What is a tumour?

Cancer nowadays is considered a pathology with a genetic component that occurs when cellular growth is out of control. Our body cells receive signals telling them when to grow and multiply and when such growth must stop. In a tumour, such cells do not respond to control signals and grow and multiply abnormally, spreading to different parts of the body, due to changes in their DNA.
The event leading to the alteration of genetic functions is called "**mutation**". When a gene is affected by mutation for different causes (biological, chemical and physical), information to the cell will not be adequate for its functions.

Is cancer hereditary?

Neoplasms are mostly pathologies caused by multiple factors generated by genetic and environmental risk factors. Most tumours are "**sporadic**", that is, they arise in the general population without any clear signs of a genetic susceptibility. In these types of tumours, **DNA alterations** (**mutations**) randomly develop in somatic cells, namely, those cells creating every organ and system of our body. These mutations develop in the DNA of a small group of cells and generate the genetic error that is perpetrated in the descendants of those cells. After having accumulated in an organ, they initially replace the healthy cells of that organ and then spread to other organs, both nearby and distant (metastasis).
There are forms of tumour called *"***familial***"*, when people affected by cancer in the family are close relatives. Cancer familiarity is a significant risk factor, especially when common environmental risk factors are also shared (lifestyles, diet, polluting agents, etc.) without a specific genetic alteration that makes the subject more susceptible to the disease.

When a new person is conceived, s/he acquires two pairs of each gene, one pair from the father and one from the mother. Any genetic mutations in the DNA of the parents are, therefore, passed on to children. If one of the parents has a mutation in one of the cancer-generating genes (hereditary tumour), **the offspring has a 50% chance of inheriting that mutation.** People inheriting a germ cell mutation are born with a copy of the mutated gene. These people **do not inherit the tumour, but only the susceptibility to develop that type of cancer more easily,** compared to the general population.

The ColonScreen® test

ColonScreen® is a diagnostic test developed by GENOMA Group that carries out a multiple genetic analysis aimed at **evaluating the susceptibility to the development of Colorectal cancer or Familial Adenomatous Polyposis.** Therefore, the test identifies patients with the risk of developing the aforesaid neoplasms by analysing their DNA.

Who should take the ColonScreen® test?

The genetic susceptibility test is addressed to people who from a thorough family history, show a high and concrete incidence of neoplasms in previous generations and, therefore, have a high risk of carrying a germinal mutation.
A hereditary form of cancer may be present when the family has:

- Several individuals affected by the same type of cancer or correlated cancers,
- Individuals affected by multiple tumours,
- Tumours that arise at a young age.

In particular:

- Colorectal cancer diagnosed ≤ 50 years;
- Endometrial or ovarian cancer with a family history of gastrointestinal cancer;
- Subjects with more than one cancer associated to the Lynch syndrome (e.g. colorectal and endometrial cancer);

- Three or more cases of colorectal, ovarian, endometrial, gastric cancer or other types of cancer associated with the Lynch syndrome in the family;
- Results of the Microsatellite or immunohistocemical instability test in the cancer showing a lower capacity of repairing DNA replication errors;
- Multiple primary tumours in the same patient;
- Several members of the family (on the same side) with colorectal cancer or other types of cancer.
- A known mutation in the family in one of the genes associated with colorectal cancer/polyposis susceptibility.

The geneticist, with the informed consensus of the person, will decide whether a DNA mutation diagnostic test is necessary.

What are the benefits of the ColonScreen® test?

The possibility to detect subjects at risk of developing a neoplasm is the best way for an early diagnosis of cancer and, therefore, to decrease mortality in that type of disease.
Members of families with high risks of inheriting cancer and especially those that were directly affected by a neoplasm, genetic counselling and may discuss his/her clinical and genetic situation with the geneticist. This assessment may lead to a genetic test to evaluate whether the patient is a carrier of a mutation that increases susceptibility to the development of a specific tumour.
If the test result is positive, the analysis may be extended to the relatives of the patient, in order to find those at risk.

The information from the genetic test may offer several **benefits**, such as:

- Finding family members with a **high risk of developing cancer;**
- The development of an adequate **medical check plan** for high-risk subjects, in order to promote early diagnosis of cancer;
- Awareness that the **genetic mutations may be transferred to** the offspring and the detection of high-risk offspring with germinal genetic mutations;
- The possibility to undergo **prevention therapies.**

How does the ColonScreen® test work?

The test is carried out with a blood sample. The DNA is isolated from the nucleated cells through a complex laboratory analysis **and amplified via PCR.** Then, with a state-of-the art technological **massive parallel sequencing** (**MPS**) process that uses ***Next Generation Sequencing*** (**NGS**) techniques with **ILLUMINA** sequencers, **12 genes** (exons and adjacent intrionic regions, ± 5 nucleotides) are completely sequenced at high reading depth (Table1). The chosen genes are often involved in hereditary susceptibility to the formation of colon cancer;

The resulting genetic sequences are analysed with an **advanced bioinformatics analysis**, to find mutations of the examined genes, if present.

HEREDITARY COLON CANCER

Results of the ColonScreen® test

"POSITIVE" – Presence of one or more mutations: the result shows that there are one or more mutations in one (or more) genes leading to hereditary susceptibility to the development of colon cancer; the test, therefore, shows a mutated copy of the gene. During a genetic consultancy session,

our geneticist thoroughly explains the test results and calculates the likelihood of developing the specific cancer associated to that mutation detected in a specific gene.
A positive result does not necessarily mean that the patient with a mutation will develop a tumour; it only shows **susceptibility to developing that type of tumour** in the patient, or rather, the person has a **higher risk level** compared to a person without that specific mutation. In fact, not all people carrying mutations develop neoplasms. Although such mutations significantly increase the chance of developing a tumour, the cancer does not develop until the normal copy of the corresponding gene is mutated during the life of the person.
Since everyone inherits two pairs of the same gene, a mutation must occur in each pair to cancel the function of such gene. The acquisition of a new mutation may, therefore, directly lead to a tumour. Identifying cancer-susceptibility mutation allows us to develop an intense clinical check plan and evaluate preventive surgery. A mutation also leads to the examination of other relatives at risk if they want to check their situation. In relatives the test is a predictive analysis, because it detects, within these families, the carriers of the mutation and people that do not carry the mutation, finding exactly those people with a high chance of developing a tumour and those whose risk is at the same level as the general population. That way, carriers can start specific check protocols for early diagnosis or prevention, while the latter will be screened with the same protocols as the general population.
Mutations that may be detected with the **ColonScreen®** may be divided into the following prognosis categories:

- **With known prognostic outcome;**
- **With benign prognostic outcome,** since they may be found in healthy individuals and have no pathological significance;
- **With uncertain prognostic outcome** they are not not known or classified by the medical and scientific community. In this case further examinations are needed to clarify the significance f the variation.

"NEGATIVE" - No mutations: The results show no mutations in the examined genes. However, a negative result does not necessarily mean that the patient does not risk developing a tumour. These people have the same chance of developing cancer as the general population because most of these types of tumours are sporadic in nature.

RISK STATISTICS

HNPCC - Hereditary Non-Polyposys Colon Cancer
The HNPCC (*Hereditary Non-Polyposis Colon Cancer* - **HNPCC**), also known as *Lynch Syndrome*, is an **autosomal, dominant cancer** with two phenotypes:
Lynch Syndrome I, with the onset of a **colon neoplasm** at around 45 years of age.
Lynch Syndrome II, which, in addition to colon cancer, includes the development of **extracolonic neoplasms,** at an endometrial, ovarian, urinary tract and biliary duct level.
The HNPCC is caused by a recurring mutation in one of the 4 genes that are currently known to be involved in the check and repair of DNA replication errors in all the cells of the body. Around **90%** of mutations occur in **MSH2** and **MLH1** genes (60% in MSH2 and 30% in MLH1) and only rarely involve PMS1 and PMS2 genes. When a mutation occurs in one of these genes, the ability to repair errors during DNA duplication decreases and, as a consequence, mutations accumulate in the cells, leading to a neoplasm.

According to statistics, the risk for the **general population** to develop **colon cancer** in life is around **6%**. For patients (both male and female) **carrying HNPCC mutations**, the risk of developing colon cancer is around **75-90%.**

Extracolonic neoplasms are a severe complication, the most common of which is **endometrial cancer**. The overall risk of this type of cancer is around 30%, compared to 3% among the **general population.** The risk of developing ovarian cancer is **3.5 times higher** and it may occur 20 years earlier compared to the reference population.

The **Familial Adenomatous Polyposis** (FAP or Adenomatosis coli or Colorectal Familiar Polyposis) is a relatively rare syndrome that occurs, usually at an early age, with the development of hundreds to thousands of adenomas on the large intestine. Without treatment, FAP progresses almost invariably with one or more colorectal carcinomas, usually when the individual is 30 to 50 years old; the onset of malignant lesions may be seen with careful endoscopic checks and prompt surgical intervention. The FAP is a "compulsory" precancerous condition, because the person that is affected and not treated will almost certainly develop invasive carcinoma.
The FAP is a hereditary, autosomal, dominant disorder, usually at high penetration. Therefore, a careful analysis of the family history could help detect subjects at risk in each phratry on which accurate endoscopic checks must be carried out. Presently, this process is easier, thanks to the detection of the gene that, once mutated, generates the disorder (APC gene, Adenomatous Polyposis Coli).
In a sub-type of patients, a mutation of the MUTYH (1p34.1) gene leads to an automosal recessive polyposis, the familial adenomatous polyposis linked to the MUTYH, which is characterised by a small increase in the risk of developing colorectal cancer and polyps/adenomas in the higher and lower gastrointestinal tract.

Parameters used for genetic variation reporting

Only the genes listed in Table 1 are analysed. Only mutations classified as "with known prognostic outcome" or "with uncertain prognostic outcome" according to the scientific literature and the classification in the Human Gene Mutation Database (HGMD) reference database updated on the day of the examination will be reported. Moreover, in line with American College of Medical Genetics (ACMG), only mutations with a Minor Allele Frequency (MAF) <5% (1000 Genomes Project), calculated as the frequency in which the least common allele in the population occurs, were considered as pathogenic or presumably pathogenic.

Target Coverage

Target Coverage is the average number of reads generated by the sequencing of each nucleotide base of the gene. Variations with a read depth (number of reads) lower than 30X are not highlighted by the bioinformatics analysis algorithm.

Accuracy of the ColonScreen® test

Present DNA sequencing techniques produce results with more than 99% accuracy. Even though this test is very accurate, the limits of the examination need to be considered.

Limitation of the ColonScreen® test

This examination analyses only genetic diseases and genes listed in Table 1 and cannot detect:

- Mutations positioned in the intrionic regions beyond ± 5 nucleotides from the breakpoints;
- Deletions, inversions, or duplications with a value of more than 20 bps;
- Germline mosaicism (i.e. mutations only in the gametes)

A **"NEGATIVE" - No mutations** result for the examined genes does not exclude the possibility that mutations are not present in a region of the genome that was not explored during the examination.

Some regions of our DNA may not be sequenced or have a lower coverage than the limitations set by GENOMA Group experts to guarantee an accurate examination of gene variations. These regions, therefore, are not included in the examination if they do not meet the requested qualitative standards.

In some cases the result of genome testing may reveal DNA variations or mutations with an unknown or unclassifiable clinical meaning within the current medical and scientific knowledge.

The interpretation of genetic variations is based upon the most updated knowledge available upon examination. Such interpretation may change in the future, when new scientific and medical information on the structure of the genome are acquired and may affect the evaluation of the genetic variations themselves.

Some pathologies may be caused or regulated by more than one or more variations in the DNA in one or more genes. Some of these variations may not be detected or validated by the scientific community and, therefore, may not be classified as pathogenic variations upon examination.

The intrinsic limitation of the NGS methodology is the lack of coverage uniformity of each examined genetic region. Due to this limitation, NGS tests may not detect specific genetic mutations in the selected genes.