Gynecologic Oncology 161 (2021) 762–768

Contents lists available at ScienceDirect

Gynecologic Oncology

journal homepage: www.elsevier.com/locate/ygyno

Mutational spectrum in clinically aggressive low-grade serous carcinoma/serous borderline tumors of the ovary—Clinical significance of *BRCA2* gene variants in genomically stable tumors

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HIGHLIGHTS

- 17 cases of FIGO stage III low-grade serous carcinoma/serous borderline tumors underwent molecular profiling.
- Tumor mutational burdens were generally low (range 3–10 mutations/Mb in 14 cases assessed).
- Microsatellites were assessed to be stable in 12/12 cases.
- The majority of tumors (11/17) harbored a clear driver mutation in forms of RTK/RAS/MAPK pathway gene mutations.
- While *BRCA2* variants were seen in 5/17 cases, further analyses suggest they are unlikely to be clinically significant.

ARTICLE INFO

Article history:
Received 21 January 2021
Accepted 15 March 2021
Available online 24 March 2021

Keywords:
Low-grade serous ovarian carcinoma
Serous borderline tumor
Homologous recombination pathway
PARP inhibitor
BRCA2

ABSTRACT

Objective. The mutational spectra of low-grade serous carcinomas (LGSCs) and serous borderline tumors (SBTs) of the ovary are poorly characterized. We present 17 cases of advanced or recurrent LGSC/SBT patients who underwent molecular profiling.

Methods. Thirteen LGSCs and four SBTs underwent targeted gene panel testing by massively parallel sequencing. Microsatellite stability and tumor mutation burdens (TMBs) were determined based on panel sequencing data.

Results. The mean TMB was 5.2 mutations/megabase (range 3–10) in 14 cases. Twelve of twelve (12/12) cases were microsatellite stable. Clear driver mutations were identified in 11 cases, namely *KRAS* (5/17), *BRAF* (2/17), *NRAS* (2/17) and *ERBB2* (2/17). Five cases harbored *BRCA2* alterations (allele fractions: 44–51%), including two classified as likely benign/benign variants, and three classified as variants of uncertain significance (VUSs), with two variants being confirmed to be germline. The three *BRCA2* VUSs were missense variants that were assessed to be of unlikely clinical significance, based on family cancer history and expected impact on protein function. Two patients received PARP inhibitors during their disease course, with neither of the patients demonstrating appreciable response.

Conclusions. The mutational spectra in 17 clinically aggressive SBT/LGSC cases demonstrate genomically stable tumors, frequently driven by the RTK/RAS/MAPK pathway. While *BRCA2* variants were identified, our data demonstrate *BRCA2* gene variants are at most VUSs and of dubious clinical significance, in contrast to disease-associated *BRCA1/2* variants that may be identified in high-grade serous carcinoma. Germline testing and PARP inhibitors are thus expected to provide limited benefit to patients with LGSC/SBTs.

1. Introduction

Ovarian serous carcinomas are dichotomized into low- and high-grade serous carcinomas, the distinction of which is based on nuclear atypia and mitotic activity [1,2]. This binary grading system well reflects their distinct clinical behavior, with significant differences in patient

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https://doi.org/10.1016/j.ygyno.2021.03.019
0090-8258/

survival [3]. As well, this binary system also appears to reflect their difference in pathogenesis and mutational landscapes. The fallopian tube is considered to be the primary site of origin for many high-grade serous carcinomas (HGSCs), thus the commonly used designation of "tubo-ovarian" [4,5]. HGSCs are defined by disease-associated *TP53* mutations and accompanying marked copy number variant (CNV) burden [2].

In contrast, low-grade serous carcinomas (LGSCs) are thought to evolve from epithelial inclusions, via stepwise evolution that may involve atypical/non-invasive micropapillary serous borderline tumors (SBTs), although the epithelial inclusions themselves may originate from the fallopian tubal epithelium [6,7]. The distinction between LGSC and SBT is in the extent of invasion, where a focus measuring at least 5 mm in the greatest dimension is required for the diagnosis of LGSC [8]. Nevertheless, the genetics of SBTs and LGSCs appear to be largely overlapping, including copy number abnormalities [9]. Both SBT and LGSC usually harbor a mutation in the RAS/MAPK signaling pathway, including varying frequencies of *KRAS* (26–69% in SBT and 21–41% in LGSC), *NRAS* (0% in SBT and 4–22% in LGSC), *BRAF* (13–53% in SBT and 16–26% in LGSC), and *ERBB2* (5–8% in SBT and 0–30% in LGSC) mutations, with a smaller number of cases showing *PIK3CA* mutations (4.8% in SBTs and 5% in LGSCs) [9–16]. Besides the known driver genes, the mutational spectra of LGSC/SBT, including the frequency of alterations in cancer predisposition and tumor suppression genes, have been sparsely studied.

With now several landmark clinical trials that have demonstrated efficacy of PARP inhibitors in HGSC patients [17], the low vs. high-grade distinction has become of even greater clinical significance. Clinically, patient selection for PARP inhibition is generally based on the *BRCA1/2* mutation status, and disease-associated ("pathogenic") *BRCA1/2* variants are present in up to 20% of HGSC patients (somatic and germline combined) [18]. However, sensitivity to PARP inhibitors has also been associated with *BRCA1* promoter hypermethylation status, as well as deficiency in the homologous recombination (HR) DNA repair pathway, assessed by examining for genomics "scars" [17]. In contrast, LGSC patients were not included in the phase III trials by design. While reports of LGSC with *BRCA1* (3/70 in the series by Norquist et al. [20]) and *BRCA2* (1/70 [20]) mutations have been reported [19,20], the association between LGSC/SBT and germline *BRCA1/2* mutations remains unclear. In discussing the hereditary breast and/or ovarian cancer (HBOC) syndrome, which is associated with germline *BRCA1/2* variants, "ovarian cancer" has generally referred to HGSC, and no clear guideline appears to be available for LGSC patients.

Given that data regarding the mutational landscape of LGSC/SBT remain limited, and selecting effective systemic therapeutic remains a clinical quandary for advanced and recurrent cases, we sought to evaluate the tumor profile and receipt of therapies of LGSC/SBT patients treated at a high volume tertiary care cancer center.

2. Materials and methods

2.1. Case selection

This study was performed in concordance with the institutional research ethics board (University of Pennsylvania Institutional Review Board Protocol No. 702679). A retrospective case-series study was conducted of patients at the University of Pennsylvania Health System (UPHS) with advanced or recurrent LGSC of the ovary, or SBT, diagnosed between April 2001 to February 2019, who underwent genomic tumor profiling by Caris Molecular Intelligence Tumor Profiling. Seventeen patients were treated at UPHS and their tumors were included. These patients were initially diagnosed with either LGSC of the ovary ($n = 13$), or SBT ($n = 4$), and underwent treatment by gynecologic oncologists at UPHS. The tumor tissue used for profiling was from primary tumor in nine patients and recurrent tumor in eight patients. Demographic, clinical treatment, and survival data were captured through electronic medical records. Hematoxylin and Eosin (H&E)-stained sections were reviewed to confirm the original diagnosis.

2.2. Molecular profiling of LGSC/SBT

Fifteen of seventeen (15/17) patients had undergone molecular profiling using a 592-gene panel profiled by Caris Molecular Intelligence Tumor Profiling. Two cases were profiled using the older, 46-gene panel from Caris (44 genes, plus separate full-gene sequencing reactions for *BRCA1* and *BRCA2*). All sequencing and initial data analysis were performed by Caris. Copy number alterations (CNAs) were reported as being amplified, if the average copy number of the entire gene is ≥6 copies, with ≥4 to <6 copies being interpreted as intermediate amplification. TMBs were determined based on panel sequencing data, identifying the total number of non-synonymous, somatic mutations identified per megabase (Mb) or genomic coding area. Microsatellite instability (MSI) status was measured by the direct analysis of known microsatellite regions sequenced in the 592 gene panel, and MSI status designation (high, stable, or equivocal) were validated using the results from a PCR-based MSI assay.

2.3. Variant classification

All reported genetic data, including all classified and unclassified variants by Caris, were reassessed and manually classified, applying the AMP/ASCO/CAP guidelines [21], using numerous publicly available databases, including COSMIC, OncoKB, ClinVar, and gnomAD, and primary research papers. Gene-specific databases were also used when available (e.g., *BRCA* exchange, ARUP *BRCA2* database). Variants annotated as clearly disease-associated (AMP/ASCO/CAP tier I or II) included the well-established hotspot, activating mutations in the receptor tyrosine kinase (RTK)/RAS/MAPK pathway. Disruptive frameshift variants in tumor suppressor genes (e.g., *NF2*) were also annotated as disease-associated. Population polymorphism data were a major criterion in assessing variant benignity. Other criteria include expected change in protein function, location of the impacted amino acid within the protein (with respect to functional domains), and any reported functional data.

3. Results

3.1. Patient characteristics and molecular profiling metadata

Tumors (nine primary and eight recurrent tumors) from 17 patients had successfully undergone molecular profiling, which included 13 patients with LGSC, and four patients with SBT (Table 1). The age at diagnosis ranged from 17 to 66 years (median age 37 years). All patients had advanced (FIGO stage III) or recurrent disease at the time of molecular profiling. Microscopically, all cases exhibited unequivocal and classic histomorphology of LGSC or SBT. All SBT cases (4/4) had at least focal areas of micropapillary growth, a feature that has been associated with more aggressive clinical behavior, compared to conventional SBTs. Two of the SBTs (SBT1 and SBT3) showed foci of microinvasion, and multiple foci of invasive implants were identified in one case (SBT1). 15/17 patients had undergone molecular profiling using a 592-gene panel, and two cases were profiled using the older, 46-gene panel (see Supplementary Data for the detailed panel sequencing data). None of the cases harbored a disease-associated *TP53* or *MDM2* gene variants. CNAs were assessed in 15/17 cases. Only a small number of CNAs met the reporting criteria; i.e., intermediate (≥4 to <6 copies) level of amplification (*MCL1* or *MAP2K1* copy numbers) were identified in two cases (Table 2). The CNA data are consistent with low CNV burden, although CNV burden was not formally assessed. TMBs were assessed in 14 cases, which were generally low, with TMBs being less than 10/Mb in 13/14 cases, and with mean TMB of 5.2 mutations/Mb (range 3–10) (Table 2 and Fig. 1). MSI status was assessed in 12 cases, all of which were stable, and no cases were found to harbor

Table 1
Patient clinical information and family history.

Study ID	Age	Stage	Medical therapy	Status (follow-up period)	Family history (relative affected and ages at diagnosis if known)
SBT1	57	IIIB	Chemotherapy, anastrozole	AWD (3.5 yrs)	Lung cancer (father)
SBT2	31	IIIA2	Chemotherapy, letrozole	NED (3.6 yrs)	Gynecological cancer NOS (mother)
SBT3	21	IIIC	None	AWD (2.2 yrs)	Glioblastoma multiforme (mother) Melanoma (aunt, age 50–60s) Cancer NOS (possible sarcoma) (uncle, age 50s)
SBT4	17	IIB	None	N/A	Breast cancer (maternal aunt, age 50s) Ovarian cancer (maternal grandmother, age 70s; maternal cousin, age 24) Gynecological cancer NOS (maternal aunt) Colon cancer (maternal great aunt) Leukemia (maternal aunt) Prostate cancer (maternal grandfather) Lymphoma NOS (paternal aunt)
LGSC1	49	IIIA	Chemotherapy	AWD (7.4 yrs)	Breast cancer (maternal grandmother) Melanoma (paternal aunt) Cancer NOS (mother) Colon cancer (paternal grandfather) Lung cancer (maternal aunt)
LGSC2	25	IIIC	Chemotherapy, tamoxifen, fulvestrant[a]	DOD (1.0 yrs)	Breast cancer (maternal grandmother, other maternal relatives NOS) Colon cancer (maternal grandfather)
LGSC3	37	III	Chemotherapy, rucaparib, trametinib, anastrozole	DOD (5.4 yrs)	No family history of gynecological cancer
LGSC4	54	IIIC	Chemotherapy	AWD (8.4 yrs)	Breast cancer (mother)
LGSC5	43	IIIC	Chemotherapy, bevacizumab	AWD (11.8 yrs)	Breast cancer (maternal grandmother) Endometrial cancer (maternal grandmother) Ovarian cancer (maternal aunt) Prostate cancer (paternal grandfather)
LGSC6	23	IIIC	Chemotherapy, trametinib, anastrozole, letrozole, tamoxifen	AWD (17.9 yrs)	Ovarian cancer (mother) Colon cancer (maternal grandmother) Lymphoma (mother)
LGSC7	66	IIIB	Chemotherapy, olaparib	DOD (3.0 yrs)	Breast cancer (mother, maternal grandmother, maternal aunt, paternal aunt) Ovarian cancer (paternal aunt)
LGSC8	22	IIIB	Chemotherapy, letrozole	NED (0.9 yrs)	No known family cancer history
LGSC9	45	IIIC	Chemotherapy	NED (1.0 yrs)	Breast cancer (mother, age 72) Colon cancer (father)
LGSC10	60	IIIB	Chemotherapy, letrozole	AWD (4.2 yrs)	Ovarian cancer (sister)
LGSC11	52	IIIC	Chemotherapy, bevacizumab, letrozole	AWD (9.1 yrs)	Prostate (father, age 67) Breast (1st cousin, age 39) Ovary (1st cousin, age 60) Kidney (1st cousin, age 70)
LGSC12	32	IIIC	Chemotherapy, letrozole	AWD (4 yrs)	No known family cancer history
LGSC13	24	III	Chemotherapy, tamoxifen	AWD (0.6 yrs)	Hemophagocytic lymphohistiocytosis (sister, brother)

Abbreviations: AWD, alive with disease; DOD, died of disease; N/A, not available; NED, no evidence of disease; NOS, not otherwise specified; yrs., years.
[a] Also was enrolled in a clinical trial veliparib vs. placebo (unrevealed).

disease-associated mutations in mismatch repair pathway, *POLE*, or *POLD* genes. All these clinical and molecular data were assessed to be in accord with the diagnosis of SBT/LGSC, whose genomes are thought to be generally stable.

Previously characterized driver mutations were seen in 11/17 cases, specifically 5/17 cases with *KRAS* (p.G12D or p.G12V), two *NRAS* (p.Q61R), two *BRAF* (p.V600E), and two *ERBB2* (p.Y772_A775dup, p.S310F) driver mutations (Table 2 and Fig. 1). Of the remaining six cases without a clear driver mutation, one case harbored a disease-associated variant in the *NF2* gene (p.Y192*, LGSC7) with a variant allele fraction (VAF) of 48%, which encodes a negative regulation of RAS [22].

3.2. BRCA2 variants

Altogether, *BRCA2* was the second most frequently varied gene, with five cases harboring *BRCA2* alterations, with VAFs ranging 44–51%, i.e., all suspicious of germline origin (Fig. 2). No *BRCA1* variants were identified. The *BRCA2* variants comprised one non-sense, one in-frame deletion, and three missense alterations (transcript NM_000059). Three patients had undergone germline testing, two of which were confirmed to be of germline origin.

The non-sense variant (p.K3326*) had been previously characterized to be a polymorphic stop codon [23–25]. While more recent analyses linked the p.K3326* variant with increased cancer risks [26,27], the variant has been shown to encode a functional protein and rescue *BRCA2*-deficient VC8 cells in vitro [28]. Accordingly, the variant remains benign/likely benign in ClinVar (38266) and OncoKB. The patient had undergone germline testing, with “negative” results, which likely did not report the p.K3326* variant (i.e., classified as benign). The family history was significant for a 1st-degree relative with a history of astrocytoma (age 19) and glioblastoma (age 45). Considering all these data, the variant remained a likely benign/benign variant (tier IV).

The in-frame deletion variant p.E1382del (c.4146_4148delAGA) lies in the in the BRC repeats, and the variant was confirmed to be germline in the patient. The variant was initially designated a VUS, with functional studies showing unclear effects [28]. However, several genetic testing institutions now designate the variant as likely benign based on their internal databases (ClinVar ID 37883). The patient's family history was notable for one maternal first cousin with history of numerous cancers (breast at age 39, ovary at age 60), but otherwise the family history was relatively unremarkable. The overall data were thus more consistent with a likely benign/benign variant (tier IV).

Table 2
The molecular data of the 17 patients.

ID	Panel size (genes)	Key variants[b] (amino acid change, VAF)	Driver	MSI	TMB (mutations/Mb)	CNAs	IHC
SBT1	46[a]	*KRAS* (p.G12D, 52%)	*KRAS*	N/A	N/A	N/A	ER/PR+
SBT2	592	*ERBB2* (p.Y772_A775dup, 36%); *BRCA2* (p.A1204V, 51%)	*ERBB2*	MSS	7	None	ER/PR+
SBT3	592	*BRAF* (p.V600E, 45%); *ERBB2* (p.S208N, 49%)	*BRAF*	MSS	4	None	ER/PR+
SBT4	592	None	None	MSS	4	None	ER/PR+
LGSC1	46[a]	*KRAS* (p.G12V, 36%)	*KRAS*	N/A	N/A	N/A	ER/PR-neg
LGSC2	592	None	None	N/A	4	None	ER/PR+
LGSC3	592	*KRAS* (p.G12V, 35%); *BRCA2* (p.K169R, 44%); *BRIP1* (p.S624L)	*KRAS*	N/A	10	None	ER+, PR-neg
LGSC4	592	*NRAS* (p.Q61R, 43%); *BRCA2* (p.A2633S, 46%)	*NRAS*	MSS	4	None	ER+, PR-neg
LGSC5	592	*KRAS* (p.G12D, 15%)	*KRAS*	N/A	N/A	None	ER+, PR-neg
LGSC6	592	none	none	MSS	5	None	ER+, PR-neg
LGSC7	592	*NF2* (p.Y192*, 48%)	none	MSS	6	*MAP2K1* intermediate	ER/PR-neg
LGSC8	592	*ATR* (p.H4R, 51%)	none	MSS	4	*MCL1* intermediate	ER/PR-neg
LGSC9	592	*CHEK2* (p. L183F, 79%)	none	MSS	8	None	ER/PR+
LGSC10	592	*ERBB2* (p.S310F, 28%); *ERBB2* (p.D277G, 22%); *BARD1* (p.C469R, 37%)	*ERBB2*	MSS	5	None	ER+, PR-neg
LGSC11	592	*NRAS* (p.Q61R, 54%)	*NRAS*	MSS	3	None	ER+, PR-neg
LGSC12	592	*KRAS* (p.G12V, 40%); *ERBB2* (p.E1021D, 48%)	*KRAS*	MSS	5	None	ER/PR+
LGSC13	592	*BRAF* (p.V600E, 36%); *ATR* (p.I219V, 51%)	*BRAF*	MSS	4	None	ER/PR+

Abbreviations: CNAs, copy number alterations; IHC, immunohistochemistry; Mbp, megabase pair; MSI, microsatellite instability; MSS, microsatellite stable; N/A, not available; neg, negative; TMB, tumor mutation burden; VAF, variant allele fraction.
[a] 44 (select genes) and full-gene sequencing for *BRCA1* and *BRCA2*.
[b] Included all disease-associated variants and select variants of uncertain significance.

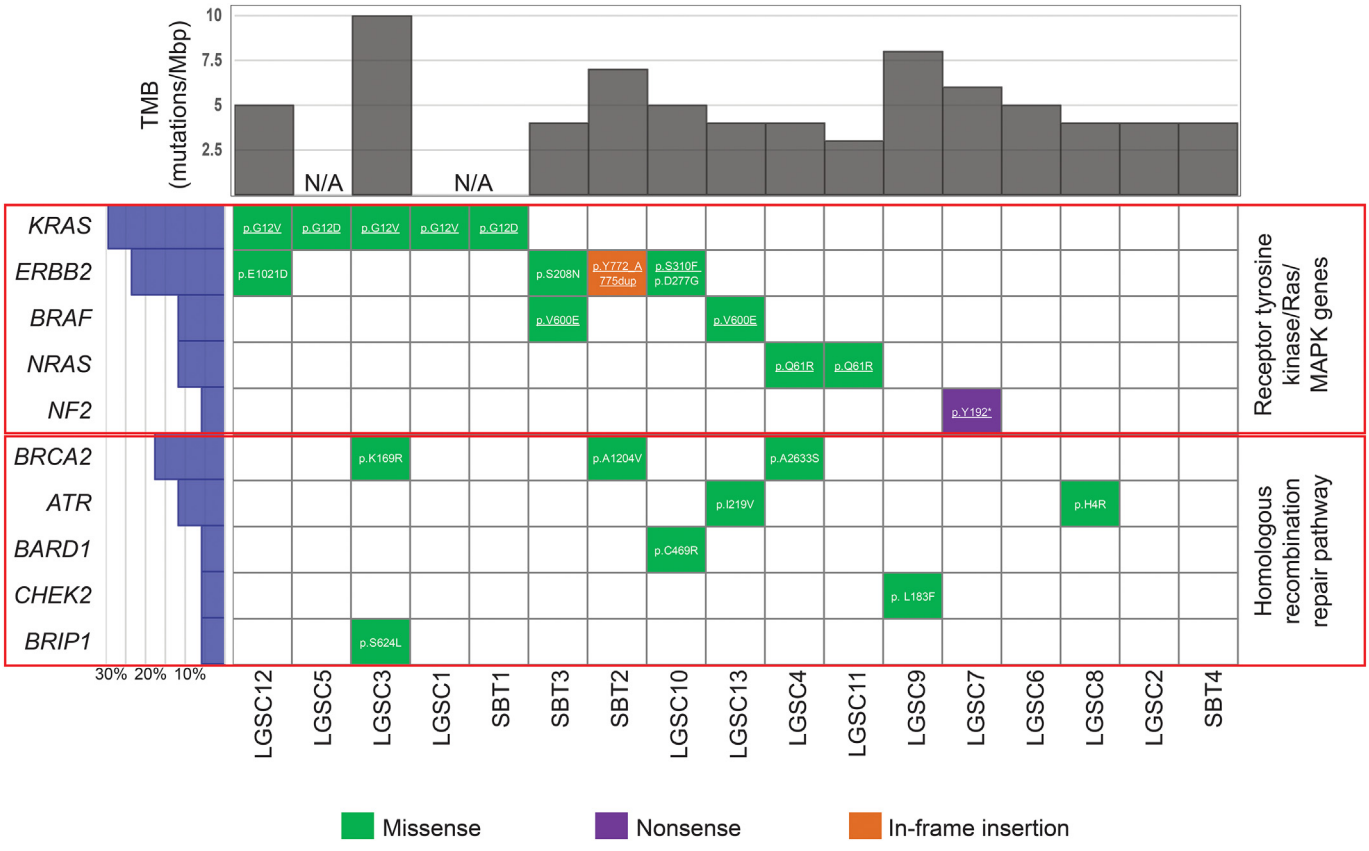
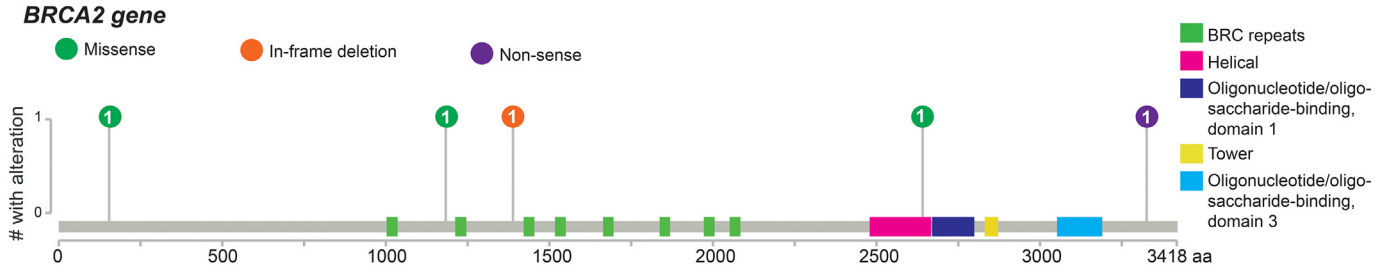


Fig. 1. Waterfall plot displaying select genetic variants in 17 cases of low-grade serous ovarian carcinoma/serous borderline tumor. Tumor mutational burdens (TMBs) are also displayed, where the data were available, based on panel sequencing data. Disease-associated variants are underlined and variants of unknown significance are not underlined. N/A = TMB not available.



Study ID	Variant (VAF)	Associated family history	AMP tier	Germline?	Comment
LGSC3	c.506A>G (p.K169R) (44%)	Limited history, with no history of GYN cancers	3 (VUS)	N/A	
SBT2	c.3611C>T (p.A1204V) (51%)	GYN cancer NOS (mother)	3 (VUS)	N/A	
LGSC11	c.4146_4148delAGA (p.E1382del) (44%)	Prostate (father, age 67) Breast (1st cousin, age 39) Ovary (1st cousin, age 60) Kidney (1st cousin, age 70)	4 (LB/B)	Yes	In-frame deletion in BRC repeat
LGSC4	c.7897G>T (p.A2633S) (46%)	Breast cancer (mother, 82)	3 (VUS)	Yes	C-terminal DNA-binding domain; SEM1-interacting region
SBT3	c.9976A>T (p.K3326*) (52%)	Glioblastoma multiforme (mother) Melanoma (aunt, 50-60s) Cancer NOS (uncle, 50s)	4 (LB/B)	No[a]	see Results

[a] The patient had undergone germline testing, with "negative" results, which likely did not report the p.K3326* variant (i.e., classified as likely benign/benign).
Abbreviations: GYN, gynecological; LB/B, likely benign/benign; N/A, not available; NOS, not otherwise specified; VAF, variant allele frequency; VUS, variant of unknown significance.

Fig. 2. *BRCA2* gene variants in five cases of low-grade serous ovarian carcinoma/serous borderline tumor.

Among the missense *BRCA2* variants, the p.A2633S (c.7897G > T) missense variant was confirmed to be of germline origin. A2633 maps to an amino acid within the C-terminal DNA-binding domain, which spans amino acids 2474–3190 [29,30], suggesting possible impact on the BRCA2 protein function. However, the patient's family history was rather unremarkable, notable for only breast cancer in her mother diagnosed at age 82. Two patients with other missense variants (p.K169R and p.A1204V) did not undergo germline testing. The two missense variants did not occur in a functional domain of *BRCA2*, and no deleterious effect on the protein function was predicted. The family histories were limited in both patients, with one patient's mother having had an unspecified gynecological cancer in her mother. Considering these data, the three missense variants were classified as VUS (tier III), but all three variants are of dubious clinical significance.

Other variants in the HR DNA repair pathway genes were *ATR* (p.H4R and p. I219V), *BARD1* (p.C469R), *CHEK2* (p.L183F), and *BRIP1* (p.S624L) variants (Fig. 1 and Table 2). The *BARD1* variant (p.C469R) occurred in the ankyrin repeat, with role in protein-protein interaction [31], and the variant was seen in a patient with family history of ovarian cancer in a 1st-degree relative (sister). Family cancer histories in other patients were less alarming (Table 1). All five variants were classified as VUSs (tier III) but are assessed to be of likely no clinical significance.

3.3. Patient survival and response to therapy

The 17 patients were followed for a median follow-up period of 3.8 years (mean 5.3 years), during which 3/17 patients succumbed to their disease (Table 1). 15/17 patients received chemotherapy, and 11/17 patients had received additional targeted therapy during their disease courses. Anti-hormonal therapy was the most commonly employed modality (10/17 patients, received letrozole, anastrozole, or tamoxifen). Among those ten patients, both estrogen (ER) and progesterone receptors (PR) were positive by immunohistochemistry in 5/10, while sole ER expression (PR-negative) was seen in four patients, suggesting reduced ER protein activity. No *ESR1* or *PGR* gene variants were identified.

VEGFR inhibition in form of bevacizumab was employed in 2/17 patients. No cases harbored *KDR* (VEGF receptor 2) amplification or variants. *VEGFB* p.R148H was seen in one patient, which was assessed to be likely benign based on minor allele frequency and assessed to be unlikely significant in the context of bevacizumab sensitivity. Two patients, including one with a *KRAS* activating mutation (p.G12V), had received a MEK inhibitor trametinib. They had stable disease during the therapy, but the treatment was discontinued in both patients due to regimen-related toxicity after one and eight months of trametinib, respectively. No patients received BRAF or HER2 inhibitors.

Two patients received a PARP inhibitor during their disease course (Table 3). One patient's (LGSC3) tumor harbored two alterations in HR repair pathway genes, specifically *BRCA2* (p.K169R) and *BRIP1* (p.S624L). The PARP inhibitor was poorly tolerated, and it is unclear whether the patient benefited from the short (three-month) trial of PARP inhibition. PARP inhibitor also appeared ineffective after treatment for five months in the other patient, who succumbed to the disease despite having stable disease during the first two months of treatment.

Table 3
Response to PARP inhibitors and molecular data.

ID	Mutations	PARP inhibitor	Response
LGSC3	*KRAS* (p.G12V, 35%) *BRCA2* (p.K169R, 44%) *BRIP1* (p.S624L)	Rucaparib	Discontinued after 3 months due to nausea/vomiting—succumbed to disease one month later.
LGSC7	*MAP2K1* copy gain	Olaparib	Initially stable disease for 2 months – progressed and succumbed to disease after 5 months of olaparib

4. Discussion

Summarizing the above results, we report the mutational spectra in 17 patients with clinically aggressive SBT/LGSC, which were found to be genomically stable and mostly driven by mutations in the RTK/RAS/MAPK pathway genes. Several variants in multiple HR pathway genes were observed, including five *BRCA2* variants, with two being confirmed to be of germline origin. However, the alterations were at most VUSs, and the reviews of the patients' personal and family history were not generally remarkable for clear *BRCA1/2*-associated cancer histories. Two patients received a PARP inhibitor for short durations, including one patient whose tumor harbored *BRCA2* VUS, but their benefits were unclear.

In this study, the clinico-pathological and molecular attributes in the 17 patients were concordant with known features of LGSC/SBT (vs. HGSC), including younger patient age (median age 37 years), rare CNAs, and the absence of *TP53* mutations. While 6/17 lacked a clear driver mutation (including one case with a potential *NF2* driver variant), this was not related to the cases undergoing limited molecular profiling, as all six cases were profiled using the larger 592-gene panel. All cases also underwent full gene sequencing of *BRCA1* and *BRCA2*, and *BRCA2* variants were identified in 5/17 cases. This frequency is difficult to compare to other studies. *BRCA1/2* variants have been reported in LGSC, with the largest study reporting frequency of 4/70 (all classified as pathogenic germline mutations in this study), using the three-tier serous tumor grading system [20]. Robust low- vs. high-grade distinction is a major challenge in these studies, and the distinction may be completely absent in some databases [32]. *BRCA1/2* mutations were not reported in an exome sequencing study, although their study was limited to eight cases [33]. VUSs also may not have been reported in previous studies, and it is difficult to estimate their frequency in SBT/LGSC patients. Both *BRCA1/2* are relatively large genes (125,951 bp and 85,405 bases, respectively, based on GRCh38/hg38), and passenger mutations can be seen, certainly in tumors with high TMBs. However, in case of SBT/LGSCs, the TMB values were low-to-modest in all cases, consistent with previous studies showing these tumors to be genomically stable [33]. Rather, all five *BRCA2* variants are suspected to be of germline origin, based on VAFs (on somatic sequencing) and available germline sequencing data. The three *BRCA2* VUSs remain as VUSs based on AMP/ASCO/CAP criteria, but correlation with family history and expected impact on protein suggests that these VUSs are unlikely to be significant. While *BRCA2* VUSs remain challenging in their clinical interpretation, our data suggest that these SBT/LGSC patients are unlikely to be associated with the HBOC syndrome. Comparing family cancer history between HGSC and LGSC patients, a study with 195 LGSC patients came to a similar conclusion [34].

Should a clearly disease-associated ("pathogenic") *BRCA1/2* variant be identified, the finding should raise the possibility of a misdiagnosed HGSC, especially in cases harboring *TP53* mutations. It is worth noting that a LGSC case has been reported in a patient with a clearly pathogenic *BRCA1* variant (c.66_67AG (185 delAG)) [19]. While the case was diagnostically compatible with LGSC and the variant is clearly an established pathogenic variant, we postulate such cases would be less likely to be associated with the deletion of the wildtype locus (i.e., loss-of-heterozygosity (LOH)) and less likely associated with HR deficiency, a scenario more commonly seen in HGSC. Our molecular profiling platform was not validated to assess LOH and HR status, but, based on VAFs, none of the 17 cases were suspicious for *BRCA2* LOH. HR deficiency can be functionally assessed by examining for genomic "scars" and examining patterns of copy number aberrations [35–37]. However, the utility of the commercially available HR assays has not been validated in LGSC/SBT, and, given the low overall CNV burdens, use of the available algorithms are likely inappropriate for LGSC/SBT. Finally, our data with PARP inhibitors in these SBT/LGSC patients are also limited, but no appreciable response was noted in two patients albeit short treatment durations (five months or shorter), including one patient with a *BRCA2* VUSs. While the utility of PARP inhibitors in LGSC/SBT is unclear, the current literature and our series data lack meaningful positive data in this regard.

The data presented here tells a cautionary tale. In correlating the histomorphology of serous tumors with molecular data, our data demonstrate that *BRCA2* gene variants can be identified in LGSCs/SBTs, but the variants, at most VUSs, are unlikely to be of clinical significance. Germline testing and PARP inhibitors are thus expected to provide limited benefit to patients with LGSC/SBTs. On the other hand, the presence of pathogenic *BRCA1/2* variants should be interpreted with care, especially in the presence of a concomitant *TP53* mutation, and such molecular findings should prompt a review of the pathological data to identify potentially mis-diagnosed HGSC patients, who are much more likely to respond to PARP inhibitors and to benefit from genetic counseling.

Author contributions

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Ronny Drapkin: supervision and writing - review & editing;
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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2021.03.019.

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