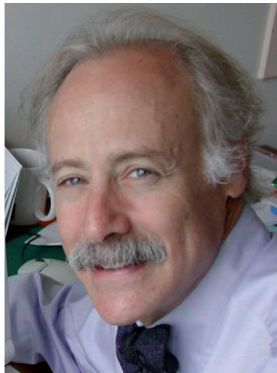


EDITORIAL

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“It is through fully understanding the human *in vivo* mechanism of action of these agents that we will be able to truly unlock the promise of cancer immune therapy”

Overcoming the logistics of immune monitoring of cancer patients during clinical trials

Marc S Ernstoff*

Cancer is a collection of complex diseases with shared and unique molecular pathways of carcinogenesis, invasion and metastases [1]. The interface between cancer and the host stroma and immune system also share unique interactions [2–4]. Cancer therapeutics attempts to leverage the differences between malignant and normal tissues and the host response to cancer: chemotherapy inhibits cell division or metabolic pathways; targeted therapy exploits the cancer-activated molecular growth pathways; antiangiogenesis treatment takes advantage of cancer-induced neovascularization; differentiation therapy drives cancer stem cells into terminal differentiation; and immune therapy focuses on regulating the inflammatory and regulatory pathways to induce an anticancer immune response. Understanding each of these pathways *in vivo* in humans will improve benefit and outcome for cancer patients. Thus, immune monitoring of patients undergoing therapy is critical to expanding this knowledge.

Immune therapy has had a long history in the treatment of cancer patients. Its roots are established in the late 19th century with the use of nonspecific bacterial stimulants pioneered by William Coley [5]. The development of antibodies as the ‘magic bullets’ was hypothesized by Paul Ehrlich about the same time [6]. In the mid-20th century, the first identification of an inflammatory cytokine, interferon, was published by Nagano and Kojima and subsequently confirmed by Isaacs and Lindenmann [7–8]. Since then, many cytokines, chemokines and regulator molecules have been discovered and used in clinical trials. Cytotoxic T cells were first described by Govaerts in 1960 and, subsequently, tumor-specific cytotoxic T cells were identified by the Boon laboratory [9–11]. Regulation of cellular immune response by T cells was first proposed by Gershon in 1970, but it was not until 2000 that Sakaguchi described the CD4⁺CD25^{high} regulatory cell [12,13]. The cellular regulatory pathways now include a variety of additional components including regulatory dendritic cells, CD8⁺ regulatory cells, suppressor B-cells, myeloid suppressor cells and numerous regulatory molecules [12].

The growing complexity of immune pathways is rich with new therapeutic targets and provides novel approaches to treating patients with cancer. Although the promise of cancer immune therapy is being realized, we have not yet been able to associate the ‘on-target effects’ of these agents and the clinical outcome. It is through fully understanding the human *in vivo* mechanism of action of these agents that we will be able to truly unlock the promise of cancer immune therapy. Immune monitoring during carefully designed clinical trials provides this knowledge. Nevertheless, monitoring of the immune system in cancer patients has its own limitations, which bear on our interpretation of the data.

Keywords: biomarkers • cancer • clinical trials • immune monitoring
• immunotherapy

*Medical Oncology Immunotherapy Group, Dartmouth-Hitchcock Medical Center, 1 Medical Center Drive, Lebanon, NH 03756, USA
E-mail: marc.s.ernstoff@hitchcock.org

Biomarkers may be prognostic or predictive. Prognostic markers are those that inform us about the likelihood of a good or bad outcome for a patient. The stage of cancer is an example of a marker of prognosis. A predictive marker should provide information with regard to likelihood of response to a treatment. An example of a predictive marker would be expression of mutated *BRAF* in melanoma, which would inform us about the utility of treatment with vemurafenib, an inhibitor of mutated BRAF. Some markers may have both prognostic and predictive value, such as estrogen receptor expression in breast cancer. Prior to adopting a biomarker as a clinical tool, it needs to be validated in prospective clinical trials [14,15]. At this time, most immune parameters have not yet been validated and are used to explore the physiology of cancer or the mechanism of therapeutic agents [16].

Many clinical trials exploring immune-based therapies incorporate immune monitoring. In so doing, the investigator has to consider the purpose of the monitoring, such as confirmation of the agent's proposed mechanism of action in humans *in vivo*, demonstration of an association of the biomarker as a predictor of outcome to treatment, or exploration of immune pathways that may be affected by treatment. Thus, the investigator has a myriad of decisions to consider, including relevant biomarkers, assay methods for each biomarker, the variance and validity of each biomarker assay, functional versus phenotypic biomarkers and historical data needed for comparisons to other trials. Then there are overlying logistical factors such as timepoints of the measurement, the biocompartment from which to obtain specimens (peripheral blood, serum or plasma; tumor tissue; surrogate tissue), how each specimen needs to be handled (assayed immediately, flash frozen, step frozen, placed in preservative or enzyme inhibition media), and the appropriate statistical approach.

As we recognize the complexity of immune monitoring, there has been a call for international harmonization and the standardization of the process [17,18]. Many feel that a single assay is unlikely to yield a useful biomarker and advocate for multiple biomarkers as a method to optimize predictive approaches [18,19]. The US FDA initiative 'Critical Path' has identified biomarker development and has laid out an approach encompassing biospecimens, analytic performance, standardization and harmonization, bioinformatics, collaboration, and data sharing to improve the field. The FDA, the International Society for Biological Therapy of Cancer-Society for Immunotherapy of Cancer, and the National Cancer Institute (USA) partnered to convene a workshop on immunotherapy biomarkers in follow up to an earlier workshop sponsored by the Society of Biological Therapy. Recommendations resulting from

the joint workshop have been published [17–20]. These recommendations address the processing and storage of samples, characterization of cellular products, assay standardization and harmonization, centralization of monitoring, analysis, reporting and assay validation.

To assist the investigator in generating informative data during the conduct of a clinical trial a carefully considered plan of immune monitoring that addresses the following areas is suggested:

- Develop a testable hypothesis;
- Assure that the assays measure the endpoint desired;
- Consider both phenotypic and functional assays;
- Consider assays that address the relevant stimulatory and regulatory pathways in your study;
- Consider measuring both host- and tumor-relevant markers that may impact on outcomes;
- Assure the assay is reproducible and has an acceptable variation;
- Address the methods for quality assurance and quality control for each assay;
- Consider assays that have been validated and harmonized across different laboratories;
- Consider the tissue compartment in which the assay needs to be measured (tumor, peripheral blood, CSF and so forth);
- Carefully plan the timepoints during the treatment regimen at which the assays are conducted to best determine kinetics of the effect;
- Prospectively plan the analysis using the appropriate statistical and bioinformatics methods;
- Assure you will have an adequate sample size to answer the immune monitoring hypothesis;
- Selection of patients, tumor burden, comorbid disease and concomitant medications may influence the outcome of the assay;
- Consider pharmacokinetic and pharmacodynamics measurements of the agent(s) being used;
- Bank specimens (cells, serum, plasma and tumor) and consider the appropriate preservatives for future assays (functional, phenotypic, DNA and mRNA).

Our understanding of immune inflammatory and regulatory pathways, as well as host-specific differences and tumor-related aspects that impact on immunity, has expanded logarithmically over the past decade. A large number of new immunotherapeutic tools have entered the clinic. It is only through careful immune monitoring

that we will better comprehend the complexity of therapy and thus improve clinical outcomes.

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