



Digestive system

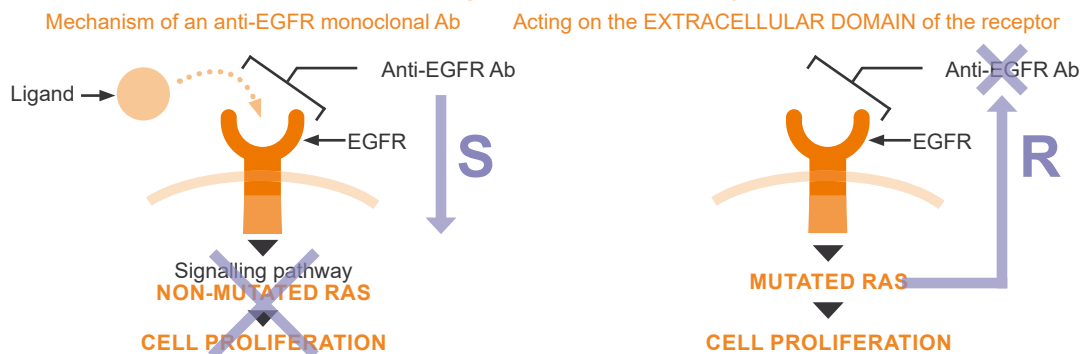
- ▶ Colorectal cancer (CRC)
- ▶ Stomach and gastroesophageal junction cancer (GEJ)
- ▶ Gastrointestinal stromal tumours (GIST)
- ▶ Pancreatic cancer

Colorectal cancer

Colorectal cancer (CRC) is the third most common form of cancer in men and second most common form in women. It is the second highest cause of mortality from cancer. It is most frequently diagnosed at an advanced stage (metastatic). As a result, compared with early diagnosis, it is associated with a poor survival rate (overall survival 90% at stage I and 13% at metastatic stage). The treatment of colorectal cancers is based on surgery, possibly combined with chemotherapy and/or targeted therapy and/or, more recently, immunotherapy.

In the 2000s, there was a treatment revolution in CRC (targeted therapy) based on monoclonal antibodies (Ab) that block the EGFR receptor. These Abs act on the extracellular domain of the receptor and can be used according to the RAS status. There is no TKI that acts on the intracellular domain of the EGFR receptor with marketing authorisation for metastatic CRC. Other molecular markers (BRAF, MSI) affect the prognosis and treatment decision for CRC (combination of anti-EGFR/ant-BRAF and immunotherapy). It is as such essential that a treatment strategy is defined based on personalised medicine.

Example of a targeted anti-EGFR therapy



S: sensitivity to the monoclonal Ab if RAS not mutated

R: resistance to the monoclonal Ab if RAS mutated

Eurofins Biomnis offers molecular theranostic and prognostic testing of CRCs: 2 NGS panels (COLO1 and COLO2), an MSI test and targeted FISH techniques to fulfil the needs of multidisciplinary clinician meetings. The list of offered analyses has been drawn up based on international guidelines (ASCO-CAP, ESMO).

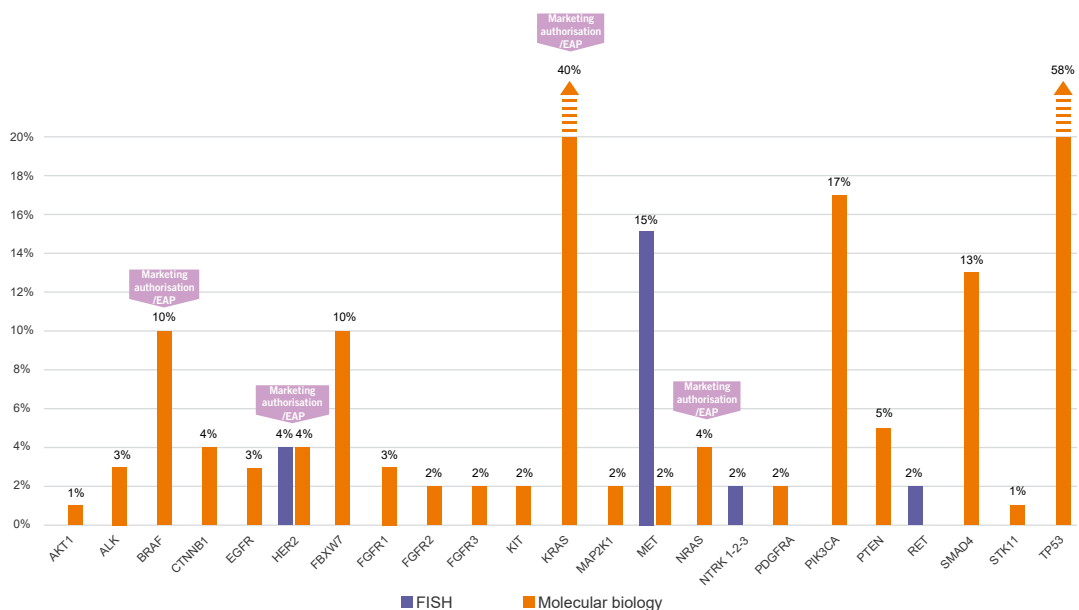
**“Targeted treatment: sensitivity and resistance” and “impact on prognosis” in colorectal cancer:
What analyses are available? What techniques are used? What are potential targets for the future?**

“COLO1” NGS panel	KRAS – NRAS – BRAF
MSI test and MLH1 hypermethylation	
FISH unitary tests – COLO-RECTAL	HER2 – MET – RET – NTRK 1-2-3
“COLO2” NGS panel	AKT1 – ALK – BRAF – CTNNB1 – EGFR – HER2 – FBXW7 – FGFR1 – FGFR2 – FGFR3 – KIT – KRAS – MAP2K1 – MET – NRAS – PDGFRA – PIK3CA – PTEN – SMAD4 – STK11 – TP53 (21 genes)

The NGS “COLO1” panel fulfils the routine needs of clinicians for prescribing targeted therapies for metastatic colorectal cancer. The KRAS, NRAS and now also BRAF statuses can be used to guide the selection of a targeted therapy (monoclonal anti-EGFR Ab and anti-BRAF Ab). This panel is complemented by the MSI test, screening for MLH1 methylation and COLO-RECTAL FISH unit tests.

The NGS “COLO2” panel approach expands the range of mutations tested to the genes involved in resistance mechanisms for targeted therapies, emerging markers for targeted therapies and molecular targets that can be used when establishing a prognosis. It can also be supplemented with targeted FISH unit tests and the MSI test.

Frequency of FISH or molecular abnormalities in colorectal cancer and available targeted therapies (marketing authorisation/EAP)



“COLO1” NGS panel

RAS (KRAS and NRAS) 40% to 50% of CRC present with an RAS mutation. The prescription of anti-EGFR (cetuximab or panitumumab) in the treatment of metastatic CRC is dependent on the status of the KRAS and NRAS genes. Molecular analysis should target exons 2 (codons 12 and 13), 3 (codons 59 and 61) and 4 (codons 117 and 146) of the KRAS and NRAS genes. Marketing authorisation for these two therapies is restricted to wild-type (wt) RAS tumours. However, the absence of RAS mutation is no guarantee of a response to these targeted therapies and only 40% to 60% of patients are responders, which implies the existence of molecular resistance mechanisms. The value of RAS in prognosis remains uncertain.

BRAF Around 10% of CRCs present with a BRAF mutation (most frequently V600E). The BRAF V600E mutation is associated with a poor prognosis, especially in metastatic CRC. The predictive role of non-response to targeted anti-EGFR therapies has not been clearly established and the presence of a BRAF mutation is not a contraindication for anti-EGFR therapy. Mutated BRAF status can lead to selection of an intensive form of chemotherapy from the first-line of treatment. Targeted anti-BRAF therapy combined with anti-EGFR therapy has also recently received marketing authorisation. In addition, the presence of a BRAF mutation means that Lynch syndrome can be ruled out. In view of the above, the inclusion of BRAF in first-line analyses alongside KRAS and NRAS is therefore justified. Note that RAS and BRAF mutations are generally mutually exclusive.

MSI tests

The mismatch repair system is another DNA repair system. Four proteins (MMR proteins) are involved in this repair system and operate in heterodimers: MSH2/MSH6 and PMS2/MLH1. Mismatches are assessed in the microsatellite regions. When DNA repair occurs, the term MMR-proficient is used. When DNA repair does not occur (via mutation) the term MMR-deficient and MSI is used (MicroSatellite Instability). Microsatellite instability is defined by a hypermutated phenotype (> 10 mutations/megabase). There can be two causes of impairment of the MMR system: constitutional (Lynch syndrome) or somatic (sporadic cancer). MMR impairment therefore takes the form of loss of MMR protein expression (in the cell), microsatellite instability (in the nucleus) and as a result a high tumour mutational burden (high TMB).

The indications for the MSI test (MMR test or RER test (Replication ERror)) in colorectal gastrointestinal oncology (with or without metastases) are:

- ▶ **Pre-screening of Lynch syndrome** (constitutional oncogenetics with oncogenetic consultations) (CRC < 70 years or CRC with individual or family history of Lynch-spectrum tumours).
- ▶ **PROGNOSTIC evaluation in non-metastatic CRC:**
 - In cases of non-mutated BRAF at a localised stage, a MSI+ status is associated with a good prognosis.
 - Regardless of the BRAF status, identification of MSI+ status can be used to adapt the treatment of stage II CRC with a poor prognostic factor. The benefits of adjuvant chemotherapy in these circumstances are not significant and so it should not be prescribed.
- ▶ **THERANOSTIC stratification using IMMUNOTHERAPY** (PD-1/PDL-1 axis) in metastatic CRC (e.g. pembrolizumab). Note that in metastatic tumours, an MSI+ status is

associated with a poor response to cytotoxic chemotherapy and a good response to immunotherapy. The PDL-1 IHC technique is a supplement to this testing.

The test can be carried out either with IHC (loss of expression of one or more MMR proteins) or using molecular biology, with various techniques available: MSI-PCR (microsatellite “consensus” panels) – Idylla™ test – NGS.

Microsatellite instability (MSI+) is present in 15% of sporadic CRCs and always present in CRC linked to Lynch syndrome. 15% of MSI+ cases are linked to Lynch syndrome. The molecular markets for sporadic MSI+ CRCs are MLH1 hypermethylation and BRAF mutation. These complementary tests should as such be carried out for MSI+ cases in order to rule out a constitutional cause (Lynch syndrome).

It is important to note that the MSI test should be carried out for metastatic and non-metastatic cases.

Targeted FISH unit COLO-RECTAL tests

- HER2** HER2 amplification is a resistance mechanism against anti-EGFR therapy. Trials are also ongoing for a targeted anti-HER2 therapy, in particular with respect to metastatic wild-type RAS/BRAF CRCs.
- MET** MET amplification is associated with resistance to anti-EGFR therapy and could mean the patient would benefit from targeted anti-MET therapy, in particular for metastatic wild-type RAS/BRAF CRCs.
- RET** CRC presenting with RET rearrangement could be a candidate for RET TKIs.
- NTRK 1/2/3** The presence of NTRK 1, 2 or 3 rearrangement could allow the use of an anti-NTRK therapy.

	Rearrangement	Amplification
HER2		●
MET		●
RET	●	
NTRK 1/2/3	●	

“COLO2” NGS panel

This panel includes the COLO1 gene panel, along with:

- HER2** The presence of HER2 mutations is a resistance mechanism against anti-EGFR therapy.
 - PIK3CA** For cases of CRC not presenting with RAS mutation, PIK3CA mutations appear to be associated with a poor prognosis and a worse response to anti-EGFR monoclonal Abs. The adjuvant use of aspirin in CRC with PIK3CA mutation has been proposed in the literature.
 - MET** MET mutations may mean the patient could benefit from targeted anti-MET therapy.
- AKT1 – ALK – CTNNB1 – EGFR – FBXW7 – FGFR1 – FGFR2 – FGFR3 – KIT – MAP2K1– PDGFRA – PTEN – SMAD4 – STK11 and TP53** are emerging potential molecular markers for targeted therapy for metastatic CRCs or molecular markers for resistance to anti-EGFR therapy, or could provide additional information for the prognosis of localised or metastatic CRCs.

Note: The POLE gene is not included in our NGS panel.

Stomach and gastroesophageal junction cancer (GEJ)

In men and women combined, stomach cancer is the fourth most common form of cancer and second leading cause of death from cancer. 80% of cases are diagnosed after the age of 65 and two-thirds at an advanced stage.

FISH unit tests	HER2 – MET – NTRK 1-2-3
MSI test and MLH1 hypermethylation	

Targeted FISH unit stomach and GEJ tests

HER2 HER2 amplification is a target for targeted anti-HER2 therapy in advanced or metastatic stomach or gastroesophageal cancer. It is observed in around 20% of stomach cancers and 30% of GEJ cancers. The IHC technique is used routinely and FISH can be used to provide greater precision in unclear (++) IHC cases. The use of HER2 amplification in prognosis remains controversial in stomach cancer.

MET MET amplification may be a candidate for anti-MET therapy.

NTRK 1/2/3 The presence of NTRK 1, 2 or 3 rearrangement could allow the use of an anti-NTRK therapy.

MSI tests

The indications for the MSI test are:

- ▶ **PROGNOSIS evaluation:** an MSI+ status (observed in around 10% of cases) is associated with a good prognosis for stomach cancer. For an operated gastric cancer with MSI+, there is no observed benefit from adjuvant chemotherapy or peri-operative chemotherapy.
- ▶ **THERANOSTIC stratification using IMMUNOTHERAPY** (PD-1/PDL-1 axis) in advanced or metastatic forms with an MSI+ test (e.g. pembrolizumab, nivolumab). Testing for PDL-1 expression using IHC is a supplement to MSI analysis. Note that EBV+ status may also be a predictive factor for the response to immunotherapy in stomach cancer.
- ▶ **Pre-screening for Lynch syndrome (broad spectrum)**

MLH1

MLH1 hypermethylation appears to be associated with a good prognosis.

Gastrointestinal stromal tumours – GISTs

GIST are rare mesenchymal tumours that usually develop in the stomach (60%) or small intestine (25%).

"GIST" NGS panel

KIT – PDGFRA – BRAF

Around 85% of localised GISTs exhibit KIT (75%) or PDGFRA (10%) mutations, which are generally mutually exclusive. These mutations result in an activation of these tyrosine-kinase receptors in the absence of their specific ligands. A BRAF mutation may also be observed in GISTs can also be used as a factor for establishing a prognosis.

Testing for KIT, PDGFRA and BRAF mutations thus has diagnostic and/or prognostic and/or theranostic value:

- ▶ **Mutated PDGFRA GIST or triple negative** (wild-type KIT-PDGFRA-BRAF) exhibit a lower risk of metastases than KIT mutated GIST. A KIT mutation is observed in around 85% of metastatic GISTs while only 2% of these tumours present with a PDGFRA mutation. In KIT and PDGFRA mutations, the location of the mutation also influences the prognosis: PDGFRA exon 12 and KIT exon 11 mutations are associated with a better prognosis and PDGFRA exon 18 (not D842V) and KIT exon 9 mutations with a worse prognosis.
- ▶ **In 2001, targeted therapy based on TKIs** (imatinib) revolutionised treatment, however primary or secondary resistance mechanisms have been reported. KIT exon 11 mutations are associated with a very good response to TKIs, while PDGFRA exon 18 (D842V) mutation is associated with primary resistance to TKIs. If there is a KIT exon 9 mutation, it is recommended that the doses of TKIs are doubled. Secondary resistance mechanisms have been reported in KIT exons 13, 14, 17 and 18. Note that wild-type GIST PDGFRA/KIT/BRAF tumours do not respond to TKIs.

Pancreatic cancer

Pancreatic cancer is a rare form of cancer with a very low survival rate (< 5% at 5 years) and so far, has not benefited from the targeted therapies, which have been used against other gastrointestinal tumours.

BRCA1/BRCA2

MSI test and MLH1 hypermethylation

FISH unit tests

NTRK 1-2-3

BRCA1/BRCA2

A major advance in treatment was the identification of mutations in the BRCA1 and BRCA2 genes involved in homologous repair (see Oncology-gynaecology-sheet ref. DS85-INTGB).

These mutations can be somatic or constitutional in nature and are associated with a better response to platinum-based chemotherapy and more recently have been a target for targeted therapy: PARP inhibitors.

In the case of constitutional mutations, an oncogenetic consultations should be conducted (predisposition to breast/ovarian/pancreatic cancer).

MSI tests

The indications for the MSI test are:

- ▶ **Theranostic stratification using immunotherapy** (PD-1/PDL-1 axis) in advanced or metastatic forms with a MSI+ test (e.g. pembrolizumab, nivolumab).
- ▶ **Pre-screening for Lynch syndrome** (broad spectrum)

Targeted FISH unit tests

NTRK 1/2/3 The presence of NTRK 1, 2 or 3 rearrangement could allow the use of an anti-NTRK therapy.

Note: As with breast and ovarian cancer, oncogenetic consultations are key for individual and family treatment.

In conclusion, the molecular biology and FISH approaches for gastrointestinal tumours are constantly changing. This document was written according to the state of knowledge in 2020.

Note: Alongside FISH and molecular analyses, Eurofins Biomnis can also test other biological parameters in relation to gastrointestinal oncology:

- Septine 9 in connection with colorectal cancer screening tests.
- Evaluation of the toxic risk of fluoropyrimidines (5-FU).
- Tumour markers such as AFP, ACE and CA19-9.
- Radioimmunological assays such as VIP, gastrine and glucagon for the pancreas and Type III procollagen (P3P) for the liver.

The evaluation of tumour mutational burden (TMB) as a predictive test for response to immunotherapy is also available from Eurofins Biomnis.

Note: Cholangiocarcinoma is not covered in this information sheet.

Molecular characteristics of genes analysed using NGS:

Gene	NM_	EXONS	COLO1	COLO2	GIST
AKT1	NM_005163.2	3		●	
ALK	NM_004304.4	21, 22, 23, 25		●	
BRAF	NM_004333.5	11, 15	●	●	●
CTNNB1	NM_001904	3		●	
EGFR	NM_005228	12, 18–21		●	
HER2	NM_004448.3	19–21		●	
FBXW7	NM_033632.3	5.8-11		●	
FGFR1	NM_023110	3, 4		●	
FGFR2	NM_000141	7, 9, 12		●	
FGFR3	NM_000142	7, 9, 14, 16, 18		●	
KIT	NM_000222	8, 9, 11, 13, 17		●	●
KRAS	NM_004985	2, 3, 4	●	●	
MAP2K1	NM_002755	2		●	
MET	NM_001127500	2, 14, 16, 19		●	
NRAS	NM_002524	2, 3, 4	●	●	
PDGFRA	NM_006206	12, 14, 18		●	●
PIK3CA	NM_006218	2, 8, 10, 14, 21		●	
PTEN	NM_000314.6	1, 3, 6–8		●	
SMAD4	NM_005359	3, 5, 6, 8, 9–12		●	
STK11	NM_000455	1, 4, 6, 8		●	
TP53	NM_000546	2, 4-10		●	

Before taking any samples, view the key information for each test (pre-analytical, turnaround time, required documents*, etc.) on www.eurofins-biomnis.com > section Test Guide > **Analysis Code**

Analysis codes

- Colorectal NGS panel 1 – 3 genes: **COLO1**
- Colorectal NGS panel 2 – 21 genes: **COLO2**
- MSI test: **MICSA**
- MLH1 hypermethylation: **MLH1**
- Targeted FISH unit tests: **MOHC4**
- BRCA 1/2 (somatic): **BRCAS**
- TMB test: **TMB**

*Required documents

- Test request form - Oncology - Solid tumours (ref. B9-INTGB)
- Histopathology report

Turnaround time (FISH and NGS): 10 days (one extra week if verification by Sanger is required)

Contact

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Literature references

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Comprehensive review of targeted therapy for colorectal cancer. Xie et al. Signal Transduct Target Ther. 2020 Mar 20;5(1):22. PMID: 32296018

Recommendations for the use of next-generation sequencing (NGS) for patients with metastatic cancers: a report from the ESMO Precision Medicine Working Group. F Mosele et al. Ann Oncol 2020 Aug 24;S0923-7534(20)39971-3. PMID: 32853681

Websites <https://www.mycancergenome.org/>
<https://www.e-cancer.fr/>
<https://www.cancer.gov/>

Abbreviations

CRC	Colorectal cancer
GIST	Gastrointestinal stromal tumour
ICI	Immune checkpoint inhibitors
IHC	Immunohistochemistry
TKI	Tyrosine-kinase inhibitor
GEJ	Gastroesophageal junction
MDCM	Multidisciplinary consultation meeting
TMB	Tumour Mutational Burden