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EDITORIAL

Biomarkers of Therapeutic Response in Melanoma and Renal Cell Carcinoma: Potential Inroads to Improved Immunotherapy

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The promise of biomarkers of response for melanoma and renal cell carcinoma is enormous, but to date, the potential remains largely untapped. High-dose interleukin-2 (IL-2) is the only regimen currently approved for the treatment of advanced melanoma, and it offers the prospect of prolonged disease-free survival for some patients. However, high-dose IL-2 is also one of the most toxic and difficult regimens ever developed for treatment of cancer. Hospitalization is required for supportive care. Although this regimen was approved by the US Food and Drug Administration a decade ago for treatment of stage IV melanoma on the basis of a collection of phase II trials, it has never been evaluated in a phase III trial. High-dose IL-2 is not offered at the majority of hospitals in the United States, and it is generally not even considered as therapy for most patients outside the United States.

An understanding of the pathogenesis of solid tumors and the mechanism of action of therapeutic agents has provided the foundation for advancement in the treatment of breast and colorectal carcinomas. To date, such effective targeted therapy has been elusive in treatment of melanoma. For patients with melanoma who are no longer surgically curable, the prospect of treatment with high-dose IL-2 offers hope. This hope is tempered by the knowledge that of 100 patients treated, only 16 will obtain objective remission, and of these, only six will be durable beyond 2 years. The situation in adjuvant therapy for treatment of melanoma is somewhat brighter; high-dose interferon alfa-2b (IFN-α-2b) benefits up to 33 of 100 patients treated in the postoperative adjuvant high-risk setting. For advanced melanoma, the same agent IFN benefits only 16% of patients, a figure remarkably similar to that for IL-2. On the basis of an understanding of the mechanism of action, biomarkers of response might allow us to select certain patients who have the capacity to respond to IL-2 or IFN-α and treat only the 16% of the population able to derive benefit. This prospect has been an impetus to the undertaking of high-throughput analyses of potential proteomic and genomic predictors of therapeutic response.

Analyses of the mechanisms of agents similar to IL-2, such as high-dose IFN-α, have been pursued in the study of tumor tissues before and after treatment. The impact of high-dose IFN-α therapy on signal transducer and activator of transcription (STAT) 1, STAT3, and STAT5 signaling and the modulation of immunologic responses by T cells and dendritic cells of patients with nodal metastasis have been demonstrated.[1-3] The study of lymphocytes in peripheral blood has also been informative. With high-dose IFN-α, reversals of STAT1 phosphorylation signaling defects have been found in 30% of patients with advanced disease.[4] This fraction of patients manifesting signaling defects is remarkably similar to the fraction deriving benefit from adjuvant therapy with high-dose IFN-α in the postoperative adjuvant setting. Blood serum is an even more accessible but previously unmined source of potential insight into mechanism. An analysis of corollaries of response to IFN by the Hellenic Oncology Group has recently demonstrated that autoimmunity directed at endocrine and other autoantigens correlates with the therapeutic benefit of IFN-α, and after 3 to 12 months of therapy, the appearance of signs of autoimmunity is associated with 50-fold reduction in frequency of relapse.[5] More recently, multiplex studies of the serum of patients treated in US Cooperative Group trials have demonstrated a correlation between long-term benefit from IFN-α and a profile of proinflammatory cytokines that may allow prediction of benefit before initiation of therapy.[6]

So what do we know about the subpopulation of patients responding to high-dose IL-2? Studies of tumor tissue in the context of high-dose IL-2 therapy have been undertaken using fine-needle aspiration biopsies, which have demonstrated a range of biologic effects, although these have not to date allowed the refinement of patient selection for IL-2 therapy.[7] The pursuit of fine-needle aspiration biopsies for tumor sampling is being undertaken only at specialized centers.

In this issue of *Journal of Clinical Oncology*, Sabatino et al[8] report the results of a proteomic analysis of the serum of patients (most of whom had metastatic melanoma) who were treated with high-dose IL-2 using a customized, multiplex antibody-targeted protein array platform to survey expression of soluble factors associated with tumor immunobiology. Soluble factors associated with clinical responses were analyzed using a multivariate permutation test, and survival outcomes were determined using Kaplan-Meier and log-rank tests. Customized Pierce SearchLight Proteome Arrays (Thermo Fisher Scientific, Rockford, IL) were used in assays of serum before and 3 hours after the fourth dose of IL-2; 16 proteins per well were measured in standard 96 well plates spotted with different monoclonal antibodies. A sandwich enzyme-linked immunosorbent assay technique was used to generate chemiluminescent signals for calculation of the levels of each analyte.

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In a training set of 10 patients, 68 of 110 analytes were identified as potentially relevant, and an independent validation set of 49 patients was then analyzed. Vascular endothelial growth factor (VEGF) and fibronectin were identified as independent predictors of nonresponse. In particular, high levels of these proteins correlated with lack of clinical response and decreased overall survival. These analyses suggested that maximal alterations in gene transcription occur early after IL-2 administration, resulting in significant alterations of cytokines and chemokines in the tumor microenvironment. To our knowledge, the impact of transcriptional changes on circulating proteins had not been evaluated before the study by Sabatino et al,[8] and changes in specific serum proteins before and after treatment revealed that high pretreatment VEGF and fibronectin levels were predictive of nonresponse to IL-2 and decreased overall survival. Responding and nonresponding patients were not separated in this assay. Combined cluster analysis of 10 potential predictors of response in the validation set was based on a multivariate permutation test. The number of differentially expressed proteins seemed to be higher than expected by chance among responders compared with nonresponders, with two sample *t* statistics for each protein. This analysis was repeated 10,000 times in the multivariate permutation test, and the proportions of the random replications that resulted in as many significant proteins as seen in the actual data were reported as the significance levels for the number of proteins. Significance of class prediction from unsupervised analysis was based on a Fisher's exact test or χ^2 test. VEGF levels not significantly modulated by IL-2 were included for the validation study on the basis of an exploratory P_2 value ($P_2 < .1$) trending toward significance when pretreatment values were compared between responding and nonresponding patients. After combining training and validation analyses, only VEGF was identified as a predictor of response to IL-2 therapy, although fibronectin maintained statistical significance after combining data sets, suggesting a potential role as an independent predictive biomarker.

To date, the majority of putative predictors of IL-2 response have been post-treatment variables, such as the height of rebound lymphocytosis, treatment-induced thrombocytopenia, development of autoimmune thyroiditis and vitiligo, and decreases in absolute number and frequency of peripheral T regulatory cells. In renal cell carcinoma, the level of expression of carbonic anhydrase IX in primary tumors has been found to be a potentially useful pretreatment predictor of IL-2 response, but prospective validation and an understanding of the mechanism through which carbonic anhydrase IX might predict clinical response to IL-2 remain to be elucidated.

Soubrane et al[9-10] have reported serum VEGF-A and VEGF-C levels to be higher in patients with high tumor burden compared with those in patients with low tumor burden.[11] Pretreatment serum VEGF-C levels have been shown to be higher in patients refractory to biochemotherapy (cisplatin, recombinant IL-2, and IFN-α) compared with those in responding patients. After treatment, serum VEGF-C levels were noted to have increased specifically in nonresponding patients with high tumor burden, whereas before treatment, serum VEGF-A levels were not significantly different between responders and nonresponders. On the other hand, after biochemotherapy, an increase in VEGF-A levels has been shown to be correlated with disease progression, whereas a decrease in serum VEGF-A levels is correlated with objective response to treatment, as assessed by WHO criteria.

In cancer tissues, in contrast to normal tissues, VEGF is produced not by endothelial cells but rather by tumor cells and/or the tumor stroma in a paracrine mode.[12-14] Therefore, one might expect higher serum VEGF levels in patients with higher tumor burdens. In their study, Sabatino et al[8] do not report a correlation of serum VEGF levels with tumor burden or previously reported parameters of response to IL-2, such as the site of metastatic disease. We do not have data on the molecular nature of the tumor tissues, which would be illuminating. Indeed, a retrospective analysis of 374 patients treated with high-dose IL-2 previously showed a correlation between the sites of metastatic disease and objective response rate (54% for patients with cutaneous or subcutaneous metastasis *v* 12% for patients with disease at other sites).[15] In addition, Sabatino et al do not report a correlation of serum VEGF levels with the serum lactate dehydrogenase, an established biomarker of tumor burden and poor outcome in melanoma that has already been incorporated into the sixth edition of the American Joint Committee on Cancer staging system for melanoma. It is possible that high serum VEGF levels are a marker of tumor burden and more advanced disease. Patients with advanced melanoma and high tumor burden benefit least from biologic therapeutic approaches, because of immunosuppression and the selective pressures in the direction of tumor immune escape. The most impressive results with immunotherapy have been obtained in the adjuvant setting after surgical resection of melanoma (with high-dose IFN-α-2b), and the role of VEGF and/or fibronectin levels in this setting has not been established but would be of interest.

The quality of the patient immune response has been shown to differ between early and advanced disease settings. T helper (Th) 1–type CD4+ antitumor T-cell function seems critical to the induction and maintenance of anti–cytotoxic T-cell lymphocyte (CTL) responses in vivo, and Th2- or Th3/T-regulatory–type CD4+ T-cell responses may subvert Th1-type cell-mediated immunity, providing a microenvironment conducive to disease progression. Patients with active melanoma or renal cell carcinoma have been shown to display strong tumor-antigen–specific Th2-type polarization. Normal donors and patients who were disease free after therapy demonstrated either weak mixed Th1- and Th2-type or strongly polarized Th1-type responses to the same epitopes.[16] Therefore, patient immune tolerance seems to impede therapy for advanced disease and may be less pronounced earlier in microscopic disease settings, in which susceptibility to immunologic intervention seems to be greatest.

To date, to our knowledge, no large adjuvant study of IL-2 has ever been undertaken, so for IL-2, this represents conjecture. However, the studies of the past several years focusing on IFN-α have been illustrative, and studies planned for anti-CTLA4 blocking antibodies in the adjuvant arena (Ipilimumab, Medarex and Bristol-Myers Squibb, Princeton, NJ) may offer the opportunity to examine factors that may predict response to this modality once again in the future.

Other analyses of pretreatment factors have correlated tumor response to IL-2 with Cw7 phenotype or low serum levels of IL-6 and C-reactive protein.[15,17] None of these factors has proven to be sufficiently predictive of clinical benefit to be useful in patient selection for IL-2 therapy. The future lies in ongoing and planned studies that will create models and define predictive biomarker signatures of responsiveness to specific therapeutic interventions (such as IFN-α-2b, anti-CTLA4 monoclonal antibodies, and IL-2) in individual patients on the basis of biomarkers selected through serologic, immunoproteomic, and immunogenomic studies.

The work of Sabatino et al[8] presented in this issue of *JCO* offers us the opportunity to refine our application of IL-2 in treatment of patients with metastatic melanoma and renal cell cancer, using serum biomarkers that are readily assessed to predict which patients are more likely to benefit from this modality. Studies of current immunotherapeutic agents, such as IL-2, IFN-α, and anti-CTLA4 blocking antibodies, may lead to a better understanding of the mechanism of action of these agents and guide selection of the appropriate populations of patients for therapy. This will improve on current therapy even if the agents at hand are no more effective overall than other agents have been in the past. The use of high-throughput protein array analyses for multiple potentially relevant biomarkers in the analysis of biomarkers of therapeutic outcome offers a range of new insights. These analyses may resolve single predictors or limited panels of predictive biomarkers, which may make it possible to individualize application of these therapies and help those patients who cannot benefit from them avoid exposure to the toxicities of these agents. This will improve the therapeutic index and acceptability of these regimens, which have been previously offered only to a few patients with advanced melanoma and renal cell cancer because of their toxicity.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Provision of study materials or patients: Ahmad A. Tarhini
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