

Clinical characteristics and molecular aspects of low-grade serous ovarian and peritoneal cancer: a multicenter, observational, retrospective analysis of MITO Group (MITO 22)

Lucia Musacchio¹, Daniela Califano², Michele Bartoletti^{3,4}, Laura Arenare⁵, Domenica Lorusso^{1,6}, Nunzia Simona Losito⁷, Gennaro Cormio^{8,9}, Stefano Greggi¹⁰, Francesco Raspagliesi¹¹, Giorgio Valabrega¹², Vanda Salutarì¹, Carmela Pisano¹³, Anna Spina², Daniela Russo², Michele Del Sesto⁷, Vincenzo Canzonieri^{14,15}, Francesco Ferrà¹⁶, Gian Franco Zannoni^{17,18}, Vera Loizzi⁹, Viola Ghizzoni¹, Claudia Casanova¹⁹, Valentina Tuninetti¹², Monika Ducceschi¹¹, Vittoria Del Vecchio⁹, Simona Scalone⁴, Domenico Priolo¹⁶, Francesco Perrone⁵, Giovanni Scambia^{1,6} and Sandro Pignata¹³✉

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BACKGROUND: Low-grade serous ovarian and peritoneal cancer (LGSC) is a rare disease and few data on the clinical and genomic landscape have been published.

METHODS: A retrospective analysis of patients diagnosed with LGSC between 1996 and 2019 was conducted in MITO centers. Objective Response Rate (ORR) to treatments, progression-free survival (PFS) and overall survival (OS) were assessed. Additionally, the tumor molecular profile of 56 patients was evaluated using the Next Generation Sequencing (NGS) FoundationOne CDX (Foundation Medicine®).

RESULTS: A total of 128 patients with complete clinical data and pathologically confirmed diagnosis of LGSC were identified. ORR to first and subsequent therapies were 23.7% and 33.7%, respectively. PFS was 43.9 months (95% CI:32.4–53.1) and OS was 105.4 months (95% CI: 82.7–not reached). The most common gene alterations were: KRAS ($n = 12$, 21%), CDKN2A/B ($n = 11$, 20%), NRAS ($n = 8$, 14%), FANCA ($n = 8$, 14%), NF1 ($n = 7$, 13%) and BRAF ($n = 6$, 11%). Unexpectedly, pathogenetic BRCA1 ($n = 2$, 4%), BRCA2 ($n = 1$, 2%) and PALB2 ($n = 1$, 2%) mutations were found.

CONCLUSIONS: MITO 22 suggests that LGSC is a heterogeneous disease for both its clinical behavior in response to standard therapies and its molecular alterations. Future prospective studies should test treatments according to biological and molecular tumor's characteristics.

CLINICAL TRIAL REGISTRATION: This study is registered under NCT02408536 on [ClinicalTrials.gov](https://clinicaltrials.gov).

British Journal of Cancer (2022) 127:1479–1486; <https://doi.org/10.1038/s41416-022-01897-1>

INTRODUCTION

Low-Grade Serous Ovarian Cancer (LGSOC) represents a rare entity accounting for 2% of all epithelial ovarian cancers (OC) and for 4.7% of serous ovarian carcinomas [1]. Due to its rarity, LGSOC has been less studied with respect to high-grade serous ovarian cancer (HGSOC) counterpart, and many clinical and molecular

aspects remain unknown. It is characterized by an indolent clinical course, with a lower biologic aggressiveness and a longer 5-year overall survival compared to HGSOC [2]. Patients with LGSOCs are often treated with cytoreductive surgery followed by platinum/paclitaxel chemotherapy. However, due to its low proliferative activity LGSOC is a relatively chemo-resistant disease and data

¹Department of Women and Child Health, Division of Gynecologic Oncology, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy. ²Microenvironment Molecular Targets Unit, Istituto Nazionale Tumori, IRCCS - Fondazione G. Pascale, Naples, Italy. ³Department of Medicine (DAME), University of Udine, Udine, Italy. ⁴Unit of Medical Oncology and Cancer Prevention, Department of Medical Oncology, Centro di Riferimento Oncologico di Aviano (CRO), IRCCS, Aviano, Italy. ⁵Clinical Trial Unit, Istituto Nazionale Tumori, IRCCS - Fondazione G.Pascale, Naples, Italy. ⁶Department of Life Science and Public Health, Catholic University of Sacred Heart, Largo Agostino Gemelli, Rome, Italy. ⁷Surgical Pathology Unit, Istituto Nazionale Tumori, IRCCS - Fondazione G. Pascale, Naples, Italy. ⁸Gynecologic Oncology Istituto Tumori Giovanni Paolo II – IRCCS, Bari, Italy. ⁹Interdisciplinary Department of Medicine, University of Bari, Bari, Italy. ¹⁰Gynecologic Oncology Unit, Istituto Nazionale Tumori, IRCCS - Fondazione G. Pascale, Naples, Italy. ¹¹Gynecologic Oncology Unit, Istituto Nazionale Tumori, IRCCS, Milan, Italy. ¹²Department of Oncology, University of Torino at Ordine Mauriziano Hospital, Turin, Italy. ¹³Department of Urology and Gynecology, Istituto Nazionale Tumori IRCCS Fondazione G. Pascale, Napoli, Italy. ¹⁴Pathology Unit, Centro di Riferimento Oncologico di Aviano (CRO Aviano), IRCCS, National Cancer Institute, Aviano, Italy. ¹⁵Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy. ¹⁶Medical Oncology Department, San Vincenzo Hospital, Taormina, Messina, Italy. ¹⁷Department of Woman, Child and Public Health Sciences, Gynecopathology and Breast Pathology Unit, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy. ¹⁸Pathological Anatomy Institute, Catholic University of Sacred Heart, Rome, Italy. ¹⁹Oncology Department, Santa Maria delle Croci Hospital, Ravenna, Italy. ✉email: s.pignata@istitutotumori.na.it

coming from retrospective single institution studies have shown an objective response rate (ORR) to both platinum and non-platinum-based chemotherapies of only 24% and 4% in first line and recurrent setting, respectively [3, 4]. Furthermore, recently reported conflicting results about chemotherapy responses suggest that LGSOC includes patients with different characteristics [5]. Because of the clinical heterogeneity of LGSOC and the lack of prospective data indicating the most effective therapeutic strategy in first line as well as in the recurrent setting, in the last few years several studies have been directed towards a better knowledge of the intrinsic disease characteristics, including genomic alterations and biomarkers expression, with the aim to identify novel molecular drivers that might be predictive of clinical behavior and/or guide the use of targeted therapies.

Molecular and genomic studies have shown that LGSOC appears to have a very low prevalence of TP53 mutations (<8%) [6, 7], loss of CDKN2A/B (15–53%) [8, 9] and often harbors activating mutations of genes involved in the mitogen-activated protein kinase (MAPK) pathway, such as KRAS (20–40%), BRAF (7–26%), ERBB2 (0–30%), and NRAS (5–33%) [10–13].

In this context, the identification of oncogenic mutations affecting MAPK genes (RAS/RAF/MEK/ERK) led to the evaluation of targeted therapy, such as MEK inhibitors (MEKi) in clinical trials, that have shown a promising activity with a better ORR and a statistically significant improvement in PFS in persistent and recurrent LGSOC when compared to the standard of care [14–16]. Additionally, hormone receptors are frequently overexpressed in LGSOC with respect to HGSOC, with reported rates of ER expression of 96% and PR expression of 58%, respectively [17, 18]. In this respect, endocrine therapies like tamoxifen and aromatase inhibitors are currently adopted options in recurrent disease and first line maintenance setting based on relatively large retrospective series and prospective clinical trials supporting this therapeutic strategy [19–21].

Finally, recent published data, in a small cohort of Asian patients, showed an “unexpected” mutational landscape of LGSOC reporting in some cases mutations in Homologous Recombination Repair (HRR) DNA pathway genes such as BRCA 1 / 2, BARD 1, ATR, BRIP1, and CHECK2 [22].

These findings suggest a heterogeneous molecular profile of this rare disease and the need to identify actionable mutations and biomarkers that might predict tumor response.

With the aim of better define clinical and molecular characteristics of LGSOCs we conducted this descriptive retrospective analysis of the data from the Multicenter Italian Trials in Ovarian Cancer (MITO) group.

MATERIALS AND METHODS

MITO 22 is an Italian, multicenter, observational, and retrospective analysis involving 8 MITO centers and coordinated by National Cancer Institute of Naples. The study was approved by the ethical committees of each participating institution and written consent was required.

Between 1996 and 2019, 171 patients diagnosed with de novo low-grade serous carcinoma of the ovary and peritoneum (LGSC) or recurrence of LGSC after surgical resection of borderline serous ovarian carcinoma were identified and enrolled in this study and demographic, clinical, surgical, and molecular data were collected. Pathologic diagnosis was based upon the two-tiered classification system for grading serous ovarian cancers published in 2004 [23], and gynecologic pathologists at the participating MITO centers with significant expertise in this grading system reviewed all cases. In addition, immunohistochemical staining for p53 and WT1 was performed to confirm the diagnosis. Given that several cases were diagnosed before 2014, the 1988 FIGO criteria were used to classify these patients [24]. Patients were included in this analysis if they had pathologically confirmed early or advanced-stage LGSC.

Patients’ data were collected into a centralized database recording demographic and clinical information, including age at diagnosis, stage according to International Federation of Gynecology and Obstetrics (FIGO) criteria, body mass index (BMI), serum CA 125 levels, type of cytoreductive

surgery, residual disease status after cytoreduction, treatment details at the time of diagnosis and at the time of subsequent relapses, response rate during primary and subsequent treatments, and date of last contact or death. Cytoreductive surgery was considered complete in case of Residual Tumor (RT) = 0, optimal if RT was <1 cm, and suboptimal if residual disease was ≥ 1 cm diameter. Eastern Cooperative Oncology Group (ECOG) definitions were used to define performance status.

Follow up data were collected until January 2021. Descriptive statistics were used to characterize the patient’s population. The follow-up was assessed from the start of first line chemotherapy to the date of the last contact or date of death. Clinical response was determined using Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1, assessed separately in each of the 8 MITO centers, and stable disease (SD) was reported for those patients who met RECIST v1.1 for a minimum of 12 weeks. Clinical Benefit Rate (CBR) is defined as the percentage of patients who achieved complete response, partial response, and stable disease. Progression-free survival (PFS) was defined as the time between the start of first line chemotherapy and first observation of recurrence or the date of last follow-up or death for any cause. Overall survival (OS) was defined as the time between the start of first line chemotherapy and the date of last follow-up or death for any cause. Median follow up was estimated by means of reverse Kaplan–Meier curve. PFS and OS curves were described according to the reverse Kaplan–Meier method. Univariate and multivariate Cox’s regression model was used to analyze the prognostic role of clinical factors on survival. Schoenfeld residuals test was used to judge the proportional-hazards assumption. Differences were considered statistically significant at p value < 0.05. All the analyses were performed with STATA 14 MP (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

Molecular analysis

For the genomic data, samples of histologically confirmed LGSC cases that had a tumor block available and sufficient tumor fraction were test with the NGS platform FoundationOne CDX (Foundation Medicine®).

Of 128 LGSCs included in our analysis, 79 tumor specimens were considered adequate for the genomic evaluation. Formalin-fixed paraffin-embedded (FFPE) samples were collected, centralized at MITO coordinating center, and stored according to Italian guidelines [25]. A 5 μ m section was cut from each FFPE block, stained with hematoxylin and eosin (H&E), and reviewed by a gynecologic pathologist with significant expertise at the National Cancer Institute of Naples.

Results from the Foundation Medicine® reports were collected, stored in the centralized datasheet, with the data points collected related to patient demographics, their cancer diagnosis and treatments, and analyzed.

The principal endpoint was to describe the mutations discovered.

RESULTS

Between 1996 and 2019, 128 patients with diagnosis of pathologically confirmed LGSC or invasive recurrence after surgical resection of borderline serous ovarian carcinoma were treated and followed up within the 8 MITO centers in Italy and enrolled in this analysis. Of the 171 identified patients, 43 were excluded from this analysis due to incomplete clinical data and follow-up information ($n = 36$) or pathologically unconfirmed diagnosis of LGSC ($n = 7$) (Supplementary Appendix 1).

Demographics and clinical characteristics at baseline of the included patients ($N = 128$) are summarized in Table 1. Median age at the time of diagnosis was 53.2 years (Interquartile range: 42.3–63.1). The majority of LGSCs were diagnosed at an advanced stage (FIGO III and IV), with 68.8% women ($n = 88$) at stage III and 7.8% ($n = 10$) at stage IV. Twenty patients (15.6%) had stage I and ten (7.8%) had stage II. Two patients with invasive recurrence after surgical resection of borderline serous ovarian cancer were classified as FIGO stage III.

Primary debulking surgery was performed in 115 patients (89.8%) while 11 patients (8.6%) underwent neoadjuvant chemotherapy (NACT) x 3–6 cycles followed by interval debulking surgery. Among these patients receiving upfront or interval cytoreductive surgery, 68.7% were completely debulked (RT = 0), while 10.2% underwent suboptimal cytoreduction (RT < 1 cm) and 19.5% received incompletely debulking (RT ≥ 1 cm).

Table 1. Patients' characteristics at diagnosis.

	(N = 128)
Age, median (IQR)	53.2 (42.3–63.1)
BMI, median (IQR)	23.4 (21.2–26.0)
Stage FIGO, n (%)	
I	20 (15.6)
II	10 (7.8)
III	88 (68.8)
IV	10 (7.8)
ECOG PS, n (%)	
0	80 (62.5)
1	4 (3.1)
Missing	44 (34.4)
CA 125 value, n (%)	
<35 U/ml	11 (8.6)
>35 U/ml	63 (49.2)
Missing	54 (42.2)
Primary debulking surgery, n (%)	
Yes	115 (89.8)
No	13 (10.2)
Interval debulking surgery, n (%)	
Yes	11 (8.6)
No	117 (91.4)
Residual Tumor, n (%)	
0	88 (68.7)
<1 cm	13 (10.2)
≥1 cm	25 (19.5)
Missing	2 (1.6)

BMI body mass index, ECOG PS Eastern Cooperative Oncology Group Performance Status, FIGO International Federation of Gynecology and Obstetrics, IQR Interquartile Range.

No fertility-sparing surgery with comprehensive staging was performed, and all patients with FIGO stage I ($n = 20$) underwent radical surgical staging.

114 patients received first line chemotherapy (Supplementary Appendix 2). Within the chemotherapy group, 103 patients (90.3%) received carboplatin intravenously every 3 weeks plus paclitaxel, 10 patients (8.8%) were treated with carboplatin monotherapy and only one patient, who refused hair losing therapies, was treated with carboplatin plus pegylated doxorubicin liposomal (PDL).

Thirty patients (26.3%) received bevacizumab in combination with first line chemotherapy and then as maintenance, and only 4 patients received hormone therapy as maintenance after first line platinum-based chemotherapy. Of these, 2 patients received tamoxifen and 2 women received letrozole.

Overall, 76 patients (66.7%) recurred and 19 (25%) underwent secondary cytoreductive surgery at the time of the first relapse, with 8 women (42.1%) completely debulked (Supplementary Appendix 3). Of the 76 patients with relapsed disease, treatment details are summarized in Supplementary Appendix 3: most of them (64.5%) received platinum-based chemotherapy at the time of first recurrence, followed by PDL monotherapy (18.4%) and topotecan (5.3%), according to platinum free interval (PFI) and based upon the treating physician's preference. Bevacizumab was administered in 16 patients (21.0%) in combination with second line platinum-based chemotherapy and as maintenance. Hormone therapy was administered in 23 patients (30.3%): 6 patients received it as active treatment and in 17 women hormone therapy

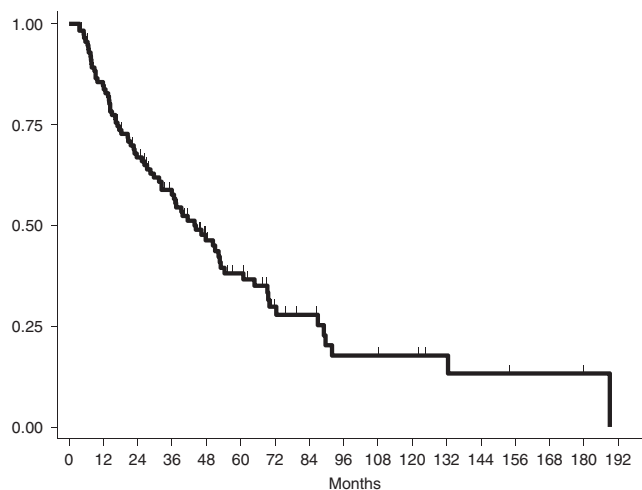


Fig. 1 Progression-Free Survival (PFS) of the overall population. The median PFS was 43.9 months (95% CI: 32.4–53.1).

was administered as maintenance treatment after second-line chemotherapy. Aromatase inhibitors were the most common drugs administered, with 11 patients (14.5%) receiving letrozole, 6 patients (8.6%) anastrozole, and 1 patient treated with exemestane.

Data on second recurrence and subsequent treatment details were available for 37 patients (Supplementary Appendix 4): most of patients (35.1%) received platinum-based chemotherapy, followed by weekly paclitaxel (18.9%) and PLD (10.8%). Hormone therapy was administered in 11 patients and 6 of them received letrozole.

Regarding the response evaluation using RECIST v1.1 criteria, in line with previous reported data [1], the objective response rate (ORR) to first line chemotherapy was of 23.7%, with 14 (12.3%) complete responses (CR) and 13 (11.4%) partial responses (PR). Stable disease (SD) was achieved in 4 patients (3.5%), with a CBR of 27.2%. Of the 30 patients receiving Bevacizumab in first line in combination with platinum-based chemotherapy and as maintenance, 22 underwent complete debulking surgery and 8 were evaluable for response, with 4 patients that achieved complete responses and 4 women that obtained partial responses.

Unexpectedly, a higher ORR of 33.7% was achieved in subsequent chemotherapy lines, with 11 (10.3%) complete responses and 25 (23.4%) partial responses. CBR was 59.9%, and 28 patients (26.2%) achieved a stable disease. Furthermore, in this disease setting we found a high rate of complete and partial responses among the 16 patients receiving Bevacizumab at the time of first recurrence. Thirteen (81.2%) women were evaluable for response, and we found 5 (31.2%) complete responses, 7 (43.7%) partial responses and 1 (6.2%) stable disease.

Response rate (RR) to hormone therapy was 8.5%, with only 2 complete responses achieved, instead CBR was 82.6% (Supplementary Appendix 5).

The survival analysis was performed on 114 patients. With a median follow up of 70.5 months (95% CI: 55.0–87.5), PFS was 43.9 months (95% CI: 32.4–53.1) (Fig. 1) and OS was 105.4 months (95% CI: 82.7–not reached) (Fig. 2).

In the multivariate Cox model (Supplementary Appendix 6) only residual tumor at the time of upfront surgery was confirmed as an independent prognostic factor for PFS, while age >50 years, primary debulking surgery and residual tumor were prognostic factors for OS.

Considering these findings, we evaluated separately the median PFS in patients completely debulked and in group of patients underwent optimal and suboptimal cytoreductive surgery, and we found a median PFS of 69.3 months (95% CI: 51.2–89.7) in the first group and a median PFS of 13.6 months (95% CI: 8.8–20.7),

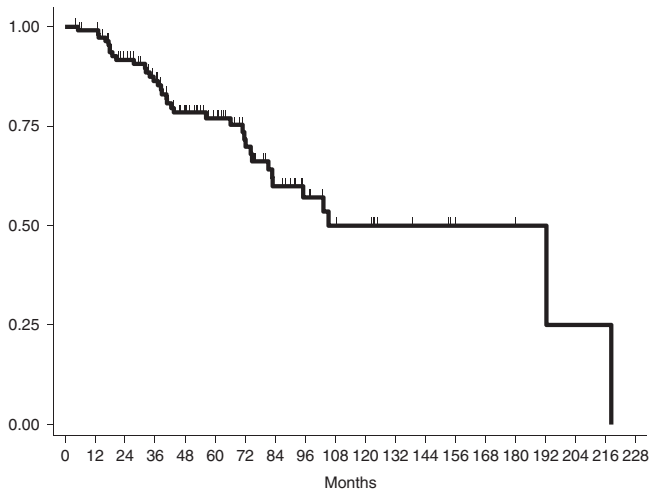


Fig. 2 Overall Survival (OS) of the overall population. The median OS was 105.4 months (95% CI: 82.7– not reached).

respectively. In addition, we assessed the PFS and OS in our patients after excluding the stage I women, and we found a median PFS of 39.2 months (95% CI: 28.5–52.3) and a median OS of 103.2 (95% CI: 81.3-not reached).

Molecular analysis

Seventy-nine of the 128 patients provided FFPE tumor: of these, 56 were considered suitable for FoundationOne CDX (Foundation Medicine®) tissue-testing and 23 failed at the mutational analysis. The reason given by Foundation Medicine® for sample failure were as follows: two specimens did not have adequate nucleated cellularity, three did not yield sufficient DNA and eighteen did not meet the minimum performance specifications in preparations for sequencing. Of the 56 patients who had the mutation analyses done, 53 (94.6%) were tissues deriving from primary tumor and 3 (5.4%) were tissues from recurrent tumor obtained at the time of secondary surgery. In this report we have focused on 45/56 patients harboring mutation of genes involved in HRR pathway, in MAPK pathway and in Endocrine-Resistance pathway.

A summary of mutations detected are listed in Table 2 and other details are summarized in Table 3.

The most common Foundation Medicine® mutations identified (including pathogenetic and variant of unknown significance) were: KRAS ($n = 12$, 21%), CDKN2A/B ($n = 11$, 20%), NRAS ($n = 8$, 14%), FANCA ($n = 8$, 14%), NF1 ($n = 7$, 13%), and BRAF ($n = 6$, 11%). Unexpectedly, pathogenetic BRCA1 ($n = 2$), BRCA2 ($n = 1$) and PALB2 ($n = 1$) mutations were found in some patients. Furthermore, about the genes involved in endocrine-resistance pathway, pathogenetic CDKN2A/B ($n = 11$), PIK3CA ($n = 2$) and AKT1 ($n = 1$) mutations were identified.

Among the 23 patients harboring mutation of genes involved in HRR-pathway, 18 women were evaluable for response: among them 3 CR (16.7%), 2 PR (11.1%) and 1 SD (5.6%) to the first line platinum-based chemotherapy were reported. Similarly, among 36 women with mutated genes of the MAPK- pathway, 30 women underwent first-line chemotherapy, of which 3 CR (10%), 5 PR (16.7%) and 1 SD (3.3%) were observed. In the group of 14 patients harboring mutation of genes involved in endocrine-resistance pathway, 1 patient had FIGO stage I and 13 women received first line chemotherapy: 3 PR (23.1%) and 2 SD (15.4%) were identified; no complete responses were recorded in this group.

Additionally, we evaluated response rate to subsequent chemotherapy lines for each group: in the HRR altered pathway group we observed 21.4% PR and 35% SD, in the MAPK altered pathway group 5% CR, 30% PR and 35% SD, and finally in endocrine-resistance altered pathway group 12.5% PR and 16.7% SD.

Table 2. Frequency of mutations in HRR-pathway, MAPK-pathway and Endocrine-Resistance pathway detected in 56 patients underwent molecular profile.

Type of mutations	N (%)	Pathogenetic, n	VUS, n
HRR- pathway			
ATR	3 (5)	0	3
BARD1	1 (2)	0	1
BRCA1	2 (4)	2	0
BRCA2	5 (9)	1	4
BRIP1	1 (2)	0	1
CHEK2	2 (4)	0	2
FANCA	8 (14)	0	8
FANCG	1 (2)	0	1
PALB2	4 (7)	1	3
RAD21	1 (2)	0	1
RAD51B	2 (4)	0	2
RAD52	1 (2)	0	1
MAPK- pathway			
BRAF	6 (11)	6	0
ERBB2	3 (5)	1	2
KRAS	12 (21)	12	0
NF1	7 (13)	4	3
NF2	2 (4)	1	1
NRAS	8 (14)	8	0
Endocrine Resistance-pathway			
AKT1	1 (2)	1	0
CDKN2A/B	11 (20)	11	0
ESR1	2 (4)	0	2
PIK3CA	3 (5)	2	1

Survival analysis was performed in each of these three groups. Since HRD is a widely recognized biomarker of platinum sensitivity, we evaluated survival outcomes in terms of PFS and OS, in the HRR-deficient group and HRR-proficient group, finding a trend of survival advantage in HRD-positive patients compared to HRD-negative patients. Specifically, the median PFS was 47.9 months (95% CI: 27.3-not reached) and 31.5 months (95% CI: 14.4–39.7) in HRD-positive and HRD-negative groups, respectively; the median OS was 74.2 months (95% CI: 66.2-not reached) in HRD-positive patients and 72.1 months (95% CI: 33.4-) in HRD-negative group. Moreover, the median PFS was 47.8 months (95% CI: 20.7–70.1) in MAPK-pathway group, and 31.5 months (95% CI: 14.5–50.4) in endocrine-resistance pathway group and the median OS was 82.7 months (95% CI: 56.3-not reached) in MAPK-pathway group and 72.1 months (95% CI: 42.5-not reached) in endocrine-resistance pathway group.

In addition, we evaluated response to hormone-therapy when administered in first, second and third treatment line in patients with mutated genes of the endocrine-resistance pathway. Of 14 patients harboring these mutations, only 7 received hormone-therapy and all showed SD as best response.

DISCUSSION

LGSC is a rare histological subtype of epithelial ovarian and peritoneal cancer. To date, few available data have highlighted the clinical aspects and the prognosis of this disease, and the most appropriate therapeutic approach has not yet been defined. In our series we describe the clinical outcome and the molecular aspects of a large cohort of LGSCs.

Table 3. Molecular details of 45/56 patients with molecular profiling.

ID	Age (Years)	Stage	Key variants	Type of alteration	MSI	TMB (Mutation/Mb)
LGSOC 1	69	III	NF1	Loss exons 13–29	MSS	3
LGSOC 2	65	III	KRAS; NF1 ^a	G12V P678T	MSS	1
LGSOC 3	36	III	KRAS	G12D	MSS	0
LGSOC 4	59	I	KRAS	G12V	MSS	1
LGSOC 5	28	III	ERBB2	A775_G776insYVM A	MSS	1
LGSOC 6	73	I	CHEK2 ^a ; BRAF	L363F V600E	MSS	0
LGSOC 7	58	I	RAD52 ^a ; KRAS	E288K G12D	MSS	1
LGSOC 8	26	IV	KRAS	Q61K	MSS	1
LGSOC 9	55	III	FANCA ^a ; PALB2	L1138V H762fs*8	N/A	N/A
LGSOC 10	58	III	RAD51B ^a	Amplification	MSS	1
LGSOC 11	53	III	FANCA ^a ; ERBB2 ^a ; NF2; CDKN2A/B	A746S amplification R57 ^a loss	MSS	1
LGSOC 12	36	III	BRAF	V600E	MSS	4
LGSOC 13	71	III	NF1	Loss exons 21–47	MSS	0
LGSOC 14	58	III	FANCG ^a ; RAD21 ^a ; ERBB2 ^a	N167S AMPLIFICATION Q57R	MSS	0
LGSOC 15	69	III	NRAS; CDKN2A/B	Q61R loss	MSS	0
LGSOC 16	54	III	NRAS	Q61R	MSS	6
LGSOC 17	32	III	BRAF	MKRN1-BRAF fusion	MSS	3
LGSOC 18	45	III	NFI	F443fs*30	N/A	N/A
LGSOC 19	66	III	RAD51B ^a ; KRAS	R159H G12D	MSS	3
LGSOC 20	43	III	BRCA1; BRAF CDKN2A/B	Loss exons 2–21 G596R loss	MSS	0
LGSOC 21	61	III	ATR ^a ; NRAS	R515H Q61R	MSS	1
LGSOC 22	59	III	BRIP1 ^a ; KRAS	P47A G12V	N/A	N/A
LGSOC 23	33	I	ATR ^a ; BRAF	E665K L485S	MSS	1
LGSOC 24	52	III	AKT1; ESR1 ^a	E114Q A59V	N/A	N/A
LGSOC 25	53	III	BARD1 ^a ; BRCA2 ^a ; PIK3CA ^a	Q730P E3002D amplification	MSS	6
LGSOC 26	65	III	NF1 ^a ; CDKN2A/B	N45S CDKN2A/B p16INK4a loss and p14ARF loss exons 2–3	MSS	1
LGSOC 27	52	III	FANCA ^a ; PALB2 ^a ; NRAS	L1143V L939W Q61R	MSS	0
LGSOC 28	68	III	NRAS	Q61R	MSS	1
LGSOC 29	67	III	NF1	S557fs*11	MSS	3
LGSOC 30	58	III	KRAS	Q61R	MSS	0
LGSOC 31	63	I	FANCA ^a ;	L1143V	MSS	1
LGSOC 32	39	III	CHEK2 ^a ; PALB2 ^a ; NRAS	L183F K957Q Q61R	MSS	1
LGSOC 33	60	II	NRAS; PIK3CA	Q61K N345K	MSS	8
LGSOC 34	26	III	CDKN2A/B	Loss	MSS	1
LGSOC 35	40	III	BRCA2; NF1 ^a	Splice site 632–2A>G V253del	MSS	1
LGSOC 36	68	III	FANCA ^a ; KRAS; CDKN2A/B	R1011C G12R loss	MSS	N/A
LGSOC 37	50	III	BRCA2 ^a ; KRAS; CDKN2A/B	I505T G12V p16INK4a loss and p14ARF loss exon 23	MSS	0
LGSOC 38	48	I	BRAF	V600E	MSS	0
LGSOC 39	75	III	PALB2 ^a ; NF2 ^a ; CDKN2A/B	Rearrangement I495V p16INK4a loss and p14ARF loss exon 23	MSS	0
LGSOC 40	33	I	KRAS	G12V	MSS	4
LGSOC 41	41	I	BRCA1; BRCA2 ^a FANCA ^a KRAS CDK2A/B PIK3CA	Rearrangement exon 10 G1771D H1000Q A146T Loss N345I M278I	MSS	0
LGSOC 42	80	III	NRAS CDKN2A	Q61R rearrangement intron 1	MSS	1
LGSOC 43	45	III	FANCA ^a ;	L1138V	N/A	6
LGSOC 44	37	III	ATR ^a ; CDKN2A/B	V1267I loss	MSS	1
LGSOC 45	25	III	BRCA2 ^a ; FANCA ^a ; ESR1 ^a	K3326 T126R H6Y	MSS	0

MSS Microsatellite stable, MSI Microsatellite instable, TMB tumor mutational burden.

^aVariant of Unknown Significance (VUS).

LGSCs are characterized by indolent course and published data showed better outcome of this disease with respect to HGSOCS. In this context, in their comparative analysis, Gockley et al. demonstrated a median OS of 90.8 months (95% CI = 78.7–106.3) and 40.7 months (95% CI = 40.08–41.5) in low-grade and high-grade ovarian cancers, respectively [2]. According to literature data demonstrating a longer OS in these patients [26], in our series we found a median OS of 105.3 months (95% CI = 82.7–not reached). On the other hand, in our population we found a longer PFS of 43.9 months (95% CI: 32.4–53.0) than that described in the literature, highlighting this finding a meaningful role of complete cytoreductive surgery on the survival outcome.

Interestingly, we found a higher ORR to standard chemotherapy than that reported in previous studies in the recurrent setting. Specifically, response rate to cytotoxic agents at the time of relapse (including second and third subsequent therapy lines) was 33%. These data are interesting and need to be interpreted with caution. Over the past decade, a series of publications have indicated that LGSC is less chemo sensitive than HGSOCS. Gershenson et al., reported an ORR of only 3.7% in a cohort of 58 patients with relapsed disease treated with conventional chemotherapy [4]. Additionally, in a study of 48 women with low-grade primary peritoneal cancer receiving chemotherapy, 66.7% of patients were noted to have persistent or progressive disease [27]. Furthermore, Schmeler et al., identified 25 women who received neoadjuvant chemotherapy, with ORR of only 4% [28]. Given these reports, some authors suggested that LGSCs are resistant to standard cytotoxic agents, and conventional chemotherapies should be abandoned, as result. In our view, this perspective is premature. Although LGSOCS is less chemotherapy sensitive respect to HGSOCS, it is not completely chemotherapy resistant. In the MILO study, the responses to chemotherapy were greater than in previous reported single institution retrospective series, with an ORR of 13% and a CBR of 73% [15]. On the other hand, we noted that in our analysis the majority of recurrent LGSOCS that achieved complete or partial response were platinum sensitive patients and received platinum-based chemotherapy plus bevacizumab at the time of relapse. Given the high rate of complete and partial responses that we found in the group of patients treated with Bevacizumab, these findings may justify the higher ORR reported in our series. Indeed, some authors suggested that bevacizumab have an antitumor activity in recurrent LGSC setting. Recently, Dalton et al., reported an ORR of 47.5% in a cohort of forty patients with recurrent low-grade serous disease treated with bevacizumab [29]. The same results were showed previously by Grisham et al., that reported an ORR of 45% among 17 serous borderline and LGSCs treated with bevacizumab alone or in conjunction with chemotherapy at the time or recurrence [30].

Another important issue to investigate is response rate to hormone therapy. The benefit of hormone therapy in LGSOCS have been widely demonstrated and our findings reinforce this issue. Gershenson et al., evaluated the activity of hormone therapy in recurrent LGSOCS showing an ORR of 9% and a CBR of 82.7% [19]. Overlapping results were found in our cohort of patients with an ORR of 8% and a CBR of 82.6%. Data on immunohistochemical evaluation of ER and PG receptors are not available in our report and may be a focus for future research.

Regarding the genomic analysis, it is known that precision medicine is growing enormously in the management of many types of cancers. The detection of “oncogenic driver” mutations and the use of targeted therapy has dramatically improved prognosis of some tumors. Due to relatively reported chemoresistance of LGSC, in the last few years growing attention has been dedicated to its molecular landscape, and published data showed that these patients often carry mutations of genes involved in MAPK-pathway [31]. These findings led to evaluate the activity of MEK inhibitors in several prospective clinical trials with conflicting

results reported. In the MILO trial, binimetinib was evaluated in persistent and recurrent LGSCs and no significant difference in the primary endpoint of PFS versus physician’s choice chemotherapy was achieved [5]. Contrary, the prospective randomized trial GOG 281 demonstrated statistically significant superiority of trametinib when compared to standard of care in terms of PFS. In addition, a post hoc analyses of the two clinical trials demonstrated that the presence of the MAPK-pathway mutation appears to be a predictive biomarker [31]. Indeed, patients with recurrent LGSOCS harboring a KRAS mutation and treated with binimetinib displayed a longer PFS (median 17.7 vs 10.8 months) and were 3.4 times more likely to have a complete or partial radiographic response to treatment when compared to those that were KRAS wild type [32]. A similar trend was found for patients treated with binimetinib harboring any MAPK mutation (ORR of 41% in those with binimetinib versus 13% in those without) [32].

In GOG 281 trial, the same treatment effect was observed. Patients harboring activating mutations in KRAS, BRAF, or NRAS (HR 0.55 [95% CI 0.28–1.07]) respect to mutation-negative patients (0.64 [0.39–1.03]) [14] had a PFS benefit with trametinib treatment.

On the contrary, in a phase II prospective trial, Farley et al., demonstrated that Selumetinib is a well-tolerated and an active treatment in recurrent LGSOCS with PFS > 6 months achieved in 63% of patients but response to this MEK inhibitor did not appear to be related with RAS/RAF mutational status [16]. However, these results should be interpreted with caution, because the analysis was carried out on a small sample and despite the molecular assessment in most patients (82%) was made on the primary tissue tumor, the concordance of BRAF or KRAS mutational activation between primary and recurrent/metastatic disease has not been adequately studied [16].

These published results are appealing suggesting that molecular subtyping of LGSOCS still remains an open field for research.

In our series, further to the more typical mutation related to LGSOCS, we found mutations of genes involved in HRR- pathway (ATR, BARD1, BRCA 1 /2, BRIP1, CHEK2, FANCA, FANCG, PLAB2, RAD21, RAD51B and RAD52) with two cases of BRCA1, one case of BRCA2, and one case of PALB2 pathogenetic mutations. In a recently published retrospective analysis evaluating the mutational spectra of 17 LGSOCS, several variants in multiple HR pathway genes were observed, including five BRCA2 variants, with two being confirmed to be of germline origin [22]. These findings could suggest that heterogeneity is strong in patients with LGSOCS identified on a morphological base, and that some mutations identify patients that could benefit from chemo or PARP inhibitors; this hypothesis should be tested in prospective trials.

Besides, in our series we found that 20% of patients ($n = 11$) harbored CDKN2A/B mutations and that patients with mutations of genes involving in hormone resistance pathway (including CDKN2A/B, AKT1, ESR1 and PIK3CA) showed a shorter PFS of 31.5 months (95% CI; 4.5; 50.4) and a shorter OS of 72.1 months (95% CI 42.5-not reached) compared to patients harboring mutations of genes involved in the other two pathways evaluated. This finding highlights the previous reported data suggesting that CDKN2A/B aberrations are enriched in OC cases with shorter survival [33] and potential drug target of CDKN2A could represent a promising avenue for therapeutic intervention to improve outcomes for these patients.

In our study, due to the small numbers, we failed to correlate the mutational landscape with the response to chemo and hormone therapy. Of course, the study suffers of the intrinsic limitation related to the retrospective nature of the data collection. However, to our knowledge, this is the largest genomic study of this rare disease reported, analyzing 324 genes. International collaboration should be undertaken to verify if a better molecular classification of LGSOCS might help in guiding patients toward chemotherapy, hormone therapy of other target-based drugs.

DATA AVAILABILITY

The datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

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ACKNOWLEDGEMENTS

The authors are grateful to Gelsomina Iovane, Margherita Tambaro and Angela Maria Trujillo for data management.

AUTHOR CONTRIBUTIONS

SP conceived the study; LM, DC and SP contributed to methodology; All authors collected the data; DC, GFZ, NSL were responsible for the assessment of the primary FFPE tumor tissue, including the estimation of tumor cellularity; LM and LA interpreted and analyzed the data; LM wrote the manuscript; LA, LM, DC and SP played a key role in interpreting the results and revised the article critically for important intellectual content; SP supervised the project and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved; All authors reviewed and approved the final version of the manuscript.

FUNDING

A specific funding was received for this work. The research leading to these results has received funding from AIRC under IG 2016 – ID. 18921 and IG 2021 – ID. 25932 projects – P.I. SP and CO-2018–12367051 (Ministero della Salute) P.I. SP; Ricerca Corrente grant M2/7 from Ministero della Salute to DC, Ricerca Corrente from Ministero della Salute to SP.

COMPETING INTERESTS

DL reports research funding from Clovis, GSK and MSD, personal interests with AstraZeneca, Clovis Oncology, GSK, Pharmamar, MSD and financial interests with Clovis, Genmab, GSK, MSD. Board of Directors, GCGI (Gynecologic Cancer Inter Group). FR reports honoraria from GSK, Pharmamar, Clovis, MSD and Roche. VS

reports honoraria from GSK, PharmaMar, Roche, MSD, Eisai, Clovis, Oncology, AstraZeneca. FP reports honoraria for educational and advisory activity from Incyte, GSK, Eli Lilly, Ipsen, Astellas, AstraZeneca, Roche, BMS, Bayer, Clovis, Pierre Fabre and grants for clinical trials to his institution from Roche, AstraZeneca, Pfizer, MSD, Bayer, Incyte Taiho, Janssen, Exelixis, Ailenor, Daiichi Sankyo. GS reports research support from MSD and honoraria from Clovis Oncology. Consultant for Tesaro and Johnson & Johnson. SP reports honoraria from AstraZeneca, MSD, Roche, Pfizer, Clovis, GSK, Pharmamar and research funding from MSD, Roche, AstraZeneca and Pfizer. LM, DC, MB, LA, NSL, GC, SG, GV, CP, AS, DR, MDS, VC, FF, GFZ, VL, VG, CC, VT, MD, VDV, SS and DP have nothing to disclose.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the ethics committee of the National Cancer Institute of Naples (22/14 OSS) and the ethics committees of the participating centers, and it was

conducted in accordance with the Declaration of Helsinki. The study participants gave written informed consent.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41416-022-01897-1>.

Correspondence and requests for materials should be addressed to Sandro Pignata.

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