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The role of clinical-pathological factors, genetic factors, and the microbiome in the prognosis and treatment response in ovarian cancer - the path to personalized therapy

SUMMARY OF THE DOCTORAL THESIS

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Published scientific papers *in the field of the PhD thesis*

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2. Simion L, Chitoran E, Cirimbei C, Stefan DC, Neicu A, Tanase B, Ionescu SO, Luca DC, Galeș L, **Gheorghe AS**, Stănculeanu DL, Rotaru V. A Decade of Therapeutic Challenges in Synchronous Gynecological Cancers from the Bucharest Oncological Institute. Diagnostics. 2023;13(12):2069. **(IF = 3.0)**

3. Rădoi VE, Țurcan M, Maioru OV, Dan A, Bohîlțea LC, Dumitrescu EA, **Gheorghe AS* (corresponding author)**, Stănculeanu DL, Thodi G, Loukas YL, Săbău ID. Homologous Recombination Deficiency Score Determined by Genomic Instability in a Romanian Cohort. Diagnostics. 2023;13(11):1896. **(IF = 3.0) - chapter 7;** https://www.mdpi.com/2075-4418/13/11/1896

4. **Gheorghe AS**, Dumitrescu EA, Komporaly IA, Mihăilă RI, Lungulescu CV, Stănculeanu DL. New Targeted Therapies and Combinations of Treatments for Cervical, Endometrial and Ovarian Cancers: A Year in Review. Current Oncology. 2022;29(4):2835-47 **(IF = 2.8) - chapter 1.2;** https://www.mdpi.com/1718-7729/29/4/231

5. **Gheorghe AS**, Negru ȘM, Preda M, Mihăilă RI, Komporaly IA, Dumitrescu EA, Lungulescu CV, Kajanto LA, Georgescu B, Radu EA, Stănculeanu DL. Biochemical and Metabolical Pathways Associated with Microbiota-Derived Butyrate in Colorectal Cancer and Omega-3 Fatty Acids Implications: A Narrative Review. Nutrients. 2022;14(6):1152 **(IF = 4.8) – Editor's Choice - chapter 2;** https://www.mdpi.com/2072-6643/14/6/1152

6. Dragomir RD, Sas I, Săftescu S, Popovici D, Margan R, **Dragomir AS**, Stanca H, Mocanu V, Pac C, Negru ȘM. Treatment experience and predictive factors associated with response in platinum-resistant recurrent ovarian cancer: a retrospective single-

institution study. Journal of Clinical Medicine. 2021;10(16):3596 **(IF = 3.0)**

7. **Gheorghe AS**, Negru ȘM, Nițipir C, Mazilu L, Marinca M, Gafton B, Ciuleanu TE, Schenker M, Dragomir RD, Gheorghe AD, Stovicek PO, Bandi-Vasilica M, Boț AC, Mihăilă RI, Zob DL, Kajanto AL, Stănculeanu DL. Knowledge, attitudes and practices related to COVID-19 outbreak among Romanian adults with cancer: a cross-sectional national survey. ESMO Open. 2020;6(1):100027 **(IF = 7.1) - chapter 6.3.4;** https://www.esmoopen.com/article/S2059-7029(20)32892-1/fulltext

II. Articles published in national indexed journals

8. **Gheorghe AS**, Preda M, Dumitrescu EA, Mahler B, Ginghină O, Stănculeanu DL. The Microbiome in Ovarian Cancer - a Narrative Synthesis of the published studies. 2023;82(3):202-209 **- chapter 2;**
https://roami.ro/wp-content/uploads/2024/07/5-review2_issue3_2023.pdf

9. **Gheorghe AS**, Stănculeanu DL. Gut microbiota during chemotherapy in patients with epithelial ovarian cancer. Romanian Archives of Microbiology and Immunology. 2022;81(2):154

10. **Gheorghe AS**, Negru ȘM, Preda M, Mihai MM, Manolescu LCS, Popa MI, Stănculeanu DL. Microbiome Implications in carcinogenesis initiation and promotion. Oncolog-hematolog.ro. 2020;51(2):17-23 **- chapter 2;** https://www.medichub.ro/reviste-de-specialitate/oncolog-hematolog-ro/implicatiile-microbiomului-in-initierea-si-promovarea-carcinogenezei-id-3188-cmsid-68

11. Stănculeanu DL, Mihăilă RI, Komporaly IA, Toma O, Kajanto L, **Gheorghe AS**, Bodilcu AL, Georgescu B, Zob DL. Actualities in epithelial ovarian cancer. Oncolog-hematolog.ro. 2019;48(3):53

12. Popa GL, **Gheorghe AS**, Preda M. The intestinal microbiota changes paradigms in hepatic cirrhosis. Infectio.ro. 2017;51(3):5-9

13. Popa GL, **Gheorghe AS**, Preda M, Popa MI. The intestinal microbiota changes paradigms in irritable bowel syndrome. Infectio.ro. 2017;50(2):5-9

14. Popa GL, **Gheorghe AS**, Preda M, Popa MI. The intestinal microbiota reconfigures the boundaries of knowledge. Infectio.ro. 2017;49(1):5-9

Working hypothesis and general objectives

The project (PhD Thesis) comprises several parts/types of studies:

1. Descriptive retrospective observational single-center descriptive study

The study "Treatment strategies in advanced ovarian cancer: primary cytoreductive surgery and adjuvant chemotherapy or neoadjuvant chemotherapy and interval cytoreduction?" aims to provide a "*real-life*" presentation of outcomes for patients with stage III-IV high-grade serous ovarian cancer who received neoadjuvant chemotherapy followed by interval cytoreductive surgery versus primary cytoreductive surgery and neoadjuvant chemotherapy. This is a topic of active debate in the scientific community. To benefit 5-year overall survival outcomes, we documented data from patients who underwent primary surgery or a first cycle of neoadjuvant chemotherapy between January 1, 2018 and December 31, 2018.

2. Observational observational cohort analytic study

The study "HRD (homologous recombination deficiency) score determined by genomic instability analysis in a group of ovarian cancer patients" tests the hypothesis that ovarian cancer patients with a high HRD score, determined by genomic instability analysis, have a better prognosis and respond more favorably to targeted therapies such as PARP inhibitors compared to patients with a low or absent HRD score. This hypothesis implies that the HRD score may serve as a predictive biomarker for the efficacy of personalized therapies and could influence both overall survival and progression-free survival in ovarian cancer patients. To assess HRD score in a Romanian patient population, we developed an observational analytic cohort study.

3. Case study and literature review

The study "The impact of PARP inhibitors on long-term survival in BRCA1-mutated advanced ovarian cancer", based on a case study and literature review of BRCA mutations in Romania, hypothesized that PARP inhibitors significantly improve long-term survival in patients with BRCA-mutated advanced ovarian cancer compared to patients who do not receive this treatment. This hypothesis suggests that treatment with PARP inhibitors has a positive impact on overall and progression-free survival in BRCA-mutated patients, providing a significant therapeutic benefit. In this chapter, particularities that may explain the prolonged response are discussed and provide a clinical perspective to academic research.

4. Proposal for an experimental analytic clinical trial

The prospective clinical trial "OVAGIOME - Ovarian cancer and VAGInal microbiOMe interactions for for Enhanced therapeutic outcomes" will ultimately aim to modulate the vaginal microbiome in patients with advanced ovarian cancer by administering vaginal probiotics concomitantly with neoadjuvant chemotherapy in order to amplify treatment response and achieve complete pathologic response following interval cytoreductive surgery. Vaginal probiotics may produce defense factors to maintain a healthy vaginal microenvironment, interact with potential pathogens, and remediate microbial dysbiosis. In addition, vaginal probiotics have the ability to regulate the body's inflammatory and immune response, and some bacterial strains exhibit properties with anti-carcinogenic potential, which could have apoptotic effects on cancer cells by modulating the expression of microRNA molecules and affecting tumor cell signaling.

1. Treatment strategies in advanced ovarian cancer: primary cytoreductive surgery and adjuvant chemotherapy or neoadjuvant chemotherapy and interval cytoreduction? - Single-center retrospective observational observational study

The study aims to provide a "real-life" presentation of outcomes for patients with stage III-IV high-grade serous ovarian cancer who received neoadjuvant chemotherapy followed by interval cytoreductive surgery compared with primary cytoreductive surgery and neoadjuvant chemotherapy. This is a topic of active debate within the scientific community. It also compares outcomes between the two treatment strategies, investigates risk factors for recurrence and assesses the impact of BRCA mutations and HRD deficiency on patient outcomes. To benefit 5-year overall survival outcomes, we documented data from patients who underwent primary surgery or a first cycle of neoadjuvant chemotherapy between January 1, 2018 and December 31, 2018.

This is an observational, retrospective study on a cohort of ovarian cancer patients treated since 2018 at the Institute of Oncology "Prof. Dr. Dr. Al. Trestioreanu" in Bucharest, Romania. The study includes patients who fulfill specific inclusion and exclusion criteria, previously presented.

The primary objective is to compare the 5-year overall survival (OS) between patients who underwent primary cytoreductive surgery and those who received neoadjuvant chemotherapy followed by interval cytoreductive surgery for stage IIIA-IVB ovarian cancer.

In this study, the distribution of patients was based on ECOG performance score, age and body mass index (BMI). The majority of patients (84%) had an ECOG score of 0, indicating a good general condition with no functional limitations. In terms of age, the majority (66%) were in the 60-69 age range. The BMI distribution showed that 67% of the patients were overweight (BMI between 25 and 29.9) and 14% were obese, reflecting a high prevalence of overweight in this sample.

The distribution of patients according to FIGO stage and histologic type reveals that the majority were diagnosed with advanced disease. Thus, 36% of the patients were stage IVA and 19% were stage IIIB and IVB. Histologically, 91% of patients had high-grade serous carcinoma, the most common form of ovarian cancer. In terms of tumor localization, the majority of patients (95%) had tumors localized to the ovary, indicating a major prevalence of ovarian involvement

The distribution of BRCA mutations and homologous recombination deficiency (HRD) status reflect the genetic profile of the patients. BRCA testing was not performed in 9% of the patients, and 67% had no BRCA mutations, while 16% had BRCA1 mutations and 8% had BRCA2 mutations. Regarding HRD, testing was not performed in 65% of the patients, and of those tested, 11% were HRD positive, which may influence treatment options with PARP inhibitors.

Variables were analyzed in each of the two groups:

The majority of patients in both groups were aged 60-69 years. There were no significant differences between the two groups ($p=0.8633$).

Most patients in both groups had a BMI between 24.9-29.9. There were no significant differences between the groups ($p=0.9795$).

The vast majority of patients (91%) had high-grade serous cancer, with little variation between groups ($p=0.1796$).

Most tumors were located in the ovary (95%), with no significant differences between groups.

Patients in the neoadjuvant chemotherapy group had a balanced distribution between stages III and IV, while the majority of patients in the adjuvant group were stage IV ($p<0.001$).

Approximately 84% of patients in both groups had an ECOG score of 0 (no significant limitations in activities of daily living) ($p=0.9863$).

In both groups, the majority of patients had no BRCA mutations. BRCA tests were not performed in a small proportion of patients in both groups ($p=0.1602$).

Positive HRD was significantly more frequent in the neoadjuvant chemotherapy group ($p<0.001$), but the low number of tests precludes the conclusion of a significant result.

Patients operated on after neoadjuvant chemotherapy tended to have lower Aletti scores, indicating that the surgeries were less complex in general, probably due to the effect of chemotherapy in reducing tumor volume before surgery. Patients operated per primam show higher scores, suggesting more complex surgery.

Surgical complications occurred in 28.99% of patients who received neoadjuvant chemotherapy followed by interval cytoreduction and in 51.61% of those who received primary cytoreduction.

The initial chemotherapy treatment (neoadjuvant or adjuvant) in all patients used the standard combination of paclitaxel and carboplatin.

In the neoadjuvant chemotherapy group, 65.22% of the patients (46) had a favorable KELIM score, while in the adjuvant chemotherapy group, it was found in 45.16% of the patients (14).

After adjuvant treatment, 47 patients (47.00%) received maintenance treatment with Bevacizumab. At that time in Romania, Olaparib was not reimbursed as maintenance treatment after first-line treatment, but only as maintenance treatment after first relapse. The combination of Olaparib plus Bevacizumab was also not available. For this reason, patients received only Bevacizumab as maintenance treatment after first-line treatment

After the first relapse, 24 patients (24.00%) received Olaparib maintenance treatment and 46 (46.00%) received Bevacizumab.

Depending on the time to first relapse, patients were considered platinum resistant (17.00%) or platinum sensitive (83.00%). This was taken into account for determining subsequent treatments.

In first-line treatment (100 patients), carboplatin + paclitaxel is the most commonly used regimen (74% of patients) and is the standard treatment for platinum-sensitive tumors. Other less common regimens included carboplatin + gemcitabine (9%), topotecan (8%) and liposomal pegylated doxorubicin (7%).

In second-line treatment (48 patients), carboplatin + paclitaxel remains the dominant treatment as percentage (54.17% of cases), but with a decrease compared to first-line. Gemcitabine and topotecan are also used (16.67% and 14.58% respectively).

In the third line of treatment (20 patients), gemcitabine was the most commonly used regimen (40%), followed by liposomal pegylated doxorubicin (20%) and topotecan (15%).

In the fourth line of treatment (8 patients), the options vary, but paclitaxel is the most used (37.5%), followed by etoposide and gemcitabine (25% each).

The median RFS (mean time to first recurrence) for the neoadjuvant chemotherapy group (N1) is 13.75 months, compared with 11.38 months for the adjuvant chemotherapy group (N2). The median RFS (time to recurrence for 50% of patients) is identical in both groups: 12 months. Patients in the neoadjuvant group have a slightly longer relapse-free survival, but the medians are the same for both groups, suggesting that the treatments offer similar benefits.

The mean PFS1 for the neoadjuvant group (N1) is 14.40 months compared with 13.83 months for the adjuvant group (N2). The median PFS1 is slightly lower for the neoadjuvant group (11 months) compared to the adjuvant group (12 months). Although the mean differences are minimal, the adjuvant-treated group has a slightly longer median PFS1, indicating a marginal benefit in this phase.

The mean PFS2 for the neoadjuvant group is 6.92 months compared with 6.23 months for the adjuvant group. Median PFS2 is almost identical between groups: 7 months (neoadjuvant) and 6 months (adjuvant). There are no major differences between groups in progression-free survival after second-line treatment.

Mean OS is similar between groups: 31.78 months for neoadjuvant and 30.74 months for adjuvant. Median OS is slightly higher in the neoadjuvant group (27 months) than in the adjuvant group (26 months). Overall survival is very similar between the two groups, with no significant differences.

The differences between neoadjuvant and adjuvant chemotherapy are minimal in terms of relapse-free survival (RFS), progression-free survival (PFS) and overall survival (OS). However, in some treatment lines, neoadjuvant chemotherapy appears to offer a slight advantage, particularly for RFS, PFS1, PFS2 and OS. The small number of patients in some phases of the study limits the statistical significance of these conclusions.

Patients treated with neoadjuvant chemotherapy appear to show slightly better survival than those operated per-primam who received adjuvant chemotherapy at certain time points, but there is not a very large difference between the two curves. The p-value ($p = 0.08$) indicates that the difference between the two groups is not considered statistically significant at a common significance threshold ($p < 0.05$). However, the p is relatively close to 0.05, which could suggest a trend towards a clinically relevant difference, but more data may be needed to confirm this.

Although the group treated with neoadjuvant chemotherapy shows a slightly longer relapse-free survival than the group treated with adjuvant chemotherapy, the difference is not large enough to be considered statistically significant.

In terms of PFS1, in patients who received either adjuvant chemotherapy (black line) or neoadjuvant chemotherapy (red line), the two survival curves are very similar, indicating that there is no significant difference in progression-free survival (PFS) between the two groups.

The p-value of 0.745 indicates that there is no statistically significant difference in survival between the two groups, so both adjuvant and neoadjuvant chemotherapy had comparable effects on PFS1.

The same is true for PFS2, with a p-value of 0.525, with no statistically significant difference between the two groups in PFS2.

Also, PFS3 is similar between the two groups, with p statistically insignificant ($p = 0.887$).The previously described numerical difference in OS on the 2 arms is not statistically supported. A p-value of 0.579 indicates that there is no statistically significant difference in overall survival between patients in the two groups.

Patients who received Olaparib as maintenance therapy at first relapse had a significantly longer overall survival compared to those who received Bevacizumab, and this difference is highly statistically significant ($p < 0.001$). Olaparib appears to be a more effective maintenance treatment in improving overall survival in this setting.

Although the results suggest a significant difference between the groups treated with Bevacizumab and Olaparib, it is important to note that the sample size was relatively small (46 patients treated with Bevacizumab and 24 with Olaparib). Thus, these findings should be interpreted with caution, and future studies with a larger sample size are needed to confirm these findings and to further evaluate the efficacy of the treatments.

The COVID-19 pandemic had multiple documented influences on the treatment of cancer patients. In the group of ovarian cancer patients analyzed, 87% were not affected by the pandemic in terms of treatment. However, 7% had their treatments delayed due to the pandemic and 6% of patients died, possibly as a result of complications associated with SARS-CoV-2 infection. Although delays occurred, these results show that most patients were able to continue therapy without major interruptions.

In an article entitled "Knowledge, attitudes and practices related to the COVID-19 pandemic among Romanian adults with cancer: a national cross-sectional study" (Authors: A.

S. Gheorghe, Ș. M. Negru, C. Nițipir, L. Mazilu, M. Marinca, B.. Gafton, T. E. Ciuleanu, M. Schenker, R. D. Dragomir, A. D. Gheorghe, P. O. Stovicek, M. Bandi-Vasilica, A. C. Boț, R. I.Mihăilă, D. L. Zob, A. L. Kajanto & D. L. Stănculeanu), published in ESMO Open, vol. 6, January 2021, we analyzed the influence of pandemic on oncology patients.

The COVID-19 pandemic has posed major challenges for cancer care providers because of the special precautions required for cancer patients, whose immune systems are often compromised. The study aimed to describe the level of knowledge, attitudes and practices related to COVID-19 among Romanian cancer patients in order to assess the impact of the pandemic and the effectiveness of response measures.

By analyzing separately patients with primary ovarian or peritoneal cancer (82 out of 1585), we obtained information on risk perceptions, fears and the influence of COVID-19 on disease progression in this diagnostic category. 67.50% of the patients (n=54) considered that their oncologic diagnosis conferred them an additional risk compared to the rest of the population to contract the new coronavirus. In contrast, 88.61% (n=70) did not believe that this risk justified delaying treatment. Almost a third, 35.00% (n=28), were more afraid of oncologic disease progression and only 7.50% (n=6) were more afraid of coronavirus infection.

The results of this retrospective study make a real-world contribution to the general debate on the optimal management of patients with advanced-stage high-grade serous ovarian cancer (FIGO III-IV). Although both primary cytoreduction followed by adjuvant chemotherapy and neoadjuvant chemotherapy followed by interval cytoreduction have shown similar outcomes in progression-free survival and overall survival, the choice between these two approaches remains a clinical challenge.

Overall survival (OS) and progression-free survival (PFS): The results suggest that there are no significant differences between the two therapeutic approaches in terms of OS and PFS, both showing comparable survival. This observation is consistent with previous clinical trials, which have indicated that both neoadjuvant and adjuvant chemotherapy are viable options for the treatment of patients with advanced ovarian cancer.

Patients who received neoadjuvant chemotherapy generally had less complex surgery (lower Aletti score). This trend may be explained by tumor volume reduction before surgery, facilitating complete cytoreduction. However, it should be noted that patients who underwent primary cytoreduction required more complex surgery, which may influence the risk of postoperative complications.

Although no major between-group differences in BRCA status were observed, HRD-positive patients tended to respond better to PARP inhibitor-based treatments, suggesting that this biomarker may play an important role in personalizing treatment for these patients.

A small percentage of patients were directly affected by delays or complications caused by the pandemic, which indicates adequate management of cancer patients during this difficult period. However, the psychological impact of the pandemic and fears of treatment interruptions were felt by a proportion of patients, emphasizing the importance of ensuring continuity of care even in a health crisis.

One of the main limitations of the study is its retrospective nature and the relatively small number of patients included, which may limit the statistical power of the conclusions. In addition, treatments and access to resources varied according to the pandemic context, which may influence the results. Also, over the past 5 years, therapeutic advances and drug approvals/refunds have generated other potential types of therapeutic conduct for clinical cases similar to those in the study. Larger prospective studies are needed to validate these findings and provide further evidence in support of one of the two therapeutic approaches.

The results of this study suggest that both primary cytoreduction and neoadjuvant chemotherapy followed by surgery are viable options for patients with advanced ovarian cancer. However, the choice between neoadjuvant chemotherapy followed by interval cytoreduction and primary cytoreduction followed by adjuvant chemotherapy for the treatment of advanced-stage high-grade serous ovarian cancer depends on a number of patient-specific clinical and prognostic factors.

Patients with BRCA mutations or homologous recombination deficiency (HRD) may have a better response to PARP inhibitor-based therapies, which are more effective after neoadjuvant chemotherapy, thus providing an additional advantage for this approach.

Primary cytoreduction is usually preferred in patients with a good general condition (ECOG 0-1), surgically resectable disease and a lower risk of complications. It is associated with a better prognosis, especially in cases where complete cytoreduction can be achieved.

In conclusion, the choice between these two approaches has to be individualized, taking into account tumor characteristics as well as the patient's general condition and available resources. The decision is based on a balance between the risks associated with each strategy and the long-term benefits for the patient.

2. HRD (homologous recombination deficiency) score determined by genomic instability in a group of ovarian cancer patients - impact on clinical practice

The Homologous Recombination Deficiency (HRD) score is a molecular analysis approach that quantifies the cellular rate of acquisition of chromosomal breaks using specific quantitative patterns called 'genomic scars'.

Cells with high HRD values have been shown to be more sensitive to poly (ADP-ribose) polymerase inhibitors and platinum therapy. Due to its strong predictive and prognostic value, the European Expert Consensus recommends BRCA and HRD testing for patients diagnosed with advanced ovarian cancer.

HRD genetic testing (including loss of heterozygosity - LOH, large-scale state transitions - LST and telomeric allelic imbalance - TAI) has prognostic value (progression-free survival and overall survival) and an impact on the comprehensive treatment plan, which has been validated by a large number of clinical trials.

The samples included in this study were paraffin-embedded biopsy tissue collected from females with a primary diagnosis of ovarian cancer. Formalin-fixed, formalin-fixed, paraffin-embedded (FFPE) tissue from female patients with a primary diagnosis of ovarian cancer outside the department in which testing was performed was requested from external pathology departments whenever the quality and/or quantity of tumor tissue was not sufficient for HRD testing.

Patient selection was made using the recommendations of the ESMO guidelines and included patients confirmed with ovarian, tubal or peritoneal cancer. In addition, 23 of the patients already had metastases. An important inclusion criterion was that the patients had to be BRCA1/2 negative on a previous tumor cell test.

Testing was performed using genomic instability scoring, which was determined by summing LOH, TAI and LST scores using the OncoScan C.N.V. platform (Thermo Fisher Scientific, Waltham, MA, USA).

Genetic material was extracted using the QIAamp DNA FFPE Tissue FFPE kit (Qiagen, Hilden, Germany). Concentration was determined using the Qubit TM dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA, USA) on a Qubit fluorometer. Extracted genetic material was tested on OncoScan CNV on an Affymetrix platform (Thermo Fisher Scientific, Waltham, MA, USA) following the manufacturer's instructions.

Oncoscan Affymetrix is a kit is based on MIP (Molecular Inversion Probe) technology and has the ability to detect "genomic scars" in degraded DNA, such as that of FFPE tumor tissue. The results are analyzed using the Chromosome Analysis Suite (ChAS) software with the GRCh37 reference genome.

For molecular tissue-based HRD tests, selection of the representative tumor area and assessment of the percentage of malignant cells, necrosis and inflammatory components are of fundamental importance. Usually, a minimum of 30% tumor component is recommended to guarantee variant detection by molecular techniques.

The study group included 100 Caucasian female patients from Romania, aged between 42 and 77 years, who were diagnosed with high-grade serous ovarian carcinoma, fallopian tube or primary peritoneal cancer. Selected patients had no somatic BRCA1 or BRCA2 mutations and received first-line chemotherapy and Bevacizumab.

The initial cohort consisted of 100 patients, of which 30 patients had samples that did not meet the criteria for HRD testing. One of the most important criteria is that the FFPE (Formalin-Fixed Paraffin-Embedded Paraffin-Embedded) samples were analyzed, prepared by the pathology laboratory and had a minimum tumor content of 30%. Another important criterion is tumor DNA integrity; the laboratory tested the concentration of extracted DNA in each sample and there were five samples that had less than 162.4 ng/µl (Qubit measurement - a method used to quantify the concentration of nucleic acids, DNA or RNA, in biological samples using

fluorometers; the Qubit system uses fluorescent dyes that specifically bind to target molecules and the intensity of the fluorescence emitted is proportional to the amount of genetic material present in the sample).

A total of 30% of the samples were thus unsuitable and unacceptable for further testing.

Data will be presented for 70 female patients for whom HRD testing was performed with the following results: 20 patients tested negative and 50 patients tested positive with a high HRD score.

Patients who had a high HRD score were followed up and therapeutic management and overall outcome will be presented.

30% of patients had an HRD score between 42 and 61. In addition, 13 patients (26%) were HRD positive with a score between 82 and 101, while 12 patients (24%) had a score between 62 and 81.

The highest HRD scores (142-161) were present in only 2% of the analyzed cases (1 patient).

The age range was 42-77 years. Thus, the mean age was 63.16 years.

The highest score was LST for each patient, but all scores together reported genomic scarring of tumor tissue more accurately than when taken separately.

The majority of patients were diagnosed with FIGO stage IIIC ovarian cancer (29, 58%; 95% CI: 43.21%-71.81%), with the highest mean HRD score 85 (85, Std. Dev. = 25.42).

Patients who were eligible for maintenance therapy with PARP inhibitors had to have a complete response (CR) or partial response (PR) from platinum chemotherapy. Of 50 patients, 35 received PARP inhibitor therapies, with median progression-free overall survival (PFS) increased from 4 months to 8.2 months (HR 0.38; 95% CI 0.35-0.47; $p < 0.001$).

The present study showed that the implementation of HRD testing is feasible, despite currently being performed on pharmaceutical tickets, and the overall turnaround time for receipt of HRD results was acceptably long for treatment decision. However, the most critical issue for performing HRD testing is the amount of tumor tissue that must be available. A considerably large number of patients did not receive a sufficient HRD test result due to lack of available

tumor tissue, and some of these patients may not have received optimal treatment because ofthis.

On the other hand, HRD testing should be started as soon as possible to allow more rapid availability of test results for firm planning of maintenance therapy. After patients underwent primary surgery and then received six cycles of chemotherapy every three weeks (a duration of approximately 126 days), the results of the present study were available after an average of 35 days, which was acceptable and in line with previously reported genome core analyses.

The lack of cost coverage by the NHS means that not all medical staff are aware of the availability of these voucher tests for their patients, which is one of the most significant drawbacks for receiving HRD results more quickly and access for all patients with high-grade ovarian cancer.

The high proportion of ineligible HRD tests - in the present article, this occurred for 30% of patients - was another significant logistical concern that was highlighted in the analysis. The absence of sufficient tumor material was the sole cause of insignificant HRD results. Since a significant proportion of tumors are already in remission by this point, the expectation that enough tumor tissue will be found in the sometimes very small biopsy specimen to perform HRD testing is often misleading. In the current study, we could not locate enough tumor tissue in 30% of patients.

Because the efficacy and approval status of maintenance therapy to be administered subsequently can be largely relied on HRD status, it is important that the quantity and quality of tumor tissue used for diagnostic workup be sufficiently high. A significant factor in shortening the turnaround time for HRD testing is the amount of tumor tissue present at the time of diagnosis. If the initial test failed, new FFPE tumor blocks may have been needed to redo the procedure, which would have prolonged the wait for the HRD test result.

The paraffin-embedding process that had been used largely damaged the FFPE DNA samples. Additional deterrents to the genetic screening process for mutation signatures can be introduced by the parameters of the paraffinization procedure.

The relationship between HRD/BRCA somatic testing and panel testing is another thing to think about. Testing for germline panels that include HRR genes cannot be omitted, as HRD results often provide inconclusive information.

However, there are significant academic initiatives underway to screen alternative HRD tests to those that are already accessible to reduce costs and improve performance.

Given that the majority of patients in our cohort had a favorable outcome and a higher survival rate, HRD testing is effective and there should be further studies with a larger number of patients to have a clearer picture of the HRD picture in Romania.

Therefore, HRD testing should be continuously optimized, taking into account the controversies of the various testing methodologies and the different cut offs, in order to maximize the benefit to the oncology patient.

Choosing a 'gold standard' for HRD testing is one of the biggest problems. The latest molecular, clinical, functional and genomic tests have benefits and drawbacks. The genomic scar HRD assays that have been successfully validated to date are valuable in estimating the magnitude of benefit of PARP inhibitors and can be used to guide therapy decision-making. They fail to address the complicated and dynamic nature of the HRD phenotype, which limits their usefulness (especially in the platinum-sensitive recurrent scenario). Therefore, to maximize the potential of PARP inhibitors in patients with HGOC, better biomarkers are needed to determine the patient's current HR status, which may require composite assays.

HRD testing in clinical practice detects 'genomic scarring' as an indirect measure of genomic instability through impaired DNA damage repair. Tests validated to date assess the percentage of LOH determined by sequencing SNPs or using a score calculated by combining three factors: SNP-LOH, telomere allelic imbalance (TAI) and LST.

PARP inhibitors act at the level of DNA single-strand breaks, preventing efficient repair, increasing genomic instability and thus leading to tumor cell death.

3. The impact of PARP inhibitors on long-term survival in BRCA1-mutated advanced ovarian cancer - Case Study and Literature Review

Progestin-free survival (PFS) of more than 109 months is a significant success for the treatment of ovarian cancer presented in this clinical case. Importantly, the patient had PFS of 65 following Olaparib treatment. This PFS aligns with growing evidence supporting the efficacy of PARP inhibitors in extending survival rates. The complete radiological remission maintained from August 2018 to February 2022 underscores the potential of using Olaparib as a maintenance therapy, redefining survival paradigms in ovarian cancer and representing a

testament to the durable potential of personalized oncology treatment offered by PARP inhibitors.

This important element has prompted clinical deliberations on the duration of Olaparib maintenance therapy, particularly in the context of the patient maintaining complete remission, given the low risk of myelodysplastic syndrome and acute myeloid leukemia as a side effect of PARP2 inhibitors. Further studies could investigate the optimal duration of treatment taking into account the well-known mechanisms of acquired resistance.

The case exemplifies the complexities and potential successes in the management of a case of advanced ovarian cancer with BRCA1 mutation, highlighting the transformative impact of PARP inhibitors in oncology. The individualized treatment approach, guided by genetic profiling and tailored to the dynamically changing disease state, allowed for prolonged survival and maintenance of quality of life. The case also underlines the need for a continuous multidisciplinary approach and the adaptability of treatment plans according to patient progression, recurrence and complications. The role of surgery (tumor cytoreductive interventions) in ovarian cancer remains a central pillar in the management of the disease, offering a clear survival benefit when optimally performed and integrated with systemic therapies such as chemotherapy, targeted treatments such as PARP inhibitors and hormonal therapy such as Tamoxifen.

The role of BRCA1 and BRCA2 mutations as biomarkers for a particularly long-lasting benefit from Olaparib is evident, albeit in a limited population (one-third of the cases in Study 19); we speculate that individual mutations may be of particular importance in this regard. The PFS of 65 months for the patient in the case presentation exceeded the median overall survival (mOS) of 51.7 months reported in the pivotal SOLO-2 trial, in which Olaparib was administered to patients with platinum-sensitive relapsed high-grade serous or endometrioid ovarian cancer.

4. Proposal for an experimental analytic clinical trial

The studies on the vaginal, gut and tumor microbiome described in Chapter 2 of the overview bring a new dimension to the ability to personalize ovarian cancer treatment and improve prognosis. This research suggests that the microbiome may influence disease progression and treatment response, providing new directions for personalized therapies. Introducing the microbiome as a biomarker and therapeutic target in ovarian cancer could

transform the way we approach ovarian cancer, contributing to the development of more effective treatments better tailored to the needs of individual patients.

We have developed a prospective clinical trial design, OVAGIOME, which focuses on the interactions between the vaginal microbiome and ovarian cancer. This proposal aims to investigate how the composition of the microbiome may influence disease progression and response to treatments such as chemotherapy and PARP inhibitors. This line of research makes a significant contribution to the emerging field of the microbiome in oncology, providing new insights for personalized therapies and introducing the microbiome as a novel biomarker in the management of ovarian cancer. The prospective clinical trial "OVAGIOME - Ovarian cancer and VAGInal microbiOMe interactions for Enhanced therapeutic outcomes" will have the following objectives: Description of the composition/diversity of the cervicovaginal microbiota in ovarian cancer patients; Interpretation of data resulting from the application of a questionnaire to assess behavioral, socioeconomic, genetic and environmental factors in ovarian cancer patients, in order to establish the causal relationship between eubiosis or dysbiosis of the vaginal microbiome and the progression of ovarian cancer; Identification of microbial biomarkers predictive for treatment response; To determine whether there is a correlation between clinico-pathologic, genetic factors and the vaginal/intestinal microbiome; To modulate the vaginal microbiome in ovarian cancer patients by administering vaginal probiotics concomitantly with neoadjuvant chemotherapy in order to amplify treatment response and achieve complete pathologic response following interval cytoreductive surgery. Vaginal probiotics can produce defense factors to maintain a healthy vaginal microenvironment, interact with potential pathogens, and remediate microbial dysbiosis. In addition, vaginal probiotics have the ability to regulate the body's inflammatory and immune response, and some bacterial strains exhibit properties with anti-carcinogenic potential, which could have apoptotic effects on cancer cells by modulating the expression of microRNA molecules and affecting tumor cell signaling.

Conclusions and personal contributions

1. Single-center retrospective observational observational study of treatment strategies inadvanced ovarian cancer: We conducted a retrospective single-center study comparing two major therapeutic strategies for patients with advanced ovarian cancer: primary cytoreductive

surgery followed by adjuvant chemotherapy versus neoadjuvant chemotherapy followed by interval cytoreduction. The study included a detailed analysis of overall survival (OS), progression-free survival (PFS), and the impact of clinico-pathologic and demographic factors on treatment response. This work provides new data on factors influencing the therapeutic decision tailored to the local context and optimizes clinical guidelines, demonstrating that both strategies can be effective, but the choice must be personalized for each patient according to the stage of disease and comorbidities, taking into account her clinical circumstances.

2. The HRD study and impact on clinical practice: In a group of ovarian cancer patients, we assessed the clinical relevance of the homologous recombination deficiency (HRD) score determined by genomic instability. We observed a significant correlation between elevated HRD scores and favorable response to PARP inhibitors. This study validates HRD as a key biomarker in the personalization of ovarian cancer treatment, supporting its widespread use to guide therapeutic decisions, particularly in BRCA mutation-negative cases, thereby expanding therapeutic options.

3. Case study on the impact of PARP inhibitors on long-term survival: In this case study, we present a patient with advanced ovarian cancer with BRCA1 mutation who received treatment with Olaparib, with significant PFS. Through a literature review, we evaluated the long-term efficacy of PARP inhibitor treatment in correlation with other clinical and genetic factors. This case study provides further evidence of the essential role of PARP inhibitors in prolonging overall survival and quality of life of patients, especially in those with BRCA1/2 mutations. Although the clinical cases have a minimal level of evidence for clinical decisions, they contribute to the literature and may provide insights into personalized treatments according to specific patient characteristics.

4. The prospective clinical trial "OVAGIOME - Ovarian cancer and VAGInal microbiOMe interactions for Enhanced therapeutic outcomes" highlights the need for further studies to validate the potential of the microbiome and to develop new clinical guidelines, including microbiome testing and the application of adjuvant probiotic-based therapies, in order to make cancer treatments as targeted, effective and personalized as possible.

5. Collaboration with multidisciplinary teams: My research has been based on collaboration with multidisciplinary teams, including colleagues from the fields of genetics, microbiology and surgical oncology. This integrated approach allows for a comprehensive

assessment of each case, contributing to the personalization of treatment and a better understanding of the interactions between clinico-pathological and genetic factors associated with prognosis. This methodology is essential for future progress in ovarian cancer management. Also, collaboration with other oncology colleagues from other academic centers has been an important component of my research. This collaboration has facilitated knowledge sharing, collaboration in conducting various studies, and is a step towards the development of multicenter studies.

6. Based on the retrospective data collection on ovarian cancer for the retrospective observational study, it is hoped that in the future a regional ovarian cancer registry could be implemented, centralizing the clinicopathological, genetic and molecular data of all patients. This registry would allow not only the monitoring of disease progression and response to treatment, but also a comparative analysis between different therapeutic approaches, thus providing a solid basis for future personalized treatment strategies. The registry can serve as a national model for real-time data collection and for improving ovarian cancer surveillance and treatment in Romania.

Selected bibliography

Machida H, Tokunaga H, Matsuo K, Matsumura N, Kobayashi Y, Tabata T, Kaneuchi M, Nagase S, Mikami M. Survival outcome and perioperative complication related to neoadjuvant chemotherapy with carboplatin and paclitaxel for advanced ovarian cancer: a systematic review and meta-analysis. European Journal of Surgical Oncology. 2020 May 1;46(5):868-75.

Feng Z, Wen H, Li R, Liu S, Fu Y, Chen X, Bi R, Ju X, Wu X. Comparison of survival between primary debulking surgery versus neoadjuvant chemotherapy for ovarian cancers in a personalized treatment cohort. Frontiers in Oncology. 2021 Feb 10;10:632195.211.

Tao, M.; Wu, X. The role of patient-derived ovarian cancer organoids in the study of PARP inhibitors sensitivity and resistance: From genomic analysis to functional testing. J. Exp. Clin. Cancer Res. 2021, 40, 338. https://doi.org/10.1186/s13046-021-02139-7.

Herzog, T.J.; Vergote, I.; Gomella, L.G.; Milenkova, T.; French, T.; Tonikian, R.; Poehlein, C.; Hussain, M. Testing for homologous recombination repair or homologous recombination deficiency for poly (ADP-ribose) polymerase inhibitors: A current per-spective. Eur. J. Cancer 2023, 179, 136–146. https://doi.org/10.1016/j.ejca.2022.10.021.

Patel, J.N.; Braicu, I.; Timms, K.M.; Solimeno, C.; Tshiaba, P.; Reid, J.; Lanchbury, J.S.; Darb-Esfahani, S.; Ganapathi, M.K.; Sehouli, J.; et al. Characterisation of homologous recombination deficiency in paired primary and recurrent high-grade serous ovarian cancer. Br. J. Cancer 2018, 119, 1060–1066. https://doi.org/10.1038/s41416-018-0268-6.

Heitz, F.; Ataseven, B.; Staniczok, C.; Denkert, C.; Rhiem, K.; Hahnen, E.; Heikaus, S.; Moubarak, M.; Welz, J.; Dagres, T.; et al. Implementing HRD Testing in Routine Clinical Practice on Patients with Primary High-Grade Advanced Ovarian Cancer. Cancers 2023, 15, 818. https://doi.org/10.3390/cancers15030818

Stanculeanu D, Median DM, Cebotaru CL, Teodorescu G, Voinea C. EP987 Prevalence and spectrum of BRCA mutations in patients with platinum-sensitive ovarian cancer from Romania – results of an observational study. Int J Gynecol Cancer 2019; 29: A52.

Vidra R, et al. Spectrum of BRCA1/2 Mutations in Romanian Breast and Ovarian Cancer Patients. Int J Environ Res Public Health 2022; 19, 4314. doi.org/10.3390/ijerph19074314.

Bolton KL, et al. Association between BRCA 1 and BRCA 2 mutations and survival in women with invasive epithelial ovarian cancer. JAMA 2012; 307(4): 382-390. doi:10.1001/jama.2012.20

Łaniewski P, Ilhan ZE, Herbst-Kralovetz MM. The microbiome and gynaecological cancer development, prevention and therapy. Nature Reviews Urology. 2020;17:232-250

Chambers LM, Bussies P, Vargas R, Esakov E, Tewari S, Reizes O, et al. The microbiome and gynecologic cancer: current evidence and future opportunities. Current Oncology Reports. 2021 Aug;23(8):92. DOI: 10.1007/s11912-021-01079-x

Rizzo AE, Gordon JC, Berard AR, Burgener AD, Avril S. The female reproductive tract microbiome—implications for gynecologic cancers and personalized medicine. Journal of Personalized Medicine. 2021 Jun 11;11(6):546. DOI: 10.3390/jpm11060546

Lehtoranta L, Ala-Jaakkola R, Laitila A, Maukonen J. Healthy vaginal microbiota and influence of probiotics across the female life span. Frontiers in Microbiology. 2022:787. DOI: 10.3389/fmicb.2022.819958

Lev-Sagie A, Goldman-Wohl D, Cohen Y, Dori-Bachash M, Leshem A, Mor U, et al. Vaginal microbiome transplantation in women with intractable bacterial vaginosis. Nature medicine. 2019 Oct;25(10):1500-4. DOI: 10.1038/s41591-019-0600-6