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The Society for Immunotherapy of Cancer Biomarkers Task Force Recommendations Review

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Abstract

The clinical successes in cancer immunotherapy have led to a critical need for biomarkers in cancer immunotherapy. It is of the utmost importance to know who is most likely to benefit from these therapies (predictive biomarkers) but also who is starting to respond (prognostic biomarkers) and how the therapy functions in order to make rational combination choices (mechanism of action biomarkers). The Society for Immunotherapy of Cancer (SITC) Biomarkers Task Force addressed the state of the art and made a series of recommendations for the field, which is summarized here.

Keywords

cancer; checkpoint blockade; sequencing; flow cytometry; validation; standardization

Introduction

The immunotherapy clinical successes across many different tumor types have signaled a revolution in the approach to cancer treatment. While the occasional immunotherapy success in earlier years was often tied to multiple immune monitoring assays to investigate whether tumor-specific antitumor immunity was generated and to test for potential biomarkers of response, the numbers of durable objective clinical responders was so low that biomarkers were impossible to identify. The improved therapies available now, including checkpoint blockade (blocking CTLA-4 and PD-1) and adoptive cellular therapies (including TIL and CAR-T cells), are significantly improving patient outcomes, leading to much greater numbers of durable responders. There are also myriad combinations of checkpoint blockade, costimulatory agonist antibodies, transferred cells, recombinant viruses, small molecules and chemotherapeutic drugs, radiation and surgery that sometimes result in further clinical improvements. All of these clinical advances lack validated biomarkers for prediction,

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prognostication and mechanism of action that could unequivocally identify patients for enrollment or identify the best combination.

For many years, the Society for Immunotherapy of Cancer (SITC, formerly the International Society for Biological Therapy of Cancer, iSBTc) has held workshops and developed white papers with recommendations on immunologic monitoring and identification of immune biomarkers [1–9] (www.sitcancer.org/research/biomarkers). Given the enormous change and progress in the field, SITC recently convened four working groups of international experts from academia, pharma, biotech and regulatory agencies to identify the current hurdles in the field, present the state of the art, and make recommendations for next steps to identify useful biomarkers, and standardize and validate them for routine clinical use so that treatments are ultimately personalized to the patients most likely to respond and benefit, and the most rational combinations are identified.

A number of candidate biomarkers have been identified which show promising signals in multiple clinical trial settings, yet still have some limitations. Some appear to be prognostic after therapy is underway but are not predictive. Others have a positive value range that overlaps with the negative group value range, not allowing for an actionable cut off value for patient selection. Among the candidate biomarkers are the absolute lymphocyte count (ALC), frequencies of circulating or tumor infiltrating regulatory T cells (Treg), circulating myeloid-derived suppressor cells (MDSC), tumor antigen – specific CD8+ T cells, “exhausted” phenotype cells (including T cells expressing multiple checkpoint molecules like CTLA-4, PD-1, LAG-3, TIM-3), $ICOS^+$ activated T cells, mutation load in tumors, and, with arguably the best standardized data to date, PD-L1 expression on tumors and the extent of $CD3^+$/$CD8^+$ immune infiltrate (including the ImmunoScore). All of these important signals continue to be evaluated prospectively and in expanding clinical settings.

Novel Technologies

Technological advances have enabled great strides in biomarker research. The data obtained for each tumor or blood sample obtained has vastly increased in recent years. Working Group “2” examined this aspect of biomarker research [10]. Molecular technologies, such as whole exome sequencing (WES), have become efficient and inexpensive, allowing high complexity data to be obtained from many patients. WES allows the comparison between tumor tissue and normal tissue to identify the tumor-specific mutations, which allows analysis of the mutation load in tumor DNA and identification of tumor-specific mutated genes which may serve as immunogenic neoantigens in their protein form. Patients who will benefit from small molecule inhibitors that block mutation-activated signaling pathways can also be identified. RNA-based gene arrays and RNA-seq are technologies that allow an unbiased approach to fully examine expressed genes and screen for potentially important regulatory pathways, from tumor cells, surrounding tissue and/or peripheral blood. Other technologies that are less widely used to date which are informing tumor and immune interactions includes epigenetic profiling. Identifying the methylation status of specific regulatory regions can help identify cell types like Treg (based in part on FOXP3 locus methylation) from very small sample sizes. T cell receptor (TCR) sequence testing allows determination of the clonality or diversity of the T cell specificities in the circulation or

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tumor infiltrate. Such TCR sequence measures have been a significant clinical outcome correlate.

In addition to molecular technologies, mass cytometry is increasingly used across institutions to perform extremely high dimensional analytes of cell surface and intracellular proteins and identify many subsets and lineages of immune cells. Protein microarrays/ seromics allows identification of the antigenic specificities of circulating antibodies which indicates B cell activation and specificity and may also be a surrogate for a $CD4^+$ T cell response. Lastly, multispectral tissue imaging is expanding quickly, allowing the detection of at least 6 proteins per tissue section in addition to spatial relationship data. Such tissue data can separate tumors infiltrated with effector cells from those with effector cells excluded from the tumor center. These technological advances have revolutionized the way in which biomarker analyses are performed and yield significantly greater depth of data from each patient specimen. A series of 12 monthly "Biomarker Technology Primers" have been published which present specific technologies, and the existing data supporting the utility of each technology presented [9, 11–21].

Systematic Evaluations

The members of this working group focused on systematic approaches to evaluate blood cells, serum and plasma, lymph nodes and tumor samples. Analyses of candidate biomarkers occurs in all of these specimen types and immune compartments, including, ALC, T cell phenotyping, Treg and MDSC phenotype and frequency measures as well as circulating protein levels. These are all currently viewed as common assessments which are tested to either confirm or refute their status as true biomarkers in particular clinical settings. At the other end of the biomarker spectrum in terms of complexity is the microbiome. Microbiome studies are also becoming incorporated more routinely, particularly those focused on the gut microbiome based on emerging data from other physiological sites. As cell therapies become more widely tested and increasingly efficacious (and now approved by regulatory agencies), identification of biomarkers of a clinically effective cellular product becomes more commonly tested to understand patient-to-patient variability in these autologous products. Genetically engineered cell therapeutics expressing specific TCRs and CARs need to be tracked *in vivo* after infusion to determine whether they proliferate *in vivo* and their degree of persistence over time, which may be critical to clinical response.

While many biomarker studies are focused on melanoma, due in part to the history of success with immune-based therapies, as well as to accessibility of skin surface tumors, this working group used gastrointestinal cancers as an example clinical setting for biomarker testing. The study of these tumors, for example hepatocellular cancer, is hindered by limited ability to access tumor biopsies and can involve complex clinical and immunologic confounders such as chronic virus infection, organ cirrhosis and unique toxicity profiles.

Baseline Measures

The optimal time point for identification of the best treatment is at baseline, or at diagnoses, before any therapies have begun. It is rare that any viable blood samples (cells or serum) are banked before therapy begins, and tumor samples from diagnosis are invariably formalin-

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fixed and paraffin-embedded (FFPE) and not suitable for many immunologic tests. However, technological advances and multiplexing can make archived FFPE tissues highly valuable for investigation of immune status and immune response. Another group of experts addressed the state of testing baseline immunity [22].

Two biomarkers from baseline tumor samples have undergone significant standardization and validation. The first is the Immunoscore, developed in colorectal cancer (originally as CD3/CD8/CD45RO but validated internationally as CD3/CD8 stains), which has greater predictive value than classical T-N-M tumor staging [23–28]. The second is the expression of PD-L1 on tumor cells [29–31]. The PD-L1 expression testing data continue to evolve as data from different assay platforms, different antibodies, tumor types and PD-1 combination trials are evaluated. While it cannot unequivocally predict PD-1 blockade clinical outcome, the expression data are informative, particularly in combination with tumor expression level and immune cell infiltrate expression data. Other active areas of biomarker investigation from baseline tumor and immune specimens with stronger existing data include MDSC frequencies, transcriptional signatures (including cytotoxic CTL-type signatures),, inhibitory molecular pathways, genetic SNP analysis and identification of tertiary lymphoid structures (which may be sites of important antigen presentation outside of secondary lymphoid structures).

Standardization and Validation

Another hurdle in the biomarker field has been a lack of standardization of assays across laboratories and groups of investigators, as well as the lack of pre-analytical and analytical validation, which can hinder the scientific confirmation that a candidate biomarker should be experimentally focused on. In addition, many clinically validated assays in medicine are not as complex as many cellular and molecular immune assays. Single genetic mutations in genes which confer sensitivity to molecular inhibitors are more straightforward than MDSC frequencies (where the phenotype in humans continues to be discussed) or characterization of tumor infiltrates. Therefore, a working group with scientific and regulatory expertise focused on this area, and ultimately created a guidance for analytical validation [32] and clinical validation [33] of immunotherapy biomarkers.

The critical parameters for pre-analytic and analytic validation of biomarker assays were described in detail, including sensitivity, specificity, linearity, precision, limits of detection, accuracy, repeatability, reproducibility and robustness. As examples, PD-L1 staining (largely on tumor cells) and the IFNγ ELISPOT assay (for the frequency of antigen-specific T cells) were presented. In the second part of the work, the critical aspects of clinical validation were presented, including analysis of clinical sensitivity and specificity, false positive and negative rates, identification of cut points, and regulatory considerations for biomarker implementation Table 1).

Summary and Conclusions

To formally convey the results of the SITC Task Force working group efforts, an open meeting was held at the NIH, and each working group made short presentations with substantial discussion sessions. The meeting also presented work based at the National

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Cancer Institute (NCI) examining biomarkers [34]. During the first session, the level of standardization and validation for DNA, RNA and protein profiling, TCR clonality, ELISPOT and PD-L1 staining was discussed. The second session presented technology updates on multispectral tissue imaging, sequencing and mutation analysis, CyTOF and blood profiling and how they can be incorporated into current trials. The third session presented data on tumor and tumor-draining lymph node profiling, autologous cellular product analysis, antigen presentation pathway inhibition as well as how complex data sets from multiple platforms can be analyzed. This addressed a critical need in the field due to the increased use of high throughput approaches and hypothesis-generating molecular and protein profiling assays. The fourth session presented baseline tumor mutation analysis, “hot” and “cold” tumors, blood cell profiling, antibody responses and tertiary lymphoid structures. The meeting also had NCI perspectives presented, including topics of novel blood phenotypic profiles that are being further investigated in cancer vaccination trials, and the profiling of extracellular vesicles (which are now more easily investigated due to advances in technology).

With guidance in development of clinically valid immune biomarkers and harnessing newer technologies helping to identify robust and reproducible signals that many in the field are testing in current clinical trials today, our ability to have truly personalized cancer immunotherapies is closer to being a reality (Figure 1). An important recent advance is the approval of PD-1 blockade with pembrolizumab for microsatellite instability-high (MSI high) tumors regardless of tumor histology. This exciting advance comes quickly after the identification of MSI-high tumors being especially responsive to PD-1 blockade and has now become an approved, actionable immunotherapy biomarker.

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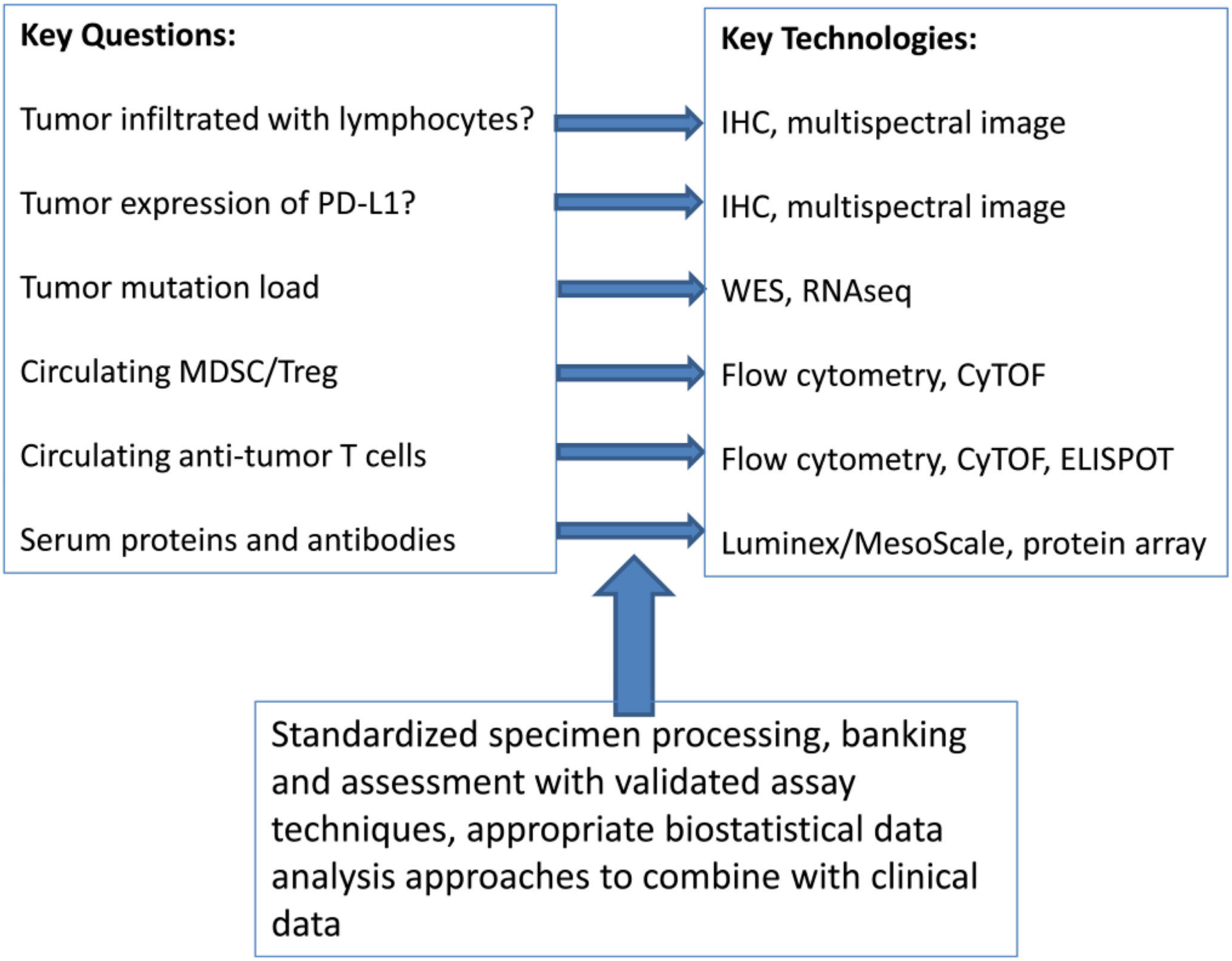

Figure 1.
High priority candidate biomarkers and technology approaches for immunotherapy to be considered in trial designs and immune monitoring assessments.

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Table 1

Evaluating the performance of a predictive biomarker[1]

1. A trial designed to assess the clinical validity of a predictive biomarker must predefine the clinically meaningful performance metrics for the predictor.
2. Guidelines for informative reporting of studies on prognostic as well as diagnostic markers exist which are applicable to cancer immunotherapy.
3. The choice of specific performance metric and the benchmark performance level that must be attained is dependent on the intended clinical use. To sort out the predictive versus prognostic value of a biomarker from a stratified design, it is necessary to evaluate the effect of an interaction between the marker and the treatment. Only specific interactions will result in a marker that can improve patient outcomes in the target population.
4. Demonstration that a predictor's output is statistically associated with the clinical endpoint is not sufficient evidence of acceptable performance. Although the presence of such an association may establish the clinical validity of the test, statistical significance does not always translate into a clinically meaningful association or provide clinically useful, or actionable, information. To establish clinical utility, as opposed to clinical validity, there must be evidence suggesting that the use of the test is likely to lead to a clinically meaningful benefit to the patient beyond current standards of care.

[1]Adapted from Dobbin, et al. JITC, 2017 (33)