

# Loss of 1p36.33 Frequent in Low-Grade Serous Ovarian Cancer



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## Abstract

**BACKGROUND:** Low-grade serous ovarian cancer (LGSOC) is a rare subtype of epithelial ovarian carcinoma. Limited data regarding the molecular-genetic background exist beyond mutations in the RAS signaling pathway. There is a growing need to better characterize these tumors due to chemoresistance and limited therapeutic options in advanced or recurrent disease. **METHODS:** We performed genome-wide copy number aberration (CNA) profiles and mutation hotspot screening (*KRAS*, *BRAF*, *NRAS*, *ERBB2*, *PIK3CA*, *TP53*) in 38 LGSOC tumor samples. **RESULTS:** We detected mutations in the RAS-signaling pathway in 36.8% of cases, including seven *KRAS*, four *BRAF*, and three *NRAS* mutations. We identified two mutations in *PIK3CA* and one mutation in *MAP3K1*, *EGFR*, and *TP53*. CNAs were detected in 86.5% of cases. None of the focal aberrations was correlated with specific clinical characteristics. The most frequently detected CNA was loss of 1p36.33 in 54.1% of cases, with a trend towards lower progression-free survival and overall survival in patients with 1p36.33 loss. **CONCLUSIONS:** Activating RAS mutations were dominant in our series, with supplementary detection of two *PIK3CA* mutations which may lead to therapeutic options. Furthermore, we detected 1p36.33 deletions in half of the cases, indicating a role in tumorigenesis, and these deletions may serve as a prognostic marker.

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## Background

Low-grade serous ovarian cancer (LGSOC) is a rare disease representing only 5%-8% of all ovarian cancers and 6%-10% of all serous ovarian cancers [1–3]. They can present *de novo* or as a recurrence from a serous borderline tumor (SBT). LGSOC presents typically in a younger patient group than high-grade serous ovarian cancer (HGSOC) with a median age at diagnosis of 43-55 years and 63 years, respectively [3–5]. Low grade tumors are more indolent, resulting in a longer overall survival (OS) compared with to HGSOC (81.8-126.2 months vs. 53.8-57 months), although low-grade carcinomas are more resistant to chemotherapy [1–4,6]. They have a <5% response rate to first-line chemotherapy compared to the 80% response rate of their high-grade counterpart [1,7]. The progression-free survival (PFS) of LGSOC is

similar compared to that of HGSOC, with a median PFS of 19.5 months [4], although higher PFS rates (25-36 months) have been described for SBT-associated cases [8,9].

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Shih and Kurman introduced a model of two different pathways leading to HGSOC and LGSOC [10]. They describe both serous tumors not only as histologically differentially graded but also as two distinct clinical, molecular, and epidemiological entities. Histological characteristics suggest that low-grade tumors often develop from low-malignant potential tumors in a pathogenic continuum with a 60% presence of SBT in LGSOC, while high-grade tumors arise *de novo* from the surface epithelium and only have a 2% incidence of concomitant SBT. Hence, the presence of an SBT is a risk factor for developing LGSOC [4,8,10,11]. While nearly all HGSOCs are characterized by genetic loss of *TP53* [12], LGSOCs are typically wild-type *TP53* and presumably arise in a stepwise fashion from serous cystadenoma, adenofibroma, or serous borderline tumors [13]. LGSOCs harbor a high rate (40%) of activating mutations of the *MAPK* pathway (Table 1).

In 2003, Singer et al. reported the first analysis of *KRAS* and *BRAF* mutations in LGSOC and reported a frequency of 36% and 32% positive tumors, respectively [14]. Since then, many studies have confirmed the presence of these mutations in LGSOC, although observed frequencies seem to vary considerably (0%-32% for *BRAF* and 15.4%-54.5% for *KRAS* mutations) (Table 1). *BRAF* mutations are more frequent in SBT and in early-stage LGSOC tumors. As such, *BRAF* mutated LGSOC tumors are often characterized by a better prognosis [3,5,14-16].

In 2014, Emmanuel et al. broadened the spectrum of mutations in the MAPK pathway by identifying *NRAS* mutations at a frequency of 15% in 20 LGSOCs with adjacent SBT [9]. Further studies

confirmed *NRAS* as a possible oncogenic driver in LGSOC (Table 1). Hunter et al. also conducted whole exome sequencing in 19 LGSOC cases and identified recurrent mutations in *KRAS*, *BRAF*, and *NRAS*, in addition to somatic mutations in *USP9X* and *EIF1AX* [7]. The latter two genes have both been linked to regulation of mTOR, suggesting that mTOR inhibitors may be useful in combination with MEK or RAF inhibitors in LGSOC. In contrast to the latter study, few other genome-wide studies have been performed. As a result, relatively little is known about chromosomal instability in LGSOC. The only more or less consistent finding is that loss of chromosome 1p has been observed in a number of studies (Table 2).

Since LGSOC is often resistant to chemotherapy, the development of novel targeted therapies has the potential to ameliorate the prognosis of LGSOC patients. The MEK inhibitor selumetinib has shown promising results in LGSOC [17]. However, to select patients for such targeted therapies, it is imperative to upfront define a subset of biomarker-positive patients that will benefit from these therapies.

The purpose of this study is to further analyze the genomic profile of LGSOC using mutational analysis and, to our knowledge, the largest copy number aberration (CNA) analysis of LGSOC.

## Methods

### Patients and Tumors

We collected fresh-frozen or paraffin-embedded tumor samples and clinical data of 38 patients with LGSOC treated at the University

**Table 1.** Overview of Mutational Analyses in LGSOC Conducted with Either Immunohistochemistry, Polymerase Chain Reaction, Hotspot Genotyping, or Whole Exome/Genome Sequencing

Author	Journal Publication Year	No. LGSOC	No. KRAS (%)	No. BRAF (%)	No. NRAS (%)	No. Other (%)
Haas et al. [36]	<i>Virchows Arch</i> 1999	6	2 (33%) codon 12			
Singer et al. [14]	<i>J Natl Cancer Inst</i> 2003	22	8 (36%) codon 12-13	7 (32%) codon 599		
Wong et al. [15]	<i>Am J Pathol</i> 2010	43	8 (19%) codon 12-13	1 (2%) codon 600		
Verezkey et al. [37]	<i>Pathol Oncol Res</i> 2011	17	4/17 (23.5%) codon 12-13	0 (0%) codon 600		0 TP53 (0%)
Schlosshauer et al. [38]	<i>Int J Gynecol Pathol</i> 2011	4		0 (0%) codon 600		
Jones et al. [30]	<i>J Pathol</i> 2012	15	4 (26.7%) codon 12	3 (20%) codon 600		1 PIK3CA 345 N/K (6.7%)
Sundov et al. [39]	<i>Diagn Pathol</i> 2013	11	6 (54.5%) codon 12-13	0 (0%) codon 600		
Grisham et al. [21]	<i>Cancer</i> 2013	19	3 (15.8%) codon 12-13	1 (5.3%) codon 600		
Farley et al. [17]	<i>Lancet Oncol</i> 2013	34	4 (41%) codon 12-13	2 (6%) codon 599		
Emmanuel et al. [9]	<i>Clin Cancer Res</i> 2014	20	7 (35%) codon 12	2 (10%) codon 600	3 (15%) 2*Q61R, Q61K	
Gershenson et al. [22]	<i>Br J Cancer</i> 2015	79	18 (22.8%) codon 12	3 (3.8%) codon 600		
Hunter et al. [7]	<i>Oncotarget</i> 2015	19	4 (21%) codon 12	3 (16%) codon 600	1 (5.3%) Q61R	0 HRAS (0%) 0 TP 53 (0%) 15% EIF1AX 11% USP9X
Sadlecki et al. [40,41]	<i>Tumor Biology</i> 2017	13, 14	2 (15.4%) codon 12	0 (0%) codon 599		
Etemadmoghadam et al. [26]	<i>Cancer Res</i> 2017	23	5 (22%) codon 12	3 (13%) codon 600	5 (22%) 3*Q61R 2*Q61K	2 NF1 (9%) 3 EIF1AX (13%) 3 USP9X (13%)
Xing et al. [27]	<i>Human Pathol</i> 2017	56			2 (3.6%) Q61R	
McIntyre et al. [28]	<i>Histopathology</i> 2017	26	9 (34.6%) Codon 12 and 61	2 (7.7%) Codon 600	1 (3.8%) Q61R	1 MAP2K1 (3.8%) 2 FGFR2 (7.7%) 1 ESR1 (3.8%)
TOTAL (%)		421	84/347 (24.2%)	27/346 (7.8%)	12/144 (8.3%)	
RANGE		4-79	15.8-54.5%	0-32%	3.6-22%	

**Table 2.** CNA analyses in LGSOC.

Author	Journal Publication year	N° LGSOC	CNA loss/gain	Candidate genes
Kuo et al. [33]	Cancer Res 2009	12	Loss: Chr 1p36, Chr 9p21.3	CHD5, MiR-34a CDKN2A/B
Birch et al. [13]	Plos One 2011	11	Loss: chr 1p	-
Emmanuel et al. [9]	Clin Cancer Res 2014	13	CNI identical between paired SBT and LGSOC samples Low CNI compared to HGSOc	-
Hunter et al. [7]	Oncotarget 2015	13	Loss: 1p, 9p, 22q Gain: 7, 8	CDKN2A/B
McIntyre et al. [28]	Histopathol 2017	26	Only report on CNI Low CNI compared to HGSOc	-
Total		70		

The most frequent CNAs are indicated. *Chr*, chromosome; *CNI*, copy number index.

Hospital of Leuven, Belgium. Written consent for the use of tumor tissue and data collection was obtained of each patient, and the study was approved by the local ethics committee (study number s55308). Eligible patients were women diagnosed with LGSOC at our institution between January 1984 and August 2015 of which we had tumor material, either fresh-frozen or paraffin-embedded, and sufficient clinical data. Tumor tissue was collected at the time of surgery being either primary debulking or interval debulking surgery. Clinical data included age at diagnosis, International Federation of Gynecology and Obstetrics (FIGO) stage, laterality, lymph node involvement, ascites, type of surgery, residual tumor after surgery, response to first-line chemotherapy, presence of concomitant borderline ovarian tumor, and recurrence. Staging and grading were performed according to FIGO 2014 classification. All tumor samples were revised by a pathologist, expert in the field of gynecological tumors (P.M.). Response Evaluation Criteria in Solid Tumors (version 1.1) [18] and Gynecologic Cancer InterGroup CA125 response criteria [19] were used to determine response to chemotherapy and to define progressive disease. PFS was calculated as the period between diagnosis and recurrence, progression, or death from any cause, whereas OS was defined as the period between diagnosis and death from any cause and censored at the last date of follow-up if the patient was still alive.

### DNA Isolation

Fresh-frozen tumor sections were obtained by cryosectioning biopsies at 10  $\mu$ m. Three to five 20- $\mu$ m-thick sections were prepared from the formalin-fixed, paraffin-embedded tissue blocks. A 5- $\mu$ m hematoxylin and eosin-stained section was used to determine regions with high tumor content. Based on the hematoxylin and eosin staining, only regions containing  $\geq 60\%$  of tumor cells were retained through macrodissection. From these regions, DNA was extracted using the DNeasy blood and tissue kit (Qiagen, Düsseldorf, Germany) for the fresh-frozen samples, and a phenol-chloroform method was used on the formalin-fixed, paraffin-embedded slides.

### Hotspot Genotyping

We selected a panel by a query of the Catalogue of Somatic Mutations in Cancer database for the most frequent mutations in ovarian cancer as reported previously and applied this in genotyping of high-grade serous ovarian cancer [20]. For *KRAS*, *BRAF*, *NRAS*, *PIK3CA*, and *PTEN*, this resulted in a coverage of greater than 97%, 94%, 97%, 79%, and 7%, respectively. We extended our panel with

following genes: *AKT2*, *CDK4*, *CTNBN1*, *EGFR*, *ERBB2*, *FBXW7*, *MAP3K1*, *MAP2K4* and *27*, *PIK3R1*, *PDGRA*, *SMAD4*, and *TP53*.

DNA was aliquoted into 384-well plates and genotyped at the Vesalius Research Center (Leuven, Belgium) using the MassARRAY Compact Analyzer (Sequenom Inc., San Diego, CA). Automated genotyping calls were generated using the MassARRAY RTTM software and were manually reviewed.

### Copy Number Variation Analysis

We performed copy number analysis through whole-genome low-coverage (shallow) sequencing on 38 samples. DNA libraries were prepared using KAPA Library Preparation Kits (KK8201, Kapabiosystems, Wilmington, MA) prior to sequencing. Samples were sequenced at low coverage (0.5 $\times$ ) on an Illumina HiSeq 2000 platform. Raw sequencing data were mapped to the human reference genome (hg19) using Burrows-Wheeler Aligner (BWA v0.7.10). Using QDNAseq v1.4.2, read counts were generated for bins of size 100 kbp; bins in blacklisted regions were removed, and bin counts were corrected for GC content and mappability applying Loess regression using QDNAseq. ASCAT (v2.0.7) was used to estimate copy number profiles from these bins. Subsequently, GISTIC (v2.0.22) was applied to identify recurrent CNAs in the cohort.

### Statistical Analysis

Descriptive statistics were used for patient characteristics. Comparison between groups on clinical parameters was performed by Mann-Whitney *U* test for continuous variables or Fisher's exact test or Chi-square test for categorical variables. Kaplan-Meier method was used to construct survival curves, and log-rank test was used to calculate the difference in survival curves between groups. Statistical analyses were performed using SAS Software (version 9.4, SAS System for Windows). All tests are two-sided, and we considered a *P* value of .05 as statistically significant.

## Results

### Clinical Outcome

We selected 38 patients for which clinical follow-up data and either paraffin-embedded or fresh-frozen tissue was available for mutation analysis. Patient characteristics are detailed in Table 3. Median age at diagnosis was 53.5 years (range 25-76 years). The majority of patients was diagnosed with a FIGO stage III-IV (92.1%), and most of them underwent primary debulking surgery (65.8%) with an 86.8% R0 (no macroscopic residual tumor) resection rate. All patients, except 2 patients with stage I disease and 2 patients treated

**Table 3.** Patient Characteristics According to Ras Mutation or Wild-Type

	Ras Mutant N = 14	Non-Ras Mutant N = 24	P Value
<b>Median age</b> (years)	58.5	39.5	.220
Range	(28-68)	(25-76)	
Mean age	53.7	47.1	
<b>FIGO stage</b>			
I	3 (21.4%)	1 (4.2%)	.105
II	-	-	
III	7 (50%)	21 (87.5%)	
IV	4 (28.6%)	2 (8.3%)	
<b>Surgery</b>			
Primary debulking	7 (50%)	18 (75%)	.163
Interval debulking	7 (50%)	6 (25%)	
<b>Residual tumor</b>			
R0	11 (78.6%)	22 (91.7%)	.337
R > 1	3 (21.4%)	2 (8.3%)	
R0-1	-	-	
<b>Association of borderline component</b>	6 (42.9%)	12 (50%)	.745
<b>Initial systemic treatment</b>			
Platinum-based chemo	13 (92.9%)	24 (100%)	.368
Hormonal treatment	1 (7.1%)	-	-
<b>Bilateral</b>	9 (64.3%)	19 (79.2%)	.217
Unilateral	5 (35.7%)	3 (12.5%)	
Unknown	-	2 (8.3%)	
<b>Lymph node involvement</b>			
Yes	6 (42.9%)	9 (37.5%)	
No	8 (57.1%)	15 (62.5%)	
<b>Ascites</b>			
Yes	6 (42.9%)	4 (16.7%)	1.000
No	8 (57.1%)	19 (79.2%)	
Unknown	-	1 (4.2%)	

with an aromatase inhibitor, received platinum-based chemotherapy in either neoadjuvant or adjuvant setting. There were no significant differences in clinical characteristics between wild-type and RAS-mutated patients (Table 3).

In 18 patients, a borderline component was also present at the time of diagnosis. The presence of a borderline component was not correlated with a younger age at the time of diagnosis (53.5 vs. 55 years). Patients with a borderline component had a longer PFS and OS rate compared to the pure LGSOC patients: 36 vs. 20.4 months and 130.8 vs. 86.4 months, respectively, but this was not statistically significant ( $P = .4$ ). SBT-associated tumors were not more likely to be diagnosed in early stage, but the majority of the entire series presented in an advanced stage. Overall, 30/38 patients relapsed (78.9%) with a median PFS of 32 months, and 17/38 succumbed to the disease (45.9%). Median follow-up was 89 months (range 13-372) with two patients lost to follow-up.

### Hotspot Genotyping

*KRAS* and *BRAF* mutations were mutually exclusive in our series. Overall, mutations in the RAS signaling pathway were identified in 14/38 samples (36.8%). We found seven *KRAS* mutations (18.4%): six samples had a *KRAS* G12 V (c.35G > T) and one sample a *KRAS* G12D (c.35G > A) mutation. One *KRAS* sample also harbored a *MAP3K1* mutation. We found four *BRAF* mutations (10.5%): three samples had a *BRAF* V600E (c.1799 T > A) and one sample a *BRAF* G469A (c.1406G > C) mutation. Activating *NRAS* mutations were identified in three samples (7.9%) in our series; all were Q61R (c.182 A > G) mutations. These are all known oncogenic mutations. All *KRAS*, *BRAF*, and *NRAS* mutations were mutually exclusive. There were no significant differences regarding age, FIGO stage, and the concomitant presence of borderline between *KRAS*, *BRAF*, and *NRAS* mutated patients.

None of the clinical characteristics—age, FIGO stage, laterality, lymph node involvement, and ascites—correlated significantly with the presence of RAS mutations. Association with borderline component was also equal between the RAS-mutated and the non-RAS-mutated group (Table 3). The median PFS of the RAS-mutated group was 33.6 months (95% CI, 13.2-44.4) compared to 31.2 months (95% CI, 16.8-52.8) in the non-RAS-mutated group ( $P = .4$ ). Median OS was 130.8 months (95% CI, 51.6-195.6) in the RAS-mutated group versus 170.4 months (95% CI, 54-372) in the non-RAS-mutated group ( $P = .6$ ). OS of the entire cohort was 137 months.

Furthermore, we found two mutations in the *PIK3CA* gene: one R93W (c.277C > T) and one H1047RL (c.3140A > GT). One patient also harbored a *BRAF* mutation besides the *PIK3CA* mutation, whereas another patient harbored an E746A *EGFR* mutation (c.2235\_2249del15). We found no mutations in *ERBB2* in our cohort of 38 LGSOC and two *TP53* mutations (c.524G > A and c.817C > T) in one sample.

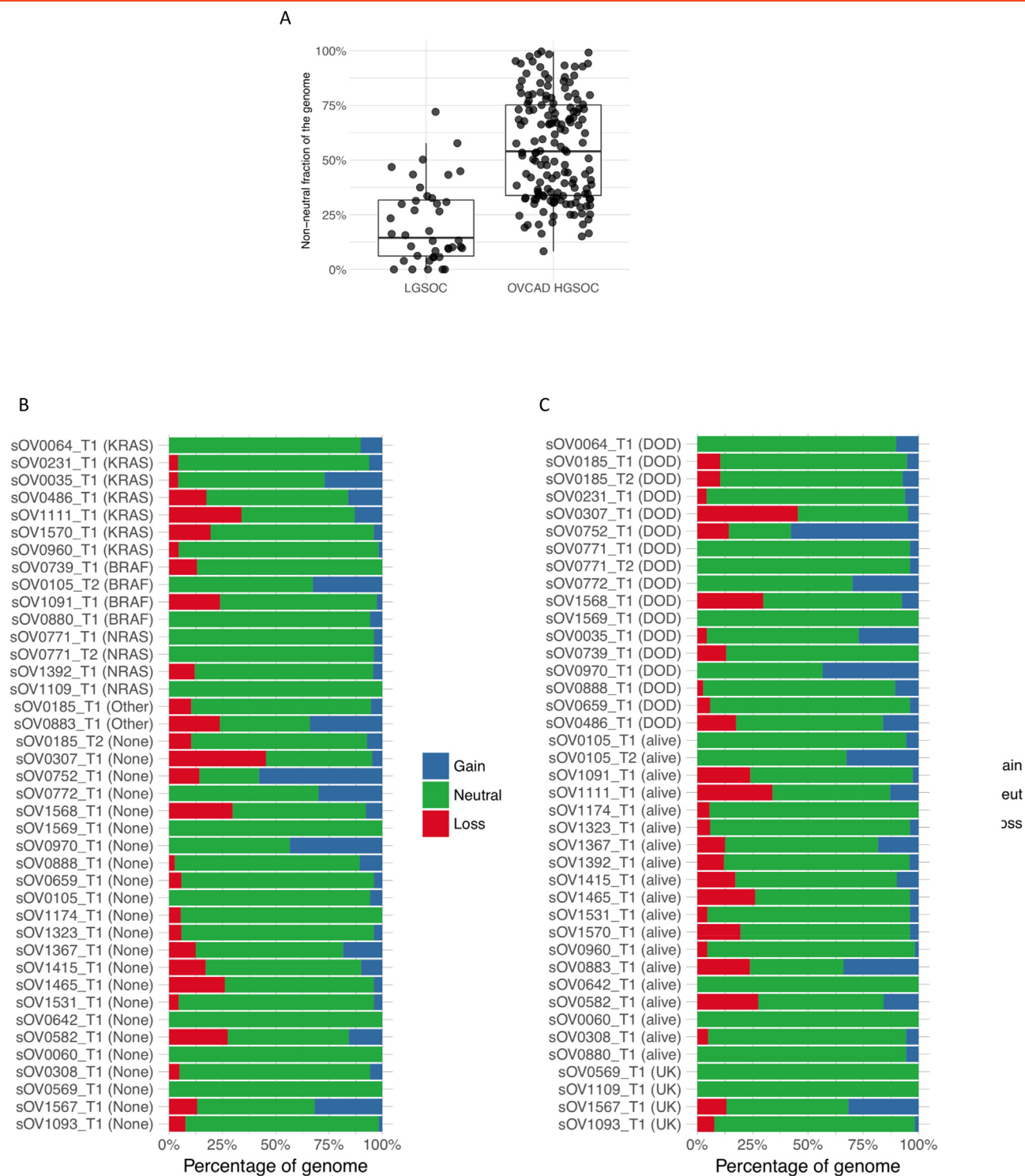
### Copy Number Variation

We conducted a genome-wide CNA analysis on 40 samples: 37 primary LGSOC tumors and 3 paired metastatic samples. CNAs were detected in 86.5% of all the primary tumor samples, whereas the remaining samples (5/37) where completely copy number-stable. Particularly, the chromosome instability index (CIN), which is the fraction of the genome affected by CNAs, was low with a median of 14.4% (IQ range: 6.1%-31.7%). This is substantially lower than in HGSOC. We compared the current dataset to a cohort of 160 stage III/IV HGSOC samples prospectively collected within the OVCAD consortium (FP6 EU-project, www.ovcad.eu) at time of diagnosis (Figure 1A). The median CIN was 53.9% in this group (vs. 14.4%), with an absolute minimum of 8.3% (vs. 0%). Importantly, CIN did also not significantly differ between *BRAF*, *KRAS*, and *NRAS* mutated samples, or between RAS-mutated and RAS wild-type samples (Figure 1B). The CIN was comparable between patients who succumbed to the disease within 60 months of diagnosis and patients who were still alive at 84 months (Figure 1C).

The most frequently reported whole chromosome aberrations were loss of 1p (33%), 6q (24%), 9p (21%), 16p/q (21%), 17p (24%), 18p/q (21%), and 22q (40%) and gain in 1q (40%), 7p/q (26%), and 8q (29%) (Figure 2). Loss of 9p (33%) and gain of 8q (44%) and chromosome 7 (39%) were slightly enriched in the samples with associated borderline component (SBT-LGSOC) compared to pure LGSOC (16%, 21%, and 21%, respectively), but these differences were not statistically significant. None of the other whole arm or focal aberrations correlated with the presence of a borderline component. Nine samples harbored a 17p deletion and 10 samples and 8q gain. Seven in each group (77.8% and 70%, respectively) succumbed to the disease ( $P = .04$  and  $P = .19$ , respectively), of which three samples had both CNAs, suggesting a correlation with a poor outcome. Loss of 15q was predominantly found in patients <45 years (43.8 vs. 9.5%,  $P = .04$ ).

We further also analyzed recurrent focal deletions and gains. The most frequent focal alteration was loss of 1p36.33 in 20/37 (54.1%) samples. We could not detect a specific enrichment of 1p36.33 loss in SBT-LGSOC cases, or any significant correlation of loss of 1p36.33 and clinical characteristics, such as age, FIGO stage, bilaterality, lymph node involvement, and the presence of ascites (Table 4). We further looked at the median PFS and OS of patients with LGSOC





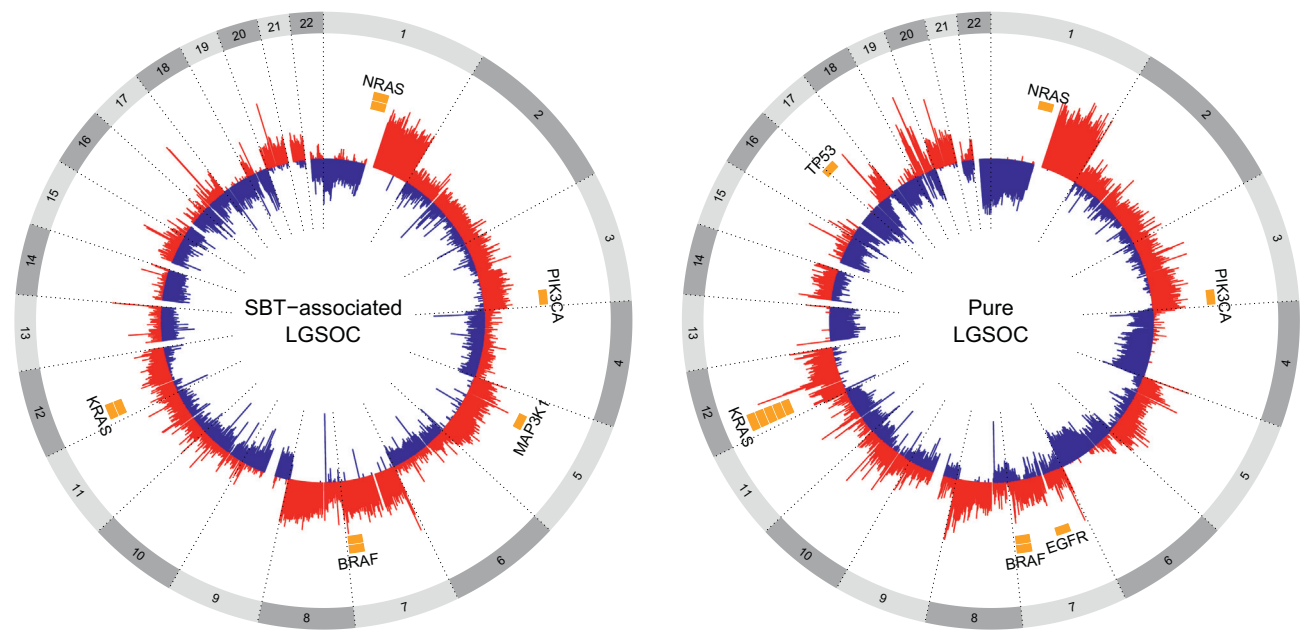
**Figure 1.** (A) Fraction of the genome altered in 38 LGSOC versus 160 high-grade serous ovarian cancer OVCAD samples. (B) Percentage of the genome gained (blue), lost (red), or neutral (green) in the Ras-mutated versus the Ras wild-type samples. (C) Percentage of the genome gained (blue), lost (red), or neutral (green) in patients who succumbed to the disease within 60 months of diagnosis (DOD = death of disease) or were still alive after 60 months.

tumors characterized by 1p36.33 loss. The median PFS of patients with the 1p36.33 alteration was 26.4 months (95% CI, 13.2-50.4) compared to 44.4 months (95% CI, 16.8-67.2) in the group of patients without 1p36.33 loss ( $P = .40$ ). Similarly, median OS in the 1p36.33 group was 130.8 months (95% CI, 51.6-195.6) compared to 172.8 months (95% CI, 52.8-372) in the group without 1p36.33

loss ( $P = .52$ ). One sample with loss of 9p harbored a homozygous deletion of the 9p21.3 region which contains *CDKN2A/2B*.

Paired Samples

In three samples, we performed genotyping and copy number analyses on the primary tumor as well as a metastatic site at time of



**Figure 2.** Circos plot showing oncogene hotspot mutations and the large-scale CNAs in the 38 tumor samples.

diagnosis (OV0105, OV0185, and OV0771). OV0771 shared the same *NRAS* mutation in both primary and metastatic sample. For the other two samples, genotyping results were discordant: OV0105 showed a *BRAF* and *PIK3CA* mutation in the metastatic sample but not in the primary tumor. OV0185 harbored a *PIK3CA* mutation in the primary but not in the metastatic sample.

**Table 4.** Patient Characteristics According to Loss of 1p36.33

	1p36.33 Loss N = 20	No 1p36.33 Loss N = 17	P Value
<b>Median age</b> (years)	47.5	55	.636
Range	(25-76)	(26-76)	
Mean age	48.3	51.8	
<b>FIGO stage</b>			
I	1 (5.0%)	2 (11.8%)	.630
II	-	-	
III	15 (75%)	13 (76.4%)	
IV	4 (20%)	2 (11.8%)	
<b>Surgery</b>			
Primary debulking	14 (70%)	10 (58.8%)	.512
Interval debulking	6 (30%)	7 (41.2%)	
<b>Residual tumor</b>			
R0	17 (85%)	15 (88.2%)	1.000
R > 1	3 (15%)	2 (11.8%)	
R0-1	-	-	
<b>Association of borderline component</b>	9 (45%)	9 (52.9%)	.745
<b>Initial systemic treatment</b>			
Platinum-based chemo	20 (100%)	13 (76.4%)	.036
Hormonal treatment	0 (0.0%)	2 (11.8%)	
No adjuvant chemotherapy	0 (0.0%)	2 (11.8%)	
<b>Bilateral</b>	16 (80%)	12 (70.6%)	.434
Unilateral	3 (15%)	5 (29.4%)	
Unknown	1 (5.0%)	-	
<b>Lymph node involvement</b>			
Yes	11 (55%)	4 (23.5%)	.092
No	9 (45%)	13 (76.5%)	
<b>Ascites</b>			
Yes	5 (26.3%)	5 (29.4%)	1.000
No	14 (73.7%)	12 (70.6%)	
Unknown	1 (5.0%)	-	

The fraction of copy number alterations was similar in the OV0105 and OV0771 paired samples, while the primary sample of OV0185 showed a higher accumulation of CNAs with respect to the paired metastasis.

**Discussion**

LGSOCs are genetically stable tumors, primarily characterized by activating mutations in the *MAPK* pathway and a low number of copy number alterations. We identified a 36.8% mutation rate in the *MAPK* pathway: 18.4% *KRAS*, 10.5% *BRAF*, and 7.9% *NRAS* mutations, consistent with earlier reports (Table 1). The lower *BRAF* mutation frequency is in concordance with the finding of Wong et al. who found only one *BRAF* mutation in 33 advanced LGSOC samples [15]. Our series consists mainly of stage III/IV patients; hence, *BRAF* mutations are not associated with early stage and a better outcome in advanced LGSOC. However, *BRAF* mutations are more frequently detected in SBTs, and these *BRAF*-mutated SBTs are less likely to recur [7,21].

Wong et al. reported an OS of 77.9 months in 8 LGSOC with either a *KRAS* or *BRAF* mutation (7 *KRAS*, 1 *BRAF*) versus 47.3 months for 25 LGSOC patients with wild-type *KRAS* and *BRAF* ( $P = .28$ ) [15]. In 79 cases of LGSOC, Gershenson et al. reported a median OS of 106.8 months (95% CI, 50.6-162.9) in *KRAS/BRAF* mutated patients (21/79) compared to 66.8 months (95% CI, 43.6-90.0) in *KRAS/BRAF* wild-type patients ( $P = .018$ ) [22], suggesting a prognostic effect of *BRAF/KRAS* mutation. In our series, median OS in the Ras-mutated group was 130.8 months compared to 170.4 months in the non-Ras-mutated group ( $P = .61$ ). Hence, the prognostic effect of *BRAF* and *KRAS* mutation cannot be confirmed. In the cohort of Gershenson, however, 69% of *KRAS/BRAF* wild-type patients and 52.4% of *KRAS/BRAF* mutated patients had gross residual disease at the time of cytoreductive surgery compared to 13.2% in our series (3 patients with a Ras mutation and 2 wild-type patients). Since residual tumor is the most widely accepted prognostic factor in ovarian cancer [23] and this holds

probably even more for LGSOC than for other more rapidly growing ovarian cancers, this could significantly impact OS data. The percentage of patients with stage III/IV disease was comparable between both series (93.8% vs. 92.1%). Hence, our higher R0 rate might explain the longer OS in our cohort. Furthermore, it is not clear how many of the patients in the series of Gershenson were SBT-associated since this might also impact PFS and OS [9]. Additionally, other reports suggest, as we do, a poorer outcome of malignancies with *KRAS/BRAF* mutations compared with wild-type *KRAS/BRAF* [24,25]. We combined all Ras-pathway mutations because the PFS and OS of the patients with an *NRAS* mutation were comparable to the PFS and OS of patients with *KRAS* or *BRAF* mutations.

More recently, *NRAS* mutations were described in LGSOC. Five studies identified 12 *NRAS* mutations (either at Q61R or Q61K) in a total of 118 cases, resulting in an average frequency of 9.3% [7,9,26–28]. We found three *NRAS* mutations in our series. The role of *NRAS* mutation in the development of LGSOC merits further investigation given new possible targeted therapies against *NRAS* and its downstream effectors [29].

Furthermore, we observed two mutations in *PIK3CA*. One patient also harbored a *BRAF* mutation besides the *PIK3CA* mutation. Jones et al. previously described a *PIK3CA* N345K (c1035T > A) mutation in one case out of 15 with pure LGSOC [30]. One patient harbored an *EGFR* mutation. *EGFR* mutations are a rarity in SBTs and have not been previously reported in LGSOC. Showell et al. found a higher nuclear and cytoplasmic staining of *EGFR* in 61 SBTs and 10 LGSOC tumors compared to benign ovarian tumors or HGSOC tumors but could not detect any mutation [31]. Activating *EGFR* mutation can upregulate the PI3K pathway. Recurrent mutations in *USP9X* and *EIF1AX* have recently been published [7,26]. Both are linked to regulation of mTOR. All these findings may suggest a role for mTOR inhibitors as an additive to treatment with MEK and RAF inhibitors.

We observed no mutations in *ERBB2* in our cohort of 38 LGSOC. We tested *ERBB2* given the recent data of *ERBB2* mutations in serous borderline tumors [32]. Our study suggests that these mutations are restricted to noninvasive serous pathology.

Unexpectedly, we detected one *TP53* mutation. Revision of both the primary surgery specimen and the recurrence specimen 2 years after diagnosis by an expert pathologist in the field of gynecological tumors (P.M.) confirmed the diagnosis of LGSOC. Both the estrogen receptor and progesterone receptor were highly positive. Birch et al. previously described a *TP53* mutation in a serous tumor of low malignant potential [13].

The presence of a borderline component is not indicative of Ras-pathway-activated tumors [9]. We did not find a significant difference in association of borderline component between the RAS-mutated group and the non-Ras-mutated group ( $P = .74$ ). SBT-LGSOC cases did show a longer PFS and OS, as demonstrated by Emmanuel et al., compared to the pure LGSOC patients: 36 vs. 20.4 months and 130.8 vs. 86.4 months, respectively. This difference was not statistically significant ( $P = .44$ ), but this might be due to the small series. Emmanuel et al. also reported a younger age at time of diagnosis for epithelial tumors associated with a borderline tumor compared to invasive epithelial carcinomas, but 80% of the latter consisted of HGSOC [9]. We did not find a difference in age at onset.

CNAs were detected in 86.5% of all the samples. The majority of cases showed low-level copy number alterations in contradiction to

HGSOC that is associated with a very high level of copy number alterations. This is in concordance with previous reports [7,9,13,28,33]. The fraction of the genome altered is similar for Ras mutated and wild-type tumors (Supplementary data S1). Associating clinical features with somatic mutations or CNAs was difficult because most patients presented with bilateral and/or advanced disease (Table 2).

The most frequently reported whole chromosome aberrations were loss of 1p (33%), 6q (24%), 9p (21%), 16p/q (21%), 17p (24%), 18p/q (21%), and 22q (40%) and gain in 1q (40%), 7p/q (26%), and 8q (29%), and are in concordance with previous studies [7,9,13]. The most significant CNA in the series of Hunter et al. [7] was loss of 9p, which was found in 53% of LGSOC cases and in only 2% of SBT cases, indicating a possible role in transition from SBT to an invasive carcinoma. We observed a loss of 9p in 33% of cases of borderline-associated LGSOC samples compared to 16% in pure LGSOC cases ( $P = .21$ ). 9p contains a candidate gene at locus 9p21.3, *CDKN2A/2B*. We found one deletion at this locus. Hunter et al. found loss of 1p, 9q, 18q, and X to be common for borderline tumors and LGSOC samples and gain of chromosomes 7, 8, and 22 to be enriched in SBTs but not in LGSOC. In our series, loss of 9p (33%) and gain of 8q (44%) and chromosome 7 (39%) were slightly enriched in the samples with associated borderline component (SBT-LGSOC) vs. 16%, 21%, and 21% in pure LGSOC samples ( $P = .21$ ,  $P = .12$ ,  $P = .26$ ). Overall, we detected similar CNAs. Discrepancy between findings may be explained by the fact that the samples in the series of Hunter et al. were not paired SBT-LGSOC samples and due to the low number of cases analyzed in both series. We analyzed the invasive component of borderline-associated cases. Thus, findings are correlative and caution should be taken to identify these CNAs as markers of transition. We could not withhold an enrichment of other CNAs in patients with an associated borderline component. This suggests that these CNAs can be present in both borderline and LGSOC but are not specific for the transition of borderline to the invasive component given their equal presence in LGSOC without borderline component. This is consistent with Emmanuel et al. who found similar CNAs in LGSOC samples and their associated SBT component [9].

Loss of 1p36.33 was the most frequent focal alteration (54.1%) with no association with a specific clinical characteristic (Table 3). Kuo et al. previously reported loss of 1p36 in LGSOC [33]. They suggested *CHD5* and *miR-34a* as possible targets. Mutation analysis however did not reveal any mutations. However, *CHD5* maps to the 1p36.31 region and *miR-34a* to the 1p36.22 region. We further localized the known loss of 1p36 in LGSOC to the 1p36.33 region. This region contains 70 genes, none of which are known cancer consensus genes (Catalogue of Somatic Mutations in Cancer). However, in a broad range of human cancers, 1p36 is a mutational hotspot which suggests that the loss of tumor suppressor activity maps to this genomic region during tumorigenesis [34]. Genes in the 1p36 region may be affected by haploinsufficiency or promotor methylation since mutations detected in this region are rare. Many tumor suppressor genes do not require classic inactivation by a two-hit manner but contribute to tumorigenesis through reduced dosage of their gene products by mechanisms such as copy number or epigenetic changes, transcriptional repression, or aberrant miRNA regulation. Additionally, 14/37 (33%) of our samples also showed a whole arm loss of 1p, further contributing to the loss of function of these TSGs. In total, 22/37 (59.5%) samples harbored a 1p36 loss.



Finally, loss of 1p36.11-21 was associated with tumor dissemination in microsatellite stable tumors of stage II-IV colon tumors [35]. In our series, PFS and OS tended to be lower in patients with the 1p36.33 alteration compared with those without.

## Conclusion

Although mutations in the Ras signaling pathway are the most frequently reported oncogenic events in LGSOC, mutation frequency of the Ras/Raf/MEK/MAPK signaling pathway could be underestimated because of incomplete gene sequencing. It is possible that mutations in genes outside of the exome targeted thus far are responsible or that larger-scale CNA, inversions, or translocations may be important. These possibilities should be tested by whole-genome sequencing of a larger cohort. Nevertheless, a large proportion of LGSOC are “wild type” for the Ras/Raf/MEK/MAPK signaling pathway. The drivers of these wild-type tumors remain undefined.

Loss of the 1p36 region is more frequent in LGSOC than mutations in the RAS signaling pathway. Mutations detected in the 1p36 regions are scarce. Hence, identifying a possible gene or pathway for targeted therapy is not that straightforward since multiple tumor suppressor genes might be affected in this region and they, at their turn, can interact reciprocally or with other pathways. Nevertheless, the focus should shift from trying to target one known mutation towards a broader approach, such as miRNA replacement therapy.

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## Authors' Contributions

Conception and design: E. Van Nieuwenhuysen, I. Vergote.

Acquisition of data: E. Van Nieuwenhuysen.

Analysis and interpretation of data: E. Van Nieuwenhuysen, Pieter Busschaert, Annouschka Laenen, D. Lambrechts, I. Vergote.

Pathological review: P. Moerman.

Writing: E. Van Nieuwenhuysen.

Review and/or revision of the manuscript: all authors.

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## Disclosure of Potential Conflicts of Interest

There are no potential conflicts of interest to disclose by any of the authors.

## Availability of Data and Materials

Research data and data not presented in the manuscript are available as supplementary data (S1). They will also be available upon request to the corresponding author.

## Ethical Approval

Written consent for the use of tumor tissue and data collection was obtained of each patient, and the study was approved by the local ethics committee of the University Hospitals Leuven (study number s55308). This study was performed in accordance with the Declaration of Helsinki.

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