

Sequencing Approaches Driving Your Oncology Research

Whitepaper



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Translational oncology provides an interface between scientific cancer research and its practical applications in the clinical setting. The findings from scientific research help to accelerate and improve the possibilities of diagnoses and therapies for patients. Every tumor is different, as is every patient. Therefore, treatments need to be individualized and adapted to the tumor to achieve the best possible therapeutic outcome for the patient. In translational oncology, science and medicine work closely together to drive oncological research and fight cancer.

Our Translational Oncology Service Category

As every tumor is different, the research possibilities are versatile. Cancer research can have many objectives – and many starting points. To foster as many research objectives as possible, we offer a wide range of products grouped into four service categories:

- ✕ Comprehensive Tumor Profiling (CTP),
- ✕ Focused Tumor Profiling (FTP),
- ✕ Liquid Biopsy, and
- ✕ TS0500.

Comprehensive Tumor Profiling (CTP)

In our Comprehensive Tumor Profiling product category, tumors are analyzed in a broad range. With our CTP Exome products, all protein-coding regions of your tumor sample are analyzed. These regions only comprise 1%-2% of the genome but cover approximately 89% of all known disease-causing mutations. If disease-causing mutations in non-coding regions are additionally of interest, our CTP ExomeXtra® products combine the advantages of whole exome sequencing and whole genome sequencing. Our proprietary ExomeXtra® enrichment is also available for tumor samples, bringing all its benefits to tumor research. These benefits include the detection of disease-relevant variants in non-coding regions, a unique CNV backbone for the genome-wide analysis and high-resolution detection of copy number variations (CNV), and the detection of selected pathogen genomes. Regular updates of our ExomeXtra® enrichment ensure constant improvement based on the latest scientific knowledge. For the most extensive and holistic view of your tumor sample, we can sequence the whole genome of your tumor sample with our CTP Genome products. For all CTP products, we have two options available: a tumor-normal comparison and a tumor-only mode. For the tumor-normal comparison, we require a tumor and a corresponding normal sample. With the tumor-normal comparison, tumor-specific mutations can reliably be identified as only those mutations that are detected in the tumor sample and not in

the normal sample. Tumor-specific mutations can also be identified in the tumor-only mode. Detected mutations are, however, compared to a database and not to a sample-specific normal sample. To provide the most specific results for tumor mutations, we recommend using a tumor-normal comparison, if possible.

Focused Tumor Profiling (FTP)

Our Focused Tumor Profiling product category enables targeted sequencing of selected tumor-relevant genes. We offer different tumor panels with varying numbers of analyzed genes. Our largest tumor panels, with approximately 780 genes, are available with our FTP TUM products. These products comprise a broad range of known tumor-associated genes. Additionally, selected pharmacogenetically-relevant genes, such as CACNA1S and NUDT15, a number of therapy-relevant fusions, and selected tumor-relevant pathogen genomes are covered. With regular updates, we incorporate the latest scientific knowledge and medical findings into our FTP TUM products. As for the CTP products, our FTP TUM products are available as a tumor-normal comparison (FTP TUM TN) and a tumor-only mode (FTP TUM T). Our molecular pathology panel, available as FTP PAT T product, covers over 60 genes highly relevant to tumor disease. Amongst others, common oncogenes, such as EGFR or KRAS, or tumor suppressor genes, such as TP53, PTEN, or BRCA1/2, are included. When fusions are of interest, we offer our FTP FUS T product to analyze more than 200 genes with a focus on fusions. In contrast to the previous tumor products, FTP FUS T is based on the analysis of RNA.

Liquid Biopsy

The blood-based analysis of circulating tumor cells or tumor-derived nucleic acids is known as liquid biopsy analysis. Since only a blood draw is necessary for a liquid biopsy, it is a non-invasive method for cancer detection and monitoring. However, as only a small fraction of the DNA circulating in the bloodstream is derived from the tumor, highly sensitive detection methods are required. To detect and analyze these small fractions of circulating tumor DNA (ctDNA), our Liquid Biopsy product category offers different products. With our LB Target products, single variants can be monitored and analyzed using a droplet digital PCR (ddPCR) approach. In contrast, LB Focus targets 31 selected cancer-associated genes, including TP53, PTEN, KRAS, and EGFR. For a broader view on your liquid biopsy sample, our LB Exploratory product allows for the analysis of 523 genes. It is based on the TruSight™ Oncology 500 Assay.

TS0500

The TruSight™ Oncology 500 Assay is not only available for ctDNA as liquid biopsy product, but also for solid samples. Both products are available in our TS0500 product category. With the TS0500 Assay, developed by Illumina, 523 cancer-relevant genes are analyzed to detect variants, identify fusions, and determine biomarkers.

As everyone has a different approach, background, and starting point, it might not be easy to quickly find the product that best suits your research questions, especially as each product has its own features, strengths, and limitations. To help you identify the product best suited for your research question, we have prepared two tables with key features – one table with analysis features and one table with sample-related features. For each of our products in the four product categories, we have indicated whether the respective analysis is feasible or whether the feature is available.

Analysis Features of Our Translational Oncology Products

One of the most pressing questions when working with tumor samples concerns mutations. Somatic mutations are genetic changes in body cells that occur after conception. Mutations play a pivotal role in cancer development. With almost all our products, we can identify somatic mutations that manifest as small nucleotide variants (SNVs) and indels, and with many products, we can additionally identify copy number variations (CNVs). Depending on the chosen approach, somatic variants are either identified based on a corresponding normal sample in a tumor-normal comparison or in the tumor-only mode based on population genetics. If fusions are the focus of your research, we offer a few dedicated products (see table 1). Please note that the number of genes analyzed to identify somatic mutations differs between the products. For further information, please have a look at the “Sample Features of Our Translational Oncology Products” section.

The determination of biomarkers is relevant in diagnosis, treatment selection, and cancer monitoring. They can also be used to identify patients eligible for clinical trials and stratify patients for the best treatment choice. We offer the determination of the tumor mutational burden (TMB), the microsatellite instability (MSI), and the calculation of the homologous recombination deficiency (HRD) score. The tumor mutational burden specifies the number of mutations in a cancer cell and is given as mutations per megabase (Mut/Mb). The microsatellite instability refers to an instability in short, repeating sequences of DNA throughout the genome, also known as microsatellites. The instability is noticeable as an altered number of repeats in these microsatellites, often caused by a deficiency in the DNA mismatch repair system. If the homologous recombination repair (HR) pathway does not work properly, cells cannot repair double-strand breaks in the DNA adequately, leading to genomic instability. This genomic instability can manifest as loss of heterozygosity (LOH), telomeric allelic imbalance (TAI), and large-scale state transitions (LST). The severity of the genomic instability is given as the HRD score. The biomarkers TMB, MSI, and HRD can be determined for many of our products (see table 1). For more information, we have dedicated articles about [TMB](#), [MSI](#), and [HRD](#) in our Blog.

Several pathogens are known to increase the risk of developing certain cancers. Human papillomaviruses are, for example, a major cause of cervical cancer, while Epstein-Barr virus infections are associated with Burkitt lymphoma, Hodgkin lymphoma, and gastric cancer. To meet this growing research field, some of our products can identify selected pathogen genomes, such as human papillomaviruses (HPV), Epstein-Barr virus (EBV), or human cytomegalovirus (CMV).

Table 1 | Possible analyses available for our products.

		Identify Biomarkers			Identify somatic mutations			Identify selected pathogen genomes associated with cancer
		TMB	MSI	HRD	SNVs & indels	CNVs	Fusions	
Comprehensive Tumor Profiling	CTP Genome TN	✓	✓	✓	✓	✓		
	CTP Genome T	✓	✓	✓	✓	✓		
	CTP ExomeXtra® TN	✓	✓	✓	✓	✓		✓
	CTP ExomeXtra® T	✓	✓		✓	✓		✓
	CTP Exome TN	✓	✓	✓	✓	✓		
	CTP Exome T	✓	✓		✓	✓		
Focused Tumor Profiling	FTP TUM TN	✓	✓	✓	✓	✓		✓
	FTP TUM T	✓	✓		✓	✓		✓
	FTP PAT T				✓			
	FTP FUS T						✓	
Liquid Biopsy	LB Target							
	LB Focus				✓			
	LB Exploratory	✓	✓		✓	✓	✓	
TS0500	TS0500 ctDNA	✓	✓		✓	✓	✓	
	TS0500 Tissue	✓	✓		✓	✓		

Sample Features of Our Translational Oncology Products

Maybe you are limited by the sample material you have available but want to maximize the value of your analysis. To comprehensively analyze and evaluate your tumor sample, we recommend using a tumor and a normal sample for a tumor-normal comparison. However, we also have many options available if you only have a tumor sample. As already indicated, we have a dedicated product category for liquid biopsy samples that meet the special requirements these sample types bring along.

Most of our dedicated tumor products are DNA-based, except our product to detect fusions, which is RNA-based.

One major aspect that comes with the different products is the target size: we can analyze single variants, some selected highly-relevant genes, most tumor-relevant genes, or up to the whole genome of your tumor sample – depending on your research question. If only a few highly relevant tumor-associated genes are pivotal for your research, a targeted tumor profiling might be the most efficient approach. It allows you to focus on what is important for your research without redirecting your attention. For broader views on your tumor sample, we have panels with increasing target size. As mentioned earlier, we can also sequence the tumor’s exome or even the whole genome. Additionally, we can apply our ExomeXtra® sequencing approaches to your tumor sample – with all its advantages like the detection of disease-causing non-coding variants, an improved CNV backbone, and the detection of selected pathogen genomes.

Table 2 | Sample features of our products.

		Comparison of tumor and normal tissue	Analysis of a single tumor sample	Possible as a liquid biopsy	DNA-based approach	RNA-based approach	Target
Comprehensive Tumor Profiling	CTP Genome TN	✓			✓		Genome
	CTP Genome T		✓		✓		Genome
	CTP ExomeXtra® TN	✓			✓		ExomeXtra®
	CTP ExomeXtra® T		✓		✓		ExomeXtra®
	CTP Exome TN	✓			✓		Exome
	CTP Exome T		✓	(✓)	✓		Exome
Focused Tumor Profiling	FTP TUM TN	✓			✓		~ 780 genes
	FTP TUM T		✓	(✓)	✓		~ 780 genes
	FTP PAT T		✓		✓		> 60 genes
	FTP FUS T		✓			✓	> 200 genes
Liquid Biopsy	LB Target		✓	✓	✓		single variants
	LB Focus		✓	✓	✓		21 genes
	LB Exploratory		✓	✓	✓		523 genes
TS0500	TS0500 ctDNA		✓	✓	✓		523 genes
	TS0500		✓		✓		523 genes

Further Relevant Products

By combining your sample's features with your desired analysis option, you can narrow down the tumor products that best suit your research question.

Nevertheless, we also have products that might be beneficial for your project outside of our Translational Oncology service category:

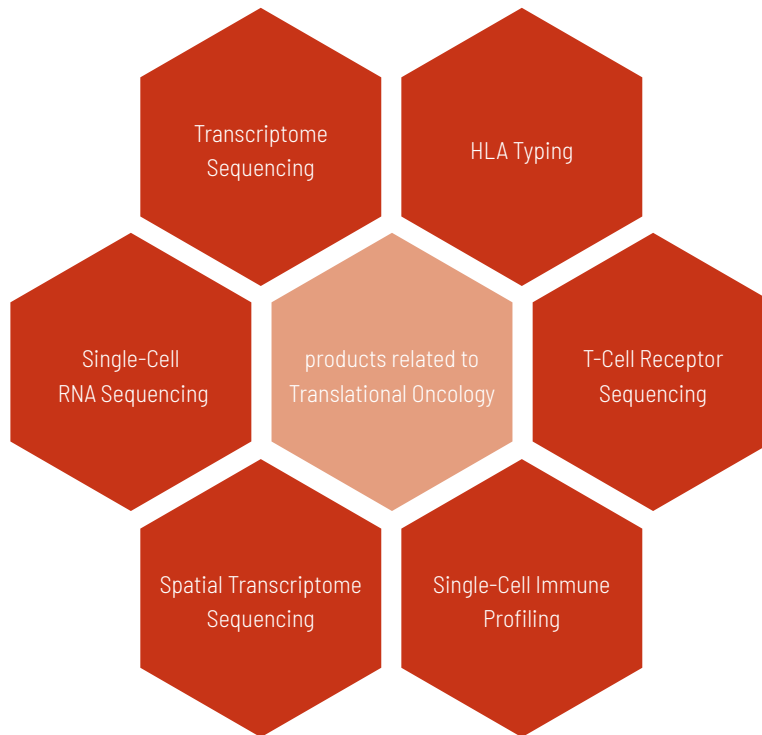


Figure 1 | Additional product categories that can be relevant for translational oncology research.

Most products in our Translational Oncology service category focus on analyzing your tumor's DNA and the subsequent detection of somatic variants and determination of relevant biomarkers. These mutations, however, influence and alter gene expression. Two already mentioned categories of genes are oncogenes and tumor suppressor genes: oncogenes are often upregulated in cancerous cells, while tumor suppressor genes are downregulated. To understand the effect and influence of detected mutations, transcriptome analyses focusing on gene expression can be very helpful. Especially in combination with a healthy tissue sample, the differential gene expression in the tumor cells can give valuable insights into the emergence, development, progression,

and treatment intervention of the tumor. With our Transcriptome Sequencing products, (differential) gene expression can be determined.

If your tumor sample is very heterogeneous, traditional transcriptome sequencing might not be sufficient to account for the tumor's diverse characteristics. With our Single-Cell RNA Sequencing products, the individual transcriptomes from thousands of cells in a single sample can be analyzed. These expression results on single-cell level can, for example, resolve the heterogeneity of your tumor sample and help to identify and understand the interaction of tumor cells, their development, and evolution.

With our Spatial Transcriptome Sequencing products, single-cell sequencing is lifted to a new dimension: The cell's transcriptome is brought into context with its tissue morphology, providing novel, valuable insights into biological architectures and tissue heterogeneity. Especially for the investigation of a tumor's heterogeneity and microenvironment, the combination of gene expression profiling with conventional staining methods and spatial information can provide valuable information on tumor progression, metastasis, and therapy responses.

When focusing on immunotherapies, our Immunology service category might include additional relevant products for your research: from HLA Typing, over T-Cell Receptor Sequencing, to Single-Cell Immune Profiling – our Immunology products can also drive your cancer research by

- ✗ supporting the characterization of an individual's response to drug therapy,
- ✗ providing important information for immunotherapies,
- ✗ enabling monitoring of immunotherapies and interventions,
- ✗ improving personalized medicine,
- ✗ and much more.

The identification and determination of the human leukocyte antigen (HLA) genes are important for cancer research, especially for the development of personalized immunotherapies and the general understanding of how tumors interact with the immune system. The HLA genes play, for example, a pivotal role in the chimeric antigen receptor (CAR) T-cell therapy, but they can also help to predict a patient's response to immunotherapies. Our HLA Typing products are based on exome sequencing results and subsequent bioinformatic analyses to determine the present HLA genes in the sample.

T-Cell Receptor (TCR) Sequencing focuses on the analysis of the T-cell receptor repertoire by identifying and quantifying the different T-cells. With our TCR Sequencing product, we provide information about the diversity, clonality, and changes in the TCR repertoire. With this information, helpful insights into, for example, the tumor heterogeneity, and the potential for personalized immunotherapies can be achieved.

Similar to the single-cell sequencing approach already described, our Single-Cell Immune Profiling products provide a detailed view of the immune cells' expression on single-cell level, combined with the cells' individual T-cell receptor (TCR) or B-cell receptor (BCR) repertoire. Investigating the diversity of immune cells with respect to the TCR and BCR repertoires provides insights into the functioning of our immune system and its response to different conditions, including the emergence, development, and progression of cancer.



About Us

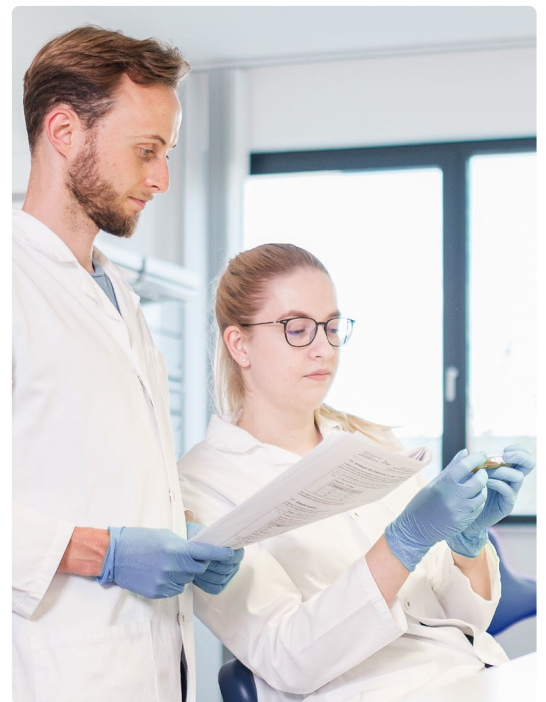
CeGaT was founded in 2009 in Tübingen, Germany. Our scientists are specialized in next-generation sequencing (NGS) for genetic diagnostics, and we also provide a variety of sequencing services for research purposes and pharma solutions. Our sequencing service portfolio is complemented by analyses suited for microbiome, immunology, and translational oncology studies.

Our dedicated project management team of scientists and bioinformaticians works closely with you to develop the best strategy to realize your project. Depending on its scope, we select the most suitable library preparation and conditions on our sequencing platforms.

We would be pleased to provide you with our excellent service.
Contact us today to start planning your next project.



For more details please visit
www.cegat.com/rps



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