GENETIC TUMOR DIAGNOSTICS
Every patient deserves to receive personalized precision medicine.
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.
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, reduce sensitivity to certain treatments, 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 (Morganti et al., 2020; Walter et al., 2020; Wu et al., 2020).

Tumor progression and increase in genetic diversity

1. Initial mutation
2. Growth
3. Further mutations
4. Metastases
OVERVIEW OF CEGAT’S 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. To choose the right test to be executed, an understanding of the available samples is needed, and, most importantly, the objective for the requested diagnostic is necessary.

Which analysis to choose?

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Solid tumor tissue</th>
<th>Liquid Biopsy</th>
<th>Solid tumor tissue</th>
<th>Solid tumor tissue</th>
<th>Liquid Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scope</strong></td>
<td>749 genes (full coding) and selected structural variants in 33 genes</td>
<td>Whole exome and transcriptome sequencing; HLA class I typing</td>
<td>Disease specific gene sets covering up to 53 genes and selected structural variants in 9 genes</td>
<td>Hot spot regions in 36 genes and TP53 full coding</td>
<td></td>
</tr>
<tr>
<td>Normal tissue comparison</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Detailed drug options list</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Detection threshold (NAF)</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>0.25%</td>
<td></td>
</tr>
<tr>
<td>Input</td>
<td>50ng DNA</td>
<td>50ng DNA</td>
<td>50ng DNA</td>
<td>20ng DNA</td>
<td></td>
</tr>
<tr>
<td>Minimal tumor fraction</td>
<td>20%</td>
<td>20%</td>
<td>20%</td>
<td>1%</td>
<td></td>
</tr>
<tr>
<td>SNV and INDELS</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CNV</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>TMB</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>MSI</td>
<td>✓</td>
<td>✓</td>
<td>✓ (extra order)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>HRD</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Fusion gene analysis / structural variants (DNA-based)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Additional option: RNA-based fusion transcript analysis (CancerFusionRx)</td>
<td>✓</td>
<td>✓</td>
<td>✓ (extra order)</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Neoantigen</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Pharmacogenetics variants (selection)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>CHIP detection</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Representation of biological pathways and coverage profile (Copy number variations)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>
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 100 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) - 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*

*Detection of mosaic variants: new biomarker CHIP (Clonal Hematopoiesis of Indeterminate Potential)*

Optional services: RNA-based fusion transcript analysis from tumor RNA analyzing >100 genes (CancerFusionRx).

For the detailed gene directory visit: [www.cegat.com/cancerprecision](http://www.cegat.com/cancerprecision)
The medical report contains the results from the genetic testing requested by 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).

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### Table: Somatic Variants

<table>
<thead>
<tr>
<th>Variant</th>
<th>Gene</th>
<th>Functional category</th>
<th>NAF</th>
<th>Pharmacogenetics</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.3847_3848delGT; p.His1047Arg</td>
<td>ARID5B</td>
<td>Missense</td>
<td>0.14</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>c.697C&gt;T; p.Gln233*</td>
<td>FGFR1</td>
<td>Complete gene</td>
<td>0.0</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

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*Note: NAF represents the frequency with which the mutated allele was detected in the sequencing data (1 is 100%). The sensitivity of copy number detection depends on the sample's tumor content and the sample's overall quality. Copy numbers are estimated on the basis of the NGS data and should be treated as estimated values. The set of candidate therapeutic relevant genes. There is no evidence for the presence of further strong amplifications or of homozygous deletions of therapeutically relevant genes.
4 Complete list of automatically detected somatic variants and chromosomal aberrations
The table includes all somatic variants (single nucleotide variants and small deletions/insertions ≤ 40bp) 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 for the referring physician 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-intern 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.
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 (Jones et al., 2015; Sun et al., 2018).

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.

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.
Understanding the molecular genetic tumor profile is essential for personalizing the patient's treatment and identifying additional treatment options. The number of markers 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 (HBV/EBV), germline variants (that explain the patient's disease), pharmacogenetic variants with implications on cancer treatments, and possible clonal hematopoiesis of indeterminate potential (CHIP).

<table>
<thead>
<tr>
<th>Tumor tissue &amp; tumor content</th>
<th>Tumor mutational burden (TMB)</th>
<th>Microsatellite instability (MSI)</th>
<th>Homologous recombination deficiency (HRD)</th>
<th>Fusions, structural variants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample of the breast cancer from MM/YYYY</strong></td>
<td>7.6 Var/Mb</td>
<td><strong>No evidence for MSI</strong>&lt;br&gt;Score 0.14</td>
<td><strong>Evidence for a possible HRD</strong>&lt;br&gt;Score 58.0</td>
<td><strong>No evidence for therapeutically relevant structural variants on DNA- and RNA-level</strong></td>
</tr>
<tr>
<td>30% (histologically)&lt;br&gt;25% (bioinformatically)</td>
<td>High ≥ 10</td>
<td>Indication of MSI ≥ 0.33</td>
<td>Indication of HRD ≥ 30</td>
<td></td>
</tr>
<tr>
<td>Required min 20%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor Drivers</th>
<th>Viral infection</th>
<th>Germline variants</th>
<th>Pharmacogenetics</th>
<th>CHIP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Identified tumor driver:</strong>&lt;br&gt;<em>BRCA2, PIK3CA</em>&lt;br&gt;<strong>Relevant genes without oncogenic alterations:</strong>&lt;br&gt;<em>BRCA1, ERBB2</em></td>
<td><strong>No evidence for an infection with HPV/EBV in the tumor sample</strong></td>
<td><strong>Detection of a pathogenic germline variant in gene</strong>&lt;br&gt;<em>BRCA2</em></td>
<td><strong>No evidence for germline variants that are likely to affect drug tolerance</strong></td>
<td><strong>No evidence for CHIP</strong></td>
</tr>
</tbody>
</table>

*CeGaT*
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. 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. This list includes drug classes and names as well as their approval (FDA/EMA) and limiting conditions.

Variants with potential therapeutic relevance:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Functional category</th>
<th>Variant</th>
<th>NAF</th>
<th>Effect on protein function</th>
<th>Therapeutic option for discussion in the MTB</th>
<th>Approved by EMA/FDA</th>
<th>Approved for current entity</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA2 (germline)</td>
<td>frameshift</td>
<td>c.3847_3848delGT;p.Val1283Lysfs*2</td>
<td>het.</td>
<td>loss of the wild-type-allele in tumor</td>
<td>inactivating PARP-Inhibitor</td>
<td>EMA* &amp; FDA*</td>
<td>EMA* &amp; FDA*</td>
</tr>
</tbody>
</table>

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

Sample report: Exemplary for the detected BRCA2 variant and BRCA2 appropriate drugs. Upper Part (A): A section from Table 1 of the report listing variants with therapeutic options. Lower Part (B): Listing drugs (Niraparib and Olaparib are shown in the section only as an example). Besides Niraparib and Olaparib, other drugs (Rucaparib, and Talazorib) are described.

For more details please see sample report
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.

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

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
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 the genes of our panel 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, CeGaTs panel is well above the minimum requirement of 1.5 Mb and ensures a robust estimate of TMB (Buchhalter et al., 2019).

TMB and MSI are reliable biomarkers for predicting immune checkpoint inhibitors efficacy

Le et al., 2015; Reck et al., 2019; Palmeri et al., 2022.

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 technique 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.
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 (Heeke et al., 2018; Nguyen et al., 2020).

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 by working in concert with HRD 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) and
- 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.
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 CeGaT’s 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.
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 neutrophic 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 includes more than 100 genes for novel fusion detection, 85 well-described fusions, and 5 specific transcript variants. 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**
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 main target of Liquid Biopsy 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.

INTEGRATION OF GENETIC TUMOR DIAGNOSTICS IN PATIENT MANAGEMENT

Our two somatic tumor panels 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 the earlier 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, an interdisciplinary MTB discusses all results. Based on all available information on the tumor (and the patient), this board will decide on the most promising treatment strategy. Finally, this individualized therapy is applied, and the patient is 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.
CancerDetect®

Ideally suited for monitoring and follow-up of certain tumor diseases

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.

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 ≥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)

Single marker or hotspot testing

<table>
<thead>
<tr>
<th>Gene</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NRAS</td>
<td>negative</td>
</tr>
<tr>
<td>EGFR</td>
<td>found</td>
</tr>
<tr>
<td>PTEN</td>
<td>negative</td>
</tr>
<tr>
<td>BRAF</td>
<td>negative</td>
</tr>
</tbody>
</table>
Patient and Indication:
- 42 years old, female with metastatic NSCLC
- no tumor biopsy available

Primary Report:
Small panel for lung cancer was negative (ctDNA concentration too low).

CancerDetect® Report:
Performing our CancerDetect® analysis revealed a tumor content of 2% in LB and detected the EGFR T790M mutation. This mutation represents one of the most common resistance mechanisms to Tyrosine Kinase Inhibition (TKI) therapy, often occurring in NSCLC patients after first-line TKI treatment. For this patient, the treatment with third-generation of EGFR Inhibitors was initiated.

Application “Relapse 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.

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.
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 or
- Genomic DNA (1-2 µg)

**Tumor tissue: (tumor content at least 20%)**
- FFPE tumor block (min. tissue size 5x5x5 mm) or
- FFPE tumor tissue slides (min. 10 slices 4-10 µm, tissue size 5x5 mm) or
- Genomic DNA (> 200 ng) or
- Fresh frozen tumor tissue or
- 3x 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

**The order form for CancerPrecision® and CancerDetect® can be downloaded here:**
[www.cegat.com/forms](http://www.cegat.com/forms)

The form can be completed electronically, printed, and signed by the patient as well as by the requesting physician.

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.
Which tumor entities can be analyzed by CeGaT’s Cancer-Precision®-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 standard 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.

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 the treating physician contact us to discuss this in more detail. Please reach out to us at 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
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.