

NGS in a patient with mCRC- can we do more?

Jovana Bjelanović,
medical oncologist,
GH “Đorđe Joanović”, Zrenjanin

Patient: K.F. 1948. year of birth

OP 22.08.2018. Resectio sigmo-rectalis, T-T anastomosis.

PH:Adenocarcinoma intestini coli invasive.G2;LNN 4/16.DUKES C. PNI+ R0

unmutated KRAS and unmutated NRAS

HT cum Capecitabine VIII c. until 28.03.2019.

17.12.2021. Nephrectomia l. sin. pp Pyelonephrosis chr.

14.02.2022 PET/CT Meta pulmonum , Lymphadenomegalia retroperitonealis.; Laesio focalis S8 hepatis

example of how treatment can go

I LINE: HT cum Folfox VI c. until do 7/6/2022 RM
HT cum Folfox II c. until 10/27/2022.RM
HT cum FOLFOX/Cetuximab V, DeGramont/Cetuximab I untill 2/22/2023.
HT cum DeGramont/Cetuximab VI until 7/27/2023. PM

II LINE: HT cum Folfiri VI c. until 12/8/2023. PM

III LINE: HT cum Campto/Erbitux XVIII c. until 11/30/2024. SD
HT cum Campto/Erbitux IV c. until 2/21/2025. PM

IV LINE: HT cum Mitomycin/Capecitabin lic. Untill 7/03/2025. PM.

V LINE: HT cum Folfiri VI until 11/25/2025. (reinduction)

VI LINE : Th c Lonsurf I

VII LINE: Th c Fruzaqla II PM until June

more than four years in total for metastatic disease
example of how treatment can go but what if!!!???

...what if!!!???

Patient: S.Ž. vear off birth 1973.

Personal history: no chronic diseases

Familly history: Father bladder cacer, Mother vulvar cancer

12.10.2023. CT of the chest, abdomen and pelvis: meta hepatitis and tumor of the rectum (In S4 of the liver, a hypodense change **26x30mm**, v.s. sec. deposit. Changes of similar characteristics are also seen in **S5 u at the level of the v.porta , in S6 subcapsular 7mm**. In the region of the rectum mass (can be seen primarily of infiltrative etiology

MR abdomen 20.12.2023. focal liver changes IV segment 3.7x2.8 cm, characteristic of sec. deposit, with **perifocal microcystic lesion co-capsular 8mm,** microcyst (less likely cystic sec.deposit) **and VIII segment 3x2cm, sec.deposit., with minor focal change medially subcapsular in VI seg. 9x7mm, sec.deposit,**

Colonoscopy was done 10/25/2023. up to 15 cm, where an obturative mass was found, biopsied

Ph report: rectal mucosa, adenoma villosum intestini crassi cum dysplasia low grade et high grade

Colonoscopy was repeated on 11/15/2023. rebiopsied large villous tumor mass of predominantly adenoma structure

Ph report: the finding corresponds to a tubulovillous adenoma of the colorectal mucosa with high-grade dysplasia

Bleeding and obstructive tumor

OP 3/20/2024. Resectio recti ant. superior sec. Hartmann

PH report: **Adenocarcinoma mucinosum invasive intestini crassi HG2
pT3 N2a(In 6/17) HG2 LVI+ PNI+**

Mutated KRAS and Wild type NRAS

29.4.2026. CT of the chest, abdomen and pelvis: PM (Progresio metaheptis)

MR of the abdomen 5/14/2024. liver with **four focal changes** that have the characteristics of sec. deposit, **increased in size:**
the largest in S4 with a diameter of 58 mm, (was 37 mm), the second in S8/S5 near the hilus with a size of 45 mm (was 30 mm), the third in S6 diameter 17 mm (was 9 mm) and fourth in S5 diameter 7 mm

5/22.2025 OK Indicated **conversion bio/ht** according to the Folfox 4 protocol with a biological drug

I LINE:

TH cum Folfox4 VI/Bevacisumab IV until 9/22/2024. PR

(reduction of volume and size of metastases in the liver-the largest in S4 has a diameter of 36 mm (was 58 mm), the second in S8/S5 near hilus measuring 29mm (was 45mm), third in S6 measuring 8mm (was 17mm) and fourth in S5 diameter 5 mm (was 7 mm))

TH cum Folfox IV / Bevacisumab IV until 12/26/2024. SD
(in the liver)

Polyneuropathia medicamentosa

Reactio allergica medicamentosa susp-OXALIPLATIN

OP 4/23/2025. Exploratio (intraoperatively verified targets in both lobes of the liver that are **in close contact with blood vessel, surgical treatment wasn't possible**)

I LINE (STILL):

TH cum Folfiri/Bevacizumab XII (VI+VI) until 1/17/2026. SD

TH cum FOLFIRI/Bevacisumab VI until July 2026. PM

7/16/2026. NMR of the pelvis: NSD

7/23/2026. NMR of the abdomen : **the confluent change affecting S3/S4/S5/S7** is significantly larger overall dimensions currently **111x72x77mm** (previously measure 89x43x50mm). The change **covers like a muff the branch of c. right lobe portswith suspected infiltration**. As a result, the dilatation of the segmental intrahepatic bile ducts with a diameter of up to 5 mm .PM is seen
7/23/2026. CT of the chest: NSD

10.8.26. OK: Indicated continuation of therapy according to the **Folfox4 protocol in VI series (reinduction) with increased premedication and caution due to suspected allergic reaction**

Icterus

Percutaneous bile duct drainage was performed

She is about to start the therapy

NEXT therapy LINE for our patient:

FTD–TPI (Trifluridine-tipiracil, marketed as Lonsurf) – Bevacizumab is recommended for patients who have previously received fluoropyrimidines, oxaliplatin, irinotecan and biologics, if available [I, A] **BUT she dos not have lung disease dominantly- indication of our health insurance fund!!!**

Regorafenib (marketed as **Stivarga**) is recommended after FTD–TPI–bevacizumab due to the better efficacy of the latter in the third line [I,A].

mOS 2 months longer! vs BSC!! (CORRECT and CONCUR tral)

Fruquintinib (marketed as **Fruzaqla**) is recommended for patients who have previously received fluoropyrimidines, oxaliplatin, irinotecan and biologics, **after progressing on FTD–TPI or regorafenib** [I,A] (FRESCO-2 trial , mOS 3month longer vs BSC!

Reinduction of the initial induction therapy may be optionally recommended after second-line therapy for patients who derived clinical benefit during firstline ChT (often oxaliplatin) and progressed while off treatment [IV, C].

Salvage ChT cannot be recommended

This is the moment where NGS could play an important role!?

It was done October 2025. on patent own arrangement

Tražena analiza: CGP-sveobuhvatno genomsko profilisanje tumora

IZVEŠTAJ br. I-01-CGP-13

PODACI O UZORKU

Oznaka uzorka: CGP_13

Eksterni broj uzorka (ukoliko postoji): 2532/24_4 (186/24, 4572/24)

Datum prijema uzorka: 28/10/2025

Tip uzorka: tkivo (fiksirano u formalinu i ukalupljeno u parafinski blok -FFPE)

Vrsta tkiva iz kojeg je uzet uzorak: rektum

Procenat tumorskog tkiva: /

sample: rectal tumor tissue

<i>Molekularni potpisi karakteristični za tumore</i>	<i>Terapije odobrene/preporučene za navedeni tip tumora</i>	<i>Terapije odobrene/preporučene u drugim tumorima</i>
Microsatelite Status (MS) – stabilan (0.0029)		durvalumab + olaparib, lenvatinib + pembrolizumab <i>strana 21/30</i>
Tumor mutational burden (TMB) – nisko (1.21 mut/Mb)		
HRDsig – negativan		
LOH – neodređen		

MS – Mikrosatelitski status; TMB – mutaciono opterećenje tumora; HRDsig – deficijencija homologe rekombinacije; LOH – gubitak heterozigotnosti

<i>Klinički značajne genomske varijante</i>	<i>VAF%</i>	<i>Terapije odobrene/preporučene za navedeni tip tumora</i>	<i>Terapije odobrene/preporučene u drugim tumorima</i>
KRAS p.G12V NM_004985.5: c.35G>T	44%	regorafenib, cetuximab <u>rezistencija</u> , panitumumab <u>rezistencija</u> <i>strana 15/30</i>	
PIK3CA p.E545K NM_006218.4: c.1633G>A	24%		alpelisib + fulvestrant, capiasertib + fulvestrant, everolimus, sirolimus <i>strana 17/30</i>
CHEK2 p.I157T NM_007194.4: c.470T>C	47%		enzalutamide + talazoparib, olaparib <i>strana 18/30</i>
APC p.Q1378* NM_000038.6: c.4132C>T	50%		
SMAD4 p.G386S NM_005359.6: c.1156G>A	34%	cetuximab <u>rezistencija</u> , panitumumab <u>rezistencija</u> <i>strana 5/30</i>	
BRAF wildtype	/	dabrafenib <u>rezistencija</u> , encorafenib <u>rezistencija</u>	encorafenib <u>rezistencija</u> , dabrafenib <u>rezistencija</u> , vemurafenib <u>rezistencija</u> <i>strana 16/30</i>

VAF-eng. Variant allele frequency (učestalost varijante)

Genomske varijante sa VAF <5% (subklonovi)	VAF%	Terapije odobrene/preporučene za navedeni tip tumora	Terapije odobrene/preporučene u drugim tumorima
GNAS p.R201H NM_000516.7: c.602G>A	4.8%		

Genomske varijante neutrdenog značaja
MSH2(NM_000251.3):c.1748A>G; VAF=48%
MAP3K13(NM_004721.5):c.1945T>C; VAF=47%
CIC(NM_015125.5):c.1634C>T; VAF=46%
MERTK(NM_006343.3):c.2612T>C; VAF=46%
FLT1(NM_002019.4):c.1556C>A; VAF=26%
KMT2D(NM_003482.4):c.2332_2358del; VAF=55%

genetic variants of undetermined significance

variant present in combination

KRAS p.G12V

VAF 44% RD 1 626

Tier I-A

Clinical studies reported that patients with colorectal cancer harboring KRAS G12V were resistant to VEGF and MTOR inhibitors and sensitive to combinations of PI3K and MEK inhibitors. A clinical study suggests that RAS-mutant solid tumor patients with higher MAPK pathway activation (e.g. KRAS G12C/R/V) may survive longer with MEK inhibitor treatment compared to those with lower MAPK pathway activation (e.g. KRAS G12A/D/S and KRAS G13D).

Therapies approved/guidelines-recommended in: **Colorectal cancer**

regorafenib

cetuximab
resistancepanitumumab
resistance

combination

KRAS p.G12V, PIK3CA p.E545K

Tier II-D

A preclinical study suggests that TP53 wild type colorectal cancer cells harboring KRAS G12V and PIK3CA E545K are more sensitive to combined MEK and PI3K/MTOR inhibitors as compared to either agent alone in a PDX model.

In a clinical study, two patients with colorectal cancer harboring a PIK3CA codon 545 mutation and a KRAS codon 12 mutation demonstrated stable disease on MTOR inhibitor therapy.

In a phase I trial, one patient with colorectal cancer harboring a PIK3CA mutation and a KRAS mutation progressed on a PI3K inhibitor. Preclinical studies suggest combined PI3K and KRAS mutations are generally associated with sensitivity to PI3K, AKT, MDM2, MEK, MTOR inhibitors and resistance to a PI3K/MTOR/ATR inhibitor and a KRAS G12C inhibitor.

No approved therapies

AVENIO Tumor Tissue CGP Kit V2 panel i navify® Mutational Profiler su predviđeni za deteciju genomskih varijanti u tumorskom tkivu i ne prave razliku između somatskih i germinativnih varijanti. S toga se u odgovarajućem kliničkom kontekstu preporučuje testiranje germinativnih varijanti za sledeće gene: APC, ATM, BAP1, BRCA1, BRCA2, BRIP1, CHEK2, FH, FLCN, MEN1, MLH1, MSH2, MSH6, MUTYH, NF1, NF2, PALB2, PMS2, POLE, PTEN, RAD51C, RAD51D, RB1, RET, SDHA, SDHB, SDHC, SDHD, SMAD4, STK11, TGFB2, TP53, TSC1, TSC2, VHL i WT1.

The test is intended for the detection of genomic variants in the tumor and does not distinguish between somatic and germline variants, therefore testing germline variants for the-above mentioned genes is recommended

Why do we need RAS status?
She has K RAS mutated tumor

FIRST-LINE THERAPY: CHOOSING THE **RIGHT** BACKBONE



What drives first-line choice?

1 MSI-H / dMMR



IMMUNOTHERAPY FIRST

Pembrolizumab
preferred
(or nivo + ipi in
selected cases)



Deep and durable responses

2 RAS/BRAF WT + LEFT-SIDED



ANTI-EGFR STRATEGY PREFERRED

FOLFOX / FOLFIRI
+ cetuximab
or panitumumab



Especially when tumor shrinkage matters

3 RIGHT-SIDED or RAS MUTANT



BEVACIZUMAB-BASED THERAPY

Doublet chemo
+ bevacizumab
(FOLFOX / CAPOX / FOLFIRI)



Standard of care across most clinical scenarios

4 BRAF V600E



NEW 2025 OPTION

Encorafenib
+ cetuximab
+ mFOLFOX6
(BREAKWATER)



Improved outcomes in
BRAF V600E mCRC



BIOLOGY FIRST. CHEMOTHERAPY SECOND.



SHE HAS TP53 50% MUTANT tumor

Mutations in **APC, TP53** contributing to tumor development, progression, and treatment resistance.

TP53 (51% frequency) are often foundational, while **ARID1A (31% frequency)** often co-occurs with them, particularly in **aggressive cases**

Key Genetic Drivers in Rectal Cancer

- **APC (Adenomatous Polyposis Coli)**: found in 66% of cases, responsible for initiating tumor growth
- **TP53 (Tumor Protein p53)**: A key **tumor suppressor** frequently mutated (51–74%) in colorectal cancer, **often leading to poor prognosis and therapy resistance**.
- **ARID1A (AT-Rich Interactive Domain-containing Protein 1A)**: A subunit of the SWI/SNF chromatin remodeling complex, mutated in ~31% of cases. It functions as **a tumor suppressor**, and its loss is associated with tumor proliferation, metastasis

Therapeutic options being investigated:

Gene ERCC4: PARP inhibitor, re-challenge on platinum

Gene ARID1A : PARP inhibitor , EZHZ2 inhibitor , ATR inhibitor, Aurora kinase inhibitor, BET inhibitor

Gene TP53 : CHK1 inhibitor , Wee inhibitor

Gene APC : wnt signaling inhibitors

SHE HAS SMAD4 34% MUTATED TUMOR

SMAD4 gene mutations or protein loss in colorectal cancer (CRC) are strongly linked to aggressive tumor behavior, advanced disease stages, metastasis, and a poorer overall prognosis

Mutation occurs in 10% of CRC cases, but total loss of SMAD4 protein expression is found in up to 30% of CRC tumors

Inherited germline mutations in SMAD4 cause Juvenile Polyposis Syndrome which significantly raises the lifetime risk of developing colorectal and gastrointestinal cancer

When SMAD4 does not work, **other signaling pathways are activated that drive cancer.** On going are testing **combinations of drugs that block those alternative pathways**, such as PI3K/Akt/mTOR inhibitors, STAT3 inhibitors, and MEK/ERK inhibitors,

Loss of the SMAD4 gene often results in a **poorer response to standard chemotherapy (such as 5-FU or irinotecan).**

Because of this, **drugs (such as RICTOR or STAT3 inhibitors) are being investigated to be given together with chemotherapy** to make the tumor sensitive to treatment again

Regulation of the TGF- β pathway: **Drugs that target proliferation are tested TGF- β signaling pathway (to which SMAD4 naturally belongs)** to influence the tumor microenvironment and prevent metastasis

Why do we need MSI/TMB ?

SHE HAS MSS TUMOR

Immunotherapy in MSI CRC

Mismatch repair-deficient (dMMR) CRCs constitute up to 15% of CRC neoplasms and are characterized by high microsatellite instability (MSI-H) by polymerase chain reaction (PCR) or lack of mismatch repair (MMR) protein expression by immunohistochemistry

These tumors demonstrate an **excellent response to immune checkpoint inhibitor (ICI) therapy**

Potential therapy options:

Pembrolizumab (first line and later)

Nivolumab+/- Ipilimumab

..being investigated:

Atezolizumab/Bevacizumab/FOLFOX(XELOX)

Atezolizumab/Bevacizumab

Immunotherapy/RT

Why do we need HRD score?

SHE HAS HRD NEGATIVE SCORE

Tumors with high HRD scores are very likely **to carry a deficient DNA damage repair system**

High homologous recombination deficiency (HRD) scores are **acting as a potential biomarker for increased sensitivity to DNA-damaging treatments like platinum-based chemotherapy and PARP inhibitors**

High HRD/genomic instability is often **linked with poorer pathologic response (pIR) to initial standard neoadjuvant chemoradiotherapy treatment**, highlighting the need for tailored strategies

Some of the combination of therapies are being investigated:

- **Olaparib+Bevacizumab**
- **Olaparib + Irinotecan:** Intermittent administration has been studied for safety
- **Olaparib + Oxaliplatin:** Preclinical data suggests synergy in inhibiting tumor growth
- **Veliparib + FOLFIRI**
- **Olaparib + Irinotecan**
- **Veliparib + Capecitabine with Radiotherapy**

Why do we need HER2 status?

SHE HAS RAS MUTATED TUMOR but...

5-8% of RAS/BRAF WT CRC, left side colon/rectum, tumor that are resistant to EGFR inhibitors

Anti-HER2 strategies:

Trastuzumab - Tucatinib, the highly selective HER2 tyrosine kinase inhibitor (TKI) in patients with treatment-refractory, HER2-positive, RAS-wt mCRC

Trastuzumab/Pertuzumab

Trastuzumab– Lapatinib

The antibody– drug conjugate **Trastuzumab deruxtecan (T-DXd)** showed convincing activity in patients with immunohistochemistry (IHC)3+ or IHC2+ and in situ hybridisation (ISH)- positive disease

CONCLUSION:

Chemotherapy has been and still it is the **backbone of systemic treatment** of advanced/metastatic CRC

We are still learning about NGS

Potential benefit: one test, one tissue sample (tissue is consumed with each test), at the beginning of treatment

The right patient-the right test-the right therapy(medicine)

TEST SMART. TREAT SMARTER.

AT DIAGNOSIS, TEST EVERY PATIENT

RAS | BRAF | MSI/MMR | HER2 | NTRK (rare)

BIOMARKER / STATUS

TREATMENT IMPLICATION

	MSI-H / dMMR	→		IMMUNOTHERAPY FIRST Pembrolizumab
	RAS/BRAF WT + LEFT-SIDED	→		ANTI-EGFR PREFERRED Cetuximab / Panitumumab
	RIGHT-SIDED OR RAS MUTANT	→		BEVACIZUMAB-BASED THERAPY Chemo + Bevacizumab
	BRAF V600E	→		ENCORAFENIB + CETUXIMAB ± mFOLFOX6
	HER2 POSITIVE	→		HER2-TARGETED THERAPY Tucatinib + trastuzumab



BIOLOGY DRIVES TREATMENT.
NOT ALL mCRC IS THE SAME.



Interreg



Cofinanțat de
Uniunea Europeană

IPA România – Serbia



Thank you for your attention!