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Inhibiting the inhibitors
Checkpoints blockade in solid tumors

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Abbreviations: APC, antigen-presenting cell; CTLA4, cytotoxic T lymphocyte-associated protein 4; FDA, US Food and Drug Administration; HCV, hepatitis C virus; PD-1, programmed cell death 1; Treg, regulatory T cell

It has been known for many years that the immune system can actively respond to (pre)neoplastic cells and prevent oncogenesis (a concept known as cancer immunosurveillance).[1] However, in most cases, antitumor immune responses are inefficient and neoplasms are allowed to establish. Indeed, effector T cells often infiltrate developing malignant lesions and may significantly influence the prognosis of the disease,[2,3] but are frequently functionally impaired.[4] As compared with circulating T cells or T cells infiltrating healthy tissues, these cells (1) secrete reduced amounts of effector cytokines, (2) exhibit limited proliferative capacity, (3) express low levels of pro-survival receptors, and (4) display a terminally differentiated phenotype. Multiple mechanisms have been associated such a functional impairment, most of which can be reconducted to the immunosuppressive nature of the tumor microenvironment.[5] Malignant cells actively secrete cytokines, metabolites and other molecules that directly inhibit the function of antitumor T lymphocytes. In addition, cancer cells can recruit immune cell populations that are capable of hindering effector responses. These include, among others, $CD4^+CD25^+FOXP3^+$ regulatory T cells and myeloid-derived suppressor cells, which mediate immunosuppressive effects via contact-dependent as well as contact-independent mechanisms.[5] Thus, neoplastic cells as well as immunosuppressive cells of the immune system express multiple inhibitory ligands on their surface that engage cognate receptors on T lymphocytes.

The CD28 family of co-receptors plays a major role in the regulation of immune responses, mostly as they deliver co-stimulatory or co-inhibitory signals to T-cells that have recognized a peptide/MHC complex on the surface of an antigen-presenting cell (APC).[6] Besides CD28, which operates as a major co-stimulatory receptor upon binding to CD80 (also known as B7-H1) or CD86 (also known as B7-H2) on APCs, this family include cytotoxic T lymphocyte-associated protein 4 (CTLA4) and programmed cell death 1 (PDCD1, commonly known as PD-1). CTLA-4, which also interacts with CD80 and CD86, is expressed on the surface of activated T cells (including Tregs) and delivers co-inhibitory signals. Along similar lines, upon interaction with its main ligands (CD274, also known as PD-L1, and PDCD1LG2), PD-1 negatively regulates multiple T-cell functions, including their activation, proliferative potential and ability to secrete effector cytokines. The most prominent physiological role of these molecules is to limit T-cell responses and avoid autoimmune diseases.[6] However, the elevated expression levels of CTLA4 and PD-1 on cancer cells led to the hypothesis that these factors could underlie a major mechanism of immune escape mediated by the inhibition of effector T-cell responses.[7]

Chronic antigenic or inflammatory stimulation induces the expression of CTLA4 and PD-1 on the surface of T cells, as demonstrated in individuals chronically infected with HIV-1 and the hepatitis C virus (HCV).[8] Similarly, these receptors are highly upregulated by tumor antigen-specific T cells, resulting in the robust impairment of effector functions. In line with this notion, the blockade of CTLA4 and PD-1 with specific monoclonal antibodies has been shown to promote tumor regression in multiple preclinical models as it stimulates the function of tumor-specific $CD4^+$ and $CD8^+$ T cells.[7] CTLA4-targeting interventions also boost immune responses by simultaneously interfering with the inhibitory activity of Tregs.[7] These data strongly suggested that the blockade of CTLA4 and/or PD-1 could offer a valid approach for the treatment of a wide variety of cancers.[9]

Monoclonal antibodies are nowadays widely employed in anticancer immunotherapy.[10,11] In 2 clinical trials published in June 2012, 2 different monoclonal antibodies targeting PD-1 or PD-L1 were given to patients affected by different solid tumors, including neoplasms that are generally refractory to immunotherapy such as lung carcinoma.[12,13] Both antibodies exhibited significant clinical activity as they induced tumor regression (objective response rate of 18–27% and 6–17% for PD-1- and PD-L1-targeting antibodies, respectively) or stabilized disease progression. Importantly, none of the patients with $PD\text{-}L1^-$ tumors receiving anti-PD-1 antibodies manifested an

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objective response, suggesting that the expression of PD-L1 on the surface of cancer cells constitutes a robust predictive biomarker that may allow clinicians to implement personalized therapeutic approaches.[13]

In a recent Phase I clinical trial, melanoma patients were treated with the hitherto experimental anti-PD-1 antibody nivolumab plus ipilimumab, a CTLA4-blocking antibody approved by US Food and Drug Administration (FDA) for use in this oncological indication in 2011.[14] The authors reported an objective response rate of 40% and evidence of clinical activity in 65% of the patients. In spite of the fact that up to 53% of patients experienced Grade III-IV adverse events (although reversible, for the vast majority), some degree of tumor regression could be observed in more than 80% of cases.

These results lend strong support to the notion that the dormant immune system can be awaken by the simultaneous inhibition of distinct immunological checkpoints. High-content single-cell analysis of the phenotype and functionality of the immune cell subpopulations involved in such a reactivation will provide further insights into the effector mechanisms that mediate cancer immunosurveillance in humans. Given the large number of functions regulated by CTLA4 and PD-1, these mechanisms are likely to involve multiple cell types. Currently, a number of biological agents including immunostimulatory cytokines such as interleukin-15,[15] are being tested to break the immunological tolerance of cancer patients and boost antitumor T-cell immunity. As it has already been demonstrated in preclinical settings,[16] the combination of immunostimulatory agents with checkpoint inhibitors may boost even further anticancer immune responses and hence constitute an optimal immunotherapeutic approach against (at least some types of) cancer.

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