MEDICOVER GENETICS

CATALOGUE

SOLID TUMOR TESTS HISTOPATHOLOGY & GENETICS

Detect&Act

Physician Information

MEDICOVER GENETICS
ABOUT US

Medicover Genetics was developed within Medicover, **a network of hospitals and diagnostic laboratories across European markets**. Our purpose is to empower people to use comprehensive and meaningful genetic tests at the forefront of their diagnostic journey, fueled by our vision to place genetics at the core of medical decisions. We want to achieve this by leveraging advancements in genomics to develop relevant diagnostic solutions, supported by professional medical interpretation, to improve people's health and well-being.

Spanning cytogenetic analyses, molecular pathology solutions, the latest in next generation sequencing (NGS) technology and microbiome sequencing, Medicover Genetics offers a **complete in-house and tailor-made portfolio produced in our laboratories in Germany** and offered internationally. Medicover is the sole testing site in Europe for Bionano's Saphyr® technology: the third-generation optical mapping solution which resolves large-scale structural variations currently missed by NGS.

Using a robust diagnostics pipeline, we make **NGS testing and variant discovery efficient, scalable** and **accessible** by converting NGS data into customized clinical reports in a timely manner, **thereby decreasing turnaround times**.

Patient support through genetic counselling is integral to our patient journey and crucial to explain complex findings to them as well as assist physicians as they support their patients. With more than **20 certified genetic counsellors** across our markets, we are able to provide this locally and in the local language.

MEDICOVER GENETICS

PURPOSE

To empower people to use comprehensive and meaningful genetic tests at the forefront of their diagnostic journey

VISION

To place genetics at the core of medical decisions

MISSION

Leverage advancements in genomics to develop relevant diagnostic solutions, supported by professional medical interpretation, to improve people's health and well-being

VALUES

Humanity | Passion | Innovation | Medical Excellence | Integrity

MEDICOVER GENETICS
WHY US

- A network of +100 histopathologists and medical institutions **makes us a leader in pathology and genetic testing** in >10 countries with foundations dating back to 1998

- Home to digital scanners, allowing a **faster and more accurate diagnosis**

- A network of laboratories and medical institutions makes Medicover Genetics **a leader in genetic testing** in Germany with foundations dating back to 1998

- A clinical team comprised of scientists, physicians and medical geneticists, several with **>20 years of experience** in genetic testing, assuring meaningful and comprehensive genetic tests

- **Up-to-date diagnostic algorithms** and gene panels based on current scientific literature and international guidelines

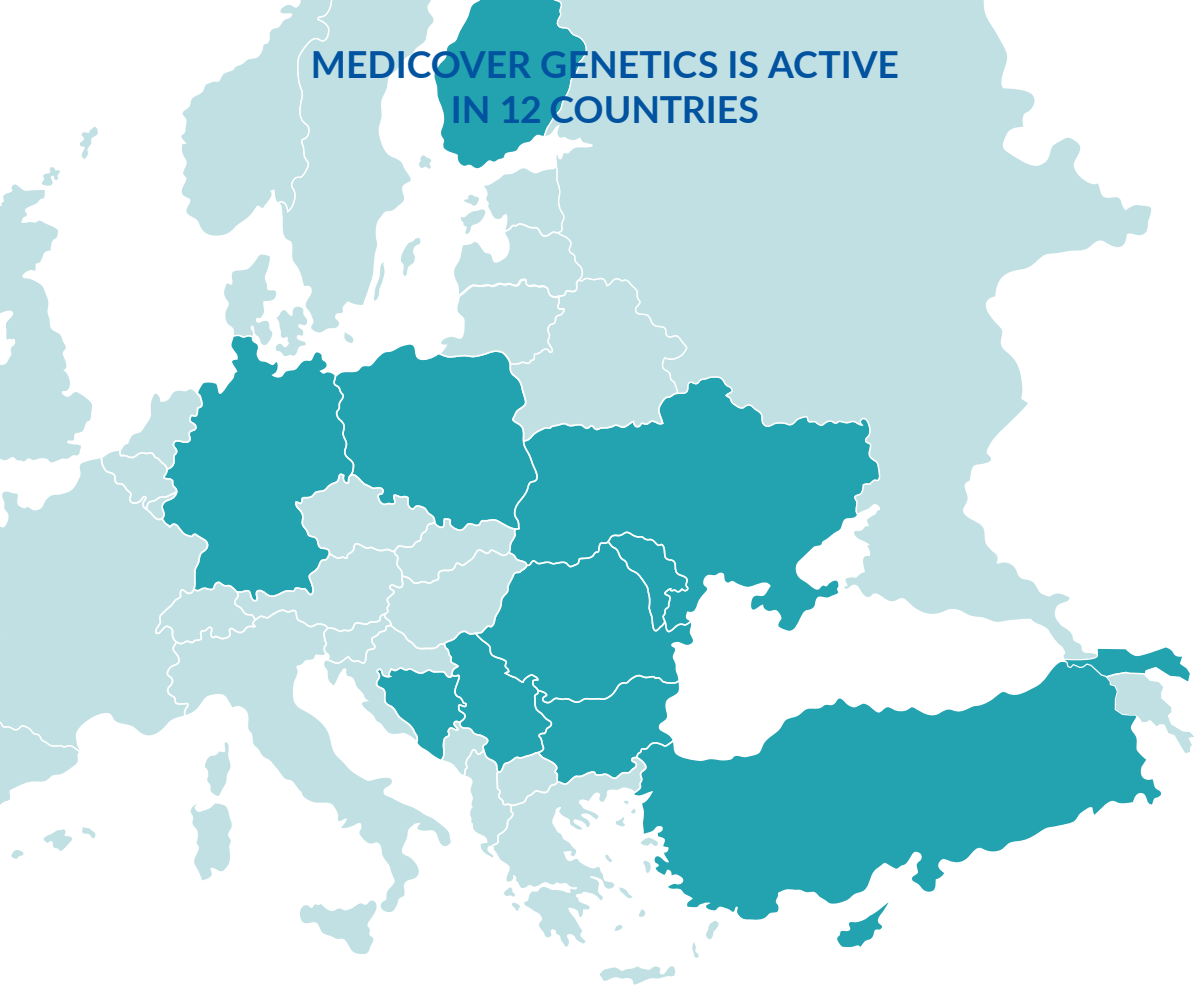
- Expertise in gene variant analysis ensuring **"no variant left behind"**

- Cutting-edge technology in sequencing and laboratory methods allows for **short turnaround times**

- **Quality** assessed by several certified bodies, including EFI, DIN EN ISO 9001, DIN EN ISO15189 accreditation for medical laboratories, DIN EN ISO/IEC 17025 accreditation for testing and calibration laboratories and a generally valid GMP (Good Medical Practice) certificate

- **Data privacy** is your right and our priority

MEDICOVER GENETICS IS ACTIVE IN 12 COUNTRIES



BOSNIA-HERZEGOVINA | BULGARIA | CYPRUS | FINLAND | GEORGIA
GERMANY | MOLDOVA | POLAND | ROMANIA | SERBIA | TURKEY | UKRAINE

MEDICOVER GENETICS
TABLE OF CONTENTS

DIAGNOSTIC PROCESS

HISTOPATHOLOGY

Step 1: Biopsy

The tissue fragment is removed from the patient during surgery and sent to the laboratory for microscopic examination to determine the diagnosis.

Step 2: Gross examination

The tissue is examined macroscopically and relevant fragments are sampled.

Step 3: Conventional and complementary stainings

- **Hematoxylin and eosin (H&E) staining:** to visualize overall cellular structure
- **Special stains:** to visualize cell morphology, detect and localize subcellular components
- **Immunohistochemistry:** to detect specific protein markers that support tumor classification, assessment of prognostic and predictive factors, and identification of biomarkers for targeted therapy

Step 4: HER2 evaluation for breast and gastric cancers

HER2 levels are evaluated using silver *in situ* hybridization (SISH).

Step 5: Pathology report

A report is delivered to the ordering physician with a summary of pathology findings and recommendations for sequencing, if appropriate.

SEQUENCING

CONSIDER ADDITIONAL HEREDITARY CANCER PANEL TESTING

Step 6: Molecular diagnostic analysis of the tumor tissue
Based on the pathology findings, specific sequencing analysis may be recommended.

Step 7: Final report with pathology and sequencing results
A final report is delivered to the ordering physician with a summary of all findings, treatment recommendations and relevant clinical trials.

TISSUE REQUIREMENTS

Tissue fragments from a biopsy:

- Stored in 10% formalin (stable for 24-72 hrs)
- Embedded in paraffin blocks (stored in dry and dark conditions)

TURNAROUND TIMES

Histopathology analysis: 7-10 working days
Genetic analysis: 7-20 working days

MEDICAL COUNSELLING

We can provide expert medical interpretation of the results for the specialist physician and the patient, where needed. This includes advice on which tests to choose, interpretation of findings, treatment options and relevant clinical trials.

SOLID TUMOR
OVERVIEW

BACKGROUND

Close to 20 million new cancer cases and 10 million deaths have occurred annually in recent years. **Solid tumors represent approximately 90% of adult cancers** and millions of histopathology slides are analyzed annually revealing information crucial for cancer diagnosis and staging. In up to 40% of patients, complex genomic alterations are identified which can serve as biomarkers to predict response to a specific therapy and/or prognosis.

Histopathological examination and genetic testing can determine the tumor profile and suggest appropriate management or treatment plans, when available.

We offer both histopathology and molecular genetic analyses for solid tumors.

Characterizing the cellular and molecular changes in a solid tumor is critical for treatment strategy. Customized treatment depends on the type and severity (stage) of the cancer and the specific genetic alterations in the tumor tissue. Genotype-directed therapy or genotype-matched clinical trials can improve patient care and survival.

Account for **~90% of adult cancers** and **50%** of cancers in **children** and **young adults**

Complex **genomic alterations** identified in up to **40% of patients**

High **clinical benefit** of genomic profiling for patients

SOLID TUMOR
TUMOR PROFILING

Tumor profiling is the investigation of molecular changes or biomarkers in tumor tissue. It allows for **individualized, more efficient** and **personalized treatment**, depending on the unique genetic alterations in the tumor. Treating a tumor based on its molecular features may result in better patient outcome, including improved clinical management and increased survival. Tumor profiling allows the investigation of genomic mechanisms involved in tumor formation, including microsatellite instability and fusion genes, as well as enabling investigation of tumor mutation burden analysis.

Microsatellites are repeated sections of DNA, 1-6bp long, that are found throughout the entire genome and account for approximately 3% of it. Due to their repeated sequences, microsatellites are prone to a high mutation rate. **Microsatellite instability** (MSI) is a cause of unique molecular alterations and hypermutated phenotypes. It is triggered by an impaired DNA mismatch repair (MMR) system, which frequently results from germline or somatic mutations or promoter hypermethylation of genes in the DNA MMR system, such as *MLH1*, *MLH2*, *MSH6*, and *PMS2*.

Tumor mutation burden (TMB) can be determined as a predictive biomarker and can support the selection of patients who may benefit from immune checkpoint inhibitor (ICI) therapy. TMB is defined as the number of somatic coding, non-synonymous variants in the tumor genome per megabase and is associated with the development of neoantigens that trigger antitumor immunity.

Gene fusions occur across a wide spectrum of tumor types. Gene fusions arise as a result of genomic rearrangements, including chromosomal inversions, interstitial deletions, duplications, or translocations, and can drive both the development and progression of cancer. Sequencing efforts have identified rare oncogenic fusions across several forms of cancer. Many of these fusions have proven to be viable targets or are the subject of promising ongoing research.

SOLID TUMOR THERAPIES

Targeted therapies use small molecules to attack the deregulated proteins that support the survival of cancer cells. In many tumors, signaling pathways regulated by protein kinases are subject to somatic mutations; thus, kinase inhbitors are the most successful targeted small molecules. Small molecule approaches are more favorable for cancers like lung, colorectal and breast, as they focus on particular molecular changes unique to a specific cancer.

Common small molecule therapies

Target	Drug	Tumor type
BCR-ABL	Imatinib, dasatinib, nilotinib, bosutinib, regorafenib, ponatinib	CML, ALL, GIST, CRC
PDGFR	Imatinib, dasatinib, nilotinib, sunitinib, sorafenib, regorafenib, erdafitinib, lenvatinib	ALL, CML, GIST, RCC, pNET, HCC, thyroid cancer, CRC, UC
PDGFR	Pazopanib	RCC, soft tissue sarcoma
EGFR	Afatinib, gefitinib, osimertinib, vandetanib, erlotinib, lapatinib, dacomitinib, neratinib	NSCLC, PDAC, medullary thyroid cancer, BrCA
FGFR	Erdafitinib, lenvatinib, pazopanib	UC, thyroid cancer, HCC, RCC, soft tissue sarcoma
HER	Afatinib, osimertinib, neratinib, lapatinib	NSCLC, BrCA
CDK 4/6	Ribociclib, abemaciclib, palbociclib	BrCA
C-KIT	Imatinib, dasatinib, nilotinib, sunitinib, sorafenib, regorafenib, erdafitinib, lenvatinib	CML, ALL, GIST, HCC, pNET, RCC, thyroid cancer, CRC, UC
C-KIT	Cabozantinib, pazopanib	Soft tissue sarcoma
SCF	Imatinib	CML, ALL, GIST

SRC	Dasatinib, bosutinib, vandetanib	ALL, CML, medullary thyroid cancer
CSF	Nilotinib, sunitinib, erdafitinib	CML, GIST, RCC, pNET, UC
DDR	Nilotinib, regorafenib	CML, CRC
C-MET	Crizotinib, cabozantinib	NSCLC, HCC, RCC
VEGFR	Sunitinib, sorafenib, axitinib, vandetanib, regorafenib, erdafitinib, lenvatinib, cabozantinib, pazopanib	RCC, HCC, medullary thyroid cancer, GIST, pNET, thyroid cancer, CRC, UC, soft tissue sarcoma
RET	Vandetanib, sunitinib, regorafenib, sorafenib, erdafitinib, alectinib, lenvatinib, cabozantinib	Medullary thyroid cancer, GIST, RCC, pNET, CRC, HCC, thyroid cancer, UC, NSCLC
TIE2	Vandetanib, regorafenib, cabozantinib	Medullary thyroid cancer, CRC, RCC, HCC
RAF	Vemurafenib, sorafenib, regorafenib, encorafenib, dabrafenib	Melanoma, HCC, RCC, thyroid cancer, CRC
PARP	Olaparib, rucaparib, talazoparib, niraparib	Ovarian cancer, BrCA
TRK	Larotrectinib, regorafenib, entrectinib, cabozantinib, lorlatinib	Solid tumor, CRC, NSCLC, HCC, RCC
BTK	Ibrutinib	MCL, CLL, SLL
MEK	Cobimetinib, binimetinib, trametinib	Melanoma
FTL	Sorafenib, sunitinib, erdafitinib, brigatinib, cabozantinib, gilteritinib	HCC, RCC, thyroid cancer, GIST, pNET, UC, NSCLC, AML
ROS1	Entrectinib, crizotinib, brigatinib, lorlatinib, ceritinib, cabozantinib	Solid tumors, NSCLC, RCC, HCC
ALK	Entrectinib, alectinib, crizotinib, brigatinib, lorlatinib, ceritinib	Solid tumors, NSCLC
IGF-1R	Brigatinib, ceritinib	NSCLC
IDH1	Ivosidenib, enasidenib	AML

26S proteasome	Bortezomib, carfilzomib, marizomib	Multiple myeloma, MCL
PI3KCA	Alpelisib	BrCA
PI3K	Duvelisib, copanlisib	CLL, SLL, follicular lymphoma

CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; pNET, pancreatic neuroendocrine tumors; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; UC, urothelial cancer; HCC, hepatocellular carcinoma; RCC, renal cell carcinoma; MCL, mantle cell lymphoma; CLL, chronic lymphocytic leukemia; SLL, small lymphocytic lymphoma; AML, acute myeloid leukemia; BrCA, breast cancer; CRC, colorectal cancer (modified from Fernandez-Rozadilla et al. 2021)

Immunotherapy has also become an important tool in the development of targeted therapies. There are numerous monoclonal antibodies (mAbs) to treat various types of cancers. These antibodies are produced specifically to block cell surface receptors that are present (ideally) exclusively on tumor cells and tumor-promoting molecules. They recognize a tumor antigen and cause cell death through various mechanisms, including apoptosis, indirect elimination by recruitment of immune cells with cytotoxic properties, or by activation of the complement cascade. Immune checkpoint inhibitors (ICIs) are mAbs that assist the immune system and have shown great benefits for patient survival. ICIs currently in use are CTLA-4, PD-1 and PD-L1 inhibitors.

Common monoclonal antibody therapies

Target	Drug	Tumor type
HER2	Adotrastuzumab, trastuzumab, pertuzumab	BrCA
EGFR	Cetuximab, panitumumab, necitumumab	CRC, HNSCC, NSCLC, PDAC, glioma, squamous NSCLC
VEGFR	Ramucirumab	Gastric cancer, NSCLC
VEGF	Bevacizumab	CRC, NSCLC, BrCA, glioblastoma, RCC

CD-20	Rituximab, ofatumumab, ibritumomab, tositumomab, obinutuzumab	Non-Hodgkin lymphoma, CLL, follicular lymphoma
CD-22	Inotuzumab	ALL
CD-52	Alemtuzumab	CLL
CD-33	Gemtuzumab	AML
CD-30	Brentuximab	Hodgkin lymphoma, anaplastic large cell lymphoma
CD19/CD3	Blinatumomab	ALL
CD38	Daratumumab	Multiple myeloma
CTLA-4	Ipilimumab	Melanoma, RCC
PD-1	Nivolumab	Melanoma, NSCLC, SCLC, RCC, UC, Hodgkin lymphoma, HNSCC, MSI-H/dMMR CRC, HCC
PD-L1	Atezolizumab, avelumab, cemiplimab, pembrolizumab, durvalumab	UC, NSCLC, BrCA, RCC, CSCC, melanoma, NSCLC, HNSCC, Hodgkin lymphoma, MSI-H cancer, gastric cancer, cervical cancer, HCC, MCC
RANKL	Denosumab	Giant cell tumor of the bone
GD2	Dinutuximab	Pediatric neuroblastoma
PDGFR	Olaratumab	Soft tissue sarcoma
SLAMF7	Elotuzumab	Multiple myeloma

BrCA, breast cancer; CRC, colorectal cancer; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; RCC, renal cell carcinoma; CLL, chronic lymphocytic leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; UC, urothelial cancer; MCL, mantle cell lymphoma; MSI-H, microsatellite instability-high; dMMR, mismatch repair deficient; MCC, Merkel cell carcinoma; CSCC, cutaneous squamous cell carcinoma (modified from Fernandez-Rozadilla et al. 2021)

OUR TEST OPTIONS

We offer both histopathology and genetic testing for solid tumors. These can be ordered separately or together:

HISTOPATHOLOGY *only*

GENETIC TESTING *only* – THREE OPTIONS:

TARGETED-THERAPY TESTS	GENE PANELS	COMPREHENSIVE PANEL
Identifies specific genomic changes that are relevant to therapy with approved therapeutic products (targeted therapies)	In addition to targeted therapies, both test options can identify additional biomarkers, microsatellite instability, and recommend clinical trials	

HISTOPATHOLOGY *and* GENETIC TESTING

GENETIC TESTING OPTIONS

		TARGETED-THERAPY TESTS	GENE PANELS	COMPREHENSIVE PANEL
	Number of genes	Up to 9 genes	Up to 29 genes	>500 genes
	Turnaround time	7-10 days	20 days	20 days
Actionability	**Approved treatments**	√	√	√
Actionability	**Available therapies**		√	√
Actionability	**Referral to clinical trial**		√	√
Markers analyzed	**Full-gene sequencing**	√*	√	√
Markers analyzed	**Fusion genes** the abnormal joining of parts of two different genes	√*	√	√
Markers analyzed	**MSI** microsatellite instability, an analysis of mutation frequency within microsatellites (short, repeated sequences of DNA)	√*	√	√
Markers analyzed	**SNV** single nucleotide variants, a DNA sequence variation that occurs when a single nucleotide in the genome sequence is altered (mutated)	√	√	√
Markers analyzed	**Other biomarkers** biological molecule that is a sign of a normal or abnormal process, or of a disease		√	√
Markers analyzed	**CNV** copy number variants, altered number of copies of a gene present in the genome			√
Markers analyzed	**TMB** tumor mutational burden, total number of mutations found in the DNA of cancer cells			√

**In special cases only*

HISTOPATHOLOGY AND TARGETED-THERAPY TESTS

Specific tumor classification using IHC

The standard markers are listed for each cancer type. Additionally, the pathologist may decide to use extra markers for thorough assessment of a tumor in some cases.

Bladder

CD44, CK20, CK7, GATA3, Ki67, p53

FGFR2, *FGFR3*

Gastrointestinal

CD34, c-KIT (CD117), desmin, DOG1, Ki67, S100

BRAF, *KIT*, *NF1*, *PDGFRA*, *SDHA*, fusion genes

Gastric

CDX-2, CG-A, CK20, CK7, HER-2, Ki67, SYN

fusion genes, MSI

Ovarian

AFP, calretinin, EMA, ER, hCG, inhibin, napsin A, OCT3/4, p16, p53, PAX8, PR, SALL4, WT1

BRCA1, *BRCA2*, fusion genes, MSI

Pancreatic

CK19, CK20, CK7, MUC5AC

BRAF, *BRCA1*, *BRCA2*, *KRAS*, *PALB2*, *SMAD4*, fusion genes, MSI

Fusion genes: ALK, NTRK, RET, ROS; IHC, immunohistochemistry; MSI, microsatellite instability; NSCLC, non-small cell lung carcinoma

Breast

E-CD, ER, GATA3, HER2, Ki67, PR

BRCA1, *BRCA2*, *PIK3CA*, fusion genes, MSI

Colon

CDX-2, CK20, MLH1, MSH2, MSH6, PMS6, villin

BRAF, *KRAS*, *NRAS*, fusion genes, MSI

Melanoma

HMB45, Ki67, Melan-A, p16, S100, SOX10

BRAF, *KIT*, *NRAS*, fusion genes

NSCLC

ALK, CG-A, EGFR, p40, PD-L1, ROS, SYN, TFF1

BRAF, *EGFR*, *ERBB2*, *KRAS*, *MET*, fusion genes

Prostate

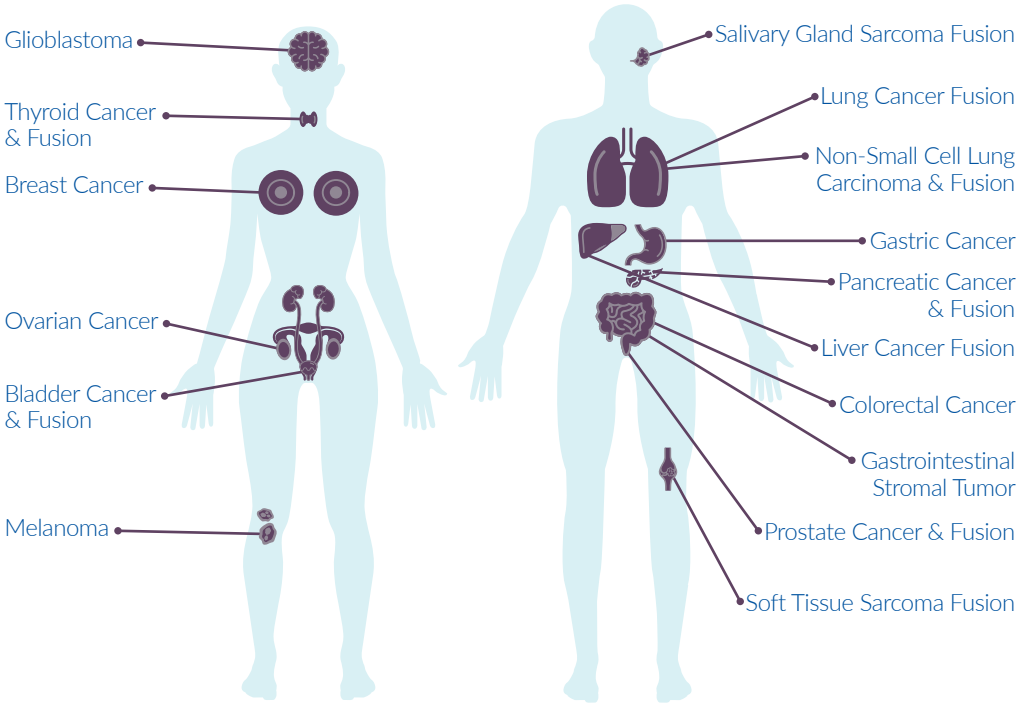
BCC-AMACR, CK34BE12, CK5/6, NKX3.1, p63, PSA, PSAP

ATM, *BRCA1*, *BRCA2*, *CDK12*, *CHEK2*, *FANCA*, *PALB2*, *PTEN*, *RAD51*, MSI

Special stains used for broad tumor identification:

Gram, Ziehl Nielsen, Giemsa, PAS, Masson's Trichome, Silver, GMS II, Alcian Blue ph 2.5, Amylase, Perls, Weigert Van Gieson, Congo Red

SOLID TUMOR GENE PANELS AND FUSION GENE PANELS



Consider Hereditary Cancer Panels Predict&Prevent if your patient's tumor has a genetic cause

For the most up-to-date information about our tests, including technical information and gene lists, please visit:
www.medicover-genetics.com

COMPREHENSIVE SOLID TUMOR PANEL

WHY CHOOSE A COMPREHENSIVE PANEL?

- Tests for **clinically actionable genomic alterations** that are often implicated in solid tumors
- Analyzes **>500 genes** in the tumor from **one sample**
- Helps diagnose a tumor of **unknown primary origin**, e.g., in metastatic cancer
- **Genomic alterations may be missed** with a specific gene panel
- The analysis includes:

523 genes — Single nucleotide variants

59 genes — Copy number variants

54 genes — Fusion genes

WHAT ARE THE ADVANTAGES?

- A clinically useful approach to **investigate genomic mechanisms involved in tumor formation**, including microsatellite instability, and enables investigation of tumor mutation burden analysis
- Knowing the **complete molecular profile** of the tumor can help to:
  - Establish a **definite diagnosis**
  - Provide information about the **behavior of a tumor**, including growth and spread
  - Recommend approved **therapy**, including **targeted therapies** or **immunotherapies**
  - **Rule out** inappropriate **therapies**
  - Select appropriate **clinical trials**

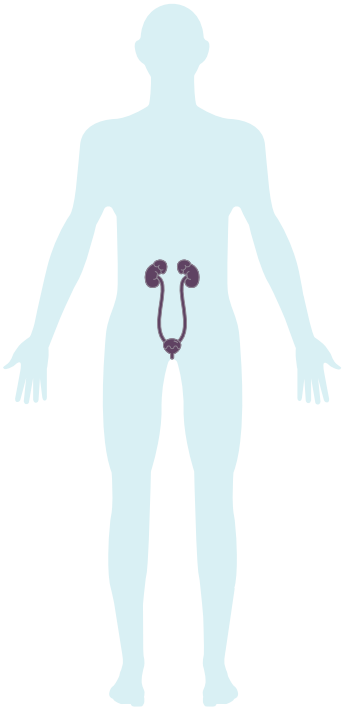
WHO IS THE TEST FOR?

Recommended for patients with **cancer of unknown primary origin and rare cancers**

BLADDER CANCER

BACKGROUND

With about 30,000 new cases every year, bladder carcinoma is one of the most frequent forms of cancer. The median age of onset is 73 years for men and 75 years for women, with men being affected three times more often. Besides genetics, other risk factors include the handling of aromatic amines, smoking, chronic urinary bladder infections and radiation therapy.



Most bladder carcinomas are diagnosed in patients with macroscopic hematuria. In >90% of cases, urothelial carcinomas, often with multifocal occurrence, are involved. Bladder carcinomas develop in two different ways and lead to **non-muscle invasive tumors (NMIBC)** that are histologically classified as papillary, and non-papillary **muscle invasive tumors (MIBC)** with an increased risk of metastasis. At the time of diagnosis, about 75% of bladder tumors are restricted to the urothelium or the lamina propria (~70% are designated Ta and are non-invasive papillary carcinomas, ~20% have spread to the lamina propria and are designated T1, and ~10% are NMIBC, designated Tis), and about 25% have already infiltrated into deeper layers of the bladder. There is a correlation between tumor grade and tumor stage: superficial tumors are low grade, invasive tumors are high grade. Due to the high recurrence rate of NMIBC, regular invasive control examinations (cystoscopy) are required.

GENOMIC ALTERATIONS

NMIBCs are characterized by activating variants in *FGFR3* occurring early in the pathogenesis as well as variants in *HRAS* (~10%). Variants in *FGFR3* are found in low-grade NMIBC in 66% of Ta and 37% of T1 tumors. *FGFR3* activation may occur due to chromosome translocations. The resulting fusion genes with *TACC3* or *BAIAP2L1* lead to the formation of potent transforming oncogenes. The detection of *FGFR3* variants in urine sediments can therefore be used as a marker for recurrence. Variants in the tumor suppressor gene *TSC1* (15%) and in *PIK3CA* (16-25%) are also found in NMIBC, the latter often correlating with variants in *FGFR3*. Furthermore, in 50% of cases chromosome 9 is deleted. As a result, the *CDKN2A* gene located in 9p21 that encodes p16 and p14ARF is affected, leading to negative regulation of signaling pathways. Further inactivating variants have been described in the *STAG2*, *KDM6A*, *CREBBP*, *EP300* and *ARID1A* genes.

MIBCs show predominantly inhibitory variants in the tumor suppressor genes *TP53* (6-20%) and *RB1* (~5%) as well as variants in the regulators of these signaling pathways, such as amplifications of *MDM2* and *E2F3* and homozygous deletions of *CDKN2A*. Variants in *TSC1* (~10%), *AKT1* and *PIK3CA* are also found in MIBC, but at a lower frequency than in NMIBC. In addition, *RAS* variants, variants in APC and deletions in *PTEN* can be detected in some cases of MIBC. MIBCs with variants in *HER2* show a good response to neoadjuvant chemotherapy, while MIBCs with variants in *ERCC2* respond well to cisplatin-based chemotherapy.

TESTS AND POSSIBLE THERAPIES

Checkpoint inhibitors are approved by the FDA for use in metastatic urothelial carcinoma (mUC). It may be helpful to determine TMB in these cases, as a high TMB is associated with a response to checkpoint inhibitors. In patients with urothelial carcinoma, variants can be detected in gene loci associated with hereditary tumor diseases and DNA mismatch repair genes.

As well as the deletion 9p21 (*CDKN2A*), MIBC tumor cells often show an increased copy number of all chromosomes, which can be detected by **UroVysion-FISH analysis** (Chr. 3, 7 and 17, locus-specific probe Chr. 9p21). The test, which has a sensitivity of 72% and a specificity of 83%, has been approved by the FDA to aid in the monitoring and diagnosis of bladder cancer. The combined morphological evaluation of urinary cytology and FISH increases the diagnostic sensitivity, this gives certainty to an initial diagnosis if cytology is unclear. For progress monitoring for previously diagnosed bladder cancer, the intervals between cystoscopy examinations may be shortened. However, a positive UroVysion test is not conclusive for the diagnosis of bladder carcinoma.

TARGETED-THERAPY TESTS

Gene	Genomic region
FGFR2	All
FGFR3	All

GENE PANEL

FUSION GENES

For the most up-to-date list of genes in our gene panels and fusion genes, please visit:
www.medicover-genetics.com

30,000 new cases per year

Men three times more affected

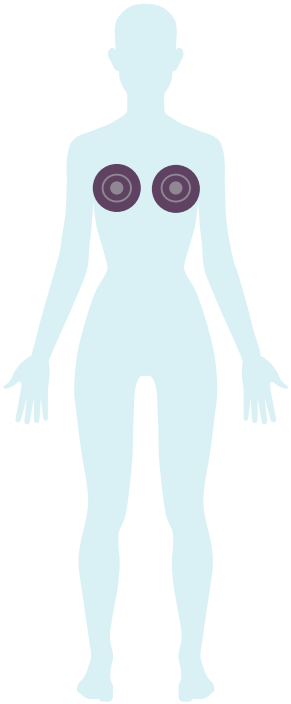
Age of onset:
73 years (men),
75 years (women)

BREAST CANCER

BACKGROUND

Breast cancer remains the most common cancer type amongst women. **Globally in 2020, 2.3 million women were diagnosed and 685,000 died.** With increasing treatment options for breast cancer, profiling the genomic landscape of breast tumors and incorporating those findings into patient care has become critical. Breast cancer is recognized as a group of heterogeneous diseases with variable clinical courses and treatment responses. Gene expression profile assays allow for recurrence risk prediction beyond clinical and pathologic presentations and refinement of adjuvant therapy.

The recent successes of **PI3K inhibitors** for the treatment of *PIK3CA*-mutated hormone receptor (HR)–positive breast cancer and of **PARP (poly (ADP-ribose) polymerase) inhibitors** in deleterious germline *BRCA1/2*-mutated tumors, have solidified the role of genomic testing to guide therapy for patients with breast cancer.



GENOMIC ALTERATIONS

In **metastatic HR-positive breast cancer**, overexpression of HR and activation of its downstream pathway are key features and anti-estrogen therapy, as monotherapy or in combination, remains the standard first-line treatment. *ESR1* gene mutations are found in approximately 20% of metastatic HR-positive breast cancers.

***PIK3CA* mutation** results in the activation of the PI3K/AKT pathway, leading to dysregulation of key cellular functions. *PIK3CA* mutations are found in different frequencies among breast cancer subtypes; HR-positive breast cancer has the highest frequency, with *PIK3CA* mutations in 40% of tumors in both metastatic and early-stage diseases. Other mutations along the PI3K/AKT pathway are also present in HR-positive breast cancer, including *AKT1* and *PTEN*. Like *PIK3CA*, these mutations are found most commonly in HR-positive breast cancer. Other common genomic alterations in HR-positive breast cancer, including *CDH1*, *GATA3*, *KMT2C*, *MAP3K1*, *MAP2K4*, *NF1*, and *ERBB2*, have been implicated as potential driver mutations, mechanisms of resistance, or prognostic markers.

In **metastatic HER2-positive breast cancer**, HER2 is encoded by *ERBB2*, and *ERBB2* amplification is a common feature of HER2-positive breast cancer. *ERBB2* amplification is significant because it predicts a benefit from HER2-directed therapies. Activating *ERBB2* mutations can result in altered cell growth and differentiation in addition to the activation of the mitogen-activated protein kinase pathway. Given that up to one-half of HER2-positive breast cancer also expresses HR at various levels, genomic alterations may be shared between these two patient populations.

***TP53* mutations** are observed in HER2-positive breast cancer in approximately 55% of both early-stage and metastatic disease. Brain metastasis remains a clinical challenge in HER2-positive breast cancers.

In **metastatic tripe-negative breast cancer** (TNBC), which is characterized by a lack of estrogen and progesterone receptors and HER2 expression, has a worse prognosis than other breast cancer subtypes. Despite the approval of ICIs for treatment of metastatic TNBC, resistance inevitably develops, and survival outcomes remain poor. Genomic profiling of TNBC has revealed that several genetic alterations are present at higher prevalence than in other breast cancer subtypes. *TP53* tumor suppressor gene mutations are found in more than one-half of metastatic TNBC tumors and reach up to more than 90% in certain reports. *TP53* mutations are frequent in early-stage TNBC as well, nearing 70% to 80%. Targeted therapy that restores *TP53* function remains an important unmet need in oncology. Other mutations include *PIK3CA*, *RB1*, and *PTEN*, but they occur at a much lower frequency.

Germline mutations of *BRCA1/2*, which are known to cause homologous recombination deficiency (HRD), are found in 10% to 20% of TNBC, and somatic mutations of *BRCA1/2* are found in 3% to 5% of TNBC. Multiple somatic mutations that can result in HRD have been implicated, and include *BRCA1/2*, *RAD51*, *PALB2*, *ATR*, *CHK1*, *WEE1*, and *PLK1*, among others. These mutations are observed in all metastatic breast cancer subtypes but in relatively low frequencies.

In addition to specific genomic alterations, evaluation of tumor **microsatellite instability** (MSI) that leads to defects in DNA mismatch repair has become standard care in metastatic solid tumors. Patients with tumors that harbor MSI are candidates for treatment with the ICI pembrolizumab. MSI has been documented in breast cancer but at a lower frequency compared with other cancers.

POSSIBLE THERAPIES

Approvals for **alpelisib and PARP inhibitors** for the treatment of metastatic breast cancer with *PIK3CA* mutations and germline *BRCA1/2* mutations, respectively, have highlighted the importance of breast cancer genomic profiles in treatment decisions. **Tumor-agnostic treatment** indicated by microsatellite instability and *NTRK* fusions provide additional treatment options for patients whose disease harbors those genetic alterations. Recognizing that current frontline treatments for breast cancer are based on well-founded evidence, tumor genomic testing should be performed in the appropriate setting to help guide therapy.

TARGETED-THERAPY TESTS

Gene	Genomic region
BRCA1	All
BRCA2	All
PIK3CA	Hotspots

GENE PANEL

MICROSATELLITE INSTABILITY

For the most up-to-date list of genes in our gene panels and fusion genes, please visit:
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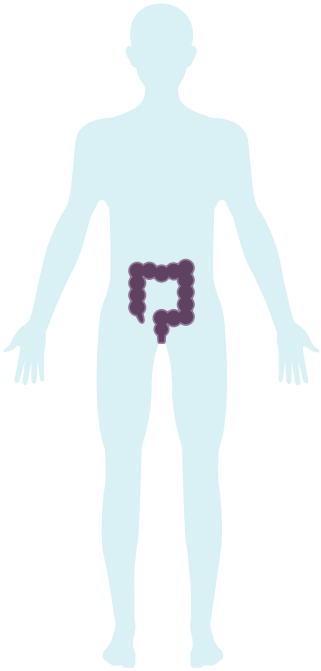
COLORECTAL CANCER

BACKGROUND

Colorectal carcinomas (CRC) account for about 14% of all tumors in adults. Histologically, >95% of colon carcinoma patients present with adenocarcinoma. Disease prognosis depends on the location (colon versus rectum) and stage at diagnosis. While 5-year survival is 85% to 90% in stage I and II, survival rates drop to about 60% in stage III and 5% in stage IV. CRCs are biologically heterogeneous. Classically, **variants in the *APC* gene and chromosomal instability** occur. Another pathway involves serrated adenomas with epigenetic promoter methylation and high microsatellite instability (MSI), and there are also mixed forms.



While the majority of CRC occur sporadically, familial clustering is seen in approximately 10% of CRC. About **2% to 3% of all CRC are due to Lynch syndrome or hereditary non-polyposis colon cancer** (HNPCC). MSI analysis is recommended on tumor material in all patients with CRC to further test for Lynch syndrome and assess response to immunotherapy with ICI. The simultaneous presence of a high MSI (MSI-H) and a variant in *BRAF* strongly suggests the presence of a sporadic tumor. This can be supported by analysis of MLH1 promoter methylation, which also results in MSI-H. Sporadic MSI-H is detectable in approximately 20% of stage II patients, which correlates with localization in the right colon, poor histologic differentiation, and mucinous adenocarcinomas. These patients have a slightly better prognosis but do no benefit from adjuvant therapy with 5-fluorouracil.

GENOMIC ALTERATIONS

Variants in *BRAF* (8-10% of CRC) are associated with a more aggressive CRC phenotype, chemotherapy resistance, MSI-H, and poorer overall survival. No improvement in overall survival and progression-free survival is seen with anti-EGFR therapy. For patients with *BRAF* variants previously treated with anti-EGFR therapy, therapy with the *BRAF* inhibitor, vemurafenib, in combination with irinotecan and cetuximab or panitumumab is recommended.

Approximately 10% to 20% of CRC patients show **variants in *PIK3CA***, which are associated with CRC in the right hemicolon, a mucinous subtype, and variants in *KRAS*. Variants in *PIK3CA* are also associated with resistance to anti-EGFR therapy. However, CRC patients with *PIK3CA* variants who start aspirin therapy after diagnosis show higher CRC-specific survival and overall survival than patients without *PIK3CA* variants.

Approximately 2% of CRC patients exhibit **HER2 overexpression**, which in >90% is from amplification of *ERBB2* and rarely from activating variants in *ERBB2*. This is associated with acquired primary and secondary resistance to anti-EGFR therapy. HER2-targeted therapies are currently being tested in HER2-positive metastatic CRC patients in clinical trials and may offer a therapeutic option where there is resistance to anti-EGFR therapy.

Kinase fusions are detected in <1% to 2% of CRC patients, primarily involve *RET*, *NTRK*, *ALK*, and *ROS*, and are usually associated with CRC in the right hemicolon, MSI-H and *RAS* wild-type, and shortened overall survival. A few studies show that patients with these fusions may benefit from targeted tyrosine kinase inhibitor therapy.

For patients for whom none of the above biomarkers are available for therapy, MSI-H or even mismatch repair deficiency (MMR-D) is a predictive biomarker for ICI. However, this limits this type of therapy to approximately 5% of metastatic CRC.

MMR-D leads to an accumulation of somatic variants and thus a high tumor mutation burden (TMB). However, this can also arise independently of an MMR-D/MSI-H, e.g., due to variants in *POLE*. Therefore, a high TMB seems to be a more appropriate marker for the response of ICI. Homozygous or hemizygous variants in *JAK1* are predictive for resistance to ICI. They are found in approximately 15% of primary CRC, but less frequently in metastatic CRC.

POSSIBLE THERAPIES

All patients with metastatic CRC should be offered analysis of the *KRAS*, *NRAS*, and *BRAF* genes. Variants in *KRAS* (~ 50% of CRC) and *NRAS* (~ 5% of CRC) result in loss of the antiproliferative effect of EGFR antibodies. Therefore, **anti-EGFR therapy** (e.g., cetuximab, panitumumab) is only effective in patients who do not have a variant in *KRAS* or *NRAS*. In addition, patients with a tumor situated in the right hemicolon show no benefit from anti-EGFR therapy despite not having a variant in *KRAS* or *NRAS*. Therefore, first-line therapy with anti-EGFR antibodies and combination chemotherapy is recommended for CRC patients who are *KRAS/NRAS*-wild-type and have the a left-sided primary tumor, whereas patients with the right-sided primary tumor and/or variants in *KRAS* or *NRAS* are advised to receive **chemotherapy in combination with bevacizumab**, if necessary.

Account for **~14%** of all **adult tumors**

5-year survival is **85-90%** (stage I&II), **60%** (stage III) and **5%** (stage IV)

Majority of CRC sporadic, **~10% familial**

TARGETED-THERAPY TESTS

Gene	Genomic region
KRAS	Exon 2-4
NRAS	Exon 2-4
BRAF	Exon 11, 15

GENE PANEL

MICROSATELLITE INSTABILITY

For the most up-to-date list of genes in our gene panels and fusion genes, please visit:
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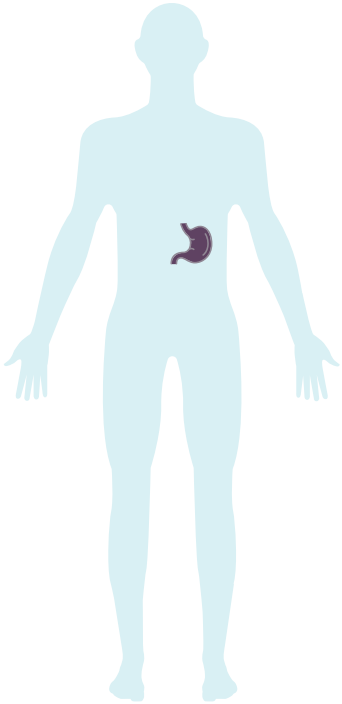
GASTRIC CANCER

BACKGROUND

Gastric cancer (GC) or stomach cancer is one of the most common malignancies and the 4th leading cause of cancer-related death. **Men are affected twice as often as women**. GC is a multifactorial disease in which both environmental and genetic factors can have an impact on its occurrence and development. The incidence rate of GC rises progressively with age, and the median age at diagnosis is 70 years; however, approximately 10% of gastric carcinomas are detected at the age of 45 or younger.

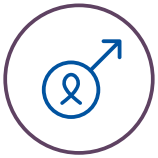
Before cancer develops, pre-cancerous changes often occur in the inner lining (mucosa) of the stomach. These early changes rarely cause symptoms, so they often go undetected. Cancers starting in different sections of the stomach can cause different symptoms and tend to have different outcomes. The location can also affect treatment options.



About **90-95% of gastric tumors are adenocarcinomas**. These cancers develop in the gland cells in the mucosa. There are two main types of stomach adenocarcinomas: intestinal and diffuse. The **intestinal type** tends to have a slightly better prognosis, and the cancer cells are more likely to have certain gene changes that might allow for treatment with targeted therapy. The **diffuse type** tends to grow and spread more quickly. It is less common than the intestinal type, and it tends to be harder to treat.

The **prognosis** is mainly determined by the stage, but also by histology, general condition and comorbidity. In early and localized stages, the therapy is curative, in metastatic stages, palliative. Therapeutic modalities are mainly surgery and medical tumor therapy. Despite some progress in the last 10 years, cancer-specific mortality remains very high, at 70%.

4th leading cause of cancer-related **death**



Men affected **twice** as often

Incidence rate **increases** with **age**

GENOMIC ALTERATIONS

If the cells have a certain amount of an immune checkpoint protein called **PD-L1**, treatment with an immune checkpoint inhibitor such as pembrolizumab (Keytruda) might be an option. For patients with **high levels of microsatellite instability** (MSI-H) or with a **high tumor mutational burden** (TMB-H), treatment with an immune checkpoint inhibitor might be recommended. If the cells have **changes in one of the *NTRK* genes**, certain targeted drugs can be an option for treatment.

POSSIBLE THERAPIES

In some people with stomach cancer, the cancer cells have too much of a growth-promoting protein called HER2 on their surface. Cancers with increased levels of HER2 are called HER2-positive. Drugs like **trastuzumab** that target the HER2 protein can often be helpful in treating these cancers.

Adding trastuzumab to chemotherapy can help some people with advanced, HER2-positive stomach cancer live longer than with chemotherapy alone. As drug only works if the cancer cells have too much HER2, samples of the cancer must be tested for HER2 before starting treatment. Several similar versions (called biosimilars) are now available as well, including Ogivri, Herzuma, Ontruzant, Trazimera, and Kanjinti.

Fam-trastuzumab deruxtecan (Enhertu) is an antibody-drug conjugate (ADC), which is a monoclonal antibody linked to a chemotherapy drug. In this case, the anti-HER2 antibody attaches to the HER2 protein on cancer cells, bringing the chemotherapy directly to them. This ADC can be used alone to treat advanced HER2-positive stomach cancer, typically after treatment with trastuzumab has been tried.

Ramucirumab is a monoclonal antibody that binds to a VEGF receptor. This prevents VEGF from binding to cells and telling them to make more blood vessels. This can help slow or stop the growth of some cancers. Ramucirumab is used to treat advanced stomach cancer, most often after at least one chemotherapy drug (or combination) stops working.

A very small number of stomach cancers have changes in one of the *NTRK* genes. This causes them to make abnormal TRK proteins, which can lead to abnormal cell growth and cancer. **Larotrectinib (Vitrakvi) and entrectinib (Rozlytrek)** are drugs that target the TRK proteins. These drugs can be used to treat advanced cancers with *NTRK* gene changes that are still growing despite other treatment.

TARGETED-THERAPY TESTS

Gene	Genomic region
MSI	Stable/unstable
NTRK1/2/3	Rearrangement

GENE PANEL

MICROSATELLITE INSTABILITY

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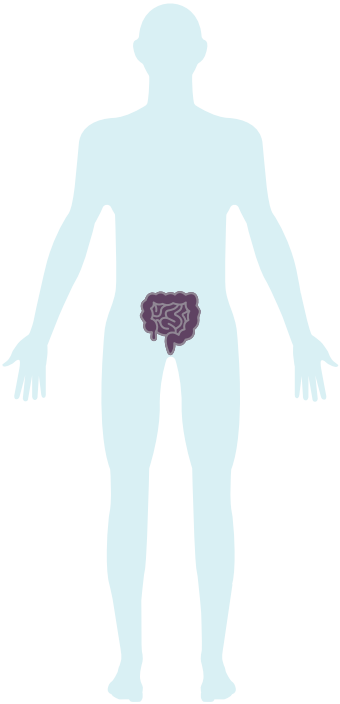
GASTROINTESTINAL STROMAL TUMOR

BACKGROUND

Although rare, gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract. The most frequent primary localizations are in the **stomach area (50-60%) and small intestine (20-30%)**, less frequently in the colorectum (5-10%) and esophagus (<1%). GISTs represent a morphological and biological continuum from randomly discovered, <10 mm benign micro-GISTs, to large sarcomas. GISTs are divided into **three morphological subgroups**: about 70% are of the spindle-cell subtype, about 10% are of the epithelioid subtype and another 20% are of the mixed spindle-cell-epithelioid subtype. Immunohistochemistry is essential for a histological diagnosis of GISTs. The antibody against CD117 (KIT receptor) or DOG1 is detectable in about 95% of all GISTs. About 70% to 80% of the cases also express the stem cell-associated antigen CD34.



GENOMIC ALTERATIONS

Despite the clinicopathological differences, most GISTs share the same genetic profile. This includes ***KIT* and *PDGRFA* variants**, which lead to the constitutive activation of tyrosine kinases. They can be detected in small tumors with a diameter <1 cm indicating that it is an early pathogenetic event.

A hereditary predisposition is very rarely observed. These are either familial GISTs with a corresponding germline variant in *KIT*, GIST in the context of Carney-Strakatis syndrome or GIST in connection with neurofibromatosis type 1.

GISTs that do not have variants in *KIT* or *PDGRFA* are classified as **succinate dehydrogenase (SDH)-deficient GIST or non-SDH-deficient GIST**. The SDH-deficient group accounts for 20% to 40% of the *KIT/PDGFRA* wild-type GIST and is characterized by a loss of expression of the SDH subunit B (SDHB), mostly due to sporadic and/or germline variants in *SDHA*, *SDHB*, *SDHC* and *SDHD* (*SDHx*). In addition to sporadic cases, the SDH-deficient group includes Carney Triad and Carney-Strakatis syndrome. The non-SDH-deficient group includes neurofibromatosis type 1 with variants in *NF1* and GIST with variants in *BRAF*, *KRAS* and *PIK3CA*.

60% begin in the **stomach**, **35%** develop in the **small intestine**

Antibody against **CD117** or **DOG1** detectable in **~95% of cases**

5-year survival rate is **83%** (depending on type & stage)

POSSIBLE THERAPIES

The frequency of *KIT* variants is about 80% to 90%. Patients with variants in the untreated primary tumor usually respond to treatment with the **tyrosine kinase inhibitor (TKI) imatinib**. The most frequent variants in *KIT* are found in exon 11 (60%) and are very heterogeneous in length and type (deletions, insertions, point mutations and combinations). Patients with a *KIT* exon 11 deletion have a higher risk of relapse than those with an exon 11 insertion or point mutation, *PDGFRA* variant, or wild type. In 10% to 15% of cases, *KIT* variants are found in exon 9 where the same six base pair insertion almost always occurs, which leads to duplication of the amino acids alanine 502 and tyrosine 503.

Exon 9 variants are predominant in GISTs with small intestine localization and about half of the cases show a response to imatinib. However, it has been shown that these patients benefit when they are treated from the start with 800 mg of Glivec (imatinib)/day instead of 400 mg Glivec/day or treatment with another TKI. In addition, variants have been described which are detected during treatment with a TKI and lead to resistance. Other *KIT* variants, in exon 13 (Lys642Glu) and exon 17 (Asn822Lys), are found rarely. For *KIT* exon 13 and 14 variants, therapy with the second-line **TKI sunitinib** is possible, while for the exon 17 variant (Asn822Lys), primary resistance can be expected. For secondary *KIT* exons 17 and 18, sunitinib is also usually ineffective. A response to sorafenib and regorafenib has been shown.

The frequency of *PDGFRA* variants is about 10% to 15%. Here too, patients with variants in the untreated primary tumor usually respond to therapy with the TKI imatinib. However, if the variant Asp842Val is found, it is associated with resistance to imatinib. Treatment with second-line TKI sunitinib or third-line TKI regorafenib is also indicated for these tumors.

TARGETED-THERAPY TESTS

Gene	Genomic region
KIT	Exon 9, 11, 13, 17
PDGFRA	Exon 12, 14, 18
SDH	All
BRAF	Exon 11, 15
NF1	All
NTRK1/2/3	Rearrangement

GENE PANEL

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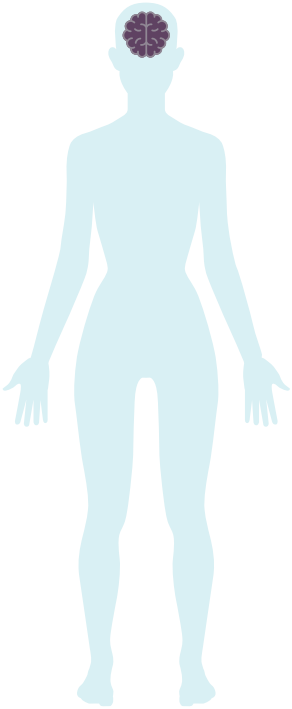
GLIOBLASTOMA

BACKGROUND

Gliomas (astrocytomas, oligodendrogliomas and glioblastomas) are the most common primary brain tumors in adults, accounting for approximately 50% of all cases.



GENOMIC ALTERATIONS

A biological and prognostic classification of WHO grade II to III gliomas is performed based on the occurrence of somatic variants in the genes *IDH1* and *IDH2*. Pathogenic **variants in *IDH1* and *IDH2*** can be detected in over 70% of primary astrocytomas, oligodendrogliomas and secondary glioblastomas. Thus, the absence of variants in *IDH1* and *IDH2* in glioblastoma is associated with primary glioblastoma. *IDH*-altered gliomas also have their own clinical phenotype: patients are significantly younger than those with *IDH* wild-type gliomas, and often have localization in the frontal lobe and a larger tumor at the time of diagnosis. Tumors with pathogenic variants in *IDH1* and *IDH2* are also associated with a more favorable prognosis than gliomas with *IDH1/2* wild-type tumors.

A further prognosis can be made based on the presence of a loss of heterozygosity (LOH) of 1p/19q. This depends on the presence or absence of pathogenic variants in the genes *IDH1* or *IDH2*, as well as the LOH of 1p/19q. **Oligodendrogliomas** with a variant in *IDH1* or *IDH2* and the presence of LOH 1p/19q (approximately 30% of WHO grade II-III gliomas) have the most favorable prognosis. **Astrocytomas** (approximately 50% of WHO grade II-III gliomas) and WHO grade IV glioblastomas with a variant in *IDH1* or *IDH2* and the presence of intact 1p/19q have an intermediate prognosis. An unfavorable prognosis is reported for astrocytomas, WHO grade IV diffuse midline glioma with histone H3 Lys27Met variant (approximately 20% of WHO grade II-III gliomas), and WHO grade IV glioblastomas with *IDH1/2* wild-type and presence of intact 1p/19q.

POSSIBLE THERAPIES

About 40% of *IDH1/2* wild-type glioblastomas show MGMT promoter methylation. **Temozolomide** is an alkylating cytostatic agent that induces G2/M cell cycle arrest, mismatching and apoptosis by adding a methyl group to the O6 position of guanine in the DNA. MGMT is an O6 methylguanine DNA methyltransferase and a so-called suicide DNA repair protein that can reverse temozolomide-induced methylation, and thus DNA damage. It does this by catalyzing the transfer of the methyl group inserted at the O6 position of guanine to the cysteine residue of its own position 145.

If methylation is present in the MGMT promoter, gene silencing occurs and MGMT is switched off. Therefore, MGMT promoter methylation positively indicates combined radiotherapy and temozolomide chemotherapy. Secondary resistance to temozolomide can be caused by variants in *TP53* and other genes of the DNA mismatch repair system.

***TERT* variants** are found in approximately 72% of *IDH* wild-type glioblastomas and 26% of variant *IDH* glioblastomas. These are often Cys228Thr and Cys250Thr variants of the promoter region. *TERT* promoter variants are associated with low survival and resistance to radiotherapy.

About 50% of glioblastomas show ***EGFR* alterations** (amplification, rearrangement or single nucleotide variant). Nearly 20% of primary glioblastomas have a deletion of exons 2 to 7, *EGFRvIII*, which is associated with *EGFR* amplification. Patients in therapy studies are first tested for *EGFR* amplification or immunohistochemically for *EGFRvIII* positivity.

GENE PANEL

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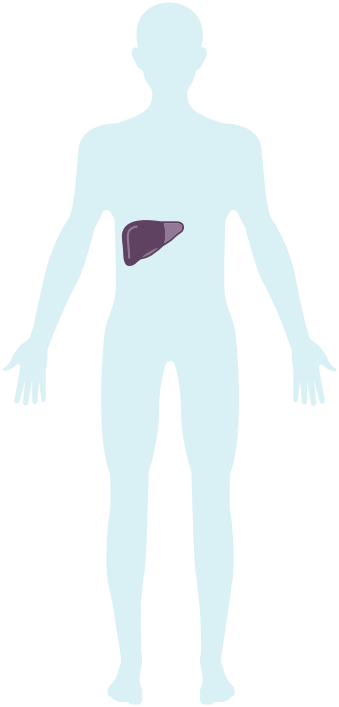
LIVER CANCER

BACKGROUND

Malignant tumors in the liver either originate in the liver (primary liver cancer) or spread from cancer sites elsewhere in the body (metastatic liver cancer).
Most cancerous tumors in the liver are metastatic.



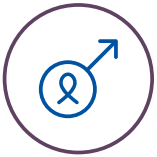
Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer in adults. HCC can have different growth patterns. Some begin as a single tumor that grows larger and later spreads to other parts of the liver. A second type seems to start as many small cancer nodules throughout the liver, not just a single tumor. This is seen most often in people with cirrhosis (chronic liver damage). Several subtypes of HCC can be classified. Most often these subtypes do not affect treatment or prognosis; however, one subtype, **fibrolamellar** (FLC), is important to recognize. FLC is rare, making up less than 1% of HCC and primarily occurs in adolescents and young adults with no history of liver disease and normal liver function. This subtype tends to have a better outlook than other forms of HCC.

Intrahepatic cholangiocarcinoma (bile duct cancer) accounts for about 10% to 20% of cancers that start in the liver. These cancers start in the cells that line the small bile ducts within the liver. Most cholangiocarcinomas, however, actually start in the bile ducts outside the liver. Cholangiocarcinomas are often treated the same way as HCC.

Hepatoblastoma is a very rare kind of cancer that develops in children, usually in those younger than 4 years old. The cells of hepatoblastoma are similar to fetal liver cells. About 2 out of 3 children with these tumors are treated successfully with surgery and chemotherapy, although the tumors are harder to treat if they have spread outside the liver.

GENOMIC ALTERATIONS

***FGFR2* fusions** are the most frequent *FGFR* fusions and are particularly common in cholangiocarcinoma; *FGFR2–TACC3* fusions have been frequently described in patients with intrahepatic cholangiocarcinoma. These fusions activate the canonical *FGFR* signaling and possess oncogenic activity. It has been demonstrated that the *FGFR2–CCDC6* fusion induces cancer cell proliferation and tumorigenesis *in vivo*.



Men about **three times** more affected

5-year survival rate is **20%** (depending on type & stage)

Most cancerous **tumors** are **metastatic**

POSSIBLE THERAPIES

Many of the targeted drugs used to treat liver cancer are **kinase inhibitors**. These drugs block several kinase proteins, which normally help tumor cells grow in one of two ways: either by helping tumor cells to grow directly, or by helping tumors to form the new blood vessels they need in order to get bigger (angiogenesis). Blocking these proteins can often help stop the growth of the cancer.

Sorafenib (Nexavar) and lenvatinib (Lenvima) can be used as the first treatment for liver cancer if it cannot be treated by surgery or if it has spread to other organs. Sorafenib may work better in people with liver cancer caused by hepatitis C.

Regorafenib (Stivarga) and cabozantinib (Cabometyx) can be used to treat advanced liver cancer, typically if other treatments are no longer helpful.

Bevacizumab (Avastin) is a monoclonal antibody that targets vascular endothelial growth factor (VEGF), a protein that helps new blood vessels to form. This drug can be used along with the immunotherapy drug atezolizumab (Tecentriq) as the first treatment for liver cancer that cannot be treated by surgery or that has spread to other organs.

Ramucirumab (Cyramza) is a monoclonal antibody that targets a VEGF receptor protein on cells, which can help stop the formation of new blood vessels. This drug can be used to treat advanced liver cancer, typically after another treatment stops working.

LIVER CANCER FUSION GENE PANEL

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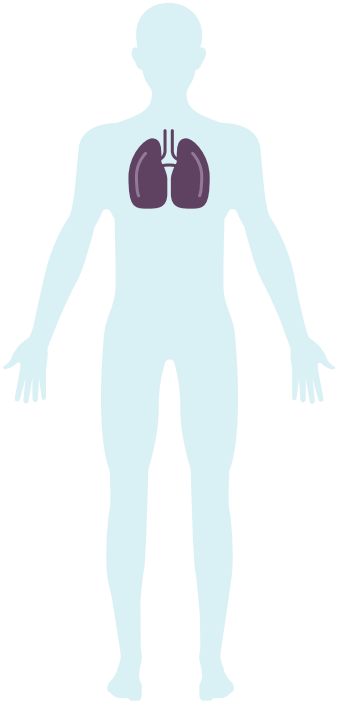
LUNG CANCER

BACKGROUND

Lung cancer is a heterogeneous group of tumors with more than 50 histomorphological subgroups and one of the most fatal forms of cancer worldwide. **Non-small cell lung carcinoma** (NSCLC) accounts for about 85% to 90% of malignant lung diseases, with adenocarcinomas (ADC, 40-50%) and squamous cell carcinomas (SCC, 20-30%) being the most frequent histological subgroups. Few patients are diagnosed at an early stage of disease (stage I or II). In more than 60% of cases there is a locally advanced or metastasized carcinoma (stage III or IV), in which resection is no longer possible. The 5-year survival rate for all stages is about 17% on average, whereas for stage IV NSCLC it is only 2% or less. The therapeutic strategy has evolved to genetically guided therapy. Therefore, therapy-relevant variants should be determined in all stage IV patients before the start of a drug-based first-line therapy.



GENOMIC ALTERATIONS

Activating **variants in the *EGFR* gene**, found in 10% to 12% of Caucasians with NSCLC and more common in non-smokers, women and patients with East Asian ethnicity, were the first molecular lesions to be targeted for treatment. *EGFR* variants are found in about 30% of ADC and rarely in SCC. A number of EGFR tyrosine kinase inhibitors (EGFR TKI) are now available, the effectiveness of which depends on the TKI and the type of *EGFR* variant.

The most frequent *EGFR* variants are deletions in exon 19 (Del19) and the exon 21 Leu858Arg variant (85-90%). Further variants are found mainly in exons 18 and 20. In the majority of patients, disease progression occurs in the course of targeted treatment due to mechanisms of primary and secondary acquired resistance to targeted drugs. In patients with an activating *EGFR* variant treated with first and second-generation EGFR TKIs, the most frequent cause of resistance (49-60%) is the occurrence of the Thr790Met missense variant in exon 20 of *EGFR*. This has led to the development of successor TKIs, for example, third-generation TKIs, which therapeutically cover both the activating variant and the resistance variant Thr790Met. In some cases, this resistance variant is detectable in the primary tumor at the time of diagnosis. Where there is disease progression under TKI treatment and suspected resistance, the Thr790Met variant should be sought in a tissue or liquid biopsy. This also allows the investigation of other resistances that occur in *EGFR* positive NSCLC (e.g., Cys797Ser).

***ALK* rearrangements**, mainly translocations, occur in about 3% to 13% of ADC. They are usually in younger patients and those who have never smoked and only in the absence of *EGFR* and *KRAS* variants. Furthermore, pleural, pericardial and brain metastases are more likely to be described here. In most cases, there is a fusion with *EML4*. ALK-TKIs are available for the treatment of patients with *ALK* rearrangements. However, *ALK* resistance variants can occur under therapy with ALK-TKI (especially crizotinib), or other signaling pathways can be switched on, e.g., by the occurrence of activating variants in *EGFR*, which should be checked with a tissue rebiopsy in case of disease progression under ALK-TKI therapy and suspected resistance.

***ROS1* rearrangements** are found in about 1% to 2% of NSCLC; they exclude *ALK*, *EGFR* and *KRAS* alterations and qualify patients for therapy with crizotinib. Lorlatinib and carbozantinib are second-generation TKIs for *ROS1* positive patients who have developed resistance to crizotinib.

Variants in *BRAF*, predominantly at amino acid position Val600, are found in approximately 6% of ADC and 4% of SCC. These can be treated with a combination therapy composed of dabrafenib and trametinib.

POSSIBLE THERAPIES

There are numerous other changes for which specific therapeutic concepts are being tested and effective inhibitors are available in clinical trials: *HER2* amplifications, *KRAS* variants, *MET* alterations, *NRG* fusions, *NTRK* fusions and *RET* translocations.

Various studies have shown that patients with these genetic changes who received a **targeted therapy**, had a significant improvement in overall survival compared to those patients in whom no suitable biomarkers could be detected and who did not receive targeted treatment.

In patients without genetic alterations for whom targeted therapies are approved, it is recommended that PD-L1 expression is determined for possible **immunotherapy with checkpoint inhibitors**. Determination of the tumor mutation burden (TMB) in patients with NSCLC can serve as a predictive biomarker to support the selection of patients who may benefit from immune checkpoint inhibitor therapy. The National Comprehensive Cancer Network (NCCN) guidelines have already recommened immunotherapy with checkpoint inhibitors (nivolumab with or without ipilimumab) as first-line therapy in metastatic NSCLC with high TMB.

The diagnosis of lung carcinoma is usually based on morphology from small biopsies. After the pathologist has determined the entity, all investigations to determine the biomarkers relevant for therapy must be carried out on this limited material. Therefore, diagnostic approaches that allow the simultaneous characterization of all relevant variants, as well as determination of TMB and microsatellite instability, are preferred.

TARGETED-THERAPY TESTS

Gene	Genomic region
EGFR	Exon 18-21
BRAF	Exon 11, 15
KRAS	Exon 2-4
ERBB2	All
MET	Exon 14-skipping
ALK	Rearrangement
ROS1	Rearrangement
RET	Rearrangement
NTRK1/2/3	Rearrangement

LUNG CANCER FUSION GENE PANEL

NON-SMALL CELL LUNG CARCINOMA GENE PANEL

NON-SMALL CELL LUNG CARCINOMA FUSION GENE PANEL

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~2.3 million people diagnosed worldwide per year

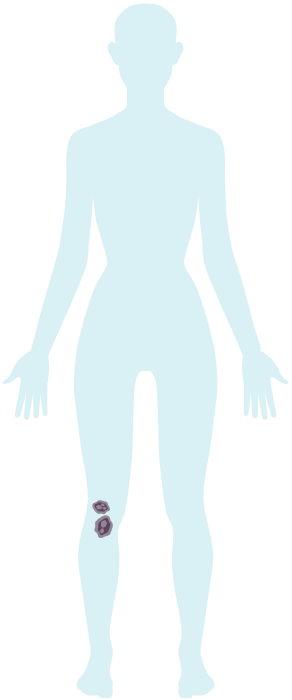
Account for **85-90%** of **malignant lung** diseases

5-year survival rate for all stages is about **17%**

MELANOMA

BACKGROUND

Malignant melanoma of the skin is the skin tumor with the **highest rate of metastasis** and is associated with more than 90% of all deaths from skin tumors. More than 90% of melanomas occur as primary tumors of the skin (**cutaneous melanomas**), 5% are found primarily in the eye, often in the area of the retina (**ocular melanomas**), and in a few cases, malignant melanomas are found in the mucous membranes.



Melanoma is generally curable if diagnosed early. An essential requirement is a resection with sufficient safety margin. However, the early tendency to metastasize is problematic. The presence and location of metastasis influences clinical presentation and prognosis. For patients with stage IIIA, IIIB and IIIC metastatic melanoma (American Joint Committee on Cancer, 2017), the 5-year survival rate is between 23% and 87%. The median survival time for patients with stage IV metastatic melanoma is estimated to be 8-12 months with large interindividual variation. Drug treatment in stage IV and non-resectable stage III includes targeted molecular therapies or immunotherapy.

GENOMIC ALTERATIONS

Variants in *BRAF* are detected in 40% to 50% of cutaneous melanomas and lead to constitutive activation of the kinase function. The most common variant in *BRAF* (Val600Glu) accounts for 70% to 88% of all *BRAF* variants. Other changes in *BRAF* occur in about 5% of all melanomas, and include fusions involving *BRAF*.

Activating **variants in *NRAS*** are found in 20% to 30% of melanomas and typically exclude a variant in *BRAF* and *KIT*. They are associated with an aggressive course and unfavorable prognosis and may possibly cause resistance in *BRAF*-altered melanoma under therapy.
About 2% to 5% of melanomas show a **variant in *KIT***. They are concentrated in two melanoma subtypes: mucosal melanoma and acral lentiginous melanoma. In about 50% of the cases, a response to imatinib or nilotinib (off-label use) is observed. This is an option for patients with *KIT* variants after unsuccessful immunotherapy with checkpoint inhibitors (ICI).

Variants in *NF1* occur in 12% to 18% of melanomas and in 46% of *BRAF/NRAS* wild-type melanomas and are associated with a high risk of death and poor overall survival. These patients have a reduced response to *BRAF* inhibitors. In patients with *NF1*-altered melanomas, pan-RAF or type 2 RAF inhibitors in combination with MEK inhibitors or PI3K/mTOR inhibitors should be considered. *NF1* variants correlate with a high tumor mutation burden (TMB), so that ICI should be considered.

In about 10% of mucosal melanomas and about 90% of uveal melanomas, variants are found at amino acid position Gln209 in *GNA11* or *GNAQ*. Both ***GNA11* and *GNAQ* variants** are associated with poor overall survival in mucosal melanoma, and variants in *GNA11* are associated with more aggressive behavior in uveal melanoma. ***CDKN2A* variants** are found in 2% of all melanomas and in 30% to 40% of familial melanomas.

Fusions involving the *NTRK1*, *NTRK2* and *NTRK3* genes are very rare in melanoma (<1%). However, they are associated with a high response rate to the TRK inhibitors larotrectinib and entrectinib.

POSSIBLE THERAPIES

Two **BRAF inhibitors** (vemurafenib and dabrafenib) have been approved by the FDA as standard therapy for advanced melanoma and show a response in approximately 50% of patients when used as monotherapy. Unfortunately, relapses often occur after completion of therapy due to acquired resistance to BRAF inhibitors. The **combination of BRAF and MEK inhibitors** (dabrafenib and trametinib or vemurafenib and combimetinib) appears to slow the development of resistance and shows prolonged progression-free and overall survival compared to BRAF inhibitor monotherapy. Melanomas with variants close to codon 600 (especially Leu597 and Lys601) also show a response to MEK inhibitors or to a combination of BRAF and MEK inhibitors. Fusions involving *BRAF* also show a response to MEK inhibitors and non-specific RAF inhibitors (e.g., sorafenib). An alternative is ICI, although no data are available on the best sequential therapy of BRAF/MEK inhibitors and checkpoint inhibitors.

For melanoma patients with non-resectable metastases, the use of ICI should be examined. **PD-1 antibodies** or their combination with ipilimumab are superior to monotherapy with ipilimumab in terms of progression-free survival. PD-L1 expression is not always a reliable marker for predicting therapy response, since PD-L1 negative tumors also respond and PD-L1 expression is inconsistent between primary tumor and metastases and between metastases in about 50% of cases. Therefore, determining the TMB is supportive since TMB is significantly higher in patients with a good response to therapy than in patients with a poor response.

Despite promising early clinical data, only a few patients with *NRAS*-altered melanoma benefit from treatment with a MEK inhibitor. However, preliminary data show tumor regression as well as a response or stable disease with a combination of MEK inhibitors and CDK4/6 inhibitors in patients with metastatic *NRAS*-altered melanoma (off label use).

TARGETED-THERAPY TESTS

Gene	Genomic region
BRAF	Exon 11, 15
KIT	Exon 11, 13, 17
NRAS	Exon 2-4
NTRK1/2/3	Rearrangement

GENE PANEL

For the most up-to-date list of genes in our gene panels and fusion genes, please visit:
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5th most common cancer among **men and women**

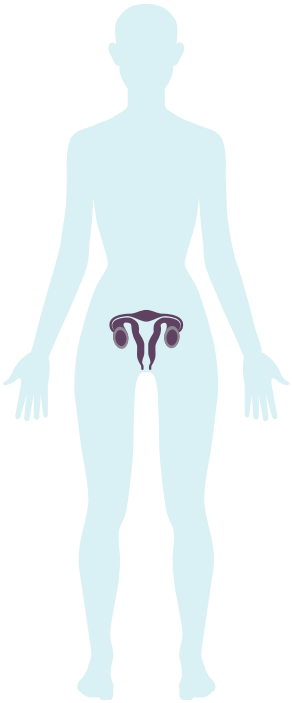
Average age of diagnosis is **65 years**

5-year survival rate is **93%**

OVARIAN CANCER

BACKGROUND

Ovarian cancer accounts for about 3.2% of all malignant neoplasms in women and is responsible for 5.3% of cancer deaths. In most cases the tumors are diagnosed at an advanced stage, so that the relative 5-year survival rate is currently an average of 41%. Overall, 75% of all ovarian cancers are fast, aggressively growing tumors (high-grade serous carcinomas, mixed carcinomas and carcinosarcoma), which in turn are responsible for 90% of the mortality from ovarian cancer.



The **risk of developing ovarian cancer increases with age** and depends on various hormonal factors. In addition, familial clusters of certain tumor diseases (especially ovarian, breast, colon and/or endometrial carcinomas) represents an established risk factor. Independent of the family history or the age of the patient, **the risk for a genetic predisposition is between 20.8% and 25.8% on average** (20.8% for causal variants *BRCA1* or *BRCA2* and 25.8% for causal variants in *BRCA1/2* or other risk genes). As a result, all women with ovarian cancer should be informed about the risk of a genetic predisposition and offered genetic counselling.

POSSIBLE THERAPIES

Patients with advanced epithelial ovarian cancer, carcinoma of the fallopian tube or primary peritoneal carcinoma (stage IIB-IV) receive platinum-based chemotherapy in combination with a taxane.

Olaparib (Lynparza®) has been approved by the EMA for maintenance therapy following a complete or partial response to first-line platinum-based chemotherapy, provided that a pathogenic or likely pathogenic variant in the *BRCA1* or *BRCA2* gene has been detected in the patient's tumor tissue or germline.

Olaparib is a PARP1 (poly (ADP-ribose) polymerase 1) inhibitor that uses the principle of "synthetic lethality". Olaparib inhibits the repair of DNA single-strand breaks according to the base excision repair mechanism. These single strand breaks are transformed into double strand breaks by the collapse of the replication fork, which in turn are repaired by homologous recombination (HR) or non-homologous end-joining (NHEJ). If HR fails due to a mutation in a DNA repair gene involved in HR, such as *BRCA1* or *BRCA2*, the error-prone NHEJ takes effect and the cells become apoptotic.

In recurrent platinum-sensitive ovarian/fallopian tube/peritoneal carcinoma with complete or partial response to platinum-containing therapeutics, olaparib can be administered as second-line maintenance therapy regardless of the *BRCA1/2* mutation status. Nevertheless, *BRCA*-mutated patients show a reduced risk of disease progression under Lynparza® compared to patients with an undetermined *BRCA* status (19.1 vs. 8.4 months).

TARGETED-THERAPY TESTS

Gene	Genomic region
BRCA1	All
BRCA2	All
MSI	Stable/unstable
NTRK1/2/3	Rearrangement

GENE PANEL

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Account for **3.2%** of all **malignant neoplasms** in **women**

5-year survival rate is **41%** (on average)

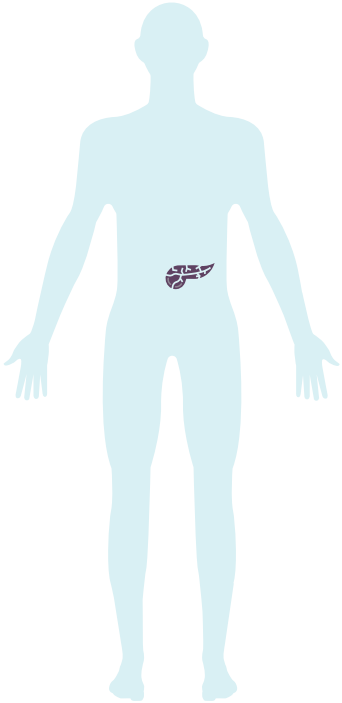
50% of **women** diagnosed **≥63 years**

PANCREATIC CANCER

BACKGROUND

Pancreatic cancer has the **lowest survival rate** of all cancers and is the 4th most common cause of cancer death. In early stages, malignant neoplasms of the pancreas often cause no symptoms or only unspecific symptoms so that the tumor is often detected late. As a result, the relative 5-year survival rate is about 8% for both men and women.

Over 95% of pancreatic malignancies are **ductal adenocarcinomas** caused by degeneration of the exocrine part of the pancreas. Exocrine pancreatic carcinoma develops mainly from premalignant precursors of the epithelium in the pancreatic duct (PanIN, pancreatic intraepithelial neoplasia). In addition, mucus-forming, cystic lesions such as mucinous cystic neoplasms (MCN) and intraductal papillary mucinous neoplasms (IPMN) are also considered precancerous for invasive pancreatic cancer.



GENOMIC ALTERATIONS

The progression from ductal epithelial cell dysplasia to adenocarcinoma is biologically characterized by the accumulation of a variety of genetic and epigenetic aberrations. The most common genetic aberrations are **variants in the *KRAS*** oncogene which are detected in >90% of patients (mostly Gly12Asp or Gly12Val in exon 2) and are considered to be initial oncogenic changes. As disease progresses, variants in the tumor suppressor genes *CDKN2A*, *TP53* (in >80%) and *SMAD4* (40-50%) may occur.

Molecular genetic analysis of cystic fluid or pancreatic secretions is used for further differential diagnostic classification. Modern high-throughput screening techniques have shown that, especially in IPMN, entity specific genetic alterations are present in the cystic fluid. Thus, **variants in *GNAS*** are only described in IPMN (41-66%) and not in MCN, SCA or solid pseudopapillary neoplasms. A molecular genetic analysis of *KRAS* and *GNAS* can therefore be used as a building block in the preoperative diagnosis of these tumors. The simultaneous determination of protein-based tumor markers, in particular carcinoembryonic antigen, further increases the sensitivity of this investigation while maintaining the same high specificity.

POSSIBLE THERAPIES

Where information about the tumor is available, the *KRAS* status in the tumor also allows prognostic and predictive statements. Patients with ***KRAS* wild-type status** in the tumor material showed a clinically more favorable course in several studies. However, more than 90% of patients have *KRAS* variants which, together with other factors, lead to an immunosuppressive environment and explains why T-cell targeted therapies such as CTLA4 or PD-L1 blockers have so far remained largely ineffective. The same is true for ***SMAD4* status**, where wild-type status is also associated with a less aggressive clinical course, although some studies also showed that the loss of *SMAD4* expression is associated with a better response to adjuvant chemotherapy. *SMAD4* could thus also serve as a predictive biomarker.

Another predictive biomarker is ***hENT1* expression** in tumor tissue. *hENT1* is crucial for the uptake of the chemotherapeutic agent gemcitabine into tumor cells. Patients with high *hENT1* expression responded significantly better to therapy with gemcitabine. Similar data were obtained for the expression of the protein **SPARC (osteonectin1)**, which is detected particularly in the tumor stroma and correlates with a worse prognosis. SPARC is the receptor for another chemotherapeutic agent—nab-paclitaxel. Patients with high SPARC expression in the tumor stroma seem to benefit more from this therapy than those with low SPARC expression. Germline variants have also been shown to be a predictive marker for therapy response. For example, patients with **germline variants in *BRCA1/2***, which lead to a deficiency in DNA repair, respond better to platinum-based therapy regimens. According to the latest data from the POLO study, patients with metastatic, platinum-sensitive pancreatic cancer and germline *BRCA1/2* variants also show significantly prolonged progression-free survival under maintenance therapy with the PARP inhibitor olaparib. About 5% to 7% of all patients with pancreatic cancer, especially those affected at a young age, are carriers of a germline variant. In order to therapeutically target the much larger collective of pancreatic tumors, studies are currently investigating treatment approaches with epigenetic therapeutics such as HDAC and EZH2 inhibitors in combination with immunotherapy and chemotherapy.

4th most common cause of **cancer death**

No symptoms/**only unspecific symptoms in early stages** resulting in late tumor detection

5-year survival rate is about **8%** (lowest of all cancers)

TARGETED-THERAPY TESTS

Gene	Genomic region
KRAS	Exon 2-4
SMAD4	All
BRCA1	All
BRCA2	All
MSI	Stable/unstable

GENE PANEL

FUSION GENES

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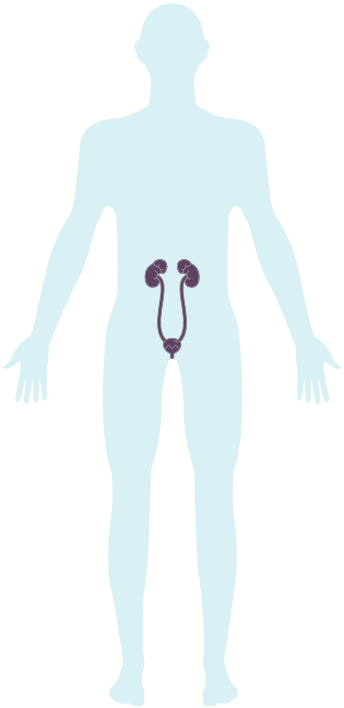
BACKGROUND

Prostate carcinoma (PCa) is the most common non-cutaneous cancer in men worldwide, with about 1.6 million cases and 366,000 deaths annually. Approximately **80% to 90% of all PCa arise in the peripheral zone** of the prostate, about 10% in the transition zone, and few in the other three areas of the prostate (central, anterior, around the urethra). **Three stages of development** have been identified: (a) intraepithelial neoplasia, which can be considered precancerous and is characterized by hyperplasia of luminal cells and progressive loss of basal cells; (b) adenocarcinoma androgen-dependent (divided into latent and clinical stages), characterized by complete loss of basal cells and luminal phenotype; the tumor is androgen-dependent and its growth can be controlled by androgen deprivation; and (c) adenocarcinoma androgen-independent (or castration-resistant).



GENOMIC ALTERATIONS AND POSSIBLE THERAPIES

With early diagnosis and treatment, patients have a good prognosis. Treatment of PCa depends on grade, stage, and age and ranges from active surveillance to a mixture of surgery, chemotherapy, radiation, and/or **androgen deprivation therapy (ADT)**. ADT is often used in combination with surgery or radiation, and often in combination with chemotherapy for metastatic disease. ADT may include two approaches: surgical castration (orchiectomy) or chemical castration with drugs that target the androgen receptor (AR) pathway.

Although most patients initially respond well to this therapy, nearly all cases recur and progress to primary castration-resistant prostate cancer (CRPC) or metastatic CRPC (mCRPC). For example, if this resistance is based on an alteration in AR, in *NCOR1/2*, or in *FOXA1*, therapy with AR inhibitors (e.g., enzalutamide) or CYP17A1 inhibitors (e.g., abiraterone) may prolong overall survival.

Approximately 25% of mCRPC patients demonstrate homologous DNA repair (HRR) deficiency and may benefit from therapy with PARP (poly (ADP-ribose) polymerase) inhibitors (PARPi).

The greatest benefit of **PARPi therapy** is shown by mCRPC patients with a change in *BRCA1* or *BRCA2*, whereas patients with variants in *ATM* and *CDK12* show limited response. However, patients with *CDK12* inactivation may benefit from therapy with immune checkpoint inhibitors alone or in combination with PARPi due to an increased neoantigen load. Variants in the other HRR genes have a relatively low prevalence in mCRPC. Combination therapy with PARPi and second-generation antiandrogens could improve treatment response.

Two PARPi for the treatment of PCa have been approved by the FDA: olaparib and rucaparib. **Olaparib** is for mCRPC patients who have previously received a second-generation hormonal agent and have a variant in an HRR gene (*BRCA1*, *BRCA2*, *ATM*, *BARD1*, *BRIP1*, *CDK12*, *CHEK1*, *CHEK2*, *FANCL*, *PALB2*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54L*); **rucaparib** is for mCRPC patients who have previously received a second-generation hormonal agent or taxane chemotherapy and have a variant in *BRCA* or *BRCA2*.

A **family history of PCa** increases the risk for PCa. In addition, PCa has been associated with hereditary breast and ovarian cancer (HBOC) syndrome (due to germline variants in homologous DNA repair genes) and Lynch syndrome (due to germline variants in DNA mismatch repair genes). Indeed, approximately 11% of patients with PCa and at least one additional primary tumor in the family carry germline variants that are associated with an increased risk of cancer. Therefore, we recommend a thorough review of personal and family history for all patients with PCa as well as patient education.

TEST RECOMMENDATIONS

Guidelines recommend tumor testing for **variants in HRR genes** (*BRCA1*, *BRCA2*, *ATM*, *CHEK2*, *PALB2*, *FANCA*, *RAD51D*, *CDK12*) and determination of **microsatellite instability** (MSI) or mismatch repair status (MLH1, MSH2, MSH2, PMS2) in all men with regional or metastatic high-risk prostate cancer. In cases of high MSI (MSI-H), therapy with immune checkpoint inhibitors may be considered, as pembrolizumab has been approved by the FDA for the treatment of non-resectable or metastatic solid tumors with dMMR or MSI-H. Targeted germline testing can be used to identify patients with family members who may be at increased risk for cancer.

A **TMPRSS2-ERG translocation** is found in approximately 15% of prostate intraepithelial neoplasia (PIN) and in approximately 50% of PCa patients. TMPRSS2-ERG-positive tumors exhibit some special features related to androgen metabolism. They have increased androgen-regulated gene expression and altered intratumoral androgen metabolism, as demonstrated by decreased testosterone concentrations and increased dihydrotestosterone (DHT)/testosterone ratios. Therefore, patients with TMPRSS2-ERG-positive PCa may benefit from novel inhibitors targeting alternative DHT biosynthesis. In the future, PARPi may also play a role in PCa patients with TMPRSS2-ERG translocation because PARP1 can interact with ERG. The resulting ERG overexpression could be inhibited by PARPi and the growth of ERG-positive tumor cells slowed.

Approximately 15% of PCa show **variants in *SPOP*** that are associated with favorable prognosis and improved progression-free survival, especially in patients with high PSA levels before treatment. There is also evidence that in *SPOP*-mutated PCa, SPOP cannot bind to PD-L1, hence, PD-L1 cannot be degraded. This may support the use of immunotherapy with immune checkpoint inhibitors in these tumors.

About **1.6 million cases** annually

~60% of cases diagnosed in **men ≥65 years**

5-year survival rate is **98%** (mostly local/regional stage)

TARGETED-THERAPY TESTS

Gene	Genomic region	Panel
BRCA1	All	*BRCA Oncomine*
ATM	All	*BRCA Oncomine*
PALB2	All	*TPv3b*
FANCA	All	*TPv3b*
RAD51	All	*TPv3b*
CHEK2	All	*TPv3b*
CDK12	All	*TPv3b*
PTEN	All	*TPv3b*
MSI	Stable/unstable	Fragment length

GENE PANEL

FUSION GENES

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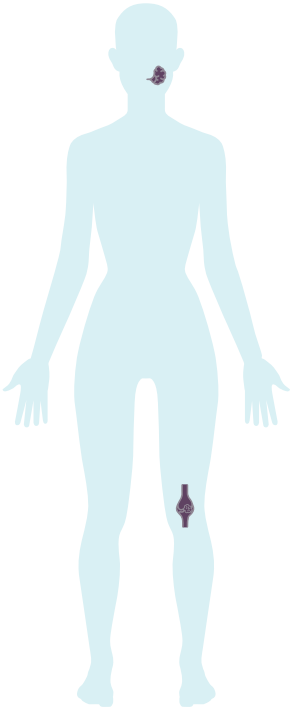
SARCOMA

BACKGROUND

Sarcomas are mesenchymal malignancies that originate from soft tissue or bone. They include more than **80 histological subtypes**, although they account for less than 1% of malignant neoplasms in adults. Subtypes are classified according to the non-neoplastic cell type or the non-neoplastic cell line they resemble or into which they differentiate (e.g., adipocytes, fibroblasts, smooth muscle, etc.). The clinical behavior within different subtypes can vary widely.



GENOMIC ALTERATIONS

Myxoid liposarcoma is a translocation-associated sarcoma that accounts for about one-third of all liposarcomas. It usually occurs in the deep soft tissue of the extremities but can occur in the retroperitoneum. Classically, myxoid liposarcomas are associated with a recurrent fusion of *DDIT3* (*CHOP*), which is most common with the *FUS* gene.

Rhabdomyosarcoma (RMS) is an aggressive malignancy defined by differentiation of skeletal muscles. It is the most common soft tissue sarcoma in children but can also occur in adults. RMS most commonly occurs in the head and neck area, the urogenital tract and the subfascial tissues of the extremities. The current WHO classification distinguishes embryonic, alveolar, spindle cell/sclerosing and pleomorphic subtypes of rhabdomyosarcoma. The second most common subtype of RMS in children, alveolar rhabdomyosarcoma (ARMS), has a worse prognosis than the most common subtype, embryonic rhabdomyosarcoma (ERMS). In contrast to ERMS, the majority of ARMS are associated with a characteristic reciprocal translocation that merges *FOXO1* with *PAX3* or *PAX7*. The identification of a *FOXO1* translocation is a reliable means to distinguish ARMS from ERMS, which has important implications for prognosis and treatment.

Low-grade fibromyxoidal sarcoma (LGFMS) is a rare tumor in young, middle-aged adults that usually occurs in the deep soft tissue of the proximal extremities and trunk. LGFMS is positive for the expression of MUC4, which can be detected immunohistochemically. MUC4 in turn is a downstream target of the translocation protein FUS-CREB3L2, which results from a t(7;16) translocation typical for LGFMS.

Dermatofibrosarcoma protuberans (DFSP) is a low-grade fibroblastic sarcoma that presents as a dermal or subcutaneous lump in young adults, usually on the trunk. DFSP has a recurrent fusion of *COL1A1* with *PDGFRB* and shows a response to tyrosine kinase inhibitors directed against PDGFRB.

Inflammatory myofibroblastic tumor (IMT) is a spindle cell tumor with intermediate malignant potential that is found in the lung, mesentery and omentum of children and young adults. More than half of the IMTs show an *ALK* gene rearrangement, which correlates immunohistochemically with the overexpression of ALK proteins. The detection of an ALK translocation has therapeutic implications, as crizotinib targets ALK in surgically untreatable/recurrent cases. Non-ALK rearranged IMTs often show fusions involving alternative kinase receptors such as *ROS1*, *PDGFRB*, *NTRK1* and *RET*.

Solitary fibrous tumor (SFT) is a fibroblastic neoplasia in adults with a broad anatomical distribution and a mostly benign clinical course; however, up to 25% of cases metastasize. Rarely, SFTs can develop into a high-grade, non-differentiated sarcoma with anaplastic and heterologous elements. SFTs are characterized by an intrachromosomal inversion in chromosome 12, which leads to a fusion of *NAB2* and *STAT6*.

Epithelioid hemangioendothelioma (EHE) is the most aggressive lesion in the family of hemangioendotheliomas. These are vascular neoplasias with borderline malignant potential that range from benign hemangiomas to clinically aggressive angiosarcomas. The diagnosis can be confirmed by identifying the *WWTR1-CAMTA1* or *YAP1-TFE3* fusion characteristic of EHE.

Clear cell sarcoma, formerly known as malignant melanoma of the soft tissue, can now be differentiated from melanoma by detecting the specific genetic aberrations *EWSR1-ATF1*, *EWSR1-CREB1* or *FUS-ATF1*. They mostly occur in the extremities of younger patients with the involvement of tendons, aponeuroses and/or fasciae. Lymph node metastases are detectable in up to 40% of patients.

Synovial sarcoma (SS) is a high-grade malignant disease that most commonly occurs in the extremities of young adults, but it can affect patients of any age at any anatomical site. SS is associated with a translocation between chromosomes X and 18, which fuses parts of the *SS18* and *SSX* genes (*SSX1* 60%, *SSX2* 38%, *SSX4* 2%). Identification of the t(X;18) translocation remains the gold standard for confirmation of the diagnosis.

Ewing sarcoma (ES) is a "small round blue cell tumor" as described by its histology. This high-grade malignancy is the second most common bone and soft tissue sarcoma in children. ES is most commonly associated with recurrent translocation between *EWSR1* and members of the ETS family of transcription factors, most commonly FLI1. Other less common merger variants have been identified, including *FUS*, *ERG*, *ETV1* and *ETV4*. While immunohistochemistry can be helpful to exclude other entities, final classification often requires the detection of characteristic ES-associated gene rearrangements.

Ewing-like sarcomas are a heterogeneous family of tumors that have morphological similarities to classic ES, but do not have a defining *EWSR1*/ETS translocation. Like ES, Ewing-like sarcoma, is an undifferentiated round-cell tumor caused by specific chromosomal translocations. The majority of Ewing-like sarcomas show a fusion between *CIC* and *DUX4*. While detection of the defining rearrangement is required for confirmation, immunohistochemical staining of the transcription factors PAX7 and WT1 may support the diagnosis of a *CIC-DUX4* sarcoma (PAX7 negative, WT1 positive) when compared to ES (PAX7 positive, WT1 negative). Current data suggest that the clinical course and resistance to therapy in patients with *CIC-DUX4* sarcoma are more aggressive compared to classic ES.

The second most common group of Ewing-like sarcomas is characterized by *BCOR1* gene rearrangements, with the majority showing fusion of *BCOR1* with *CCNB3*. The immunohistochemistry for CCNB3 supports the diagnosis. Furthermore, mergers of *EWSR1* with non-ETS partners are detected.

Extraskeletal myxoid chondrosarcoma (ESMC) is a rare sarcoma that typically occurs intramuscularly in the extremities of middle-aged adults. In most cases, ESMC is associated with a reciprocal translocation between *EWSR1* and *NR4A3*, although other rare fusion variants have been reported.

Alveolar soft tissue sarcoma (ASPS) is a rare malignant sarcoma of unknown histogenesis that is most likely to develop in the lower extremities and head and neck of young children. It is associated with a translocation between *ASPSCR1* and *TFE3*.

Desmoblastic small round cell tumor (DSRCT) is a highly aggressive, treatment-resistant sarcoma that typically occurs in the abdomen of young men and is associated with a recurrent rearrangement of *EWSR1* and *WT1*.

POSSIBLE THERAPIES

Despite this heterogeneity, the therapeutic standard for the treatment of most metastatic soft tissue sarcomas (STS) remains **anthracycline-based chemotherapy** although there is significant toxicity and variable efficacy. Regardless of the recent development of several novel chemotherapeutic agents, the prognosis for metastatic STS remains poor: 2-year median survival of 38% and median survival time of 18 months.

SALIVARY GLAND SARCOMA FUSION GENE PANEL

SOFT TISSUE SARCOMA FUSION GENE PANEL

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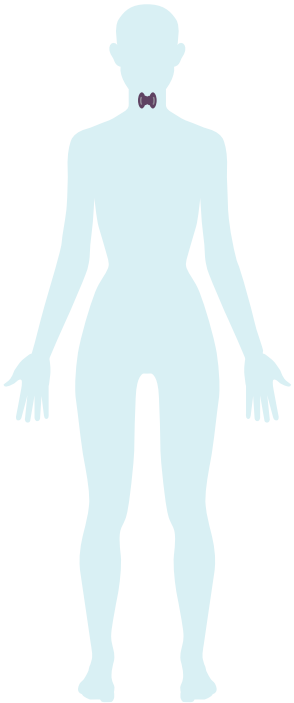
THYROID CANCER

BACKGROUND

Thyroid carcinomas are the most common endocrine tumors and are responsible for 1% to 5% of tumors in women and <2% in men. It is a heterogeneous disease that originates from two different epithelial cell types: follicular cells and parafollicular calcitonin-producing cells. Most **thyroid carcinomas develop from follicular cells** (follicular cell-derived thyroid carcinoma). These include papillary (PTC; 65-80%), follicular (FTC; 10-30%), Hürthle cell and poorly differentiated (PDTC; <2%) and anaplastic thyroid carcinomas (ATC; <2%). PTC and FTC together are called **differentiated thyroid carcinomas** (DTC) and account for 90% to 95% of all thyroid carcinomas. In general, these are slow-growing tumors with an excellent prognosis: 20-year survival rates are >90% with conventional therapy consisting of resection with or without thyroidectomy with radioactive iodine therapy and suppressive thyroid hormone therapy.

Medullary thyroid carcinomas (MTC) develop from parafollicular calcitonin-producing cells and can be part of multiple endocrine neoplasia type 2 (MEN2), which is a hereditary disposition for the development of MTC.



GENOMIC ALTERATIONS AND POSSIBLE THERAPIES

In the majority of cases, DTC is caused by molecular changes in so-called **driver genes such as *BRAF* and *RAS*** or by the creation of fusion genes. These changes lead to the pathological activation of the MAPK signaling pathway, which plays a key role in the regulation of many cellular processes. By accumulating further molecular genetic alterations, e.g., in *TERT*, tumor suppressor genes or the phosphoinositide-3-kinase signaling pathway, DTC can change into dedifferentiated PDTC or ATC. However, a proportion of patients with DTC show more aggressive disease progression with a recurrent or metastatic course and an insensitivity to radioiodine therapy.

Papillary thyroid carcinoma (PTC) is by far the most common entity within the group of epithelial thyroid carcinoma accounting for about 65% to 80%. It is characterized by chromosomal rearrangements involving the *RET* gene (*RET*/PTC rearrangements or *RET* fusion genes) as well as pathogenic variants within the *RAS* or *BRAF* proto-oncogenes, all of which ultimately lead to pathological activation of the MAPK signaling pathway. Variants in the *BRAF* (Val600Glu, Lys601Glu), *NRAS* (codon 12, 13 and 61), *HRAS* or *RET* genes are found in almost 70% of all cases of PTC.

Tumors with variants in *BRAF* are usually undifferentiated and associated with more aggressive tumor characteristics such as a poorer response to radioactive iodine therapy. *BRAF* Val600Glu is found in 45% to 59% of PTC: primarily in the classical papillary and tall cell variant and in up to 80% of patients with recurrent or metastatic PTC.

PTC with variants in *NRAS*, *HRAS* and *KRAS* are more frequently differentiated and are found in 10-20% of the follicular variant of PTC. Pathogenic variants in *BRAF* and *RAS* are almost never found together in PTC tumors. Pathogenic variants in the TERT promoter (Cys288Thr; Cys250Thr) are also found in up to 10% of all PTC. They are associated with a poorer clinical outcome, especially in combination with *BRAF* variants.

Follicular thyroid carcinoma (FTC) is the second most common entity (10-30%). FTC has a different variant profile with a relatively higher proportion of pathogenic *RAS* variants (about 30-40% of all FTCs), whereas *BRAF* Val600Glu and other *BRAF* variants are rarely found (0-4%). Interestingly, changes are relatively frequent in another candidate driver gene, *EIF1AX* (5-7%). In addition, *TERT* variants are also found in FTC and seem to be associated with a significantly worse prognosis.

Medullary thyroid carcinomas (MTC) account for about 3% to 5% of thyroid carcinomas. About 25% of MTC are hereditary and show germline variants in the *RET* gene. In addition, somatic variants can also be detected in *RET* in up to 66% of sporadic MTC. In 75% to 95% of the cases, a *RET* Met918Thr variant is found, which is associated with a more aggressive course, as is the rarer Ala883Phe variant (high risk variants). The simultaneous occurrence of several somatic variants in the *RET* gene is also associated with a worse prognosis. In up to 81% of sporadic, *RET* wild-type MTC, variants are found in *HRAS*, especially Gln61Arg, and less frequently in *KRAS*. The role of Ras variants is less well characterized, but there are data that suggest tumors with somatic *RAS* variants are less aggressive than those with high-risk *RET* variants, but more aggressive than those with non-high-risk RET variants. The mTOR signal pathway is also activated, both in hereditary and sporadic MTC. There is evidence that high mTOR activity is associated with more invasive sporadic MTC and a higher rate of lymph node metastasis.

GENE PANEL

FUSION GENES

For the most up-to-date list of genes in our gene panels and fusion genes, please visit:
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Women three times more affected

5-year survival rate is **98%** (about two-thirds of cases diagnosed at local stage)

About **two-thirds of cases** in people **20-55 years**

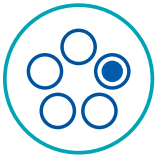
MEDICOVER GENETICS
OUR PORTFOLIO

Medicover Genetics offers an extensive genetic testing portfolio to help health care professionals identify the most appropriate genetic test for the patients. Our mission is to shorten the diagnostics journey by creating opportunities for physicians and patients to find the right information about genetic disorders, genetic testing and associated genes.

Our portfolio includes the following categories:

Hereditary cancer panels

Somatic tumor diagnostics



Rare disease panels

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