Department of Medicine, Imperial College London, London W12 0NN, UK
[5] Dept of Gynaecological Oncology, Glasgow Royal infirmary, Glasgow G31 2ER, UK
[6] Early Clinical Development, IMED Biotech Unit, AstraZeneca, Cambridge, UK
[7] Institute for Cancer Research, Sutton, SM2 5NG, UK
*Contributed equally to the work

Corresponding Author:
Name: Prof Robert Brown
Affiliation: Department of Surgery and Cancer, Imperial College London, London W12 0NN, UK
Mailing Address: Hammersmith Hospital, IRDB 4th Floor, Du Cane Road, London W12 0NN, UK
Phone: +44 020 7594 1804
Fax: +44 020 7594 2129
Email: b.brown@imperial.ac.uk

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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 carboplatinum monotherapy. Ovarian tumors (n=250), with high-grade serous (HGSOC) subgroup analysis (n=179), were classified as HRD positive (HRD score {greater than or equal to}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 58 and p=0.035, respectively). An alternative exploratory HRD score threshold ({greater than or equal to}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.

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) [8], telomeric allelic imbalance (TAI) [9], and large-scale state transition (LST) [10]. Each individual measure has been shown to be associated with response to platinum-based therapy in either triple negative breast (TNBC) or ovarian cancer [9-11], and the combined score has been shown to be a better predictor of homologous recombination deficiency than any of the individual scores [12].

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 ≥42 or a tumor *BRCA1/2* mutation) from HR non-deficient 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 et al. 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 $\geqslant$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 tumours 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 end-points in this study included CA125 response, 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 ≥42 (5^{th} 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 ≥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 ≥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.

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 **Supplemental Figure 1**. Tumour 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 pathological 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. Progression free survival (PFS) was determined according to RECIST version 1.0 [20]. CT scans were carried out at baseline and after six cycles of treatment and also carried out if CA125 rose or clinical progression was suspected. PFS was the time from randomisation until PD or death from any cause (whichever occurred first).

Molecular Analysis

DNA from patient samples was extracted from three to five 10 micron 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, Madison, WI). 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. 10 μL of RNase A (A1973, Promega, Madison, WI) 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 was 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 et al [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, LOH) and the combined HRD score is the sum of the individual biomarker scores [7, 13]. An HRD score threshold of 42 (5^{th} 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 have a high HRD score (≥42) or a tumor *BRCA1* or *BRCA2* (tBRCA) mutation and HR non-deficient (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 5^{th} percentile level of HRD scores observed in *BRCA1/2* deficient tumors (HRD score ≥42) to the 1^{st} 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 Figure 2**. 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 non-amplified 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 (ORs) and Wald confidence intervals (CIs) from logistic regression models. Associations with PFS and OS were assessed with hazard ratios 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 is shown in **Supplementary Table 1**. 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 1**). *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 $p=1.6\times10^{-4}$; OR 5.50, 95% CI 1.89-16.0) compared to 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 **Figure 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), and in the subgroup of HGSOC patients (p= 0.0016 and p=0.032, respectively; **Supplemental Table 2**). 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 ($p = 5.0\text{x}10^{-4}$) (**Supplemental Table 2**). A receiver operating curve (ROC) was used to compare sensitivity and specificity of different thresholds as predictors of CA125 response (**Supplementary Figure 2**). *CCNE1* amplification was not significantly associated with CA125 response in either the overall cohort or the HGSOC subgroup (**Supplemental Table 2**).

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, performance status), HRD status remained significantly associated with response in the overall cohort ($p=3.6x10^{-4}$, **Supplemental Table 3**). Similarly, HRD status (≥42) retained statistical significance in the HGSOC subset after adjusting for clinical variables (p=0.0050). In these multivariable analyses of the overall cohort and HGSOC subset (**Supplementary Table 3**), 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 ($p = 9.4x10^{-4}$) (**Supplementary Table 4**). tBRCA was significantly associated with CA125 response in the full cohort (p=0.049), but not the HGSOC subset after adjusting for clinical factors (**Supplemental Table 5).**

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 ($p=2.1x10^{-4}$ 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 ($p= 1.4x10^{-4}$and $p= 3.3x10^{-4}$, respectively; **Table 1**) and multivariate ($p=3.0x10^{-6}$and $p=3.1x10^{-4}$, respectively) Cox proportional hazards models (**Supplementary Table 6**). Improvements in median PFS and OS were similar to those observed for the pre-specified threshold (**Supplementary Figure 3**).

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 (**Figure 2**). HRD status was associated with a 7 month improvement in PFS (18.9 months for HR deficient vs 11.6 months for non-deficient) and a 20 month improvement in OS (48.5 months for HR deficient vs 28.1 months for non-deficient) (**Supplementary Table 7**). 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 (**Figure 3 and Supplementary Table 8**).

In multivariate Cox PH analyses including all patients, HRD status remained significantly associated with both PFS ($p=2.1x10^{-5}$) 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 ($p=2.2x10^{-4}$ 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 non-mutant patients, HRD status was significantly associated with PFS (p=0.0023, hazard ratio 0.50, 95% CI 0.31-0.80) and OS (p=0.015; hazard ratio 0.47, 95% CI 0.25-0.91) in the entire cohort (N=216), and in HGSOC patients (N=150; PFS p=0.017, hazard ratio 0.55, 95% CI 0.33-0.92; OS p=0.037, hazard ratio 0.49, 95% CI 0.24-0.99).

CCNE1 amplification was associated with PFS ($p=1.8\times10^{-4}$) 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 (**Supplemental Table 9**).

In Cox PH analyses of the full cohort adjusted for clinical factors, HRD and *CCNE1* amplification, HRD was associated with both PFS and OS ($p=7.3\times10^{-4}$ 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) (**Supplemental Table 9**).

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 (hazard ratio 1.13, 95% CI 0.79–1.62) or HRD positive (hazard ratio 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 overall survival there were no significant differences in OS rates in the HRD negative (hazard ratio 1.54, 95% CI 0.96–2.45) or HRD positive (hazard ratio 0.61, 95% CI 0.25–1.48) groups, but the effect of dose intensification on overall survival 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 intra-patient 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 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 SCOTROC-4 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 gynaecological pathologists and *TP53* mutation status. While we recognize the important of defining histotype in this heterogeneous disease, some non-high grade serous tumours (endometrioid and mucinous) can have defective homologous repair as determined by the HRD score (see Supplementary Table 1). Since, the SCOTROC4 trial was all epithelial ovarian cancer we had a predetermined analysis plan that would analyse all available tumours 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 tumour development pathways.

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

298 statistical significance. This result is consistent with previously published observations in both TNBC and
299 ovarian cancer [3-5, 13, 23], and supports the hypothesis that HRD status (as defined by HRD score in
300 combination with *BRCA1/2* mutation screening) predicts sensitivity to DNA damaging agents.

301 An exploratory analysis of an alternate HRD score threshold at the 1st percentile (≥33) of HRD
302 scores in *BRCA1/2* deficient tumors showed that HRD status remained significantly associated with
303 CA125 response, while the fraction of biomarker positive to biomarker negative patients increased with
304 the reduction in the HRD threshold. In a companion diagnostic context, such a threshold adjustment
305 would enable more patients to receive drug benefit, although will also increase the number of patients
306 receiving treatment with limited benefit.

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

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

318 HRD status as defined by a three biomarker HRD score in combination with *BRCA1/2* mutation
319 screening provided significant improvement over clinical variables in identifying patients with ovarian
320 cancer who had improved response to platinum monotherapy, and was prognostic in this setting. HRD
321 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 which 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 which target this defect may provide an opportunity to improve outcomes for patients with *CCNE1* amplified ovarian tumors.

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REFERENCES

1. Dann RB, DeLoia JA, Timms KM, Zorn KK, Potter J, Flake DD, 2nd, et al. BRCA1/2 mutations and expression: response to platinum chemotherapy in patients with advanced stage epithelial ovarian cancer. Gynecol Oncol. 2012;125:677-82.
2. Byrski T, Huzarski T, Dent R, Gronwald J, Zuziak D, Cybulski C, et al. Response to neoadjuvant therapy with cisplatin in BRCA1-positive breast cancer patients. Breast Cancer Res Treat. 2009;115:359-63.
3. Domchek SM, Aghajanian C, Shapira-Frommer R, Schmutzler RK, Audeh MW, Friedlander M, et al. Efficacy and safety of olaparib monotherapy in germline BRCA1/2 mutation carriers with advanced ovarian cancer and three or more lines of prior therapy. Gynecol Oncol. 2016;140:199-203.
4. Mirza MR, Monk BJ, Herrstedt J, Oza AM, Mahner S, Redondo A, et al. Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer. N Engl J Med. 2016.
5. Swisher EM, Lin KK, Oza AM, Scott CL, Giordano H, Sun J, et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. Lancet Oncol. 2017;18:75-87.
6. Network CGA. Integrated genomic analyses of ovarian carcinoma. Nature. 2011;474:609-15.
7. Timms KM, Abkevich V, Hughes E, Neff C, Reid J, Morris B, et al. Association of BRCA1/2 defects with genomic scores predictive of DNA damage repair deficiency among breast cancer subtypes. Breast Cancer Res. 2014;16:475.
8. Abkevich V, Timms KM, Hennessy BT, Potter J, Carey MS, Meyer LA, et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. Br J Cancer. 2012;107:1776-82.
9. Birkbak NJ, Wang ZC, Kim J-Y, Eklund AC, Li Q, Tian R, et al. Telomeric Allelic Imbalance Indicates Defective DNA Repair and Sensitivity to DNA-Damaging Agents. Cancer Discovery. 2012;2:366-75.

10. Popova T, Manie E, Rieunier G, Caux-Moncoutier V, Tirapo C, Dubois T, et al. Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. Cancer Res. 2012;72:5454-62.
11. Telli ML, Jensen KC, Vinayak S, Kurian AW, Lipson JA, Flaherty PJ, et al. Phase II Study of Gemcitabine, Carboplatin, and Iniparib As Neoadjuvant Therapy for Triple-Negative and BRCA1/2 Mutation-Associated Breast Cancer With Assessment of a Tumor-Based Measure of Genomic Instability: PrECOG 0105. J Clin Oncol. 2015;33:1895-901.
12. Mills GB, Timms KM, Reid JE, Gutin AS, Krivak TC, Hennessy B, et al. Homologous recombination deficiency score shows superior association with outcome compared with its individual score components in platinum-treated serous ovarian cancer. Gynecologic Oncology. 2016;141:2-3.
13. Telli ML, Timms KM, Reid JE, Hennessy B, Mills GB, Jensen KC, et al. Homologous Recombination Deficiency (HRD) Score Predicts Response to Platinum-Containing Neoadjuvant Chemotherapy in Patients with Triple Negative Breast Cancer. Clin Cancer Res. 2016.
14. Mayr D, Kanitz V, Anderegg B, Luthardt B, Engel J, Lohrs U, et al. Analysis of gene amplification and prognostic markers in ovarian cancer using comparative genomic hybridization for microarrays and immunohistochemical analysis for tissue microarrays. American Journal of Clinical Pathology. 2006;126:101-9.
15. Etemadmoghadam D, deFazio A, Beroukhim R, Mermel C, George J, Getz G, et al. Integrated genome-wide DNA copy number and expression analysis identifies distinct mechanisms of primary chemoresistance in ovarian carcinomas. Clin Cancer Res. 2009;15:1417-27.
16. Etemadmoghadam D, Weir BA, Au-Yeung G, Alsop K, Mitchell G, George J, et al. Synthetic lethality between CCNE1 amplification and loss of BRCA1. Proc Natl Acad Sci U S A. 2013;110:19489-94.

17. Au-Yeung G, Lang F, Azar WJ, Mitchell C, Jarman KE, Lackovic K, et al. Selective Targeting of Cyclin E1-Amplified High-Grade Serous Ovarian Cancer by Cyclin-Dependent Kinase 2 and AKT Inhibition. Clin Cancer Res. 2017;23:1862-74.
18. Banerjee S, Rustin G, Paul J, Williams C, Pledge S, Gabra H, et al. A multicenter, randomized trial of flat dosing versus intrapatient dose escalation of single-agent carboplatin as first-line chemotherapy for advanced ovarian cancer: an SGCTG (SCOTROC 4) and ANZGOG study on behalf of GCIG. Annals of Oncology. 2013;24:679-87.
19. Rustin GJ1, Vergote I, Eisenhauer E et al Definitions for response and progression in ovarian cancer clinical trials incorporating RECIST 1.1 and CA 125 agreed by the Gynecological Cancer Intergroup (GCIG). Int J Gynecol Cancer. 2011;21(2):419-23. doi: 10.1097/IGC.0b013e3182070f17
20. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000;92:205-16.
21. Eggington JM, Bowles KR, Moyes K, Manley S, Esterling L, Sizemore S, et al. A comprehensive laboratory-based program for classification of variants of uncertain significance in hereditary cancer genes. Clin Genet. 2014;86:229-37.
22. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17:405-24.
23. Kaklamani VG, Jeruss JS, Hughes E, Siziopikou K, Timms KM, Gutin A, et al. Phase II neoadjuvant clinical trial of carboplatin and eribulin in women with triple negative early-stage breast cancer (NCT01372579). Breast Cancer Res Treat. 2015;151:629-38.

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

		Overall Cohort		HGSOC Subset	
Variable	Levels	Hazard Ratio (95% CI)	P-Value	Hazard Ratio (95% CI)	P-Value
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*	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*	Amplified	1.82 (1.15-2.88)	0.015	1.72 (1.04-2.85)	0.043
	Not Amplified	*Ref*		*Ref*	

*CCNE1 Amplification Status was determined for 248 out of 250 patients in the full cohort, and 178 out of 179 patients in the HGSOC sub-cohort.

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

			PFS		OS	
Variable	**Levels**	**Patients N (%)**	**Hazard Ratio (95% CI)**	**P-Value**	**Hazard Ratio (95% CI)**	**P-Value**
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*/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 < 2cm	54 (24)	1.35 (0.80-2.30)		1.41 (0.69-2.86)	
	Macroscopic > 2cm	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 < 2cm	48 (28)	1.37 (0.77-2.44)		1.11 (0.52-2.36)	
	Macroscopic > 2cm	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)	

*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.

Overall Cohort (N=225)					
		PFS		**OS**	
Variable	**Levels**	**Hazard Ratio (95% CI)**	**P-Value**	**Hazard Ratio (95% CI)**	**P-Value**
***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*/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 ≤ 2cm	1.10 (0.66-1.83)		1.19 (0.59-2.38)	
	Macroscopic > 2cm	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)					
		PFS		**OS**	
Variable	**Levels**	**Hazard Ratio (95% CI)**	**P-Value**	**Hazard Ratio (95% CI)**	**P-Value**
***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 ≤ 2cm	1.07 (0.61-1.87)		0.94 (0.45-1.97)	
	Macroscopic > 2cm	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)	

Figure Legends

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.

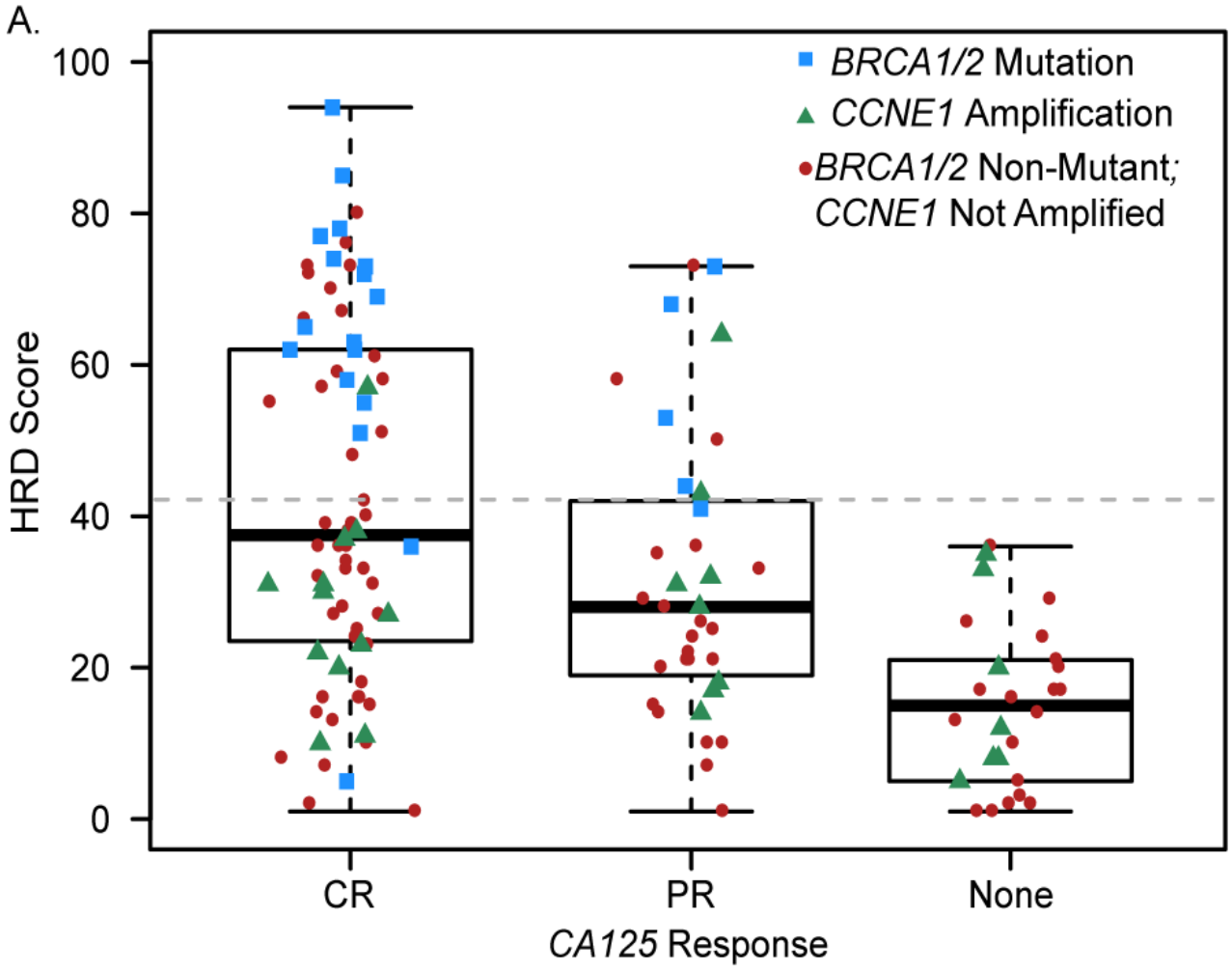
Figure 2. Biomarker status and survival in overall SCOTROC4 cohort

Kaplan-Meier Survival curves for the overall cohort (N=250) according to (A) HRD status, (B) tBRCA mutation status, and (C) *CCNE1* amplification. PFS, Progression Free Survival,; OS, Overall Survival. Details of numbers of events and median survival with 95% CI are shown in **Supplemental Table 7.**

Figure 3. Biomarker status and survival in 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. PFS, Progression Free Survival,; OS, Overall Survival. Details of numbers of events and median survival with 95% CI are shown in **Supplemental Table 8.**

Figure 1


A.
100
80
60
40
20
0
HRD Score
BRCA1/2 Mutation
CCNE1 Amplification
BRCA1/2 Non-Mutant;
CCNE1 Not Amplified
CR
PR
None
CA125 Response

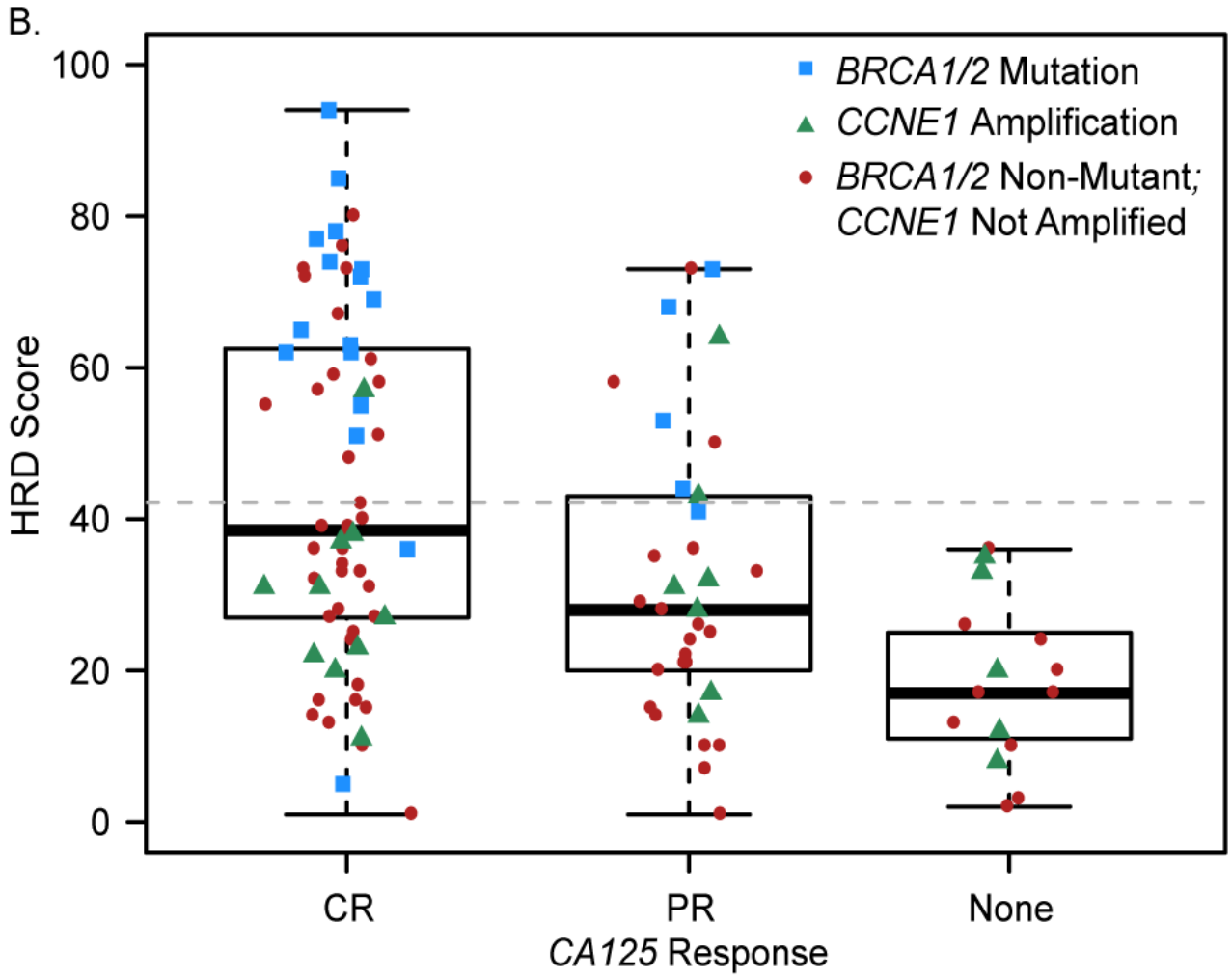

B.
100
80
60
40
20
0
HRD Score
BRCA1/2 Mutation
CCNE1 Amplification
BRCA1/2 Non-Mutant;
CCNE1 Not Amplified
CR
PR
None
CA125 Response

Figure 2

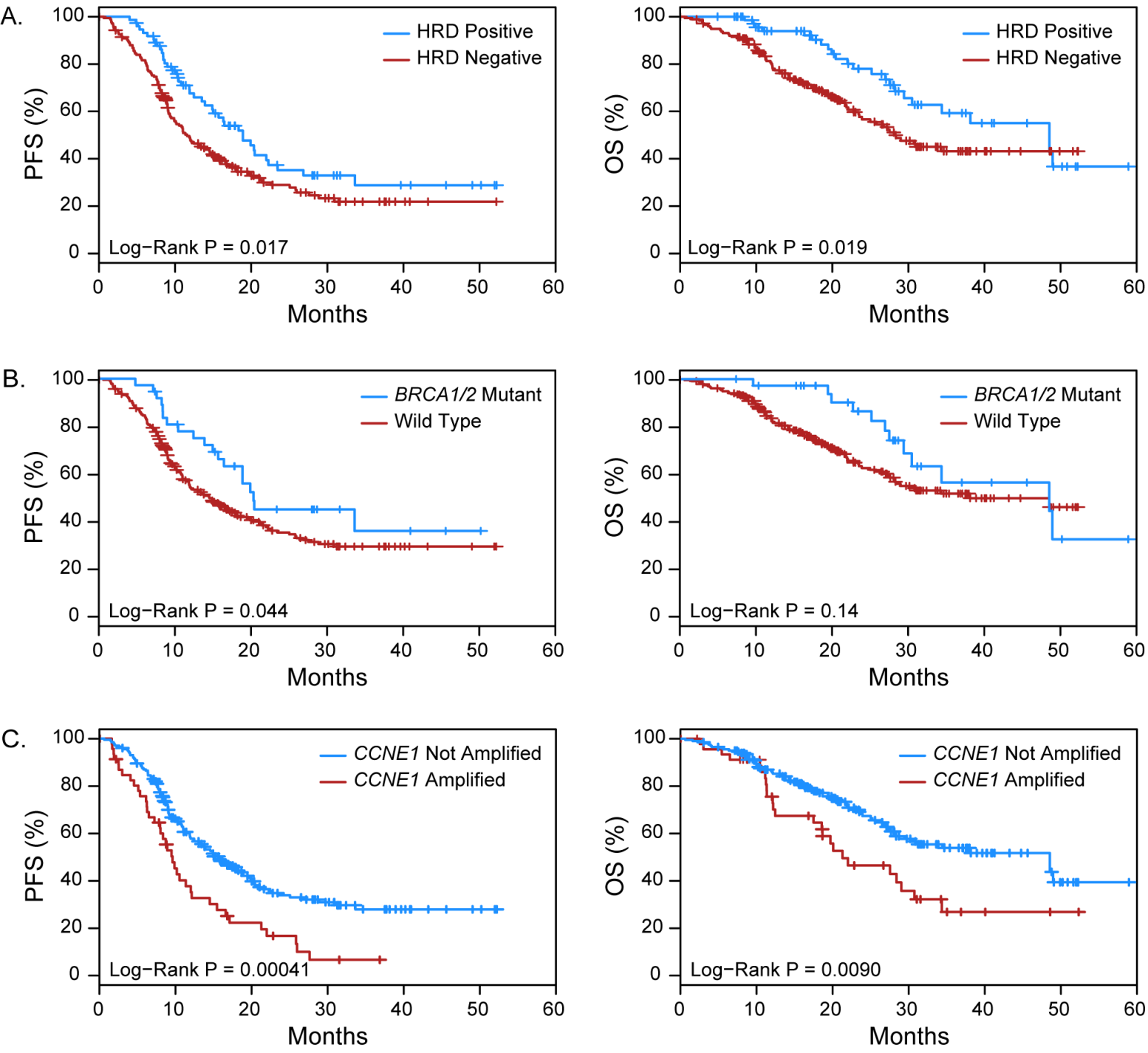
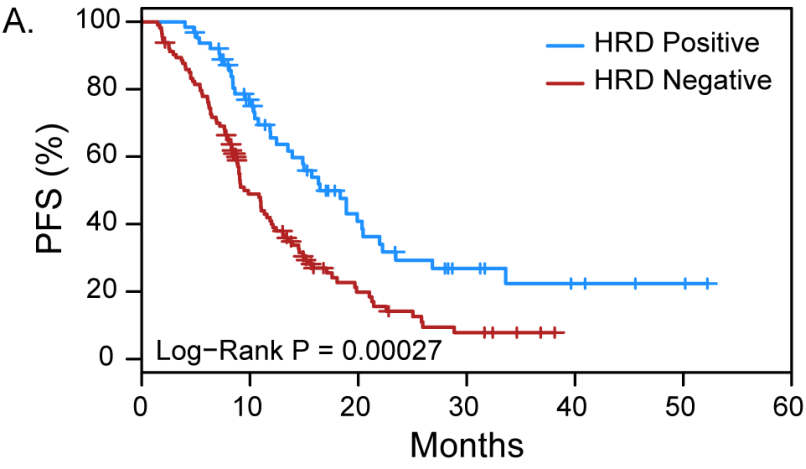
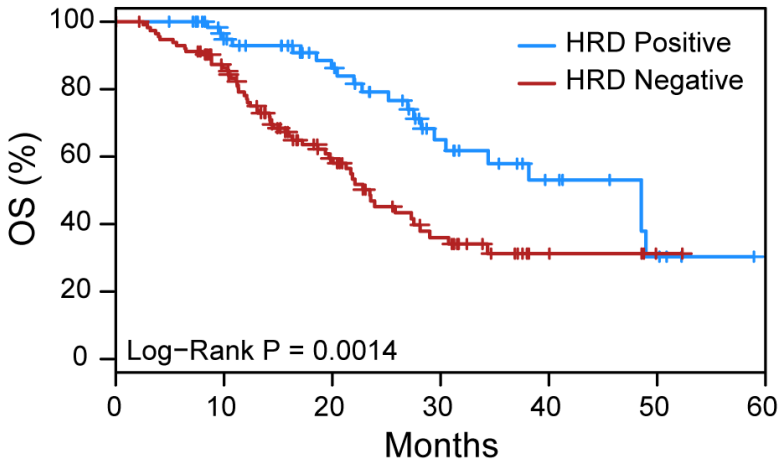
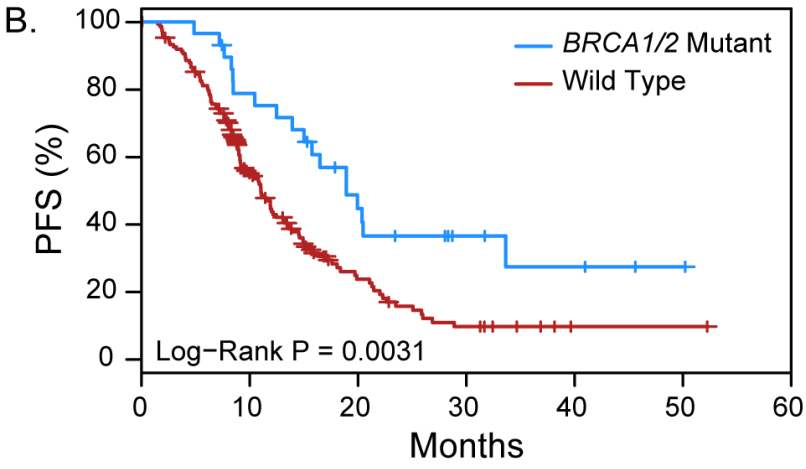
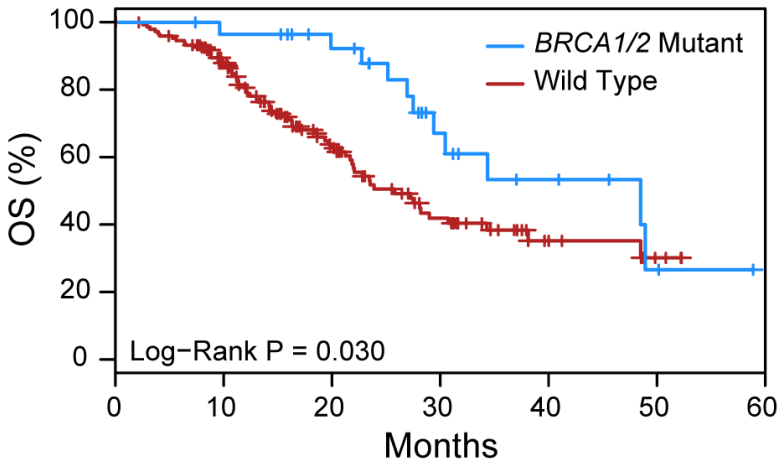

A.
PFS (%)
HRD Positive
HRD Negative
Log−Rank P = 0.017
Months
OS (%)
HRD Positive
HRD Negative
Log−Rank P = 0.019
Months
B.
PFS (%)
BRCA1/2 Mutant
Wild Type
Log−Rank P = 0.044
Months
OS (%)
BRCA1/2 Mutant
Wild Type
Log−Rank P = 0.14
Months
C.
PFS (%)
CCNE1 Not Amplified
CCNE1 Amplified
Log−Rank P = 0.00041
Months
OS (%)
CCNE1 Not Amplified
CCNE1 Amplified
Log−Rank P = 0.0090
Months

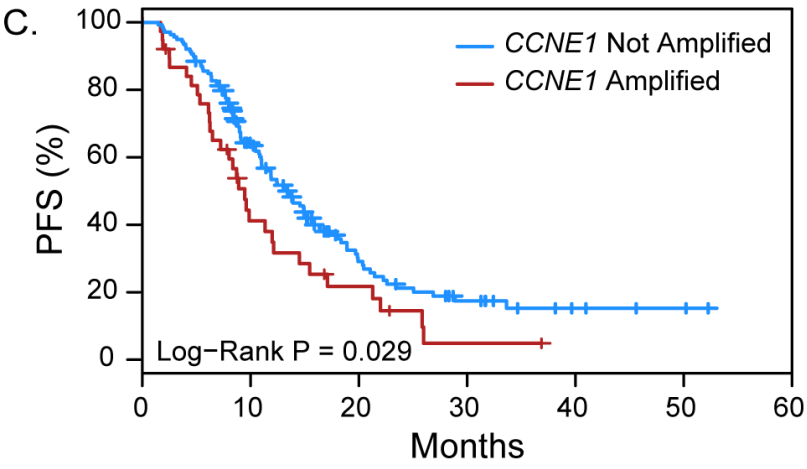
Figure 3

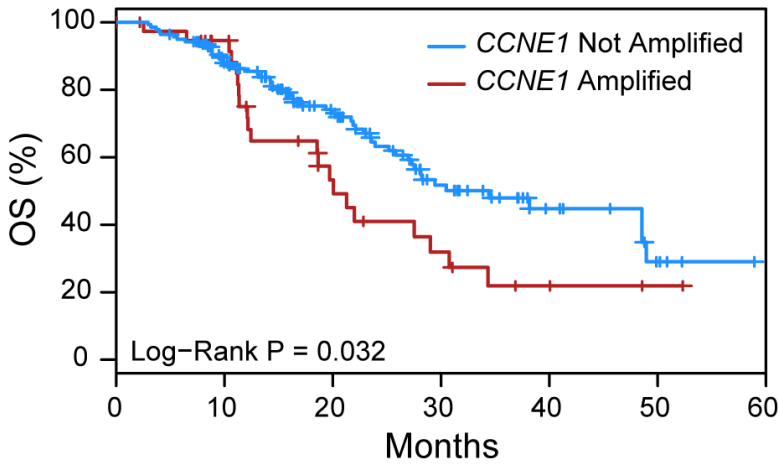

A.
PFS (%)
HRD Positive
HRD Negative
Log−Rank P = 0.00027
Months


OS (%)
HRD Positive
HRD Negative
Log−Rank P = 0.0014
Months


B.
PFS (%)
BRCA1/2 Mutant
Wild Type
Log−Rank P = 0.0031
Months


OS (%)
BRCA1/2 Mutant
Wild Type
Log−Rank P = 0.030
Months


C.
PFS (%)
CCNE1 Not Amplified
CCNE1 Amplified
Log−Rank P = 0.029
Months


OS (%)
CCNE1 Not Amplified
CCNE1 Amplified
Log−Rank P = 0.032
Months