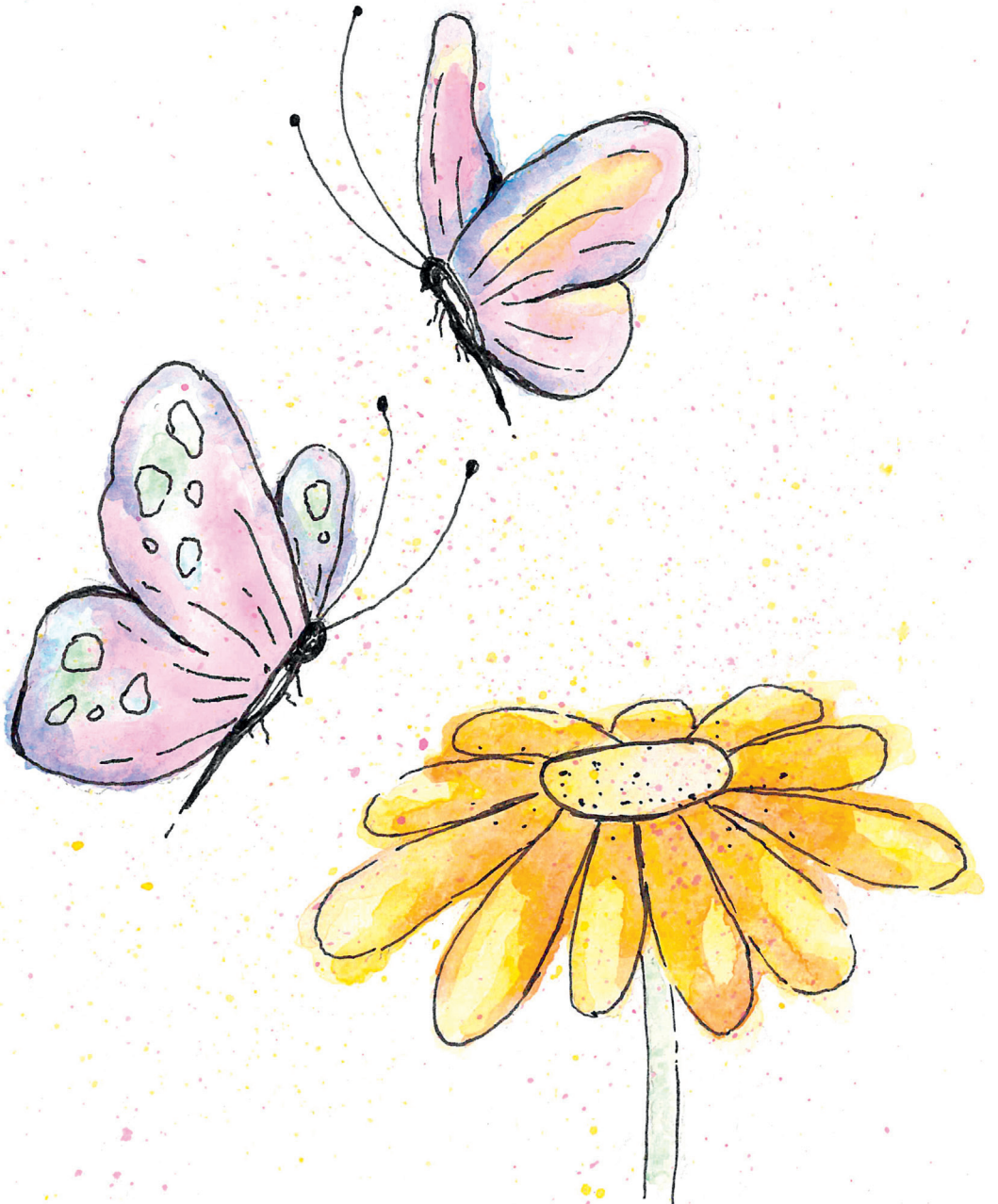
RAD51 as biomarker for the identification of homologous recombination deficient gynaecological carcinomas

Wijk, L.M. van



Chapter 3

The RECAP test rapidly and reliably identifies homologous recombination-deficient ovarian carcinomas

Lise M. van Wijk[1], Sylvia Vermeulen[1], Matty Meijers[1], Manuela F. van Diest[1], Natalja T. ter Haar[2], Marthe M. de Jonge[2], Nienke Solleveld-Westerink[2], Tom van Wezel[2], Dik C. van Gent[3], Judith R. Kroep[4], Tjalling Bosse[2], Katja N. Gaarenstroom[5], Harry Vrieling[1], Maaike P.G. Vreeswijk[1]

[1] Department of Human Genetics, Leiden University Medical Center, Leiden, the Netherlands
[2] Department of Pathology, Leiden University Medical Center, Leiden, the Netherlands
[3] Department of Molecular Genetics, Erasmus Medical Center, Rotterdam, the Netherlands
[4] Department of Medical Oncology, Leiden University Medical Center, Leiden, the Netherlands
[5] Department of Gynecology, Leiden University Medical Center, Leiden, the Netherlands

Cancers **2020**, *12*(10), 2805; doi: 10.3390/cancers12102805

Simple Summary

The sensitivity to PARP inhibitors is related to tumor-specific defects in homologous recombination (HR) and extends beyond *BRCA1/2* related deficiencies. A robust method to identify HR-Deficient (HRD) carcinomas is therefore of utmost clinical importance. In this study, we evaluated the use a functional test (the RECAP test) for the identification of HRD ovarian tumors.

Forty-nine epithelial ovarian carcinomas (EOC) were analyzed by the RECAP test. Thirty-nine of these tumors were of the high-grade serous (HGSOC) histological subtype. Ten out of these 39 HGSOC samples showed HRD (26%), whereas ovarian carcinomas of other histological subtypes (*n* = 10) were all HR-proficient. Eight out of 9 sequenced HRD tumors showed pathogenic *BRCA1/2* variants or *BRCA1* promoter hypermethylation.

This study shows that the RECAP test is a reliable and rapid test to identify functional deficiencies in HR and a good alternative to DNA-based HRD tests.

Abstract

Recent studies have shown that the efficacy of PARP inhibitors in epithelial ovarian carcinoma (EOC) is related to tumor-specific defects in homologous recombination (HR) and extends beyond *BRCA1/2* deficient EOC. A robust method with which to identify HR-Deficient (HRD) carcinomas is therefore of utmost clinical importance. In this study, we investigated the proficiency of a functional HR assay based on the detection of RAD51 foci, the REcombination CAPacity (RECAP) test, in identifying HRD tumors in a cohort of prospectively collected EOCs.

Of the 39 high-grade serous ovarian carcinomas (HGSOC), the RECAP test detected 26% (10/39) to be HRD, whereas ovarian carcinomas of other histological subtypes (*n* = 10) were all HR-Proficient (HRP). Of the HRD tumors that could be sequenced, 8/9 showed pathogenic *BRCA1/2* variants or *BRCA1* promoter hypermethylation, indicating that the RECAP test reliably identifies HRD, including but not limited to tumors related to *BRCA1/2* deficiency. Furthermore, we found a trend towards better overall survival of HGSOC patients with RECAP-identified HRD tumors compared to patients with HRP tumors.

This study shows that the RECAP test is an attractive alternative to DNA-based HRD tests and further development of a clinical grade RECAP test is clearly warranted.

Keywords: Epithelial Ovarian Carcinoma - Homologous Recombination Deficiency – RECAP test – RAD51- BRCA1 – BRCA21.

1. Introduction

Surgical cytoreduction combined with platinum-based chemotherapy has traditionally been the standard of care in the treatment of epithelial ovarian carcinoma (EOCs) patients [1,2]. However, a series of recent clinical trials showed that poly (ADP-ribose) polymerase inhibitor (PARPi) as maintenance treatment of EOC patients with platinum-sensitive cancers results in significant improvement of both progression-free survival (PFS) and overall survival (OS) in newly diagnosed and recurrent EOC [3-11]. This led to FDA and EMA approval of various PARPi as maintenance therapy in patients with platinum-sensitive EOC, both in the primary and recurrent setting. Although the presence of pathogenic variants in *BRCA1* or *BRCA2* is the best genetic predictor for a good clinical response to PARPi therapy, multiple studies have shown that PARPi efficacy is also observed in patients with non-*BRCA1/2* deficient carcinomas [4,5,9,12]. Since current evidence suggests that a deficiency in homologous recombination (HR) underlies sensitivity to PARPi, a search for biomarkers that correlate with HR Deficiency (HRD) was initiated with the aim of developing a single method that can identify all patients who could potentially benefit from PARPi treatment [13-16] (Table S1).

Most of the currently available HRD tests are DNA-based. Using gene-specific analyses, the percentage of HRD high-grade serous ovarian carcinomas (HGSOC) is estimated to be as high as 50% [17]. In addition to the presence of pathogenic variants in *BRCA1* and *BRCA2* (20%), inactivation of other genes involved in the HR pathway (6%) and the epigenetic silencing of *BRCA1* (10%) or *RAD51C* (3%) have also been reported in HGSOC [13,17,18]. The prevalence of HRD in other histological subtypes of EOCs such as low grade serous, endometrioid, clear cell and mucinous OC is still unclear [19]. In addition to gene-specific analyses, more complex DNA-based approaches, such as the identification of 'genomic scars', are now being explored as potential biomarkers of HRD. These methods focus on identifying mutational signatures caused by the use of alternative, error-prone repair pathways to repair DNA double-strand breaks (DSBs) in the absence of HR, and include copy number-based methods incorporating loss of heterozygosity (LOH) [20-23], telomeric allelic imbalances (TAI) and large-scale state transitions (LST) [21,23-25]. Similar but more complex whole genome sequencing-based approaches include HRDetect, a weighted model that includes features such as microhomology-mediated deletions, base substitution, rearrangement signatures, and LOH [26]. HRD-associated genomic scars have been shown to facilitate identification of both *BRCA1/2* deficient (m*BRCA*) as well as *BRCA1/2*-unrelated (wt*BRCA*) HRD [12,20,23,26].

The predictive value of DNA-based HRD status (defined by the presence of pathogenic variants in *BRCA1/2* and/or a specific genomic scar) for PARPi treatment benefit in EOC

patients has been evaluated in a number of clinical studies. These studies revealed that although both *BRCA* mutation status and HRD status provided information regarding the magnitude of the potential treatment benefit of a PARPi in a given patient population, these biomarkers did not sufficiently discriminate between patients that would or would not benefit from treatment [4,5,9,12,27]. Overall, while these DNA-based tests provide valuable proof of concept for the existence of an additional group of HRD tumors beyond those related to *BRCA1/2*, DNA-based approaches also suffer from a number of important drawbacks regarding the identification of HRD tumors. Firstly, they cannot identify all EOC patients who would benefit from treatment with PARPi [4]. Secondly, the interpretation of modern sequencing data is far from straightforward, as gene-based analyses often detect variants of uncertain significance (VUS) without a clear relationship to HRD, and signature-based approaches identify a relatively large fraction (18%) of HR-intermediate cases in EOC [26]. Thirdly, DNA-based assays are relatively complex, costly and time-consuming.

As an alternative to DNA-based HRD tests, functional assays that assess the ability of replicating tumor cells to accumulate RAD51 protein at DNA DSBs were developed for use in breast, ovarian and endometrial cancer [28-37]. A major advantage of this approach is that RAD51-based tests detect the current HR status of the tumor, irrespective of underlying genetics. Furthermore, the "static" HRD status as measured by DNA-based approaches may overestimate the number of tumors that are "functionally" HRD because they make no account for undetected reversion mechanisms [16,38-41]. Studies describing RAD51-based tests have shown that they are able to identify HRD tumors, including but not limited to *BRCA1/2*-related tumors, and can detect phenotypic reversion of the HRD phenotype in *BRCA*-related tumors [28-35,37,41]. In EOC, impairment of HR, as assessed in primary cultures established from ascitic fluid, correlates with PARPi sensitivity both *in vitro* and in clinical studies [30,31]. A recent study using low passage, primary patient-derived tumor and ascites cells confirmed the predictive power of a functional HR score for platinum sensitivity [35].

The REcombination CAPacity test (RECAP) test is a RAD51-based functional test which was previously successfully used for the analysis of HR function in solid breast and endometrial carcinomas [36,37]. In contrast to other RAD51-based tests, the RECAP test enables the assessment of HR on primary tumor tissue without the need to dissociate tissue and culture tumor cells, greatly reducing the time required to perform the test.

In the current study, additional quality controls are described that enable the use of the RECAP test in both solid EOC tumors and ascites-derived EOC tumor cells, taking into account future clinical implementation in routine diagnostic pathology. We demonstrate

that the RECAP test allows the rapid and reliable determination of the functional HR status of EOC, and then explore its correlation with best overall therapy response and overall survival (OS).

2. Results

2.1 REcombination CAPacity (RECAP) Test

The RECAP test has been described in detail in earlier publications [35-37], but in this study we adapted the protocol to make it suitable for direct use with solid tumor and ascites specimens, removing the need for tumor dissociation and cell culture procedures (Figure 1). The RECAP test can include up to 25 tumor specimens simultaneously, and works equally well on fresh or thawed cryopreserved samples (Figure S1). The use of cryopreserved samples greatly increases the clinical utility of the RECAP test, allowing one e.g., to collect tumor tissues from different centers and perform the test in one laboratory. The entire procedure (quality assessment and RECAP test) for a capacity of 25 samples can be completed within two weeks and represents a 25-hours hands-on workload for one person (Table S2). The cost price of the RECAP test is more than 10-fold lower compared to DNA-based alternatives (Table S3) and can be completed in less time than a *BRCA* NGS gene panel (Table S2).

2.2 Identification of Homologous Recombination-Deficient Tumors Using the RECAP Test

In total, 50 (33 solid and 17 ascites) tumor specimens (71% of the collected specimens) met the quality criteria and were therefore eligible for the RECAP test (Figure 2A, Table S4). Of the 50 specimens analyzed, RECAP scores could be determined for 49 tumor specimens (98%) obtained from 48 patients (33 solid tumors and 16 ascites specimens; hereafter referred to as 'RECAP specimens'), as one ascites specimen had to be excluded due to an insufficient number of GMN^+ cells (<40) (Figure S2B, Table S4). Two tumor specimens were obtained from the same patient (case 18 and 28): one at primary cytoreductive surgery (solid tumor) and one at recurrence (ascites).

Based on the RECAP test, ten (20%) of the 49 tumor specimens were found to be HRD (i.e., a RECAP score 0-20%), 37 (76%) were HRP (i.e., a RECAP score 51-100%) and two tumors (4%) were HRI (i.e., a RECAP score 21-50%). Examples of immunofluorescent stained slides are presented in Figure S3. Median RECAP scores differed by 6% (range: 0-34%) between two independent observers, with a high interrogator reliability for final HR group assignment ($\kappa = 0.71$). Thirty-nine of the 49 tumors (80%) were HGSOC, and of these tumors 26%

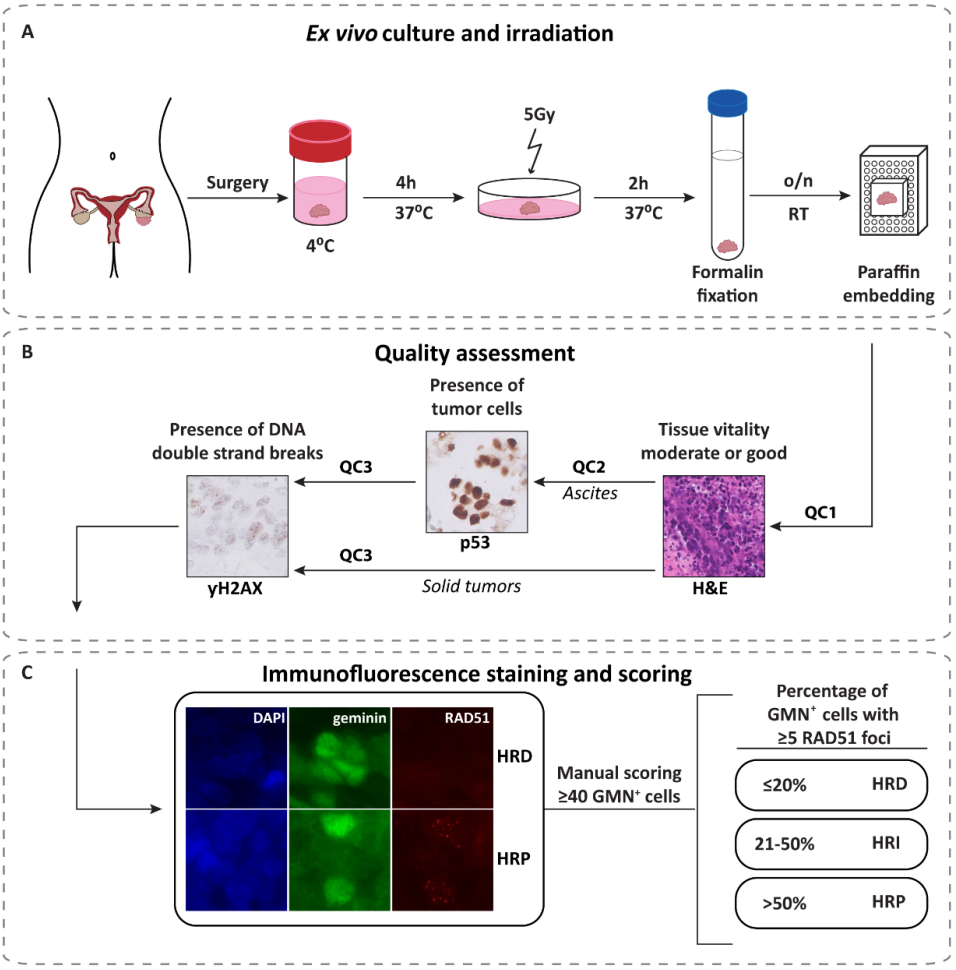


Figure 1. RECAP test procedure. A) After surgery, tumor specimens were placed in OSE medium and incubated for 4h at 37°C on a 60 rpm rotating platform prior to irradiation with 5Gy. Tumor specimens were fixed two hours after irradiation and embedded in paraffin. B) H&E slides were evaluated for tissue quality (QC1) by an experienced pathologist. For ascites specimens an additional p53 IHC staining was performed to confirm the presence of tumor cells (QC2). All tumor specimens were evaluated for the presence of DNA double stand breaks based on yH2AX IHC (QC3). C) A RAD51/GMN IF was performed on tumor specimens that passed the quality assessment. The RECAP score indicates the percentage of GMN$^+$ cells (≥40 cells) with RAD51 foci (≥5 foci). The whole procedure can be performed on multiple specimens simultaneously. Abbreviations: o/n = overnight; RT = room temperature; QC = quality control; H&E = Hematoxylin and Eosin; IF = immunofluorescence; GMN$^+$ = geminin-positive; HRP = HR-Proficient; HRI = HR-Intermediate; HRD = HR-Deficient.

(10/39) were HRD (Figure 2A). The remaining ten tumors (20%) were of other histological subtypes and were all HRP, thus HRD was restricted to HGSOC in this set of tumors.

2.3 Genetic Alterations in HR Genes

In an effort to determine the molecular basis for loss of HR functionality in tumors displaying HRD, (epi)genetic analyses were performed in nine HRD tumors. One HRD tumor could not be analyzed due to an insufficient amount of tumor DNA. Pathogenic variants in *BRCA1, BRCA2,* or *BRCA1* promoter hypermethylation were detected in eight out of nine (89%) HRD tumors in this particular cohort. Six HRD tumors harbored pathogenic variants in *BRCA1* with concomitant LOH of the wild-type allele (cases 40, 41, 42, 44, 45 and 47; Figure 2B; Table S5). One HRD tumor carried a pathogenic variant in *BRCA2* with LOH of the wild-type allele (case 43; Figure 2B; Table S5), while *BRCA1* promoter hypermethylation was observed in another HRD tumor (case 49). No pathogenic variants in *BRCA1*, *BRCA2* or 13 additional HR-related genes, nor *BRCA1* promoter hypermethylation or large genomic rearrangements in *BRCA1,* were detected in the remaining HRD tumor (case 48) nor in the two HRI tumors (case 38 and 39). No pathogenic variants in *BRCA1* and/or *BRCA2,* or *BRCA1* promoter hypermethylation, were identified in any of the HRP tumors available for DNA analysis (Figure 2B).

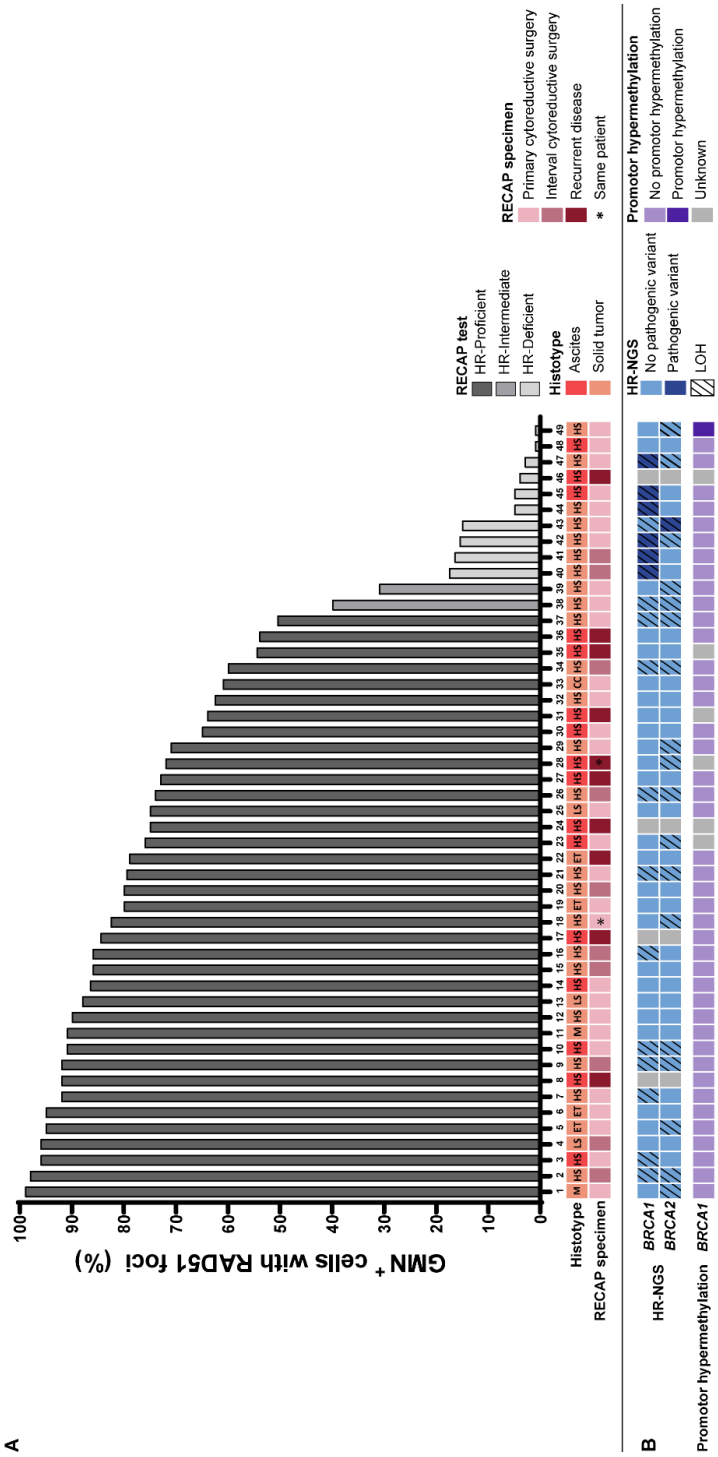


Figure 2. RECAP scores of 49 EOC tumor specimens. A) RECAP scores were calculated as the average percentage of GMN$^+$ cells with ≥5 RAD51 foci scored by two independent observers, and specimens were classified as HRP (51%-100%), HRI (21%-50%) or HRD (0-20%). B) NGS analysis identified seven HRD tumors harboring a pathogenic variant in *BRCA1* or *BRCA2* with concomitant LOH. No pathogenic variants in *BRCA1* or *BRCA2* were identified in HRI and HRP tumors. *BRCA1* promoter hypermethylation was observed in case 49. Pathogenic variants in other HR genes were tested in the HRD and HRI tumors, but none were identified. Abbreviations: HR = Homologous Recombination; HRP= HR-Proficient; HRI = HR-Intermediate; HRD = HR-Deficient; RECAP = REcombination CAPacity; GMN$^+$ = geminin-positive; NGS = next-generation sequencing; HS = high-grade serous; LS = low-grade serous; M = mucinous; CC = clear cell; ET = endometrioid; LOH = loss of heterozygosity.

2.4 Clinicopathological Characteristics

Characteristics of the 48 patients for which the functional HR status of the tumor could be established are shown in Table 1 and Table S6. The mean age of the patients was 62.5 (±1.7 SEM) years and 38/48 (79%) of the patients had been diagnosed with FIGO stage III or IV disease. The majority of patients were diagnosed with HGSOC 38/48 (79%). Tumor specimens were obtained from 33 ovarian tumors (i.e., solid) and from 16 ascites specimens.

Table 1. Clinicopathological characteristics stratified for homologous recombination capacity of the tumor.

Characteristics		Total cohort *n* = 48	HRP *n* = 36	HRI *n* = 2	HRD *n* = 10
Age at diagnosis	*Mean (±SEM)*	62.5 (±1.7)	63.6 (±1.9)	76.5 (±9.2)	55.9 (±3.2)
FIGO stage	*I (I; IA; IC)*	4 (8.3%)	3 (8.3%)		1 (10%)
	IIB	6 (12.5%)	6 (16.7%)		
	III (IIIA; IIIC)	31 (64.6%)	22 (61.1%)	2 (100%)	7 (70%)
	IV	7 (14.6%)	5 (13.9%)		2 (20%)
Histologic subtype	*High-Grade Serous*	38 (79.2%)	26 (72.2%)	2 (100%)	10 (100%)
	Low-Grade Serous	3 (6.3%)	3 (8.3%)		
	Endometrioid	4 (8.3%)	4 (11.1%)		
	Clear cell	1 (2.0%)	1 (2.8%)		
	Mucinous	2 (4.2%)	2 (5.6%)		
Tumor specimen type*	*Solid tumor*	33 (67.3%)	24 (66.7%)	2 (100%)	7 (70%)
	Ascites	16 (32.7%)	13 (36.1%)		3 (30%)
Tumor specimen obtained*	*Primary disease*	39 (79.6%)	28 (75.7%)	2 (100%)	9 (90%)
	Recurrent disease	10 (20.4%)	9 (24.3%)		1 (10%)
Primary treatment strategy	*Staging*	3 (6.3%)	3 (8.3%)		
	Primary cytoreductive surgery	24 (50%)	18 (50%)	1 (50%)	5 (50%)
	Neoadjuvant chemotherapy	21 (43.7%)	15 (41.7%)	1 (50%)	5 (50%)
Residual tumor after cytoreductive surgery**	*Complete (0 cm)*	25 (53.2%)	16 (45.7%)	2 (100%)	7 (70%)
	Optimal (<1 cm)	18 (38.3%)	17 (48.6%)		1 (10%)
	Not optimal (>1 cm)	4 (8.5%)	2 (5.7%)		2 (20%)
Previous cancer(s)	*Ovarian and/or breast*	15 (31.2%)	10 (27.8%)		4 (40%)
	None	33 (68.8%)	26 (72.2%)	1 (50%)	6 (60%)

* Two tumor specimens were derived from one patient. ** One patient with an HRP tumor did not undergo cytoreductive surgery. Abbreviations: HRP = HR-Proficient; HRI = HR-Intermediate; HRD = HR-Deficient; FIGO = International Federation of Gynecology and Obstetrics.

Twenty-four of the 48 (50%) patients underwent primary cytoreductive surgery followed by adjuvant platinum-based chemotherapy, while 21 of the 48 (44%) started on neoadjuvant platinum-based chemotherapy followed by interval cytoreductive surgery (Figure S4). In 39/48 (80%) patients, RECAP specimens were obtained at primary or interval cytoreductive surgery (i.e., primary disease) and in 10/48 (20%) patients at recurrent disease. A complete (i.e., no macroscopic rest tumor) or optimal cytoreduction (i.e., tumor rest <1cm in diameter) at initial surgery was achieved in 43/47 (92%) patients. One patient did not undergo interval cytoreductive surgery because of progressive disease after NACT (Table 1).

2.5 The RECAP test as a Biomarker for Platinum-Based Therapy Response

Although this study was not primarily designed to assess the relationship between the RECAP score and clinical response to platinum-based therapy, a subgroup of patients was available that allowed exploration of the potential differences in platinum-based therapy response between patients with HRD and HRP tumors. Patients were included in this analysis if they met the following criteria: 1) RECAP classification of HRP or HRD, 2) the RECAP specimen was obtained at initial diagnosis of ovarian cancer (at primary or interval cytoreductive surgery), 3) the patient received platinum-based chemotherapy after the RECAP specimen was obtained, and 4) follow-up after first-line treatment was available (Figure S5).

When considering the HGSOC patient group (Table 2), which included all of the HRD cases, we found a trend towards a better OS for patients with HRD tumors compared to patients with HRP tumors (Kaplan Meier p = 0.061) (Figure 3A). When other histological subtypes (all non-HRD) were included, this trend was weakened but maintained (Kaplan Meier p = 0.143) (Figure 3B).

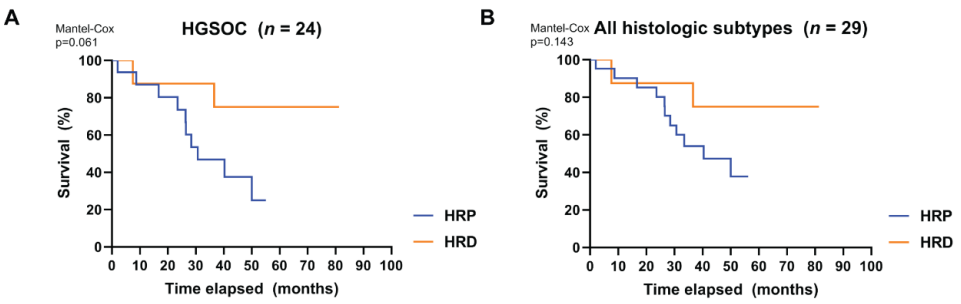


Figure 3. Overall survival of patients with high-grade serous ovarian carcinoma (HGSOC) only and diverse histological subtypes. Kaplan Meier estimates show the overall survival (%) over time (months) in a subgroup of patients whose tumors were analyzed by the RECAP test (see Materials and Methods). A) Overall survival of 24 patients with HGSOC. B) Overall survival of 29 patients with tumors of diverse histological subtype. A Log-rank (Mantel-Cox) test was performed on both groups.

Table 2. Correlation between RECAP score and clinical response.

Clinical Parameters		Diverse histologies			HGSOC		
		HRP *n* = 21	**HRD *n* = 8**	**HRP vs. HRD**	**HRP *n* = 16**	**HRD *n* = 8**	**HRP vs. HRD**
Best overall therapy response - as measured with CT scans/CA125 levels	*Complete Response (CR)*	17 (81.0%)	7 (87.5%)	$p = 0.483$	12 (75.0%)	7 (87.5%)	$p = 1.000$
	Partial Response (PR)	3 (14.3%)			3 (18.8%)		
	Progressive Disease (PD)	1 (4.7%)	1 (12.5%)		1 (6.2%)	1 (12.5%)	
Disease Free Survival (DFS) - Time between start of treatment and progressive disease	*Months (median)*	16.0	23.8	$p = 0.341$	15.4	23.8	$p = 0.232$
Overall therapy response last check-up - as measured with CT scans/CA125 levels	*Complete Response (CR)*	6 (28.6%)	5 (62.5%)	$p = 0.092$	3 (18.8%)	5 (62.5%)	$p = 0.058$
	Partial Response (PR)	1 (4.7%)	1 (12.5%)		1 (6.2%)	1 (12.5%)	
	Stable Disease (SD)	2 (9.5%)			2 (12.5%)		
	Progressive Disease (PD)	12 (57.2%)	2 (25.0%)		10 (62.5%)	2 (25.0%)	
Overall Survival (OS)	*Yes*	10 (47.6%)	6 (75%)	$p = 0.238$	6 (37.5%)	6 (75%)	$p = 0.193$
	No	11 (52.4%)	2 (25%)		10 (62.5%)	2 (25%)	

DFS and follow-up time were tested with the Mann-Whitney U test. Other clinical parameters were tested with Fisher's exact test. Abbreviations: HGSOC = High-grade serous ovarian carcinoma; HRP = HR-Proficient; HRD = HR- Deficient; CT = computed tomography.

However, patients with HRP or HRD tumors did not differ significantly in terms of best overall therapy response following first-line platinum-based chemotherapy ($p = 1.000$ HGSOC only; $p = 0.483$ all histological subtypes). Median follow-up of the patients included in this analysis was 36.6 months. No difference was observed regarding DFS between patients with HRP and HRD tumors ($p = 0.232$ HGSOC only; $p = 0.341$ all histological subtypes).

3. Discussion

The development of a functional biomarker test that allows reliable identification of HRD ovarian malignancies is of broad oncologic interest, as it would facilitate the optimal selection of patients who are most likely to benefit from treatment with PARPi. Using an optimized RECAP test, we investigated the occurrence of HRD in 49 prospectively collected EOC. A substantial fraction of all EOCs in the current study consisted of HGSOC, and of these 26% were classified as HRD. The prevalence of HRD tumors amongst HGSOC that were related to *BRCA1/2* deficiency (including *BRCA1* promoter hypermethylation) was broadly in line with previous reports (Table S1) [17]. However, extended genetic analyses, including *RAD51C* promoter hypermethylation and mutation analysis in HR-related genes, suggest that the proportion of HRD in HGSOC may be as high as 50% [12,17,42], a finding also reported by others using either DNA signature-based approaches or a RAD51-based functional test [9,12,30,31]. Yet, using a similar functional HRD test, our group and Tumiati *et al*. observed a somewhat lower frequency of HRD (~30%) in HGSOC [35]. In our study, only one out of nine HRD tumors (11%) available for sequencing could not be explained by a *BRCA* defect, which is somewhat lower than expected based on previous studies [9,12]. As different methods have been used to identify HRD tumors, several factors may contribute to the observed differences in the prevalence of HRD tumors found in these studies. Firstly, patient cohorts differed in several aspects, such as the inclusion of platinum-sensitive primary and recurrent disease. Secondly, the "static" HRD status as measured by DNA-based approaches may overestimate the number of tumors that are "functionally" HRD because of, for example, undetected reversion mechanisms [16,38-41]. However, the RECAP test may possibly underestimate the frequency of HRD tumors, e.g., due to the inability to identify tumors that are unable to complete HR because formed RAD51 filaments are unstable [43].

The need for improved characterization of HR status in EOC has emerged in recent PARPi trials in which patient response was clearly related to HR status [4,5,9,12,44]. As expected, all trials reported significant benefits in EOC patients with underlying *BRCA1/2* defects (both germline and somatic) following PARPi treatment. Intriguingly, however, the PFS hazard ratios (HR) found in posthoc subanalysis clearly indicated that not only EOC patients with wt*BRCA* high HRD scores benefit from PARPi but also a subset of EOC patients with wt*BRCA* tumors showing low HRD scores using DNA-based methods (MyChoice® HRD test). Although unlikely based on available evidence, this outcome could be due to a PARPi response in otherwise HRP EOC. A more likely explanation is that current DNA-based assessment of HR status is not yet sufficiently accurate. Some of the HRD tumors might not be functional HRD and a number of functional HRD tumors might be missed when only DNA-based approaches are considered.

Although to date, no clinical trials have evaluated RAD51 foci formation as a biomarker for therapy response, several retrospective studies have reported that patients with HRD tumors (defined by a RAD51 functional test) show higher platinum sensitivity and improved survival rates [31,35]. Although our study was not designed or powered to reliably allow the predictive power of HRD as detected by the RECAP test to be assessed, we nevertheless found a trend towards better overall survival of HGSOC patients carrying HRD tumors compared to HRP tumors. Formally, we cannot rule out that this better overall survival is driven by the high frequency of *BRCA*-related HRD tumors (seven out of eight) for which improved therapy outcome has previously been reported.

Additional studies will be required to determine whether the RECAP test is a reliable, comprehensive and efficient biomarker test for a PARPi response. Ideally, these studies should include a comprehensive comparison of the performance of various HRD biomarker tests with respect to sensitivity, specificity, take-rate and costs.

The RECAP test as described here has many advantages over DNA-based tests in terms of cost, speed of turnaround and simplicity of analysis once implemented. The cost per sample for the RECAP test is more than a 10-fold lower than the costs of running a *BRCA* NGS gene panel (Table S3). In addition, the RECAP test can be completed in less time than a *BRCA* NGS gene panel (Table S4). Another major strength of the RECAP test is that it allows identification of HRD tumors independently of *BRCA* status, while correctly assessing *BRCA*-related tumors with reversion mutations as being HRP [36,37,41], in contrast to DNA-based tests [16,38-40]. In our study, the RECAP test identified all tumors with *BRCA* defects as HRD, further underlining the high reliability of the test. As some laboratories nowadays offer *BRCA* tumor testing to identify patients for follow up germline *BRCA* testing, the use of the RECAP test as a prescreen for *BRCA* testing would substantially reduce the number of tumors to be sequenced to identify these patients.

The RECAP test is not without its limitations. It relies on fresh or cryopreserved tumor specimens and requires induction of DSBs by ionizing radiation or chemical compounds such as platinum-based compounds or PARPi [29,30,32,33,36,37]. The percentage of samples that did not pass our stringent quality control (29%) is higher than reported for DNA-based analyses (15%) [4,9].

Initial set-up and implementation in a routine diagnostic setting might therefore be challenging in some laboratories. Recently, an attractive adaptation of the RECAP procedure was proposed that maintains the advantages of the test while avoiding the drawbacks. This new approach is innovative because it now allows assessment of RAD51 foci in FFPE breast tumor samples directly [45,46]. We now propose to establish

whether the use of FFPE material can serve as a reliable substitute for fresh tumor tissue, comparing fresh tumor specimens and matching archival FFPE tumor blocks on RAD51 scores, ideally in large cohorts of different tumor types. Should the FFPE-based method prove to be as reliable as the current RECAP test, the use of RAD51 as a biomarker for the identification of HRD tumors will become feasible in many more diagnostic laboratories, facilitating the rapid and reliable identification and selection of patients who may derive most benefit from PARPi treatment.

In conclusion, we show that functional analysis of HR status in EOC by the RECAP test enables fast and reliable identification of tumors with a deficiency in HR. The RECAP test is therefore an attractive alternative to DNA-based HRD tests and warrants further development as a clinical grade test.

4. Materials and methods

4.1 EOC Patient Material

Fresh tumor tissue or ascites fluid from patients with primary or recurrent EOC who underwent cytoreductive surgery or drainage of ascites fluid at Leiden University Medical Center (LUMC) was prospectively collected if sufficient material was available for research. In total, 70 specimens (43 solid tumors and 27 ascites) were obtained from 66 patients between June 2010 and July 2017.

After surgical removal of the tumor, macroscopic dissection was performed for diagnostic purposes at the Department of Pathology. When available, a tumor tissue fragment (minimum of 0.5-1cm^3) was transferred to OSE medium (Wisent Bioproducts, cat. 316-030-CL) supplemented with 10% Fetal Bovine Serum (FBS) (Bodinco), 1% penicillin (100 U/ml) and streptomycin (0.1 mg/ml), kept at 4°C and processed within 24 hours of surgical resection (Figure 1A). Ascites was collected in fluid drainage bags, kept at 4 °C and used within 24 hours after fluid drainage. Surplus tumor specimens were cryopreserved to enable comparison of test outcomes in fresh versus cryopreserved specimens (see below).

All specimens were coded with a unique research code. The study protocol has been approved by the Medical Ethics Committee of the LUMC on 7th February 2011 and 24th May 2017 (P10.226 and G17.041) and specimens were handled according to the Code for Proper Secondary Use of Human Tissue in the Netherlands as established by the Dutch Federation of Medical Scientific Societies.

4.2 Cryopreservation and Thawing of Tumor Specimens

Surplus tumor specimens were cryopreserved in ampules containing 500 µl Recovery Cell Culture Freezing Medium (Gibco, cat. 12648010). Cells isolated from ascites were collected and cryopreserved at approximately 10 million cells per ampule containing 500 µl of Recovery Cell Culture Medium. Ampules were placed in a freezing container (Nalgene Mr. Frosty, Sigma, cat. C1562) overnight at -80ºC before transfer to liquid nitrogen storage. Cryopreserved tumor specimens were thawed quickly in a 37ºC water bath. Solid tumor specimens were washed in pre-warmed (37ºC) OSE medium containing 40% FBS by gentle rotation of the tube for 5 minutes and subsequently transferred to 2.5 ml OSE medium supplemented with 10% FBS and pen/strep, and next incubated on a 60 rpm rotation platform at 37ºC in a 5% CO_2 humidified atmosphere overnight prior to irradiation. After thawing, cells from ascites were collected by centrifugation (1000 rpm, 5 minutes), washed for 5 min in the presence of pre-warmed (37ºC) OSE medium containing 40% FBS, collected by centrifugation and supplemented in culture medium for subsequent use (see below).

4.3 REcombination CAPacity (RECAP) Test

The RECAP test was performed according to our previously published method [37], but was adapted for ascites specimens as follows: Tumor cells were obtained by centrifugation (5 minutes, 1000 rpm) of one liter of ascites fluid, cell pellets were then washed in lysis buffer (155 mM NH_4CL/21 mM Tris) to remove red blood cells, and after a second round of centrifugation, cell pellets were washed in OSE medium supplemented with 10% FBS and penicillin-streptomycin (pen/strep). Approximately ten million cells were seeded in 20ml OSE medium supplemented with 10% FBS and pen/strep prior to incubation and irradiation. After fixation, cells were centrifuged (1000rpm, 5 minutes), washed in 70% ethanol and embedded in Cytoblock gel (ThermoFisher Scientific, cat. 7401151). In addition, all tumor specimens were subjected to quality assessment.

4.4 Quality Assessment

To determine whether the tissue quality and the number of tumor cells was sufficient for analysis in the RECAP test, three quality assessments were applied as summarized in Figure 1B and Figure S2A.

First, the quality of the tumor specimen was assessed by a pathologist (TB) using a hematoxylin and eosin (H&E) stained section of the irradiated tumor used in the RECAP test (quality control 1, QC1). The tissue quality (1 - 2 = poor, 3 – 4 = moderate and 5 – 6 = good) was determined on the basis of the sum of the tissue vitality (1 = poor, 2 = moderate and 3 = good) and tumor percentage (0 = <5%, 1 = 5 - 20%, 2 = 21 - 50% and 3 = >50%). When a tumor specimen had a total tissue quality score of ≤ 2, cryopreserved tissue was thawed and the procedure was repeated. If the total tissue quality score of this sample was also ≤ 2, the specimen was excluded from further analysis (Table S4).

To aid in the identification of tumor cells in ascites specimens, we included a p53 immunostaining as a second quality control (QC2) since over 98% of HGSOC have been reported to be *TP53*-mutant [47]. Immunohistochemistry staining for p53 (Agilent Dako, cat. M7001) was performed as described previously [48]. A pathologist (TB) used a combination of H&E and p53-IHC stained slides to either include (sufficient cancer cells available) or exclude (insufficient cancer cells available) ascites cases from the study (Table S4).

Third, yH2AX immunostaining was included as a proxy for the presence of DNA DSBs (quality control 3, QC3). Immunohistochemistry was performed as described previously [48]. The primary yH2AX antibody (mouse, monoclonal, Sigma-Aldrich, cat. 05-636) was diluted 1:40.000 in block buffer and incubated at RT o/n. The secondary antibody (BrightVision poly-HRP-anti-Mouse, VWRDPVVM0110HRP, Immunologic) was incubated for

one hour at RT according to manufacturers' protocol. A pathologist (TB) scored the slides based on the presence (inclusion of the sample) or absence (exclusion of the sample) of yH2AX foci.

A total of 70 EOC specimens (43 solid tumors and 27 ascites specimens) (Figure S2A) were collected from 66 patients. Among these tumor specimens 47/70 passed quality control according to QC1 (Materials and Methods, 30 solid and 17 ascites). Of the fresh specimens that failed QC1, 11 (3 solid and 8 ascites) could still be included by using cryopreserved material (Figure S1), resulting in a total of 58/70 (33 solid and 25 ascites) tumor specimens that passed QC1. Seventeen of the 25 ascites specimens passed QC2 (Materials and Methods, p53 staining). All tumor specimens were positive for yH2AX foci and therefore passed QC3.

4.5 Immunofluorescence Staining for RAD51 and Geminin

Immunofluorescence staining for RAD51 (GeneTex, cat GTX70230) and geminin (ProteinTech, cat. 10802-1-AP) was performed as described previously [37], with the following modifications: Tissue sections were incubated at 60^{0}C overnight on SuperFrost Plus microscope slides (75 × 25mm, VWR, Cat. 631-0108) prior to immunofluorescence staining and no EdU immunostaining was performed (Figure S3).

4.6 Scoring of RECAP Tumor Specimens

The scoring of RECAP tumor specimens was blinded for genetic data (*BRCA* status). Tissue sections stained for DAPI, geminin (G2/S phase marker) and RAD51 were manually scored using a Zeiss Axio Imager D2 microscope with a HXP 120C light source. DAPI was used to localize tumor cells in the tissue section based on morphology. Cells were considered geminin-positive (GMN$^+$) if the nucleus was homogenously stained. GMN$^+$ cells were considered RAD51$^+$ if there were at least 5 nuclear foci visible. A minimum of 40 GMN$^+$ cells, randomly chosen in vital tumor areas (defined by the lack of necrosis visible with DAPI) were scored. Specimens with less than 40 GMN$^+$ cells were excluded from the analysis (Figure 1C and Figure S2B).

The RECAP score is the average percentage score, for two independent observers, of GMN+ cells with RAD51 foci. Tumors were allocated to one of three groups: HR-Proficient (HRP; 51-100%), HR-Intermediate (HRI; 21-50%) or HR-Deficient (HRD; 0-20%). The two hour post-irradiation incubation time point and the thresholds for HR status assignment were determined in a previous study on breast tumors [37]. In this and subsequent studies on breast and endometrial carcinomas these settings allowed unequivocal discrimination between *BRCA* wildtype and *BRCA1/2* deficient tumors (including those

with promoter hypermethylation of *BRCA1*) while identifying an additional group of HRD tumors not related to *BRCA1/2* [36,37]). In EOC, with these settings RAD51 foci formation was observed in the majority of replicating tumor cells in *BRCA* wildtype tumors while correctly identifying *BRCA1/2* deficient tumors. The Cohen kappa coefficient (k) was used to measure interobserver and intertest agreement.

4.7 Tumor DNA Isolation

Tumor DNA was isolated from formalin-fixed paraffin-embedded (FFPE) tissue blocks either by taking three 0.6 mm tumor cores or by microdissection of tumor areas with at least 70% tumor cells (10 mm slides). Fully automated DNA isolation was performed using the Tissue Preparation System (Siemens Healthcare Diagnostics) as described previously [37]. An H&E slide (5µm) was prepared for each FFPE tissue block to determine tumor percentage prior to tumor DNA isolation. The Qubit dsDNA HS Assay Kit was used for DNA quantification according to manufacturer's protocol (Qubit 2.0 Fluorometer, Life Technologies).

4.8 Next-Generation Sequencing

Next-generation sequencing (NGS) was performed using 40 ng of tumor DNA per sample isolated from FFPE tissue blocks. The mean tumor cell percentage of included samples was 62% (range: 10% – 90%). All tumors (HRD, HRI and HRP) have been sequenced for *BRCA1* and *BRCA2* and analyzed for promoter hypermethylation of *BRCA1*. The non-BRCA HRD tumor and the two HRI tumors were subsequently analyzed for pathogenic variants in 13 additional HR-related genes and large genomic rearrangements in *BRCA1*.

The custom Ampliseq HDR15v1-panel (Thermo Fisher) was used for variant detection in the coding exons of the following HR-associated genes: *ATM* (not covered by design: exon 25 (1225-1231), exon 36 (1813-1821), exon 52 (2576-2596)), *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CDK12* (not covered by design: exon 1 (294-302)), *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *PPP2R2A*, *RAD51B*, *RAD51C* (not covered by performance, exon 5 (236-241)), *RAD51D* (not covered by performance, exon 5 (130-160)), and *RAD54L*. Details on request (TVW, NS). Mutation and LOH analysis of the NGS data was performed as described previously by de Jonge *et al.* [49]. Variants were categorized using the 5-tier pathogenicity classification according to Plon *et al*: Class 1 = benign, Class 2 = likely benign, Class 3 = variant of unknown significance (VUS), Class 4 = likely pathogenic, and Class 5 = pathogenic [50].

4.9 BRCA1 Promoter Hypermethylation by MS-MLPA

Analysis of *BRCA1* promoter hypermethylation by MS-MLPA was performed as described previously [37].

4.10 BRCA1 MLPA

Copy number variant (CNV)-MLPA was performed as described previously [49].

4.11 Clinical Response Evaluation and Follow-Up

Clinical follow-up data were retrospectively collected for all patients whose tumor specimens had an informative RECAP score. Ovarian cancer staging was performed according to the 2014 International Federation of Gynecology and Obstetrics (FIGO) guidelines [51]. The study was conducted in accordance with the Declaration of Helsinki and the Dutch Code of Good Conduct.

Primary therapy response after first-line treatment and overall therapy response at the last check-up were assessed by physical and gynecological examination, measurement of tumor marker CA125 and/or computed tomography (CT) scans according to the RECIST version 1.1 guideline [52]. In patients with CA125 levels < 35 KU/L [53] and no clinical signs of tumor by physical and gynecological investigation, a standard CT scan was not always performed after first-line treatment (Figure S4). Primary therapy response after first-line treatment was noted as a complete or partial response, or as stable or progressive disease according to the WHO criteria. The best overall therapy response is defined as the best recorded response from the start of the treatment to disease progression/recurrence.

Disease-Free Survival (DFS) was defined as the period between start of treatment, i.e primary cytoreductive surgery, staging procedure or start of platinum-based chemotherapy, and the first observation of recurrent or progressive disease or death due to any cause, whichever occurred first. A patient was considered platinum-sensitive when no recurrence or progression was noted for ≥6 months after the last chemotherapy. When recurrence or progression occurred within <6 months after the last chemotherapy, a patient was considered platinum-resistant. Overall Survival (OS) was determined from the date of start of treatment i.e. date of primary surgery or start of platinum-based chemotherapy to the date of death from any cause. Follow-up time was calculated from the date of start of treatment until the last check-up before cut-off for the final analysis or the date of death from any cause (Figure S4).

4.12 Statistical Analysis

Statistical analysis of clinical data was performed with SigmaStat 3.5 and Graphpad Prism 8.0. Student's t-tests were performed on numerical data when normality criteria were met, otherwise Mann-Whitney U tests were performed. Chi-square tests were performed on categorical data when normality criteria were met and Fisher's exact tests were performed when data were not normally distributed. Kaplan-Meier plots of follow-up

were generated in Graphpad Prism 8.0 for OS. Images were produced with GraphPad Prism 8.0 and Adobe Creative Suite CS6.

5. Conclusions

In this manuscript we describe the use of the REcombination CAPacity (RECAP) test to identify HRD ovarian carcinomas. We found that all HRD ovarian carcinomas in our cohort were of the high-grade serous (HGSOC) histological subtype. The RECAP test showed that 26% (10/39) of HGSOCs were HRD. Of the HRD tumors available for sequencing, 8/9 showed pathogenic *BRCA1/2* variants or *BRCA1* promoter hypermethylation, indicating that the RECAP test matches and exceeds the detection capacity of DNA-based tests, but more rapidly and at lower costs. Furthermore, we found a trend towards better overall survival of HGSOC patients with HRD tumors compared to patients with HR proficient tumors. Overall, we show that the RECAP test is rapid, reliable and as such a good alternative to DNA-based tests.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conceptualization, Dik C. van Gent, Harry Vrieling and Maaike P. G. Vreeswijk; Data curation, Lise M. van Wijk, Natalja T. ter Haar, Marthe M. de Jonge, Judith R. Kroep, Tjalling Bosse and Katja N. Gaarenstroom; Formal analysis, Lise M. van Wijk, Sylvia Vermeulen, Matty Meijers, Manuela F. van Diest, Natalja T. ter Haar, Marthe M. de Jonge, Nienke. Solleveld-Westerink and Tom van Wezel ; Funding acquisition, Dik C. van Gent, Harry Vrieling and Maaike P. G. Vreeswijk; Investigation, Lise M. van Wijk, Sylvia Vermeulen, Matty Meijers, Manuela F. van Diest, Natalja T. ter Haar, Marthe M. de Jonge and Katja N. Gaarenstroom; Methodology, Lise M. van Wijk, Manuela F. van Diest, Dik C. van Gent, Judith R. Kroep, Tjalling Bosse, Katja N. Gaarenstroom, Harry Vrieling and Maaike P. G. Vreeswijk; Project administration, Maaike P. G. Vreeswijk; Supervision, Harry Vrieling and Maaike P. G. Vreeswijk; Visualization, Lise M. van Wijk; Writing – original draft, Lise M. van Wijk, Harry Vrieling and Maaike P. G. Vreeswijk; Writing – review & editing, Sylvia Vermeulen, Natalja T. ter Haar, Marthe M. de Jonge, Nienke Solleveld-Westerink, Tom van Wezel , Dik C. van Gent, Judith R. Kroep, Tjalling Bosse and Katja N. Gaarenstroom.

Funding

Grant support: Alpe d'HuZes EMCR 2014-7048

Acknowledgements

We would like to thank Tessa Rutten (Pathology department, Leiden University Medical Center, Leiden, the Netherlands) for her excellent work in collecting data about the workload and cost price of the *BRCA* next-generation sequencing panel. This work was supported by The Dutch Cancer Society KWF Alpe d'HuZes (grant EMCR:2014-7048 to D.C. van Gent and M.P.G. Vreeswijk).

References

1. Lheureux, S.; Gourley, C.; Vergote, I.; Oza, A.M. Epithelial ovarian cancer. *Lancet* **2019**, *393*, 1240-1253, doi:10.1016/S0140-6736(18)32552-2.
2. Colombo, N.; Sessa, C.; Bois, A.D.; Ledermann, J.; McCluggage, W.G.; McNeish, I.; Morice, P.; Pignata, S.; Ray-Coquard, I.; Vergote, I., et al. ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease. *Int J Gynecol Cancer* **2019**, 10.1136/ijgc-2019-000308, doi:10.1136/ijgc-2019-000308.
3. Ledermann, J.; Harter, P.; Gourley, C.; Friedlander, M.; Vergote, I.; Rustin, G.; Scott, C.; Meier, W.; Shapira-Frommer, R.; Safra, T., et al. Olaparib maintenance therapy in platinum-sensitive relapsed ovarian cancer. *N Engl J Med* **2012**, *366*, 1382-1392, doi:10.1056/NEJMoa1105535.
4. Mirza, M.R.; Monk, B.J.; Herrstedt, J.; Oza, A.M.; Mahner, S.; Redondo, A.; Fabbro, M.; Ledermann, J.A.; Lorusso, D.; Vergote, I., et al. Niraparib Maintenance Therapy in Platinum-Sensitive, Recurrent Ovarian Cancer. *N Engl J Med* **2016**, *375*, 2154-2164, doi:10.1056/NEJMoa1611310.
5. Coleman, R.L.; Oza, A.M.; Lorusso, D.; Aghajanian, C.; Oaknin, A.; Dean, A.; Colombo, N.; Weberpals, J.I.; Clamp, A.; Scambia, G., et al. Rucaparib maintenance treatment for recurrent ovarian carcinoma after response to platinum therapy (ARIEL3): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet* **2017**, *390*, 1949-1961, doi:10.1016/S0140-6736(17)32440-6.
6. Pujade-Lauraine, E.; Ledermann, J.A.; Selle, F.; Gebski, V.; Penson, R.T.; Oza, A.M.; Korach, J.; Huzarski, T.; Poveda, A.; Pignata, S., et al. Olaparib tablets as maintenance therapy in patients with platinum-sensitive, relapsed ovarian cancer and a BRCA1/2 mutation (SOLO2/ENGOT-Ov21): a double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Oncol* **2017**, *18*, 1274-1284, doi:10.1016/S1470-2045(17)30469-2.
7. Moore, K.; Colombo, N.; Scambia, G.; Kim, B.G.; Oaknin, A.; Friedlander, M.; Lisyanskaya, A.; Floquet, A.; Leary, A.; Sonke, G.S., et al. Maintenance Olaparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N Engl J Med* **2018**, *379*, 2495-2505, doi:10.1056/NEJMoa1810858.
8. Friedlander, M.; Matulonis, U.; Gourley, C.; du Bois, A.; Vergote, I.; Rustin, G.; Scott, C.; Meier, W.; Shapira-Frommer, R.; Safra, T., et al. Long-term efficacy, tolerability and overall survival in patients with platinum-sensitive, recurrent high-grade serous ovarian cancer treated with maintenance olaparib capsules following response to chemotherapy. *Br J Cancer* **2018**, *119*, 1075-1085, doi:10.1038/s41416-018-0271-y.
9. González-Martín, A.; Pothuri, B.; Vergote, I.; DePont Christensen, R.; Graybill, W.; Mirza, M.R.; McCormick, C.; Lorusso, D.; Hoskins, P.; Freyer, G., et al. Niraparib in Patients with Newly Diagnosed Advanced Ovarian Cancer. *N Engl J Med* **2019**, *381*, 2391-2402, doi:10.1056/NEJMoa1910962.
10. Coleman, R.L.; Fleming, G.F.; Brady, M.F.; Swisher, E.M.; Steffensen, K.D.; Friedlander, M.; Okamoto, A.; Moore, K.N.; Efrat Ben-Baruch, N.; Werner, T.L., et al. Veliparib with First-Line Chemotherapy and as Maintenance Therapy in Ovarian Cancer. *N Engl J Med* **2019**, *381*, 2403-2415, doi:10.1056/NEJMoa1909707.
11. Ray-Coquard, I.; Pautier, P.; Pignata, S.; Pérol, D.; González-Martín, A.; Berger, R.; Fujiwara, K.; Vergote, I.; Colombo, N.; Mäenpää, J., et al. Olaparib plus Bevacizumab as First-Line Maintenance in Ovarian Cancer. *N Engl J Med* **2019**, *381*, 2416-2428, doi:10.1056/NEJMoa1911361.
12. Hodgson, D.R.; Dougherty, B.A.; Lai, Z.; Fielding, A.; Grinsted, L.; Spencer, S.; O'Connor, M.J.; Ho, T.W.; Robertson, J.D.; Lanchbury, J.S., et al. Candidate biomarkers of PARP inhibitor sensitivity in ovarian cancer beyond the BRCA genes. *Br J Cancer* **2018**, *119*, 1401-1409, doi:10.1038/s41416-018-0274-8.

13. Network, C.G.A.R. Integrated genomic analyses of ovarian carcinoma. *Nature* **2011**, *474*, 609-615, doi:10.1038/nature10166.
14. Walsh, T.; Casadei, S.; Lee, M.K.; Pennil, C.C.; Nord, A.S.; Thornton, A.M.; Roeb, W.; Agnew, K.J.; Stray, S.M.; Wickramanayake, A., et al. Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing. *Proc Natl Acad Sci U S A* **2011**, *108*, 18032-18037, doi:10.1073/pnas.1115052108.
15. Ruscito, I.; Dimitrova, D.; Vasconcelos, I.; Gellhaus, K.; Schwachula, T.; Bellati, F.; Zeillinger, R.; Benedetti-Panici, P.; Vergote, I.; Mahner, S., et al. BRCA1 gene promoter methylation status in high-grade serous ovarian cancer patients--a study of the tumour Bank ovarian cancer (TOC) and ovarian cancer diagnosis consortium (OVCAD). *Eur J Cancer* **2014**, *50*, 2090-2098, doi:10.1016/j.ejca.2014.05.001.
16. Patch, A.M.; Christie, E.L.; Etemadmoghadam, D.; Garsed, D.W.; George, J.; Fereday, S.; Nones, K.; Cowin, P.; Alsop, K.; Bailey, P.J., et al. Whole-genome characterization of chemoresistant ovarian cancer. *Nature* **2015**, *521*, 489-494, doi:10.1038/nature14410.
17. Konstantinopoulos, P.A.; Ceccaldi, R.; Shapiro, G.I.; D'Andrea, A.D. Homologous Recombination Deficiency: Exploiting the Fundamental Vulnerability of Ovarian Cancer. *Cancer Discov* **2015**, *5*, 1137-1154, doi:10.1158/2159-8290.CD-15-0714.
18. Flaum, N.; Crosbie, E.J.; Edmondson, R.J.; Smith, M.J.; Evans, D.G. Epithelial ovarian cancer risk: A review of the current genetic landscape. *Clin Genet* **2020**, *97*, 54-63, doi:10.1111/cge.13566.
19. Torre, L.A.; Trabert, B.; DeSantis, C.E.; Miller, K.D.; Samimi, G.; Runowicz, C.D.; Gaudet, M.M.; Jemal, A.; Siegel, R.L. Ovarian cancer statistics, 2018. *CA Cancer J Clin* **2018**, *68*, 284-296, doi:10.3322/caac.21456.
20. Abkevich, V.; Timms, K.M.; Hennessy, B.T.; Potter, J.; Carey, M.S.; Meyer, L.A.; Smith-McCune, K.; Broaddus, R.; Lu, K.H.; Chen, J., et al. Patterns of genomic loss of heterozygosity predict homologous recombination repair defects in epithelial ovarian cancer. *Br J Cancer* **2012**, *107*, 1776-1782, doi:10.1038/bjc.2012.451.
21. Timms, K.M.; Abkevich, V.; Hughes, E.; Neff, C.; Reid, J.; Morris, B.; Kalva, S.; Potter, J.; Tran, T.V.; Chen, J., 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, doi:10.1186/s13058-014-0475-x.
22. Kanchi, K.L.; Johnson, K.J.; Lu, C.; McLellan, M.D.; Leiserson, M.D.; Wendl, M.C.; Zhang, Q.; Koboldt, D.C.; Xie, M.; Kandoth, C., et al. Integrated analysis of germline and somatic variants in ovarian cancer. *Nat Commun* **2014**, *5*, 3156, doi:10.1038/ncomms4156.
23. Marquard, A.M.; Eklund, A.C.; Joshi, T.; Krzystanek, M.; Favero, F.; Wang, Z.C.; Richardson, A.L.; Silver, D.P.; Szallasi, Z.; Birkbak, N.J. Pan-cancer analysis of genomic scar signatures associated with homologous recombination deficiency suggests novel indications for existing cancer drugs. *Biomark Res* **2015**, *3*, 9, doi:10.1186/s40364-015-0033-4.
24. Popova, T.; Manié, E.; Rieunier, G.; Caux-Moncoutier, V.; Tirapo, C.; Dubois, T.; Delattre, O.; Sigal-Zafrani, B.; Bollet, M.; Longy, M., et al. Ploidy and large-scale genomic instability consistently identify basal-like breast carcinomas with BRCA1/2 inactivation. *Cancer Res* **2012**, *72*, 5454-5462, doi:10.1158/0008-5472.CAN-12-1470.
25. Birkbak, N.J.; Kochupurakkal, B.; Izarzugaza, J.M.; Eklund, A.C.; Li, Y.; Liu, J.; Szallasi, Z.; Matulonis, U.A.; Richardson, A.L.; Iglehart, J.D., et al. Tumor mutation burden forecasts outcome in ovarian cancer with BRCA1 or BRCA2 mutations. *PLoS One* **2013**, *8*, e80023, doi:10.1371/journal.pone.0080023.

26. Davies, H.; Glodzik, D.; Morganella, S.; Yates, L.R.; Staaf, J.; Zou, X.; Ramakrishna, M.; Martin, S.; Boyault, S.; Sieuwerts, A.M., et al. HRDetect is a predictor of BRCA1 and BRCA2 deficiency based on mutational signatures. *Nat Med* **2017**, *23*, 517-525, doi:10.1038/nm.4292.
27. Staaf, J.; Glodzik, D.; Bosch, A.; Vallon-Christersson, J.; Reuterswärd, C.; Häkkinen, J.; Degasperi, A.; Amarante, T.D.; Saal, L.H.; Hegardt, C., et al. Whole-genome sequencing of triple-negative breast cancers in a population-based clinical study. *Nat Med* **2019**, *25*, 1526-1533, doi:10.1038/s41591-019-0582-4.
28. Willers, H.; Taghian, A.G.; Luo, C.M.; Treszezamsky, A.; Sgroi, D.C.; Powell, S.N. Utility of DNA repair protein foci for the detection of putative BRCA1 pathway defects in breast cancer biopsies. *Mol Cancer Res* **2009**, *7*, 1304-1309, doi:10.1158/1541-7786.MCR-09-0149.
29. Graeser, M.; McCarthy, A.; Lord, C.J.; Savage, K.; Hills, M.; Salter, J.; Orr, N.; Parton, M.; Smith, I.E.; Reis-Filho, J.S., et al. A marker of homologous recombination predicts pathologic complete response to neoadjuvant chemotherapy in primary breast cancer. *Clin Cancer Res* **2010**, *16*, 6159-6168, doi:10.1158/1078-0432.CCR-10-1027.
30. Mukhopadhyay, A.; Elattar, A.; Cerbinskaite, A.; Wilkinson, S.J.; Drew, Y.; Kyle, S.; Los, G.; Hostomsky, Z.; Edmondson, R.J.; Curtin, N.J. Development of a functional assay for homologous recombination status in primary cultures of epithelial ovarian tumor and correlation with sensitivity to poly(ADP-ribose) polymerase inhibitors. *Clin Cancer Res* **2010**, *16*, 2344-2351, doi:10.1158/1078-0432.CCR-09-2758.
31. Mukhopadhyay, A.; Plummer, E.R.; Elattar, A.; Soohoo, S.; Uzir, B.; Quinn, J.E.; McCluggage, W.G.; Maxwell, P.; Aneke, H.; Curtin, N.J., et al. Clinicopathological features of homologous recombination-deficient epithelial ovarian cancers: sensitivity to PARP inhibitors, platinum, and survival. *Cancer Res* **2012**, *72*, 5675-5682, doi:10.1158/0008-5472.CAN-12-0324.
32. Naipal, K.A.; Verkaik, N.S.; Ameziane, N.; van Deurzen, C.H.; Ter Brugge, P.; Meijers, M.; Sieuwerts, A.M.; Martens, J.W.; O'Connor, M.J.; Vrieling, H., et al. Functional ex vivo assay to select homologous recombination-deficient breast tumors for PARP inhibitor treatment. *Clin Cancer Res* **2014**, *20*, 4816-4826, doi:10.1158/1078-0432.CCR-14-0571.
33. Patterson, M.J.; Sutton, R.E.; Forrest, I.; Sharrock, R.; Lane, M.; Kaufmann, A.; O'Donnell, R.; Edmondson, R.J.; Wilson, B.T.; Curtin, N.J. Assessing the function of homologous recombination DNA repair in malignant pleural effusion (MPE) samples. *Br J Cancer* **2014**, *111*, 94-100, doi:10.1038/bjc.2014.261.
34. Shah, M.M.; Dobbin, Z.C.; Nowsheen, S.; Wielgos, M.; Katre, A.A.; Alvarez, R.D.; Konstantinopoulos, P.A.; Yang, E.S.; Landen, C.N. An ex vivo assay of XRT-induced Rad51 foci formation predicts response to PARP-inhibition in ovarian cancer. *Gynecol Oncol* **2014**, *134*, 331-337, doi:10.1016/j.ygyno.2014.05.009.
35. Tumiati, M.; Hietanen, S.; Hynninen, J.; Pietilä, E.; Färkkilä, A.; Kaipio, K.; Roering, P.; Huhtinen, K.; Alkodsi, A.; Li, Y., et al. A functional homologous recombination assay predicts primary chemotherapy response and long-term survival in ovarian cancer patients. *Clin Cancer Res* **2018**, 10.1158/1078-0432.CCR-17-3770, doi:10.1158/1078-0432.CCR-17-3770.
36. Meijer, T.G.; Verkaik, N.S.; Sieuwerts, A.M.; van Riet, J.; Naipal, K.A.T.; van Deurzen, C.H.M.; den Bakker, M.A.; Sleddens, H.F.B.M.; Dubbink, H.J.; den Toom, T.D., et al. Functional ex vivo assay reveals homologous recombination deficiency in breast cancer beyond BRCA gene defects. *Clin Cancer Res* **2018**, *24*, 6277-6287, doi:10.1158/1078-0432.CCR-18-0063.
37. de Jonge, M.M.; Auguste, A.; van Wijk, L.M.; Schouten, P.C.; Meijers, M.; Ter Haar, N.T.; Smit, V.T.H.B.; Nout, R.A.; Glaire, M.A.; Church, D.N., et al. Frequent Homologous Recombination Deficiency in High-grade Endometrial Carcinomas. *Clin Cancer Res* **2019**, *25*, 1087-1097, doi:10.1158/1078-0432.CCR-18-1443.

38. Sakai, W.; Swisher, E.M.; Karlan, B.Y.; Agarwal, M.K.; Higgins, J.; Friedman, C.; Villegas, E.; Jacquemont, C.; Farrugia, D.J.; Couch, F.J., et al. Secondary mutations as a mechanism of cisplatin resistance in BRCA2-mutated cancers. *Nature* **2008**, *451*, 1116-1120, doi:10.1038/nature06633.
39. Norquist, B.; Wurz, K.A.; Pennil, C.C.; Garcia, R.; Gross, J.; Sakai, W.; Karlan, B.Y.; Taniguchi, T.; Swisher, E.M. Secondary somatic mutations restoring BRCA1/2 predict chemotherapy resistance in hereditary ovarian carcinomas. *J Clin Oncol* **2011**, *29*, 3008-3015, doi:10.1200/JCO.2010.34.2980.
40. Christie, E.L.; Fereday, S.; Doig, K.; Pattnaik, S.; Dawson, S.J.; Bowtell, D.D.L. Reversion of BRCA1/2 Germline Mutations Detected in Circulating Tumor DNA From Patients With High-Grade Serous Ovarian Cancer. *J Clin Oncol* **2017**, *35*, 1274-1280, doi:10.1200/JCO.2016.70.4627.
41. Meijer, T.G.; Verkaik, N.S.; Deurzen, C.H.M.v.; Dubbink, H.-J.; Toom, T.D.d.; Sleddens, H.F.B.M.; Hoop, E.O.D.; Dinjens, W.N.M.; Kanaar, R.; Gent, D.C.v., et al. Direct Ex Vivo Observation of Homologous Recombination Defect Reversal After DNA-Damaging Chemotherapy in Patients With Metastatic Breast Cancer. *JCO Precision Oncology* **2019**, 10.1200/po.18.00268, 1-12, doi:10.1200/po.18.00268.
42. Lu, J.; Wu, D.; Li, C.; Zhou, M.; Hao, D. Correlation between gene expression and mutator phenotype predicts homologous recombination deficiency and outcome in ovarian cancer. *J Mol Med (Berl)* **2014**, *92*, 1159-1168, doi:10.1007/s00109-014-1191-9.
43. Whelan, D.R.; Lee, W.T.C.; Yin, Y.; Ofri, D.M.; Bermudez-Hernandez, K.; Keegan, S.; Fenyo, D.; Rothenberg, E. Spatiotemporal dynamics of homologous recombination repair at single collapsed replication forks. *Nat Commun* **2018**, *9*, 3882, doi:10.1038/s41467-018-06435-3.
44. Swisher, E.M.; Lin, K.K.; Oza, A.M.; Scott, C.L.; Giordano, H.; Sun, J.; Konecny, G.E.; Coleman, R.L.; Tinker, A.V.; O'Malley, D.M., et al. Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial. *Lancet Oncol* **2016**, 10.1016/S1470-2045(16)30559-9, doi:10.1016/S1470-2045(16)30559-9.
45. Cruz, C.; Castroviejo-Bermejo, M.; Gutiérrez-Enríquez, S.; Llop-Guevara, A.; Ibrahim, Y.H.; Gris-Oliver, A.; Bonache, S.; Morancho, B.; Bruna, A.; Rueda, O.M., et al. RAD51 foci as a functional biomarker of homologous recombination repair and PARP inhibitor resistance in germline BRCA-mutated breast cancer. *Ann Oncol* **2018**, *29*, 1203-1210, doi:10.1093/annonc/mdy099.
46. Castroviejo-Bermejo, M.; Cruz, C.; Llop-Guevara, A.; Gutiérrez-Enríquez, S.; Ducy, M.; Ibrahim, Y.H.; Gris-Oliver, A.; Pellegrino, B.; Bruna, A.; Guzmán, M., et al. A RAD51 assay feasible in routine tumor samples calls PARP inhibitor response beyond BRCA mutation. *EMBO Mol Med* **2018**, *10*, doi:10.15252/emmm.201809172.
47. Zhang, Y.; Cao, L.; Nguyen, D.; Lu, H. TP53 mutations in epithelial ovarian cancer. *Transl Cancer Res* **2016**, *5*, 650-663, doi:10.21037/tcr.2016.08.40.
48. de Jonge, M.M.; Ritterhouse, L.L.; de Kroon, C.D.; Vreeswijk, M.P.G.; Segal, J.P.; Puranik, R.; Hollema, H.; Rookus, M.A.; van Asperen, C.J.; van Leeuwen, F.E., et al. Germline *BRCA*-associated Endometrial Carcinoma is a Distinct Clinicopathologic Entity. *Clin Cancer Res* **2019**, *25*, 7517-7526, doi:10.1158/1078-0432.CCR-19-0848.
49. de Jonge, M.M.; Ruano, D.; van Eijk, R.; van der Stoep, N.; Nielsen, M.; Wijnen, J.T.; Ter Haar, N.T.; Baalbergen, A.; Bos, M.E.M.M.; Kagie, M.J., et al. Validation and Implementation of BRCA1/2 Variant Screening in Ovarian Tumor Tissue. *J Mol Diagn* **2018**, *20*, 600-611, doi:10.1016/j.jmoldx.2018.05.005.
50. Plon, S.E.; Eccles, D.M.; Easton, D.; Foulkes, W.D.; Genuardi, M.; Greenblatt, M.S.; Hogervorst, F.B.; Hoogerbrugge, N.; Spurdle, A.B.; Tavtigian, S.V., et al. Sequence variant classification and reporting: recommendations for improving the interpretation of cancer susceptibility genetic test results. *Hum Mutat* **2008**, *29*, 1282-1291, doi:10.1002/humu.20880.

51. Prat, J.; Oncology, F.C.o.G. Staging classification for cancer of the ovary, fallopian tube, and peritoneum. *Int J Gynaecol Obstet* **2014**, *124*, 1-5, doi:10.1016/j.ijgo.2013.10.001.
52. Schwartz, L.H.; Litière, S.; de Vries, E.; Ford, R.; Gwyther, S.; Mandrekar, S.; Shankar, L.; Bogaerts, J.; Chen, A.; Dancey, J., et al. RECIST 1.1-Update and clarification: From the RECIST committee. *Eur J Cancer* **2016**, *62*, 132-137, doi:10.1016/j.ejca.2016.03.081.
53. Rustin, G.J.; van der Burg, M.E.; Griffin, C.L.; Guthrie, D.; Lamont, A.; Jayson, G.C.; Kristensen, G.; Mediola, C.; Coens, C.; Qian, W., et al. Early versus delayed treatment of relapsed ovarian cancer (MRC OV05/EORTC 55955): a randomised trial. *Lancet* **2010**, *376*, 1155-1163, doi:10.1016/S0140-6736(10)61268-8.

Supplementary Materials

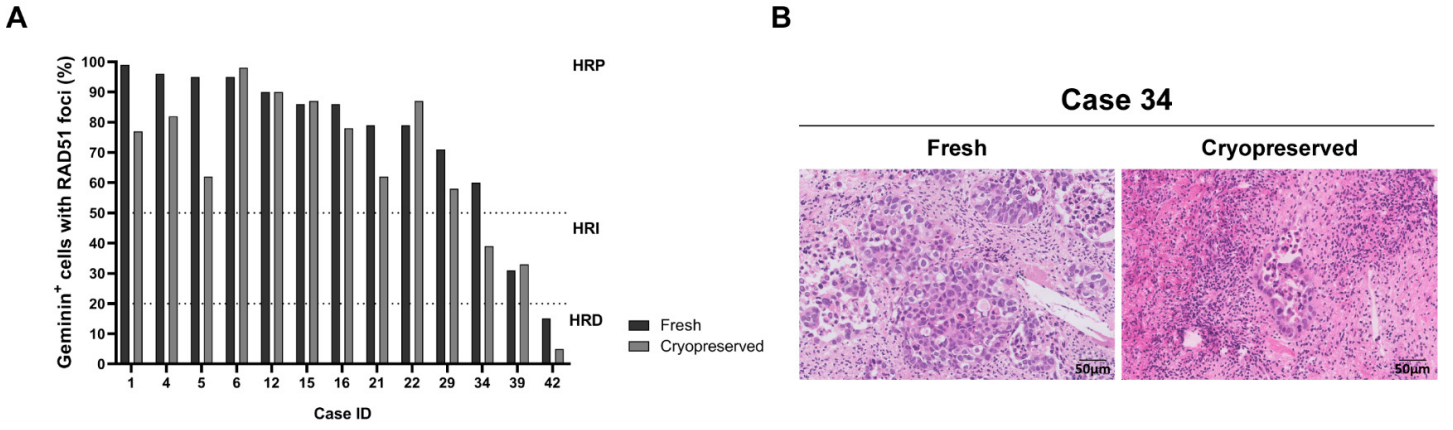


Figure S1. Validation of the RECAP test using fresh and cryopreserved tumor specimens. Case IDs correspond to case IDs described in Figure 2, Figure S3, Table S5 and Table S6. **A**) Thirteen tumor specimens were selected to assess whether cryopreserved tumor tissue can substitute for fresh tumor tissue when determining HR status using the RECAP test. All thirteen cases (matched fresh and cryopreserved) passed the quality assessment, and the HR group assignment based on the RECAP score was highly concordant between fresh and cryopreserved tumor specimens (12/13). RECAP scores for cryopreserved tumor specimens displayed a median score difference of 9% (range: 2% - 24%) between two independent observers, with a high interrogator reliability for final HR group assignment (κ = 1). **B**) Only one specimen was assigned to another HR group, i.e. case 34 was categorized as HRI using cryopreserved tissue (RECAP score 39%), but as HRP using the fresh specimen (RECAP score 60%). This discrepancy may have been caused by tumor heterogeneity, as multiple tumor fields were visible in the fresh specimen (left) but only one tumor field was present in the cryopreserved specimen (right), as visualized in the H&E slides. Abbreviations: HRP = HR-Proficient; HRI = HR-Intermediate; HRD = HR-Deficient; GMN$^+$ = geminin-positive; H&E = Hematoxylin and Eosin.

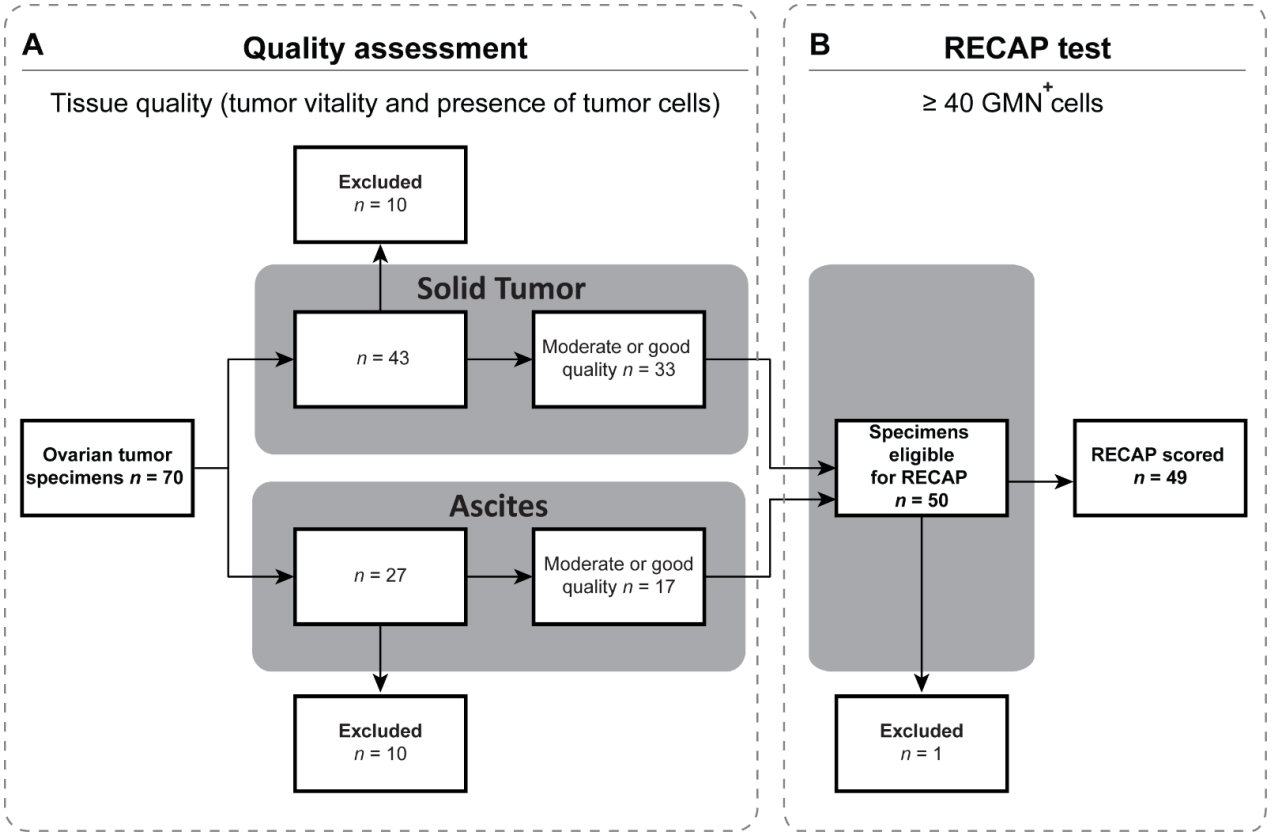


Figure S2. Flowchart illustrating inclusion criteria for RECAP specimens and take-rate. A) Quality assessment was performed as described in Figure 1 and Materials & Methods. Ten solid tumors were excluded due to poor tissue quality. Ascites specimens were excluded due to poor tissue quality (*n* = 2) and lack of tumor cells (*n* = 8). **B)** One ascites specimen was excluded. In total, 49 tumor specimens were scored by the RECAP test. Abbreviations: RECAP = REcombination CAPacity; GMN = geminin-positive.

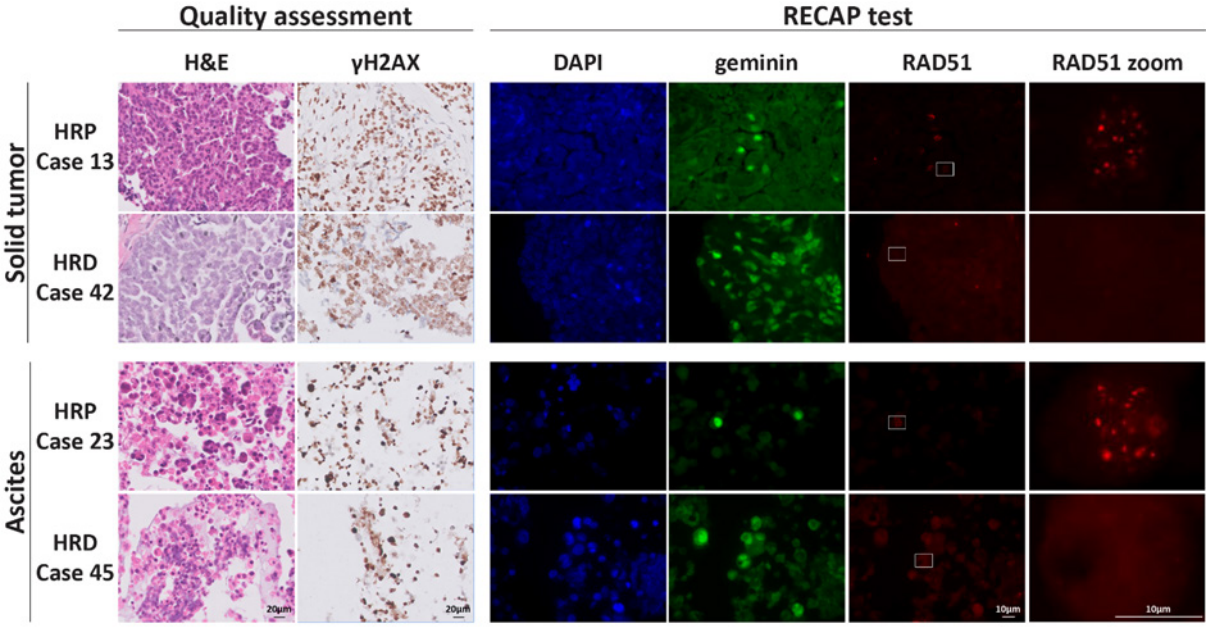


Figure S3. Microscopy illustration of HRP and HRD solid and ascites ovarian tumor specimens classified by the RECAP test. Tumor tissue quality (H&E) was approved by an experienced pathologist and the presence of DNA DSBs was confirmed by γH2AX immunohistochemistry. DAPI was used to identify tumor cells based on morphology. Geminin, a G2/S phase marker, was used to identify cells in G2/S phase. The RAD51 zoom images represent enlargements of the cells surrounded by white boxes in the RAD51 column. Cases correspond with cases described in Figure 2, Figure S1, Table S5 and Table S6. Abbreviations: HRP = HR-Proficient; HRD = HR-Deficient; RECAP= REcombination CAPacity; H&E = Hematoxylin and Eosin.

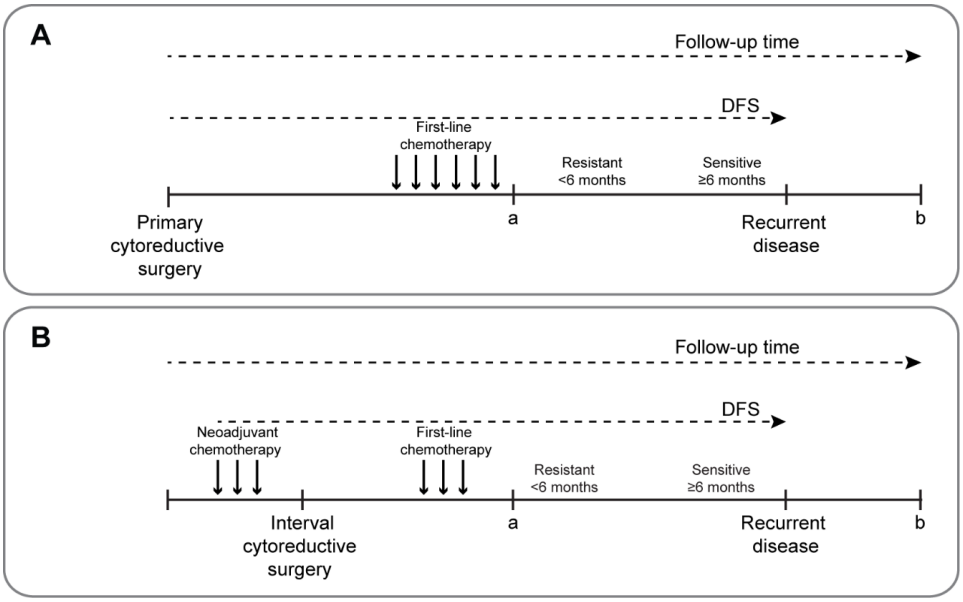


Figure S4. Timeline treatment procedure EOC patients. A) Treatment procedure for EOC patients undergoing primary cytoreductive surgery combined with first-line chemotherapy. **B)** Treatment procedure for EOC patients undergoing neoadjuvant chemotherapy combined with interval cytoreductive surgery and first-line chemotherapy. RECAP tumor specimens were obtained during the primary cytoreductive surgery, interval cytoreductive surgery or during recurrent disease. Clinical treatment outcome (indicated with 'a' and 'b') was measured directly after first-line chemotherapy treatment (a = best overall therapy response) and at the last check-up or death (b = OS). The follow-up time was measured as the time between the start of treatment and the last check-up or death. DFS was measured as the time between the start of treatment and recurrence/progression of disease. A patient is considered platinum-sensitive when no recurrence or progression occurs for ≥6 months after the last chemotherapy. When recurrence or progression occurs in <6 months after the last chemotherapy, a patient is considered platinum-resistant. Abbreviations: EOC = epithelial ovarian carcinoma; DFS = Disease Free Survival; OS = Overall Survival.

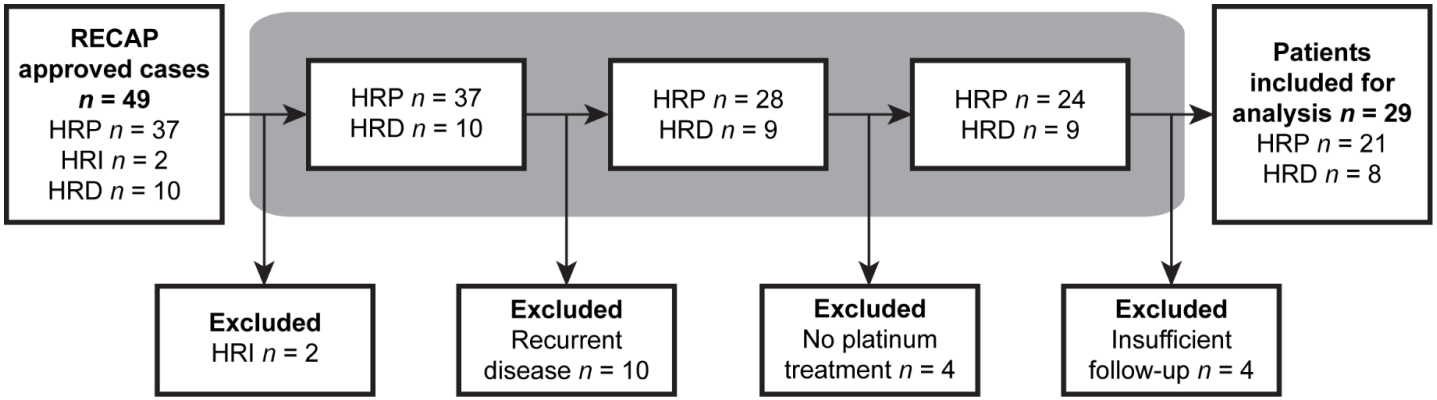


Figure S5. Flowchart illustrating patient inclusion for comparison of RECAP scores with therapy response. To determine if the RECAP score was predictive for the clinical response to platinum treatment, patients were included in the analysis based on the following criteria: i) the classification based on the RECAP score was HR-Proficient or HR-Deficient, ii) the tumor specimen was obtained during primary disease (primary or interval surgery), iii) the patient received platinum-based chemotherapy and iv) complete follow-up data after first-line chemotherapy was available. Abbreviations: HR = Homologous Recombination; HRP = HR-Proficient; HRI = HR-Intermediate; HRD = HR-Deficient; RECAP = REcombination CAPacity.

Table S1. Identification of HR-Deficient tumors by functional and genomic analyses.

HR test	Article	Year	Trial	Type of analyses	Patient cohort	Sample type	Number of samples/ patients	Output	Therapy response in patients
Functional assay RAD51 foci formation	*Mukhopadhyay, A., et al*	2010		N.A	EOC, enriched for advanced stage and high-grade EOCs	Ascitis	25	64% (n=16) HRD	N.A.
	Mukhopadhyay, A., et al	2012		*BRCA1* IHC	EOC stage II/IV, PPC	Ascitis	50	52% (n=26) HRD, from which 35% (8/23) negative for BRCA1 staining	HRD associated to platinum sensitivity and an increased PFI
	Patterson, M.J., et al	2014		N.A.	EOC, not selected	malignant pleural effusions	4	0% HRD	N.A.
	Shah, M.M., et al	2014		N.A.	OC	Human ovarian carcinoma cell lines, pre-selected	4 cell lines	25% (n=1) HRD cell lines(A2780ip2) with BRCAness profile, harbouring PTEN mutation	N.A.
						PDX from naïve omental tumor nodules	8 PDX lines	38% (n=3) HRD PDX lines	N.A.
	Tumiati, M., et al	2018		WGS/WES/Targeted WES	HGSOC	Dissociated tumor cells and ascites	21	29% (n=6) HRD, one tumor with *BRCA1* pathogenic variant	HRD associated to platinum sensitivity, good response to primary therapy and improved OS
				LOH				No strong correlation between LOH and HRD	
Genomic scars/ mutational signatures	*Abkevich, V., et al*	2012		Genome-wide LOH	EOC, not selected	Snap-frozen tissue samples	640 (total of three cohorts)	33% (211/640) HRD tumors based on pathogenic variants in *BRCA1* and/or *BRCA2, BRCA1* methylation or low expression of *BRCA1/2*	HRD scores associated to improved PFS and OS in one patient cohort (n=435)
				BRCA1/BRCA2 NGS				Number of long LOH regions was significantly higher in *BRCA1/2* deficient tumors	
				RAD51C promoter methylation				HRD scores were significantly higher among samples with *RAD51C* promoter methylation	
				Genome-wide LOH and AI	HGSOC, grade 2 or 3	Snap-frozen tissue samples	47	62% (29/47) tumors had high levels of LOH/AI	Patients with high level LOH/AI showed a longer PFS

Table S1. continued

HR test	Article	Year	Trial	Type of analyses	Patient cohort	Sample type	Number of samples/ patients	Output	Therapy response in patients
Genomic scars/ mutational signatures	*Wang, Z.C., et al*	2012		*BRCA1* promoter methylation				20% (9/44) with *BRCA1* promoter methylation	Patients with *BRCA1* promoter hypermethylation had similar survival characteristics to patients without defects in *BRCA*
	Birkbak, N.J., et al	2013		Total number of exome mutations (TCGA)	HGSOC, unselected	Snap-frozen tissue samples	316	11% (34/316) were *mBRCA* with high Nmut	High whole-exome mutation burden (Nmut) in tumors with BRCA pathogenic variants associated to improved PFS and OS
				gBRCA1/BRCA2 or *tBRCA1/2* (TCGA)				22% (n=70) *mBRCA*	
	Lu, J., et al	2014		Gene expression of 116 genes	HGSOC, stage III or IV	TCGA database	308	52% (159/308) were HRD	HRD scores were associated to longer PFS and improved OS
	Pennington, K.P., et al	2014		NGS for HR gene panel	EOC, PFTC or PPC, partially selected	Snap-frozen or FFPE tissue samples	390	31% (121/390) had a mutation in one or more of 13 HR-related genes	Patients with mutations in HR-related genes had a better response to primary platinum chemotherapy and improved OS than patients without mutiations in HR-related genes.
	Ruscito, I., et al	2014		*BRCA1* promoter methylation	HGSOC, unselected	Snap-frozen tissue samples	257	15% (38/257) tumors with *BRCA1* promoter methylation	No impact on PFS and OS rates.
	Konstantinopolous, P.A.	2015		Gene expression of 116 genes	HGSOC, stage II till IV	TCGA database	489	20% tumors with *BRCA1/2* somatic or germline pathogenic variant	
								6% tumors with pathogenic variants in HR-related genes	
				BRCA1 and *RAD51C* promoter methylation				10% tumors with *BRCA1* promoter methylation, 2% with *RAD51C* promoter methylation	
	Mirza, M.R., et al	2016	ENGOT-OV16/ NOVA	HRD Myriad score (LOH, TAI and LST)	EOC, platinum-sensitive, selected	FFPE tissue sections	296	55% (162/296) HRD tumors, from which 29% (47/162) with a *BRCA* pathogenic variant	Clinical benefit was observed in both HRD and HRP groups. HRD status was not sufficiently precise to predict Niraparib sensitivity.
	Coleman, R.L., et al	2017	ARIEL3	*BRCA1/BRCA2* NGS and LOH	HGSOC, OEC, PFTC or PPC, platinum-sensitive, selected	FFPE tissue sections	354	63% (354/564) HRD tumors, based on presence of *BRCA* pathogenic variant or LOH.	BRCAwt patients with high genomic LOH showed increased PFS compared to BRCAwt with low genomic LOH after rucaparib treatment. However, HRD status was not sufficiently precise to predict Rucaparib sensitivity.

Table S1. continued

Genomic scars/ mutational signatures	*Swisher, E.M., et al*	2017	ARIEL2	*BRCA1/BRCA2* NGS and LOH	HGSOC, recurrent, platinum-sensitive		192	21% (40/192) patients had a *BRCA* pathogenic variant	PFS was longer in the subgroups with *BRCA* pathogenic variants and LOH high compared to the LOH low subgroup after treatment with rucaparib.
								43% (82/192) patients had a LOH high score	
	Domcheck, S.M. et al	2017	Study 42, NCT01078662	*BRCA1/BRCA2* NGS	*gBRCA1/2* EOC patients	FFPE tissue sections	154	All patients had a *gBRCA* pathogenic varia	Notable antitumor activity in patients with g*BRCA1/2*m advanced ovarian cancer to olaparib treatment.
	Stronach, E.A., et al	2018	SCOTROC4	HRD score (LOH, TAI and LST)	EOC stage IC to IV, PFTC or PPC	FFPE tissue sections	250	30% (n=74) HRD tumors, from which 14% (n=34) with somatic *BRCA* pathogenic variant	HRD score and *BRCA* pathogenic variants significantly associated to CA125 complete response after carboplatin treatment.
								36% (n=64) HRD HGSOC only, from which 16% (n=29) with *BRCA* pathogenic variants	
	Hodgson, D.R., et al	2018		NGS for HR gene panel	Relapsed serous OC, grade 2 or 3 with platinum sensitivity	Blood samples and FFPE tissue sections	209	53% (111/209) tumors harboured *BRCA1/2* pathogenic variant	Patients with *BRCA* pathogenic variants gained most benefit from olaparib.
			Study 19, D0810C00019; NCT00753545	*BRCA1* promoter methylation				10% (21/209) tumors were *wtBRCA*, but harboured pathogenic variants in at least one HR-related gene	21/95 patients with *BRCA*wt, but pathogenic variants in HR-related genes gained similar benefits from olaparib as patients with BRCA pathogenic variants.
				HRD Myriad score (LOH, TAI and LST)				70% (139/199) obtained a Myriad HRD score, 73% (101/139) of these patients harboured a *BRCA* pathogenic variant	wt*BRCA* patients with HRD status were only marginally more likely to benefit from olaparib.
	Tsibulak, I., et al	2018		*BRCA1/2* mRNA-expression levels	EOC, primary debulking,	Snap-frozen tissue samples	201	18% (36/201) tumors harboured a *BRCA1* pathogenic variant, 6% (11/201)	Low *BRCA1* levels were associated to an impr
				Targeted NGS using the TruSight Cancer sequencing panel					Low *BRCA2* levels were associated to an improved OS and longer PFS.
				BRCA1 promoter methylation				11% (21/199) tumors with *BRCA1* promoter methylation	
	González-Martín, A. et al	2019	PRIMA/ ENGOTOV26/ GOG-3012	HRD Myriad score (LOH, TAI and LST)	Newly diagnosed, platinum-sensitive, HGSOC or endometrioid grade III or IV, selected	FFPE tissue sections	733	373 (50.9%) patient had an HRD score, 223 (30.4%) of these patients had pathogenic variants in *BRCA1/2*	Among the patients with an HRD score, the PFS was significantly longer in the niraparib treated group than in the placebo group. This correlation was also observed in the whole patient cohort and not restricted to patients with an HRD score.

Abbreviations: HRD = homologous recombination deficient; EOC = epithelial ovarian carcinoma; HGSOC = High grade serous ovarian carcinoma; PPC = primary peritoneal carcinoma; OEC = ovarian endometrioid carcinoma; PFTC = primary fallopian tube carcinoma; PDX = patient-derived xenograft; CNV = copy number variation; AI = allelic imbalance; wt = wildtype; PFI = progression free interval; PFS = progression free survival.

Table S2. Estimated workload of *BRCA* next-generation sequencing gene panel testing versus the RECAP test.

	***BRCA* NGS/HR gene panel**					**RECAP test**					
Process	**Activity**	**Hands-on time per sample**	**Hands-on time per full run (50 samples)**	**Complete time per sample**	**Complete time per full run (50 samples)**	**Process**	**Activity**	**Hands-on time per sample**	**Hands-on time per full run (25 samples)**	**Complete time per sample**	**Complete time per full run (25 samples)**
General	Tissue embedding	10 min	90 min	10 min	90 min	**General**	Tissue embedding	10 min	45 min	10 min	45 min
	H&E slicing	10 min	120 min	10 min	120 min		Paraffin cube slicing	10 min	60 min	10 min	60 min
	H&E staining (machine)	10 min	10 min	30 min	30 min	**Quality assessment**	H&E staining (machine)	10 min	10 min	10 min	10 min
	Pathologist H&E review (mark tumor area)	5 min	90 min	5 min	90 min		γH2AX IHC staining	150 min	150 min	1338 min	1338 min
DNA isolation	DNA punch or microdissection	10 min	120 min	10 min	120 min		p53 IHC staining	150 min	150 min	1338 min	1338 min
	DNA isolation (machine, using Tissue Preparation System)	30 min	45 min	240 min	240 min		Pathologist H&E, γH2AX and P53 review (5 min per slide)	15 min	135 min	15 min	135 min
	DNA concentration determination	10 min	90 min	10 min	90 min	**RECAP**	Slicing tumor tissue	10 min	250 min	10 min	250 min
	DNA quality determination (qPCR)	5 min	10 min	210 min	300 min		Preparation culture system	15 min	30 min	15 min	30 min
NGS	Dilute samples	10 min	90 min	10 min	90 min		Cryopreservation tissue	20 min	60 min	20 min	60 min
	DNA repair	5 min	10 min	20 min	30 min		Irradiation	2 min	18 min	10 min	90 min
	Library preparation (amplification, digestion, ligation)	45 min	90 min	300 min	300 min		Tissue fixation	10 min	60 min	10 min	60 min
	Library purification and normalisation	20 min	60 min	240 min	240 min		RAD51/geminin IF staining	150 min	150 min	378 min	378 min
	Ion S5 CHEF	30 min	30 min	480 min	480 min		IF slide scoring	15 min	375 min	15 min	375 min
	S5 sequencing	10 min	10 min	180 min	180 min						
Data analysis	Pipeline	NA	NA	960 min (o/n)	960 min (o/n)						
	Quality control	5 min	180 min	5 min	180 min						
	Analysis	30 min	1200 min	30 min	1200 min						
Total	**2-3 weeks for full run**	**4h 5 min**	**37h 25min**	**45h 50 min**	**79h**	**Total**	**2 weeks for full run**	**9h 27 min**	**24h 53 min**	**53h**	**69h 29 min**

The workload for both the *BRCA* NGS gene panel and the RECAP test are estimations based on working procedures in the Leiden University Medical Center (LUMC), Leiden, the Netherlands. Abbreviations: NGS = next-generation sequencing; RECAP = Recombination CAPacity; IF = immunofluorescence; min = minutes; h = hours; H&E = Hematoxylin and Eosin; NA= Not Applicable; o/n= overnight.

Table S3. Estimated cost prices for a *BRCA* next-generation sequencing gene panel versus the RECAP test.

	***BRCA* NGS/HR gene panel**				**RECAP test**				
Subject	**Product**	**Costs per sample**	**Costs per full run (50 samples)**	**Costs per sample in full run**	**Subject**	**Product**	**Costs per sample**	**Costs per full run (25 samples)**	**Costs per sample in full run**
General	Paraffin cube (embedding, cassette, foams)	€ 3,00	€ 150,00	€ 3,00	**General**	Paraffin cube (embedding, cassette, foams)	€ 3,00	€ 75,00	€ 3,00
	Glassware (microscope slides, coverslips)	€ 0,30	€ 15,00	€ 0,30		Buffers (antigen retrieval, washbuffers, formalin)	€ 7,50	€ 7,50	€ 0,30
	H&E machinal costs	€ 0,11	€ 5,50	€ 0,11		Disposables (pipet tips, wells-plate, falcon tubes)	€ 0,20	€ 5,00	€ 0,20
	Disposables (pipet tips, wells-plate, falcon tubes)	€ 0,20	€ 10,00	€ 0,20		Glassware (microscope slides, coverslips)	€ 0,30	€ 7,50	€ 0,30
DNA isolation	VERSANT Tissue Preparation Reagents Kit	€ 5,20	€ 260,00	€ 5,20	**Quality assessment**	H&E machinal costs	€ 0,11	€ 2,75	€ 0,11
	Qubit™ dsDNA HS Assay Kit	€ 0,79	€ 39,33	€ 0,79		Primary antibodies (γH2AX and p53)	€ 0,05	€ 1,25	€ 0,05
	Components of qPCR mix	€ 0,50	€ 25,00	€ 0,50		Secondary antibodies (DAB+ substrate chromogen system)	€ 1,00	€ 25,00	€ 1,00
	Uracil-DNA Glycosylase	€ 0,28	€ 13,92	€ 0,28		Mounting medium (Surgipath Micromount)	€ 0,50	€ 12,50	€ 0,50
	Ion AmpliSeq™ Library Kit 2.0	€ 70,69	€ 3.534,41	€ 70,69	**RECAP**	OSE medium	€ 0,25	€ 6,25	€ 0,25
	Ion Library TaqMan™ Quantitation Kit	€ 7,04	€ 352,11	€ 7,04		DNAse	€ 0,76	€ 18,88	€ 0,76
	Ion 540™ Chip Kit	€ 1.135,89	€ 1.135,89	€ 22,72		Primary antibodies (RAD51 and geminin)	€ 1,80	€ 45,00	€ 1,80
	Ion 540™ Kit-Chef (2 sequencing runs per initialization)	€ 615,59	€ 615,59	€ 12,32		Secondary antibodies (Alexa Fluor 488 and Alexa Fluor 555)	€ 0,10	€ 2,50	€ 0,10
						ProLong Gold Antifade mount with DAPI	€ 1,30	€ 32,50	€ 1,30
Total		€ 1.839,59	€ 6.156,75	**€ 123,15**	**Total**		€ 16,87	€ 241,63	**€ 9,67**

The total cost prices for both the *BRCA* NGS gene panel and the RECAP test are indications based on European market prices in August 2020. Abbreviations: NGS = next-generation sequencing; RECAP = Recombination CAPacity; H&E = Hematoxylin and Eosin; Excl = exclusive.

Table S4. Excluded tumor specimens based on tissue quality, *p53* status or the RECAP test.

	Sample type	Tumor specimen obtained	Number of patients	Total number of patients (n=21)
Exclusion QC1 tissue quality	**Solid tumors**	**Primary disease**	10	10
	Ascites	**Primary disease**	1	1
		Recurrent disease	1	1
Exclusion QC2 p53 status	**Ascites**	**Primary disease**	5	8
		Recurrent disease	3	
Exclusion RECAP test	**Ascites**	**Primary disease**	1	1

Tumor specimens were obtained during primary disease (primary or interval cytoreductive surgery) or during recurrent disease. Tumor specimens were subjected to a stringent quality assessment as described in the Materials & Methods. Abbreviations: QC = quality control; RECAP = REcombination CAPacity.

Table S5. Pathogenic variants in *BRCA1* and *BRCA2* identified in HR-Deficient ovarian carcinomas.

Case ID	Chromosome: Position	Gene	HGVSCoding	HGVSProtein	Exon/ Intron	Pathogenicity	Coverage	Variant Frequency	Type	*BRCA1* LOH	*BRCA2* LOH
40	17:43048721	*BRCA1*	NM_007294.3, c.5333-36_5406+400del	p.Asp1778GlyfsTer27	22	Class 5-clinical pathogenic	>1000	Unknown	deletion of exon 22	Yes	Not informative
41	17:41245529	*BRCA1*	NM_007294.3, c.2019delA	p.Glu673AspfsTer28	10	Class 5-clinical pathogenic	1980	0,919	frame-shift	Yes	Not informative
42	17:41276080	*BRCA1*	NM_007294.3:c.34C>T	p.Gln12Ter	2	Class 5-clinical pathogenic	1264	0.959	nonsense	Yes	Yes
43	13:32913703	*BRCA2*	NM_000059.3:c.5213_5216delCTTA	p.Thr1738IlefsTer2	11	Class 5-clinical pathogenic	1988	0.896	frame-shift	Yes	Yes
44	17:41244063	*BRCA1*	NM_007294.3:c.3485delA	p.Asp1162ValfsTer48	10	Class 5-clinical pathogenic	1987	0.613	frame-shift	Yes	Not informative
45	17:41226438	*BRCA1*	NM_007294.3:c.4575_4585delAGAGGAGCTCA	p.Gln1525HisfsTer2	14	Class 5-clinical pathogenic	1343	0,881	frame-shift	Yes	Not informative
47	17:41209068	*BRCA1*	NM_007294.3:c.5277+1G>A	Unknown	Intron	Class 5-clinical pathogenic	1687	0,808	Splice-site	Yes	Yes

Case IDs correspond to case IDs in Figure 3, S Figures S4 and S5 and Table S6. Abbreviations: LOH = Loss Of Heterozygosity.

Table S6. Extended clinicopathological characteristics of the ovarian cohort stratified for homologous recombination repair capacity as determined by the RECAP test.

		RECAP specimen characteristics						Clinical follow-up																	
	Case ID	Specimen type	Specimen obtained NACT= neoadjuvant chemotherapy	Tumor vitality H&E poor = 1 moderate = 2 good = 3	H&E Tumor % <5% = 0 5-20% = 1 21-50% = 2 >50% = 3	Total tissue quality score (2-6)	RECAP score (%)	Included for analysis NP = no platinum treatment FD = insufficient follow-up data RD = recurrent disease HRI = HR-Intermediate score	Age at diagnosis	Disease history prior to RECAP specimen inclusion	Histologic subtype HS = high-grade serous LS = Low-grade serous M = mucinous ET = endometrioid CC = clear cell	FIGO stage	Neoadjuvant chemotherapy C = Carboplatin P = Paclitaxel	Type of surgery CPS = cytoreductive primary surgery CIS = cytoreductive interval surgery	Residual tumor after surgery (cm)	Adjuvant chemotherapy C = Carboplatin P = Paclitaxel	Chemotherapy courses (total)	Primary therapy outcome CR = complete remission PR= partial remission SD = stable disease PD = progressive disease	Primary platinum response sensitive = no recurrence or recurrence > 6 months after last chemotherapy resistant = recurrence < 6 months after last chemotherapy	Progression after primary therapy or recurrence	Disease Free Survival (EFS) Time between start of treatment and progressive disease (months)	Treatment recurrent or progressive disease C = Carboplatin P = Paclitaxel CX = caelyx	Status last check-up CR = complete remission PR = partial remission SD = stable disease PD =	Deceased	Follow-up time Time between start of treatment and last check-up/ death (months)
HR-Proficient	1	Solid tumor	Surgical staging	3	2	5	99	No, NP	56	None	M	I	No	Surgical staging	0	N/A	N/A	CR	N/A	No	45,9	N/A	CR	No	45,9
	2	Solid tumor	Cytoreductive interval surgery	2	1	3	98	No, FD	72	None	HS	IV	Yes, C/P	CIS	0	C/P	8	PR	Unknown	Unknown	Unknown	Unknown	PD	Yes	32,2
	3	Ascites	Cytoreductive primary surgery	3	3	6	96	Yes	67	None	HS	IIIC	No	CPS	<1	C/P	6	CR	sensitive	Yes	17,3	C/P	PD	Yes	28,4
	4	Solid tumor	Cytoreductive interval surgery	3	2	5	96	No, NP	30	None	LS	IIIC	Yes, C/P	CIS	<1	N/A	3	PD	resistant	Yes	1,5	/Tamoxifen/Letro	PD	Yes	19,6
	5	Solid tumor	Surgical staging	3	3	6	95	No, NP	62	None	ET	IA	No	Surgical staging	0	N/A	N/A	CR	N/A	No	43,9	N/A	CR	No	43,9
	6	Solid tumor	Cytoreductive primary surgery	2	2	4	95	Yes	49	None	ET	IIB	No	CPS	0	C/P	6	CR	sensitive	No	49,1	N/A	CR	No	49,1
	7	Solid tumor	Cytoreductive primary surgery	2	3	5	92	Yes	69	None	HS	IIIC	No	CPS	<1	C/P	6	CR	sensitive	Yes	28,0	C/CX	PD	Yes	50,0
	8	Ascites	Recurrent disease	3	3	6	92	No, RD	64	Ovarian	HS	IIB	No	CPS	<1	C/P	6	CR	sensitive	Yes	19,5	C/P + C/CX	PD	Yes	38,4
	9	Solid tumor	Cytoreductive interval surgery	2	0	2	92	Yes	51	None	HS	IV	Yes, C/P	CIS	0	C/P	6	CR	resistant	Yes	9,1	Tamoxifen	PD	Yes	23,5
	10	Ascites	Before NACT	3	1	4	91	Yes	65	None	HS	IV	Yes, C/P	CIS	<1	C/P	6	PR	resistant	Yes	6,6	C/CX	PD	Yes	8,7
	11	Solid tumor	Surgical staging	2	1	3	91	No, NP	58	None	M	IA	No	Surgical staging	0	N/A	N/A	CR	N/A	No	37,0	N/A	CR	No	37,0
	12	Solid tumor	Cytoreductive primary surgery	3	3	6	90	Yes	77	None	HS	IIIC	No	CPS	0	C/P	6	CR	sensitive	No	52,8	N/A	CR	No	52,8
	13	Solid tumor	Cytoreductive primary surgery	3	3	6	88	Yes	59	None	LS	IIIC	No	CPS	<1	C/P	6	CR	sensitive	Yes	12,1	C/B/ gemcitabine	PD	No	55,0
	14	Ascites	Before NACT	2	0	2	87	Yes	69	None	HS	IIIC	Yes, C/P	CIS	<1	C/P	6	CR	sensitive	Yes	14,8	C/CX/APR-246	PD	Yes	26,4
	15	Solid tumor	Cytoreductive interval surgery	3	2	5	86	Yes	55	None	HS	IIIC	Yes, C/P	CIS	<1	C/P	6	CR	resistant	Yes	8,5	P/bevacizumab	SD	No	35,3
	16	Solid tumor	Cytoreductive interval surgery	3	3	6	86	Yes	64	None	HS	IIIC	Yes, C/P	CIS	0	C/P	9	CR	resistant	Yes	25,2	C/CX/APR-246	SD	Yes	40,3
	17	Ascites	Recurrent disease	3	0	3	84	No, RD	69	Ovarian	HS	IIIC	Yes, C/P	CIS	0	C/P	6	PR	resistant	Yes	8,2	CX/avelumab	PD	Yes	23,6
	#18	Solid tumor	Cytoreductive primary surgery	1	2	3	82	Yes	71	None	HS	IIIC	No	CPS	>1	C	6	PR	resistant	Yes	8,0	None	PD	Yes	16,7
	19	Solid tumor	Cytoreductive primary surgery	3	3	6	80	Yes	73	None	ET	IIB	No	CPS	0	C	6	CR	sensitive	No	56,2	N/A	CR	No	56,2
	20	Solid tumor	Cytoreductive interval surgery	2	2	4	80	No, FD	48	None	HS	IV	Yes, C/P	CIS	0	C/P	6	Unknown	Unknown	Unknown	Unknown	Unknown	CR	Unknown	4,9
	21	Solid tumor	Cytoreductive primary surgery	2	1	3	79	Yes	79	None	HS	IIIC	No	CPS	<1	C/P	6	CR	resistant	Yes	10,3	Unknown	PD	Yes	26,5
	22	Solid tumor	Recurrent disease	2	3	5	79	No, RD	76	Ovarian	ET	IIB	No	CPS	0	C/P	6	CR	sensitive	Yes	62,7	C/P	PD	Yes	65,2
	23	Ascites	Before NACT	3	2	5	76	Yes	76	None	HS	IIIC	Yes, C	None	N/A	C	2	PD	resistant	Yes	1,2	None	PD	Yes	1,9
	24	Ascites	Recurrent disease	3	2	5	75	No, RD	56	Ovarian	HS	IIIC	No	CPS	<1	C/P	6	CR	sensitive	Yes	38,4	C/CX	PD	Yes	56,2
	25	Solid tumor	Cytoreductive primary surgery	2	1	3	75	Yes	39	None	LS	IIIC	No	CPS	<1	C/P	6	CR	sensitive	Yes	10,3	CX/binimetinib	PD	Yes	33,4
	26	Solid tumor	Cytoreductive interval surgery	3	2	5	74	Yes	58	Breast	HS	IIIC	Yes, C/P	CIS	<1	C/P/ bevacizumab	6	CR	sensitive	Yes	16,0	C/P	PD	Yes	30,7
	27	Ascites	Recurrent disease	3	0	3	73	No, RD	53	Ovarian and breast	HS	IIIC	Yes, C/P	CIS	<1	C/P	6	CR	sensitive	Yes	13,5	C/CX/ tocilizumab	PD	Yes	25,8
	#28	Ascites	Recurrent disease	3	3	6	72	No, RD	71	Ovarian	HS	IIIC	No	CPS	>1	C	6	PR	resistant	Yes	8,0	None	PD	Yes	16,7

Table S6. continued

	*29	Solid tumor	Cytoreductive primary surgery	3	3	6	71	Yes	69	None	HS	IIIC	No	CPS	>1	C/P	6	CR	sensitive	Yes	23,4	C	PD	No	33,1
	30	Ascites	Before NACT	3	2	5	65	No, FD	73	None	HS	IIIC	Yes, C/P	CIS	<1	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	PD	Yes	14,6
	31	Ascites	Recurrent disease	3	3	6	64	No, RD	71	Ovarian	HS	IIIC	No	CPS	<1	C/P	6	CR	resistant	Yes	8,7	CX	PD	Yes	29,8
	32	Solid tumor	Cytoreductive primary surgery	2	3	5	62	Yes	74	Breast	HS	IIB	No	CPS	0	C/P	6	CR	sensitive	No	55,1	N/A	CR	No	55,1
	33	Solid tumor	Cytoreductive primary surgery	2	3	5	61	Yes	54	None	CC	IIB	No	CPS	0	C/P	6	CR	sensitive	No	32,4	N/A	CR	No	32,4
	34	Solid tumor	Cytoreductive interval surgery	3	2	5	60	Yes	78	None	HS	IIIC	Yes, C	CIS	0	C	6	CR	sensitive	No	6,0	N/A	CR	No	6,0
	35	Ascites	Recurrent disease	3	0	3	55	No, RD	63	Ovarian	HS	IIIC	Yes, C/P	CIS	0	C/P	6	CR	sensitive	Yes	13,8	C/P/ bevacizumab	PD	Yes	28,5
	36	Ascites	Recurrent disease	3	3	6	54	No, RD	73	Ovarian	HS	IIIC	No	CPS	<1	C/P	6	PR	sensitive	Yes	12,0	C/CX	PD	Yes	28,2
	37	Solid tumor	Cytoreductive primary surgery	3	3	6	51	Yes	69	None	HS	IV	No	CPS	<1	C/P	6	PR	sensitive	No	41,9	N/A	PR	No	41,9
HR-Intermediate	38	Solid tumor	Cytoreductive primary surgery	1	2	3	40	No, HRI	83	Basal cell carcinoma and breast	HS	IIIA	No	CPS	0	C/P	3	CR	sensitive	No	14,6	Unknown	CR	No	14,6
	39	Solid tumor	Before NACT	2	2	4	31	No, HRI	70	None	HS	IIIC	Yes, C/P	CIS	0	C/P/avelumab	6	SD	resistant	Yes	6,9	Avelumab	PD	Yes	23,3
HR-Deficient	40	Solid tumor	Cytoreductive interval surgery	3	0	3	17	Yes	55	None	HS	IIIC	Yes, C/P	CIS	0	C/P	6	CR	sensitive	Yes	16,1	C/P/Olaparib	PR	No	59,9
	41	Solid tumor	Cytoreductive interval surgery	2	2	4	16	Yes	71	Breast	HS	IV	Yes, C/P	CIS	>1	C/P	4	PD	resistant	Yes	4,5	None	PD	Yes	7,5
	42	Solid tumor	Cytoreductive primary surgery	3	3	6	15	Yes	48	None	HS	IIIC	No	CPS	>1	C/P	6	CR	sensitive	No	71,0	N/A	CR	No	71,0
	43	Solid tumor	Cytoreductive primary surgery	2	0	2	15	Yes	66	None	HS	IV	No	CPS	0	C/P	6	CR	sensitive	Yes	19,4	None	CR	No	70,3
	44	Solid tumor	Before NACT	3	3	6	5	No, FD	57	None	HS	IIIC	Yes, C/P	CIS	0	C/P	6	Unknown	Unknown	Yes	Unknown	Unknown	PD	Yes	18,3
	45	Ascites	Before NACT	3	0	3	5	Yes	55	None	HS	IIIC	Yes, C/P	CIS	0	C/P	6	CR	sensitive	Yes	15,1	Dexamethason	PD	Yes	36,6
	46	Ascites	Recurrent disease	3	0	3	4	No, RD	65	Ovarian	HS	IIIC	No	CPS	0	C/P	6	CR	resistant	Yes	9,7	C/P/ bevacizumab	PD	Yes	21,6
	47	Solid tumor	Cytoreductive primary surgery	2	1	3	3	Yes	44	Breast	HS	IIIC	No	CPS	<1	C/P	6	CR	sensitive	Yes	28,2	C/P	CR	No	81,3
	48	Ascites	Before NACT	3	0	3	1	Yes	39	None	HS	IIIC	Yes, C/P	CIS	0	C/P	7	CR	sensitive	No	52,5	N/A	CR	No	52,5
	49	Solid tumor	Cytoreductive primary surgery	2	3	5	1	Yes	59	Breast	HS	IC	No	CPS	0	C/P	6	CR	sensitive	No	39,1	N/A	CR	No	39,1

Case IDs correspond to case IDs in Figure 2, Figures S1, S3 and Table S5. Abbreviations: N/A = not applicable; # = same patient; * = this patient received NACT with cytoreductive surgery (0 cm tumor residue) 3 months after primary cytoreductive surgery.