IMMUNOHISTOCHEMICAL AND MOLECULAR GENETIC PROFILING IN DETERMINING PATHOGENETIC VARIANTS OF MALIGNANT EPITHELIAL OVARIAN TUMORS

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INTRODUCTION

Ovarian cancer (OC) is one of the most aggressive and lethal malignant neoplasms of the female reproductive system. Its asymptomatic course in early stages and frequent diagnosis at advanced stages contribute to poor clinical outcomes. The 5-year survival rate for patients diagnosed at an advanced stage is approximately 29.2% [1]. Furthermore, 70–90% of patients with advanced OC experience disease recurrence within 18 months of initial diagnosis [2].

Despite significant advances in treatment, including contemporary surgical techniques and systemic therapies, overall survival rates remain low. This underscores the urgent need to improve personalized therapeutic strategies for OC.

Histologically, OC comprises several morphologically and biologically distinct subtypes. The most common include high-grade serous carcinoma (HGSC), endometrioid carcinoma (ENOC), clear cell carcinoma (CCC), and low-grade serous carcinoma (LGSC). Each subtype is characterized by unique molecular and genetic features that influence its biological behavior, treatment sensitivity, and prognosis [3–6].

Immunohistochemical markers, such as WT-1 (Wilms Tumor Protein), p53, Napsin A, and progesterone receptors (PR) [7–9], together with molecular genetic testing (e.g., mutations in TP53, BRCA1/2, RAD51C, KRAS, CDK12, and PIK3CA), are increasingly critical for accurate diagnosis, risk stratification, and the selection of optimal therapeutic approaches.

In particular, assessing the status of BRCA1/2 and other defects in the homologous recombination repair (HRR) system is essential for determining eligibility for PARP (poly (ADP-ribose) polymerase) inhibitors and other targeted therapies, which have significantly improved outcomes in selected patient subgroups [10, 11].

Integrating comprehensive molecular profiling into routine clinical practice marks a shift toward personalized medicine, where treatment plans are tailored to the tumor's unique biological characteristics. Therefore, incorporating immunohistochemical and molecular-genetic analyses into standard clinical workflows represents a crucial step in enhancing the diagnosis and treatment of malignant ovarian tumors.

Objective of the study: to investigate immunohistochemical and molecular markers in tumor samples representing various pathomorphological types of OC, and to evaluate their diagnostic significance and potential role in guiding optimal personalized treatment strategies.

MATERIALS AND METHODS

A retrospective analysis was conducted on tumor samples from 37 patients diagnosed with OC who received inpatient and outpatient care at the Lviv Oncological Regional Treatment and Diagnostic Center between 2020 and 2024.

Inclusion criteria were as follows: age ≥18 years, histopathologically confirmed diagnosis of OC, availability of high-quality biopsy material, and an overall functional status of ECOG 0 or 1, as assessed by the Eastern Cooperative Oncology Group (ECOG) scale. ECOG 0 indicated full functional capacity with no restrictions in daily activity, while ECOG 1 reflected mild disease-related symptoms with preserved ability to walk and perform light work. Within the study cohort, 14 patients (37.8%) had an ECOG performance status of 0, and 23 patients (62.2%) had an ECOG of 1.

The mean age was 60.7 ± 0.9 years, ranging from 45 to 77. All patients underwent diagnostic assessment and received treatment following institutional protocols.

Diagnostic laparoscopic restaging was performed for all patients, including assessment of the peritoneal carcinomatosis index (PCI). Tumor samples were obtained via the following methods (Table 1):

- primary cytoreductive surgery: 18 samples (48.6%);
- biopsy of intraperitoneal metastatic lesions: 15 samples (40.5%);
- trephine biopsy of supraclavicular lymph node metastases: 4 samples (10.9%).

Initial histological analysis was performed using standard hematoxylin and eosin (H&E) staining in the histopathology laboratory of the Lviv Oncological Regional Treatment and Diagnostic Center.

Tumor samples obtained during primary cytoreduction, laparoscopic surgery, or trephine biopsy of distant metastatic lesions were sub-

I.R. HRYTSAY
oncologist of chemotherapy, Municipal Non-Profit Enterprise of the Lviv Regional Council "Lviv Oncological Regional Treatment and Diagnostic Center", postgraduate student of the Department of Oncology and Radiology, Danylo Halytsky Lviv National Medical University, Lviv
ORCID 0009-0007-0751-6981

O.A. PETRONCHAK
pathologist, "Western Ukrainian Histological Laboratory", Lviv
ORCID 0000-0001-7703-3036

N.A. VOLODKO
MD, professor, head of the Department of Oncology and Radiology, Danylo Halytsky Lviv National Medical University, gynecological oncologist at the Gynecological Department No. 1, Municipal Non-Profit Enterprise of the Lviv Regional Council "Lviv Oncological Regional Treatment and Diagnostic Center", Lviv
ORCID 0000-0002-4478-5554

Contacts:
Hrytsay Iryna Romanivna
Municipal Non-Profit Enterprise of the Lviv Regional Council "Lviv Oncological Regional Treatment and Diagnostic Center"
Lviv, Yaroslava Hasheka St., 2a
Tel: +38(096)-228-42-77
E-mail: hrytsay0204@gmail.com

Table 1. Clinical features of patients in the study group

Feature	Subset	Patients, n (%)
ECOG	0	14 (37.8%)
	1	23 (62.2%)
Studied material	Primary tumor Metastasis Distant metastasis	18 (48.6%) 15 (40.5%) 4 (10.9%)
Histological subtype	HGSC	19 (51.4%)
	ENOC	7 (18.9%)
	CCC	7 (18.9%)
	Unclassified tumors	4 (10.8%)
Disease stage	IIIC	30 (81.1 %)
	IV	7 (18.9%)
Treatment received	NACT + surgery + ACT	16 (43.2%)
	Surgery + ACT	20 (54.1,5%)
	PCT	1 (2.7%)

Notes: ACT – adjuvant chemotherapy, NACT – neoadjuvant chemotherapy, PCT – polychemotherapy

mitted to the Western Ukrainian Histological Laboratory (Lviv, Ukraine) for immunohistochemical evaluation. Upon receipt, a pathomorphologist assessed the quality of the tissue specimens, followed by the preparation of additional sections from paraffin-embedded blocks for microscopic analysis and confirmation of the morphological subtype of OC.

Immunohistochemical (IHC) analysis was conducted using the Vitro Master Diagnostica system. A tailored panel of primary monoclonal antibodies was selected based on the histological subtype of OC and the clinical relevance of the biomarkers for diagnosis, prognosis, and treatment planning [12–14]. The antibody panel included:

- WT-1 (Clone 6F-H2, Master Diagnóstica): mouse monoclonal antibodies targeting WT1 protein, a key marker for the serous subtype of OC;
- p53 (Clone SP5, Master Diagnóstica): rabbit monoclonal antibodies used to detect p53 protein expression. Aberrant staining patterns are indicative of TP53 mutations, commonly associated with HGSC;
- Progesterone receptor (Clone 16, Leica Biosystems): BOND™ monoclonal antibodies for detecting PR expression, which has prognostic implications and may influence therapeutic decision-making;
- Napsin A (Clone IP64, Leica Biosystems): mouse monoclonal antibodies used for identifying Napsin A expression, a marker particularly relevant for differentiating CCC from other epithelial OC subtypes.

IHC results were evaluated semiquantitatively across at least 10 high-power fields (magnification × 400). A positive reaction was defined by the presence of brown cytoplasmic or nuclear staining in tumor cells, indicating moderate to strong marker-specific expression.

The final diagnosis was established independently by two experienced pathologists, in line with the latest WHO classification of female genital tumors [15–17].

Following the immunohistochemical confirmation of tumor subtype, molecular genetic profiling was performed using next-generation sequencing (NGS) on the Illumina platform (USA). An extended gene panel was employed to detect both somatic and germline mutations. The panel included homologous recombination repair (HRR) genes; TP53 (entire coding region and exon-intron boundaries); common mutation "hot spots" in BRAF, ERBB2, KRAS, NRAS, and PIK3CA [18].

Genetic variants were interpreted in line with international guidelines from the American College of Medical Genetics and Genomics (ACMG), the ENIGMA Consortium, and the Association for Clinical Genomic Science (ACGS) [19].

Data were processed using Microsoft Excel and Statistica 12 (StatSoft, USA; license: AGAR 909 E415822FA). Statistical analysis included descriptive statistics (mean ± standard error) and non-parametric testing using the Pearson chi-square (χ^2) test to assess differences between groups. All results were presented as arithmetic means (M) with standard errors (M ± m).

The study design was reviewed and approved by the Bioethics Commission of the Danylo Halytsky Lviv National Medical University (protocol № 8 dated October 12/2022). All patients provided written informed consent to participate in the study.

RESULTS

The results of marker expression obtained through immunohistochemical analysis of epithelial ovarian tumor samples, previously evaluated histologically, are summarized in Table 2.

In serous carcinomas (n = 24), a high expression level of WT-1 was observed in 83.3% of cases, alongside aberrant p53 expression in 54.2% of samples. These findings reflect disruptions in cell cycle regulation and DNA repair mechanisms, which are commonly associated with aggressive tumor behavior and poorer prognosis in ovarian cancer [20].

PR expression was positive in 54.2% of serous carcinoma cases, suggesting a potential responsiveness to hormone therapy.

Table 2. Immunohistochemical panel for determining morphopathogenetic types of malignant epithelial tumors

Carcinomas	WT-1	p53abnormal	p53normal	PR	Napsin A
Serous carcinomas (n = 24)	83.3% (n = 20)	54.2% (n = 13)	45.8% (n = 11)	54.2% (n = 13)	-
Endometrioid carcinomas (n=7)	14.3% (n = 1)	14.3% (n = 1)	85.7% (n = 6)	85.7% (n = 6)	-
Clear cell carcinomas (n=6)	-	-	-	-	100% (n = 6)

In contrast, endometrioid carcinomas (n = 7) exhibited a distinct marker profile: only 14.3% of samples were positive for WT-1 and showed abnormal p53 expression, whereas 85.7% retained normal p53 expression and demonstrated PR positivity, which may have important implications for personalized treatment strategies.

The clear cell carcinoma group displayed a classic immunohistochemical profile, with all cases demonstrating positive expression of Napsin A, while consistently negative for WT-1 and PR, and maintaining wild-type p53 expression.

Representative microscopic images of immunohistochemical staining using the analyzed marker panel illustrate both the intensity and absence of marker expression in tumor cells (Figures 1–4).

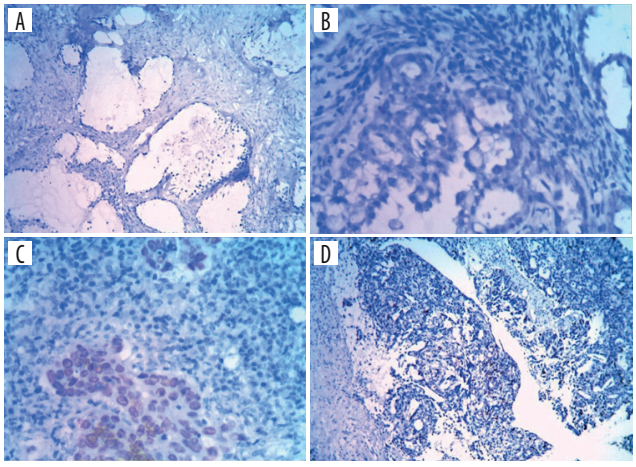

Figure 1. Immunohistochemical expression patterns of p53 in various morphopathogenetic subtypes of OC
A – HGSC: complete absence of p53 expression (null/"zero" pattern) (magnification × 10);
B – CCC: wild-type p53 expression pattern (magnification × 10 and × 40);
C – HGSC: p53 overexpression with intense nuclear staining in > 80% of tumor cells (magnification ×40);
D – ENOC: wild-type p53 expression pattern (magnification × 10 and × 40).

The diagnostic value of IHC analysis was particularly evident in cases of unclassified tumors lacking an identified primary site. In this subgroup, diagnoses had previously been based solely on histological features. While the histogenesis of these disseminated tumors could not initially be established, applying the IHC panel enabled a definitive determination of the clinical and pathomorphological subtype of OC in 100% of cases (Table 3).

In two patients, an initial histological diagnosis of HGSC was revised to LGSC based on IHC results: WT-1(+), PR 90%, normal p53 expression, and Napsin A(–) [21]. This reclassification was further supported by molecular genetic profiling, which revealed a KRAS mutation, consistent with LGSC [22].

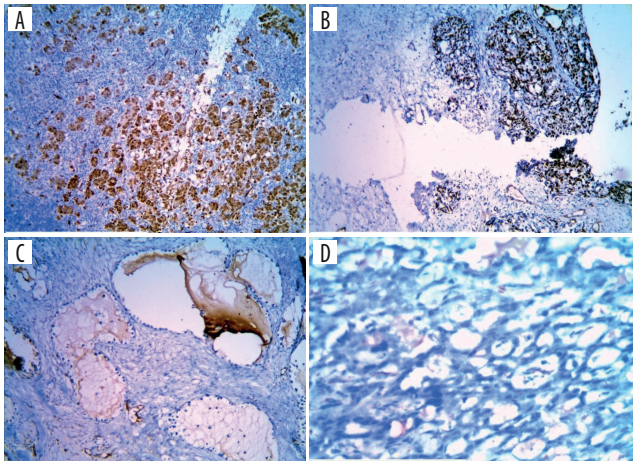

Figure 2. Immunohistochemical expression patterns of WT-1 in various morphopathogenetic subtypes of OC
A – LGSC: positive nuclear WT-1 expression (magnification × 10);
B – HGSC: positive nuclear WT-1 expression (magnification × 10);
C, D – CCC: negative WT-1 expression in tumor cells (magnification × 10 and × 40).

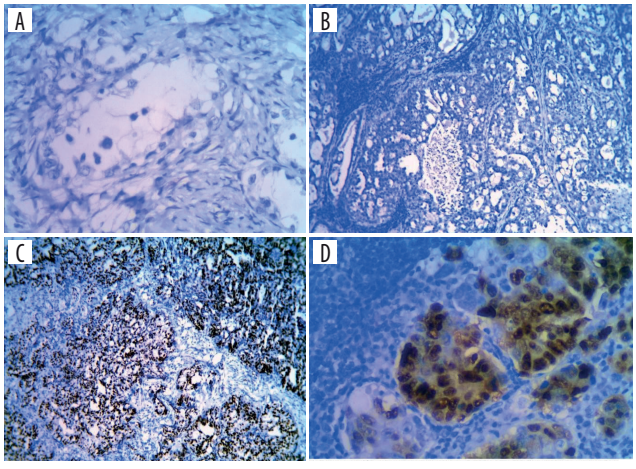

Figure 3. Immunohistochemical expression of PR in various morphopathogenetic subtypes of OC
A – CCC: negative PR expression (magnification × 40);
B – HGSC: negative PR expression (magnification × 10);
C – ENOC: strong to moderate nuclear PR positivity in approximately 85% of tumor cells (magnification × 10);
D – LGSC: strong to moderate nuclear PR positivity in approximately 80% of tumor cells (magnification × 40).

In another case, a tumor initially diagnosed as HGSC was reclassified as ENOC after IHC analysis demonstrated WT-1 negativity and strong PR positivity (+++).

Molecular genetic profiling across different OC subtypes revealed the presence of various mutations, including those in HRR pathway genes. The distribution of identified

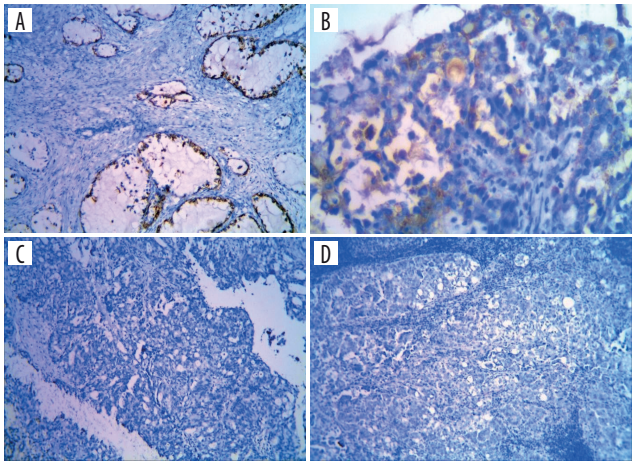

Figure 4. Immunohistochemical expression of Napsin A in various morphopathogenetic subtypes of OC
A, B – CCC: positive cytoplasmic staining for Napsin A in tumor cells (magnification × 10 and × 40);
C – ENOC: negative Napsin A expression in tumor cells (magnification × 10);
D – HGSC: negative Napsin A expression in tumor cells (magnification × 10).

Table 3. Comparative characteristics of morphological and immunohistochemical features of tumors, n (%)

Diagnostic method / OC morphotype	Histological study	Immunohistochemical study
HGSC	19 (51.4%)	21 (56.8%)
ENOC	7 (18.9%)	7 (18.9%)
CCC	7 (18.9%)	6 (16.2%)
LGSC	0	3 (8.1%)
MC	0	0
Unclassified tumors	4 (10.8%)	0

genetic alterations in HGSC, ENOC, and CCC is illustrated in Figures 5–7.

An additional analysis was conducted to compare the results of IHC evaluation of p53 protein expression with molecular genetic findings on TP53 gene mutations in the group of patients diagnosed with HGSC (Table 4). Aberrant p53 expression, either complete loss or strong diffuse nuclear staining, is expected to correlate with the presence of a TP53 gene mutation.

TP53 gene mutations were identified in 11 cases (52.4%) using NGS, while aberrant p53 protein expression was observed in 14 cases (66.7%) via IHC analysis. Among the 11 mutation-positive cases, 9 (81.8%) also exhibited abnormal p53 staining, suggesting a generally strong, but not absolute, correlation between TP53 mutation status and p53 protein expression patterns.

Notably, in 2 cases with TP53 mutations, p53 expression remained within normal limits, indicating that not all genetic alterations in TP53 lead to detectable protein-level changes on IHC. Conversely, in 5 cases (50%) without a TP53 mutation,

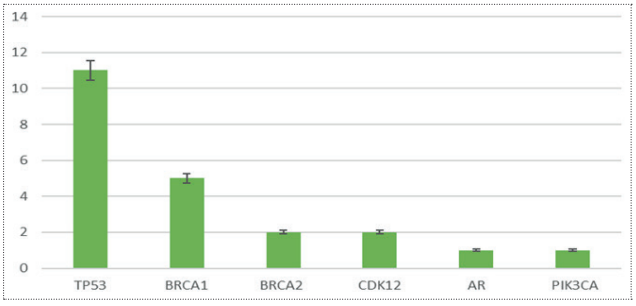

Figure 5. Distribution of detected genetic mutations in HGSC cases (n = 21)

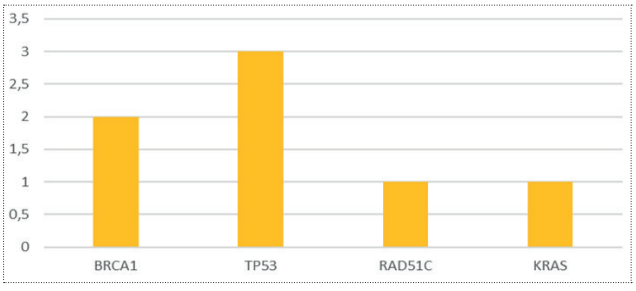

Figure 6. Distribution of detected genetic mutations in ENOC cases (n = 7)

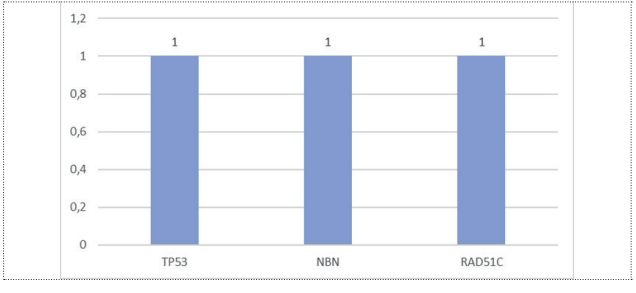

Figure 7. Distribution of detected genetic mutations in CCC cases (n = 6)

aberrant p53 expression was observed, which may reflect alternative mechanisms of p53 dysregulation, such as epigenetic changes or alterations in upstream regulatory pathways, or may highlight limitations in the sensitivity of the molecular testing platform in detecting certain mutation types.

DISCUSSION

OC is currently understood to encompass five histogenetically distinct malignant neoplasms: HGSC, LGSC, ENOC, MC, and CCC. These subtypes differ clearly in their cellular origin, molecular profile, clinical behavior, and therapeutic response, yet they have traditionally been grouped as a single disease entity.

While histological verification remains the global standard at the time of initial diagnosis, histology alone is often insufficient in clinical practice. Due to the pronounced heterogeneity and distinct mutational landscape of OC, an integrated diagnostic

Table 4. Characteristics of TP53 gene and p53 protein alterations in the HGSC subtype, n (%)

Molecular profiling of the TP53 gene	IHC p-53 normal wild-type	IHC p53 abnormal / aberrant / mutation-type (no expression)	IHC p53 abnormal / aberrant / mutation-type (hyperexpression)
Mutation, n = 11	2 (18.2%)	7 (63.6%)	2 (18.2%)
No mutation, n = 10	5 (50%)	3 (30%)	2 (20%)
Total	7	10	4

approach is required to ensure accurate classification and guide personalized therapy.

In this study, we applied a multimodal diagnostic strategy consisting of:

1. Histological subclassification based on morphological features.
2. Verification of subtype using an extended IHC panel (WT-1, p53, Napsin A, PR).
3. Molecular profiling, including assessment of HRD and TP53 mutational status [23].

The IHC findings revealed distinct expression patterns across OC subtypes, supporting its high diagnostic value in refining morphological subtype classification and in identifying the origin of tumors previously categorized as unclassified.

Among patients with HGSC, TP53 mutations were detected in 52.4% of cases, which, although lower than international estimates of up to 90%, remains consistent with its established role in HGSC carcinogenesis. The utility of IHC analysis of p53 as a molecular surrogate for TP53 mutations has been supported by prior studies, including Köbel et al. (2016) [24].

The binary IHC scoring system has proven effective in correlating abnormal p53 expression (p53abn) with TP53 mutations. This pattern includes:

- strong nuclear staining in > 80% of tumor cells (overexpression);
- complete absence of staining (null / "zero" pattern);
- cytoplasmic staining without nuclear expression.

Conversely, heterogeneous (wild-type) p53 expression typically correlates with wild-type TP53, as also described by M.H. Chui et al. (2021) [16].

In our cohort, the partial discrepancy between TP53 mutations and abnormal p53 expression may be attributed to:

1. Post-translational modifications affecting p53 stability, localization, or function.
2. Pathological protein interactions altering p53 behavior in the absence of direct gene mutations.
3. Activation of alternative signaling pathways that dysregulate p53 activity.
4. Mutations in other cell cycle regulators (e.g., MDM2, CDKN2A) that indirectly modulate p53.

These findings reinforce the central role of the p53 pathway in the pathogenesis of HGSC and highlight the importance of integrated histological, immunohistochemical, and molecular diagnostics for optimal patient stratification and therapy planning.

Interestingly, the frequency of PR expression in HGSC in our study (54%) was higher than that reported by Hongyi Li et al., 2021 [25]. This observation may require further investigation, as it could affect hormonal therapy responsiveness in a subset of HGSC patients.

In summary, deep molecular and immunohistochemical profiling not only enhances diagnostic accuracy but also facilitates the implementation of personalized therapy, including the use of targeted agents. Continued research and integration of emerging biomarkers are essential to further refine treatment strategies in OC.

CONCLUSIONS

1. A multimodal diagnostic approach combining histological assessment, immunohistochemical profiling, and molecular genetic testing significantly improves the accuracy of OC subtype identification. This is particularly important for differentiating between HGSC and LGSC, as well as for correctly identifying endometrioid and clear cell carcinomas – distinctions that directly influence treatment decisions.
2. Immunohistochemical analysis of p53 using a binary scoring system provides high sensitivity and specificity in predicting TP53 mutations, a hallmark of HGSC. However, discrepancies between TP53 mutational status and p53 protein expression underline the relevance of alternative regulatory mechanisms, such as post-translational modifications and interactions with other signaling pathways, in influencing p53 behavior.
3. The detection of mutations in TP53, BRCA1/2, RAD51C, KRAS, CDK12, and other key genes supports personalized therapy planning. In particular, identifying HRD allows for the targeted use of PARP inhibitors, improving response rates and patient prognosis across multiple OC subtypes.
4. The integration of histological, immunohistochemical, and molecular diagnostics not only enhances the accuracy of OC classification but also opens the door to individualized treatment strategies. This approach holds promise for improving outcomes and minimizing recurrence. Ongoing research will further expand the landscape of targeted therapies and support the development of novel biomarkers, ultimately refining and optimizing the care of patients with OC.

Conflict of interest.
There is no conflict of interest.