"CAROL DAVILA" UNIVERSITY OF MEDICINE AND PHARMACY, BUCHAREST
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MULTIGENIC TESTING IN PATIENTS WITH SOLID TUMORS – A STEP FORWARD TOWARDS PERSONALIZED TREATMENT

SUMMARY OF THE PhD THESIS

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TABLE OF CONTENTS:

SUMMARY OF THE PhD THESIS

The PhD thesis is structured in two parts. The general part consists of two chapters, which present data related to identifiable mutations in malignant solid tumors, techniques used for their identification, information concerning targeted therapy, as well as data regarding the impact of mutations on the development of thrombosis. The personal contributions section includes the results of the three studies conducted during the PhD research.

Introduction

Metastatic cancer is incurable and is a leading cause of death globally, including in developed countries. GLOBOCAN data estimated approximately 10 million deaths in 2022, of which lung cancer was responsible for 1/5 (20%) of all recorded deaths, followed by colorectal cancer (9% of deaths), breast cancer (7%) and gastric cancer (7%) [1].

Oncological treatments have undergone considerable evolution in recent years due to the development of genomic sequencing techniques, having laid the foundation for personalized therapy. In recent years, the medical community has witnessed significant development of targeted therapies based on molecular biomarkers, the concept of treating based on molecular characteristics rather than histopathology having taken root in modern medical practice. The medical community is now in a position to analyze and understand tumors and use their molecular characteristics to develop new individualized treatment options that can increase patient survival and quality of life.

Precision medicine has already transformed cancer management in both common and rare malignancies through targeted therapies, and is intended to further improve patient response to treatment [2]. While 5 years ago ESMO recommended that multigene testing by NGS be performed in 5 types of solid tumors, over the course of the last year the guidelines have been updated and it is now preferable that these comprehensive tests be performed in many other solid tumors where more targetable mutations can be identified, extending also to cancers where the primary tumor cannot be diagnosed by conventional methods. Furthermore, following the approvals of agnostic therapies, most cancer cases can be tested for less common mutations, in case therapies for those respective mutations might be available [3].

The present thesis aims to verify the utility and role that genomic sequencing tests can have in the clinical practice of a university hospital in Romania, on a heterogeneous group of solid tumors. The motivations for pursuing this topic was brought on by the advances in

precision therapy and by the fact that at the time of conducting the research for this thesis, in Romania, multigene testing panels implications and usage in clinical practice was not as extensive. In 2019 (the year this research began), in Romania, large multigene testing panels were not reimbursed and the experience with next-generation sequencing was not as extensive, therefore the clinical implications were not as well known as they are today. The thesis also aims to identify whether the existence of certain mutations can generate side effects or if they can modify the multimodal management of certain cancers, subsequently identifying if they impact the overall survival of patients. Thus, 3 clinical studies were conducted in support of this thesis.

I. GENERAL PART

Chapter 1. Cancer genomics: from basic research to personalized medicine

Changes occur in the DNA structure of cells as a result of exposure to a variety of environmental factors, such as ultraviolet radiation, smoking, or harmful chemicals. Changes can also occur on a genetic basis, due to errors in the replication and repair of cellular DNA. The somatic mutations that occur can accumulate over time, and can ultimately lead to the onset of carcinogenesis. The changes vary depending on their type, from minor changes, such as a single DNA base, to complex genetic changes that can affect a series of genes. In cancers, somatic mutations are most often identified, whereas only a small percentage of cancers are influenced by genetic changes that occur in germ cells - inherited mutations known as germline mutations [3].

After investigating the entire genome of malignant cells, it was observed that most tumors accumulate, during their evolution, a multitude of somatic mutations, and that some of them may be directly involved in carcinogenesis. This led to the development of databases with the aim of analyzing genes that can influence tumor evolution. Genetic changes that have an influence in this process are called “driver” mutations, and those that are not associated with cancer are called “passenger” mutations [4]. A multitude of studies conducted in recent times have identified a substantial number of genes involved in the evolution of cancer and, at the same time, mutations that have no clear role in its development have been discovered, the latter being called variants of unknown significance.

Mutational heterogeneity between different tissue types must also be taken into account. For example, in tissue harvested from metastases we can detect certain alterations that may not be present in the primary tumor, and vice versa, or in the case of primary synchronous tumors

the mutational profile of each may be different. Therefore, the origin of the tissue must be taken into account when evaluating the role of mutations in the cancer development process [4].

There has been an increasingly stronger link between research and clinical practice in recent years, especially after the advances made at molecular level, determining the need to create international, public databases, such as "The International Cancer Genome Consortium" (ICGC) or "The Cancer Genome Atlas" (TGCA), and also to create accessible tests for genome analysis. These databases support the development of therapeutic targets and the identification of biomarkers useful in clinical medical practice [5].

Chapter 2. Personalized therapy for solid tumors

Since the late 1990s targeted molecular therapy, whether small molecular agents or monoclonal antibodies that act as signal transduction inhibitors, has been the basis of precision medicine in cancer therapy, attracting attention by offering hope that with future development it could replace systemic chemotherapy. With a different toxicity profile, but generally with fewer adverse effects, targeted molecular therapy is often used in the treatment lines of various types of oncological pathologies, significantly improving survival and quality of life. Despite the major disadvantage of treatment resistance, more and more strategies are being developed to combat this side effect. Genetic testing and patient enrollment in clinical trials can aid in identifying resistance mechanisms and mutations, as well as in discovering new therapeutic targets and, consequently, new treatment options. NGS makes it possible to sequence the entire genome [6,7].

Furthermore, mutations identified in solid tumors not only influence cell proliferation and molecular mechanisms in cancer development, but also the thrombotic profile of oncological patients. It is important to identify them not only for therapeutic management, but also to establish thrombotic risk and possible personalized prophylactic interventions.

II. SPECIALISED PART – PERSONAL CONTRIBUTIONS

Chapter 3. Working hypothesis and general objectives

In the era of personalized treatment, multigenic testing is increasingly used to identify therapeutic targets in oncological patients. The present thesis, through the data extracted from the 3 clinical trials that were conducted, aims to verify the effectiveness of performing these

tests, to outline the role they can play in the management of solid cancers and also to identify whether certain mutations can generate side effects that can impact survival.

Chapter 4. General research methodology

4.1 Research directions:

In order to outline ways to personalize oncological therapy management based on multigenic testing, this thesis pursued three research directions. The first direction focused on performing and analyzing the results of multigenic testing using the next generation sequencing technique in patients with metastatic solid tumors who had progressed after one or more lines of treatment, and were managed within the Oncology Department of the "Elias" University Emergency Hospital. This allowed the identification of new therapeutic targets and the possibility of enrolling patients in clinical trials or changing therapeutic management in cases where this was achievable. The impact on survival in patients for whom the therapeutic decision was guided by the multigenic test result was also analyzed. The second research direction aims to verify whether the presence of BRCA mutation in patients with ovarian tumors influences the choice of the initial therapeutic strategy in cases with advanced malignancies and also to determine the impact it has in the choice of interval cytoreduction as opposed to primary cytoreduction. The third research direction focuses on the interrelationship between KRAS mutation status and the possibility of adverse events such as thrombosis in patients with colorectal cancer.

4.2 Data processing and statistical analysis:

Data from the three studies were collected from patients' medical records, the electronic medical system of the "Elias" University Emergency Hospital, from patients discharge notes, and from the reports of the genetic tests performed. The database was processed using Microsoft Office Excel 2013 and Microsoft Office Word 2013 and for statistical analysis the SPSS Statistics V.26 program (IBM Corp., Armonk, NY) was used. Overall survival and PFS were analyzed using the Kaplan-Meier method, with disease progression immaging being assessed based on RECIST 1.1 criteria. For the other studies, the Student's t-test, the chi-square test, and univariate or multivariate Cox regression models were also used. The statistical significance threshold was found at a $p < 0.05$.

Chapter 5. Study I : The role of genetic profiling by next-generation sequencing in advanced solid tumors: The experience of a single center in Romania

5.1 Introduction (Working hypothesis and objectives): Genetic testing plays an important role in the diagnosis and treatment choice of cancer patients, but the specific details regarding its exact role and the optimal timing for its implementation are still being clarified.

Objectives: To identify the practicality of genetic testing in patients diagnosed with advanced or metastatic solid tumors; To determine the optimal time to perform genetic testing so that the benefit is maximized; To analyze data collected from a single center in Bucharest in comparison with data from the specialized literature [8].

5.2 Materials and methods

We conducted a prospective, single-center study that included patients treated at the Oncology Clinic of Elias University Emergency Hospital during 2019-2023. Inclusion criteria were: age over 18 years; patients diagnosed with solid cancer; metastatic or locally advanced neoplastic disease; progression after one or more lines of treatment; recent biopsy (less than 6 months) from a progressive lesion; ECOG performance status of 0-2.

Genetic testing was performed on tumor tissue or blood samples. Genetic analysis was performed using the FoundationOne CDx panel. For tissue testing, the Foundation One CDx test was used. The tests were obtained free of charge through a grant received from ROCHE. For liquid testing, the FoundationOne Liquid CDx test was used.

5.3, 5.4 Results and discusssion

Between 2019 and 2023, 75 FoundationOne tests were performed on 66 patients. The test success rate was 80% (60 tests). Failure was most commonly caused by two main reasons: insufficient DNA in the sample, or insufficient tumor tissue in the sample, the percentage of failed tests being similar to other studies, that have shown a failure rate of approximately 20% [9].

Following the testing, we identified 254 genetic alterations, of which 173 (68%) were classified as pathogenic or most likely pathogenic. Of these, 81 were alterations of unknown significance at the time of testing, and 68 (26.7%) were identified as potentially targetable by either an approved agent or by an agent being evaluated in phase II or III clinical trials. The

number of alterations that corresponded to the type of cancer for which there were valid therapeutic options was 23 (9%), excluding results for TMB, MSI or PD-L1.

Of the 66 patients tested, 55 (83%) had at least one genetic alteration, while the remaining 11 patients either had failed tests or had no genetic alteration identified (2 patients). The most common genetic alteration was identified in the TP53 gene in 53% of successful tests, followed by KRAS mutations in 25% of successful tests and BRCA1/2 in 20%.

Of the nine patients with high TMB scores, only seven received immune checkpoint inhibitors, as one patient died shortly after the test result, and one received targeted therapy based on the decision of the multidisciplinary team based on the NGS test result. Indeed, retrospective data suggest that malignant tumors with high TMB are more likely to respond to immune checkpoint inhibitors (ICIs) [10].

At least one actionable genetic alteration was found in 37 patients, however 18 of them did not receive treatment based on the results. Of the remaining patients with at least one potential treatment mutation, 10 (15%) received targeted therapy, 5 (8%) received immune checkpoint inhibitor treatment, and 4 (6%) were enrolled in clinical trials. The percentage of 15% of patients who received targeted therapy is consistent with other data in the literature [11,12]. The number of patients who benefited from immunotherapy is slightly lower than the data presented in other similar studies, where approximately 20% of patients who were tested also benefited from treatment with immune checkpoint inhibitors [8,11,13,14]. There were only 4 patients who were included in a clinical trial based on the results of molecular testing. This low number is most likely due to the lack of clinical trials in Eastern European countries and the limited possibilities to enroll patients in clinical trials abroad, due to personal or financial reasons.

We analyzed the reasons why 18 out of 37 patients with at least one targetable mutation did not receive mutation-matched therapy, and compared them with data from the literature. The main reasons were: deterioration of the clinical condition that did not allow the administration of oncologic treatment; patient death shortly after the validation of the genetic test; impossibility of enrollment in existing clinical trials for various logistical, financial reasons, or failure to meet all inclusion criteria; stable disease under the treatment that the patient was following at the time of the results, not requiring a change in the therapeutic line at that time.

For 21 (32%) patients included in the study, NGS testing played an important role, as it influenced the decisions subsequently made by the multidisciplinary team. This influence was

evident in two main ways: treatment adjustment to target the actionable mutations identified by this testing method, and identification of acquired resistant mutations. This enabled the personalization of therapeutic strategies, ensuring that the treatment was better tailored to the specific genetic characteristics of each patient's tumor.

The median PFS for patients who received tailored targeted therapy was 10.1 months (with a 95% confidence interval of 6 to 13 months) [8], slightly lower than in other similar studies, where the median PFS for patients who received mutation guided treatment was approximately 12 months [15,16]. The observed difference may be explained by variability in patient characteristics or treatment strategies used between different studies.

5.5 Conclusions

The study highlights the challenges of integrating personalized medicine in oncology and the importance of adapting protocols and multidisciplinary teams. Genetic testing allows for precise selection of patients eligible for targeted therapies. The results of the study show that it is also useful in subsequent lines of treatment, increasing patient survival and quality of life. However, it is recommended that this testing be performed as early as possible in the evolution of the disease.

Chapter 6. Study II: The impact of BRCA mutations on the efficacy of neoadjuvant chemotherapy in advanced ovarian cancer

6.1 Introduction (Working hypothesis and general objectives): Patients with BRCA mutations have better survival if they undergo neoadjuvant chemotherapy and then cytoreductive surgery.

Specific objectives: The present study aims to verify whether the presence of a pathogenic mutation in the BRCA 1 or 2 genes impacts the effectiveness of neoadjuvant chemotherapy [17].

6.2 Materials and methods:

We conducted a retrospective, observational study, which included patients diagnosed, treated and followed up within the Department of Medical Oncology at "Elias" University Emergency Hospital in Bucharest, over a ten-year period, between January 2014 and March 2024. The study focused on patients diagnosed with ovarian cancer in advanced stages of the disease (stages III-IV). The patients in the study were diagnosed with serous ovarian carcinoma and underwent one of the two therapeutic options used for the management of locally advanced

ovarian cancer: neoadjuvant chemotherapy, followed by interval cytoreduction or primary cytoreduction, followed by adjuvant chemotherapy based on platinum salts and taxanes.

6.3 Results

A total of 79 patients were included in this analysis. In order to better understand the impact of BRCA mutations, comparisons were made between the BRCAmut and BRCAwt groups.

While evaluating surgical options, clear differences were observed between the two groups. More patients in the BRCAmut group (60%, n=15) were able to undergo primary cytoreduction, without the need for neoadjuvant chemotherapy. In contrast, in the other group without mutations, the percentage was lower, 48.7% (n=19) of patients being operated on primarily. For the remaining patients, preoperative chemotherapy was required, followed by interval cytoreduction: 51.3% (n=20) for BRCAwt and 40% (n=10) for BRCAmut. When surgery was not possible, patients underwent neoadjuvant treatment. In both groups, the majority received 3 cycles of preoperative chemotherapy (70% of BRCAmut patients (n=7) and 55% of BRCAwt patients (n=11). However, a notable difference was that more BRCAwt patients required additional cycles of treatment (up to six cycles).

When overall survival (OS) and PFS were analyzed, patients who underwent primary debulking surgery (PDS) had a better prognosis than those who were treated with neoadjuvant chemotherapy followed by interval debulking surgery (IDS). However, the differences were not statistically significant.

The OS of patients with BRCA mutations whom underwent interval cytoreduction (IDS) was greater than of those who underwent PDS. Specifically, patients who underwent primary cytoreduction had a median survival of 50 months (95% confidence interval 37.4-62.59 months), while those treated with IDS lived, on average, 71 months (95% confidence interval 54.97-87.02 months). The difference between the two groups was statistically significant, with a p-value of =0.043.

In contrast, in patients without BRCA mutations, the results differed, the median survival in the PDS group was 48 months (95% confidence interval; 44.92-51.07 months), while the survival in the IDS group was lower, at 38 months (95% confidence interval 22.54-53.45 months). In this case as well, the difference was statistically significant (p=0.03).

Analyzing the Kaplan-Meier curves for progression-free survival (PFS) for patients with and without mutations in the BRCA 1 or 2 genes, taking into account the therapeutic

management approach (PDS or IDS), it was observed that the differences were not statistically significant.

A multivariate analysis to identify factors that may influence the risk of death and disease progression was performed, indicating that the group of patients without BRCA mutations had a 3.4-fold higher risk of death compared to the BRCAmut group (HR = 3.482, 95% confidence interval: 1.982-6.520, p< 0.001). Also, the time to oncologic disease progression was shorter as well (HR=2.993, p<0.001).

No statistically significant differences were observed in OS or PFS according to ECOG performance status, disease staging (stage III vs IV), CA 125 marker value, age (>65 years or <65 years) or number of chemotherapy cycles performed in the neoadjuvant setting (3 vs 6 cycles).

On the other hand, it was proven that the degree of resection had a significant impact on survival. The group of patients who had complete resection (R0) had a 2.3-fold lower risk of death than the group who had residual disease after surgery (R1), (HR=2.332, 95% confidence interval:1.897-2.882, p<0.001). Also, the R1 group had a higher probability of oncological disease progression, HR=1.684, 95% confidence interval:1.415-2.045, p=0.004.

In order to determine if there was any difference in OS or PFS between the types of management approached (IDS vs PDS), a Cox regression analysis was performed and it was observed that there were no significant differences between the two strategies. Both surgical approaches can be good therapeutic options, as long as the particularities of each patient are taken into account [17].

6.5 Conclusions:

These data highlight the importance of BRCA mutation status in the therapeutic management of ovarian cancer. The results of this study show that the survival of patients with BRCA mutations was comparatively higher in the group that received neoadjuvant chemotherapy followed by cytoreductive surgery than in the case of patients who underwent primary surgery, although there were no significant differences in PFS between the two therapeutic strategies. On the other hand, BRCAwt patients had a better survival in the group that received primary cytoreductive surgery followed by chemotherapy. It should be noted that the increase of the number of neoadjuvant chemotherapy cycles beyond 3 does not bring any benefit on survival or resectability. Of course, the selection criteria for one of the described therapeutic strategies should not be based solely on BRCA mutation status. It is important to take into account not only the molecular or genomic characteristics of the tumor, but also the

clinical and biological characteristics, accessibility to treatment (approvals and indications in Romania), patient preferences and quality of life. A well-established algorithm must be established for each patient when deciding on the therapeutic strategy. These aspects require further research, especially in relation to the genomic profile of each patient.

Chapter 7. Study III: The role of KRAS mutations in colorectal cancer-associated thrombosis

7.1 Introduction (Working hypothesis and general objectives): KRAS mutation, through certain pathophysiological mechanisms, could increase the risk of developing thrombosis among patients with colorectal cancer.

Specific objectives: This study aims to verify whether there is any relationship between KRAS mutation status and the occurrence of thromboembolic events, taking into account other variables, and whether the occurrence of thrombosis impacts survival [18].

7.2 Materials and methods:

This paper is a retrospective study that included patients who were treated and followed up in the Medical Oncology Department of the "Elias" University Emergency Hospital in Bucharest, over a ten-year period, between January 2012 and October 2022. The study selected patients diagnosed with colorectal cancer, who had KRAS testing performed.

For patients diagnosed with stage II or III colorectal cancer, surgery was initially performed, followed by, if indicated according to the guidelines, adjuvant chemotherapy or oncological follow-up. For metastatic patients, combination chemotherapy based on fluoropyrimidines was initiated, to which a targeted treatment was added depending on the location of the tumor (left or right colon) and on the RAS mutation status, prescribing either bevacizumab, cetuximab or panitumumab for those who did not have a mutation in the respective gene.

7.3 Results:

Of the 130 patients included, 45 (34%) developed some form of venous thrombosis, of which deep peripheral venous thrombosis represented the majority of occurences. 27 patients who were administered anti-VEGF agents had activating KRAS mutations, while the remaining 5 patients were classified as KRAS wild type, out of the total of 32 (24%) who received this type of treatment.

The median overall survival was 55 months (SD± 7.66, 95% confidence interval 39.9-70.0) for patients who developed venous thrombosis, whereas for those without thrombosis, the median was 68 months (SD± 14.1, 95% confidence interval 40.33-95.66).

The survival rate at 12-14 months between the groups that developed thrombosis and those without thrombosis was not significantly different. However, it is observed that the difference in survival increases between the two groups over time. Thus, the survival rate at 24 months in the thrombosis group was 78% compared to 90% in the other group. At 3 years, the OS is 65% and 82%, respectively. Over time, the survival of patients who developed thrombosis remained lower than that determined among patients without thrombosis.

A univariate Cox regression showed that the KRAS mutation did not represent a major risk for overall survival (HR = 1.72, p = 0.23). The univariate analysis showed that advanced age (over 65 years), poor performance status (ECOG score greater than 2), metastatic stage and a Khorana score greater than or equal to 2 can impact survival. However, evaluating the data from the multivariate Cox regression analysis, we observe a significant influence on overall survival mainly by the ECOG performance status with a HR = 1.32 and p = 0.01, age (HR = 1.38, p = 0.05) and Khorana score (HR = 3.13, p = 0.02).

The presence of KRAS mutation caused a significant decrease in the median time to the development of venous thrombosis, from 48.2 months (SD ± 17.03, 95% confidence interval 14.60-81.39) for the entire group to 12 months (SD ± 3.51, 95% confidence interval 5.11-18.88) for the cohort with KRAS mutations. For those without mutations, the median was not reached at the end of this study.

A univariate regression analysis was performed to identify the correlation between KRAS mutation and increased risk of thrombosis, resulting in an odds ratio (OR) of 2.75 (95% confidence interval: 1.55 – 4.90, p = 0.001) for the development of thrombosis in general. For deep vein thrombosis and pulmonary thromboembolism, an odds ratio of 3.12 (95% confidence interval: 2.53 – 5.03, p = 0.002) and 1.75 (95% confidence interval: 1.23 – 3.75, with a p = 0.045) were determined, respectively.

After performing a logistic regression analysis, a statistically significant relevance was observed between the KRAS mutation status and the incidence of deep vein thrombosis or pulmonary thromboembolism, even after adjustment for Khorana score, administration of anti-VEGF monoclonal antibody and clinical stage. The chance of developing deep vein thrombosis is 3 times higher for patients with a KRAS mutation than for those without the mutation. The

chance of developing pulmonary thromboembolism is lower than that for developing DVT, but it is 1.6-1.8 times higher for patients having the mutation, with a statistically significant p.

An analysis was also performed to identify the probability of developing thrombosis over time, in relation to the presence or absence of KRAS mutations. It is observed that at 6 months the probability of developing thrombosis for KRASmut patients was 8%, while for those with KRAS wild type it was lower (7%). At 24 months, in the first group it was almost 66%, respectively 22%.

Regarding the well-known risk factors for the development of thrombosis, a multivariate Cox regression analysis was performed and did not suggest any association between bevacizumab treatment or cancer staging and VTE. However, ECOG performance status greater than or equal to 2, male gender, Khorana score greater than 2 and KRAS mutation were strongly associated with the development of thrombosis, with $p<0.05$ [18].

7.5 Conclusions:

The results of the present study show that KRAS mutation among patients with colorectal cancer is an independent factor for the occurrence of VTE. Although there are conflicting opinions regarding this association, other factors must be taken into account, such as those related to the tumor microenvironment, clinical or biological factors, as they may contribute to the coagulation process. The link between genetic mutations in solid tumors and thrombosis development emphasizes the need for personalized strategies for thromboprophylaxis.

8. General conclusions and personal contributions

The first study aimed to highlight the role that NGS testing can have for patients diagnosed with metastatic solid tumors, after having progressed on one or more lines of standard oncological treatment and for whom therapeutic options in subsequent lines were limited. NGS testing proved that, excluding failed tests, 96.5% of patients had at least one genetic alteration and 56% had a pathogenic mutation that could be targeted either using an approved therapeutic agent or using an agent under evaluation in phase 2 or 3 clinical trials. A fairly large number, considering that the population included in the study did not have or had limited subsequent therapeutic options, according to national guidelines. It is also well known that the response to subsequent lines of treatment is very low, and the advantage of targeted therapy in these situations is significant, with better treatment response rates than chemotherapy. In the study

group, for one third of patients, the change in oncological treatment was influenced by the results of multigenic testing. The average PFS of these patients was 10.1 months, an encouraging value considering the characteristics of the group. The first advantage of this study was that NGS testing added considerable value for metastatic patients with progressive disease after several lines of treatment, in guiding and personalizing oncological therapy, significantly contributing to improving their survival and quality of life.

For almost one third of patients, it was not possible to administer a personalized treatment for several reasons: deterioration of the clinical and biological condition that did not allow the administration of oncologic treatment, patient death, the impossibility of enrolling in clinical trials for logistical, financial reasons or failure to meet all inclusion criteria, the inability to conduct the respective clinical trials in Romania or non-reimbursement of drugs in Romania for the location of the primary tumor.

A second advantage of this study was that it highlighted the importance of performing multigene testing as early as possible in advanced disease, in order to allocate time to find the best therapeutic options for subsequent lines of treatment.

Another important factor highlighted in this study was the 20% failure rate of testing, which was due to the inadequate amount of tumor DNA in the submitted sample, to inadequate fixation technique, or to insufficient material. This leads to the need for better interdisciplinary collaboration between clinicians regarding samples collected for NGS, regarding biopsy and fixation techniques.

Given all the information obtained from this analysis, personalized medicine is promising in the practice of medical oncology, but still requires improvement. It is clear that there is a benefit for a certain group of patients and it is crucial to develop a better algorithm for their selection. Testing should be performed as early as possible in the therapeutic course, ideally at the time of diagnosis of advanced disease. In addition, patients should have rapid access to evidence-based clinical trials. Access to trials must be improved, especially in developing countries, where it is often limited due to costs or the lack of the necessary infrastructure for their implementation.

The second study demonstrated that BRCA mutation has an impact on survival depending on the therapeutic and surgical management used among patients with stage III/IV ovarian cancer. The OS of those with BRCA mutation who received neoadjuvant chemotherapy followed by IDS was 21 months longer than the OS of those that had a primary debulking surgery. In contrast, patients without BRCA mutations had a better OS if they underwent

primary cytoreduction followed by adjuvant treatment. It was also observed in the IDS group that patients with a BRCA mutation had a 1.8-fold higher survival benefit compared to wild-type. Of course, this may be due to the better response to chemotherapy, but also to the treatment with PARP inhibitors, which has changed the therapeutic management in these types of cancers. No major difference in survival was observed between FIGO stages III and IV. These facts reinforce that mutations in the BRCA genes play an essential role in the prognosis of patients diagnosed with ovarian carcinoma, and have a stronger impact than other clinical factors such as staging. The major advantage of this study was that it demonstrated that, for patients with a BRCA mutation, NACT+IDS reduces morbidity, facilitating a less extensive surgical intervention. Also, the presence of this mutation was associated with higher complete resection rates.

Another important aspect of this study was that it proved that increasing the number of neoadjuvant chemotherapy cycles does not bring any benefit to overall survival. It is therefore important that after each evaluation of treatment response, usually performed every 3 cycles, the case is re-discussed with expert surgeons in the field, in order to establish the opportunity to perform interval cytoreduction. This approach helps avoid the accumulation of chemotherapy-related toxicities and the emergence of treatment resistance.

All these results reveal the benefits that neoadjuvant therapy adds for patients with BRCA mutations and that depending on them, individualized decisions can be made for the management of the disease, practically achieving the objective of this research.

The results of the third study demonstrated that there is a correlation between the presence of KRAS mutation and the occurrence of DVT/VTE, which was statistically significant, regardless of other variables such as the Khorana score, Bevacizumab administration or clinical staging. At 1 year, the occurrence of DVT was 31% higher in the KRAS mutant group compared to the wild-type group. Basically, contrary to expectations, the relationship between KRAS mutation and the occurrence of thrombosis was not influenced by bevacizumab therapy, as previously suggested in the literature. As seen in other trials, in this research, the survival of patients that developed thrombosis was inferior to those without thrombotic events. Given these data, the objectives of this study were achieved. The study showed that this mutation is an independent factor in the development of VTE in patients with colorectal cancer, but other factors that may contribute to this process should also be taken into account.

Given the results of this PhD thesis, I recommend the following for clinical practice:

- Apart from tumors for which NGS testing is currently reimbursed in Romania and for which the optimal timing of its performance is well established, testing should be performed as early as possible in the evolution of the disease and especially for rare tumors, or for those that do not have many therapeutic options in subsequent lines.
- Better collaboration between clinicians, pathologists and geneticists to reduce the failure rate of multigene testing.
- Based on these results, a greater number of patients should be included in clinical trials.
- Expanding the network of clinical trials in Romania and facilitating easy access to them for all eligible patients.
- Establishment of molecular multidisciplinary boards.
- Assessing BRCA status at diagnosis, for epithelial ovarian cancers, as the presence of this mutation may help in the selection of patients for neoadjuvant therapy.
- Establishing surgical resectability criteria that also take BRCA status into account, considering that the presence of this mutation is associated with a higher complete resection rate.
- Mandatory surgical reevaluation after 3 cycles of neoadjuvant therapy in ovarian cancers.
- Including KRAS mutation in thrombotic risk assessment scores and tailoring thromboprophylaxis based on individual risk profiles.

In conclusion, genetic profiling of tumors not only brings an advantage in the personalized selection of systemic therapies, but can also contribute to establishing a better therapeutic plan that includes surgery, prophylaxis and management of adverse reactions.

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1. **Popa AM**, Stejeroiu MA, Iaciu C, Olaru M, Orlov Slavu C, Parosanu A, Stanciu IM, Pirlog C, Pavel S, Nitipir C. Correction: Role of Tumor Molecular Profiling With FoundationOne®CDx in Advanced Solid Tumors: A Single-Centre Experience From Romania. Cureus. 2025 Feb 14;17(2):c211. doi: 10.7759/cureus.c211. Erratum for: Cureus. 2023 Dec 18;15(12):e50709. doi: 10.7759/cureus.50709. PMID: 39958407; PMCID: PMC11829126. Impact factor: 1
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2. **Popa AM**, Cotan HT, Iaciu CI, Nitipir C. The Impact of BRCA Mutation on the Efficacy of Neoadjuvant Chemotherapy in Advanced Ovarian Cancer. Chirurgia (Bucur). 2024 Dec;119(6):687-700. doi: 10.21614/chirurgia.3055. PMID: 39737823. Impact factor: 0.8. ESCI
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3. Emilescu RA, Jinga M, Cotan HT, **Popa AM**, Orlov-Slavu CM, Olaru MC, Iaciu CI, Parosanu AI, Moscalu M, Nitipir C. The Role of KRAS Mutation in Colorectal Cancer-Associated Thrombosis. Int J Mol Sci. 2023 Nov 29;24(23):16930. doi: 10.3390/ijms242316930. PMID: 38069251; PMCID: PMC10707331. Impact factor: 4.9, SCIE, ISI
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     Chapter 7 pages 94-113