Emerging Immunologic Biomarkers: Setting the (TNM-Immune) Stage

Janis M. Taube

The cooperation of tumor-infiltrating lymphocytes and tertiary lymphoid tissue in early-stage colorectal carcinoma further corroborates the strong immune influences on tumor progression and patient outcome. Immune factors in the tumor microenvironment may warrant inclusion in pathology reports and staging systems for prognostication and prediction of therapeutic response. *Clin Cancer Res; 20*(8); 2023–5. ©2014 AACR.

In this issue of *Clinical Cancer Research*, Di Caro and colleagues from the Department of Immunology and Inflammation in Rozzano, Italy, explore the relationship between tumor-infiltrating lymphocytes (TIL), tertiary lymphoid tissue (TLT), and patient outcome in colorectal carcinoma (1).

The most common classification system for the staging and prognostic significance of cancer is the American Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC) tumor–node–metastasis (TNM) classification. The current system stratifies for patient survival, though patients within single TNM stages may demonstrate a range of clinical outcomes, indicating the potential for further refinement. In the current system, histologic features such as the depth of tumor invasion are used to predict disease progression, which is a "tumor-autonomous assumption" (2). Conspicuously, the potential role of the local tumor microenvironment in promoting or curtailing tumor progression is not represented (3). Furthermore, although the TNM stage of a patient is often used to drive treatment decisions, the current system does not incorporate factors that may predict response to therapy.

The presence of TIL, specifically those with cytotoxic, memory, or Th1 phenotypes within a tumor, is recognized to be of positive prognostic significance in a broad range of solid tumor types, including colorectal carcinoma, melanoma, and non–small cell lung carcinoma (NSCLC; ref. 4). In stage I–III colorectal carcinoma, the spatial organization (centrally within tumor parenchyma and at the peripheral invasive edge where the tumor interfaces with stroma) of specific TIL subsets has been shown to have greater prognostic significance than TNM stage (5). These findings have led investigators to advocate for the inclusion of an “Immuno-score” indicating the presence of specific immune infiltrates as a component of new tumor classification schemes. Such a TNM Immune system (TNM-I) system is currently being validated by an international consortium (2).

In addition to substantiating the relationship of TIL and better prognosis in early-stage colorectal carcinoma, Di Caro and colleagues studied the impact of TLT on patient outcome. The relationship between the presence of tertiary lymphoid tissue and improved patient outcome was first described in NSCLC (6). TLT is an organized structure, consisting of B-cell dense follicles, supported by interfollicular dendritic cells, and surrounded by T-cell zones. The presence of high endothelial venules (HEV) in these structures has also been noted, and these entities are thought to facilitate recruitment and subsequent T-cell activation and differentiation for a sustained antitumor adaptive immune response (6). Di Caro and colleagues performed a standardized quantitative analysis of area occupied by TLT at the invasive margin compared with the total digitized tumor surface. They identified TLT in 79% of 351 different specimens analyzed, and demonstrated that like dispersed TIL, having a high density of TLT was associated with better prognosis in patients with stage II disease.

Some of the most interesting findings from Di Caro and colleagues emerged when they looked at the relationship between TIL and TLT. They found that the density of TLT at the invasive margin correlated with the density of dispersed TIL only in patients who did not experience relapse, suggesting that a clinical benefit requires synchronization of these two components. The authors went on to provide additional evidence that TLT mediates the recruitment of lymphocytes to the local tumor microenvironment in a murine model of inflammation-mediated colorectal carcinogenesis. They adoptively transferred GFP-labeled splenocytes and demonstrated that after 24 hours, the GFP-labeled cells localized to TLT in the azozymethane and dextran sulfate sodium (AOM/DSS) mice, while very few were seen in the lymphoid tissue of control mice. In addition, they demonstrated increased HEV density within the TLT of AOM/DSS mice compared with lymphoid tissue in control mice.
further supporting the notion that these structures facilitate lymphocyte recruitment. The supporting role of TLT in the local immune response has been inferred (6, 7), and this report now provides functional evidence for the role of these tertiary lymphoid structures in providing access for TIL into the tumor microenvironment. Although the presence of TLT implies the local generation of a specific antitumor immune response, it is still unclear what proportion of the response is actually generated at this site versus another location, such as a draining lymph node. It also remains to be determined whether the B-cell component of TLT indicates a humoral contribution to the immune response.

A better understanding of the tumor–host interaction also has a number of implications for cancer immunotherapy. A growing body of evidence suggests an association between an inflamed tumor environment and response to immunotherapies, including cancer vaccines and checkpoint blockade. This makes sense mechanistically, as many of these agents, for example, anti-PD-1, likely protect or potentiate an ongoing immune response, as opposed to generating a *de novo* antitumor immune response (8). Patients whose tumors contain TLT may be better positioned to respond to this novel class of agents because their tumors can support trafficking of TIL. Many features of the immune contexture in tumors are being investigated for their relationship to therapeutic response (9), and the findings of Di Caro and colleagues support the inclusion of TLT as one of these features.

These findings also call attention to tumors with a non-inflamed phenotype. As Di Caro and colleagues have shown, patients with early-stage disease who relapsed were more likely to have low TIL and TLT densities. These tumors may avoid the immune system through immune exclusion or ignorance and may require additional intervention to generate an inflamed phenotype (10). Tumors lacking TLT likely have less local capacity for T-cell trafficking, broad tumor antigen exposure, and rapid response to potential tumoral antigen shift, resulting in less efficient and effective antitumor immunity. It is also not evident whether current immunotherapeutic regimens demonstrate significant activity in tumors lacking activated TIL. Thus in this group of patients, therapies may need to be sequenced. For example, traditional cytotoxic agents, targeted inhibitors, or cancer vaccines may be used first to induce the presence of activated TIL and even TLT (10, 11). Once the tumor has established a gateway for T-cell influx and subsequent priming, an immunotherapeutic regimen such as checkpoint blockade may be administered to protect the resultant immune response.

In the era of personalized medicine, it is likely that a parameter representing the immune microenvironment will be included in future surgical pathology reports and staging systems (Fig. 1). Many of the original findings leading to the current Immunoscore were generated in studies of colorectal carcinoma. The functional significance of various immune cell subsets in different cancer types will...
require additional investigation, as early studies indicate that some immune cell subsets, such as regulatory T cells, have ambivalent prognostic importance in different tumor types (4). In addition, the current Immunoscore focuses on the host’s adaptive immune response to tumor, and does not include features of potential adaptive immune resistance by tumor, such as PD-L1 expression, which could protect tumors from immune destruction (8). Other important practical considerations include how best to characterize the local immune milieu within tumors, i.e., with routine hematoxylin and eosin review of TIL grade (8, 12), by immunohistochemical analysis of immune cell subsets, and presence of ILT (2, 4, 5), or molecularly using gene expression signatures (4, 9). Thresholds will have to be determined as well as standardized methods for quantification that translate across institutions. Once these issues are addressed, however, the AJCC/UICC staging system, at least for specific tumor types, may indeed transition to TNM-I.

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