Commentary

Immunoscore *vs*. Microsatellite instability as prognostic biomarkers in colorectal cancer: who wins?

Constantin N. Baxevanis

Cancer Immunology and Immunotherapy Center, St Savas Cancer Hospital, Athens, Greece

Correspondence to: Constantin N. Baxevanis, Ph.D. Cancer Immunology and Immunotherapy Center, St Savas Cancer Hospital, 171 Alexandras Ave. 11522 Athens, Greece. Email: baxevanis@ciic.gr.

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Colorectal cancer (CRC) incidence rates decreased since 2001 due to improved screening tests and treatments (1,2). However, CRC still remains one of the main causes of cancer-related deaths world-wide (2). Thus, improved knowledge derived from ongoing research is required to achieve further improvements. Retrospective analyses of large CRC patient cohorts have identified specific patterns of immune activation in the tumor microenvironment which were associated with patients' overall survival. The type, density, and location of immune cells intratumorally established the Immunoscore (IS). The IS combined with the expression of genes encoding T helper 1 (Th1) cytokines (IFNγ, IL2) and cytotoxic mediators (granzymes, granulysin) delineate the immune contexture (IC). Both, the IS and IC have been generally associated with favorable clinical outcome, supporting a major role of T-cell-mediated immunity to restrain tumor progression (3-6). Studies have shown that the abundance of tumor-infiltrating T cells is associated with specific molecular features of colorectal tumors, including those with high-level microsatellite instability. Microsatellite-instable (MSI) tumors, which are characterized by the loss of DNA mismatch repair activity, are to be found in about 15% of all CRC patients; 3% of MSI tumors are associated with Lynch syndrome and the other 12% are caused by epigenetic hypermethylation of the promoter of the MLH1 gene (7). MSI patients have high immune infiltrates and immune-related gene signatures, and a better prognosis than patients with microsatellite-stable (MSS) colorectal tumors (7). The former group of patients also show highly increased mutation rates and expression of immunogenic frameshift neuropeptides. This may explain the extensive infiltration of the tumor by activated neoantigen-specific T cells, resulting in an anti-tumor immune response and enhanced patient survival in MSI patients in contrast to MSS patients (6,8,9). Nevertheless, so far there is no integrated study to assess the prognostic significance between intratumoral genomic alterations and immune patterns with the presence or absence of MSI in patients with CRC.

In a recent tissue of Immunity, Mlecnic et al. performed a comprehensive analysis of the tumor microenvironment, immune gene expression and mutational status in CRC patients in relation to their microsatellite status (10). They also characterized the presence of preexisting tumor-reactive T lymphocytes within CRC tumors, relative to MSI status of the tumor as well as to the type of the intratumoral adaptive immune response and made correlations with patients' clinical outcome (10). The authors could identify a high number of genes which were upregulated in MSI compared to MSS tumors. These genes were mainly associated with antigen presentation pathways, IFNγ signaling, Th1-related cytokines, chemokines and chemokine receptors and leucocyte migration. Moreover, MSI tumors *vs*. MSS tumors, had a higher infiltration of cells and effector molecules which are associated with Th1-immunity, including cytotoxic cells, granzymes, Th1 and T follicular helper cells, dendritic cells and neutrophils. Nevertheless, these differences in immune-related gene expression as well as in immune infiltrates were not absolute, since there were MSI tumors which had low expression of these immune markers (similar to MSS tumors) and, vice versa, there were MSS tumors which expressed these genes at high levels similar to MSI tumors. The clinical follow-up in these patients revealed that disease-free survival (DFS) was dependent on the levels of expression of the immune signatures, irrespective of the MSI or MSS

status. In the same lines, clinical outcome analyses based on IS and microsatellite status, revealed that patients with high infiltration by cytotoxic and memory cells (i.e., with IS3 and IS4) had significantly improved DFS, and overall survival (OS) irrespective of having MSI or MSS tumors. More important, the IS remained significant in multivariate analysis for clinical outcome, in marked contrast to clinicopathological parameters and microsatellite status. High immune infiltrates were associated with high mutational rates in MSI tumors, resulting in immunoediting. This was derived from a lower number of neoepitopes for frameshift and missense mutations to what was expected and this decrease was more pronounced in MSI *vs.* MSS tumors. The authors could also isolate peripheral T lymphocytes from HLA-A2.1+ patients specifically producing IFNγ in response to a mutated TGFBR2 neoepitope.

Neoantigens possess a central role in tumor immunity acting both, as cancer response predictive biomarkers and as potent tumor-specific immunogens. There is now increasing evidence to suggest that immunogenic tumors harbor higher rates of mutations and consequently increased numbers of neoepitopes. There are several studies to demonstrate that the numbers of mutations as well as of neoantigens per tumor correlate with the response to immune checkpoint-based immunotherapy in melanoma, NSCLC, and colorectal cancers with MSI (11). However, when characterizing common neoantigens between clinical responder patients, van Allen et al. found that neoantigen epitopes which were associated with clinical benefit were private events without recurrent features, suggesting that no single common antigen or mutation correlated with clinical benefit (12). Thus, the best option for determining clinical benefit from immune checkpoint inhibitors will mostly derive from integrative analyses combining exome and transcriptome analyses with tumor immunogenicity and immune infiltrates. The work by Mlecnik et al., has come to confirm exactly this, namely that response to immunotherapies depends on genomic and immune signatures comprising the frequency and number of mutations as well as the strength and the type of intratumoral network immune interactions and not just from the presence of individual mutations. To this end, by analyzing MSI *vs.* MSS tumors from CRC patients they provided convincing evidence to show (I) an association between high mutational load with increased numbers of effector memory T lymphocytes in MSI tumors; (II) that genomic and immune alterations in MSI tumors are tightly connected with an active preexisting adaptive immune response by demonstrating a more frequent immunoediting against neoantigens in MSI tumors; (III) that WNT/β-catenin mutations do not influence immune gene signatures or immune infiltrates and are to be found at similar frequencies among tumors irrespective of their microsatellite status. Importantly, the authors could also demonstrate a direct correlation between PD1/PDL1 expression with the Immunoscore in tumors which was independent of their microsatellite status. As expression of immune checkpoints intratumorally is associated with preexisting T cell immunity to tumor-specific neoepitopes (11,12), the authors proposed that CRC patients with increased Immunoscores (i.e., IS3 and IS4) will most likely benefit from immune checkpoint therapies independent of their microsatellite status.

Surely, there are multiple factors which may influence tumor evolution as well as therapy-induced clinical outcomes. Indeed, the tumor microenvironment is composed by various interacting cell populations which generate a complex network of cytokines and chemokines with their specific receptors, all of which can induce dramatic changes in gene-expression profiles and immune infiltration (13,14). Therefore, it will be important to perform integrated analyses encompassing genomic and epigenetic alterations combined with detection of immune-related signatures and characterization of immune infiltrates in order to be able to have a comprehensive picture of the dynamic changes which occur within the tumor microenvironment. By performing such types of analyses, Mlecnic et al. shed more light in our understanding of the interaction between immune lymphocytes and malignant cells in the microenvironment of colorectal tumors. The authors demonstrated that high mutational loads and high levels of immune infiltrates within MSI tumors result in immunoediting, which is to understand from the drop in the number of neoepitopes per mutation, from what was anticipated. In addition, CRC patients had functionally active T cells recognizing such neoepitopes representing natural immunity against tumor-specific mutated antigens. Although intratumoral preexisting T cells specific for the cancer may be turned off by adaptive immune resistance (15), blocking antibodies to PD1 or PDL1 can reverse this situation providing efficient immunotherapy modalities for cancer treatment. Given the correlation between PD1/PDL1 expression and the Immunoscore and considering that early stage cancers appear more frequently to have high Immunoscore vs. late stage cancers, the authors suggested that the patients at an early stage will mostly benefit from immune checkpoint therapy.

Thus, although this study does not question the role of MSI status in the tumors of CRC patients as a marker for increased immune infiltrates, higher frequencies of frameshift mutations, and favorable clinical outcome, still it demonstrates that a significant number of patients with MSS having high Immunoscore , behave similarly. In the same lines, patients with MSI-positive tumors but with low Immunoscore have unfavorable clinical outcome. These data emphasize the utmost important role of preexisting adaptive immunity, which comprises Immunoscore, as

a stronger predictor of CRC patient survival than MSI. Moreover, the results from this study place emphasis on the role of preexisting immunity as predictive biomarker for the clinical outcome of immunotherapies mostly based on immune checkpoint inhibition and propose that for successful anti-tumor immune effect, the activation of the immune system at the level of adaptive immune response will be important. Thus, immunotherapeutic protocols effectively reinvigorating preexisting antitumor T cell immunity may be quite successful by delaying or even preventing primary tumors to disseminate and develop metastases.

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Footnote

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