

# A “Big Data” View of the Tumor “Immunome”

Nicholas P. Restifo<sup>1,2,\*</sup>

<sup>1</sup>Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

<sup>2</sup>Center for Regenerative Medicine, National Institutes of Health, Bethesda, MD 20892, USA

\*Correspondence: [restifo@nih.gov](mailto:restifo@nih.gov)

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**Tumors are complex masses containing not just neoplastic cells but also stromal cells, neovasculature, and a gamut of immune cells. In this issue, Bindea et al. (2013) identify a surprising new “immune landscape” associated with prolonged disease-free survival.**

The amount of data that is available to every member of society with a high-speed Internet connection has increased at such a rate that it boggles the mind. One recent estimate is that the amount of data coursing through the global Internet in 2013 will amount to more than 500 billion gigabytes of information. This represents an increase of 10-fold over the amount of data in the past 5 years alone. This “Big Data Revolution” is likely to affect fields as diverse as weather forecasting and crime fighting, and the conduct of immunobiology is certainly no exception.

Bindea et al. (2013) have used this newfound ability to generate and to manipulate large amounts of data from varieties of sources to cast new light on the tumor microenvironment. In this issue of *Immunity*, they offer an extraordinarily high-resolution view of the immune cells found within surgically excised tumor masses.

It has long been appreciated that established tumors are complex masses that contain not only neoplastic cells but also nontransformed cellular elements such as stromal cells, the neovasculature, and a gamut of immune cells. Tumor masses have been compared to rogue organ systems that are comprised of collections of cells that compete with each other and with normal tissue in a Darwinian struggle for survival (Khong and Restifo, 2002). The complexity of these constantly evolving systems is apparent, and elucidating all of the cellular constituents—let alone their interactions with each other—is well out of reach of even the most sophisticated teams of pathologists.

Human tumors are triggered by a myriad of causes and evolve over periods measured in years and decades, and they are not easily modeled in animals. Individual scientists pursuing reductionist

approaches can provide informative narratives about particular cells, pathways, and molecules, but a full and unbiased embrace of the complexity of the human system provides a much-needed bird’s-eye view of this ongoing research. The development of high-throughput techniques such as whole-genome analysis, multicolor immunohistochemistry, and multiparameter flow cytometry—along with the bioinformatics tools useful for analyzing the resultant data—has put a high-resolution view of tumor complexity within reach.

Previous work from Galon’s group has elucidated the major importance of cytotoxic and memory T cells in determining disease-free survival in cohorts of patients (Galon et al., 2006; Pagès et al., 2005). The authors have cogently argued that a patient’s “immunoscore,” based on the quantification of specific patterns of immune activation in the tumor microenvironment, can yield information that is highly associated with patient prognosis. Currently, patients with colorectal cancer are usually given prognoses based on a staging system established by the American Joint Committee on Cancer (AJCC). This system is called the TNM system (for tumors, nodes, metastases) because it employs measurements such as the degree of invasion of the intestinal wall, the amount of tumor involvement of nearby lymphatic structures, and the presence or absence of distant metastases, to assign patients to stages, numbered I–IV. However, reports from the Galon group have used Cox multivariate analysis to claim that the predictive value of the immunoscore exceeds that of the TNM system currently in place (Mlecnik et al., 2011).

These data are consistent with a plethora of observations from other studies (Fridman et al., 2012), and in addition, they fit with findings that clinically

effective immunotherapies to date all depend on these endogenous T cells. Approaches to cancer immunotherapy that result in objective tumor regressions include the adoptive transfer of tumor-infiltrating lymphocytes (Restifo et al., 2012) as well as antibodies to immune “checkpoints” CTLA4 and PD1-PDL1 (Topalian et al., 2012; van Elsas et al., 2001).

Here, Bindea et al. (2013) use systems-biology approaches to quantify 28 different immune cell subpopulations (both innate and adaptive) in situ in human tumors. This approach relies on analysis of whole-genome expression; the analysis is backed up with PCR arrays, multicolored immunohistochemistry, and even mouse models. The authors have previously identified the importance of cytotoxic and memory T cells, but what is substantially new in the present data set is the addition of key roles identified for T follicular helper (Tfh) and B cells via microarrays and immunohistochemistry, and these new data add significantly to the algorithms used for predicting patient disease-free survival. These particular immune cells are identified and quantified with a set of leukocyte-subset-specific signature genes that were obtained by the use of highly purified, flow-sorted cells.

The authors confirmed these data by using highly sensitive quantitative PCR to further investigate the expression of 81 relatively cell-specific genes, most of them encoding membrane receptors, to study 153 patients with colorectal cancer. Using this approach, the authors found that the genes identifying the “adaptive immune clusters,” defined here as T, B, and Tfh cells, were highly correlated with patient disease-free survival. Specifically, patients with elevations in genes related to MHC-II, B cell costimulation, T cells, and Tfh cells experienced increased

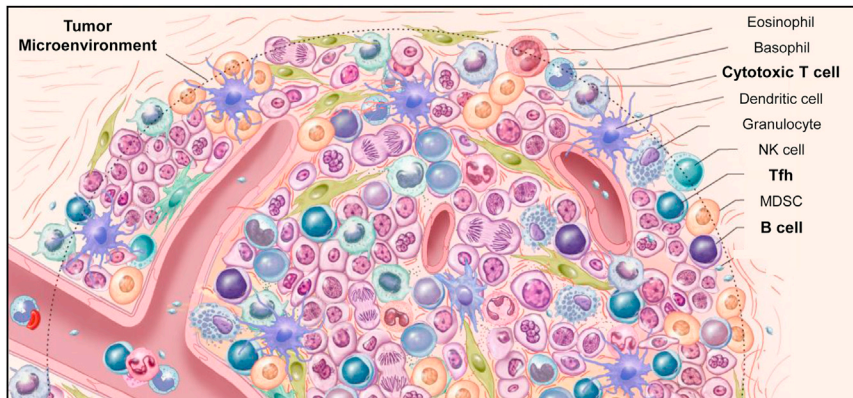


**Figure 1. Immune Cells in a Tumor**

Tumor masses are complex mixtures of cells that contain stromal cells, vasculature, and a wide array of immune cells. An unsupervised correlation matrix of the intra-tumor “immunome” reveals that, of the 28 cell types that were specifically analyzed, CTL, Tfh, and B cells (shown in bold) had the strongest correlations with increased disease-free survival, and these cell types were furthermore correlated with each other.

survival, whereas there was no significant correlation with MHC-I- and inflammation-related genes. Furthermore, the expression of markers for Tfh cells and that of markers for B cells were correlated with each other in an unsupervised analysis. Importantly, the pattern changed over time. With the exception of Tfh cells, most T cell subpopulations marked by CD8, CD45RO, CD57, and FOXP3 ebbed with tumor progression. In contrast, the density of B cells increased with tumor stage, as did the density of innate immune cells such as neutrophils, mast cells, iDCs, pDCs, and macrophages.

Underpinning the current conclusions was the finding that one chemokine in particular, CXCL13, and one cytokine, IL-21, were found to be critical and positively correlated with disease-free survival. Critics might complain that these conclusions are based on vignettes of large amounts of primary data, and still unresolved is the clear and rigorous delineation of a “training set” and a “test set.” Despite these shortcomings, the authors deploy considerable bioinformatic and statistical knowhow in the form of their CluePedia and ClueGO software (Bindea et al., 2013). More importantly for immunologists, they also show how the major components of their findings exist in plausible immune networks (Figure 1). In addition, the authors find that a tumor mechanism (chromosomal deletion of CXCL13) is associated with a decrease in CXCL13 production and decreased densities of Tfh and B cells.

Patients with more Th17 cells trended toward having a worse prognosis. It was

perhaps striking that there was a notable lack of evidence in these new data sets for a major negative role of immune cell subsets that classically mediate immunosuppression. For example, T regulatory cells were not predictive in this data set. Cells that might be designated as myeloid-derived suppressor cells (MDSCs), such as monocyte populations, were not specifically looked at, although neutrophils, macrophages and dendritic cell subsets, each of which might have components deemed to be MDSCs, were notably absent as prognostic predictors. Thus, these negative cellular influences did not proclaim themselves as predictors of negative outcomes in these patient cohorts, a surprising finding given the wealth of data indicating the importance of cellular immunosuppression in the tumor microenvironment in animal models.

The authors assemble a large amount of information in an effort to visualize the “immune landscapes” that are associated with progression and recurrence of colorectal cancer in their patient cohorts. Changes in the immune landscape help us to understand the evolution of the immune response that the authors observe with tumor progression. Given these new findings, one might conclude that immunotherapies involving Tfh cells and IL-21 might be of great interest. Based on recent work from a diversity of research laboratories, these suggestions are intriguing.

Tfh cells are a CD4<sup>+</sup> T cell subset that constitutively expresses CXCR5, a G protein-coupled seven-transmembrane receptor for CXCL13 (the central chemo-

kine identified in this analysis). Tfh cells might be involved in the formation of secondary lymphoid structures that can be visualized in some human tumor samples. Tfh cells also trigger the secretion of IL-21, a key component of the immune signature found here. In addition to helping to form follicular structures, IL-21 through its action via STAT3 might be critical in retaining tumor-stimulated CD8<sup>+</sup> T cells in less-differentiated and more potent states (Hinrichs et al., 2008).

There seems little doubt that careful quantification of tumor-infiltrating immune cells can help determine a patient’s prognosis in colorectal cancer, and this alone represents a major advance. It is still too early to know whether this detailed information about the tumor immune microenvironment can be used to guide the treatment of patients with cancer. Nevertheless, “big data”—carefully crunched with innovative software tools—might ultimately serve as a great illuminator of the biology of the antitumor response and thus spur new and more effective cancer immunotherapies.

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