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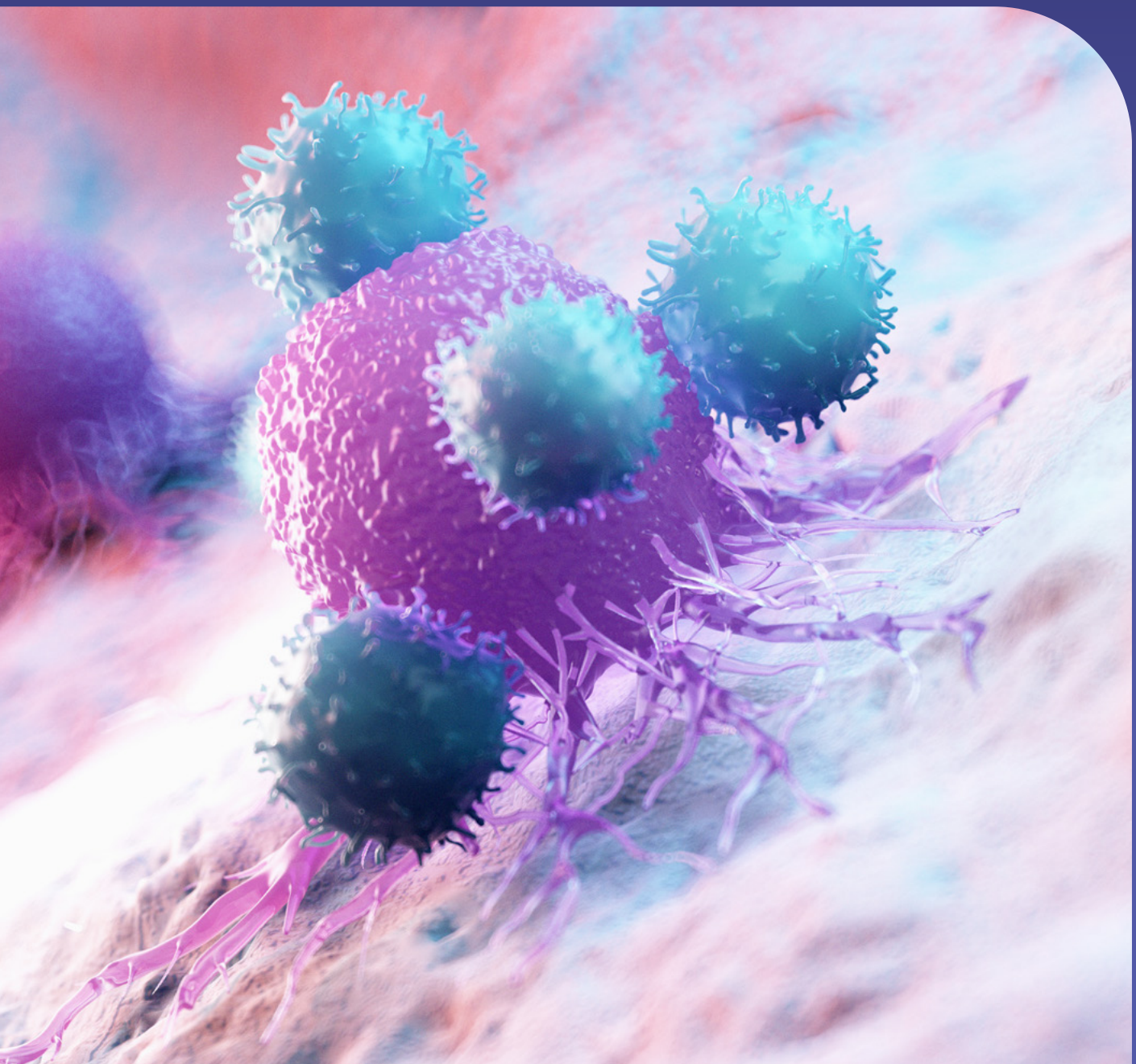
Immuno-oncology clinical trials:

Considerations in managing speciality & central laboratory services for success



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Immuno-oncology: Navigating laboratory testing in the challenging journey from translational research to commercialization

Delivering the right treatment for the right patient at the right time in immuno-oncology requires experienced laboratory expertise. Since the 2011 FDA approval of the first checkpoint inhibitor (CKI) ipilimumab approved for late-stage melanoma, immuno-oncology has gained momentum as a premier therapeutic strategy within precision medicine.

Improvements in patient stratification with increasing clinical efficacy and survival rates may augment the likelihood of regulatory approvals.

With such promise, the number of immuno-oncology trials in the 2020 development stage grew to 4,720, a 233% increase with respect to 2017.[1] The immuno-oncology market is also expected to expand at a compound annual growth rate (CAGR) of 13.2% from 2022 to 2027, with an estimated value reaching USD 8.37 billion by 2027.[2]

In this burgeoning field, integrated clinical laboratory and diagnostic solutions are needed for the challenging journey from translational research to commercialization. Scientific expertise for insightful protocol advice, operational excellence, speed, agility, and the right diagnostic tools must be forthcoming if these therapies are to achieve their full potential. Access to a sizable patient database is also desirable to enable additional early insight through analysis. Furthermore, global multicenter trials require that complex testing must be performed impeccably and in harmony at diverse global locations.

To ensure the best experience and outcomes, sponsors need to partner with specialty central laboratories uniquely experienced in immuno-oncology clinical trial execution. This guide provides insight into the testing capabilities and other laboratory features drug developers should consider when selecting a suitable partner for an immuno-oncology trial.

Overview

- With the ever-expanding possibility for specificity and design, immune-based therapies are pouring into the clinical research funnel
- In immuno-oncology clinical trials, finding the proper resources to achieve your goals can be a challenge
- Three main testing methodologies are needed for immuno-oncology: immunohistochemistry (IHC) for solid tumors, flow cytometry (FCM) for immunophenotyping, and next-generation sequencing (NGS)
- Partnering with a specialty central lab that can help you generate early insights for protocol optimization and can ramp up to commercial scale will enable you to minimize expenditures while keeping timelines intact — resulting in bringing groundbreaking therapies to patients sooner

Did you know...

- Cerba Research has trials in the immuno-oncology field with 80%+ of trials having specialty testing, such as IHC (e.g. programmed- death ligand-1 (PD-L1))?
- Our immuno-oncology trials are phase I/first-in human studied in various solid tumors, such as melanoma, colorectal cancer (CRC), and non-small cell lung cancer (NSCLC).
- We also have experience with CKIs, T lymphocyte stimulants, PD-1 antagonists, anti-CTLA4s and TIGIT protein inhibitors.
- With over 35 years of experience, Cerba Research is a leader in immuno-oncology clinical trials, wielding global solutions including a vast range of biomarker assays.

Immuno-oncology demands comprehensive specialty laboratory solutions that delivers early biological insights

Careful, knowledgeable planning and execution are required for immuno-oncology trials. Healthcare providers and researchers must manage toxicity, demonstrate efficacy, and engage in follow-up activities. At the same time, for a developer, the value of obtaining early biological insights that help identify the right patients, the right treatments, the right dosages — and the right durations to optimize protocols and streamline complex trials — cannot be overstated. At the heart of this activity are biomarkers.

It takes global access to proven laboratories and demographically rich data to drive the development of advanced biomarker strategies that facilitate downstream decisions. Knowledgeable and accessible scientists who are dedicated experts and understand the immuno-oncology space are key. Open communication, transparency, and commitment to one-on-one relationships ensure that each study is regarded as a unique opportunity, from translational research through commercialization.

At Cerba Research, clients engage with us early on for immuno-oncology protocol advice, customizing assays and integrating novel laboratory solutions in their trial to ensure their program reaches their endpoints appropriately and goes to market swiftly.

Cerba Research's access to biobanked human specimens provides a clear advantage when identifying novel and existing immuno-oncology-related pathways, including spatial immuno-oncology IHC simplex and multiplex panels, analysis of the tumor microenvironment, a strong tumor infiltrating lymphocytes (TILs), investigation for tumor mutational burden (TMB) and a vast array of next-generation sequencing (NGS). Such biomarkers can then be developed further to stratify patients into treatment groups, measure treatment efficacy, formulate hypotheses, and hopefully improve the trial's probability of success.

Biomarker strategies continue to advance, increasing precision in immunotherapies

The push and pull between immune activation and dampening of the immune response may determine some cancer outcomes. The results of these complex interactions within the tumor microenvironment and the response to immunotherapies are hard to predict, so measurements of multiple biomarkers are often required.[3] Some therapeutics prove ineffective in as many as 75% of the patients they are expected to help.[4, 5] More approaches must be developed to identify which patients are likely to benefit from any given therapy. The right biomarkers can streamline immunotherapeutic and personalized medicine programs and likely enhance drug development success rates.[6]

Biomarkers are increasing in importance and evolving quickly in Immunotherapeutics to:

- Guide treatment and dose selection
- Characterize mode of action or resistance mechanisms
- Stratify patients and/or determine appropriate inclusion-exclusion criteria
- Likely predict drug safety and efficacy profile
- Provide prognostic value
- Monitor disease longitudinally

Cerba scientists may recommend off-the-shelf biomarkers or propose well-established, research-use-only, or laboratory developed tests which have gone through various levels of fit-for-purpose validation. They can then walk the investigator through the necessary steps, guiding design and customization to provide innovative, flexible solutions. Our large assay portfolio enables targeted approaches or broad immune profiling with multifactorial biomarkers.

The required trio of specialty analysis that supports a constellation of biomarkers in immuno-oncology

A single platform will not suffice for immuno-oncology clinical trials. A complete program that allows for a holistic understanding of patient status and tumor immune susceptibility provides experienced guidance and a customized solution for three types of specialty testing: FCM, IHC, and NGS.

Flow cytometry (FCM)

FCM is a powerful tool capable of rapidly detecting and measuring thousands of cells with high sensitivity and specificity, providing a snapshot of a patient's immune profile. In addition to cell surface markers, FCM can also detect intracellular antigens such as cytokines and phosphorylated signaling proteins. This methodology allows for a functional analysis of the immune system, while likely to help with therapeutic strategies (e.g. predictive value). The simultaneous use of many biomarkers generates data that is multi–faceted, highly complex, and multi-dimensional in nature. Immune profiling by FCM produces a large amount of information from a single blood sample. The result is a granular breakdown, for example, of lymphocytes and various subsets, down to T-cell memory subsets and activated versus nonactivated markers **(Fig. 1).** Clinical researchers can use this technique to gain deeper insight into how patients are responding to immuno-oncology therapies. It enables rigorous immune profiling such as immunophenotyping and helps identify which treatments are most suitable by characterizing key immune cell surface and intracellular markers.

These studies demand highly skilled scientists to develop and appropriately validate both off-the-shelf FCM and exploratory use assays. Therefore, the lead time for assay development to validation must be considered. Further, for global trials, a standard approach is critical, including instrument and assay process standardization, such as globally harmonizing our standard operating procedures (SOPs). You can also read all about FCM method validation protocols as published by our Cerba Research authors.[7]

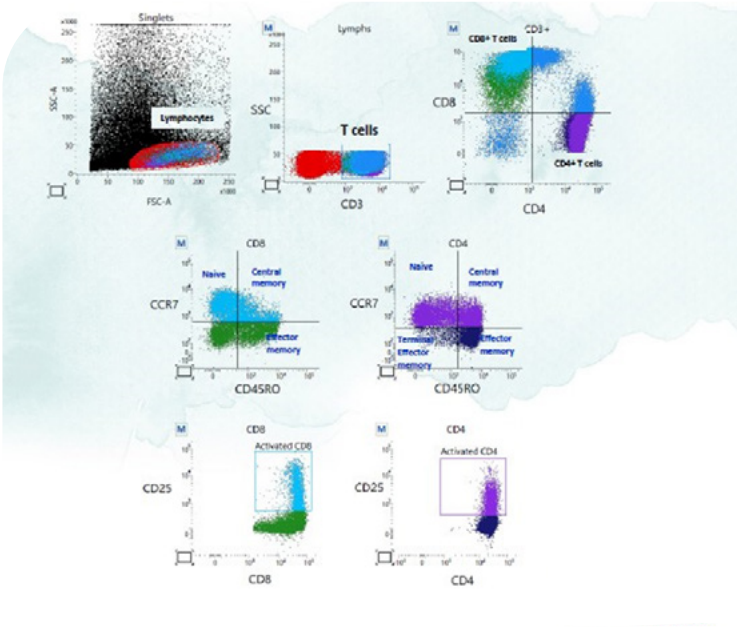

Figure 1 Profiling via 10-color immunophenotyping provides a detailed breakdown of lymphocyte subtypes.

Immunohistochemistry (IHC)

IHC is a cost-effective screening tool that may profile tissue (e.g. formalin-fixed paraffin-embedded tissue or FFPE). It is an antibody-mediated approach that allows detection of the target of interest in tissue through fluorescent or chromogenic revelation for quantification and cellular localization. This technique has been used for the diagnosis and classification of tumors such as multiple myeloma (e.g. Kappa, Lambda) and breast cancer (e.g. HER2, ER, PR). In addition, IHC conveys structural information about the tumor and its microenvironment, demonstrating the localization of immune cells in relation to the tumor or other immune cell populations. It can also reveal the expression of activation/deactivation biomarkers as part of immune cell profiling and oncogene evaluation. Cerba Research offers a vast array of immuno-oncology biomarkers by IHC. Did you know that we have 250+ IHC biomarkers already available? Did you also know that we have PD-L1 stains with multiple clones (22C3, SP142, and SP263) already validated at different levels?

Multiplex IHC, the combination of several biomarkers on a single FFPE slide, is an advanced version that allows for the detection of biomarkers in one precious tissue section. The ability to detect more biomarkers per slide is increasingly important as:

- Demand for more biomarkers is growing
- Biopsy size limits the number of likely sections (e.g. tissue is the issue)
- Some data cannot be obtained from circulating markers, such as spatial context, organization and distances between various cell populations
- PD-L1, an important immuno-oncology biomarker, can only be detected by IHC
- Patient-centric approach: view and review multiple biomarkers on one slide - no need for patients to undergo additional invasive, often painful rebiopsies
- Verify co-expression and spatial organization: view multiple targets within a preserved tissue architecture
- Immune profiling: characterize tumors and identify predictive biomarkers for immuno-oncology response
- Move from pre-clinical to clinical: use IHC to validate targets, select patients, and characterize efficacy and response. This creates an iterative feedback loop where the ability to predict responses from IHC improves as healthcare providers incorporate the data associated with patient outcomes

Available IHC immuno-oncology multiplex panels

Did you know that we have 250+ IHC biomarkers already available for analysis? Did you also know that, apart from PD-L1 simplex, we also have rich immuno-oncology multiplex panels, such as:

- Immuno-oncology tumor microenvironment (CD3/CD8/FoxP3/EP4R/PanCK)
- PD-L1 localization (CD68/PD-L1/PanCK)
- Activated cytotoxic T-cells phenotype (Ki-67/Granzyme B/CD8)
- CKI panel (CD3/CD8/PD-1/PD-L1/Custom)
- Activated T-cell subtypes phenotype (NKp46/CD8/Granzyme B/TCR)

Example: Multiplex IHC for Complex Phenotyping

The utility of multiplex IHC (mIHC) in a Cerba Research immunophenotyping study focused on myeloid-derived suppressor cells (MDSC). These drivers of the tumor microenvironment are immature myeloid cells that protect cancer from the patient's innate and adaptive immune system[8]— and from immunotherapy. They are distinctly heterogeneous, making phenotyping complex.

For complex phenotyping, FCM is a natural choice. However, it will only provide a view of the circulating immune system status at the whole-body level. This may not reflect the populations at the tumor site. For example, a cell population may be abundant, but because of a casein kinase 1 (CK1) activation, cells will stay away from the tumor; on the IHC panel, cells will be present but stop on the same line a few micrometers from the tumor. While FCM can tell you about the abundance, activation, and variations over time of several finely phenotyped populations, only mIHC can give you information about how cells behave at the tumor site level.

In this case, the Cerba IHC team (Cerba Research Montpellier) developed a multiplex IHC panel specifically to investigate the microenvironment of a non-small-cell lung cancer (NSCLC) tumor **(Fig. 2).** The five-plex panel enables the identification of both polymorphonuclear and mononuclear MDSC on a single NSCLC specimen.

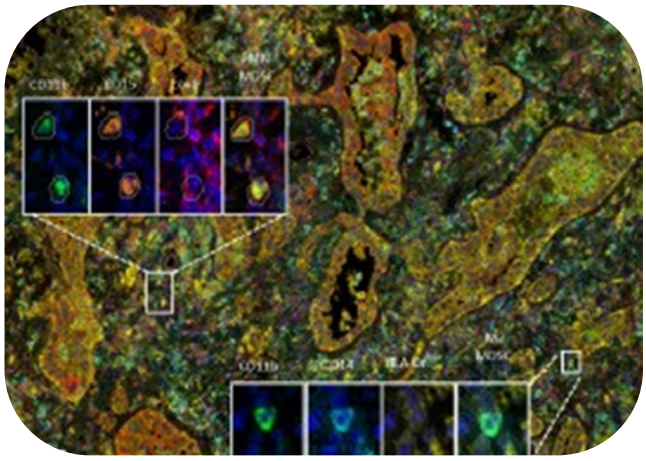

Figure 2 MDSC multiplex IHC panel developed at Cerba Research applied to non- small-cell lung cancer tissue. The high level of phenotyping allows the distinction between subtypes on a single tissue section. Shown: polymorphonuclear (PMN) MDSC and mononuclear (M) MDSC.

Broad panel NGS assays

NGS is a sequencing technology that offers ultra-high throughput, scalability, and speed. The technology can detect millions of small fragments of DNA from the entire genome or from targeted regions. NGS revolutionized oncology trials, allowing central laboratories to perform a wide range of applications with broad panel NGS assays for both solid tumors and hematological malignancies. It can also be used for an in-depth analysis and surveillance of the patient's immune repertoire, including T and B cell receptors (TCR and BCR) sequencing.

Several solid tumor studies have suggested that patients with a higher tumor mutational burden (TMB) experience increased clinical benefit from CKIs. This also suggests that TMB may be a useful biomarker for immuno-oncology response. Also note that to guide the selection of CKIs, NGS methods can provide information about TMB, DNA mismatch repair deficiency (dMMR) and microsatellite instability (MSI).[9]

Cerba Research offers a vast array of broad panel assays capable of detecting gene alterations relevant to immuno-oncology. These assays can also determine important biomarkers such as tumor mutational burden (TMB), microsatellite instability (MSI), and mismatch repair (MMR) status — all of which are often used to predict response to checkpoint inhibitors (CKIs). When multiple genes need to be analyzed, next-generation sequencing (NGS) is especially valuable, as it conserves limited biopsy tissue.

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Check out some of our broad panel NGS offerings already available for use in your immuno-oncology trial from Cerba Research:

- TCR Seq
- BCR Seq
- Cerba OncoSign (638 genes)
- Cerba OncoSign FFPE (45 genes)
- Cerba OncoSign ctDNA (45 genes)
- Cerba Extended Panel for hematological malignancies
- ACTOnco® (Over 400 genes)
- Comprehensive Solid Tumor Molecular Profile (523 genes)
- Liquid Biopsy Molecular Profiling (523 genes)
- and more

Cerba Research's capacity for high throughput, with the ability to sequence 1,000+ whole human genomes in a week — coupled with one of the largest catalogs of clinical NGS broad panel assays — helps ensure that sponsors reach milestones and preserve development timelines, whether they need whole genome sequencing, one of our broad panel NGS assays or customizing a panel to suit their clinical trial.

Immuno-oncology biomarker guidelines against select solid tumor types

Check out the table below that outlines PD-L1, TMB, MMR and MSI as immuno-oncology biomarkers against select solid tumor types where these biomarkers are recommended to be tested by means of the most commonly deployed technics. All this also aligned with what Cerba Research can offer.

As briefly explained above, TMB is an important immuno-oncology biomarker that is now incorporated in various National Comprehensive Cancer Network® (NCCN) treatment guidelines. Indeed, determining TMB is now integrated in the NSCLC, breast, bladder, cervical, colon, head & neck, melanoma, ovarian, pancreatic and prostate cancer treatment guidelines.[10] To guide the selection of CKIs, NGS can provide valuable information about TMB status.

NanoString® for Immuno-onology trials

Cerba Research serves as a reference laboratory for NanoString® and is equipped to perform protein analysis using key immuno-oncology panels. These include the immune cell profiling panel, the immuno-oncology drug target panel, and the immune activation status panel. We can also use the GeoMx immune pathways panel which is designed for targeted profiling of the tumor, tumor microenvironment, and tumor immune status.

We can profile up to 73 RNA targets designed for immuno-oncology research.

Client experience and success hinge on the quality of project management

In immuno-oncology studies, researchers are coordinating many protocols at multiple study sites while acquiring large quantities of data from many samples that require specialty testing in diverse geographical locations. This is an intricate process with the potential for delays at every turn. A laboratory organization that has harmonized SOPs is necessary.

For a successful program and satisfactory experience, project managers (PM) must be positive, approachable, and highly committed to understanding clients' needs and working with them to deliver their projects on time and on budget.

They also require a robust training profile coupled with scientific knowledge. Many of Cerba Research's PMs have M.Sc. or Ph.D. degrees, enabling them to develop and implement the right solutions to achieve the right results.

To meet client timelines, whether for a newly initiated or rescue study, an organization must establish seamless communication among sponsors, investigators, labs, shippers, and suppliers. Organizations must also be flexible. Cerba Research typically requires eight weeks for study startup but can be faster, depending on your trial requirements. Recently, a phase III trial was up complexity and running in less than two weeks in order to meet accelerated timelines.

In addition to our fast study start up when needed, did you know that our experienced PMs are currently executing on more than 25 immuno-oncology trials with monoclonal antibodies, small molecules, antibody-drug conjugates and even prodrugs?

Experienced advice and record keeping ensure proper validation and assist with regulatory submissions

Study designs in immuno-oncology are often adaptive, multiarmed studies; ensuring proper validation across the spectrum of protocol variations is essential. The typical specialty testing always requires detailed sample management, including shipping and associated compliance records, and labeling to maintain sample integrity and chain of custody. Appropriate interim reports are needed for the product to be considered for fast-track FDA approval. A unified global study database that allows easy access to data anywhere on a secure, user-friendly platform is convenient and saves time. Cerba Research's clinical trial database reports are quickly visible, allowing sponsors and study teams to search results and address queries in real-time. Additionally, reliable biobanking/biospecimen management can help with validation and compliance.

Select I/O Biomarkers	Select solid tumor type [1,2]	Most commonly deployed [1,2]	Additional Assay(s) [1]	Cerba Research NGS*	Cerba Research IHC*,**
PD–L1	Biliary, Bladder, Breast, Cervical, Colon, H&N, Kidney, Lung, Melanoma, Prostate	IHC	N/A		✓ (clones 22C3, SP142, SP263, PD–L1 multiplexed)
TMB	Biliary, Breast, Cervical, Colon, H&N, Ovarian, Pancreatic, Prostate	NGS	N/A	✓	
dMMR/MSI–H	Biliary, Bladder, Breast, Cervical, Colon, H&N, Ovarian, Pancreatic, Prostate	NHS, IHC	PCR	✓	✓

PD-L1=A co-regulatory molecule that can be expressed on tumor cells and inhibit T-cell-mediated cell death. T-cells express PD-1, a negative regulator, which binds to ligands including PD-L1 (CD274) or PD-L2 (CD273).[1]

TMB=patients with H-TMB experience increased clinical benefit from CKIs in various solid tumors[2]
dMMR/MSI-H=have been identified as biomarkers of response to CKIs in various solid tumors[2]

1. NCCN guidelines 2023;

2. Yip S et al. Curr Oncol 2019,;26(2):e241.54;+Cerba Research Data In-house mostly available through the ACTOnco®/Cerba France/Cerba US NGS panels or Cerba Montpellier/NY (IHC) *Validation level may vary. IHC=immunohistochemistry, NGS=next-generation sequencing, PDL1= programmed death-ligand 1: TMB=tumor mutational burden: dMMR= mismatch repair-deficient. MSI-H=microsatellite instability-high; H&N=head & neck cancers, HCC=hepatocellular carcinoma.

HCC= There is no established role for microsatellite instability (MSI), mismatch repair (MMR), tumor mutational burden (TMB), or PD-L1 testing in HCC at this time.[1]

Global footprint and standardization enable clients to stay with the same laboratory for later-stage trials

Reaching a global patient base can be challenging. Global, harmonized labs are a necessity to achieve timely, comparable processing of samples in multiple locations, especially when the time comes to scale up. While Cerba Research provides the individual attention ideal for early clinical stages, the company also has the global capacity for larger, later-stage clinical trials. A network of scientific experts spanning five continents ensures that everything from sample preparation to instrument setup through data acquisition and analysis is performed consistently at all sites engaged in that study.

Operational support for immuno-oncology trials depends on experience and commitment

Smooth study execution relies on accurate and timely laboratory and fulfillment services. At Cerba Research, motivated individuals personally committed to improving health via diagnostic services and 35 years of experience handling complex trial logistics equate to fast, dependable turnaround with 24 to 48 hour service, even for remote sites. Custom test kits ensure proper protocol execution across multiple investigator sites and rapid kit manufacturing — reorders taking only five business days. Keeping this function in-house not only speeds up the process, but it also helps guard against shortages or conflicting timelines.

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Cerba Research is a leading specialty laboratory service provider with the capacity and breadth of a global central laboratory network. Our highly qualified scientists provide insight on the latest biomarkers, assays and testing approaches and develop innovative solutions for unique challenges across all research phases, to pharmaceutical, biotechnology, medical device, government, public health, and CRO organizations.

For more information: info@cerbaresearch.com