# THE CURRENT BIOMARKER LANDSCAPE IN IMMUNO-ONCOLOGY

*Dr. El Mustapha Bahassi, Associate Director of Clinical Laboratories at Medpace, highlights the biomarker landscape in immuno-oncology clinical research.*

Despite the recent success of immunotherapy in effectively treating a variety of advanced and metastatic cancers[1], only a fraction of treated patients show durable responses[2]. Notably, several failures have been reported in Phase 3 trials when these therapies are used as a single agent frontline therapy in patients with no prior biomarker-based stratification[3-7]. The first successful wave of immunotherapy included antibodies against the cytotoxic T-lymphocyte–associated antigen 4 (CTLA4), programmed death-ligand 1 (PD-L1) and programmed death-1 (PD-1), but the number of immuno-oncology (I-O) targets is growing and the number of possible combinations, either of agents directed towards these targets or combined with conventional treatments, is rapidly expanding. Therefore, the development of new and efficient predictive biomarkers to guide the choice of agents and combinations is a high priority.

## I. CENTRAL LABORATORIES AND THE EVOLVING WORLD OF IMMUNO-ONCOLOGY

Today's central laboratories must adapt to the quickly evolving world of immunotherapy and respond to the needs of the clinical community by providing biomarkers in both early clinical development and later clinical validation. To this end, central laboratories must not only invest in novel technologies and platforms to support their biomarker development efforts but also bring onboard scientific expertise, including medical, research, and pharmaceutical talent. In addition to an integrated scientific multidisciplinary approach, a successful central laboratory should also have a global reach to facilitate building strong partnerships with the wider pharmaceutical industry and to provide seamless and efficient services.

Medpace Inc. and its wholly owned Medpace Reference Laboratories (MRL) have been proactive in implementing testing capabilities that match the spectrum of immuno-oncology clinical development approaches needed to support pharmaceutical industry partners and have been involved in many I-O programs. A large and specialized team of MDs, PhDs, and pharmacists are engaged early on in study development to design the best possible strategy, one that will increase the chances of success and minimize complications in later clinical development. MRL is currently present in four locations globally (Americas, Europe, and Asia Pacific), allowing a robust cross validation of processes and platforms across labs. This helps maintain a consistent high-quality deployment of assays on a global scale, and provides assurances about data consistency and comparability throughout the entire clinical trial study. MRL has also built a robust logistics infrastructure that allows for smooth and efficient sample management processes.

## II. CURRENT IMMUNO-ONCOLOGY BIOMARKERS AND DETECTION METHODS

The field of I-O biomarker research aims to understand the relationship between the immune system, the tumor, the tumor microenvironment, and the host. The unique interactions of these factors, as well as I-O biomarker presence and prevalence, contributes to the balance of activation versus suppression of the antitumor immune response[8-10]. For each patient, the

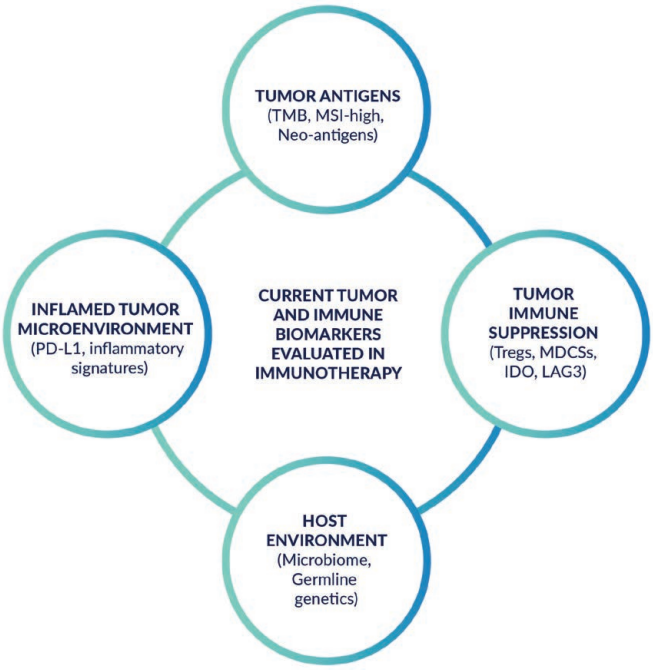
interactions of the immune system, cancer cells, and therapy are complex and unique[10]. Therefore, the goal of I-O biomarker development is to enable a more personalized approach to treatment by identifying patients who are likely to respond to specific immunotherapies[10-12].

## II.1. IMMUNO-PHENOTYPING AND RECEPTOR OCCUPANCY ASSAYS BY FLOW CYTOMETRY

Flow cytometry has become a critical component in I-O studies. The application of flow cytometry for immune characterization includes not only routine T-, B-, and natural killer (NK) cell analysis, but also differential dendritic cell subset activation profiles, monocytes, and ex vivo predictive assays. Ex vivo predictive assays are particularly critical with respect to receptor occupancy when the correlation of the activation profile with the response of the participant to the drug is being examined. MRL is actively engaged in the validation and implementation of biomarkers in I-O clinical trials and has a team of flow cytometry experts, as well as a global cross-site and cross-instrument standardized platforms. The team has forged strong partnerships with major providers to allow high flexibility in panel design. These panels are utilized for immune-phenotyping and receptor occupancy assays to confirm mechanism of action and for biomarker testing early in clinical development.

## II.2. TUMOR MUTATION BURDEN AND MICROSATELLITE INSTABILITY BY NEXT GENERATION SEQUENCING

Despite impressive durable responses, immune checkpoint inhibitors do not provide a long-term benefit to the majority of patients with cancer. Understanding the relationships between genomic markers and patient response and resistance to checkpoint blockade therapy will aid in the identification of novel biomarkers for patient stratification and resistance mechanisms, resulting in more targeted therapy and enhanced benefits for patients with cancer. MRL is fully invested in the emerging area of neoantigens as I-O genomic biomarkers. Neoantigens are newly formed antigens that have not been previously recognized by the immune system. They can arise from altered peptides formed as a result of tumor mutations or viral proteins[13,14]. Neoantigens can be recognized by the immune system as nonself and, as such, can elicit an immune response[15]. Neoantigen-specific T cells have been identified in several human cancers. High tumor mutation burden (TMB) and/or microsatellite instability-high (MSI-H) or mismatch repair deficient (dMMR) status may be associated with increased neoantigen production[16,17]. Tumors with a high burden of neoantigens are more sensitive to immunotherapy, indicating that neoantigens may be a potential I-O biomarker. As immunogenic neoantigens can be challenging to identify directly, TMB may potentially be used as a surrogate to indirectly assess neoantigen load[18,19]. In support of this testing, MRL has acquired both Next Generation Sequencing (NGS) platforms and is building bioinformatics expertise to respond to and support this testing. Other biomarkers can be tested using NGS, including inflammatory or other gene related signatures and immune repertoire and antigen/self-recognition, which involves measuring VDJ rearrangements in immunoglobulin (Ig) heavy chains and human leukocyte antigen (HLA), respectively.




**Tumor and immune biomarkers being evaluated to predict better outcomes to immunotherapy**

(TMB= tumor mutational burden; MSI-high=microsatellite instability high; PD-L1= programmed death ligand 1; Tregs= regulatory T cells; IDO=indoleamine-2,3 dioxygenase; LAG-3= lymphocyte activation gene-3)

## II.3. IMMUNE CHECKPOINT SURFACE MARKERS BY IMMUNOHISTOCHEMISTRY

Immune checkpoints are important regulators in the maintenance of immune homeostasis and prevention of autoimmunity. They consist of inhibitory and stimulatory pathways that maintain self-tolerance and assist with immune response. In cancer, immune checkpoint pathways are often activated to inhibit the nascent anti-tumor immune response. Immune checkpoint therapies act by blocking or stimulating these pathways to enhance the body's immunological activity against tumors. CTLA-4, PD-1, and PD-L1 are the most widely studied and recognized inhibitory checkpoint pathways. Drugs blocking these pathways are currently utilized for a wide variety of malignancies, and have demonstrated durable clinical activities in a subset of cancer patients. MRL has forged a strategic partnership with an outside laboratory with pathology expertise, which is integrated within Medpace networks to provide immunohistochemistry (IHC) support to its clients seamlessly and with the same level of analytical rigor found in MRL. These IHC biomarker assays for respective PD-1/PD-L1 inhibitors are designed to screen for the presence of specific PD-1/PD-L1 epitopes, as well as to estimate the percentage of T cells or tumor cells expressing this receptor or ligand.

## II.4. CYTOKINE RELEASE BY MSD AND CAR PERSISTENCE BY QPCR

The emergence of chimeric antigen receptor (CAR)-T cell approaches, initially using T cells engineered to identify and eliminate $CD19^{+}$ lymphocytes in hematological malignancies, has required a different set of biomarkers, which necessitates a robust and efficient validation process. These biomarkers primarily involve the measurement of cytokines produced during cytokine release syndrome and the detection of persistent CAR molecules in the circulation of treated patients. In support of CAR-T cell therapy, MRL provides cytokine release syndrome measurement by ELISA and MSD. Several qPCR platforms are also available to measure CAR persistence in circulation, not only at baseline but also dynamically over time.

## III. CONCLUSIONS

To date, PD-L1 protein expression is the only validated I-O biomarker. Unfortunately, the correlation between identifying the level of PD-L1 protein in a tumor as a way of confirming a druggable target and using that information to identify potential responders to treatment is not nearly as strong as it would be, for example, in the context of a traditional targeted therapy.

The level of genetic instability within a tumor, whose measurement may correlate with the degree of lymphocytic infiltration, is the next chapter in the I-O biomarker story. In late November, an I-O drug maker filed for a US regulatory approval using a biomarker other than PD-L1. The FDA granted the firm a priority review for a supplemental Biologics License Application for the treatment of previously treated patients with advanced microsatellite instability-high (MSI-H) cancer.

Ultimately, efforts to contextualize genomic correlates of response into the larger understanding of tumor immune biology will build a foundation for the development of novel biomarkers and therapies able to overcome resistance to checkpoint blockade. Combining mutational burden information with a gene expression profile (presumably with PD-L1 as a component) could improve the predictive power of I-O biomarkers.

Many proteomic and genomic approaches are being evaluated in a variety of tumor types and with various immunotherapeutic agents. Having supported oncology drug development from preclinical studies to clinical trials, and post-market introduction of the therapy and associated diagnostics, global contract research organizations, like Medpace, have a unique perspective on the novel developments in the field of I-O. A large number of novel biomarkers are still needed to support I-O therapy trials, and central laboratories are posed to play a pivotal role in the development and commercialization of these I-O therapies.

## IV. REFERENCES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