Profiling of Immune Response to Guide Cancer Diagnosis, Prognosis, and Prediction of Therapy

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Introduction

The meeting was held to provide an overview of the most recent scientific achievements in the field of immunologically relevant biomarkers. These markers could be used to assess the risk of tumor recurrence or to predict the response to treatment, including immune-based therapies. Another focus was the emerging field of genetic variation in cancer and how these markers can influence clinical outcome. Finally, the meeting addressed issues relevant to study design, statistical approaches, and tissue resource availability. Improvements in these areas are crucial for clinical validation to facilitate the translation of biomarkers into the clinic.

In the introductory session, the controversial role of inflammation in cancer was discussed by Giorgio Trinchieri (NCI, Fredrick, MD), who emphasized the bipolar interaction between immune response and cancer. Animal and clinical studies repeatedly showed that the immune system can either foster tumor growth or induce regression. Inflammation will promote tumor development and progression when negative regulatory mechanisms predominate. Both tumor cells and the surrounding host cells can take part in this process. If the inflammation is chronic, it likely will support malignant growth. This was discussed by Lisa M. Coussens (University of California, San Francisco, CA) who presented elegant animal models strongly supportive of the role of adaptive, B cell–dependent immune complexes in facilitating neoplastic progression and squamous carcinoma growth, as well as T cell–dependent effects on macrophages that regulate pulmonary metastasis of mammary cancers. Because the inflammatory process that is associated with cancer progression is usually unique to the tumor microenvironment, immunotherapeutic intervention can selectively target tumors and the immune cells that infiltrate the tumors. Effective intervention should either elicit a strong immune stimulation or reduce tumor-related immunosuppressive factors to induce tumor regression. John Kirkwood (University of Pittsburgh, Pittsburgh, PA) emphasized the need of studying the immune response markers at time points that are relevant to the research question and the assessed clinical markers. Large clinical trials to evaluate the effectiveness of biological therapies have shown that long-term assessment of survival may provide the ultimate evidence of success or failure. However, these trials provide little insight into the mechanisms that caused the outcome. A need was identified for prospective accrual of biological materials at critical time points for assessments of therapy response. Remarkably, Kirkwood and coworkers observed that pretreatment, but not posttherapy levels of proinflammatory cytokines interleukin (IL)-1α, IL-1β, IL-6, tumor necrosis factor (TNF)-α, and chemokines macrophage inflammatory protein (MIP)-1α and MIP-1β, correlated with prolonged relapse-free survival in melanoma patients treated with interferon (IFN)-α.

Immune Response as Prognostic Signatures in Cancer

Although the presence of a malignancy implies a failure of the host response, many patients will develop an innate and adaptive immune response in the course of their disease. Thus, an analysis of the endogenous immune response to the tumor may yield both novel prognostic markers and insights into the mechanism of the tumor escape from the immune system.

It is now well-documented that T-cell infiltrates correlate with reduced incidence of recurrent disease and improved overall survival (OS) in patients with some types of cancer. Jérôme Galon (Institut National de la Santé et de la Recherche Médicale, Paris, France) highlighted the concept of a T-cell infiltrate prognostic signature in colon carcinoma (CRC). Comprehensive analysis using different platforms including quantitative reverse transcription-PCR (RT-PCR), large-scale cytometric analysis, and tissue microarrays in independent cohorts showed a strong correlation with adaptive T-helper 1 (Th1) polarization and a cytotoxic T-cell immune response with better prognosis in all CRC stages. Enhanced expression of CD8 effector T cell, CD45RO memory T cell, and adaptive Th1 cell markers (CD8, T-box transcription factor 21, IFN regulatory factor-1, IFN-γ, CD3-ζ, granulysin, and granzyme B) were associated with both longer disease-free survival (DFS) and OS. High density of infiltrating T cells within both the central region and the invasive margin improved the accuracy of the survival predictor when compared with a single-region analysis. Importantly, this system classified early stage [tumor-node-metastasis (TNM) stages I and II] patients into two subgroups, e.g., a poor prognosis group that would benefit from adjuvant therapy and another group that would not. Thus, these markers could improve prognosis independent of the Union Internationale Contre le Cancer (UICC)–TNM classification in CRC.

Accumulating evidence indicates the existence of negative immunoregulation that suppresses the innate and adaptive antitumor response and attenuates tumor-specific killing. The suppression of CD4+ and CD8+ lymphocyte and B-cell functions is mediated by regulatory T (Treg) cells, which comprise the minor population of CD4+ T cells that coexpress CD25 antigen (the IL-2R α-chain). High levels of CD4+CD25+ T cells have been identified in ovarian and other carcinomas as discussed by George Coukos (University of Pennsylvania, Philadelphia, PA). These tumor-associated Treg cells

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are also characterized by expression of the transcriptional repressor FoxP3. Increased numbers of intratumor Treg cells and FoxP3 content was significantly associated with tumor progression and worse outcome. Quantification of FoxP3 by either quantitative RT-PCR or immunohistochemistry may enable the identification of a specific patient subgroup ($Foxp3^{high}$) that would benefit from therapy modulating Treg cell function.

Contradictory to the findings in solid tumors, Treg cells were found to be predictors of improved survival in Hodgkin's lymphoma and follicular lymphoma. This discrepancy may be due to mixed populations of Treg cells that share markers with either memory or activated T cells, as discussed by Theresa Whiteside (University of Pittsburgh, Pittsburgh, PA). Treg cells are heterogeneous and can be divided into three different functional subsets (Tr1, $CD4+CD25^{neg}IL10+$ transforming growth factor β1+; nTreg, $CD4+CD25^{high}Foxp3^{high}$; and IL-4–dependent Th3 cells). Whiteside and colleagues showed that Tr1 and nTreg cell numbers increase with stage in head and neck cancer and could be a useful predictor of outcome.

The phenotypical and functional changes associated with immune suppression in lymph nodes of melanoma patients were discussed by Richard Essner (John Wayne Cancer Center, Santa Barbara, CA). Essner and colleagues found a significant association between cytokine expression and the tumor status of sentinel lymph nodes (SLN) downstream of residual primary melanoma. In metastasis-free SLNs, higher levels of INF-γ, IL-2, and granulocyte macrophage colony-stimulating factor (GM-CSF) were present when compared with non-SLN (NSLN). In patients with early stages of melanoma, SLNs expressed higher levels of the immunosuppressive cytokine IL-10 compared with either adjacent NSLNs or SLNs from patients without residual melanoma at their primary resection site. Moreover, tumor-bearing SLNs expressed significantly higher levels of other immunosuppressive cytokines and chemokines, e.g., IL-13, leptin, lymphotoxin receptor β, and MIP1β but significantly less of IL-11Rα. Data presented by Masahiro Seike and Anuradha Budhu (NCI, Bethesda, MD) also emphasized the value of cytokine profiling for outcome prediction in early-stage lung and in liver cancer. These results imply that selective postoperative adjuvant immunotherapies could be applied to restore regional and systemic tumor-directed immunity. Clinical trials with locally administered GM-CSF in melanoma are ongoing.

Identification of Predictors of Response

Michael Atkins (Beth Israel Deaconess Medical Center, Boston, MA) discussed the relationship between the expression of carbonic anhydrase IX (CAIX) and the response to immunotherapy in human renal cell cancer. CAIX expression by primary renal cell cancers was found to be significantly associated with an improved response to IL-2 therapy. Current data strongly suggest that the response to therapy and its relationship to CAIX are related at least in part to the tumor phenotype and not just to yet-to-be-defined factors within a patient's genetic background. Xiao-Song He (Stanford University, Stanford, CA) showed that the clearance of chronic hepatitis C virus infection after treatment with IFN-α can be predicted by an *in vitro* assay of lymphocytes stimulated with IFN-α. The levels of induction of IFN-stimulated genes (ISG) in this assay strongly correlated with treatment outcome. The assay also suggested a role of host genetic factors in the response to IFN-α. In particular, lymphocytes from African American individuals, who respond poorly to IFN-α therapy, had lower levels of ISG expression and less STAT-1 phosphorylation when compared with Caucasian patients. These data have potentially important implications for cancer patients to identify ISG associated with IFN-α treatment outcome. Francesco Marincola (NIH, Bethesda, MD) presented his strategy to study the active phase of the immune response in cancer patients using a serial noninvasive biopsy approach. By studying the dynamic phase of an immune therapy, he observed that tumor rejection is associated with the expression of ISGs and immune effector functions in natural killer cells. These cells show similarities to T cells that have been activated by both antigen exposure and secondary proinflammatory stimuli such as IL-2 or Toll-like receptor agonists. He also noted that the transcriptional signatures associated with tumor rejection in immunotherapy are almost identical to those described by others in the context of acute allograft rejection, flares of autoimmunity, and clearance of pathogens.

Common Genetic Variations as Biomarkers of Outcome

Although the association of genetic markers with disease risk has been intensively studied, few have evaluated the applicability of these markers to predict the response to therapy and disease outcome. Recent investigations have shown that common genetic variants, including polymorphisms in the mannose-binding lectin (*MBL2*) and the chemokine receptor 5 (*CCR5*) genes, can significantly alter host susceptibility to bacterial and viral infections. *MBL2* polymorphisms also influence lung cancer risk and survival, which was highlighted by Brid Ryan and Sharon Pine (NCI, Bethesda, MD). Other studies identified genetic variants that may account for a large fraction of the disease in the general population, e.g., single nucleotide polymorphisms in the 8q24 region and prostate cancer. These findings provide novel evidence for a causative link between interindividual genetic variations and human cancer.

Howard Kaufman (Columbia University, New York, NY) discussed the finding that a *CCR5* gene polymorphism affects the survival of melanoma patients receiving immunotherapy. A 32-bp deletion polymorphism (CCR5Δ32) in *CCR5* that abrogates receptor function occurs in Europeans at an allele frequency of ~10%. Kaufman reported that melanoma patients that carry at least one copy of CCR5Δ32 have higher survival rates than other patients in the unstratified analysis. However, significant association between CCR5Δ32 and poor outcome was observed in melanoma patients undergoing immunotherapy. The finding, although preliminary, could have implications for cancer therapy because it indicates that CCR5Δ32 and other functional polymorphisms in receptors with similar function may account for a substantial fraction of nonresponders in immunotherapy trials. Angela DeMichele (University of Pennsylvania, Philadelphia, PA) discussed her finding that a functional promoter polymorphism (−174G>C) in the *IL-6* gene is associated with breast cancer outcome. DeMichele and coworkers observed that homozygous carriers of the G allele, which encodes increased *IL-6* transcription, had poor DFS and OS. This association was influenced by the estrogen receptor status of the tumors. Possible mechanisms of the effect include increased aromatase expression and resistance to chemotherapy in carriers of the GG genotype. Wen-Kai Weng (Stanford University, Stanford, CA) described two IgG fragment C receptor (FcγR) polymorphisms that alter the response of B-cell lymphoma patients to Rituximab, which is a chimeric anti-CD20

monoclonal antibody. Rituximab selectively targets CD20-positive B cells by mechanisms that may include both antibody-dependent cellular cytotoxicity (ADCC) and complement-mediated cytotoxicity. Weng and coworkers discovered that the FcγRIIa His131Arg and the FcγRIIIa Val158Phe polymorphisms are independent predictors of therapy response and progression-free survival. These two FcγR polymorphisms have been shown to be functional as they decrease the affinity of some IgG subclasses to FcγR and affect therapy-induced ADCC. Thus, further optimization of the Fc portion of Rituximab could lead to enhanced affinity of the modified antibody to the FcγR variants and to increased clinical efficacy. Xifeng Wu (University of Texas, M.D. Anderson Cancer Center, Houston, TX) presented data from a large, systematic analysis of common genetic variants in bladder cancer and how the combined effect of these variants may influence cancer risk and disease outcome. Pursuing the hypothesis that inflammation-enhancing genetic variants are risk factors, these variants were found to be susceptibility markers for the disease by either their individual effects or by joint effects. Further analysis lead to the discovery of predictive markers among these variants that can classify patients with Bacillus Calmette-Guerin therapy into poor and good responders. Lastly, Julie Ellerhorst (University of Texas M.D. Anderson Cancer Center, Houston, TX) emphasized the significance of complement component C4 genotypes for disease outcome in metastatic renal cancer.

Clinical Evaluation: Are We Ready?

Potential barriers to marker development were discussed in the last session chaired by Sheila Taube (NCI, Rockville, MD). One potential barrier is the availability of clinical specimens that permit optimization and validation of new assays. Elizabeth Hammond (University of Utah, Salt Lake City, UT) discussed a key role of biospecimens in "translating" basic science discoveries into clinical application. Hammond stressed that although large numbers of specimens are available in various repositories, they differ in quality because standardized procedures for collection and storage are still not in place across the various collection sites. Efforts should concentrate on standardization and quality control. One of the biggest challenges in clinical research is availability of human samples that have been obtained at multiple time points relevant to disease stage and response to therapy. Ena Wang (NIH, Bethesda, MD) discussed the work that aimed at development of technologies for the collection, amplification, and analysis of material using minimally invasive strategies. She showed that the application of serial biopsy analyses could improve the interpretation of clinical results during the conduct of clinical trials. The importance of appropriate study design and sample size for marker discovery and validation in clinical trials was discussed by Kevin Dobbin (NCI, Rockville, MD). The examination of new prognostic markers in clinical investigations is frequently flawed because the study lacks a clear hypothesis or is statistically underpowered to reach valid conclusions. Common pitfalls in the interpretation of high dimensional profiling studies were discussed. It was concluded that efficient development of tumor markers will depend on future investigations with statistically valid study designs, better standardization of tissue resources, and assay refinement to provide data in a format that would allow interstudy comparisons.

Summary and Recommendations

The meeting highlighted the importance of the role that patterns of inflammatory cells, immune modulators, and variations in the host genetic background play in influencing clinical outcome in cancer patients. Immunologic criteria have been shown to be strong indicators of risk, prognosis, and response to treatment, and they could be routinely measured and included in the pathologic reports. However, future work is needed to establish and validate their value as markers to predict clinical outcome in well-designed prospective clinical trials. Efforts should concentrate on comprehensive analysis of lymphocytic reactions, including CD8+ T lymphocytes, myeloid cells, Treg cell responses, cytokines and chemokines, and functional genetic variations in immune regulatory genes that could identify groups with the best outcome in various malignancies. As these variables become proven clinically useful metrics, they could improve current measures of TNM staging criteria for prognosis and classification of a high-risk population of tumors influencing current strategies for effective cancer treatments. As therapy improves and therapeutic options increase, new markers for prognosis and for guiding individualized therapy will become more important in tumor management.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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