Case Report

# Genomic Profiling - A Need for Clinical Decision? – Case Reports

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**ABSTRACT:** Cancer is still an important health issue worldwide due to increased incidence and mortality. Personalized medicine is the future of cancer treatment. Development in technology improved technical skills in DNA/RNA sequencing. NGS technology in solid-tumor samples can describe DNA or RNA analysis by including the entire genome to detect clinical relevant mutations. Genetic results may be considered having a dynamic impact because of heterogenous molecular alterations depending of time and treatment influence. We conducted a retrospective study of all NGS tests made in the last five years for the patients from 'Sf. Nectarie' Oncology Center, Craiova, Romania. We selected three relevant clinical cases where NGS analysis was performed and the results changed the perspective of the clinical decision. Our aim is to evaluate the importance of NGS results in clinical approach. Although medicine known an important development during the last decades, only a few patients can benefit of advanced personalized treatments. It is still hard to identify the alterations or gene mutations because of genetic tests are not easily available and only a small proportion of patients carries genetic alterations.

***KEYWORDS:*** *NGS (Next Generation Sequencing), digestive cancer, genetic mutations.*

## Introduction

Based on GLOBOCAN data from 2020, there were diagnosed approximately 19.3 million new cases and 10 million cancer deaths. Female breast cancer occurred the most commonly diagnosed cancer, followed by lung, colorectal, prostate and gastric cancer [1].

Despite the development of prevention, screening programs and also of patient management or treatment, the incidence of cancer is continuously increasing, being the second leading cause of death worldwide [2].

Colorectal cancer is the first most common cancer, with an estimated number of approximately 880 000 deaths in 2018 [3].

Prognosis and treatment response is based on various risk factors, including genetic mutations and genetic susceptibility [4].

According to National Cancer Institute's (NCI's) Surveillance, Epidemiology, and End Results (SEER) program and the Centers for Disease Control and Prevention's (CDC's) National Program of Cancer Registries, in the United States of America (USA) the incidence of advanced newly diagnosed cases is higher in past decades [5].

With a 5-year survival rate of approximately 10% in USA, pancreatic cancer is one of the most common mortality causes of cancer [6].

Although is already recognized that pancreatic cancer mostly has chemoradiation resistance, BRCA mutation disease treated with PARP inhibitors may benefit of a valued management. This aspect is actually continuously developed through clinical trials in order to improve patient outcomes [7].

Personalized medicine is the future of cancer treatment. Development in technology improved technical skills in DNA/RNA sequencing [8].

Contemporary forensic sciences are actually based on DNA analysis. Starting from Sanger sequencing of DNA extracting technique, Next generation sequencing (NGS) is now one of the most used tools to describe the molecular field [9].

In the past few years, NGS technologies had known a rapid development progress in order to understand genetic characteristics of tumor cells [10].

Cancer genome is an extremely generous research domain that changed carcinogenesis and also the futures of therapeutic and diagnosis instruments. NGS technologies provide to sequence a very large number of genes with high throughput and speed [11].

The genetic and epigenetic description through NGS technology changed not only therapeutic management, but also the clinical diagnosis overview [12].

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NGS technology in solid-tumor samples can describe DNA or RNA analysis by including the entire genome to detect clinical relevant mutations. There are a lot of benefits by using this type of method including deeper investigation of tumor heterogeneity, quantitative and sensitive detection of genomic alterations and an also the posibility of concomitant screening of multiple genes in different samples [13].

Genetic results may be considered having a dynamic impact because of heterogenous molecular alterations depending of time and treatment influence [14].

## Aim

We conducted a retrospective study of all NGS tests made between January 2018 and May 2023 for the patients from 'Sf. Nectarie' Oncology Center, Craiova, Romania.

In the mentioned period of time were performed NGS analysis for patients diagnosed with lung, breast, colorectal, gastric, pancreatic, ovarian cancer, but also hepatocellular carcinoma, cholangiocarcinoma and soft tissue sarcoma.

From the total of 324 NGS analyses, we selected only those patients with digestive cancers.

The aim of our study is to evaluate the importance of NGS results in clinical approach.

From the 65 patients that performed NGS analysis, we highlight three relevant clinical cases that had benefit of another therapeutic management based on genomic characteristics.

## Case presentations

### Case 1

A 55-year-old female, after investigating a sever abdominal pain that radiated in the back for more than three months, loss of appetite and unintended weight loss, through a CT scan, had a high suspicion of locoregional pancreatic cancer.

The biological investigation revealed increased values for tumor markers carcinoembryonic antigen (CEA) 35.77ng/ml and CA19-9 440.9U/ml before any therapeutic strategy.

All other labor tests had normal values.

Due to all investigations that did not revealed distant metastases, subtotal splenopancreatectomy was made.

The histopathological (HP) examination showed a pancreatic adenocarcinoma, moderately differentiated, with lymphovascular and perineural invasion-pT3pN2, four pathological lymph nodes with tumor cells from 24 lymph nodes examinated.

Because of a fluid accumulation near the site of the surgical incision detected with postoperative CT scan, systemic treatment was delayed.

Six cycles adjuvant chemotherapy FOLFIRINOX (leucovorin, 5-fluorouracil, irinotecan, oxaliplatin) was administrated.

Although the imaging examinations made after adjuvant treatment did not showed any oncological lesions, six months later, recurrent locoregional disease and multiple liver metastases were established during a MRI.

The biological investigation also showed increased tumor markers CA19-9=79.74U/ml, CEA=9.56ng/ml.

Systemic palliative treatment with erlotinib and gemcitabine as first line for metastatic adenocarcinoma was initiated.

The results were positive with partial response (PR) on CT scan and decrease of tumor markers values.

The patient was treated for 9 months until progression disease (PD) thorough peritoneal metastases was established. 9 cycles of palliative chemotherapy FOLFIRI (leucovorin, 5-fluorouracil, irinotecan) were administrated, well tolerated.

The patient accused pelvic severe pain for 1 month, while anti-inflammatory medication had no significant benefit.

The gynecological examination raised the suspicion of a bilateral ovarian carcinoma and peritoneal carcinomatosis with anterior rectal and rectovaginal mucosal infiltration.

The CT scan also evaluated PD.

Because of the differential diagnosis between primary ovarian cancer and Krukenberg metastases from the pancreatic adenocarcinoma, laparoscopic biopsy with HP examination of ovarian tissue was made.

It confirmed Krukenberg tumors and third line palliative chemotherapy was initiated with albumin-bound paclitaxel and cisplatin.

Only 5 cycles of double combination was administrated, due to peripheral neuropathy to cisplatin.

The patient continued albumin-bound paclitaxel, with PR.

After 12 administration of monotherapy, the imaging evaluation showed PD with new liver metastases and ascites.


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Due to clinical oncological guidelines, there were no more treatment options, although Eastern Cooperative Oncology Group (ECOG) performance status of the patient was good.

We decided in tumor board to make NGS testing from the Krukenberg tumor.

The result showed stable microsatellite status, TMB 3 Muts/Mb and BRCA2 gene alteration.

The multidisciplinary commission decided to initiate PARP-inhibitor Olaparib.

Progression free survival during targeted therapy was 10 months.

As adverse events during treatment with Olaparib we report cutaneous rush, asthenia, mild nausea and grade II anemia.

No serious adverse event was reported.

## Case 2

A 65-year-old female, after several months with alternating constipation and diarrhea, decided to undergo a colonoscopy.

She was diagnosed through biopsy with sigmoid adenocarcinoma.

After completing the investigation in order to instead a diagnosis, the surgery was done.

The postoperative stage was locoregional advanced sigmoid colon cancer pT4apN2b.

Because of the advanced stage and good performance status, the adjuvant chemotherapy CAPOX was well tolerated.

During surveillance, 3 months after the first CT scan showed no oncological lesions, tumor markers values significantly increased, as in Figure 1.

An abdominal ultrasound (US) was performed and a huge tumor mass included in the left ovary was detected.

The MRI confirmed the US aspect.

Bilateral anexectomy with a suspect mesenteric lymph node was made.

Although the patient was treated with standard adjuvant chemotherapy, during the surveillance, ovarian metastasis and distant lymph nodes were diagnosed with magnetic resonance imaging (MRI).

After bilateral adnexectomy, which confirmed metastatic disease from the primary adenocarcinoma, the patient was treated with first line chemotherapy for metastatic disease FOLFIRI and targeted therapy Bevacizumab for mutated KRAS.

Six months later, there was radiological (liver and peritoneal metastasis) and biochemical progression disease and the patient underwent second line chemotherapy, with a progression free survival only of 8 months.

The NGS was tested from fresh peritoneal biopsy and the tumor mutational burden was 16 Muts/Mb.

Also, high microsatellite instability was detected. The patient benefit of a clinical trial based on NGS results.

Through the clinical trial, the patient was treated with immunotherapy.

During the radiological evaluations, at the beginning, it was a pseudoprogression common for this treatment, followed by a decrease in tumor burden, as in Figure 2.

At this moment, CT and MRI confirm stable disease (Figure 3).

After 11 months of immunotherapy, the patient has stable disease, with no clinically important adverse events.

Only a grade II elevated transaminases, remitted with symptomatic and corticoid treatment.

The quality of life was significantly improved.

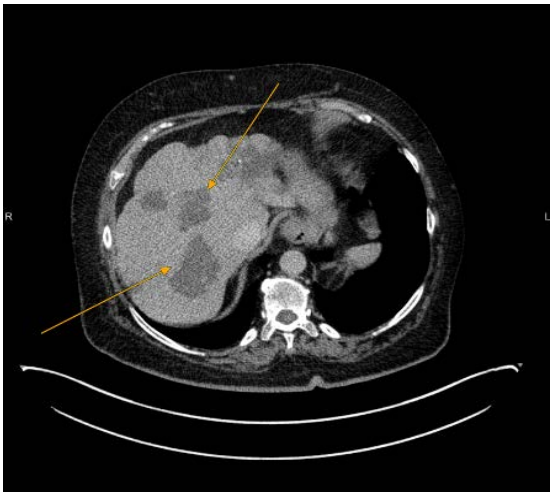

***Figure 1. Target lesions-baseline CT scan.***

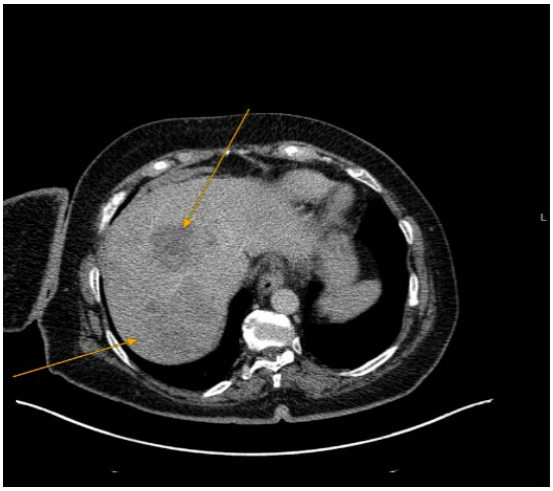

***Figure 2. CT scan. Pseudoprogression during immunotherapy.***

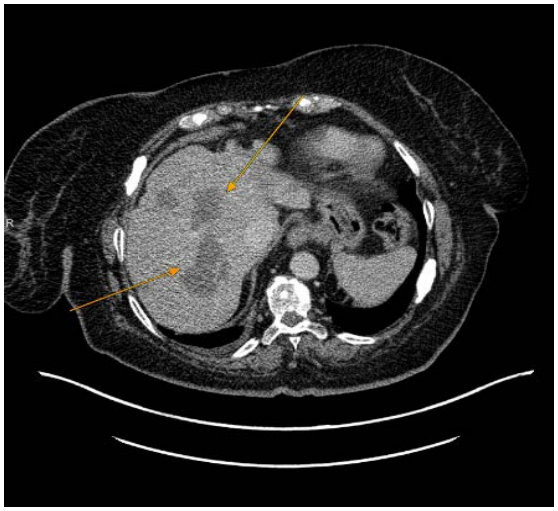

***Figure 3. CT scan.***
***Partial response after 10 weeks.***

## Case 3

A 73-year-old female accusing severe abdominal pain and cramping sensations, constipation started within three days, without any other symptoms related before, presented in emergency department in a county hospital.

After the clinical and biological examination and the CT imaging (Figures 4 and 5), bowel obstruction through rectal tumor stenosis was diagnosed.

She was immediately operated on and a laparoscopic protective colostomy.

During the hospitalization, the colonoscopy described an ulcerated, vegetative rectal tumor, that obstructed 70% of the rectum.

A biopsy was made, and the HP examination related to rectal adenocarcinoma, well differentiated.

There were no distant metastases established on imaging investigations.

Some surgery complications occurred and the patient needed an extended hospitalization.

The patient presented in the oncology department 2 months later.

We recommended to repeat the CT scan before the oncological treatment that revealed a PD with new liver metastasis of 3.2/3.1cm in the fifth segment (Figures 6-8).

Biological evaluation showed increased tumor markers values CEA=6.41ng/ml CA19-9=28.05 U/ml at baseline (Figure 9).

The chemoradiation treatment was established.

The patient was treated with CAPOX (capecitabine, oxaliplatin) and due to molecular evaluation, targeted therapy with vascular endothelial growth factor (VEGF) Bevacizumab.

A total irradiation dose of 50,4 Gy (1.8 Gy in 28 fractions) on the tumor volume and regional lymph nodes was made. 6 weeks after irradiation, a MRI revealed a dissociated response.

The rectal tumor decreased from 9.9cm to 8.5mm, but the liver metastasis increased from 3.2/3.1cm to 10.3/6.6cm.

Tumor markers values also correlated with PD as in Figure 9.

The tumor board decision was NGS testing and starting second line treatment for metastatic disease.

NGS result showed KRAS G12C mutation an MYC amplification.

Due to a clinical trial, the patient benefits from a new molecule KRAS G12C inhibitor and the standard second line therapy.

The treatment is still ongoing and the patient already has PR.

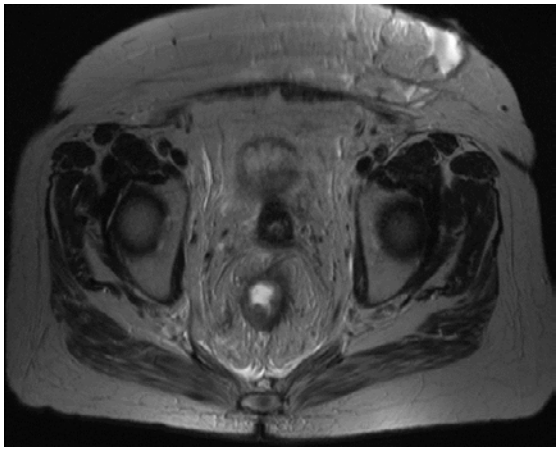

***Figure 4. Axial T2-hypointense T2 slight thickening of the rectal wall, no lymphadenopathy or invasion of the mesorectal fascia.***

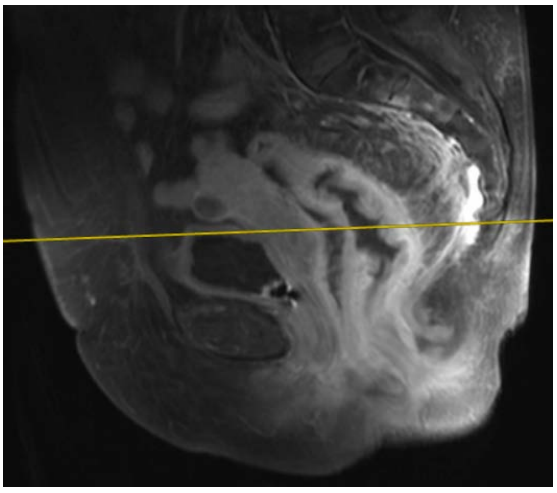

***Figure 5. Sagittal T1 FS-homogenous enhancement of the rectal wall.***


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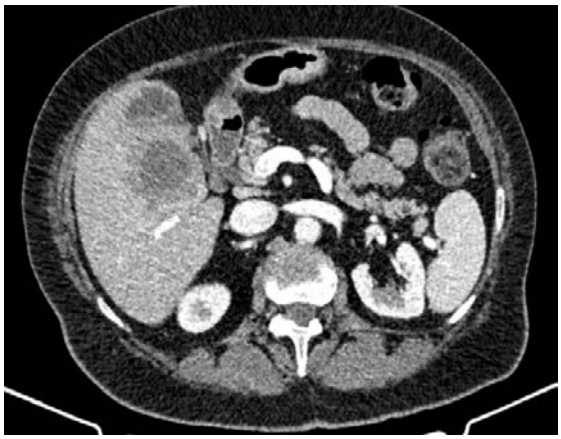
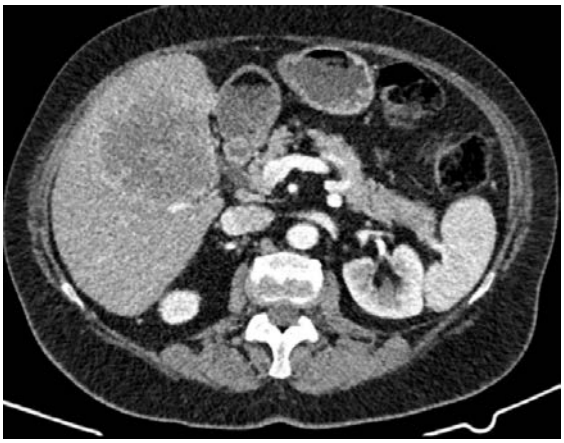
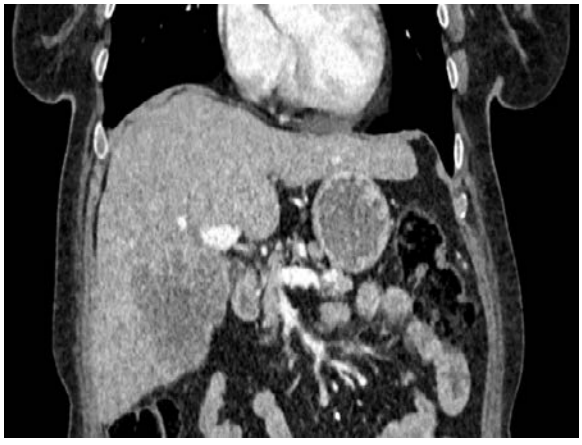


*Figure 6. CT axial plan, arterial phase-liver metastasis enhancing less than surrounding liver centrally with typically peripheral enhancement following contrast localized in the right hepatic lobe.*

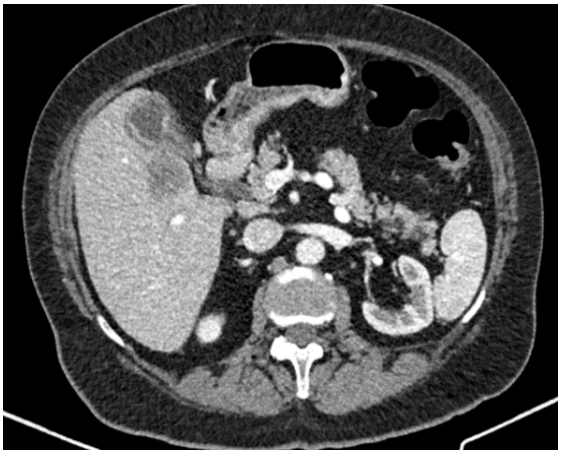


**(A)**

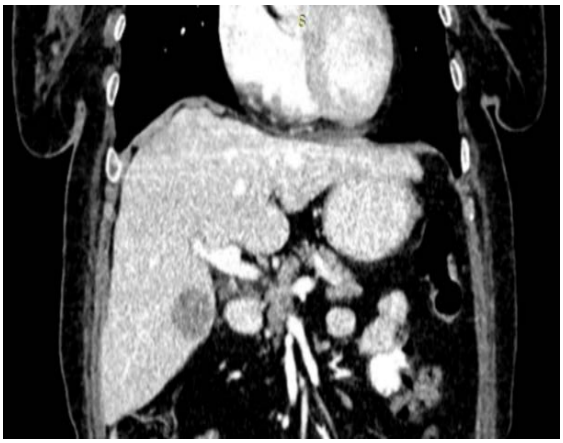


**(B)**

*Figure 7. A-CT axial and B-coronal scan of the abdomen, arterial phase-dimensional progression of the hepatic metastasis localized in the right lobe (no new metastases).*



**(A)**



**(B)**

*Figure 8. A-CT axial and B-coronal scan of the abdomen, arterial phase-Important dimensional regression of the hepatic metastasis localized in the right lobe (no new metastases).*

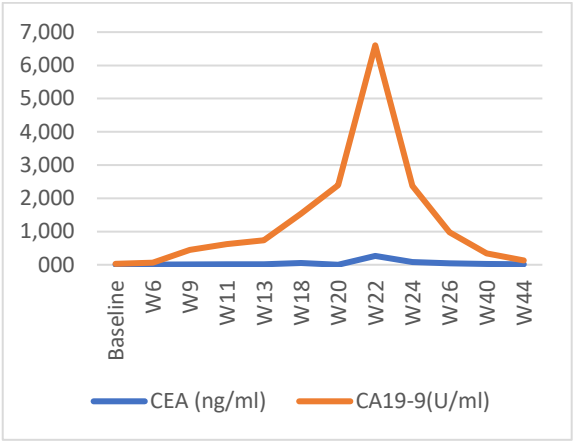



*Figure 9. CEA and CA19-9 levels during the treatment.*

## Discussion

CRC represents a heterogenous genomic disease based of the multiple different alterations that may occur such as genomic rearrangements, point mutations or gene fusions.

These all genetic changes may have an important contribution to the disease progression, metastasis or even resistance to treatment [15].

While many cancer research centers study to explore NGS technology at its best, especially in CRC, there is still a limitation of data in the field.

However, it is important to understand that for more clinical application additional studies are required [16].

After breast and ovarian cancer, BRCA gene mutation is more common in pancreatic cancer than other malignant tumors.

In addition, because of the heterozygous nature of this pathology, it is important to identify those subgroups of patients with molecular and biological specific characteristics in order to apply personalized treatment [17].

Shao et. al. realized a recent meta-analysis combined with a literature review that highlights a prevalence in pancreatic cancer of germline BRCA1 mutation 1.1% and BRCA2 mutation 4.1%, respectively [18].

Because of the relevant benefits of PARP inhibitors in breast or ovarian cancer with germline BRCA mutation, FDA approved Olaparib also as maintenance treatment in metastatic pancreatic cancer [19].

Although medicine known an important development during the last decades, only a few patients can benefit of advanced personalized treatments.

It is still hard to identify the alterations or gene mutations because of genetic tests are not easily available and only a small proportion of patients carries genetics alterations [20].

Colorectal cancer (CRC) is still a worldwide heath issue because of the high incidence and mortality.

KRAS is one of the most usually identified oncogenes in different types of cancer.

KRAS glycine-to-cysteine amino acid substitutions at codon 12 (KRAS G12C) remains a domain that must be further studied because of the currently unknown mechanism of acquired resistance to treatment.

In CRC KRAS G12C mutations is detected in approximately 3% and is more common in lung adenocarcinoma 13%, respectively [21].

There are several clinical trials that study the opportunity of treating KRAS G12C in cancer. Its particularity of resistance is based on genetic, molecular and cellular mechanisms in the tumor microenvironment [22].

## Conclusions

NGS use in clinical practice is not usually performed based on its costs.

This analysis represents a tool to describe tumor mutated genes, epigenetics and trascriptomics that influenced the perspective of oncological approach in the past years.

Due to the advantage of detecting billions of nucleotides, this technology changed in many oncological cases the clinical decision.

Tumor molecular profiling remains a complex domain that may be taken into consideration for diagnosis, prognosis or treatment options in cancer.

The major challenge is finding the best way to integrate NGS analysis in patient care management.

### Author contributions

Ana-Maria Ciurea and Michael Schenker equally contributed to this study and thus share first authorship.

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### Conflict of interests

None to declare.

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