

# Genetic Tumor Diagnostics

*Every patient deserves to receive  
personalized precision medicine.*

# CancerPrecision®

**CancerPrecision® is the first choice genetic diagnostics for cancer patients. The panel provides a better understanding of tumor behavior and its likelihood of responding to treatment. In this way, tumor profiling contributes to medicine tailored to the patient, often leading to a better outcome or reduced adverse effects.**

There is no 'one-size-fits-all' in cancer medicine, as every patient's tumor is unique. Thus, it is crucial to understand the disease history and every single tumor as best as possible. CancerPrecision®, our most extensive cancer panel, covering more than 700 tumor-associated genes and therapy-relevant fusions in more than 30 genes, provides a valid diagnosis and guidelines for action to determine the most effective therapy to increase the chances of recovery. All the analyzed data are listed in a comprehensive report, including a graphical representation of results and detailed information on applicable drugs. Thus, by choosing CancerPrecision®, you will receive a high level of professionalism and the best support to find the most effective treatment for each patient.

# Genetics in Tumor Development

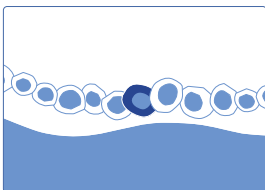
Tumors develop from cells as a consequence of mutations in genes. Mutations usually occur randomly and are typically corrected by the cell's DNA repair systems. However, exposure to carcinogens, such as tobacco smoke or UV radiation increases the number of mutations. A not correctly repaired mutation can result in altered protein function, leading to dysfunction of cellular processes and possibly the beginning of cancer. Spontaneously obtained mutations are called somatic mutations. The genetic alterations initially drive the loss of growth control, the proliferation of cell clones with reduced mortality rate, and finally the distribution of tumor cells to distant organs (metastases). The somatic mutations acquired by tumors in the course of the disease are individual and differ not only between different cancer entities but also from patient to patient – every tumor is unique. Thus, cancer is a multifactorial and heterogeneous genetic disease.

## NGS-Guided Oncogenetics – A New Era of Cancer Management

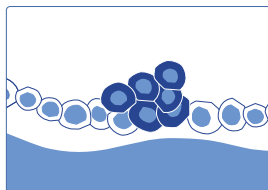
The individual set of tumor-specific mutations helps the tumor to survive and develop resistance against therapeutic agents. To choose a promising treatment strategy, a deep and accurate look into the molecular underpinnings of individual tumors is required. The use of next-generation sequencing (NGS) has initiated a new era in cancer therapy and is a driving force to change the future in personalized precision medicine. Through NGS analysis of genetic mutations, we can tailor the oncological treatments to each patient's features and each cancer genomic alterations to maximize the curative effect, minimize damage to healthy tissues, and optimize resources.<sup>1-3</sup>

## Tumor Progression and Increase in Genetic Diversity

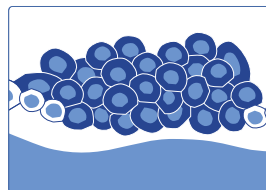
1. Initial mutation



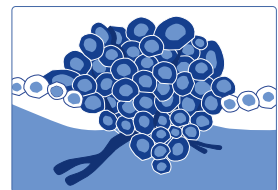
2. Growth



3. Further mutations



4. Metastases





# Genetic Tumor Diagnostics

The decision-making basis for choosing the best possible analysis strategy

Genetic analysis of the tumor is essential for choosing the optimal treatment. The table below describes the variety of genetic molecular profiling tests offered. The choice of test depends on the samples available and, in particular, on the objective of the desired diagnosis.



**CancerPrecision®**  
Full molecular analysis



**CancerDetect®**  
Analysis of hotspot regions & monitoring



**CancerNeo®**  
Identification of neoantigens



**CancerEssential®**  
Analysis of specific selected genes

Sample type	Solid tumor tissue	Liquid biopsy	Liquid biopsy	Solid tumor tissue	Solid tumor tissue
Scope	> 700 genes (full coding) and selected structural variants in > 30 genes		Hot spot regions in 36 genes and TP53 full coding	Whole exome and transcriptome sequencing	Disease specific gene sets covering up to 54 genes and selected structural variants in 9 genes
Normal tissue comparison	✓		✗	✓	✗
Detailed drug options list	✓		✓	✓	✓
Detection threshold (NAF*)	5%		0.25%	5%	5%
Minimal tumor fraction	20%		1%	20%	20%
SNV and INDELS	✓		✓	✓	✓
CNV	✓		✗	✓	✗
TMB	✓		✗	✓	✗
MSI	✓		✗	✓	✓**
HRD	✓		✗	✓	✗
Fusion gene analysis / structural variants (DNA-based)	✓		✗	✓	✓
Additional option: RNA-based fusion transcript analysis (CancerFusionRx®)	✓**	✗	✗	✓**	✓**
HPV/EBV infection	✓		✗	✗	✗
HLA Typing	✓		✗	✓	✗
Neoantigen	✗		✗	✓	✗
Pharmacogenetics variants (selection)	✓		✗	✓	✗
CHIP detection	✓		✗	✓	✗
Representation of biological pathways and coverage profile (Copy number variations)	✓		✗	✓	✗

\* Novel allele frequency

\*\* Extra order







# CancerPrecision®

First choice genetic diagnostics for cancer patients

## Enables Targeted Treatment for Your Patients

There is no 'one-size-fits-all' in cancer medicine, as every patient's tumor is unique. Thus, it is crucial to understand the disease history and every single tumor as best as possible. Comprehensive genomic tumor profiling helps to detect clinically relevant mutations in cancer-associated genes of solid tumors and provides valuable information for selecting the most efficient treatment for each patient.

*CancerPrecision® provides an optimal molecular genetic tumor profiling using NGS and forms the basis for personalized, biomarker-based cancer therapy.*

We at CeGaT have fully committed ourselves to this aim. With our long-term experience in genetic diagnostics, we have optimized our somatic tumor diagnostics to identify the somatic alterations that promote tumor growth, are responsible for drug resistance, and represent potential therapeutic targets. By using NGS technology, we analyze a panel of **more than 700 tumor-associated genes and selected therapy-relevant fusions in more than 30 genes**. Optional targeted RNA-based fusion analysis allows the detection of fusion transcripts with *de-novo* and known partners in more than 150 genes. Variations in these genes are known to significantly impact tumor pathogenesis, progression, and metastasis. Concerning immunotherapies, we determine, tumor mutational burden (TMB), microsatellite instability (MSI), and viral infection (HPV, EBV). In addition, we determine the homologous recombination deficiency (HRD) status, which provides key information, allowing for the prediction of PARP inhibitor- and platinum-based chemotherapy response caused by synthetic lethality. After a processing time of 2-3 weeks, the generated data are summarized in a comprehensive report using current scientific knowledge to support you, as the treating physician, in finding efficient treatment for each patient.

## Key Facts of CancerPrecision®

- ✗ Full sequencing and analysis of more than 700 tumor-associated genes and fusions in more than 30 genes
- ✗ High average sequencing of 500-1,000x coverage allows detection of therapy-relevant variants in subclones
- ✗ Sensitivity: > 96%\*, Specificity: > 99.9%
- ✗ Analysis of tumor mutational burden (TMB), microsatellite instability (MSI), and viral infection (HPV, EBV) – essential biomarkers for immunotherapies
- ✗ Analysis of homologous recombination deficiency (HRD) – an essential biomarker for PARP inhibition
- ✗ Analysis of single nucleotide variants (SNVs), insertions and deletions (INDELs), translocations, and copy number variants (CNVs)
- ✗ Besides therapy-relevant somatic (tumor-specific) mutations also disease causing and therapy-relevant germline variants are reported
- ✗ Selected pharmacogenetically relevant germline variants necessary for drug dose adjustment in your patients
- ✗ A list of all eligible drugs, with EMA and/or FDA approval, for which corresponding biomarkers could be detected in the tumor
- ✗ Detection of mosaic variants:  
CHIP (Clonal Hematopoiesis of Indeterminate Potential)

**Optional services:** RNA-based fusion transcript analysis from tumor RNA analyzing more than 150 genes (CancerFusionRx®).



For the detailed gene directory visit:  
[www.cegat.com/cancerprecision](http://www.cegat.com/cancerprecision)

\* Based on a high-quality sample with 20% tumor content for detecting a somatic heterozygous variant.

# Medical Report

The medical report contains the results from the genetic testing requested by you, as the referring physician. Each single medical report is prepared and discussed by an interdisciplinary team of molecular biologists and medical doctors to guarantee the highest quality.

## 1 Patient details

Name, date of birth, sex of the patient, and external ID, suspected diagnosis or indication for molecular genetic testing.

## 2 Results – Overview

On the first page, we provide an overview of the major characteristics of the tumor depicted in boxes. The results which are important for a patient's individual therapy decision are highlighted in colour. Separate boxes present TMB, MSI, and HRD score of the analyzed tumor sample, give summary information on copy number alterations and state details on detected germline variants. Moreover, we provide evidence for possible clonal hematopoiesis of indeterminate potential (CHIP) and for an HPV or EBV infection.

### 3 Findings of therapeutic relevance

In this section, the details of variants/biomarkers with therapeutic relevance are listed. For each gene, the somatic change is depicted in detail as well as the effect of the variant on the protein. In addition, for each variant/biomarker, the therapeutic option(s) are listed including EMA/FDA approval.

## PGX

If a pharmacogenetically relevant variant is detected, the gene, including the change and the effect on the protein, is presented. In addition, the affected therapy, including the resulting phenotype, is listed (not shown in this sample report). Additionally, a separate non-tumor treatment-specific PGX report can be requested.

## CHIP

In case of evidence for possible clonal hematopoiesis of indeterminate potential, the gene, the variant, and the transcript, including the NAF in blood and tumor sample, are listed (not shown in this sample report).

[illegible][illegible]



#### 4 Complete list of automatically detected somatic variants and chromosomal aberrations

The table includes all somatic variants (single nucleotide variants and small deletions/insertions ( $\leq 40$ bp)) detected automatically within the sequenced regions. Moreover, the detected copy number alterations (deletions and/or amplifications) of large genomic segments are listed. The table shows the altered chromosomal region, the functional category, and estimated copy numbers of the chromosomal region. If applicable, the genes with therapeutic relevance are displayed.

#### 5 Recommendations

In this section, clinical recommendations are given. These are, for example, recommendations of genetic counseling in cases in which pathogenic germline variants had been identified.

#### 6 Graphical representation of important biomarkers (TMB and HRD)

The tumor mutational burden (TMB) of the patient sample is visualized. The graphic shows the TMB of the patient in comparison with the mutational load published for a variety of different tumor entities. Homologous Recombination Deficiency (HRD) score of the patient is visualized. The graphic shows the HRD score of the patient compared to house-internal cohorts used in HRD score validation.

#### 7 Copy number profile

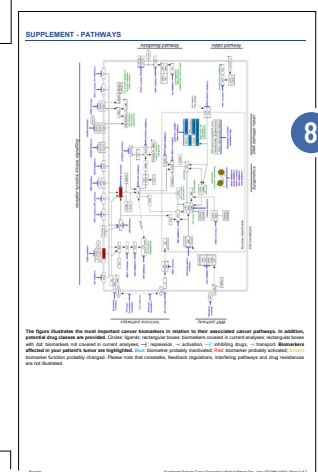
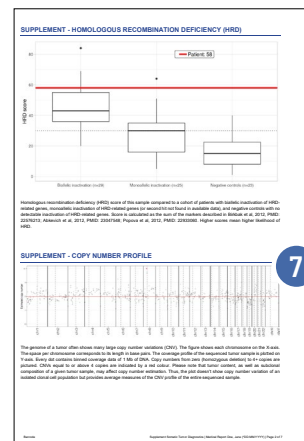
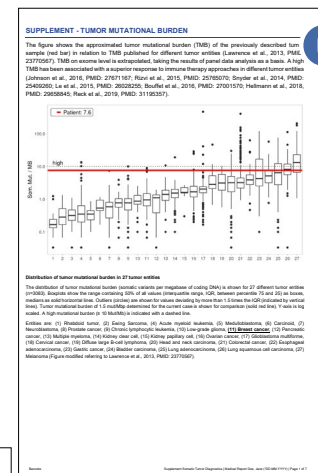
The figure shows the normalized coverage profile of the sequenced tumor sample with a resolution of 1 Mb.

#### 8 Pathway illustration

Graphical display of all relevant signaling pathways in tumors with highlighting of activated/inactivated changes in the patient. Potential therapeutic measures are shown together with their targets.

#### 9 Therapeutic strategies (FDA/EMA approved)

Selection of eligible drugs based on detected variants. This list includes drug classes and names as well as their approval (FDA/EMA) and limiting conditions.



**POSSIBLE THERAPEUTIC STRATEGIES**

Please note that the provided information on potential drugs is only a specific selection and makes no claim of completeness.

Relevant therapeutic drugs due to the homologous recombination deficiency and the variant BRCA2 (genotype): c.3847\_3848delCT; p.Val1223.IysV2, NM\_000593.4

Drug name	Drug class	Approved indication	Relevant to patient's condition	Relevant to patient's condition
Platinum-based chemotherapy	Chemotherapy	Approved for platinum-based chemotherapy	Platinum-based chemotherapy is a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance. Platinum-based chemotherapy is a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance.	Platinum-based chemotherapy is a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance. Platinum-based chemotherapy is a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance.
PARP inhibitors	PARP inhibitors	Approved for BRCA2-deficient ovarian cancer	PARP inhibitors are a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance. PARP inhibitors are a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance.	PARP inhibitors are a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance. PARP inhibitors are a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance.
Immunotherapy	Immunotherapy	Approved for BRCA2-deficient ovarian cancer	Immunotherapy is a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance. Immunotherapy is a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance.	Immunotherapy is a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance. Immunotherapy is a standard treatment for BRCA2-deficient ovarian cancer. The patient's condition is characterized by a BRCA2 mutation, which is associated with a high risk of platinum resistance.

# Tumor to Normal Tissue Comparison

## The only accurate way to determine somatic variants

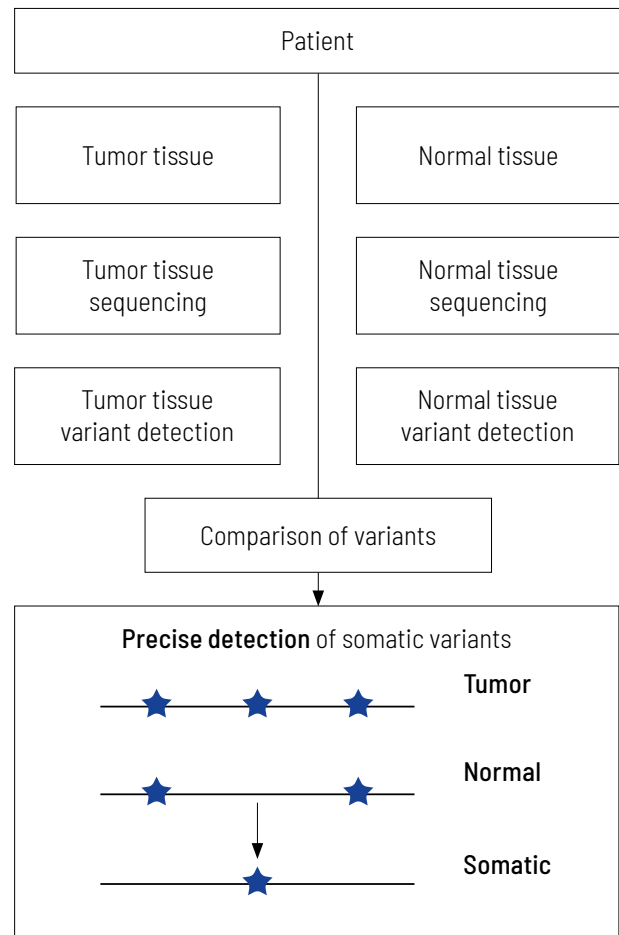
Precise information on tumor genetics is needed for correct interpretation. In tumor diagnostics, it is highly important to discriminate between variants that are restricted to the tumor (somatic variants) compared to those also present in the healthy tissue (germline variants).

The only accurate way to determine variants in the healthy tissue is to sequence the matching normal tissue together with the tumor tissue. Methods trying to replace the sequencing of normal tissue by bioinformatics approaches fail to clearly distinguish between germline and somatic variants, especially when the tumor content of the sample is high.<sup>4,5</sup> Sequencing just tumor tissue can lead to an incorrect calculation of the TMB, which in the worst case may result in patients not receiving optimal treatment.<sup>6</sup>

Comparing tumors with matching normal tissue is mandatory for obtaining meaningful results. Diagnostic tests that do not analyze tumors and matching normal tissue usually give non-accurate results. We at CeGaT always sequence tumor tissue and matching normal tissue for our CancerPrecision® Diagnostic.

**Therefore, we always sequence DNA from the tumor as well as from normal tissue (mostly blood).**

The sequencing data of both tissues are compared, and thereby truly somatic variants are determined.



# Comprehensive Tumor Profile at a Glance

Understanding the molecular genetic tumor profile is essential for personalizing the patient's treatment and identifying additional treatment options. The number of biomarkers we address in our CancerPrecision® report is very high. Therefore, the results are grouped into major categories and presented as boxes. The boxes with treatment-relevant findings are highlighted in color.

We present individual boxes for tumor content, TMB, MSI, and HRD scoring of the analyzed tumor sample, structural variant findings, tumor driver mutations, evidence for viral infections (HPV/EBV), germline variants (that explain the patient's disease), pharmacogenetic variants with implications on cancer treatments, and possible clonal hematopoiesis of indeterminate potential (CHIP).

<b>Tumor tissue &amp; tumor content</b>  Sample of the breast cancer from MM/YYYY  30% (histologically) 25% (bioinformatically)  Required min 20%	<b>Tumor mutational burden (TMB)</b>  7.6 Var/Mb  High $\geq 10$	<b>Microsatellite instability (MSI)</b>  No evidence for MSI  Score 0.14  Indication of MSI $\geq 0.33$	<b>Homologous recombination deficiency (HRD)</b>  Evidence for a possible HRD  Score 58.0  Indication of HRD $\geq 30$	<b>Fusions, structural variants</b>  No evidence for therapeutically relevant structural variants on DNA- and RNA-level
<b>Tumor Drivers</b>  Identified tumor driver: <i>BRCA2</i> , <i>PIK3CA</i>  Relevant genes without oncogenic alterations: <i>BRCA1</i> , <i>ERBB2</i>	<b>Viral infection</b>  No evidence for an infection with HPV/EBV in the tumor sample	<b>Germline variants</b>  Detection of a pathogenic germline variant in gene <i>BRCA2</i>	<b>Pharmacogenetics</b>  No evidence for germline variants that are likely to affect drug tolerance	<b>CHIP</b>  No evidence for CHIP



# Variants with Potential Therapeutic Relevance

## Guidance on potentially effective drugs

For each gene, the somatic change is depicted in detail, and the resulting therapeutic options are stated, including the EMA/FDA approval (A). These options are the basis for discussion in a molecular tumor board (MTB). At the end of the medical report, in the appendix/supplement, we provide

an extensive list of possible therapeutic strategies for each identified somatic changes (B). This list includes drug classes and names as well as their approval (FDA/EMA) and limiting conditions.

## Variants with Potential Therapeutic Relevance:

A)	Gene	Functional category	Variant	NAF	Effect on protein function	Therapeutic option for discussion in the MTB	Approved by EMA/FDA	Approved for current entity
	BRCA2 (germline)	frameshift	c.3847_3848delGT; p.Val1283Lysfs*2  and loss of the wildtype-allele in tumor	het.	inactivating	PARP-Inhibitor	EMA* & FDA*	EMA* & FDA*

B)	Drug name	Tumor entity	Approval	Approval limited to biomarkers/others	Approval in combination with other drugs
Niraparib PARP-Inhibitor	fallopian tube carcinoma	EMA	advanced or relapsed cancer, prior response to platinum-based chemotherapy		
		FDA	cancer associated with homologous recombination deficiency (HRD) adult patients, advanced or recurrent cancer, prior response to platinum-based chemotherapy		
	ovarian cancer	EMA	advanced or relapsed cancer, prior response to platinum-based chemotherapy		
		FDA	cancer associated with homologous recombination deficiency (HRD) adult patients, advanced or recurrent cancer, prior response to platinum-based chemotherapy		
	primary peritoneal carcinoma	EMA	advanced or relapsed cancer, prior response to platinum-based chemotherapy		
		FDA	cancer associated with homologous recombination deficiency (HRD) adult patients, advanced or recurrent cancer, prior response to platinum-based chemotherapy		
Olaparib PARP-Inhibitor	breast cancer	EMA	germline BRCA1/2 variant, HER2-negative or HR-positive adult patients, locally advanced or metastatic breast cancer, prior endocrine or chemo therapy		
		FDA	deleterious or suspected deleterious germline BRCA mutation, HER2-negative or HR-positive adult patients, high risk or metastatic cancer, prior adjuvant or neo-adjuvant therapy		
	fallopian tube carcinoma	EMA	deleterious or suspected deleterious germline or somatic BRCA mutation adult patients, advanced (FIGO stages III and IV) cancer, prior response to platinum-based chemotherapy		
		FDA	cancer associated with homologous recombination deficiency (HRD) adult patients, advanced cancers, prior response to platinum-based chemotherapy	Bevacizumab	

**Sample report:** Exemplary for the *BRCA2* variant detected and the resulting therapeutic options in a breast cancer patient. Top panel (A): An excerpt from Table 1 of the findings, listing variants with therapeutic relevance. Lower part (B): An excerpt of the drug listing. In addition to the drugs shown, other drugs are also described.

For more details, please see sample report:  
[www.cegat.com/cancerprecision](http://www.cegat.com/cancerprecision)



# Pathway Illustration

For a detailed understanding of altered signaling

Cancer arises due to aberrant cell behavior concerning cell growth and survival. Both processes become uncontrollable in the course of tumor development. Typically, all cellular processes are strongly regulated and controlled by a complex network of signaling pathways.

Our medical report provides a comprehensive view of the network of cancer-associated signaling pathways and their molecular “key players” and all relevant genetic alterations and available drug classes to:

- ✗ understand the interactions between the different signaling pathways and
- ✗ counteract possible tumor bypass strategies.

Tumors contain mutations in genes that have key roles in these complex signaling pathways. In this context, a single genetic alteration can affect multiple pathways. Thus, it is crucial to understand the interplay of signaling pathways, which are affected by the genetic variants, next to detecting disease-associated mutations.

This approach helps to identify possible bypass strategies of a given tumor to consider all possible therapeutic options, including effective combination therapies.

## Considered signaling pathways

- ✗ Signaling via receptor tyrosine kinases
- ✗ Cell cycle
- ✗ DNA damage repair
- ✗ Hormone pathways
- ✗ Wnt pathway
- ✗ Hedgehog pathway
- ✗ Hippo pathway
- ✗ Apoptosis pathway
- ✗ Epigenetic regulators

# TMB Determination and MSI Prediction

The basis for therapeutic decisions on immunotherapies with checkpoint inhibitors

Tumor mutational burden (TMB) — the number of somatic mutations per megabase (Mut/Mb) — is a reliable predictive biomarker for responses to treatment with immune checkpoint inhibitors. The higher the number of genetic variations within a tumor cell, the more mutated proteins are expressed. These mutated proteins are processed into short fragments (peptides) presented on the surface of tumor cells. Such mutated peptides are called neoantigens. Neoantigens are highly immunogenic. This means they are very effectively recognized by immune cells, particularly T cells. T cells are able to eliminate tumor cells upon antigen recognition directly. Therefore, the higher the number of mutations, the higher the chance that neoantigens are presented on tumor cells, and thus the more efficient is tumor eradication by T cells.

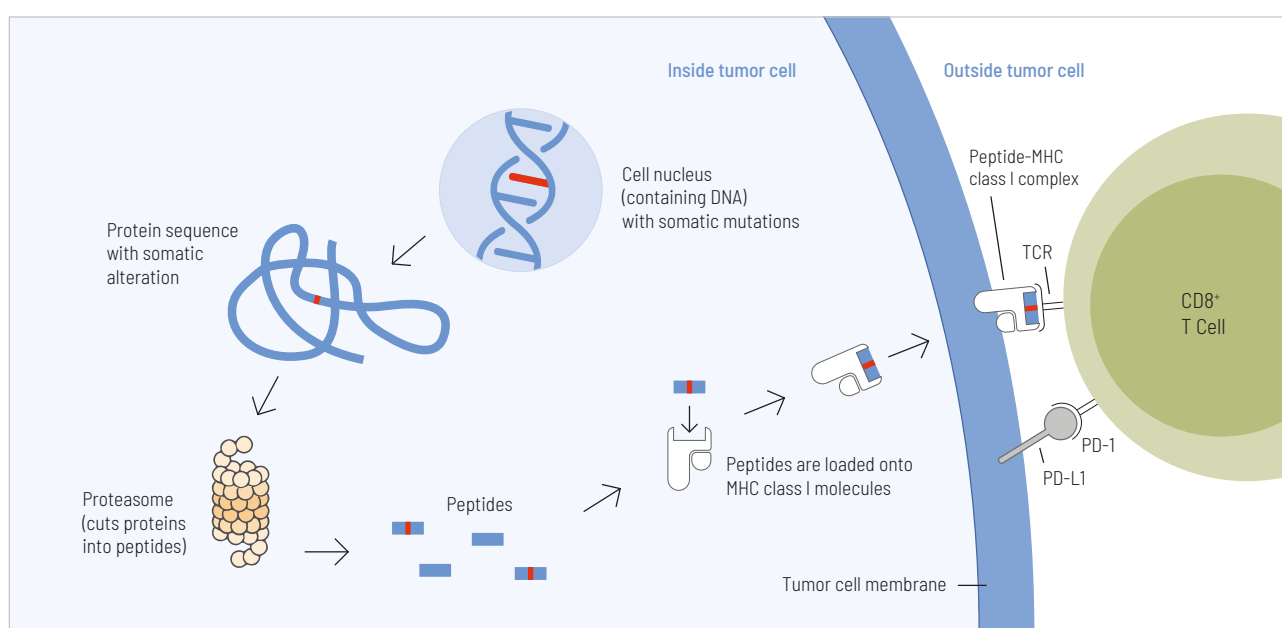
By sequencing a large gene set of both normal and tumor tissue with high sensitivity, we are able to calculate TMB precisely. This metric is used to categorize tumors into low and high mutational load. We list the classification of TMB, as well as the exact mutation rate of the tumor sample. When calculating TMB, the size of the panel is crucial for the precision of the results. With a size of 2.2 Mb, CancerPrecision® is well above the minimum requirement of 1.5 Mb and ensures a robust estimate of TMB.<sup>7</sup>

*TMB and MSI are reliable biomarkers for predicting immune checkpoint inhibitors efficacy.<sup>8-10</sup>*

MSI (microsatellite instability) is another crucial parameter for response to immune checkpoint blockade.

Microsatellites are small repetitive sequences of DNA located throughout the genome. The size of microsatellites can be altered due to failures of the DNA mismatch repair machinery.

Traditionally, MSI is detected through a comparison of satellite regions in tumor and normal tissue via PCR. However, we at CeGaT can predict the MSI status via NGS. This calculation was validated with hundreds of matched normal and tumor sample pairs across various cancer types, testing more than 2,500 target microsatellite foci.



Presentation of tumor cell-derived somatic peptides. Somatic mutations frequently arise in cancer and permanently alter the genomic information. These genetic changes can result in the expression of proteins with altered amino acid sequences. These peptides that carry a somatic change and thus display a particularly strong immunostimulatory potential can be presented on the tumor cell surface and cause an effective anti-tumor immune response.






# HRD – Homologous Recombination Deficiency

Healthy cells ensure a stable and error-free genome by using different DNA repair mechanisms. Homologous recombination repair (HR) is a DNA repair pathway that acts on DNA double-strand breaks. In case of homologous recombination deficiency (HRD), this pathway is defective so that mutations, chromosomal aberrations, and other errors can accumulate in the genome. Through the resulting genomic instability, HRD facilitates tumor development and has been shown to play a role in various cancers, most prominently in breast and ovarian tumorigenesis.<sup>11,12</sup> Loss-of-function genes involved in this pathway can sensitize tumors to PARP inhibitors and platinum-based chemotherapy, which target the destruction of cancer cells through synthetic lethality. To identify tumors where these medications are applicable, reliable determination of the HRD status is of utmost importance.

HR-deficient tumors are often caused by germline or somatic mutations in *BRCA1* or *BRCA2*. Therefore this pattern has formerly been referred to as *BRCAness*. Moreover, mutations in other HR genes such as *RAD51C*, *ATM*, and *PALB2* have been shown to cause HRD. It has to be mentioned that not every genetic defect in HR genes necessarily leads to HRD in the tumor. On the contrary, HRD can be caused without a detectable HR gene mutation, such as promoter methylation of *BRCAness* genes. Thus, if one tries to detect the mutations only in *BRCAness* genes, a potential HRD may remain undetected.

To ensure that HR-deficient tumors are not overlooked, we calculate the HRD score as part of every CancerPrecision® analysis independent of tumor entity.

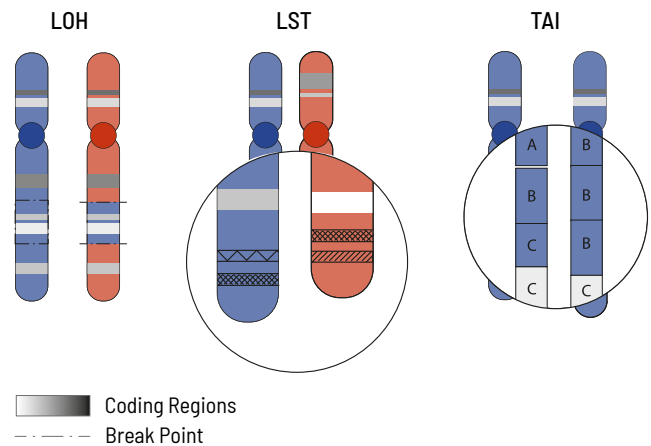
The HRD score measures overall genomic instability based on the number of INDELs, substitutions and rearrangements occurring on a genome-wide level. The responsible mutations do not have to be precisely identified for this. To calculate the HRD score of the tumor sample the mutation pattern is used and calculated from three typical HRD events:

-  Loss of heterozygosity (LOH)
-  Large-scale state transition (LST)
-  Telomeric allelic imbalance (TAI)

**LOH** is the irreversible loss of a single parental allele, which is especially severe in cases where defective gene versions are retained. LOH regions are defined as larger than 15 Mb but less than the whole chromosome.

**LST** counts the number of transition points between abnormal chromosome regions that generate chromosomal gains or losses larger than 10 Mb.

**TAI** occurs when the telomeric end of a chromosome is severely shortened in one of the two paternal chromosomes which causes an allelic imbalance in this region. This imbalance occurs because the repetitive DNA sequences in telomere regions are especially sensitive to HRD.



The HRD score is reported in our CancerPrecision® diagnostic report together with any identified somatic mutations and selected gene fusions as well as TMB, MSI and CNVs to provide a most comprehensive tumor analysis.

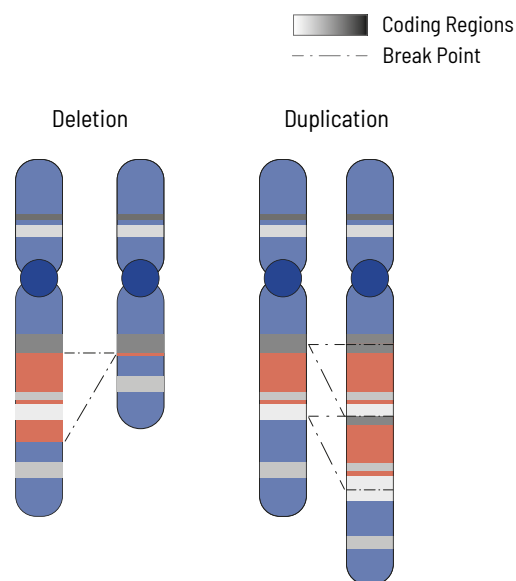
# CNV Analysis

## Determination of deletions/amplifications for highest therapeutic yield

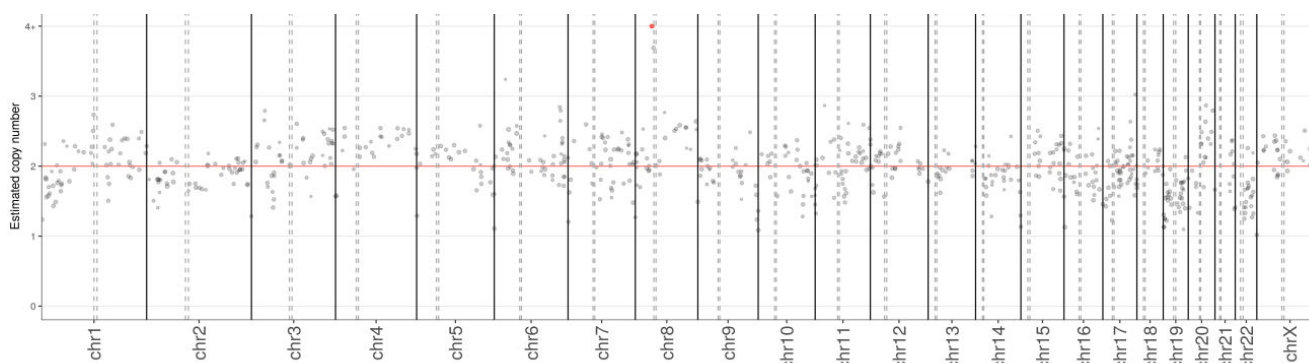
Cellular processes are tightly regulated. This regulation depends on the correct function of genes. In tumors, the copy number of genes is frequently altered, thus impairing the affected genes correct function. Increasing the copy number of a gene can increase its activity, while (partial) deletion can result in a loss of function. Therefore, chromosomal aberrations leading to copy number changes can also have therapeutic consequences.

In tumors, copy number variations (CNVs) are frequent due to the overall genomic instability. Here large chromosomal parts are often either deleted or amplified. Understanding these deletions/amplifications and knowing the genes in the affected region with therapeutic relevance is important. Therefore, deletions and amplifications are detected based on the NGS data obtained.

Deletions and amplifications are listed with the affected genes of therapeutic relevance at the beginning of the report. A complete CNV-profile of the analyzed regions is shown in the report's appendix.



CNVs often play an important role in tumor genetics. Knowing the changes in CNVs assists in choosing the optimal treatment. Therefore CNV analysis is an integral part of our somatic tumor diagnostics.



The genome of a tumor often shows many large copy number variations (CNV). The figure shows each chromosome on the X-axis. The space per chromosome corresponds to its length in base pairs. The coverage profile of the sequenced tumor sample is plotted on Y-axis. Every dot contains binned coverage data of 1 Mb of DNA. Copy numbers from zero (homozygous deletion) to 4+ copies are pictured. CNVs equal to or above 4 copies are indicated by a red colour. Please note that tumor content, as well as subclonal composition of a given tumor sample, may affect copy number estimation. Thus, the plot doesn't show copy number variation of an isolated clonal cell population but provides average measures of the CNV profile of the entire sequenced sample.

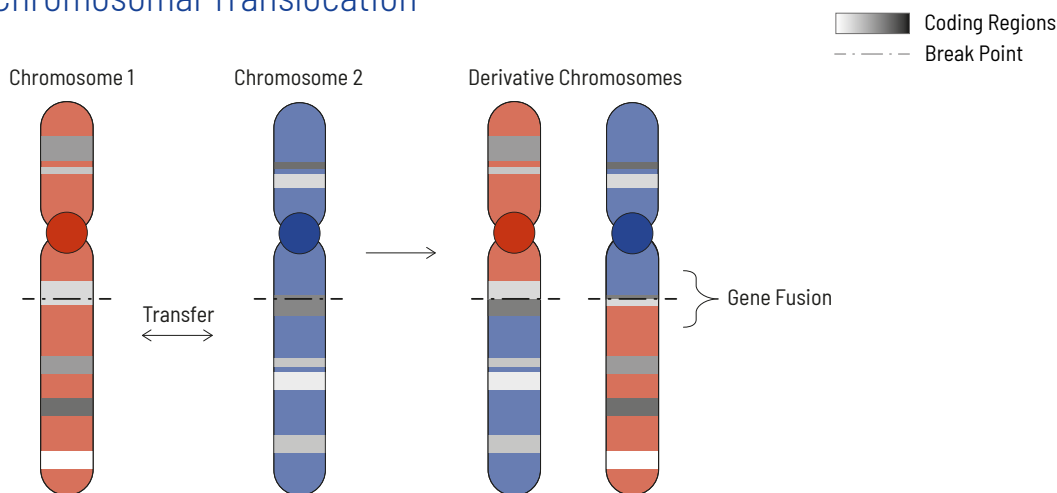
# CancerFusionRx®

## RNA-based identification of fusion transcripts

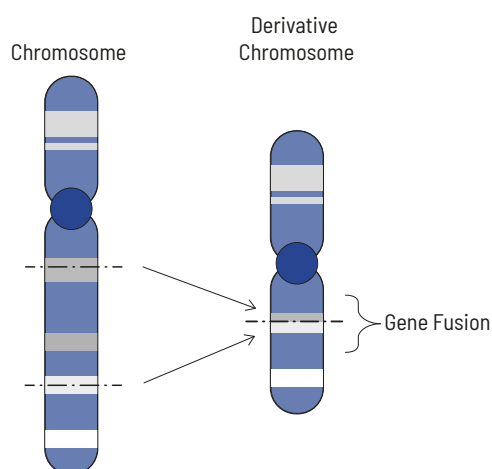
Chromosomal rearrangements frequently occur in all types of cancer. As a result, gene fusions can occur in the cancer genome. Fusions are major drivers of cancer and are therefore most relevant for treatment decisions. Conventional PCR-based methods will not detect a fusion when the other partner is not known (frequently relevant for neurotrophic tyrosine kinase, NTRK fusions). Even whole transcriptome analyses are not sensitive enough, especially when the tumor content is low.

To detect all known and previously described as well as novel gene fusions with a therapeutic option, we developed a next-generation targeted enrichment on RNA-basis. The design currently includes over 150 genes for fusion detection and over 120 exon-exon-specific enrichments with known breakpoints. This method is superior to DNA-based methods and also to whole RNA-based approaches. We strongly recommend completing the genetic tumor diagnostic by RNA enrichment for fusions for the most complete understanding of the tumor's biology.

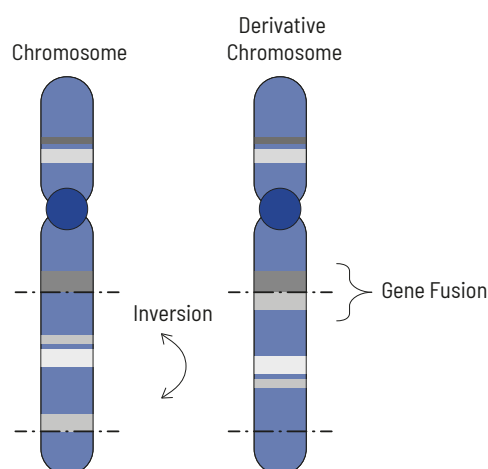
### Chromosomal Translocation



### Interstitial Deletion



### Chromosomal Inversion



Read our Application Note for more details:  
[www.cegat.com/cancerfusionrx](http://www.cegat.com/cancerfusionrx)





# The Power of Liquid Biopsy Testing

## Access to tumor by blood analysis

Liquid biopsy analysis represents an optimal alternative testing procedure in cases where tumor tissue is unavailable, e.g., irresectable tumors or poor patient conditions. The basis of liquid biopsy (LB) analysis is cell-free DNA (cfDNA) which is increasingly released into the bloodstream by necrotic and apoptotic cells in patients with cancer and other types of diseases. Circulating cfDNA is released from both normal and tumor cells. The fraction of tumor-derived cfDNA (circulating tumor DNA/ctDNA) depends on tumor entity, tumor stage, tumor burden, and other factors and therefore is not the same in every patient. Since only a fraction of circulating cfDNA is derived from the tumor, highly sensitive methods are required to detect minimal ctDNA concentrations.

CeGaT has established and validated extensive liquid biopsy panels assessing cell-free DNA. CancerPrecision® uses liquid biopsy testing to provide a complete genetic profile of the patient's tumor, supporting the choice of the best possible therapy. A tumor content of at least 20% is required for this analysis. Since the content of ctDNA can be less than 20%, CeGaT also offers CancerDetect®, a duplex UMI\*-based panel. CancerDetect® identifies sequence variants in actionable, most prevalent driver mutations with an allele frequency above 0.25%. This highly sensitive detection of tumor-specific biomarkers represents an excellent tool for monitoring treatment response and minimal residual disease. In addition, the method's high sensitivity makes failure much less likely, even with a low tumor fraction in the sample.

\* Unique molecular identifier

### Advantages of liquid biopsy

- ✗ Non-invasive
- ✗ Rapid and precise diagnosis
- ✗ Real-time monitoring of therapies and early detection of relapse or progression

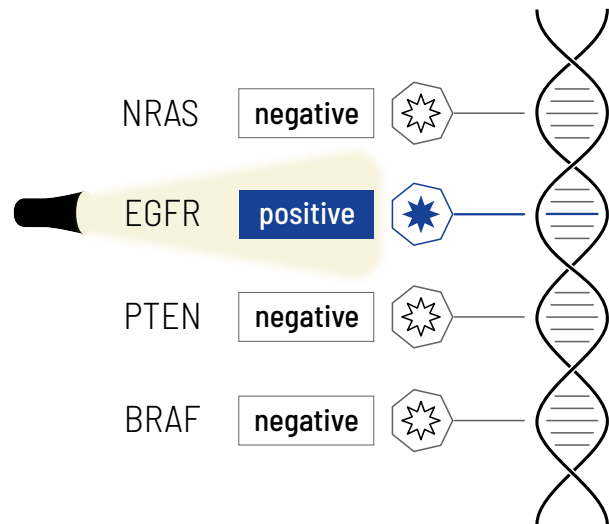
# CancerDetect®

Highly sensitive detection of actionable variants from liquid biopsy with low tumor content

Liquid biopsy analysis detects cell-free DNA (cfDNA) released into the bloodstream by necrotic and apoptotic cells and thus offers an optimal alternative when tumor tissue is unavailable. However, only a fraction of circulating DNA originates from the tumor itself. Therefore, highly sensitive methods are required to detect these minimal ctDNA concentrations. We at CeGaT established our CancerDetect® panel using duplex UMI-based technology, which detects sequence variants in actionable, most prevalent hotspots.

Moreover, due to non-invasive and repeatable sampling, CancerDetect® represents a great approach to monitoring. By very sensitive detection of tumor-specific biomarkers, the analysis of ctDNA can be used as a surrogate marker for treatment response during medical follow-up.

## Single Marker or Hotspot Testing



## Key Facts of CancerDetect®

- ✗ Liquid biopsy enables the detection of variants with potential therapeutic relevance in patients where the tumor is inaccessible, allowing to gain information about the tumor and to address treatment
- ✗ Highly sensitive and accurate detection of actionable, most prevalent driver mutations in 36 genes with very low allele frequencies by using duplex UMI-based technology (NAF  $\geq 0.25\%$ )
- ✗ High coverage: 50,000–100,000x raw coverage
- ✗ Simple, non-invasive, and repeatable sampling provides best conditions for longitudinal follow-up testing and disease monitoring
- ✗ By very sensitive detection of tumor-specific biomarkers, the analysis of ctDNA can be used to track tumor dynamics in real time and intervene or adjust treatment if necessary (e.g., at acquired drug resistance)







# CancerDetect® Sample Case

## Patient and Indication:

- ✗ 42 years old, female with metastatic non-small-cell lung cancer (NSCLC) with an *EGFR* L858R mutation in the primarius
- ✗ Relapse after initial response to afatinib treatment
- ✗ Recurrent tumor inoperable, no tumor biopsy possible

## Primary finding:

The result of an analysis of cell-free DNA using a standard lung cancer panel remained negative.

## CancerDetect® finding:

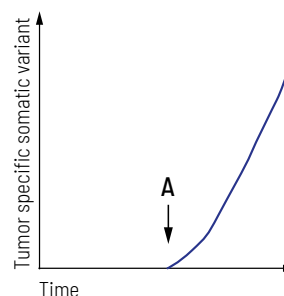
Our CancerDetect® analysis revealed a 2% tumor content in the liquid biopsy and detected the known *EGFR* L858R mutation, as well as an additional *EGFR* T790M resistance mutation. The *EGFR* T790M mutation represents one of the most common resistance mechanisms to tyrosine kinase inhibitors (TKIs) and typically occurs in NSCLC patients after first-line TKI treatment. In this patient, treatment was adjusted with the third-generation *EGFR* inhibitor osimertinib.



# CancerDetect® Monitoring Applications

## Application "Relapse early detection":

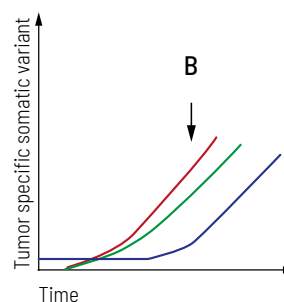
After surgery/treatment, the patient was considered tumor free. Regular LB testing revealed tumor progression showing that an increase in a tumor-specific variant accompanied the recurrence of the tumor. This biomarker's highly sensitive detection may detect the tumor's relapse earlier than conventional imaging techniques.



A = time of clinically-detectable recurrence or relapse

## Application "Monitoring during treatment":

After treatment, the patient underwent strong regression showing stable disease. Longitudinal monitoring provides an up-to-date molecular profile of the tumor and detects emerging treatment resistance in time. Here, two additional subclones occur besides the primary tumor mutation, forcing the treatment to be adjusted appropriately.



B = time of clinically-detectable recurrence or relapse and acquired resistance



## Identify neoantigens for personalized cancer vaccine design

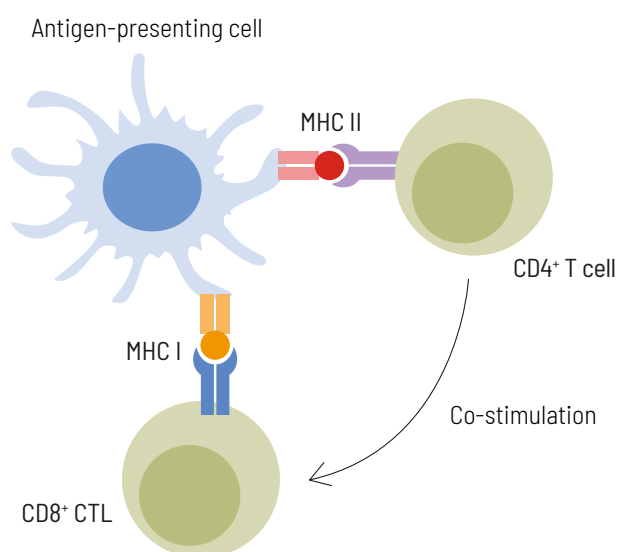
The personalization of cancer treatment is evolving rapidly. Developing a personalized cancer vaccine is a promising strategy for cancer treatment. Body cells constantly present peptides on their surface, indicating the current state of the cell. Immune cells can recognize if a cell presents normal or abnormal peptides and use this information to determine and eliminate defective cells. Abnormal peptides, so-called neoantigens, arise from DNA mutations in tumor cells and are targets for therapy using personalized vaccines.

CancerNeo® enables the analysis of a patient's tumor exome to detect tumor-specific (somatic) mutations, identifies the HLA-types, and predicts neoantigens. The expression of these neoantigens is confirmed by whole RNA sequencing (transcriptome) from the same tumor sample. Thus, our CancerNeo® provides the insights required for the design of personalized cancer vaccines – a powerful tool to boost the immune system's response to cancer cells.

## Neoantigen Prediction

Neoepitopes can be recognized by CD8+ cytotoxic T cells (CTL) and by CD4+ T helper cells. Neoepitopes recognized by CD8+ T cells are typically 8-12 amino acids long and presented by HLA class I molecules, while neoepitopes recognized by CD4+ T helper cells are usually longer (13-18 amino acids in length) and presented by HLA class II molecules. For successful immunotherapy, neoantigen selection should include short and long peptides to activate both, CD4+ and CD8+ T cells. Immune responses of each individual patient can be monitored during anti-cancer vaccination by flow cytometry-based analysis of neoepitope-specific T cell activation.

Our cancer immunology experts use in silico algorithms based on exome sequencing and HLA typing to predict and select up to 12 eligible neoantigen epitopes individualized for each patient. The selected peptides are predicted to activate not only cytotoxic T cells but also T helper cells. Therefore, in addition to short peptides (8-12 amino acids) potentially binding to HLA class I molecules also long peptides (~17 amino acids) potentially binding to HLA class II molecules are included. To confirm the expression of the selected neoepitopes, transcriptome data by RNA sequencing of the tumor sample is done in parallel. In cases of no availability of RNA, information about neoepitope expression is retrieved from protein expression databases.



## Key Facts of CancerNeo®

- ✕ Whole exome sequencing of tumor/normal tissue using CeGaT's ExomeXtra®
- ✕ Detailed assessment of somatic variants detected in more than 700 tumor-relevant genes and fusions in more than 30 genes
- ✕ Medical report with:
  - Validated list of variants with potential therapeutic relevance
  - Treatment options based on somatic variants
  - TMB determination/MSI prediction/ HRD score calculation
  - Detection of copy number variants (CNVs)
  - A List of all eligible drugs, with EMA and/or FDA approval, for which corresponding biomarkers could be detected in the tumor
  - Determination of pharmacogenetically relevant germline variants affecting the metabolism of certain tumor drugs or anesthetics
  - Assessment of the evidence for CHIP (Clonal Hematopoiesis of Indeterminate Potential)
- ✕ Tumor whole RNA sequencing with rRNA depletion
- ✕ HLA typing
- ✕ Prediction of HLA class I restricted peptide epitopes (neoepitopes) spanning tumor-specific variants from sequencing data
- ✕ Selection of most relevant neoepitopes for HLA class I and HLA class II
- ✕ Second medical report with selected peptides for formulation of a vaccine

### Optional services:

- ✕ RNA-based fusion transcript analysis (CancerFusionRx®) covering over 150 genes for fusion detection and over 120 exon-exon-specific enrichments with known breakpoints
- ✕ Immunohistochemical (IHC) analyses: PD-L1, CAR-T cell panel, HLA class I and class II staining (external)
- ✕ MGMT\* promoter methylation analysis

\* O6-Methylguanine-DNA-Methyltransferase



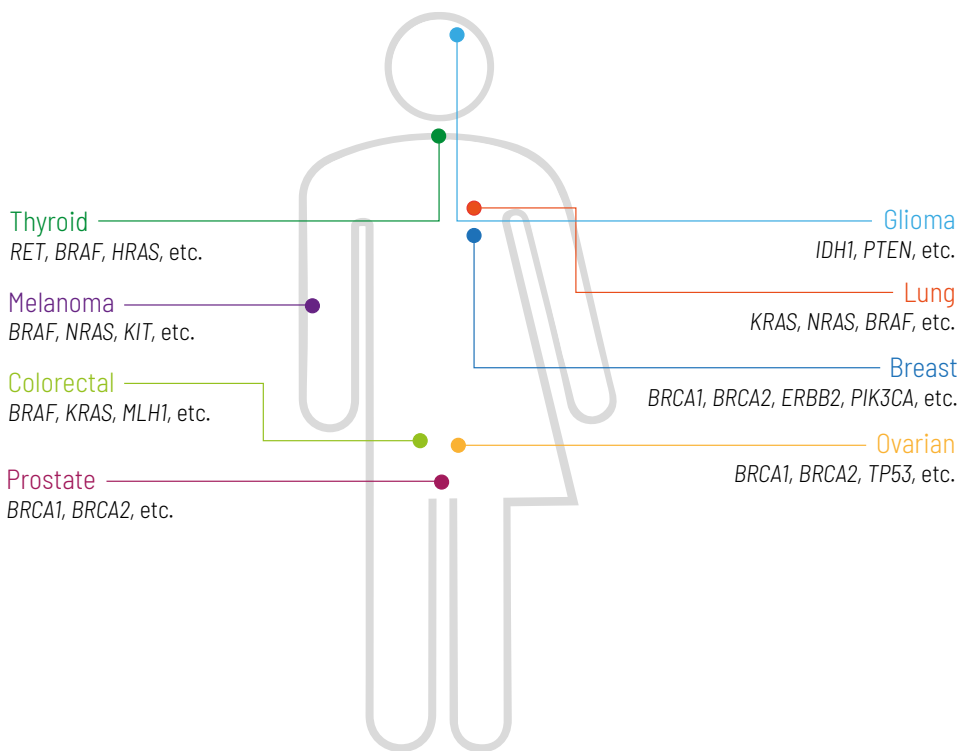
# CancerEssential®

Cost-effective analysis for the identification of actionable variants in solid tumor entities

CancerEssential® has been developed in cooperation with oncologists and pathologists in order to provide a fast, cost-efficient and high-quality analysis of the most important driver mutations within the most common tumor entities. The detection of these specific mutations in tumor tissue has a clear treatment consequence with respect to approved and targeted treatment.

## Key Facts of CancerEssential®

- ✕ Multigene panel testing
- ✕ Analysis of single nucleotide variants (SNVs), selected fusion genes, and microsatellite instability (MSI) status
- ✕ High average sequencing coverage: 1,000x
- ✕ Sensitivity\*: > 99.8%; Specificity: > 99.9%
- ✕ A list of all eligible drugs, with EMA and/or FDA approval, for which corresponding biomarkers could be detected in the tumor



Tumor entities and most common tumor-associated genes.

\* Based on a high-quality sample with 20% tumor content for detecting a somatic heterozygous variant.



## Detailed tumor therapy recommendation

The treatment options for tumor diseases depend on many factors and achieve different results for each patient. Our therapy recommendation is focusing on targeted treatment options related to genetic alterations or molecular pathology markers. Considering the results of our NGS-based somatic tumor diagnostics and detailed therapeutic patient history provided to us, we prepare a comprehensive report that

clearly lists the tumor profile and corresponding targeted treatment options, including off-label therapy options, clinical trials in requested countries, as well as already approved therapies (in-label). We give clinical evidence in a tabular form for the listed off-label therapy options, including detailed information on the trial data, trial settings, biomarkers, drugs, results, and publications.

## CancerAdvice Sample Case

Treatment recommendation for a patient with Astrocytoma, WHO-Grade II, who underwent several cycles of temozolomide and/or radiotherapy. Besides the therapeutic history (not shown below), the recommendation report lists the molecular tumor profile based on CeGaT findings, and other

important biomarkers (A), followed by recommended treatment options (B) and literature evidence (C). The literature evidence summarizes key facts from the corresponding clinical trial / case report including detailed information about setting and outcome.

A)

Tumor Profiling

Tumor Profiling Next Generation Sequencing

Gene	Functional category	Variant	NAF	Effect on protein function
IDH1	missense	c.395G>A; p.Arg132His	0.36	function changed
ATRX	stop_gained	c.1375A>T; p.Lys459*	0.62	inactivating
MYCN	amplification	complete gene, non focal (≥5 copies)	N/A	activating
SETD2	frameshift	c.2756_2757delAG; p.Glu919Valfs*15	0.05	inactivating
TP53	missense	c.742C>T; p.Arg248Trp	0.71	function changed

**NAF:** *Novel allele frequency*, the frequency with which the mutated allele occurs in the sequencing data (1 is 100%). The observed frequencies are influenced by the tumor content as well as copy number alterations and do not directly correlate with the variant's frequency in the tumor. The somatic alterations were classified with respect to their functional effect on protein levels in the following categories: inactivating/activating/function altered, likely inactivating/activating/function altered, unknown and benign (details in the methods section of the sequencing report).

**Remark:** The results shown in this table are based on the patient's sequenced sample. For full details please refer to the corresponding sequencing report from CeGaT listed below in section *Additional Information*.

Tumor Profiling NGS Signatures/Scores

Biomarker	Result	Interpretation
TMB	>5 mut/mb	low
HRD	13	negative
MSI	0.17	negative

TMB: Tumor mutational load, HRD: Homologous recombination deficiency, MSI: Microsatellite instability

Remark: The results shown in this table are based on the patient's sequenced sample. For full details please refer to the corresponding sequencing report from CeGaT listed below in section Additional Information.

Tumor Profiling Immunohistochemistry Analysis / Other Biomarkers

Biomarker	Result	Interpretation	Comment	Analysed at (material)
p53 (IHC)	-	positive	Mutation present	xx.xx.xxxx (FFPE-ID)
IDH1 R132H (IHC)	-	positive	IDH1 mutation present	xx.xx.xxxx (FFPE-ID)
ATRX (IHC)	-	negative	ATRX loss	xx.xx.xxxx (FFPE-ID)
Ki-67/Mib-1	10%	-	-	xx.xx.xxxx (FFPE-ID)
MGMT promoter methylation	31%	positive	Methylated MGMT-promoter	xx.xx.xxxx (FFPE-ID)

B)

## Possible Treatment Options

Option	Biomarker	Study-ID (study name)	Drug	Phase	Localisation
1	<b>IDH1</b> c.395G>A, p.R132H, NAF 36%	NCT04056910	Ivosidenib + Nivolumab	2	USA: Pennsylvania, Pittsburgh
2	<b>IDH1</b> c.395G>A, p.R132H, NAF 36%	NCT05484622	Vorasidenib + Pembrolizumab	1	USA: California: LA, San Francisco; Florida: Miami; Illinois: Chicago; Massachusetts: Boston; New York; North Carolina: Durham; Texas: Huston
3	<b>IDH1</b> c.395G>A, p.R132H, NAF 36%  <b>ATRX</b> c.1375A>T; p.Lys459*, NAF: 62%  <b>ATRX (IHC)</b> negative	NCT03212274	Olaparib	2	USA: California: Costa Mesa, Orange; Connecticut: New Haven; Florida: Coral Gables, Deerfield Beach, Miami, Plantation; Kansas: Fairway, Kansas City, Overland Park, Westwood; Maryland: Baltimore; Massachusetts: Boston; Missouri: Creve Coeur, Kansas City, Lee's Summit, North Kansas City, Saint Louis; New Hampshire: Lebanon; New York; Ohio: Columbus; Oklahoma; Pennsylvania: Pittsburgh; Tennessee: Nashville; Texas: Houston; Wisconsin: Madison
4	<b>IDH1</b> c.395G>A, p.R132H, NAF 36%	NCT05345002	Retifanlimab + All-trans retinoic acid	2	USA: Pennsylvania

## Perspective in Label / Off Label Therapies

Option	Biomarker	Drug Class	Drug	Availability
5	<b>IDH1</b> c.395G>A, p.R132H, NAF 36%	IDH1 Inhibitor	Ivosidenib or Olutasidenib	Off Label

If several drugs of the same option are listed, this is understood to be a combination therapy.

C)

Literature Evidence 6/6		Tumor type	glioma
Literature Evidence 4/6		Tumor type	glioblastoma
Literature Evidence 2/6		Tumor type	glioma
<b>Appendix - Literature Evidence</b> Please note that the references provided may not match the patient's current situation or diagnosis. Results from clinical study data or case reports from patients with other tumor entities whose tumors have the same or similar biomarkers as your patient are also listed below. In addition, the results of clinical studies or case reports can also be listed that have not been stratified by biomarkers, but which could be a therapeutic approach for your patient according to the molecular profile of the patient's tumor. Please note that it may here have been limited to the most important sources of literature and that there is no claim to completeness.		8.2% GBM	resection), with the most months) before the date of therapy and not be in
<b>Literature Evidence 1/6</b> <b>Tumor type</b> glioma <b>Study ID/Name</b> NCT03684811 Phase 1b/2 - <b>cohort:</b> IDH1R132X-mutated glioma that relapsed or progressed on or following standard therapy and had measurable disease - <b>staging:</b> 58% WHO III, WHO IV: 27% - <b>pretreatment:</b> on or following standard therapy; 42% receiving ≥3 prior regimens <b>Setting</b> - <b>drug dosage:</b> olutasidenib, 150 mg orally twice daily (BID) in continuous 28-day cycles - <b>MBI:</b> 88% contrast-enhancing, 12% contrast non-enhancing - <b>cohort size:</b> n=26 - <b>endpoints:</b> The primary endpoints were dose-limiting toxicities (DLTs) (cycle 1) and safety in phase I and objective response rate using the Modified Response Assessment in Neuro-Oncology criteria in phase II. <b>Biomarker</b> IDH1 (function changed) <b>Drug Class/Name</b> IDH1 inhibitor: olutasidenib ORR: 8% PR 6% (2/25) (both enhancing at baseline GBM) SD: 40% (10/25) SD >4 months: 32% (8/25) DCR: 48% CBR: 48% <b>Outcome</b> mPFS: 1.9 months mPFS low grade glioma: 16.9 months (n=4) 12-month PFS rate: 20.8% mOS: 17.2 months 12-month survival rate: 67.9% <b>genetic profile Patients with PR:</b> IDH1 R132H, TP53m, (1x ATRXm) <b>Publication</b> I de la Fuente et al., 2023, PMID: 35639513		oligoma or astrocytoma IO; AG-120) 500 mg QD, y in Cohort 1. ostoperatively, VOR.	) daily in 28-day cycles

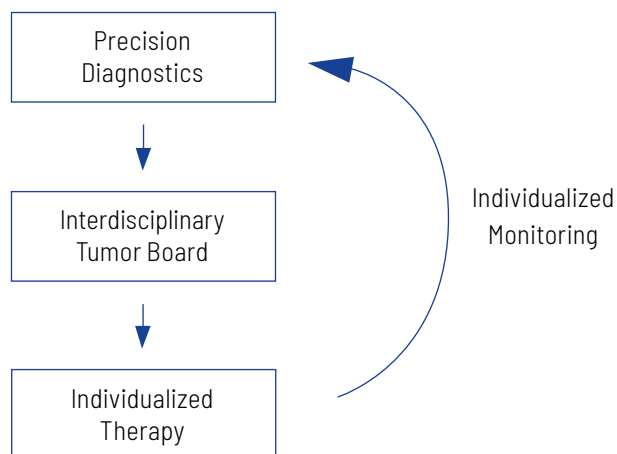
# Integration of Genetic Tumor Diagnostics In Patient Management

Our somatic tumor analyses accompany you and your patients optimally - from diagnosis to monitoring.

Time is most precious in cancer patient treatment. The earlier a cancer is detected and an effective treatment is used, the better the chances for the patient.

Understanding the molecular detail of the tumor as early as possible offers the chance to use an effective treatment at an early stage. In our opinion, the best strategy is to start with a detailed diagnostic approach that includes our CancerPrecision® analysis to address the genetic changes of the tumor.

Then, the results should be discussed in a local interdisciplinary MTB. This board decides on the most promising treatment strategy based on all available information on the tumor and the patient. Finally, this individualized therapy should be applied, and the patient should be monitored for success during treatment. Depending on the individual case, this monitoring should include genetic markers, like our CancerDetect®, to address potential resistance mechanisms or detect recurrences early.



# Sample Requirements and Process Overview

A tumor is altering over time. Thus, we recommend sending us the most recent tumor sample. In some cases, a collection and analysis of more than one sample can be reasonable.

## Our Standard Sample Requirements Are:

### Normal tissue:

- ✗ 1-2 ml EDTA blood (recommended sample type) or
- ✗ Genomic DNA (1-2 µg)

### Tumor tissue: (tumor content at least 20%)

- ✗ FFPE tumor block (min. tissue size 5 x 5 x 5 mm) (recommended sample type) or
- ✗ FFPE tumor tissue slides (min. 10 slices 4-10 µm, tissue size 5 x 5 mm) or
- ✗ Genomic DNA (> 200 ng) or
- ✗ Fresh frozen tumor tissue or
- ✗ 3 x 10 ml cfDNA tubes for liquid biopsy

Other sample material sources are possible on request.

**Please note:** In case of insufficient sample quality or tumor content, the analysis might fail.

## Checklist of Required Materials:

- ✗ Tumor tissue sample
- ✗ Normal tissue sample
- ✗ Completed order form
- ✗ Additional medical information on patient and tumor

If you have more than one option of tumor samples, please get in touch with us (tumor@cegat.com), and we will assist you in choosing the optimal specimen for your patient.

## Process of Examination



Receipt of tumor tissue, normal tissue, and order form



Sequencing of tumor and normal tissue using next-generation sequencing platforms (Illumina NovaSeq)



Detection and annotation of germline and somatic biomarkers through bioinformatic analysis of sequencing data



Analysis and interpretation of mutations by an interdisciplinary team of experts, including human geneticists and oncologists



Summary of results in a comprehensive medical report

### myCeGaT Portal

Order your genetic analyses online in the myCeGaT portal with immediate effect. To do so, scan the QR code or visit [my.cegat.com](https://my.cegat.com)





# Frequently Asked Questions

## **Which tumor entities can be analyzed by CeGaT's CancerPrecision® tumor diagnostic?**

Our comprehensive somatic tumor panel can analyze all tumor entities. CancerPrecision® covers all genes that are known to play a role in tumor development, growth, and survival and might be dysregulated in tumors in general due to mutations. Besides, the composition of CancerPrecision® is regularly updated according to the latest scientific findings to ensure that all relevant cancer-related genes are always analyzed.

## **When is the right time for genetic tumor diagnostics?**

The sooner, the better! The longer the life span of a tumor is, and the more therapies it outlasts, the more resistance mechanisms and survival strategies are developed by the tumor and the more difficult the treatment becomes. Ideally, a comprehensive genetic tumor analysis is performed after the first diagnosis. Knowledge about cancer-specific genetic alterations is crucial for choosing the best treatment.

## **Which tumor tissue should be used for the analysis – the primary tumor or the latest tumor tissue/ tumor tissue of multiple metastases?**

Tumors acquire changes over time and with every therapy. To ensure that the genetic analysis provides the most recent molecular profile of the tumor, we strongly recommend analyzing the latest tumor tissue, e.g., after relapse or progression during or after treatment or new metastases. Thus, the analysis and the subsequent treatment decision made by an oncologist consider possible resistance and escape mechanisms that the tumor might acquire as a result of therapy. Please note that there is always the possibility of analyzing several tumor samples (e.g., Double Best Analysis). For further questions, please contact [tumor@cegat.com](mailto:tumor@cegat.com).

## **What if the latest tumor cannot be removed by surgery?**

In cases where resection of newly grown tumor mass is not possible because of a hard-to-access localization in the body or because a new operation is considered too risky for the patient, we offer an analysis of liquid biopsies (e.g., blood).

In such cases, we ideally recommend the concurrent analysis of the available tumor tissue from original resection and a freshly obtained liquid biopsy sample. This approach enables robust tumor profiling based on the tumor tissue. Furthermore, that offers the chance to obtain a

comprehensive and recent picture of the tumor's genetic profile based on the analysis of the ctDNA. For liquid biopsy analysis, special blood collection tubes are required. Please get in touch with us.

## **Sampling of LB during treatment: The patient wants to perform LB analysis and is currently under treatment. When is the best time to take samples?**

First of all, the sampling should not affect the course of therapy. Since this question is complex and cannot be answered in general, we recommend that you, as the treating physician contact us to discuss this in more detail. Please reach out to us at [tumor@cegat.com](mailto:tumor@cegat.com).

## **My patient had the tumor tested and treated accordingly but got a recurrence. What should I do?**

Optimal treatment of a tumor can require adapting after a certain time. For example, the tumor might be able to adapt to the treatment by finding an escape mechanism. In such a case, a recurrence can happen, and treatment needs to be adapted. Therefore, a new sample should be analyzed to identify the changes that lead to tumor growth during treatment. Then, based on the latest findings, the treatment can be adjusted.

## **Why do you need normal tissue?**

Every human differs from other humans in tens of thousands of germline variants. Every cancer results from genetic changes in the tumor genome (somatic mutations) – cancer is considered a genetic disease. To separate somatic mutations from germline variants the individual germline variants need to be identified. When sequencing only tumor tissue this is done bioinformatically, but has been proven to result in a substantial number of mistakes. When sequencing the matched normal tissue sample (the sample from the same patient as the tumor tissue) the germline variants are directly identified, leaving no room for mistakes.

### When can I expect results?

After sample and payment receipt, our current turn around time (TAT) is 2-3 weeks. We require this time for sample processing in the lab (DNA Isolation, library prep, NGS), analysis of NGS data, and for our diagnostics team to examine the data and write the medical report.

### The samples sent in for analysis failed. What happened?

Our tumor diagnostic service relies on high-quality data. However, sometimes the amounts or quality of tumor DNA is insufficient. In these cases, we request alternative sample material. If no alternative sample material is available, the analysis needs to be cancelled.

Please note: for our tumor diagnostic analyses a tumor content of 20% is a prerequisite. In certain cases like analyzing liquid biopsy or fresh frozen tumor tissue, the tumor content can be just determined after NGS. Samples with tumor content below 20% cannot be used for evaluation, however, we have to invoice the analysis with the full price.

### What about logistics?

CeGaT provides sample collection boxes that contain instructions and the appropriate collection equipment. Also, the boxes can be used for shipment. The sample collection boxes are free of charge and can be ordered by email, mail, or phone.

Depending on your needs, the following collection kits can be ordered from our logistics department:

- ✗ EDTA kit (contains EDTA tube)
- ✗ FFPE kit (contains Slidetrays for FFPE-slides and Bubble wrap for FFPE Block)
- ✗ LB kit (contains Streck® tubes)
- ✗ EDTA + FFPE kit (contains EDTA tube and Slidetrays for FFPE-slides and Bubble wrap for FFPE Block)
- ✗ LB + EDTA kit (contains Streck® tubes and EDTA tube)

### What are the costs for the analysis?

We are happy to provide prices on request. For German customers, we can also provide information regarding reimbursement. Don't hesitate to get in touch with us at [tumor@cegat.com](mailto:tumor@cegat.com).

### References

1. Morganti, S. *et al.* Role of Next-Generation Sequencing Technologies in Personalized Medicine. In *P5 eHealth: An Agenda for the Health Technologies of the Future*, edited by G. Pravettoni & S. Triberti (Springer International Publishing, Cham, 2020), pp. 125–154.
2. Walter, C. *et al.* Sequencing for an interdisciplinary molecular tumor board in patients with advanced breast cancer: experiences from a case series. *Oncotarget* **11**, 3279–3285; 10.18632/oncotarget.27704 (2020).
3. Wu, T.-M. *et al.* Power and Promise of Next-Generation Sequencing in Liquid Biopsies and Cancer Control. *Cancer control : journal of the Moffitt Cancer Center* **27**, 1073274820934805; 10.1177/1073274820934805 (2020).
4. Jones, S. *et al.* Personalized genomic analyses for cancer mutation discovery and interpretation. *Science translational medicine* **7**, 283ra53; 10.1126/scitranslmed.aaa7161. (2015).
5. Sun, J. X. *et al.* A computational approach to distinguish somatic vs. germline origin of genomic alterations from deep sequencing of cancer specimens without a matched normal. *PLoS computational biology* **14**, e1005965; 10.1371/journal.pcbi.1005965 (2018).
6. Nassar, A. H. *et al.* Ancestry-driven recalibration of tumor mutational burden and disparate clinical outcomes in response to immune checkpoint inhibitors. *Cancer cell* **40**, 1161–1172.e5; 10.1016/j.ccell.2022.08.022 (2022).
7. Buchhalter, I. *et al.* Size matters: Dissecting key parameters for panel-based tumor mutational burden analysis. *International journal of cancer* **144**, 848–858; 10.1002/ijc.31878 (2019).
8. Le, D.T. *et al.* PD-1 Blockade in Tumors with Mismatch-Repair Deficiency. *The New England journal of medicine* **372**, 2509–2520 (2015).
9. Reck, M. *et al.* Nivolumab plus ipilimumab versus chemotherapy as first-line treatment in advanced non-small-cell lung cancer with high tumour mutational burden: patient-reported outcomes results from the randomised, open-label, phase III CheckMate 227 trial. *European journal of cancer (Oxford, England : 1990)* **116**, 137–147. (2019).
10. Palmeri, M. *et al.* Real-world application of tumor mutational burden-high (TMB-high) and microsatellite instability (MSI) confirms their utility as immunotherapy biomarkers. *ESMO open* **7**, 100336. (2022).
11. Heeke, A. L. *et al.* Prevalence of Homologous Recombination-Related Gene Mutations Across Multiple Cancer Types. *JCO precision oncology* **2018**; 10.1200/PO.17.00286 (2018).
12. Nguyen, L., W M Martens, J., van Hoeck, A. & Cuppen, E. Pan-cancer landscape of homologous recombination deficiency. *Nature communications* **11**, 5584; 10.1038/s41467-020-19406-4 (2020).

# About Us

**CeGaT is a global provider of genetic analyses for a wide range of medical, research, and pharmaceutical applications.**

Founded in 2009 in Tübingen, Germany, the company combines state-of-the-art sequencing technology with medical expertise – with the aim of identifying the genetic causes of diseases and supporting patient care. For researchers and pharmaceutical companies, CeGaT offers a broad portfolio of sequencing services and tumor analyses. CeGaT generates the data basis for clinical studies and medical innovations and drives science forward with its own insights.

The owner-managed company stands for independence, comprehensive personal customer service, and outstanding quality. CeGaT's laboratory is accredited according to CAP/CLIA, DIN EN ISO 15189, DIN EN ISO/IEC 17025, and thus meets the highest international standards. To obtain first-class results, all processes are carried out in-house under scientific supervision. We would be pleased to provide you with our award-winning service.



CeGaT GmbH  
Paul-Ehrlich-Str. 23  
72076 Tübingen  
Germany

Phone: +49 7071 56544-55  
Fax: +49 7071 56544-56  
Email: [tumor@cegat.com](mailto:tumor@cegat.com)



Accredited by DAKkS according to  
DIN EN ISO 15189:2014



CLIA CERTIFIED ID: 99D2130225