Journal of Biological Regulators and Homeostatic Agents 2025, 39(2), 3392.
https://doi.org/10.54517/jbrha3392

Article

Integration of clinical data with results from next-generation sequencing technology used to detect somatic and germline variants in patients with high-grade serous ovarian cancer

Patrycja Aleksandra Bukłaho[1,*], Joanna Kiśluk[1], Witold Bauer[2], Jacek Nikliński[1]

[1] Department of Clinical Molecular Biology, Medical University of Bialystok, 15-089 Bialystok, Poland
[2] Clinical Research Centre, Medical University of Bialystok, 15-089 Bialystok, Poland
*** Corresponding author:** Patrycja Aleksandra Bukłaho, patrycja.buklaho@sd.umb.edu.pl

CITATION

Bukłaho PA, Kiśluk J, Bauer W, Nikliński J. Integration of clinical data with results from next-generation sequencing technology used to detect somatic and germline variants in patients with high-grade serous ovarian cancer. Journal of Biological Regulators and Homeostatic Agents. 2025; 39(2): 3292.
https://doi.org/10.54517/jbrha3292

ARTICLE INFO

Received: 5 March 2025
Accepted: 13 March 2025
Available online: 14 April 2025

Abstract: Ovarian cancer represents a significant global health issue, posing a significant challenge for the field of medicine. One strategy to improve the prognosis for women around the world is to implement modern technologies and apply the concept of personalized medicine. This study employed an in-depth case study of patients diagnosed with high-grade serous ovarian cancer. Advanced genetic testing methods and a comprehensive review of the patient's medical history were utilized to identify new potential markers for early detection of the disease and eligibility for treatment. In this study, next-generation sequencing (NGS) technology was utilized for analysis. Tissue panel tests were performed to detect somatic mutations, while whole exome sequencing (WES) was conducted on blood samples to detect germline mutations. The results obtained were then analyzed in the context of the patient's medical history to identify patients with a familial predisposition to cancer and to look for an association with comorbidities. The utilization of genetic testing and the analysis of patients' medical histories facilitated the identification of somatic and germline variants in genes associated with carcinogenesis. This approach led to the identification of ovarian cancer-specific and novel variants. Furthermore, germline variants associated with comorbidities were identified. The utilization of contemporary molecular biology methodologies can markedly enhance the diagnostic accuracy of patients and allow the development of new diagnostic tests.

Keywords: ovarian cancer; HGSOC; NGS; WES; personalized medicine

1. Introduction

The incidence and mortality rates of cancer are rising at an alarming rate on a global scale. Furthermore, cancer is the leading cause of death worldwide [1]. In terms of ovarian cancer (OC), it is the seventh most common malignant neoplasm in women and the eighth leading cause of mortality [2]. In 2020 OC was the third most prevalent and most lethal gynaecological cancer globally [3,4]. It is also important to note that up to 75% of these cancers are diagnosed at an advanced stage, resulting in relatively low 5-year survival rates of 40% and 20% in stage III and IV, respectively [5]. The most frequently occurring subtype of OC is epithelial ovarian cancer (EOC), which accounts for up to 95% of all cases [6]. EOC is a heterogeneous group comprising five histological subtypes: high-grade serous ovarian carcinoma (HGSOC), low-grade serous carcinoma (LGSC), endometrioid carcinoma (EC), clear cell carcinoma (CCC) and mucinous carcinoma (MC) [5]. HGSOC is the most commonly diagnosed histological subtype of ovarian cancer, accounting for 70% of all EOC cases [6]. HGSOC is undoubtedly a significant issue in women's health. This cancer is

distinguished by its highest degree of aggressiveness among OC subtypes [7]. Additionally, a significant proportion of patients develop chemoresistance, despite an initial response to treatment [8]. The differences in the course of the disease and the question of whether the therapy used will be successful in women with HGSOC can be attributed to the significant heterogeneity of this type of cancer due to differences at the molecular level [9]. An estimated 50% of HGSOC tumors have features of homologous recombination deficiency (HRD), including BRCA1/2 mutations. The detection of these genetic alterations in a patient's tumor tissue allows the use of a molecularly targeted treatment—a poly ADP-ribose polymerase inhibitor (PARPi) [10].

The technology that makes such genetic testing possible is next-generation sequencing (NGS). In the era of personalized medicine, NGS is playing a major role in oncology, allowing the most effective therapy to be selected for each patient, as well as testing family members and identifying high-risk individuals [11]. The extensive scope of analysis enabled by NGS technology allows for the distinction between targeted sequencing (TS) and whole-exome sequencing (WES). TS encompasses a panel of genes whose regions associated with a particular disease entity are assessed in a single analysis. In contrast, WES permits the analysis of all protein-coding fragments, i.e., exons in all human genes known to date [12]. In an era such as that of personalized medicine, it is of the utmost importance to adopt an individual approach to each case with the same clinical diagnosis. The conceptual foundation of the study entailed an exhaustive examination of cases of HGSOC, encompassing advanced genetic testing utilizing NGS technology, with the integration of the resulting data with the patient's comprehensive medical history. Panel tests for tumor tissue and WES analysis for blood were performed, allowing the detection of somatic and germline variants. Preliminary analysis of the information contained in the patients' medical histories identified four groups of comorbidities, namely obesity, cardiovascular diseases, autoimmune diseases, and biliary tract diseases, which were most common in the study group. The objective of the present study was twofold: Firstly, to assess familial hereditary predisposition to cancer; and secondly, to attempt to establish a molecular link between cancer and comorbidities. WES was selected for the identification of germline variants due to its comprehensive ability to analyze all protein-coding regions, offering a broader scope than standard germline testing. WES provides critical insights into potentially actionable mutations that might not be detected through targeted gene panels. This broader view can guide treatment decisions earlier, identify hereditary mutations before they manifest clinically, and detect variants related to drug resistance or other non-cancer-related conditions, which could significantly impact therapeutic outcomes.

2. Materials and methods

2.1. Patients

The study cohort comprised 20 patients with HGSOC. Prior to the commencement of the study, informed consent was obtained from all patients, a medical history was taken, and data were pseudonymized. The biological material was biobanked at the Biobank of the Medical University of Bialystok as part of the MOBIT

project. The study was approved by the Bioethics Committee at the Medical University of Bialystok (reference number: APK.002.54.2022). Patients were selected for inclusion in the study based on the availability of relevant biological material, including intraoperatively collected tissue from the tumor, archived in the form of paraffin blocks, and a whole blood sample collected in EDTA. Furthermore, clinical data was incorporated as a valuable source of information.

2.2. DNA isolation

The ReliaPrep™ FFPE gDNA Miniprep System (Promega, USA) kit was employed for the isolation of DNA from formalin-fixed and paraffin-embedded (FFPE) tumor tissues. In turn, the isolation of genetic material from blood was conducted using the NucleoSpin Dx Blood kit (Macherey-Nagel, Germany), in accordance with the manufacturer's instructions. Subsequently, the DNA isolates were quantified utilizing a NanoDrop™ 2000c spectrophotometer (Thermo Scientific, USA). Additionally, the coefficients 260/230 and 260/280 were evaluated. Samples exhibiting the requisite quality, as indicated by an A260/A280 ratio exceeding 1.8 and an A260/A230 ratio exceeding 1.5, were deemed suitable for subsequent analysis.

Afterwards, the concentration of the isolated DNA was determined using a Qubit 3.0 Fluorimeter with the Qubit dsDNA HS Assay Kit (Invitrogen, USA).

2.3. Next-generation sequencing

Targeted gene panels were used to identify somatic molecular alterations. The VariantPlex Solid Tumor (Archer, USA) is an NGS panel that is capable of detecting single nucleotide variants (SNVs), copy number variants (CNVs), and insertions/deletions (indels) in 67 genes that have been identified as being associated with the development of solid tumors. Furthermore, analyses were conducted using the VariantPlex BRCA + PALB2 v.2 (Archer, USA). This panel is employed to identify the most prevalent sequence variants specific to ovarian cancer. The library preparation kits employ the use of Anchored Multiplex PCR (AMP), a target enrichment method that employs molecular barcoded adapters and single, nested, gene-specific primers for amplification, thereby facilitating open-ended capture of DNA. This is especially crucial for FFPE material, which is frequently characterized by degraded nucleic acids. The utilization of AMP chemistry ensures the generation of reliable results. In accordance with the manufacturer's guidelines, the prepared VariantPlex BRCA + PALB2 libraries were subjected to sequencing with 1.5 million reads per sample, while the VariantPlex Solid Tumor libraries were sequenced with 2.5 million reads per sample. Next-generation sequencing was performed on the MiSeq sequencer running on the Illumina platform.

To identify germline sequence variants, whole-exome sequencing (WES) was conducted. The libraries for sequencing were prepared using the Illumina DNA Prep with Enrichment protocol (Illumina, USA). Next-generation sequencing was performed on the NovaSeq 6000 sequencer running on the Illumina platform.

2.3.1. Targeted gene panels sequencing data analysis

The sequencing data were converted to de-multiplexed FASTQ files and subsequently processed using Archer Analysis, a software package that enables the

comprehensive secondary analysis of sequencing data, including read trimming/cleaning, de-duplication, error correction, alignment, and mutation calling. This software is provided by the reagent manufacturer and is capable of performing these functions. For the purposes of this study, variants with an allele frequency (AF) ≥ 2% were selected for analysis.

2.3.2. Whole-exome sequencing data analysis

Whole-exome sequencing data were processed using the Sarek pipeline (nf-core/sarek v3.4.2) [13–16]. The pipeline follows GATK4 Best Practices for preprocessing. Raw sequencing reads were aligned to the human reference genome (GRCh38) using the Burrows-Wheeler Aligner (BWA-MEM2) [17]. Post-alignment processing included duplicate marking and base quality score recalibration. Variant calling for single nucleotide variants (SNVs) and short insertions/deletions (indels) was performed using the Genome Analysis Toolkit (GATK) HaplotypeCaller. Variants were filtered to retain those with a minimum read depth of 30×. Datasets with less than 85% of the exome covered at ≥ 30× read depth were excluded. The remaining datasets were of high quality, with 98.9% of targeted bases covered at ≥ 30× read depth. Variant annotation was conducted using SnpEff19 [18] and the Variant Effect Predictor (VEP) and further annotated against the Catalogue of Somatic Mutations in Cancer (COSMIC) database.

For this study, coding regions, including flanking intronic regions, of 70 genes related to ovarian cancer and 34 additional genes related to comorbidities were evaluated. Variants with a minor allele frequency (MAF) ≤ 2% were initially explored. Functional classification of mutations followed guidelines from the Association for Molecular Pathology (AMP), the American Society of Clinical Oncology (ASCO), and the College of American Pathologists (CAP). The tumor mutation burden (TMB) was calculated for the entire exome region of each sample using established methods. Variants were classified according to standards from the American College of Medical Genetics and Genomics (ACMG) and AMP, with categories such as “pathogenic,” “likely pathogenic,” “variant of uncertain significance” (VUS), and “(likely) benign.” Hypomorphic variants, showing low to moderate cancer association in large studies, were also included. Classification incorporated empirical pathogenic variant burdens and MAF levels from the Exome Aggregation Consortium (ExAC) database. The significance of comorbidities and the classification of variants was retrieved from the ClinVar database.

Figure 1 shows a flow chart of the study.

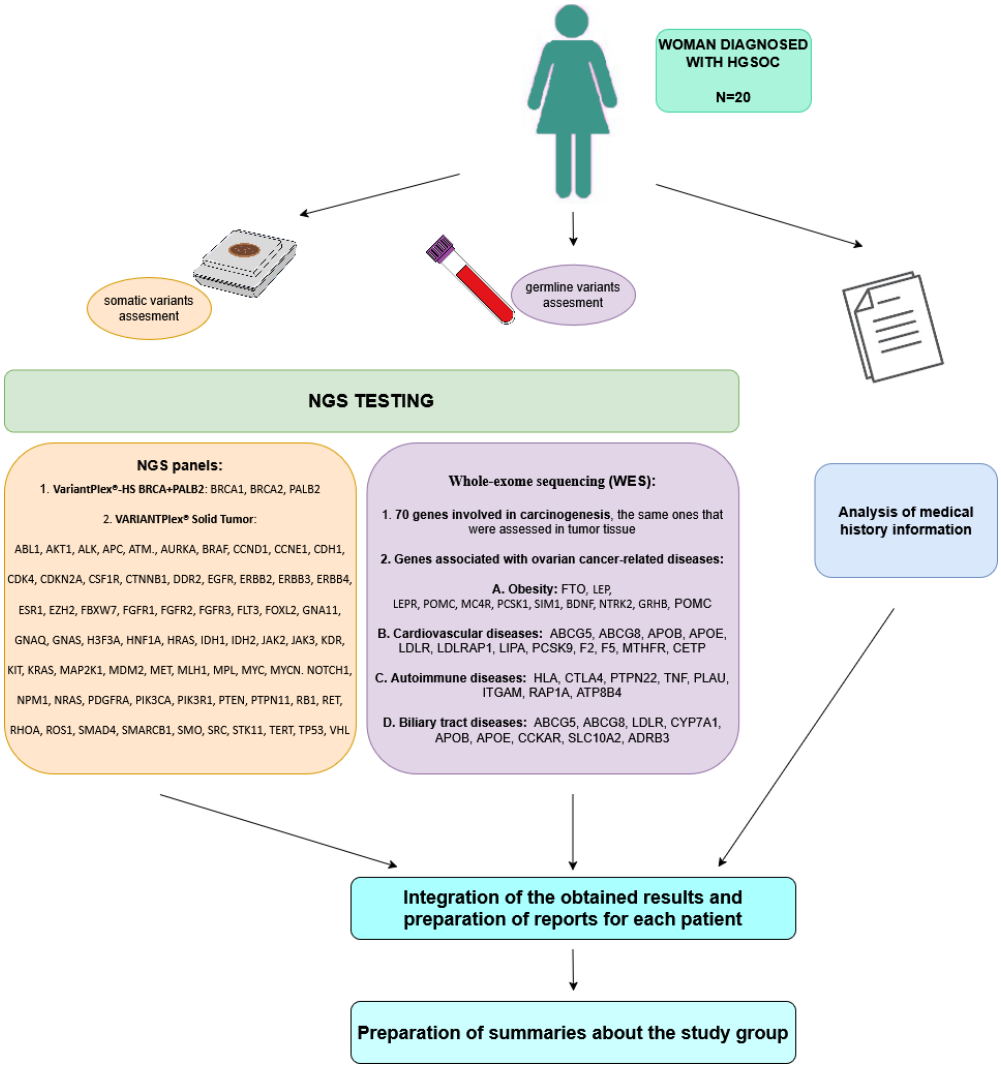

Figure 1. Summary of the study process, considering its elements.

3. Results

3.1. Clinico-pathological characterization

The median age of the female subjects under analysis was 64 years (range 39 to 88 years). The majority of patients (as many as 16) had been diagnosed with stage III cancer, with one patient having been diagnosed with stage I, two with stage II, and one with stage IV. Fifteen of the 20 women reported the presence of additional health conditions, collectively referred to as “comorbidities” (75% of patients). The questionnaires included information about a family history of cancer in 10 of the women (50% of patients). All patients belonged to the Polish population.

3.2. Somatic variants by panel tests

The utilization of panel tests enabled the identification of 122 somatic variants in cancer-related genes within the study group. With regard to the genes with the greatest confirmed importance in ovarian cancer, 7 variants were identified within the *BRCA1* gene, 16 within *BRCA2*, and 7 within *PALB2*.

3.3. Germline variants by whole-exome sequencing

A WES analysis revealed a total of 177 variants across all 20 subjects: 92 in genes linked to cancer, 53 in genes associated with cardiovascular disease, 13 in genes associated with obesity, 14 in genes linked to autoimmune disorders, and 5 in genes associated with biliary diseases.

A comprehensive list of all identified variants can be found in the repository [19].

3.4. Patient reports

A comprehensive data set on each patient was compiled, and a detailed report was generated for each individual, encompassing all available clinical information and a comprehensive catalogue of identified sequence variants. With respect to somatic variants, those that meet the criteria for molecularly targeted treatment in women diagnosed with ovarian cancer are highlighted. In the context of germline variants, those classified as pathogenic, probably pathogenic, or of uncertain clinical significance, in addition to variants associated with drug resistance, are presented. Furthermore, variants identified in both the patient's tumor tissue and blood, irrespective of their clinical significance, are presented.

4. Discussion

In the analysis of genetic predisposition to a particular disease entity within a specific population, consideration of founder mutations is of paramount importance. In the context of Poland, three specific founder mutations have been identified within the *BRCA1* gene. These are designated as 5382insC (c.5266dupC; p.Gln1756Profs), C61G (300T > G; c.181T > G), and 4153delA (c.4035delA; p.Glu1346Lysfs) [20,21]. In the study group, one patient's BRCA1 c.181T > G (p.Cys61Gly) variant was detected in tissue and blood, and one patient's BRCA1 c.4035del (p.Glu1346LysfsTer20) also in tissue and blood, and one patient's BRCA1 c.5266dup (p.Gln1756ProfsTer74) only in tumor tissue. It is estimated that founder mutations account for approximately 80% of pathogenic variants in the Polish population [20]. However, recurrent mutations are also included in clinical practice tests: 185delAG (c.68_69delAG; p.Glu23Valfs) and c.5370C > T (c.5251C > T; p.R1751*) [21]. Nevertheless, in the present study, these two variants were not identified in any of the patients, and each subsequent study reveals new recurrence variants specific to the population of the region. In their article, Kluska et al. emphasize the efficacy of NGS testing. The researchers succeeded in detecting new recurrent mutations among Polish patients with hereditary breast/ovarian cancer [22]. In the present study, two variants mentioned by Kluska et al [22]. were identified. The first of these, BRCA1 c.1067A > G (p.Gln356Arg), was found to be present in the blood of two patients and, moreover, also in the tumor tissue. The second variant, BRCA1 c.1695dup (p.Lys566fs), was germinal in one patient and somatic in three. With regard to the BRCA2 gene, one patient was found to have a variant designated as a 'BRCA2 c.658_659del (p.Val220fs)', which has been characterized as a recurrent mutation in Polish women diagnosed with breast cancer [23]. This variant was also identified within the tumor.

The study also sought to identify potential novel mutations that may be associated with hereditary ovarian cancer. In consideration of all detected sequence variants in

seven patients (35% of patients), the same variants were identified in tumor tissue and blood in the *BRCA1*, *BRCA2*, and *PALB2* genes, which are the genes most strongly associated with ovarian cancer and responsible for hereditary predisposition to the disease. The mother of one patient had a history of breast cancer, the father of another had testicular cancer, and the third patient had a family history of liver cancer in the father and suspected cancer in the mother, which may indicate a familial predisposition to cancer (15% of patients). In contrast, in the remaining four cases, there was no information about a family history of cancer. It is noteworthy that one patient exhibited the presence of two PALB2 c.2014G > C (p.Glu672Gln) and PALB2 c.2993G > A (p.Gly998Glu) variants in both the tumor and blood samples. However, these variants are classified as benign and benign/likely benign, respectively.

In eight women, variants of both somatic and germline nature were identified in other genes known to be involved in the development of solid tumors. In one patient, the DDR2 c.1323G > A (p.Met441Ile) variant was observed in conjunction with a variant in the PALB2 gene. Another patient exhibited the ERBB2 c.2446C > T (p.Arg816Cys) and KDR c.1444T > C (p.Cys482Arg) variants. It is noteworthy that an identical variant in the *KDR* gene was also identified in one more case. In this patient, no variants were identified in the *BRCA1*, *BRCA2*, or *PALB2* genes. Additionally, a medical history review revealed that the patient's mother had a diagnosis of ovarian cancer. These findings suggest that the presence of mutations in multiple genes associated with carcinogenesis could potentially contribute to an increased risk of hereditary ovarian cancer. However, further research is necessary to clarify the exact role these mutations play in hereditary predisposition. Larger studies are required to substantiate these initial observations and to fully understand the complex interactions between these genetic variants.

Another key objective of the study was to establish a molecular link between the most prevalent comorbidities and ovarian cancer. Despite an exhaustive WES analysis of 34 genes with a proven association with ovarian cancer comorbidities, no pathogenic or likely pathogenic variants were identified. Nevertheless, a number of variants of uncertain significance and conflicting classifications of pathogenicity were identified in the patient's blood. It seems probable that the combined effect of these variants and lifestyle factors may result in the development of a disease that subsequently increases the risk of ovarian cancer.

A total of 85% of patients (N = 17) were found to have at least one variant of uncertain significance or conflicting classifications of pathogenicity in genes associated with cardiovascular disease. Conversely, information on such conditions was present in the medical history of only 8 patients. The discrepancy may be attributed to the absence of an association between these variants and the progression of the disease in terms of its clinical presentation, or alternatively, to the failure to document this information during the patient interview.

In a patient with a body mass index (BMI) of 36.98, corresponding to class II obesity, a POMC c.706C > G (p.Arg236Gly) variant was identified. This variant is currently referred to as 'conflicting classifications of pathogenicity'. In contrast, in a woman with a BMI of 41.40 (class III obesity), a variant of uncertain significance, FTO c.601G > A (p.Val201Ile) and LEPR c.2260G > A (p.Val754Met), with conflicting classifications of pathogenicity, was detected. It is noteworthy that the first

patient also exhibited two variants in the *PALB2* gene, while the second patient displayed a variant in BRCA1. In these cases, the co-occurrence of variants associated with ovarian cancer and with a comorbid condition such as obesity was observed.

These observations, although based on a limited number of subjects, appear to corroborate the hypothesis that there may be a molecular link between cancer and other diseases that frequently co-occur.

The MTHFR c.665C > T variant (p.Ala222Val) was identified in as many as 45% of the patients under investigation. The ClinVar database indicates that this variant is responsible for methotrexate toxicity. Oral chemotherapy with methotrexate represents a promising avenue of treatment for recurrent ovarian cancer [24]. The awareness of the existence of this sequence variant in a patient may offer valuable insights for the selection of an appropriate treatment. It is pertinent to underscore that the variant in the MTHFR gene was identified as a consequence of its association with cardiovascular disease and thus incorporated into the WES analyses.

The present study was conducted on a small study group in order to ascertain whether the integration of clinical data and those obtained from advanced molecular studies could be a suitable direction for larger-scale research. It is hypothesized that significant information could be provided by studies divided into subgroups according to comorbidities, which would allow an attempt to determine whether the most frequently detected variants or their complex interactions cause a significant increase in the risk of developing ovarian cancer. The study was performed on archival material. It was not possible to integrate the results obtained with information on patients' response to treatment and survival time. The patients in the study group are of Polish origin; however, it should be noted that even within a single nation, there exist subpopulations with varying frequencies of the genetic variants.

5. Conclusion

Ovarian cancer is unquestionably one of the most thoroughly characterized cancers at the molecular level. Nevertheless, there is a lack of literature concerning the interaction of somatic and germline variants and the molecular connection to the pathogenesis of comorbidities.

It has been demonstrated that the integration of NGS analysis results with patient clinical data yields valuable insights and emphasizes the distinctive nature of each clinical case. This approach has the potential to markedly enhance diagnostic accuracy and the selection of appropriate treatment in the context of personalized medicine. The identification of hitherto uncharacterized sequence variants in ovarian cancer, present in both tissue and blood, may prompt a rethink of the approach to classifying cancer as hereditary. Furthermore, it is crucial to consider the existence of molecular differences across populations and to take into account the varying frequencies of specific genetic variants. It is crucial to consider the genetic burden of lifestyle-related comorbidities in ovarian cancer when developing prevention strategies. Additionally, it is vital to emphasize the significance of obtaining a comprehensive medical history from patients by healthcare professionals and educating them on the importance of the information they provide for their diagnostic and therapeutic processes, as well as for their families.

It is our contention that the utilization of the potential afforded by contemporary molecular biology, specifically NGS technology, including both panel tests and WES, coupled with an individualized and comprehensive approach to each medical case, constitutes a cornerstone of personalized medicine. This approach has the potential to confer substantial benefits to the health of patients and to enhance the efficiency of healthcare systems. It is our belief that establishing a molecular-level correlation between comorbidities and cancer would significantly enhance our understanding of the disease, thereby facilitating the development of dedicated tests to assess susceptibility to cancer within a given population. However, further studies in larger cohorts are needed to confirm the clinical utility of performing NGS technology on a broad scale.

Author contributions: Conceptualization, PAB; methodology, PAB, JK and WB; software, WB; validation, PAB, JK and WB; formal analysis, PAB and WB; investigation, PAB, JK and WB; resources, PAB, JK and WB; data curation, WB; writing—original draft preparation, PAB; writing—review and editing, JK; visualization, PAB; supervision, JN; project administration, PAB; funding acquisition, PAB. All authors have read and agreed to the published version of the manuscript.

Institutional review board statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Bioethics Committee of the Medical University of Bialystok (protocol code: APK.002.54.2022 and date of approval: 20.01.2022).

Conflict of interest: The authors declare no conflict of interest.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2021; 71(3): 209-249. doi: 10.3322/caac.21660
2. Gaona-Luviano P, Medina-Gaona LA, Magaña-Pérez K. Epidemiology of ovarian cancer. Chinese Clinical Oncology. 2020; 9(4): 47-47. doi: 10.21037/cco-20-34
3. Huang J, Chan WC, Ngai CH, et al. Worldwide Burden, Risk Factors, and Temporal Trends of Ovarian Cancer: A Global Study. Cancers. 2022; 14(9): 2230. doi: 10.3390/cancers14092230
4. Ali AT, Al-ani O, Al-ani F. Epidemiology and risk factors for ovarian cancer. Menopausal Review. 2023; 22(2): 93-104. doi: 10.5114/pm.2023.128661
5. Sambasivan S. Epithelial ovarian cancer: Review article. Cancer Treatment and Research Communications. 2022; 33: 100629. doi: 10.1016/j.ctarc.2022.100629
6. Punzón-Jiménez P, Lago V, Domingo S, et al. Molecular Management of High-Grade Serous Ovarian Carcinoma. International Journal of Molecular Sciences. 2022; 23(22): 13777. doi: 10.3390/ijms232213777
7. Chandrasekaran A, Elias KM. Synthetic Lethality in Ovarian Cancer. Molecular Cancer Therapeutics. 2021; 20(11): 2117-2128. doi: 10.1158/1535-7163.mct-21-0500
8. Matthews B, Bowden N, Wong-Brown M. Epigenetic Mechanisms and Therapeutic Targets in Chemoresistant High-Grade Serous Ovarian Cancer. Cancers. 2021; 13(23): 5993. doi: 10.3390/cancers13235993
9. Bukłaho PA, Kiśluk J, Nikliński J. Diagnostics and treatment of ovarian cancer in the era of precision medicine - opportunities and challenges. Frontiers in Oncology. 2023; 13. doi: 10.3389/fonc.2023.1227657
10. Bound NT, Vandenberg CJ, Kartikasari AER, et al. Improving PARP inhibitor efficacy in high-grade serous ovarian carcinoma: A focus on the immune system. Frontiers in Genetics. 2022; 13. doi: 10.3389/fgene.2022.886170

11. Hussen BM, Abdullah ST, Salihi A, et al. The emerging roles of NGS in clinical oncology and personalized medicine. Pathology-Research and Practice. 2022; 230: 153760. doi: 10.1016/j.prp.2022.153760
12. Gorcenco S, Ilinca A, Almasoudi W, et al. New generation genetic testing entering the clinic. Parkinsonism & Related Disorders. 2020; 73: 72-84. doi: 10.1016/j.parkreldis.2020.02.015
13. Hanssen F, Garcia MU, Folkersen L, et al. Scalable and efficient DNA sequencing analysis on different compute infrastructures aiding variant discovery. NAR Genomics and Bioinformatics. 2024; 6(2). doi: 10.1093/nargab/lqae031
14. Garcia M, Juhos S, Larsson M, et al. Sarek: A portable workflow for whole-genome sequencing analysis of germline and somatic variants. F1000Research. 2020; 9: 63. doi: 10.12688/f1000research.16665.2
15. Ewels PA, Peltzer A, Fillinger S, et al. The nf-core framework for community-curated bioinformatics pipelines. Nature Biotechnology. 2020; 38(3): 276-278. doi: 10.1038/s41587-020-0439-x
16. Di Tommaso P, Chatzou M, Floden EW, et al. Nextflow enables reproducible computational workflows. Nature Biotechnology. 2017; 35(4): 316-319. doi: 10.1038/nbt.3820
17. Vasimuddin Md, Misra S, Li H, et al. Efficient Architecture-Aware Acceleration of BWA-MEM for Multicore Systems. Proceedings of the 2019 IEEE International Parallel and Distributed Processing Symposium (IPDPS); 2019. doi: 10.1109/ipdps.2019.00041
18. Cingolani P, Platts A, Wang LL, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff. Fly. 2012; 6(2): 80-92. doi: 10.4161/fly.19695
19. Bukłaho PA, Kiśluk J, Bauer W, et al. Results from studies using next-generation sequencing to detect somatic and germline variants in patients with high-grade serous ovarian cancer. Available online: https://doi.org/10.5281/zenodo.14710360 (accessed on 7 April 2025).
20. Gronwald J, Cybulski C, Huzarski T, et al. Genetic testing for hereditary breast cancer in Poland: 1998–2022. Hereditary Cancer in Clinical Practice. 2023; 21(1). doi: 10.1186/s13053-023-00252-6
21. Kowalik A, Siołek M, Kopczyński J, et al. BRCA1 founder mutations and beyond in the Polish population: A single-institution BRCA1/2 next-generation sequencing study. PLOS ONE. 2018; 13(7): e0201086. doi: 10.1371/journal.pone.0201086
22. Kluska A, Balabas A, Paziewska A, et al. New recurrent BRCA1/2 mutations in Polish patients with familial breast/ovarian cancer detected by next generation sequencing. BMC Medical Genomics. 2015; 8(1). doi: 10.1186/s12920-015-0092-2
23. Rogoża-Janiszewska E, Malińska K, Cybulski C, et al. Prevalence of Recurrent Mutations Predisposing to Breast Cancer in Early-Onset Breast Cancer Patients from Poland. Cancers. 2020; 12(8): 2321. doi: 10.3390/cancers12082321
24. Shet D, Gatty RC. Impressive response to oral metronomic chemotherapy in ovarian cancer. Current Problems in Cancer: Case Reports. 2022; 8: 100197. doi: 10.1016/j.cpccr.2022.100197