

# Identification of Immunologic Biomarkers Associated with Clinical Response after Immune-Based Therapy for Cancer

Yushe Dang and Mary L. Disis

*Tumor Vaccine Group, Center for Translational Medicine in Women's Health,  
University of Washington, Seattle, Washington, USA*

The identification of immunologic biomarkers associated with clinical response after immune intervention for cancer is an area of intensive investigation. The field would benefit from a more systemic and directed approach for biomarker identification and evaluation. Lessons can be learned from other fields, such as cancer diagnostics, as to how to develop response-associated biomarkers. Studies in both human *in vitro* models as well as murine models of cancer can significantly inform and streamline the choice of candidates. Adoptive T-cell therapy is an interesting model for exploring potential immunologic surrogates that may predict clinical response. Most likely the clinical effectiveness of immune-based treatments will be predicted by panels of markers rather than single assays of a specific immune effector cell.

**Key words:** immune; biomarker; immunotherapy; clinical response

## Introduction

Methodologic advances in the quantitation of human immunity and the development of cancer immune-based therapies that induce a clinical response underscore the need to identify immunologic biomarkers associated with therapeutic effect. The identification of biomarkers, that indicate improved outcome would add to a better understanding of the potential mechanisms of action of immune therapy. Moreover, functional biomarkers would also allow a more rapid testing of novel immunotherapeutic approaches based on biomarker rather than clinical end points.

The discovery and validation of immunologic biomarkers for cancer therapy is complex. While the focus of the assessment of immune-based therapies has been on the effector population potentially mediating the response, elements in the tumor microenvironment as well

as host-related factors have also been shown to impact clinical outcome. Therefore, assessment of immunologic biomarkers in the context of active immunotherapy requires a broad-based approach potentially evaluating multiple biomarkers in a hypothesis-driven fashion. Discovery and validation of immunologic biomarkers of clinical response must be initiated at the earliest stages of product development.

## Approach to Determining Immunologic Biomarkers of Clinical Response

Pepe and colleagues have outlined the phases of biomarker development for the early detection of cancer.<sup>1</sup> These described phases could also be adapted to the development of immunologic biomarkers of clinical response (Table 1). Phase I of biomarker development entails the discovery or identification of potential markers that are either linked to the mechanism of action of the proposed therapy or have shown predictive capacity in animal models. Studies

Address for correspondence: Dr. Yushe Dang, 815 Mercer Street, University of Washington, Seattle, WA 98109. Voice: 206-616-8447; fax: 206-685-3128. ydang@u.washington.edu

**TABLE 1.** Assessment of Immunologic Biomarkers of Clinical Response

Phases of biomarker development	Description	Phases of clinical development	Research stage
Phase I preclinical	Identification of lead candidate biomarkers	Preclinical	Hypothesis generating
Phase II clinical assay/validation	Assay standardization for clinical use, feasibility	Feasibility trials and phase I	
Phase III retrospective evaluation	Identification of candidate surrogates of clinical response	Phase I and phase II	
Phase IV prospective evaluation	Preliminary validation, assessment of ruggedness	Randomized phase II and phase III	Hypothesis validating
Phase V implementation	Final validation	Phase III and phase IV	

Adapted from Pepe *et al.*<sup>1</sup>

at this phase are preclinical and are conducted in either human *in vitro* systems or in biologically relevant animal models. The end point of such studies would be the identification of lead candidate biomarkers that could be put forth for further evaluation.

Phase II is the first application of the proposed biomarker in a clinical trial. This phase assesses the feasibility of the marker to be standardized as a clinical assay. The assessment of the biomarker must be reproducible and demonstrate reasonable precision and accuracy.<sup>2</sup> Information is collected on the performance characteristics of the biomarker, and preliminary data may be generated as to the utility of the marker to predict clinical outcome. Over the last decade many forms of cancer immune-based therapeutics have been applied in earlier stages of disease. For example, rather than treating advanced-stage disease with a clinical end point of tumor regression, cancer vaccines are increasingly being used in the adjuvant setting. In this case, the clinical end point might be prevention of disease relapse. For this reason, the collection of data on clinical outcome may take place over months and years rather than weeks. Retrospective evaluation of immunologic assays, in relation to clinical outcome, is critical in identifying candidate biomarkers associated with clinical response (phase III). The ability to identify such candidates with some assurance will depend not only on assay performance but also on the

comprehensive collection of valid clinical data, which will facilitate both univariate and multivariate analysis of immunologic and clinical response.<sup>2,3</sup> The phases of development spanning preclinical to retrospective analysis are all hypothesis generating. If assessment of immunologic biomarkers is prospectively planned and early clinical trials are designed to include a statistically based evaluation of multiple markers, then it is reasonable to assume promising candidates could be identified to advance into phase II and phase III clinical trials.

### Immunologic Biomarker Discovery

Identification of immunologic biomarker candidates is greatly facilitated by an assessment of both the tumor and peripheral blood of the target patient population. For example, recently a type I adaptive gene expression signature has been identified for several cancers by virtue of analysis of the tumor and the correlation of expression patterns to clinical outcome.<sup>4,5</sup> Upregulation of interferon-associated genes appears to positively impact risk of relapse and survival in selected cancer populations.<sup>4,5</sup> Moreover, infiltration of CD3<sup>+</sup> T cells into the tumor bed appears, in several studies, to be a critical predictor of survival.<sup>6</sup> Such studies would suggest that the development of immunologic assays to either assess changes induced in tumor-specific type I

adaptive immunity with an experimental immunotherapeutic agent or even the development of radiographic methods to assess T-cell penetration of the tumor bed would be reasonable approaches.

Evaluation of immunity detected in the peripheral blood of cancer patients may also give some clues to appropriate biomarkers to assess in clinical trials. Analysis of PBMCs as well as serum could be useful in biomarker identification. In a recent study of over 20 patients assessing immunity directed against HER-2/neu, melanoma antigen (MAGE), and carcinoembryonic antigen (CEA), cancer patients, in general, had lower type I T-cell responses against tumor versus viral antigens.<sup>7</sup> This investigation would suggest that evaluation of restoration of tumor specific type I T-cell responses may be a reasonable biomarker to develop for immunotherapeutic clinical trials. A serologic assessment of tumor-specific immunity in patients with ovarian cancer demonstrated that the presence of IgG antibody immunity specific for selected tumor antigens could predict improved survival compared to those patients without an immune response. Moreover, immunity to multiple ovarian cancer antigens, rather than immunity restricted to a single antigen, appeared to impart a more favorable prognosis.<sup>8</sup> Enhanced cross-priming at the site of the tumor, mediated by local antigen-presenting cells, is presumed to be the major mechanism by which tumor immunity is elicited. Therefore, evaluation of immune responses to multiple tumor antigens may be a promising approach to immunologic monitoring. Indeed, epitope spreading has already been described to be a marker predictive of improved outcome after cancer vaccination in a few studies.<sup>9,10</sup>

Most immune-based therapies are focused at enhancing specific effector responses, thus eliciting an antitumor response. For this reason, most clinical trials focus on measuring the effectors presumably being induced. However, immunologic markers of immune suppression are as important, or even more im-

portant, to monitor during therapy. Multiple studies have shown the prognostic importance of CD4<sup>+</sup>FOXP3<sup>+</sup> T regulatory (Treg) cells infiltrating the tumor. While most investigations have suggested a deleterious role for Treg in inhibiting tumor regression, recent studies have suggested that Treg increases in the tumor could be beneficial.<sup>11,12</sup> Clinical evaluation of sera is also a potentially useful source to determine whether patients are benefiting from immune-based therapies. Transforming growth factor- $\beta$  (TGF- $\beta$ ) has been shown to be elevated in the serum of many types of cancers. TGF- $\beta$  is secreted by the tumor stroma as well as Tregs to limit tissue destruction and suppress immunity. Therapies that focus on enhancing cross-priming at the tumor site may result in the expansion of a multiclonal, type I, tumor-specific T-cell repertoire which may be able to reverse tumor- or Treg-induced immune suppression. Indeed, a recent study presented preliminary evidence that serum TGF- $\beta$  levels are inversely correlated with the magnitude of the IFN- $\gamma$ -producing tumor-specific T-cell response elicited with an HER-2/neu vaccine.<sup>13</sup>

Murine models of cancer have been a valuable tool in modeling human cancer therapies. Molecular profiling of these murine tumors in transgenic animals suggests a strong genetic similarity with human tumors.<sup>14</sup> Moreover, recent evidence indicates that the murine immunologic repertoire is similar to that induced in humans.<sup>15</sup> Evaluation of targeted therapies in genetically engineered mouse models expressing targets, such as HER-2/neu and epidermal growth factor receptor (EGFR), have demonstrated the ability to predict specific human toxicities that have eventually been related to the targeted therapy.<sup>16</sup> Murine models have also been useful for identifying biomarkers associated with clinical response to immunotherapeutic agents. The magnitude of the immune response, both CD8<sup>+</sup> and antibody immunity, elicited against neu with a neu-specific vaccine, predicted clinical efficacy in a transgenic mouse model of neu-mediated breast cancer.<sup>17</sup>

Moreover, using a DNA-based vaccine strategy, these investigators demonstrated that immunization was also associated with a decrease in circulating myeloid suppressor cells (MSC). Human clinical trials of the vaccine are planned with immunologic monitoring poised to measure both immune effectors (cytotoxic T lymphocyte and antibodies) as well as immune suppressors (MSC). Studies in mice have the ability to explore both the tumor and peripheral blood for biomarker identification. Evaluating a vaccine directed against a self-tumor antigen in the 9L gliosarcoma model, investigators demonstrated that key immunologic biomarkers included an elevated tumor-specific peripheral blood Th1 response, tumor-infiltrating CD8<sup>+</sup> T-cell response, and decreased level of Treg in the tumors of immunized mice.<sup>18</sup>

The data presented above suggest mining for immunologic biomarkers can occur in both human and mouse, that multiple candidate markers should be identified to progress to phase II and III biomarker testing, and that markers of both immune stimulation as well as immunosuppression should be considered for clinical development.

### **Immunologic Biomarker Assay Validation and Exploration of Clinical Utility**

The first step in candidate biomarker prioritization is the determination of whether the marker assessment can be scaled up to allow evaluation of a multitude of samples and whether the measurement of the marker can be made clinically reproducible. In short, one must develop assay performance characteristics. Quality control monitoring and assay validation are composed of several analytical measures. First, one must establish the accuracy of the assay, which refers to the correctness and exactness of the test result. Accuracy is described as the closeness of a test result to the true value. To evaluate accuracy one must have a comparison to a standard, which is often difficult to

accomplish in studies of immune-based therapies. Surrogate assessment of the assay in a nontumor system is often used to define assay performance within single laboratories. A second parameter to measure is precision, or the reproducibility of the test. Precision is described as the closeness of the test results to one another when using the same specimen and is expressed as a standard deviation and coefficient of variation of multiple sample runs. Sensitivity is the limit of detection of a method or the capacity of the method to detect minimal levels of immunity with some assurance. Specificity is the ability of a method to measure only the specific response or cell type being tested. The reliability of a method describes the ability of the test to maintain accuracy, precision, sensitivity, and specificity despite changes in external factors, such as technicians, instruments, or reagents. Only those tests that can be defined as reliable should proceed for further assessment in the context of a clinical trial. The feasibility of the application of a biomarker assay broadly across a large population and the ability to limit variability inherent in the assay are often limiting factors that will result in prioritization of one biomarker over another for clinical development.

As discussed above, clinical trials of cancer vaccines are more recently being conducted in the adjuvant setting, evaluating time to relapse as the primary outcome measure. Such studies require large numbers of patients to determine the clinical significance of potential biomarkers, and often these phase I/II studies are underpowered to allow adequate evaluation of immunologic markers.<sup>19</sup> Retrospective analyses have suggested that epitope spreading and the magnitude of the immune response elicited may be of clinical interest.<sup>9,10,13</sup> However, further prospective studies need to be performed. Adoptive T-cell therapy, i.e., infusion of tumor-competent T cells to induce tumor regression, offers a model system for rapid assessment of immunologic biomarkers. The assessment of potential marker candidates in the context of adoptive T-cell therapy offers

several advantages over other types of immune-based treatments: (1) in general, the product infused is fairly well defined (T cells) and the proposed mechanisms of action are related to the infused product, (2) tumor regression is temporally related to the T-cell infusion so that there may be a more direct correlation of assay results with response, (3) target cells in the infused product may be followed over time to assess the evolution of immunity with clinical response. Clinical trials of adoptive T-cell therapy have provided several candidate biomarkers as potential surrogates for indicating clinical efficacy.

The magnitude of tumor-specific T cells in the infused product has been shown to be related to clinical response.<sup>20</sup> Our group has been evaluating the infusion of HER-2/neu-specific T cells generated *ex vivo* in patients with refractory breast and ovarian cancer after patients have received an HER-2/neu-specific vaccine. The number of HER-2/neu-specific T cells detected in the infusion products varied greatly. To date, we have seen a 40% response rate. The total number of HER-2/neu-specific T cells infused was 43-fold higher in responding patients than that in nonresponding patients.<sup>21</sup> The clinical efficacy of adoptive T-cell therapy has also been associated with the proliferative potential of the T cells infused. It has been reported that objective responses in melanoma patients treated with adoptively transferred T cells derived from tumor-infiltrating lymphocyte were correlated with the telomere length of the transferred cells. Telomere length of infused T cells in patients with tumor regression is significantly longer than that in patients without responses and is a presumed marker for long-lived antigen-specific T cells.<sup>22,23</sup> A recent case report of a single melanoma patient showed that after infusion of an NY-ESO-1-specific CD4<sup>+</sup> T-cell clone, the frequency of NY-ESO-1-specific CD4<sup>+</sup> T cells rapidly increased, as detected by quantitative PCR assay. The transferred T cells mediated a durable clinical remission.<sup>24</sup> The persistence of multi-clonal tumor-specific T cells post infusion could

be a major factor in tumor regression. We performed kinetic analysis of HER-2/neu-specific T-cell responses post T-cell infusions using an IFN- $\gamma$  enzyme-linked immunospot (ELISPOT) assay. We found that multiclonal HER-2/neu-specific T cells in responding patients were persistent over 6 months after infusion. Both tumor antigen-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell immunity was maintained. T cell receptor V $\beta$  (TCR V $\beta$ ) repertoire analysis showed the outgrowth of several HER-2/neu-specific clones in the CD4<sup>+</sup> population of one patient 220 days post infusion.<sup>25</sup> The degree of T cell persistence in peripheral blood of adoptively transferred T cell clones was also significantly correlated with tumor regression in patients with melanoma.<sup>26</sup>

Finally, the ability of transferred T cells to penetrate into solid tumor deposits may be associated with tumor regression. In a trial of adoptive T-cell transfer in melanoma, tumor biopsy samples were found to contain minimal lymphocytic infiltration. After T-cell treatment, the biopsy samples contained large areas of necrotic tumor and areas of dense lymphocytic infiltrates. By immunohistochemical staining, the infiltrated cells were shown to express the same TCR V $\beta$  subtype as the infused T cells.<sup>27</sup> Infusion of prelabeled T cells into the patients allows the direct localization of the infused T cells. One patient with metastatic HER-2/neu overexpressing breast cancer was treated with indium-111-labeled, autologous, HER-2/neu-specific T cell clones. Imaging revealed that the infused T cells accumulated in the bone marrow, which correlated with the disappearance of bone marrow-residing disseminated tumor cells. Unfortunately, the infused T cells were unable to penetrate into solid metastases, this inability was associated with tumor progression.<sup>28</sup>

## Conclusions

Exploratory studies of novel immunotherapeutic agents must include evaluation of potential biomarkers suitable for clinical application.

It is clear that more than one marker will be needed to assess response and that markers should evaluate both immune-potentiating and immune-suppressive effects. Moreover, immune biomarkers must take into consideration not only the elicited immune effector populations but also tumor environmental and host factors. The identification of immunologic biomarkers that could function as potential surrogates indicating clinical response would significantly enhance the translation of immune-based therapies for cancer.

### Conflicts of Interest

The authors declare no conflicts of interest.

### References

1. Pepe, M.S., R. Etzioni, Z. Feng, *et al.* 2001. Phases of biomarker development for early detection of cancer. *J. Natl. Cancer Inst.* **93**: 1054–1061.
2. Walker, E.B. & M.L. Disis. 2003. Monitoring immune responses in cancer patients receiving tumor vaccines. *Int. Rev. Immunol.* **22**: 283–319.
3. McShane, L.M., D.G. Altman, W. Sauerbrei, *et al.* 2005. Reporting recommendations for tumor marker prognostic studies (REMARK). *J. Natl. Cancer Inst.* **97**: 1180–1184.
4. Galon, J., A. Costes, F. Sanchez-Cabo, *et al.* 2006. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* **313**: 1960–1964.
5. Kreike, B., M. van Kouwenhove, H. Horlings, *et al.* 2007. Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. *Breast Cancer Res.* **9**: R65.
6. Zhang, L., J.R. Conejo-Garcia, D. Katsaros, *et al.* 2003. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. *N. Engl. J. Med.* **348**: 203–213.
7. Inokuma, M., C. dela Rosa, C. Schmitt, *et al.* 2007. Functional T cell responses to tumor antigens in breast cancer patients have a distinct phenotype and cytokine signature. *J. Immunol.* **179**: 2627–2633.
8. Goodell, V., L.G. Salazar, N. Urban, *et al.* 2006. Antibody immunity to the p53 oncogenic protein is a prognostic indicator in ovarian cancer. *J. Clin. Oncol.* **24**: 762–768.
9. Butterfield, L.H., A. Ribas, V.B. Dissette, *et al.* 2003. Determinant spreading associated with clinical response in dendritic cell-based immunotherapy for malignant melanoma. *Clin. Cancer Res.* **9**: 998–1008.
10. Salazar, L., V. Goodell, M. O'Meara, *et al.* 2009. Persistent immunity and survival after immunization with a HER-2/neu (HER2) vaccine. *J. Clin. Oncol.* **27**: 3010.
11. Salama, P., M. Phillips, F. Gricu, *et al.* 2009. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. *J. Clin. Oncol.* **27**: 186–192.
12. Schreiber, T.H. 2007. The use of FoxP3 as a biomarker and prognostic factor for malignant human tumors. *Cancer Epidemiol. Biomarkers Prev.* **16**: 1931–1934.
13. Disis, M.L., D. Wallace, T.A. Gooley, *et al.* 2009. Concurrent trastuzumab and HER-2/neu specific vaccination in patients with metastatic breast cancer. *J. Clin. Oncol.* in press.
14. Herschkowitz, J.I., K. Simin, V.J. Weigman, *et al.* 2007. Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. *Genome Biol.* **8**: R76.
15. Lu, H., K.L. Knutson, E. Gad & M.L. Disis. 2006. The tumor antigen repertoire identified in tumor-bearing neu transgenic mice predicts human tumor antigens. *Cancer Res.* **66**: 9754–9761.
16. Roberts, R.B., C.L. Arteaga & D.W. Threadgill. 2004. Modeling the cancer patient with genetically engineered mice: prediction of toxicity from molecule-targeted therapies. *Cancer Cell.* **5**: 115–120.
17. Cipriani, B., A. Fridman, C. Bendtsen, *et al.* 2008. Therapeutic vaccination halts disease progression in BALB-neuT mice: the amplitude of elicited immune response is predictive of vaccine efficacy. *Hum. Gene Ther.* **19**: 670–680.
18. Driessens, G., L. Gordower, L. Nuttin, *et al.* 2008. Therapeutic efficacy of antitumor dendritic cell vaccinations correlates with persistent Th1 responses, high intratumor CD8+ T cell recruitment and low relative regulatory T cell infiltration. *Cancer Immunol. Immunother.* **57**: 1745–1756.
19. Atkins, M.B., D. Carbone, G. Coukos, *et al.* 2007. Report on the ISBTC mini-symposium on biologic effects of targeted therapeutics. *J. Immunother.* **30**: 577–590.
20. Cheever, M.A. & W. Chen. 1997. Therapy with cultured T cells: principles revisited. *Immunol. Rev.* **157**: 177–194.
21. Disis, M., L. Salazar, A. Coveler, *et al.* 2009. Phase I study of infusion of HER-2/neu (HER2) specific T cells in patients with advanced stage HER2 overexpressing cancers who have received a HER2 vaccine. *J. Clin. Oncol.* **27**: 3000.
22. Dudley, M.E., J.C. Yang, R. Sherry, *et al.* 2008. Adoptive cell therapy for patients with metastatic

- melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J. Clin. Oncol.* **26**: 5233–5239.
23. Zhou, J., X. Shen, J. Huang, *et al.* 2005. Telomere length of transferred lymphocytes correlates with in vivo persistence and tumor regression in melanoma patients receiving cell transfer therapy. *J. Immunol.* **175**: 7046–7052.
24. Hunder, N.N., H. Wallen, J. Cao, *et al.* 2008. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. *N. Engl. J. Med.* **358**: 2698–2703.
25. Dang, Y., L. Salazar, A. Coveler, *et al.* 2009. Phase I study of infusion of HER-2/neu specific T cells in patients with advanced stage HER-2/neu overexpressing cancers who have received a HER-2/neu vaccine. *J. Immunol.* **182**: 41.3.
26. Robbins, P.F., M.E. Dudley, J. Wunderlich, *et al.* 2004. Cutting edge: persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. *J. Immunol.* **173**: 7125–7130.
27. Dudley, M.E., J.R. Wunderlich, P.F. Robbins, *et al.* 2002. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. *Science* **298**: 850–854.
28. Bernhard, H., J. Neudorfer, K. Gebhard, *et al.* 2008. Adoptive transfer of autologous, HER2-specific, cytotoxic T lymphocytes for the treatment of HER2-overexpressing breast cancer. *Cancer Immunol. Immunother.* **57**: 271–280.