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White Paper

Biomarker and Bioanalytical Assays for Immuno-oncology Drug Development

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Introduction:

Cancer has long been considered a cell cycle disease where mutations in tumors that control DNA replication, cell cycle proteins, and DNA repair mechanisms lead to uncontrolled cell growth.[1] Over the last decade, however, there has been a paradigm change in cancer research, emphasizing a tumor's interaction with the body's immune system. Specifically, mutations in cancerous cells facilitate a shift of the immune system's balance between recognition of self and non-self antigens, to evade the immune system and continue to grow.[2] This has led to the development of a new class of cancer therapies, termed immuno-oncology or immunotherapy, that specifically hinders immune effector evasion by cancers, reinvigorating or potentially expanding anticancer immune responses. Moreover, clinical treatments previously thought to be unresponsive to standard cancer therapies have shown lasting responses to immunotherapy agents.[3]

What is Immunotherapy?

Immunotherapy involves strengthening or stimulating the immune system's ability to recognize a tumor or providing a missing immune effector function. All innate and adaptive immune mechanisms of the body's immune system have been shown to participate in tumor recognition and control. NK cells recognize unique ligands on the first tumor cells and macrophages, and dendritic cells process their antigens and release cytokines to attract T cells and B cells.[4] This stimulation of the adaptive immune system initiates a robust cytokine response, and production of antibodies leads to the elimination of tumor cells and immune memory to specific antigens.

Cancer cells have however adapted to evade this immune response. If tumor cells are not eliminated, remaining cells continue to mutate to generate new antigens that are not detected by previous immune responses. Also, tumors take advantage of inhibitory T cell signals intended to protect the body from recognizing self-antigens. As shown in Figure 1, many tumor cells upregulate antigens such as programmed death ligand 1 (PD-L1) and B7. These ligands bind to corresponding receptors on T cells (PD-1 and CTLA-4) which inhibit cytokine responses and adaptive immune responses of T cells allowing the tumor cells to evade immune system detection. Although both are immune checkpoints, CTLA-4 downregulates the early activation of naïve and memory T cells, while PD-1 limits the activity of T cells in the periphery during an inflammatory response.

Immunotherapy aims to stimulate the immune system to respond to cancer cell adaptations or to gain new responses. There are several forms of immunotherapy categorized into passive and active therapy.

Key Concepts:

– 1.6 million Americans are expected to be newly diagnosed with cancer this year

– Over 240 immunotherapy drugs are in development that alter the body's own immune system to target tumor cells.

– Robust quantitation of biomarkers in a regulated bioanalytical environment for immunotherapy drug development studies is crucial for understanding the mechanism of action of new drugs, profiling immune response and efficacy to therapy, and stratifying subjects for studies.

– Multiple types of assays including cell sorting, flow cytometry, multiplexed biomarkers, and gene expression, are necessary for regulatory approval of a new immunotherapy candidate.

The first passive treatment used the infusion of effector cells such as cytotoxic T cells, NK cells, or IL-2 and INF-α cytokines for indications of malignant melanoma and renal cell carcinoma.

The second generation of passive immunotherapy, immune checkpoint inhibitor antibodies, bind to PD-L1, PD-1, or CTLA-4 and prevent the inhibitory signals decreasing tumor killing (Figure 2). These antibodies lead to an increase in cytokines that promote tumor killing. Several of these antibodies are FDA approved for melanoma and lung cancer and continue to be studied for other cancers. Even newer immunotherapies initiate active responses by removing and modifying T cells or developing vaccines to recognize patient tumor-specific antigens. Regardless of the modality of treatment, bioanalytical assays and biomarkers characterizing the immune system responses are necessary during development of these therapies to determine their mechanism of action (MOA), measure their efficacy, and identify patients who would benefit from the treatment.

Clinical Drug Development of Immunotherapies

Immunotherapy of cancer is expected to become increasingly utilized as the standard of care alongside traditional approaches such as chemotherapy or radiotherapy. The U.S. FDA has recently approved the first immunotherapy agents.[5] The currently approved therapies are Alemtuzumab (anti-CD52), Atezolizumab (anti-PD-L1), Avelumab (anti-PD-L1), Ipilimumab (anti-CTLA-4), Ofatumumab (anti-CD20), Nivolumab (anti-PD-1), Pembrolizumab (anti-PD-1), Rituximab (anti-CD20), and Durvalumab (anti-PD-L1). Furthermore, it's estimated that more than 240 immunotherapy medicines and vaccines are in development with over 1300 clinical studies in progress (Table 1).

Table 1. Classification of Current Clinical Studies for Immunotherapy Drugs

Database Details[a]	Phase 1	Phase 2[c]	Phase 3[d]
Studies	611	641	67
Completed	505	53	4
In progress	62	548	55
Suspended/Terminated	44	41	9
Drugs[b]			
Small Molecule	231	286	49
Oral Formulation	217	273	36
Biological Therapeutic	555	604	60
Monoclonal Antibody	413	493	51
Vaccine	40	105	5
Radiation Therapy	28	63	6
Peptide	19	31	2
Oligonucleotide	14	16	1
Diagnostic	10	14	1
Antibody conjugated	7	10	0

a. Data obtained from Clarivate Analytics Database search, Immuno-oncology and clinical development, 02-Nov-2018

b. Drugs may have more than one molecular type description,

c. Includes Phase 1/2 studies

d. Includes Phase 2/3 includes

However, challenges exist for the development of these therapies since they rely on personalized immune responses that can vary from individual to individual and the mechanism of their action is often unknown. This necessitates the need to develop tools to identify patients who can benefit from a particular form of immunotherapy treatment.[6–8] Bioanalytical methods such as biomarker assays, cell-based assays, flow cytometry, gene expression, and immune response assays which are outside of the typical pharmacokinetic (PK) assays used for most drug approvals need to be developed and validated. Also, biomarkers used to determine and quantitate the mechanism of action and efficacy of these drugs continue to be discovered. As immuno-oncology drugs currently on the market are expanded for more cancers, new targets and drugs are developed, and these agents are combined with existing chemotherapy agents, the need for these assays will continue to grow (Figure 3). Below, we describe the critical biomarkers and bioanalytical methods required for these agents and considerations required for conducting these assays in a regulated environment.

Figure 1. Proposed mechanism of immune suppression by tumor cells

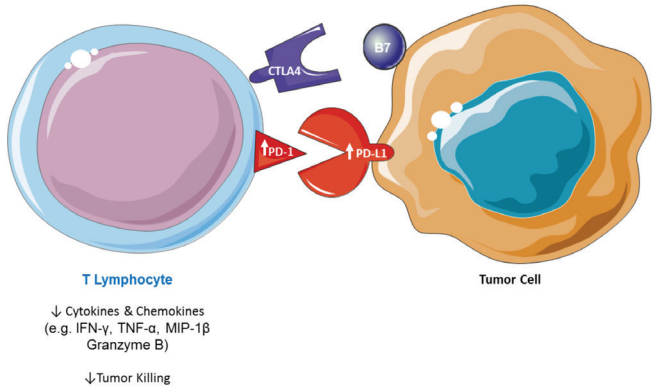
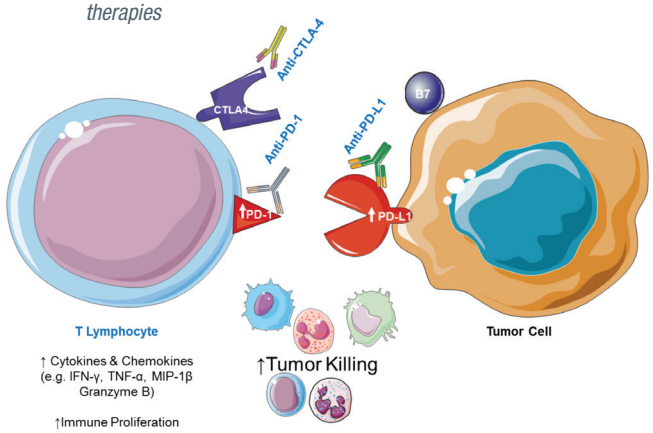


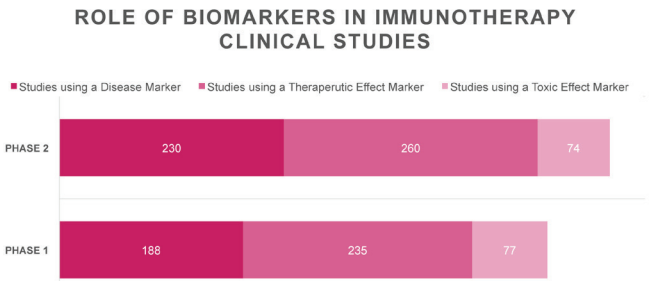
Figure 2. Proposed mechanism of action of current antibody immunotherapy therapies



Biomarkers and Bioanalytical Assays for Immunotherapy

The primary goal of immunotherapy now is to use precision medicine to determine which drug will show the best efficacy for the individual patient and their specific cancer. Immunologic biomarkers spanning DNA, cells, cytokine responses, and tissues characterizing mechanism, efficacy, and patient stratification must be measured during trials. These biomarkers then help elucidate the target population of a drug and whether modifications in its formulation or mechanism are necessary for other populations. This decision making requires robust bioanalytical assays using multiple technologies and modalities that are properly validated with consideration for analyte stability, reference material, controls, specificity, and biological variability in the context of cancer.

Figure 3. Number of Immunotherapy Studies using a Biomarker by Phase and Biomarker Role



Data obtained from Cortellis database, Clarivate Analytics search, Immuno-oncology and clinical development, 02-Nov-2018

Immunologically responsive tumors that express the checkpoint ligands are more likely to respond to checkpoint blockade antibody therapies.[9] Therefore, it is essential to perform biomarker studies to characterize different classes of tumors and provide guidance for therapeutic strategies by measuring CTLA-4, PD-1, and PD-L1 from tumor sites. To then characterize the mechanism and immune response produced by these therapeutics, cytokines and chemokines must be determined in serum, intracellularly, and in tissue. The most common cytokines measured include macrophage inflammatory protein-1β (MIP-1β, CCL4), TNF-α, IFN-γ, and IL-2. As shown in Figure 4, these cytokines are altered in melanoma and other cancers when measured in serum. In some patients, prior interventions to achieve local, productive inflammation in the tumor microenvironment are required in combination with checkpoint blockade antibodies to enhance the clinical response for immunologically-ignorant tumors. These inflammatory stimulants also require measurement of biomarker cytokine responses.

The biomarkers that will require quantitation during immunotherapy trials are expected to increase as attempts are made to widen the subsets of patients eligible for immunotherapy. For example, biomarkers that indicate a favorable response to anti-CTLA-4 therapy include ICOS which is expressed on the cell surface of activated T cells and was shown to increase in a dose-dependent manner in patients with treatment with ipilimumab. Other biomarkers such as lactate dehydrogenase (LDH), C-reactive protein (CRP), vascular endothelial growth factor (VEGF), and GM-CSF are associated with improved clinical outcomes in patients treated with anti-PD-1 and anti-CTLA-4 therapy.

Biomarkers that quantitate safety and toxicity are also becoming increasingly important. Despite the remarkable success of checkpoint blockade therapies, the side effects of stimulating the immune system have proven challenging in many populations. Immunoassays and novel biomarkers based on genome sequencing are being developed to anticipate the personalized responses to these therapies.

Progress to identify and validate biomarker candidates has also been limited by the use of unstandardized assays and variable results. Since immunotherapy studies require measuring changes in biomarkers that involve DNA, cells, cell surface receptors, and tissue, many assay platforms must be used which together provide a comprehensive understanding of a drug's mechanism and efficacy. The most common technology, its applications, and specific biomarkers measured are described in Table 2. Proper consideration must be given to reference material, quality controls, analyte and sample collection and stability, assay specificity, and biological variability.

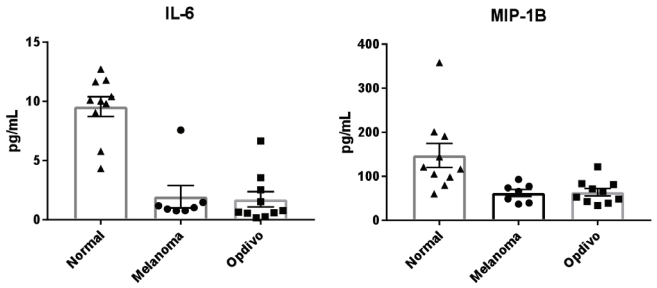
Using Cell Sorting to Obtain Enriched Samples

Analysis of tumor and immune cells is necessary in development of an immunotherapy to determine if the patient is a candidate for the therapy and if they are responding to treatment. This includes analysis of tumor mutational burden, check point inhibitor and tumor antigen status.[10,11] However, obtaining tissue biopsies for studies is often times difficult and whole blood analysis may not detect rare tumor cells. Therefore, cell sorting is an effective method to detect and enrich for circulating tumor cells for downstream experiments. These methods are highly sensitive and can isolate as few as 3 tumor cells in 7.5 mL of blood with greater than 99% purity (Figure 5).

Table 2. Bioanalytical assays and biomarkers necessary for immunotherapy drug development

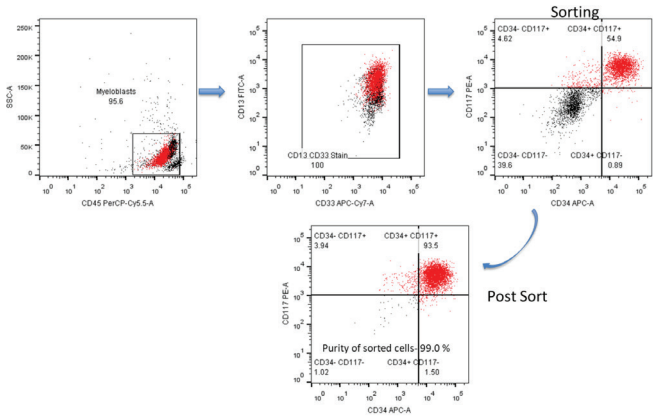
Assay	Biomarkers	Purpose	Matrices
ELISA	Anti-PD-1, Anti-PD-L1, etc.	Antibody Titer (PK) Primary Endpoint	Serum and plasma
ELISA	cytokines, chemokines	Secondary Endpoint/ MOA	Serum and plasma
ELISA	PD-1, PD-L1, CTLA-4, other targets	Patient Stratification	Serum and plasma
Flow Cytometry	CD4+, ICOS+ T cells, CD40, CTLA-4, Circulating tumor cells, CD22, B and T lymphocyte attenuator, CD37, B-raf	Secondary Endpoint/ MOA	PBMCs, whole blood, cells from ascites and pleural effusions
ELISPOT	IFN-γ, granzyme B	Secondary Endpoint/ MOA	PBMCs
qPCR, microarray	STAT4, TBX21, MAGE-A3, chemokines, neoantigen signature	Secondary Endpoint/ MOA, Patient Stratification	DNA and RNA from tumor, lymph nodes, and PBMCs

Figure 4. Serum concentrations of cytokine biomarkers in melanoma patients after treatment with nivolumab (Opdivo™)



Data for demonstration purposes only. Not determined from a clinical study.

Figure 5. Sorting of cells allows highly pure isolation of rare tumor cells and immune response profiling



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