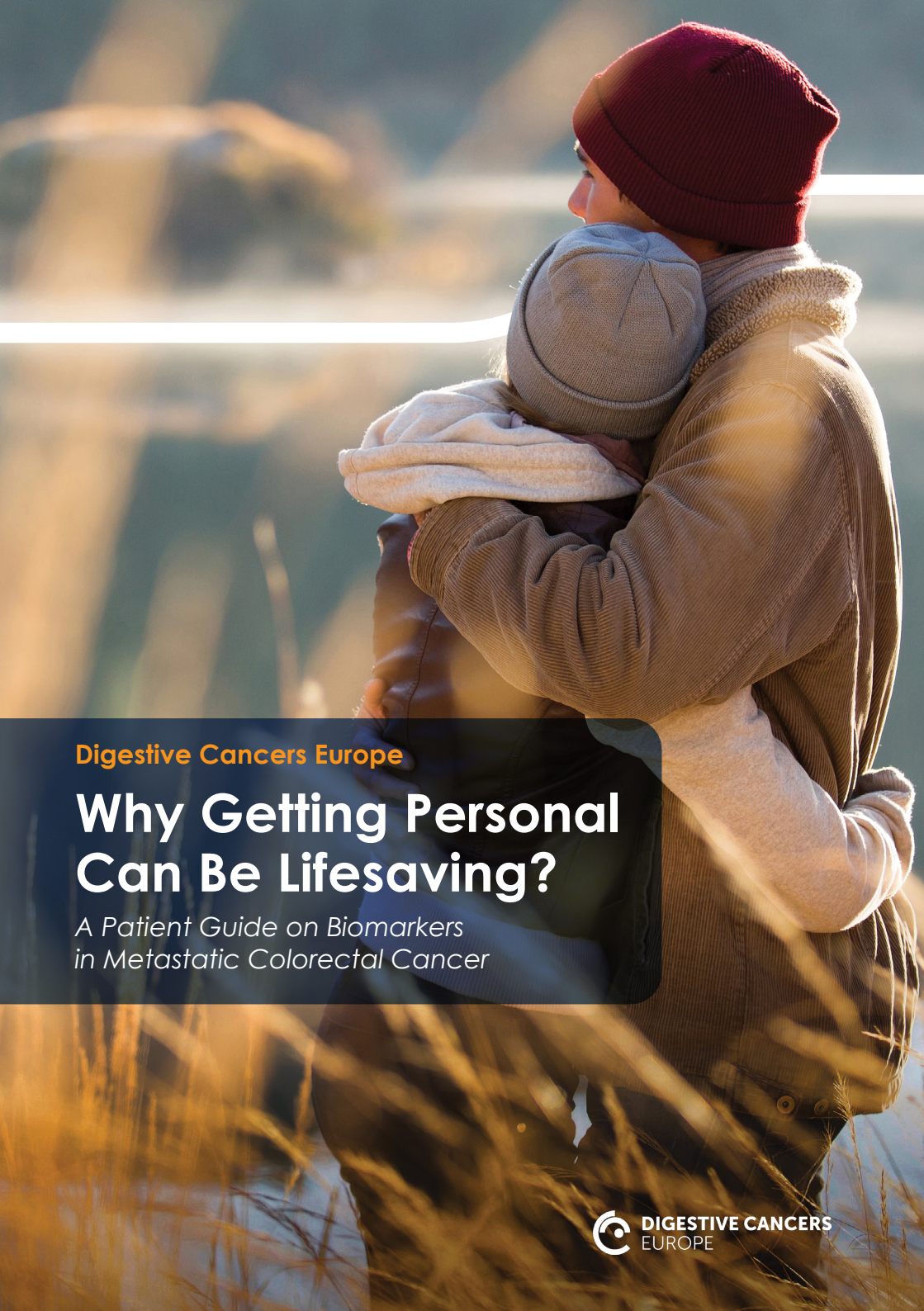


Digestive Cancers Europe

Why Getting Personal Can Be Lifesaving?

A Patient Guide on Biomarkers in Metastatic Colorectal Cancer

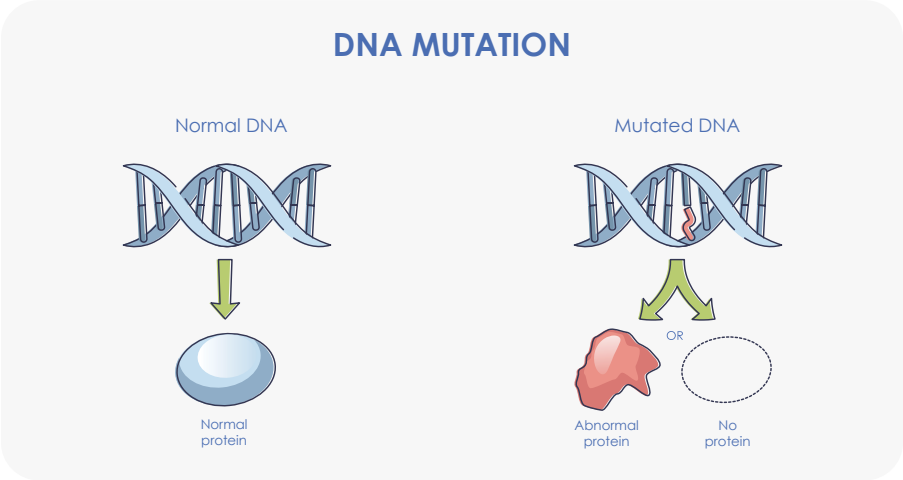
DIGESTIVE CANCERS EUROPE

Table of Contents:

What is a biomarker?

A biomarker is a biological molecule found in blood, other body fluids, or tissues. They give **an indication of a normal or abnormal process, or of a condition or disease.** Biomarkers are widely used in medicine to evaluate different physiological and disease processes. They are a tool to measure how the body is doing. A biomarker can be a change in DNA, RNA, or protein.

The human body is made of cells. Each cell contains our DNA that is used to produce RNAs and proteins. The DNA is the macromolecule containing all the information necessary to make our body functioning and RNA is the molecule reading this information and traducing it into proteins. Alterations in the DNA can be reflected in the sequence of the DNA itself, in the RNAs and the proteins, thus in how the cells and the organs work.



A type of DNA alteration is called a mutation. This happens when a fragment of DNA has changed. When this mutation is present only in the DNA of tumour cells it is called a **somatic mutation**. In colorectal cancer, most of the DNA mutations are somatic, which means they do not affect your family members. Only a small percentage of patients with colorectal cancer have mutations **(germline mutation)** that can be passed on to their offspring.

DNA mutations and consequently RNA and protein alterations can be captured by measuring biomarkers.

Why it can be important to know your tumour biomarkers?

Each tumour is unique. Knowing your tumour biomarkers is like knowing the fingerprints of your hand. It is a characterization of your tumour that can provide your doctor with a great deal of information and help you to find the most suitable treatment.

Which information can a biomarker provide?

A biomarker can be **diagnostic** and help to identify the cancer type. There are **prognostic** biomarkers used to learn about the estimated course of cancer if it goes without treatment.

Predictive biomarkers can predict whether the body will respond to a specific treatment or not. **Pharmacodynamic** biomarkers help with dosing decisions. Finally, **recurrence** biomarkers are used to monitor if the cancer is coming back after it has been treated. Some biomarkers can cover different information at the same time.

Who should be tested for biomarkers?

A biomarker test should be conducted for colorectal cancer patients with advance or metastatic disease to identify their tumour characteristics and determine the most appropriate treatment strategy.

All patients with colorectal cancer should be tested for Microsatellite Instability (MSI), regardless of their stage of the disease. If you want to learn more about MSI go to page 10.

New treatments may be developed for specific biomarkers in the future. Testing recommendations should evolve as science advances.

How are biomarkers tested?

Biomarkers can be tested directly on a tumour sample isolated during surgery, during a colonoscopy or biopsied as an outpatient procedure. Some biomarkers are tested using a blood sample through what is called **liquid biopsy.**

Different laboratory techniques can be used to test biomarkers:

- **Immunohistochemistry (IMC)** allows the detection of biomarkers directly on tumour samples.
- **Sequencing** and **Next Generation Sequencing (NGS)** can read the DNA sequence of one or multiple biomarkers at a time. This is usually performed with DNA extracted from the tumour sample.
- Other techniques that can be used to test your tumour biomarkers are **Retro transcriptase PCR (Rt-PCR)** or **Fluorescent in Situ Hybridization (FISH)**. Both techniques can identify the number of copies of specific biomarkers using fluorescence. Rt-PCR detects the RNA and FISH both the DNA and RNA.

When should biomarkers be tested?

Biomarkers should be tested on **diagnosis of advance colorectal cancer** (except for MSI). You may also be retested during therapy as your biomarkers profile may change through your journey.

Acquired resistance to some therapies may indicate that your tumours now have a different biomarker profile, and your doctor may decide to re-test your biomarkers.

What are some common biomarkers?

The most common tumour biomarkers

BRAF

BRAF is a gene producing a protein having the same name, BRAF. The BRAF protein is produced when the cell needs to grow. **An irregularity in the production of BRAF can cause the cells to grow continuously**, leading to cancer development.

BRAF mutations are **somatic mutations** found in the tumours of **8-12%** of patients with metastatic colorectal cancer. The most common mutation in *BRAF* is called V600E, although other less frequent *BRAF* mutations do exist.

The *BRAF* V600E mutation is a **prognostic biomarker of aggressive** tumour growth. The mortality risk for patients with a *BRAF* mutation is more than two times higher than for those with a normal *BRAF* gene. Therefore, knowing about a BRAF gene mutation indicates the need for a **rapid start of care with effective treatment** due to the aggressiveness of the disease.

An anti BRAF treatment has been approved, given in combination with anti-EGFR treatment (encorafenib + cetuximab).

The *BRAF* mutation is also a predictive biomarker, which means it predicts that your tumour is **unlikely to respond to treatment with EGFR inhibitors** when given alone or in combination with chemotherapy.

There is a great deal of research and new treatments being developed for *BRAF* gene mutations today. You should discuss this with your medical team.

The European Society of Medical Oncology (ESMO) recommends that **all colorectal cancer patients should be tested for BRAF mutations at the time of diagnosis.** Also, *BRAF* mutations should be assessed along with *KRAS* and *NRAS* mutations.

CEA

CEA stands for Carcinoembryonic Antigen. It is a biomarker produced by cell tumours that can be measured in the blood. It can be used to monitor the progression of your disease. The levels of CEA in your blood should decrease after your tumour has been removed by surgery or after drug treatment. If this does not happen, it may be a sign that your tumour was not completely removed or has come back.

Also, if your CEA levels do not go down during chemotherapy, it could mean that the cancer is not responding to your current treatment.

It is important to remember that **CEA biomarker testing has some limitations because it is not always associated with cancer progression or recurrence.** This is because it only measures protein levels and these levels can be influenced by factors that are not related to the cancer.

If the CEA is negative, it does not guarantee that cancer has not come back and if it is positive, it does not mean that cancer has come back. Test results may need to be repeated or confirmed by additional testing and the results should be discussed with your healthcare team.

HER2

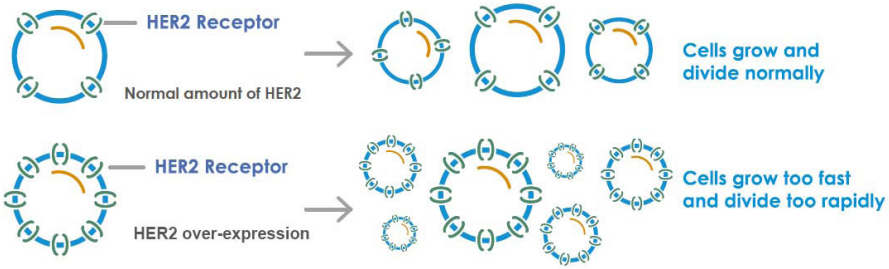
HER2, also called *ERBB2*, is a gene producing a protein that is present on the surface of all **human epithelial cells**, the cells covering the surfaces of organs in the body and function as a protective barrier.

This protein is a receptor transmitting the message of growing or not growing from the outside of the cells to the inside.

In about 8% of rectal cancers and 3% of colon cancers, the *HER2* gene can be amplified, meaning that there is an overproduction of the protein.

HER2 amplification and overexpression are predictive biomarkers for **poor response to treatment with EGFR inhibitors**. Although recent evidences support the use of anti HER2 treatment in metastatic colorectal cancer with HER2 over-expression or amplification. Enrollment in clinical trials is highly recommended.

HER2 mutations can also be acquired over time as a resistance mechanism to therapies such as EGFR inhibitors — so repeating HER2 testing at disease progression or recurrence can be useful.



KRAS

KRAS is a gene producing the KRAS protein which has a major role in the **regulation of the cell cycle.** Approximately **40% of colorectal cancer patients** have tumour mutations in the *KRAS* gene. The most common KRAS mutations are located in a specific part of the gene called exons, numbers 2, 3 and 4.

Knowing your KRAS status is important for treatment decisions. Tumours with KRAS mutations are **unlikely to respond to EGFR inhibitors,** such as panitumumab and cetuximab, so if you have one of these mutations, you should not receive these drugs.

KRAS mutations can also be acquired over time as a resistance mechanism to therapies such as EGFR inhibitors — so repeating KRAS testing at disease progression or recurrence can be useful.

MSS/MSI-H

The DNA holds mismatch repair (MMR) genes which are involved in **repairing our genetic code** if it is wrongly duplicated during the process of cell replication. The major MMR genes are *MLH1*, *MSH2*, *MHS6*, *PMS2* and *EPCAM1*. When the MMR process is working properly the cells are defined as **MicroSatellite Stable (MSS)**, while a mutation in MMR genes may cause MMR deficiency, this can lead to **microsatellite instability (MSI-high or MSI-H).**

MSI-H/MMR deficiency occurs in **15% of all colorectal cancers,** and in **4% of stage IV/metastatic colorectal cancers.** MSI-H tumours are much rarer in rectal cancer (found in 0.5-1% of patients). The mutations associated with MSI-H/MMR deficiency are in **95-97% of the cases only occurring in your tumour cells** and not all the cells of your body (somatic mutation).

Although 3-5% of MSI-H/MMR deficiency patients have an associated **Lynch Syndrome,** which means that the MMR mutation is affecting all the cells of their body and their family member might also have it (germline mutation). Lynch Syndrome patients are at higher risk for developing other cancers and should speak with a genetic counsellor. For more information on Lynch Syndrome read this brochure for further detail.

Importantly, **all colorectal cancer patients should be tested for MSI-H/MMR deficiency.** MSI-H is relatively uncommon in stage IV colorectal cancer but knowing this about your tumour is important as it qualifies you for immuno-therapy with **immune checkpoint** inhibitors.

NRAS

NRAS is a gene producing the NRAS protein which has a major role in the **regulation of the cell cycle.** Approximately **5% of colorectal cancer** patients have tumour mutations in the *NRAS* gene. The most common *NRAS* mutations are located in a specific part of the gene called exons, numbers 2,3 and 4.

Knowing your NRAS status is important for treatment decisions. Tumours with NRAS mutations are **unlikely to respond to EGFR inhibitors,** such as panitumumab and cetuximab, so if you have one of these mutations, you are should not receive these drugs.

NRAS mutations can also develop over time and become a resistance mechanism to therapies such as EGFR inhibitors — so repeating NRAS testing at disease progression or recurrence can be useful.

NTRK

There are 3 *NTRK* genes (1, 2 and 3) that produce three similar proteins: TRK-A, TRK-B, and TRK-C. In normal situation, the TRK proteins function as receptors, and their role is to **receive signals from neighbouring cells** to instruct cells to grow and divide. In less than 1% of colorectal cancer patients, *NTRK* genes can fuse with other non-related genes which then produce what is called **NTRK fusion proteins** that promote uncontrolled cell growth and division in cancer cells.

Usually, the NTRK fusion phenomenon occurs when tumor cells are also MSI-H/MMR deficient.

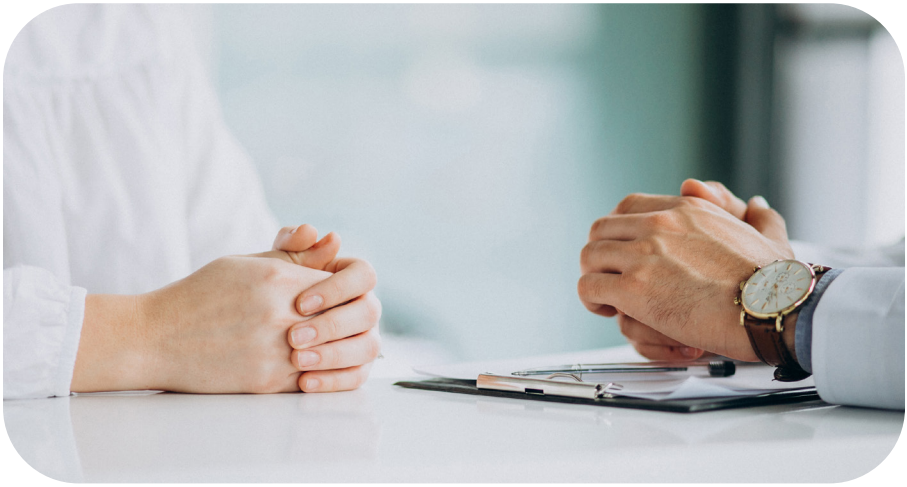
There are drugs available that target **NTRK fusion proteins,** such as larotrectinib and entrectinib. Next-generation sequencing (NGS), PCR, FISH and immunohistochemistry are common methods of detecting NTRK fusions. **It's important to note that NTRK mutations are different from NTRK fusions, and the drugs mentioned above may not work against NTRK mutations.**

PIK3CA

The *PIK3CA* gene is responsible for producing a protein, which is one part of an enzyme called phosphatidylinositol 3-kinase (PI3K). PI3K regulates many cellular processes including proliferation, migration or movement of cells, apoptosis or programmed cell death, and survival. The mutation is a **driver mutation** that pushes the **growth of cells.**

The routine analysis of this biomarker is not recommended but, if you have comprehensive biomarker panel testing done on your tumour through Next Generation Sequencing (NGS) or you are **participating in a clinical trial,** PIK3CA can be analyzed.

Some studies show that, mutation in a specific part of *PIK3CA* (exon 20) may predict **resistance to EGFR-antibody therapy.** Although *PIK3CA* is not considered a predictive biomarker.



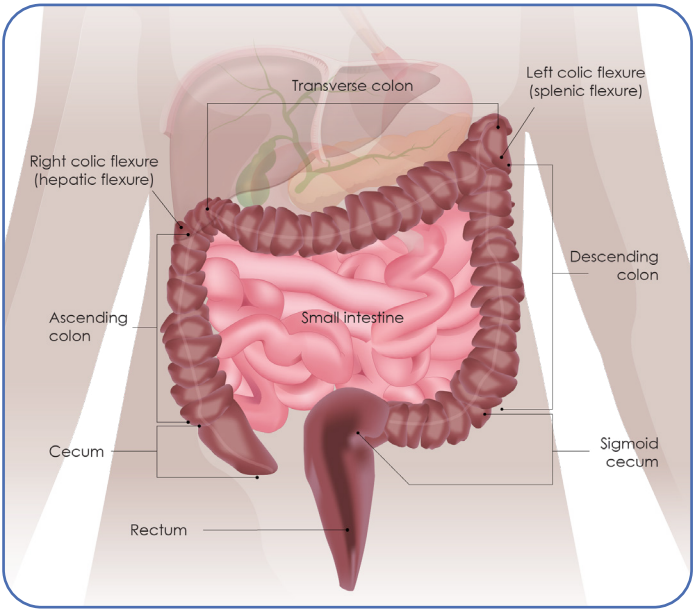
SIDEDNESS

The colon is the last part of our intestine. The parts of the colon on the right side of our abdomen are the cecum, ascending colon, and hepatic flexure. On the left side, we have splenic flexure, descending colon, and sigmoid.

The right or left location of the tumour can influence its characteristics and treatment.

In the **right-sided tumours,** their flat histology and unspecific symptomatology (anaemia, vomiting) can make them more difficult to detect at an early stage. Mutations associated with MSI-H/MMR deficiency are commonly observed. This predicts a **positive response to checkpoint inhibitors and immunotherapy treatments.**

The **left-sided tumours** have a polypoid-like morphology and associate with symptoms like change in bowel habits, blood in the stool, and bowel obstruction. They are usually diagnosed at an earlier stage and **have a better prognosis.**



Moreover, **sidedness influences first line treatment in *RAS* and *BRAF* no mutated tumors .** In right sided tumors there is a limited befit from anti-EGFR drugs in combination with chemotherapy, while left sided tumors benefit from anti-EGFR drugs in combination with chemotherapy.

Additionally, the transverse colon crossed both the right and the left side. Taking into account the embryological origin of the transverse colon,

proximal (closer to the liver) transverse is considered right sided whereas distal (closer to the bladder) transverse is left sided. Only 5% of colon cancers occur in the transverse colon.

Pharmacodynamic biomarkers

DPYD

DPYD is a gene producing a protein with the enzymatic function of **eliminating the 5-Fluorouracil (5-FU) from our body.** 5-FU is a commonly used drug for the treatment of cancers. Mutations in this gene are associated with less ability to metabolize drugs such as 5-Fluorouracil (5FU) and similar or fluoropyrimidine-based chemotherapies (capecitabine, FOLFOX, FOLFIRI or FOLFIRINOX).

Patients with less-efficient enzyme activity who are treated with 5-FU or regimes based on 5FU or similar drugs **may experience severe side effects** like neutropenia (low white blood cells increasing, the risk of infections), diarrhoea, and mucositis (inflammation and ulceration from mouth to anus) and in extreme cases, life-threatening complications.

The European Medicines Agency (EMA) has recommended that **patients should be tested for the lack of the DPYD enzyme before starting cancer treatment with 5-FU** or with the related medicines, capecitabine and tegafur.

UGT1A1

UGT1A1 gene produces an enzyme involved in transforming small lipophilic molecules such as hormones, bilirubin, or drugs into water-soluble metabolites that our body is then able to excrete. Mutation in this gene can associate with **increased toxicity due to the worst ability to excrete irinotecan,** a drug used for colorectal cancer treatment.

This biomarker should be tested when your levels of **conjugated bilirubin** are low and if you are a candidate for a high dose irinotecan treatment (>180 mg/m2).

Emerging biomarkers and treatments

As science is evolving, new biomarkers are emerging as new possible targets for drug developments and treatment design. You might have these biomarkers analyzed if Next Generation Sequencing was used to profile your tumour or if you are participatin g in a clinical trial.

Some of these biomarkers are **MET, HER3, PTEN, TGF-a, amphiregulin and epiregulin and EGFR.**

Also, the analysis of **circulating tumour DNA (ctDNA)** is emerging as a new tool for the molecular profiling of tumours. It has a role as a prognostic marker especially in adjuvant settings and for the detection of recurrence diseases.

The use of these biomarkers and their implication in cancer progression and treatment is still under study. You might want to discuss their relevance in your specific case with your doctor.

Also, be informed that **there are clinical trials investigating new drugs targeting already well-defined biomarker mutations.** Being informed of your biomarker mutations can help you figure out which clinical trial may be good for you.

Biomarkers are a powerful tool in the fight against colorectal cancer. You have the right to know if you have been tested for biomarkers and what the results are. Receiving a cancer diagnosis can be scary and there is a lot of information to process. Talking with your medical team about your biomarker analysis can help you understand what will come next and help you to explore all the treatment opportunities that may fit your personal case.

Glossary

Acquired resistance
This occurs when a tumour no longer responds to a treatment that worked previously. This happens when 1) new tumour cells acquire new mutations or 2) a group of preexisting cells with a mutation able to resist the treatment grow stronger.

Apoptosis
This is the death programmed or triggered by the organism itself, to control its development and growth, which can be physiological in nature and is triggered by genetically controlled cell signals. Apoptosis has a very important function in organisms, as it makes possible the destruction of damaged cells, preventing the appearance of diseases such as cancer, which is the consequence of an indiscriminate replication of a damaged cell.

Circualating Tumour DNA (ctDNA).
It is the DNA of the tumour that might be present in the bloodstream of patients.

DNA
This is a molecule containing the genetic code of the cells. It is a double helix molecule composed of the combination of four nucleotides: adenine (A), cysteine (C), guanine (G) and thymine (T) conjugated with sugar and a phosphate. The order, or sequence, of these bases, determines the information available for building and maintaining an organism.

Exon
This is the part of a gene sequence that is used to produce RNA and proteins.

Enzyme
This is a protein involved in the chemical reactions of the body.

Immunotherapy
This is a type of cancer treatment that helps your immune system fight cancer.

Immune checkpoint
This is a group of proteins that are key regulators of the immune response against external cells and for the preservation of self-tolerance. Cancer cells can express, on their surface, specific immune checkpoint proteins that inhibit the immune system T cells to attack and kill them.

Gene
A gene is a piece of DNA containing the information to produce RNA and proteins. An international research effort called the Human Genome Project, which worked to determine the sequence of the human genome and identify the genes that it contains, estimated that humans have between 20,000 and 25,000 genes.

Gene mutation
It is an alteration in the sequence of the DNA. Some mutations may not have much effect, others may be beneficial, or may be dangerous, leading to pathology such as cancer.

Germline mutation
It is a mutation present in your germline cells (the cells that become sperm or oocytes when they mature). This mutation can be the only mutation that can be passed on to your offspring. A germline mutation can also affect all the cells of your body.

Protein
A protein is a molecule involved in the physiological functions of the body. Proteins also create the structure of certain body tissues and organs. They are made of amino acids. The sequence of amino acids determines each protein unique 3-dimensional structure and its specific function.

Somatic mutation
This is a mutation in a somatic cell. These mutations do not involve the germline and consequently do not pass on to offspring.

Wild type (WT)
A term used to indicate that a gene or a protein does not have a mutation.

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For further information:

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