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Immune Checkpoint Therapy and the Search for Predictive Biomarkers

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Abstract

Immune checkpoint therapy has started a revolution in the field of oncology. The concept that the immune system plays a critical role in anti-tumor responses, which has been around for decades, has finally been proven and firmly established with elegant preclinical studies and dramatic clinical responses in patients as a result of antibodies that block inhibitory T cell pathways. However, the clinical responses being achieved are only in a subset of patients and more work is needed to provide a better understanding of the mechanisms that elicit tumor rejection, which will enable identification of appropriate biomarkers, reveal new targets, provide data to guide combination studies and eventually dictate a platform that allow more patients to derive clinical benefit, including cures, with immune checkpoint therapy.

Introduction

The promise of targeting the immune system to treat cancer is finally being realized with immune checkpoint therapy (1, 2). However, biomarkers to select which patients will respond to therapy remain elusive. In contrast, the era of genomic medicine led to the identification of driver mutations, such as BRAF, which led to the development of drugs to target BRAF mutations and even combination therapies to target BRAF and downstream resistance mechanisms such as MEK (3, 4). These studies led to significant clinical responses and established the driver mutation, for example BRAF, as the predictive biomarker by which to choose patients for treatment. The clinical success of these genomically-targeted agents laid the foundation for other cancer therapies, including the prerequisite to identify predictive biomarkers for selection of patients for treatment. However, the framework used for identification of predictive biomarkers for genomically-targeted agents presents a challenge for immunotherapy. As opposed to mutated genes in tumors that remain constant, the immune response is dynamic and changes rapidly. Therefore, the issues facing the field of cancer immunotherapy is not how to identify a single biomarker to select only a few patients for treatment but, how to measure an evolving immune response, recognize the immune response that contributes to clinical benefit and drive every patient's immune response in that direction through combination therapies. The specificity, adaptability and memory response that are inherent to the immune system gives

us the opportunity to measure multiple components, not just a single biomarker, that can be targeted over time to provide curative treatments for many patients.

Definition of Biomarkers

Biomarker studies are undoubtedly important to understand biologic processes that contribute to clinical outcomes (benefit and/or toxicities) as a result of therapy (5). Pharmacodynamic biomarkers provide information regarding whether an agent has “hit its target”. These biomarkers can be used to determine dose, frequency and possible combination strategies for a particular therapy. Prognostic biomarkers provide information regarding clinical outcome. These biomarkers are useful for informing patients about risk of recurrence or median survival. These biomarkers can also be used for prospective stratification of clinical trials. Predictive biomarkers are those that can accurately predict a response to a give treatment. Surrogate biomarkers are those that may be used as a substitute for a clinically meaningful endpoint. There are intense investigations ongoing to identify appropriate biomarkers for immune checkpoint therapy.

The soldiers of anti-tumor immune responses: T cells

T cell responses are known to play a significant role in tumor rejection. In human tumors, infiltrating T cells were shown to correlate with improved survival outcomes for patients (6–8) and first-generation immunotherapy studies were based on the concept of trying to turn T cell responses “on”. In 1982, the T cell receptor protein structure was identified (9), which enabled subsequent work (10) demonstrating that T cell activation requires interaction between T cell receptor and foreign antigen bound to major-histocompatibility complex (MHC) expressed on antigen-presenting cells (APCs). Initially, vaccines comprised of tumor-associated antigens that could bind to MHC and interact with T cell receptor were tested in clinical trials to stimulate an anti-tumor immune response. However, these trials did not provide significant clinical benefit (11). Although T cells require T cell receptor stimulation as the first signal to begin the process of T cell activation or turning “on” of T cell responses, it was not sufficient to drive the process to completion. In the early 1990s, it was shown that productive T cell activation requires CD28 co-stimulation (12). Therefore, to turn T cell responses “on”, two signals were needed: signal 1 via the T cell receptor interaction with MHC bound to antigen and signal 2 via interaction between CD28 molecules expressed on T cells and B7 expressed on APCs. Tumor cells inherently lack expression of B7 molecules, which may explain why tumor cells escape the immune response. However, T cells that are activated via appropriate interaction with APCs may not require further activation signals when encountering tumor cells. The knowledge that T cell activation requires T cell receptor signaling and CD28 co-stimulation provided a critical piece of information for understanding T cell responses.

However, subsequent research revealed that T cell responses are tightly regulated. In July 1994, two scientists, Drs. Jim Allison and Jeff Bluestone, independently presented data at a Gordon Research Conference demonstrating that a molecule known as CTLA-4 (cytotoxic lymphocyte antigen-4), which was expressed after T-cells had been activated or turned “on”, acted as an inhibitory molecule to turn T-cell responses “off” (13, 14). The data was very

controversial since the field viewed T-cells as requiring “on” signals provided by T-cell receptor and CD28 co-stimulation but had not conceived of the idea that the “on” signals eventually led to upregulation of an “off” signal, which meant that the vaccines used to turn on T-cell immune responses inevitably induced the “off” signal as well. In July 1995, at the International Congress of Immunology meeting, Dr. Allison went on to present data showing that CTLA-4 was an inhibitory immune checkpoint that could be blocked with a monoclonal antibody to enhance anti-tumor immune responses and tumor rejection in murine tumor models. These data were published (15) and Dr. Allison’s novel concept of blockade of an inhibitory immune checkpoint pathway to unleash the immune system became a paradigm shift in cancer therapy.

Immune checkpoint therapy

Clinical trials with anti-CTLA-4 demonstrated tumor regression in patients regardless of tumor types, with Phase I/II trials indicating clinical responses in patients with melanoma, renal cell carcinoma, prostate cancer, urothelial carcinoma, and ovarian cancer (16). Two Phase III clinical trials with anti-CTLA-4 (ipilimumab, BMS) were conducted in patients with advanced melanoma and demonstrated improved overall survival for patients treated with ipilimumab (17, 18). Importantly, these trials indicated long-term durable responses with greater than 20% of treated patients living for more than 4 years, including a recent analysis indicating survival of 10 years or more for a subset of patients (19). The FDA approved ipilimumab as treatment for patients with melanoma in 2011.

The clinical success of anti-CTLA-4 opened a new field termed “immune checkpoint therapy” (1, 2). Additional pathways that regulate T cell responses are being targeted as cancer treatments. One such pathway is PD-1 (programmed death 1) and its ligand PD-L1. Similar to CTLA-4, PD-1 is expressed on activated T cells; however, unlike CTLA-4, PD-1 inhibits T cell responses by interfering with T cell receptor signaling as opposed to outcompeting CD28 for binding to B7. PD-1 also has two ligands, PD-L1 and PD-L2. PD-L2 is predominantly expressed on antigen presenting cells while PD-L1 can be expressed on many cell types including cells comprising the immune system, epithelial cells and endothelial cells. Antibodies targeting PD-L1 have shown clinical responses in multiple tumor types including melanoma, renal cell carcinoma, non-small cell lung cancer (20) and bladder cancer (21). Similarly, Phase I clinical trials with a monoclonal antibody against PD-1 demonstrated clinical responses in multiple tumor types including melanoma, renal cell carcinoma (RCC) and non-small cell carcinoma (NSCLC) (22). In addition, a large Phase I clinical trial with an anti-PD-1 antibody known as MK-3475 was shown to lead to response rates across all doses (2 mg/kg or 10 mg/kg) of ~37–38% in patients with advanced melanoma, including patients who had progressive disease after prior ipilimumab treatment (23), which led to FDA-approval of MK-3475 (pembrolizumab, Merck). Phase III clinical trials with anti-PD-1 (nivolumab, BMS) also led to FDA-approval of nivolumab for patients with melanoma (24, 25), NSCLC (26, 27) and RCC (28).

Since CTLA-4 and PD-1 regulate different inhibitory pathways on T cells, combination therapy with antibodies targeting both molecules was tested and shown to improve anti-tumor responses in a pre-clinical murine model (29). A Phase I clinical trial with anti-

CTLA-4 (3mg/kg, ipilimumab, BMS) in combination with anti-PD-1 (1mg/kg, nivolumab, BMS) also demonstrated tumor regression in ~50% of treated patients with advanced melanoma, all with tumor regression of 80% or more (30). Data from the Phase III clinical trial led to FDA-approval of combination therapy with ipilimumab plus nivolumab for patients with metastatic melanoma (31). There are ongoing clinical trials with anti-CTLA-4 plus anti-PD-1, or anti-PD-L1, in other tumor types with preliminary data indicating promising results (32, 33), which highlight this novel combination as an effective immunotherapy strategy for cancer patients.

Immune monitoring and biomarkers

The clinical responses observed with immune checkpoint therapy agents (anti-CTLA-4, anti-PD-1 and anti-PD-L1) occur in only a subset of patients and investigations are underway to identify predictive, prognostic and pharmacodynamic biomarkers. Although immune monitoring studies in peripheral blood would be preferable due to the ease of obtaining peripheral blood samples, the majority of data to date indicate that an understanding of the tumor microenvironment would first be necessary to help in identifying potential biomarkers that are relevant for anti-tumor immune responses (1, 2, 16, 34–39). It is possible that biomarkers identified in the tumor microenvironment will then provide a roadmap for identifying biomarkers in peripheral blood samples. Many studies have shown that tumor-infiltrating T cells correlate with clinical outcomes to immune checkpoint therapy (34–37). More recently, other studies have indicated that certain genes or pathways may exclude T cells from the tumor microenvironment thereby leading to resistance to immune checkpoint therapy (38, 39).

We have relied on tissue-based and pre-surgical studies to obtain pre-treatment and post-treatment tumor tissues for unbiased gene expression studies followed by additional confirmatory studies. For example, in our first pre-surgical clinical trial with anti-CTLA-4, a comparison of pre-treatment and post-treatment tumor tissues revealed a significant increase in expression of the genes related to the inducible costimulator (ICOS) pathway, which led to additional studies identifying an increase in frequency of ICOS⁺ T cells in both tumor tissues and blood samples (40). ICOS is another member of the CD28/CTLA-4 immunoglobulin superfamily whose expression is increased upon T cell activation. We found that an increase in frequency of ICOS⁺ CD4 T cells correlated with clinical benefit in patients (41). We proposed that ICOS served as a biomarker of T cell activation in the setting of anti-CTLA-4 immunotherapy and established ICOS⁺ CD4 T cells as a pharmacodynamic biomarker of anti-CTLA-4 treatment (42). More importantly, we also found that ICOS⁺ T cells were effector T cells that produced IFN γ and could recognize tumor antigens (43). Our work also demonstrated that ICOS enables signaling through the PI3K pathway to increase T-bet expression, which enables Th1 anti-tumor immune responses (44). We hypothesized that combination therapy targeting ICOS plus CTLA-4 blockade could improve anti-tumor immune responses and tumor rejection, which was demonstrated in murine tumor models (45). Therefore, ICOS serves as both a pharmacodynamic biomarker and a novel target.

Biomarkers are also being explored for anti-PD-1 and anti-PD-L1 therapy. Since PD-L1 can be expressed on tumor cells, initial studies focused on expression of PD-L1 on tumors as a

predictive biomarker for anti-PD-1 or anti-PD-L1 therapy. However, recent data indicate that patients whose tumors were deemed to be PD-L1-negative can also respond to anti-PD-1 and anti-PD-L1 treatments (21, 46). Since PD-L1 expression on tumor cells, and other cells including immune cells and stromal cells, can be regulated by IFN γ expression, it is doubtful that PD-L1 expression, either on tumor cells or immune cells, can be used as a predictive biomarker since PD-L1 expression is dynamic and will change over time (1, 2). Patients' tumors can be evaluated at a specific time by immunohistochemical studies and deemed to be PD-L1-negative for that moment but, after treatment with immunotherapy agents that act to stimulate a strong T cell response with IFN γ production, the tumor cells can become PD-L1⁺, which may enable anti-PD-1 and anti-PD-L1 antibodies to be clinically beneficial in these patients.

Since patients whose tumors were scored as PD-L1-negative did respond to anti-PD-1 and anti-PD-L1 therapies, it is clear that PD-L1 expression is not a predictive biomarker that can be used to select patients for treatment with anti-PD-1/PD-L1 antibodies. Although PD-L1 expression may not serve as a predictive biomarker, it may serve as a prognostic biomarker since patients with PD-L1⁺ tumors tend to have higher response rates with anti-PD-1 and anti-PD-L1 therapies as compared to patients with PD-L1-negative tumors (21, 46). However, the Phase I clinical trial with anti-CTLA-4 plus anti-PD-1 demonstrated that both PD-L1-positive patients and PD-L1-negative patients had similar response rates in the setting of combination therapy (30). Similar data were reported for a combination study with anti-PD-1 plus pazopanib or sunitinib (47). These data highlight the concept that an effective immunotherapy strategy will drive a strong T cell response with IFN γ production that will make baseline expression of PD-L1 irrelevant for correlation with clinical outcomes.

Although PD-L1 expression in tumors is usually dynamic, with increased expression occurring in the setting of T cell infiltration and IFN γ production by tumor-infiltrating cells, there is a setting in which PD-L1 expression has been found to be constitutive. In Hodgkin's lymphoma, Reed-Sternberg cells are known to harbor amplification of chromosome 9p24.1, which encode PD-L1 and PD-L2 and lead to their constitutive expression. Anti-PD-1 (nivolumab) was shown to elicit an objective response rate of 87% in a cohort of 20 patients with Hodgkin's lymphoma (48). Therefore, in malignancies that harbor amplification of chromosome 9p24 or upregulate PD-L1 or PD-L2 in response to an oncogenic signal, which provides a constitutive biomarker, it may be possible to predict clinical responses to anti-PD-1/PD-L1 therapies.

Other predictive biomarkers that have been explored include the number of mutations within a given tumor, which has been termed "mutational load" (49). Although greater numbers of mutations will likely provide a higher chance of generating neoantigens, which can be recognized by T cells, the data to date indicate that mutational load also functions as a prognostic biomarker rather than a predictive biomarker. In reported studies, patients with higher mutational load tended to have a greater chance of responding to immune checkpoint therapy such as anti-CTLA-4 or anti-PD-1; however, there were some patients who had lower numbers of mutations but still had measurable clinical benefit with immune checkpoint therapy (50, 51). Recently, investigators have also reported that the microbiome within subjects may affect anti-tumor immune responses and investigations are ongoing to

determine whether the presence of specific microorganisms in patients can act as predictive biomarkers for clinical benefit with immune checkpoint therapy (52, 53).

Although predictive biomarkers would be very helpful in selecting appropriate patients for treatment with immunotherapy, and avoiding toxicities in patients who are not likely to benefit, this may not be possible due to the dynamic nature of the immune system. We need additional studies focused on understanding the interplay between immune responses and the tumor microenvironment, which will provide data regarding mechanistic pathways that drive or impede anti-tumor immune responses and eventual tumor rejection. These data will guide the development of more effective immunotherapy strategies for cancer patients.

Ongoing translational research

The field of cancer immunotherapy is still in the arena of exploratory biomarkers (5) and as such, we need to first identify relevant biomarkers and assays before we can routinely implement validated assays. Assays that allow simultaneous measurement of multiple parameters may be advantageous. Since many existing immune monitoring assays incorporate phenotypic and functional aspects of different subsets of cells, one should keep in mind that the selection of phenotypic and functional assays relies on having knowledge ahead of time regarding these areas of immunology to inform selection of these assays; therefore, these are knowledge-driven analyses. However, a central disadvantage of the knowledge-driven approach is that the result will be only as good as the body of knowledge and as such, genes that are not known to be involved in the phenotype or function cannot be considered. An alternative to knowledge-based identification of immune correlates is a data-driven approach, in which genome-wide analyses of gene expressions are carried out on different subsets of cells, such as T and B cells. Subsequently, correlations between expressed genes and corresponding phenotype and/or function can be made to enable identification of biomarkers in an unbiased manner (54). Since the ideal assay(s) for identifying immunologic events that correlate with clinical benefit remain to be established, we need to re-examine our current immune monitoring strategies and design novel assays that will enable us to integrate data from: gene expression studies; epigenetics studies; multiparametric flow cytometry and CYTOF studies; immunohistochemical and tumor architectural studies; mutational load and neoantigen studies; and studies related to the microbiome. We will also need to measure immune responses longitudinally, especially those within tumor tissues, in order to obtain laboratory data and identify relevant biomarkers that correlate with clinical outcomes.

Conclusions

The dramatic anti-tumor responses seen in some patients as a result of treatment with anti-CTLA-4 provide a significant subset of patients who can be investigated for potential biomarkers that correlate with clinical outcome. The next generation of immunotherapy agents, such as anti-PD-1/PD-L1, has also led to significant anti-tumor responses. We must conduct careful studies in treated patients so that we can gain knowledge to develop even more effective therapies. The era of mechanistic studies in patients are upon us and it is imperative that we apply the same innovative strategies that were used to identify relevant

genes and pathways in murine models to experimental clinical trials aimed at understanding human immune responses. The concept of “reverse translation” can be applied whereby we conduct small clinical trials in patients to obtain appropriate pre-treatment, on-treatment and post-treatment tumor tissues and blood samples for in-depth laboratory studies, which lead to the generation of hypotheses that can be tested in murine models, thereby providing details about the precise biologic pathways that elicit tumor rejection (Figure 1). These types of studies will undoubtedly enable us to identify biomarkers, gain insights into mechanisms, recognize adaptive pathways and prioritize combination immunotherapy strategies. We are optimistic that instead of establishing specific biomarkers to include and exclude patients for treatment, the field of cancer immunotherapy will continue to move forward to provide clinical benefit for a greater number of patients based on an understanding of the principles of anti-tumor immune responses. We may not have identified predictive biomarkers for cancer immunotherapy but, it is due to the dynamic nature of the immune system, which gives us an opportunity to continuously learn about evolving human immune responses so that we may implement effective monotherapy and/or combination therapy strategies based on longitudinal studies of each patient’s immune response.

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Integrating Laboratory and Clinical Research “Reverse Translation”

Figure 1.

Depiction of “reverse translation” whereby we start by conducting immunotherapy clinical trials in patients to obtain appropriate tumor tissues and blood samples for exploratory studies, which enables hypothesis generation and subsequent experiments in animal models for hypothesis testing, with data from both the patients and animals providing a greater understanding and leading to development of more effective therapies.