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Effect of HIPEC according to HRD/*BRCA*wt genomic profile in stage III ovarian cancer: Results from the phase III OVHIPEC trial

Simone N. Koole[1,2] | Philip C. Schouten[3] | Jan Hauke[4] | Roel J. C. Kluin[5] | Petra Nederlof[6] | Lisa K. Richters[4] | Gabriele Krebsbach[4] | Karolina Sikorska[7] | Maartje Alkemade[8] | Mark Opdam[8] | Jules H. Schagen van Leeuwen[9] | Henk W. R. Schreuder[10] | Ralph H. M. Hermans[11] | Ignace H. J. T. de Hingh[12] | Constantijne H. Mom[13] | Henriette J. G. Arts[14] | Maaike van Ham[15] | Peter van Dam[16] | Peter Vuylsteke[17,18] | Joyce Sanders[6] | Hugo M. Horlings[6] | Koen K. van de Vijver[19] | Eric Hahnen[4] | Willemien J. van Driel[1] | Rita Schmutzler[4] | Gabe S. Sonke[2] | Sabine C. Linn[2]

[1]Department of Gynecology, The Netherlands Cancer Institute, Center of Gynecologic Oncology Amsterdam, Amsterdam, The Netherlands

[2]Department of Medical Oncology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

[3]Department of Molecular Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

[4]Faculty of Medicine and Center for Familial Breast and Ovarian Cancer and Center for Integrated Oncology (CIO), Cologne, University Hospital Cologne, Cologne, Germany

[5]Genomics Core Facility, Netherlands Cancer Institute, Amsterdam, The Netherlands

[6]Department of Pathology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

[7]Department of Biometrics, The Netherlands Cancer Institute, Amsterdam, The Netherlands

[8]Core Facility of Molecular Pathology and Biobanking, The Netherlands Cancer Institute, Amsterdam, The Netherlands

[9]Department of Obstetrics & Gynecology, Sint Antonius Hospital, Nieuwegein, The Netherlands

[10]Department of Gynecological Oncology, University Medical Center Utrecht, Utrecht, The Netherlands

[11]Department of Gynecology and Obstetrics, Catharina Hospital, Eindhoven, The Netherlands

[12]Department of Surgical Oncology, Catharina Hospital, Eindhoven, The Netherlands

[13]Department of Obstetrics and Gynecology, Amsterdam University Medical Center, Center of Gynecologic Oncology Amsterdam, Amsterdam, The Netherlands

[14]Department of Gynecological Oncology, University Medical Center Groningen, Groningen, The Netherlands

[15]Department of Gynecological Oncology, Radboud University Medical Center, Nijmegen, The Netherlands

[16]Department of Gynecologic Oncology, University Hospital Antwerp, Antwerp, Belgium

[17]Department of Medical Oncology, UCL Louvain, CHU Namur Sainte-Elisabeth, Namur, Belgium

[18]University of Botswana, Gaborone, Botswana

[19]Department of Pathology, University Hospital Ghent, Ghent, Belgium

Abbreviations: BRCA, breast cancer susceptibility gene; CI, confindence intervals; CNV, copy-number variation; CRS, cytoreductive surgery; FFPE, formalin-fixed paraffin-embedded; FIGO, federation for gynecology and obstetrics; GCIG, Gynecologic Cancer InterGroup criteria; HEBON, hereditary breast and ovarian cancer; HIPEC, hyperthermic intraperitoneal chemotherapy; HR, hazard ratio; HRD, homologous recombination deficiency; HSP, heat-shock proteins; IARC, International Agency for Research on Cancer; IP, intraperitoneal; MLPA, multiplex ligation-dependent probe amplification; NGS, next generation sequencing; OS, overall survival; PARP, platinum or poly (adenosine diphosphate [ADP]-ribose) polymerase; RECIST, response evaluation criteria in solid tumors; RFS, recurrence-free survival.

Philip C. Schouten and Jan Hauke have contributed equally to this study.

Willemien J. van Driel and Rita Schmutzler have contributed equally to this study.

Gabe S. Sonke and Sabine C. Linn have contributed equally to this study.

Substantial parts of this manuscript overlap with the thesis of Dr. S.N. Koole entitled "HIPEC for ovarian cancer", previously published limitedly on paper, and at https://www.publicatie-online.nl/publicaties/simone-koole/.

Correspondence
Sabine C. Linn, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands.
Email: s.linn@nki.nl

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Abstract

The addition of hyperthermic intraperitoneal chemotherapy (HIPEC) with cisplatin to interval cytoreductive surgery improves recurrence-free (RFS) and overall survival (OS) in patients with stage III ovarian cancer. Homologous recombination deficient (HRD) ovarian tumors are usually more platinum sensitive. Since hyperthermia impairs BRCA1/2 protein function, we hypothesized that HRD tumors respond best to treatment with HIPEC. We analyzed the effect of HIPEC in patients in the OVHIPEC trial, stratified by HRD status and *BRCA*m status. Clinical data and tissue samples were collected from patients included in the randomized, phase III OVHIPEC-1 trial. DNA copy number variation (CNV) profiles, HRD-related pathogenic mutations and *BRCA1* promotor hypermethylation were determined. CNV-profiles were categorized as HRD or non-HRD, based on a previously validated algorithm-based *BRCA1*-like classifier. Hazard ratios (HR) and corresponding 99% confidence intervals (CI) for the effect of RFS and OS of HIPEC in the *BRCA*m, the HRD/*BRCA*wt and the non-HRD group were estimated using Cox proportional hazard models. Tumor DNA was available from 200/245 (82%) patients. Seventeen (9%) tumors carried a pathogenic mutation in *BRCA1* and 14 (7%) in BRCA2. Ninety-one (46%) tumors classified as *BRCA1*-like. The effect of HIPEC on RFS and OS was absent in *BRCA*m tumors (HR 1.25; 99%CI 0.48-3.29), and most present in HRD/*BRCA*wt (HR 0.44; 99%CI 0.21-0.91), and non-HRD/*BRCA*wt tumors (HR 0.82; 99%CI 0.48-1.42), interaction *P* value: 0.024. Patients with HRD tumors without pathogenic *BRCA1/2* mutation appear to benefit most from treatment with HIPEC, while benefit in patients with *BRCA1/2* pathogenic mutations and patients without HRD seems less evident.

KEYWORDS
HIPEC, homologous recombination deficiency, ovarian cancer

What's new?

Serous ovarian cancers that are homologous recombination deficient (HRD) often are sensitive to platinum-containing chemotherapy. Whether hyperthermic intraperitoneal chemotherapy (HIPEC) with cisplatin benefits patients with HRD tumors, however, remains unclear. In this study, an algorithm-based HRD classifier was validated using data and tissue derived from the randomized, phase III OVHIPEC-1 trial. Interval cytoreductive surgery and HIPEC was found to prolong recurrence-free and overall survival moin patients with HRD/*BRCA1* wild-type ovarian cancers. Responses of *BRCA1/2*-mutated and HR-proficient ovarian cancers to HIPEC were less pronounced. The HRD classifier is a promising tool for identifying ovarian cancer patients who may benefit from HRD relying treatment modalities.

1 | INTRODUCTION

Epithelial ovarian cancer has the highest mortality of all gynecologic tumors in the western world. The majority of patients are diagnosed with International Federation for Gynecology and Obstetrics (FIGO) stage III disease.[1-3] Standard treatment consists of maximal cytoreductive surgery (CRS) in combination with platinum-based chemotherapy. The 10-year survival of women with stage III or IV ovarian cancer is 10% to 15% and did not improve in the past 20 years, despite extensive CRS and (neo-)adjuvant intravenous chemotherapy.[4,5] The peritoneal surface is the primary site of disease recurrence in the majority of patients and therapeutic approaches that specifically target the peritoneal surface are therefore required.[6,7] Delivering chemotherapy intraperitoneally (IP) maximizes drug exposure at the peritoneal surface. Hyperthermic intraperitoneal chemotherapy (HIPEC) is a single approach in which heated chemotherapy is administered directly into the abdominal cavity at the end of complete or near-complete CRS. The multicenter randomized phase III OVHIPEC trial showed improved recurrence-free

survival (RFS) and overall survival (OS) after interval CRS with HIPEC using cisplatin as compared to interval CRS, in patients with stage III ovarian cancer.[8]

Up to 50% of high-grade serous ovarian cancers are homologous recombination deficient (HRD) due to germline or somatically acquired breast cancer susceptibility gene-1 (*BRCA1*) or *BRCA2* mutations, epigenetic inactivation of *BRCA1*, or other *BRCA*-independent defects in the HR pathway.[9,10] In the absence of homologous recombination, these tumors rely on error-prone DNA repair mechanisms such as nonhomologous end-joining to repair DNA double-strand breaks (DSB) that are induced by platinum-containing chemotherapy.[11] As these mechanisms cause genomic instability and increased cell death, HRD tumors are sensitive to platinum-containing chemotherapy, including HIPEC.[12] Hyperthermia may act synergistically with platinum-based chemotherapy as heat causes depletion of the BRCA1 and BRCA2 proteins and impairs BRCA1/2 protein function, thereby transiently inducing HRD.[13,14] Ovarian cancers may constitute a spectrum ranging from completely homologous recombination deficient to completely homologous recombination proficient, with intermediate phenotypes.

Homologous recombination deficient cancers frequently harbor the same characteristic genomic scars as germline *BRCA1* mutated (*gBRCA1m*)-associated cancers. These scar patterns consist of specific gains and losses in DNA copy number variation (CNV), which can be measured by various methods, including comparative genomic hybridization (CGH) and (low-coverage) next generation sequencing.[15-20] We used a previously established and validated *BRCA1*-like algorithm to classify CNV profiles as HRD or non-HRD.[21]

We hypothesize that patients with HRD tumors might predominantly benefit of treatment with HIPEC. Since the novel HRD *BRCA1*-like classifier not only identifies *BRCA1* mutated tumors, but also a subset of *BRCA*wt tumors that are HRD, we stratified our analysis for three different groups: tumors that harbor *BRCA1/2*m, tumors that are HR impaired without a *BRCA1/2* mutation (HRD/*BRCA*wt), or HR proficient (nonHRD) tumors. To test whether patients with HRD tumors respond better to interval CRS with HIPEC, we estimated the effect of HIPEC in patients who participated in the phase III OVHIPEC trial and stratified the results by HRD status and *BRCAm* status.

2 | MATERIALS AND METHODS

2.1 | Patients

The multicenter, randomized, open-label, phase III OVHIPEC-1 trial included 245 patients with FIGO stage III ovarian, fallopian tube, or peritoneal cancer. The trial accrual period was between 2007 and 2016. Because of the extent of disease at diagnosis, patients were ineligible for primary CRS and received three cycles of neo-adjuvant carboplatin-paclitaxel chemotherapy followed by interval CRS. Full eligibility criteria have been published elsewhere.[8] During surgery, patients were randomly assigned (1:1) to interval CRS with or without HIPEC. All patients received three additional cycles of carboplatin-paclitaxel after surgery. For this ancillary pathology study, we analyzed available tissue samples from patients in the OVHIPEC-1 trial. More detailed information on tissue selection can be found in the supplementary files.

2.2 | DNA isolation

Formalin-fixed paraffin-embedded (FFPE) tissue was collected at three time points: (1) before neo-adjuvant chemotherapy; (2) during interval CRS before the administration of HIPEC; and (3) at disease recurrence. After central review by two specialized pathologists (KVdV, JS), DNA was isolated from FFPE tumor samples containing more than 30% tumor cells, using Qiagen AllPrep DNA/RNA kit. One valid sample per patient, preferably derived at interval CRS, was selected for this analysis.

2.3 | Low coverage next generation sequencing

All available DNA samples were sequenced low coverage, to distinguish amplifications or deletions on a minimal resolution of 20 kb. The amount of double-stranded DNA was quantified using the Invitrogen Qubit dsDNA HS Assay Kit (Fisher Scientific Ltd, Leicestershire, UK) and fragmented to lengths of about 160 base pairs using a Covaris, then purified using 2× AMPure XP beads (Beckman Coulter, cat no A63881). The DNA library was adapted for sequencing using the KAPA HTP library preparation kit (KAPA Biosystems, KK8504). Samples were sequenced single-read 65 base pair, 13 to 14 samples per lane, on an Illumina HiSeq2500. Reads where aligned against the GRCh38 reference genome using BWA 0.7.17, mem algorithm. Reads, per 20 kb on the genome, were counted and compared against reference-based, predicted mappability, thereafter gc correction took place and this yielded the 2log ratios for analyses. The sequencing coverage and quality statistics for each sample are summarized in Table S1.

2.4 | BRCA1-like classification

CNV profiles were classified as BRCA1-like (HRD) or non-BRCA1-like (non-HRD), using a previously trained and validated, shrunken-centroids classifier specific for ovarian cancer patients.[21] In short, the 20 kb resolution copy number profiles were mapped to the 1 MB resolution input for the classifier. The 2log ratios were averaged per 1 MB, centered and scaled to conform the next-generation sequencing data to the oligonucleotide array CGH data, the classifier was trained on. This correction is similar to quantile normalization, and was performed by fitting a linear regression model with Gaussian distribution and the identity link function using the glm R function to the sorted location-wise average of the training set and to this dataset. The centering of the current dataset

is then corrected by subtracting the alpha coefficient of the model. Subsequently the scaling is corrected by multiplying by the beta coefficient. The 1 MB mapped, platform-corrected samples are subsequently segmented using the uniseg function from the cghseg R package, and are classified with the pamr R package.[22,23] These methods were implemented in a dockerfile, the image of which can be run as docker container. The classifier assigns a discriminative score, between 0 (non-BRCA1-like) and 1 (BRCA-like), to any new DNAcopy number profile. The previously validated cutoff value of 0.5 was used for these analyses.[21] More detailed description of the validation of the *BRCA1*-like classifier can be found in the supplementary files.

2.5 | Panel mutational sequencing

All DNA samples were centrally analyzed in an accredited laboratory (Center for Familial Breast and Ovarian Cancer, Cologne, Germany) using targeted next generation sequencing (NGS) covering the entire coding regions and exon-flanking sequences (±15 nt) of *BRCA1* (NM_007294.4), *BRCA2* (NM_000059.3), and 25 non-*BRCA1/2* cancer predisposition genes (*ATM*, NM_000051.3; *BARD1*, NM_000465.4; *BRIP1*, NM_032043.3; *CDH1*, NM_004360.5; *CHEK2*, NM_007194.4; *FAM175A*, NM_139076.3; *FANCM*, NM_020937.4; *MLH1*, NM_000249.3; *MRE11A*, NM_005591.3; *MSH2*, NM_000251.2; *MSH6*, NM_000179.2; *MUTYH*, NM_001128425.1; *NBN*, NM_002485.4; *NF1*, NM_001042492.2; *PALB2*, NM_024675.4; *PMS2*, NM_000535.6; *PTEN*, NM_000314.8; *RAD50*, NM_005732.4; *RAD51C*, NM_058216.3; *RAD51D*, NM_002878.3; *RECQL*, NM_002907.3; *SMARCA4*, NM_001128849.1; *STK11*, NM_000455.5; *TP53*, NM_000546.5; *XRCC2*, NM_005431.2).[24,25] For NGS, we employed a customer-tailored SureSelect gene panel (Agilent, Santa Clara). Sample preparation was performed using the SureSelect XT Low Input Reagent Kit (Agilent) and SureSelect XT HS and XT Low Input Enzymatic Fragmentation Kit (Agilent) with 70 ng of input DNA. Sequencing was performed on a NextSeq500 platform (Illumina, San Diego) using the NextSeq500/550 Mid Output Kit v2.5 (Illumina).

Bioinformatic analyses were carried out using the SeqNext module of the SeqPilot Software Package, Version 5.1.0 Build 503 (JSI medical systems GmbH, Ettenheim, Germany). The filters were selected in such a way that only variants at positions which were covered by at least 50 total reads and a variant fraction of at least 5% of the Fwd and Rev reads were recorded. Variant classification was performed in accordance with the regulations of the international ENIGMA consortium (https://enigmaconsortium.org) as previously described in detail.[26] All genetic variants were classified using a 5-tier variant classification system as proposed by the International Agency for Research on Cancer (IARC) Unclassified Genetic Variants Working Group, namely, deleterious = class 5, likely deleterious = class 4, variant of uncertain significance (VUS) = class 3, likely benign = class 2 and benign = class 1. Class 4/5 germline variants were subsequently defined as "mutations."

2.6 | BRCA1 promotor hypermethylation multiplex ligation-dependent probe amplification

The *BRCA1* promotor methylation status was determined using the ME001 kit (Version D3, MRC Holland, the Netherlands) using manufacturers protocol. The optimal input was 80 ng double-strength DNA (Qubit based), minimum input was 25 ng dsDNA. All samples were diluted in a Tris-EDTA buffer (10:0.1).

2.7 | Germline mutational status

Germline mutational status (g*BRCA1* mutation, g*BRCA2* mutation, no pathogenic g*BRCA* mutation or status unknown) was derived from the clinical patient files. In addition, we crosslinked the OVHIPEC patient-set with the national hereditary breast and ovarian cancer (HEBON) database to obtain additional hereditary information and germline *BRCA* status. For patients with a sequenced tumor *BRCA* mutation and a reported germline mutation, the sequenced variant was assigned to the germline status. Sequenced tumor *BRCA* mutations in the absence of a known germline mutation were labeled as tumor mutations, irrespective of the mutation/variant allele frequency.

2.8 | Clinical endpoints

Recurrence-free survival (RFS) was defined as the time from randomization to disease recurrence or progression, on the basis of the Response Evaluation Criteria in Solid Tumors (RECIST) criteria version 1.1, a rise in CA-125 level according to the Gynecologic Cancer InterGroup criteria (GCIG), or death from any cause, whichever occurred first.[27] Overall survival (OS) was defined as the time from randomization to death from any cause. Patients alive at last follow-up were censored at that time.

2.9 | Statistics

All randomized patients from the OVHIPEC-1 trial are included in these analyses if sufficient DNA samples for CNV sequencing was available. Baseline and treatment characteristics are presented per treatment arm using the exact test for categorical variables. Baseline characteristics were compared of the included patients, and of the patients who dropped out of this analysis because of insufficient tumor material.

The effect of HIPEC was evaluated in three mutually exclusive subgroups defined by *BRCA*m and HRD status: *BRCA1/2 mut* vs HRD/*BRCAwt* vs non-HRD/*BRCAwt*. Treatment effects per subgroup together with 99% confidence intervals (CI) are displayed in a forest plot. Hazard ratios for the effect of treatment arm (HIPEC vs no HIPEC), and BRCA-subgroup (*BRCA1/2 mut* vs HRD/*BRCAwt* vs non-HRD/*BRCAwt*) for RFS and OS were explored in univariate and multivariate Cox proportional hazard models. Results of the model are reported with corresponding CI fitted for RFS and OS, and interaction *P* values (alpha 0.05). Kaplan-Meier estimates are

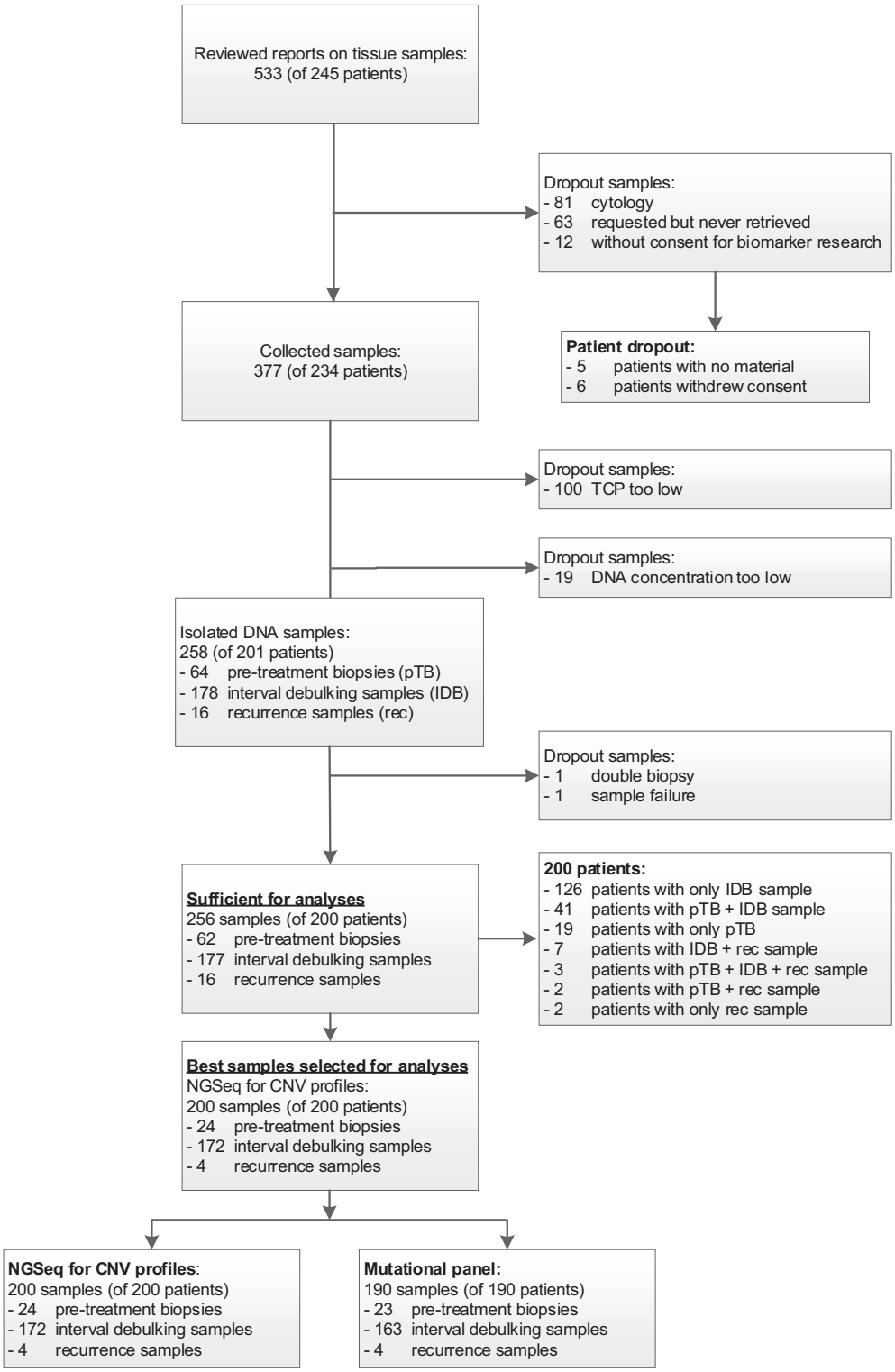

FIGURE 1 CONSORT diagram for tissue availability

compared using log-rank tests. All analyses were performed using R-statistics (R 3.6.3 GUI 1.70).

3 | RESULTS

Tissue samples with sufficient DNA-samples for CNV-sequencing were available for 200/245 (82%) patients included in the OVHIPEC-1 trial (Figure 1). Reasons for missing samples included no informed consent for biomarker analyses, complete pathologic response after neo-adjuvant chemotherapy, low quality of the retrieved DNA, and nonresponse from participating sites (Figure 1). Baseline characteristics of these 200 patients were largely similar to the 45 patients for whom no DNA sample was available, except for lower likelihood of (near-)complete pathologic response (Table S2). Baseline and treatment characteristics among patients included in this ancillary side study were well balanced across the arms of the study (Table 1).

TABLE 1 Baseline characteristics per treatment arm

	Surger n = 106	Surgery plus HIPEC n = 94	*P* value[a]
Histological type (%)			.199
High-grade serous	93 (88%)	85 (90%)	
Low-grade serous	2 (2%)	4 (4%)	
Carcinosarcoma	4 (4%)	1 (1%)	
Clear-cell	4 (4%)	0	
High-grade endometrioid	1 (1%)	1 (1%)	
Low-grade endometrioid	0	2 (2%)	
High-grade mucinous	1 (1%)	1 (1%)	
Metastasis gastro-intestinal tumor	1 (1%)	0	
Surgery result			.771
Complete resection	69 (65%)	59 (63%)	
Suboptimal resection, tumor nodule <2.5 mm	22 (21%)	20 (21%)	
Suboptimal resection, tumor nodule >2.5 mm < 1 cm	13 (12%)	12 (13%)	
Incomplete/no resection	2 (2%)	3 (3%)	
Pathologic response (%)			.267
Complete/ near complete	2 (2%)	5 (5%)	
Partial to no response	87 (82%)	79 (84%)	
Nonmeasurable[b]	17 (16%)	10 (11%)	
TP53 mutation (%)			.097
Yes	90 (85%)	73 (78%)	
No	10 (9%)	17 (18%)	
Unknown[b]	6 (6%)	4 (4%)	
BRCA mutation (%)[c]			.958
g*BRCA1*[c]	7 (7%)	6 (6%)	
tumor *BRCA1*	3 (3%)	4 (4%)	
gBRCA2[c]	5 (5%)	5 (5%)	
tumor *BRCA2*	3 (3%)	1 (1%)	
*BRCA*wt	84 (77%)	75 (80%)	
No panel mutation or germline information available	4 (4%)	3 (3%)	
BRCA1 hypermethylation (%)			.258
BRCA1 hypermethylated	7 (7%)	10 (11%)	
Not *BRCA1* hypermethylated	87 (82%)	75 (80%)	
Unknown	12 (11%)	9 (10%)	
Other mutation variants (%)			.168
NF1	0	2 (2%)	
FANCC	0	2 (2%)	
PMS2	2 (2%)	0	
ATM	2 (2%)	0	
MUTYH	1 (1%)	0	
MSH6	1 (1%)	0	
NBN	1 (1%)	0	
CDH1	1 (1%)	0	
RECQL	1 (1%)	0	

TABLE 1 (Continued)

	Surger n = 106	Surgery plus HIPEC n = 94	*P* value[a]
RAD51C	0	1 (1%)	
SMARCA4	0	1 (1%)	
No mutation or *BRCA1* hypermethylation found	70 (66%)	63 (67%)	
No more tumor material available, no clinical information on mutation status	4 (4%)	3 (3%)	
BRCA1 profile (%)			.835
BRCA1-like profile	47 (44%)	44 (47%)	
Non-*BRCA1*-like profile	59 (56%)	50 (53%)	
Median time to recurrence, months (IQR)	10.7 (9.2-12.5)	13.8 (10.8-17.0)	.03[d]
Median time to death, months (IQR)	33.9 (28.2-41.9)	45.7 (37.0-65.1)	.037[d]

[a]Exact test *P* value.
[b]Pathologic response could not be measured, because of missing surgical specimens; not included in statistical test.
[c]For 3/13 gBRCA1 mutation carriers, no tumor material for sequencing was available for panel testing. For all other germline BRCA1/2 mutation carriers, the pathologic variant was confirmed with tumor panel sequencing.
[d]Kaplan-Meier based survival estimates, *P* value from log-rank test.

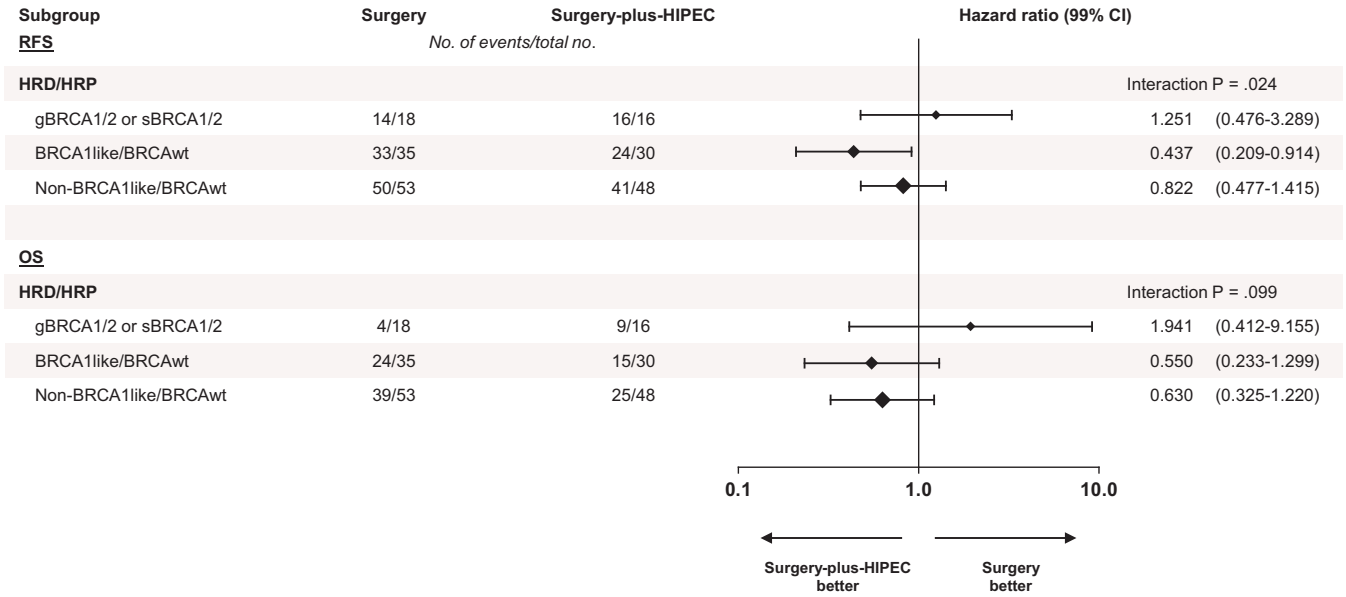

FIGURE 2 Exploratory subgroup analysis for recurrence-free survival and overall survival. Reported *P* values resulted from the multivariable cox model from Table 2 [Color figure can be viewed at wileyonlinelibrary.com]

Data on germline mutational testing was retrieved for 108/200 (54%) patients. Panel sequencing results were available for 190/200 (95%) of the samples, because DNA was insufficient in the remaining 10 patients. The prevalence of a tumor mutation in *BRCA1* was 17/190 (9%) and in *BRCA2* 14/190 (7%). Deleterious tumor mutation variants in other possibly tumor predisposition genes were found in 15/190 patients (*NF1*, *ATM*, *MUTYH*, *PMS2*. *FANCC*, *MSH6*, *NBN*, *CDH1*, *RECQL*, *RAD51C*, *SMARCA4*). All gene variants found are listed in Tables 1 and S4. Three of the 10 patients in whom panel sequencing results were unavailable were known carriers of a germline pathogenic *BRCA1* mutation.

Subgroup analysis was performed to analyze the predictive effect of *BRCAm* and/or HRD tumor on the effect of HIPEC. For patients with tumor or germline pathogenic *BRCA1/2* mutations, the HR for the effect of HIPEC was 1.25 (99%CI 0.48-3.29) for RFS and 1.94 (99%CI 0.42-9.16) for OS. For the HRD/*BRCA*wt group hazard ratios for RFS and OS are 0.44 (99%CI 0.21-0.91) and 0.55 (99%CI 0.23-1.30), respectively. HR for the non-HRD/*BRCA*wt group was 0.82 (99%CI 0.48-1.42) for RFS and 0.63 (99%CI 0.32-1.22) for OS (Figure 2). *P* values for interaction derived from the Cox models were .024 for RFS and .099 for OS (Figure 2).

A significant independent beneficial effect remained for treatment with HIPEC (HR 0.676 [95% CI 0.467-0.979], $P = .038$ and having a *BRCA1/2* mutation in multivariable analysis for OS (HR 0.513 [95%CI 0.274-0.961], $P = .037$, Table 2). Kaplan-Meier curves for RFS and OS are presented for all tested subgroups in Figures 3 and S2.

TABLE 2 Univariable and multivariable analysis for recurrence-free survival and overall survival

	Univariable			Multivariable[a]		
	HR	95% CI	*P* value	HR	95% CI	*P* value
Recurrence-free survival						
CRS	ref.					
CRS + HIPEC	0.719	0.534-0.968	.030	0.709	0.525-0.957	.025
BRCA1-like/*BRCA*wt	ref.					
Germline and/or tumor *BRCA1/2*m	0.753	0.483-1.173	.210	0.710	0.454-1.110	.133
Non-*BRCA1*-like	1.160	0.831-1.621	.383	1.111	0.794-1.554	.540
Overall survival						
CRS	ref.					
CRS + HIPEC	0.675	0.467-0.977	.037	0.676	0.467–0.979	.038
BRCA1-like/*BRCA*wt	ref.					
Germline and/or tumor *BRCA1/2*m	0.519	0.277-0.972	.041	0.513	0.274–0.961	.037
Non-*BRCA1*-like	1.216	0.815-1.813	.338	1.201	0.806-1.790	.369

[a]Terms for interaction included in the single model for RFS, and the single model for OS are treatment arm (HIPEC vs no HIPEC) and *BRCA*-subgroup (*BRCA1/2*m vs *BRCA1*-like/*BRCA*wt vs non-*BRCA1*-like).

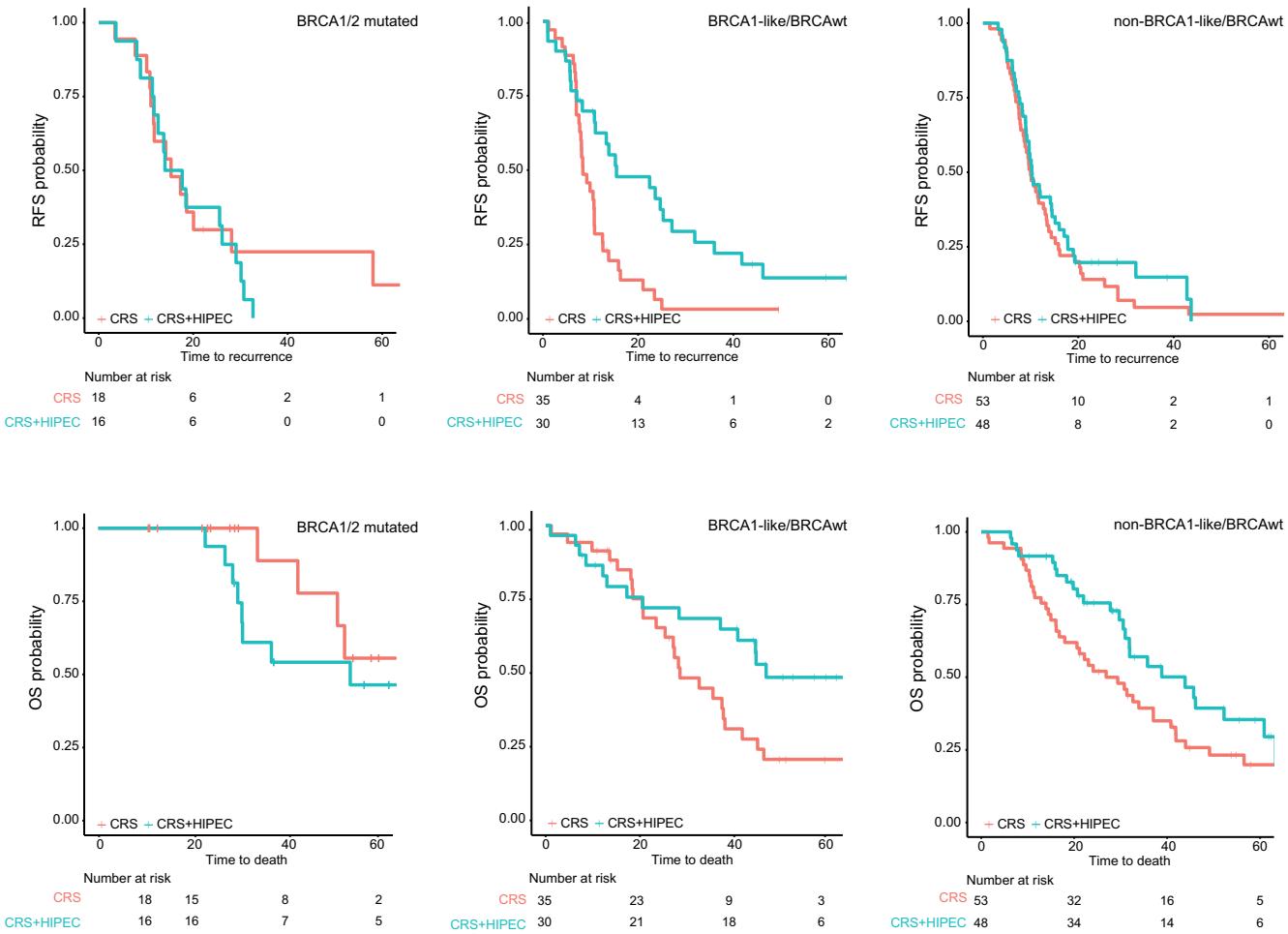

FIGURE 3 Kaplan-Meier curves for *BRCA*mut, *BRCA1*-like/*BRCA*wt and non-*BRCA1*-like patients for RFS and OS by treatment arm [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

We hypothesized that patients with HRD or HR impaired tumors were most likely to benefit from treatment with HIPEC. Our results show that HIPEC may not add additional benefit over intravenously administered platinum for patients with *BRCA1/2*m tumors. Patients with stage III ovarian cancer whose tumor harbor a *BRCA1*-like HRD genomic profile without pathogenic *BRCA1/2* tumor mutations, seem to experience most benefit on RFS and OS of the addition of HIPEC to interval cytoreductive surgery. Although we could not confirm our hypothesis based on these analyses, it might provide evidence that HRD assessed with the ovarian cancer *BRCA1*-like classifier is a potential tool for selection of ovarian cancer patients for specific treatments, such as HIPEC.

Patients with *BRCA1/2*m tumors are particularly sensitive to the (neoadjuvant) platinum, and HIPEC might not further improve effects over intravenously administered chemotherapy. This observation might be enhanced by the neoadjuvant administration of the chemotherapy, possibly inducing resistance to platinum. Since this subgroup was particularly small (n = 34), and the number of events is low, more data are required to study the effect of intraperitoneal chemotherapy and HIPEC in this specific subgroup before drawing final conclusions.

The HRD/*BRCA*wt tumors derive significant benefit of HIPEC. The HRD or HR impaired phenotype in these patients is explained by other mechanisms than *BRCA1* dysfunction alone and may result in impaired or intermediate BRCA1 or BRCA2 protein function. Possibly, an intermediate intrinsic ability to repair double-strength DNA breaks can be further hampered with hyperthermia leading to significant tumor cell kill and eventually the observed recurrence free and overall survival benefit. Hyperthermia has shown to deplete BRCA1 and BRCA2 protein function, and upregulate mammalian heat-shock proteins (HSP).[13,28] The heat-induced *HSP90* inhibition disrupts DNA damage repair pathways, and induces further BRCA1/2 protein degradation.[14] It thereby sensitizes cells to the DNA damage caused by platinum-containing chemotherapy. Poly(adenosine diphosphate [ADP]-ribose) polymerase (PARP)-inhibitors inhibit DNA repair pathways and cause apoptosis of cancer cells, particularly in HR deficient cells, such as cells with BRCA1/2 protein disfunction. Hyperthermia might therefor sensitize for PARP-inhibition too.[29,30] The fact that hyperthermia degrades *BRCA1* and *BRCA2* function, might lead to the hypothesis that HIPEC might also sensitize HRD/*BRCAwt* tumors which generally show more average sensitivity to platinum.

For patients with a non-HRD signature, the trends for effect of HIPEC for both RFS and OS were less convincing. Probably, these tumors are HR proficient and thus well capable of DS DNA damage repair, despite hyperthermia. The exploratory nature of our analyses prohibits firm conclusions and these patients might actually benefit from HIPEC, given the HR <1 and broad confidence intervals (HR 0.82 and 0.63 for RFS and OS, respectively).

Maintenance therapy with either PARP-inhibition or bevacizumab was not part of standard of care in the Netherlands during the conduct of the OVHIPEC-1 study. PARP-inhibitor maintenance therapy for recurrent ovarian cancer is reimbursed in the Netherlands since May 2018 and because OVHIPEC-1 accrued patients between 2007 and 2016, only a small minority of the patients will have been treated with PARP-inhibition. Therefore, second line treatment with PARP-inhibition will presumably only have had a small effect on our results.

The population included in this ancillary pathology study, comprised 190 of 245 (82%) of the total OVHIPEC-1 study population. Within this group, deleterious germline/tumor *BRCA1* or *BRCA2* mutations were identified in 34 (17%) patients (Table 1). Both the total prevalence of pathogenic *BRCA1* and *BRCA2* mutations, and the proportion of germline mutants could possibly be slightly higher in the total OVHIPEC-1 intention-to-treat population than we observed in this dataset. This may be due to two reasons. Standard hereditary testing was not performed for all patients at the onset of this trial (2006). Patients included early in the trial were less likely to be tested for germline mutations. We were not able to determine (blood-derived) germline mutational status. We relied on germline mutational status obtained from the patient file or the HEBON database for 54% of the individuals included in this analysis. In previously published ovarian cancer cohorts, germline and somatic mutations in *BRCA1/2* have been observed in 22% to 27% of tumors.[10,25] On the other hand, the overall HR for RFS is 0.72 in the population included in this study, which is slightly higher than de early reported 0.66 in the total trial cohort.[8] This suggests the effect of HIPEC is somehow better in the remaining group, that was excluded because of insufficient tissue.

The developed *BRCA1*-like HRD classifier had a sensitivity of 100% in recognizing pathogenic tumor *BRCA1* mutations. Of the patients with an HRD tumor, 29% had *BRCA1* or *BRCA2* deleterious mutations, 13% had other HRD related gene mutations and 10% had *BRCA1* promotor hypermethylation. The remaining 48% of HRD tumors, possibly represent tumors with other aberrations in the HRD pathway (see Figure S1 and Table S3). This resembles the adequacy of the *BRCA1*-like classifier in breast cancer, and the results of the classifier in an earlier ovarian cancer dataset. Within the AGO-TR1 dataset, the detection rate of the *BRCA1*-like classifier for BRCA1 mutations and promoter hypermethylation was 95.6%.[16,20,31]

This analysis has some limitations. First, although material was available for the vast majority of the trial population (82%), tumors that were most sensitive to neo-adjuvant chemotherapy were underrepresented due to missing tumor tissue. A relatively large proportion of these patients carried a germline *BRCA* mutation. Second, the OVHIPEC-1 trial did not include blood-sample collection. As a result, we were unable to collect blood-derived reference DNA to determine germline mutational status. Third, the power of our study is limited and numbers are low. Trends for the effect of HIPEC in the different subgroups are hypothesis generating and the results should be confirmed in independent datasets. Other additional hypotheses regarding the optimal temperature, chemotherapy agent and concentration and the duration of HIPEC need to be evaluated in future studies.

We show that the developed HRD classifier is a potential tool for selection of ovarian cancer patients who benefit from treatment with HIPEC. The algorithm-based classifier was able to identify HRD tumors based on a *BRCA1*-like profile, with a sensitivity of 100% in

recognizing tumor *BRCA1* mutations. Whether this HRD classifier is also predictive for platinum sensitivity, PARP-inhibitor resistance or PARP-inhibitor sensitivity, should be further explored.[32]

5 | CONCLUSIONS

Ovarian cancer patients with HRD/*BRCA1*wt status appear to have the largest effect on RFS and OS after treatment with interval CRS and HIPEC, while benefit of the addition of HIPEC in patients with *BRCA1/2*m seems less evident. For patients with non HRD tumors, the effect of HIPEC for both RFS and OS were less convincing. This HRD classifier is a potential tool for selection of ovarian cancer patients who benefit from treatment with HIPEC, and it may also predict the effect of other treatment modalities relying on HRD in ovarian cancer. These results should be further explored in future research.

AUTHOR CONTRIBUTIONS

Conceptualization: Simone Koole, Philip Schouten, Willemien van Driel, Gabe Sonke, Sabine Linn. Data curation: Simone Koole, Philip Schouten, Jan Hauke, Roel Kluin. Formal analysis: Simone Koole, Karolina Sikorska. Funding acquisition: Sabine Linn. Investigation: Simone Koole, Philip Schouten, Jan Hauke, Roel Kluin, Petra Nederlof, Lisa Richters, Gabriele Krebsbach, Maartje Alkemade, Mark Opdam, Joyce Sanders, Hugo Horlings, Koen van de Vijver. Methodology: Simone Koole, Philip Schouten, Jan Hauke, Roel Kluin. Project administration: Simone Koole, Eric Hahnen, Willemien van Driel, Rita Schmutzler, Gabe Sonke, Sabine Linn. Resources: Petra Nederlof, Lisa Richters, Gabriele Krebsbach, Maartje Alkemade, Mark Opdam, Jules Schagen van Leeuwen, Henk Schreuder, Ralph Hermans, Ignace de Hingh, Constantijne Mom, Henriette Arts, Maaike van Ham, Peter van Dam, Peter Vuylsteke, Joyce Sanders, Hugo Horlings, Koen Van de Vijver, Eric Hahnen, Willemien J. van Driel. Software: Philip Schouten, Jan Hauke, Roel Kluin. Supervision: Eric Hahnen, Willemien van Driel, Rita Schmutzler, Gabe Sonke, Sabine Linn. Validation: Karolina Sikorska. Visualization: Simone Koole. Writing: original draft: Simone Koole. Writing: review & editing: all authors. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST

Philip Schouten is partner employed by AstraZeneca, with no direct connection to this work. Ignace de Hingh has an unrestricted research grant from Roche and RanD Biotechnology for unrelated research. The grant is payed to the institute. Rita Schmutzler has a grant from Amgen and AstraZeneca, and received honoraria from presentations from AstraZeneca and Janssen-Cilag. She reports AdBoards from: AstraZenca, GSK, Clovis Oncology and MSD. Gabe Sonke reports consulting work for Biovica ans Seagen. Research support in paid to the institution from Agendia, AstraZeneca, Merck, Novartis, Roche and Seagen. Sabine Linn has been an advisory board member for AstraZeneca, Cergentis, IBM, Novartis, Pfizer, Roche and Sanofi, and has received unrestricted institutional research support of unrestricted educational funding from Agendia, Amgen, AstraZeneca, Bayer, Daiichi Sankyo, Eurocept Pharmaceuticals, Genentech, Immunomedics, Merck, Roche, Sanofi and TESARO. Sabine Linn has a patent application pending on a BRCA-like ovarian cancer classifier. All other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.

ETHICS STATEMENT

Institutional review board approval was obtained from all participating hospitals. All patients included in this analysis gave written informed consent for biomarker research. Trial registration number: Clinicaltrial.gov: NCT00426257, EudraCT number: 2006-003466-34.

ORCID

Simone N. Koole https://orcid.org/0000-0002-8909-1935
Jan Hauke https://orcid.org/0000-0001-8236-4075
Eric Hahnen https://orcid.org/0000-0002-1152-8367
Sabine C. Linn https://orcid.org/0000-0001-5541-0347

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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