Transcriptional profiling of whole blood: a rich source of immune biomarkers in cancer

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Immunotherapy is at the forefront of cancer treatment, and biomarkers are urgently needed to predict patient response to therapy. Recently, we discovered a 4-gene peripheral blood mRNA signature for prolonged survival in patients treated with tremelimumab. Peripheral blood mRNA is a readily accessible and under-utilized source of clinically relevant biomarkers.

The tumor environment can suppress T cells and other immune cells through release of humoral factors and direct cell-cell contact. Malignant cells may also induce myeloid cells to secrete pro-angiogenic factors. These signals are strongest within the local tumor microenvironment. However, particularly in advanced tumors, the systemic immune system including peripheral blood leukocytes may also be affected. Peripheral blood has the advantage of being easily accessible in the clinical setting and is a practical source of biomarkers. Immune cells in the peripheral blood are migratory cells whose transcriptional profile may be influenced by the presence of tumor cells either residing in solid tissue as well as those present in blood. The transcriptional profile of peripheral blood may, in turn, reflect the degree of tumor-mediated immunosuppression and impact potential response to immunotherapy.

In our recent publication, “Blood mRNA Expression Profiling Predicts Survival in Patients Treated with Tremelimumab,” we identified a panel of 4 gene transcripts predictive of survival in patients treated with tremelimumab. Tremelimumab (ticilimumab, Pfizer), an IgG2 antibody that targets cytotoxic T-lymphocyte associated protein (CTLA-4), an inhibitory marker expressed on T cells. Tremelimumab has established activity in melanoma and is comparable in terms of toxicity and proposed mechanism of action to the FDA approved CTLA-4 blocking antibody, Yervoy (ipilimumab, Bristol Myers Squibb). Tremelimumab is currently being administered in experimental protocols as part of combination regimens for melanoma but it has not yet been submitted for FDA approval since it has yet to show superiority to dacarbazine. However, Yervoy was never directly tested against either dacarbazine or tremelimumab so the relative efficacies of the 2 antibodies remain unknown.

In order to identify a genomic signature of prolonged survival in patients receiving tremelimumab therapy, peripheral blood samples were obtained from 218 patients enrolled in a multi-center Phase II clinical trial of tremelimumab between 2005 and 2006. Reverse transcription polymerase chain reaction (RT-PCR) was performed on whole peripheral blood samples to quantify mRNA levels of 169 candidate genes selected on the basis of prior studies of gene expression in cancer patients, and on the known mechanisms of action of CTLA-4. We found that 4 genes, cathepsin D (CTSD), phospholipase A2 group VII (PLA2G7), thioredoxin reductase 1 (TXNRD1), and interleukin 1 receptor associated kinase 3 (IRAK3) predicted overall survival in this population. These results were then confirmed in a validation set of 260 patients enrolled in the treatment arm of a multi-center Phase III study comparing tremelimumab and chemotherapy between 2006 and 2007 (P = 0.001). Gene signature scores added significantly to standard clinical predictors in a multivariable model (P < 0.001). A limitation to this study is that unfortunately blood was not available from the control arm of the Phase III study so that the determination could not be made as to whether the 4-gene panel is a predictive or a prognostic marker (or both).

Importantly, this work (refer to Fig. 1) provides evidence to the feasibility of using genomic blood biomarkers to predict outcomes in metastatic melanoma. Expression levels of the 169 genes measured correlated very closely between the 2 populations at baseline. Further, relative expression levels of the genes between patients who survived more than 1 y and patients who survived less than 1 y were also closely matched. Thus, gene expression correlated reproducibly with outcome in 2 independent populations. Blood is a readily accessible source of biomarkers, and relatively small amounts are sufficient
for RNA extraction. Thus, peripheral blood seems a very promising resource for future research seeking to define novel, clinically applicable biomarkers.

Biomarkers are urgently needed to predict responsiveness to immunotherapy. Although immunotherapy does not have the toxicities of conventional chemotherapy, the potential for severe life threatening auto-immunity makes CTLA-4 blockade difficult to tolerate. Further, most patients do not respond or respond only transiently to CTLA-4 blockade, so that targeted patient selection would provide greatly improved clinical benefit. Antibodies targeting programed cell death 1 (PD-1) have a more favorable risk benefit ratio but many patients progress or respond only transiently and treatment can induce severe auto-immune toxicity, particularly if given in combination with ipilimumab. Prior studies have identified many immune correlates in the blood of patients responding to Yervoy®, including alterations in the numbers, phenotype, and clonal distributions of CD4+ and CD8+ T cells. There is likewise evidence that characteristics of the immune microenvironment within the tumor context may predispose particular patients to beneficial responses to immunotherapy. A biomarker defined based on sampling the immune milieu in peripheral blood based on gene expression studies prior to initiation of therapy is conceivable and would be ideal for clinical applications.

RNA in whole blood is likely primarily reflective of the transcriptional activity of peripheral blood leukocytes. Our research is thus in line with reported findings that alterations in peripheral blood suppressor myeloid cell populations correlate with, and are predictive of, response to CTLA-4 blockade. Furthermore, the genes identified in our panel to correlate inversely with survival, CTSD and IRAK3, have established roles in cancer growth and immunosuppression. IRAK3, is primarily expressed by myeloid cells and suppresses toll-like receptor signaling, diminishing the perception of “danger signals” by the immune system. CTSD is expressed by most mammalian cells and is overexpressed in melanoma. Similar to vascular endothelial growth factor (VEGF), another proposed marker for poor response to both Yervoy® and IL-2, CTSD favors angiogenesis and metastasis.

As the paradigm in cancer treatment shifts toward immunotherapy, understanding the complex interplay between cancer cells and the host immune system becomes increasingly critical. We know that this interaction occurs and is reflected by changes in phenotype and activation status of peripheral blood leukocytes. Assessing gene expression is a straightforward way to assess the systemic immune milieu and may be relevant to the development of clinically useful predictive biomarkers, both those specifically for CTLA-4 blockade as well as markers more broadly relevant to immunotherapeutic regimens in general.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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