

Tumor-associated macrophages – additional effectors at anti-PD-1/PD-L1 therapy?

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Immunotherapies that target programmed cell death protein 1 (PD-1) or one of its ligands, programmed cell death ligand 1 (PD-L1), are a recent breakthrough in treatment of human malignant diseases (1), including non-small cell lung cancers (NSCLCs) (2). Monoclonal antibody drugs that target the interaction between PD-1 and PD-L1 have shown dramatic and/or durable responses in a subset of NSCLC patients (3-6), leading to FDA approvals of three agents (nivolumab, pembrolizumab, and atezolizumab) for treatment of metastatic NSCLC patients. Other agents that also target this pathway, such as durvalumab (7) and avelumab (8), are currently under clinical development.

In an illustration of how these drugs work to eliminate tumor cells (*Figure 1*), the two most prominent actors are tumor cells themselves and tumor infiltrating cytotoxic T cells (1). In this context, tumor cells that express PD-L1 suppress immune reactions of PD-1 positive activated T cells through the PD-L1/PD-1 interaction, thus the blockade of this pathway by immunotherapeutic drug(s) enables T cells to counterattack tumor cells.

On the other hand, it is also true that tumor immune microenvironment not only contains cytotoxic T cells but also consists of heterogeneous cell populations (9) including natural killer cells, dendritic cells, regulatory T cells, myeloid-derived suppressor cells, and tumor-associated macrophages (TAMs). Several studies have identified that PD-1 is expressed in some of these immune cells other than cytotoxic T cells, and, reportedly, the PD-1

inhibits function of these immune cells (10-12). However, the roles of PD-1, expressed by these immune cells, on tumor maintenance and tumor development are not fully understood. In addition, it is also unclear to date how immunotherapies that target PD-1 or PD-L1 affect these PD-1 positive immune cells (other than T cells). In a recent study, Gordon *et al.* has reported that both mouse and human TAMs express PD-1, PD-1 expression negatively correlates with phagocytic potency of TAMs, and blockade of PD-1/PD-L1 pathway *in vivo* reduced tumor growth and lengthened the survival of mice in macrophage dependent fashion using *in vitro* and *in vivo* colon cancer models (13).

Macrophages are among the most abundant normal cells in the tumor microenvironment (14). Most tissue macrophages arise from yolk sac/fetal liver progenitors, and aside from that, the lung residential macrophages are from three distinct lineages (yolk sac/fetal liver/and bone marrow) that arrive at different times, reside in different locations (alveolar/interstitial, or peripheral/central), according to a recent lineage-tracing study in mice (15). On the other hand, macrophages involved in pathogen responses appear to come from circulating bone marrow monocytes (14).

When considering the roles of macrophages in tumors, although their functions are exceptional diverse, researchers often simply divide macrophages into two subtypes; M1 macrophages (classically activated macrophages) and M2 macrophages (alternatively activated macrophages) (16). These subtypes correspond to “tumor killing” and “tumor

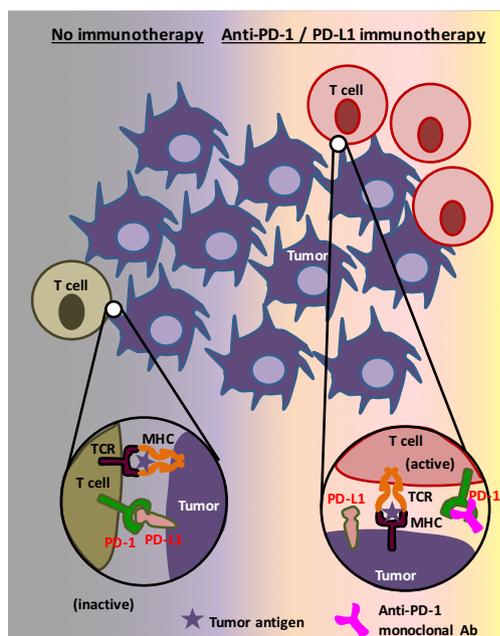


Figure 1 Model of action of anti-PD-1/PD-L1 immunotherapy in tumor cells—T cells interaction theory. Tumor cells that express PD-L1 suppress cytotoxic function of PD-1 positive activated T cells through the PD-L1/PD-1 interaction. Therefore, the blockade of this pathway by anti-PD-1 drugs or by anti-PD-L1 drugs enables T cells to counterattack tumor cells.

promoting”, respectively, since M1 macrophages have roles to promote inflammation, while M2 macrophages suppress inflammation and facilitate tissue repair.

In clinical research settings, the roles of TAMs, as a prognostic factor, have been extensively studied in many tumor types, including NSCLCs. Although the results still have some contradictions, recent two meta-analyses (17,18) conclude that TAMs in tumor islet (higher density of M1 TAMs) predict better prognosis, while TAMs in stroma (higher density of M2 TAMs) predict worse prognosis in NSCLCs. These results suggest a dual role of TAMs in tumor development and maintenance, providing a rationale to target immune suppressive TAMs (M2 TAMs) as a part of immunotherapies for cancers. In fact, for patients with solid malignancy or lymphoma, there are several ongoing trials (e.g., NCT02216409) that target CD47, an “immune checkpoint” of macrophages, the inhibition of which facilitates macrophages to phagocyte tumor cells.

In the study by Gordon *et al.* (13), colon cancer mouse cell lines were subcutaneously injected into immunocompetent mice to assess the expression and the

roles of PD-1 on TAMs. They observed that around 50% of macrophages in the tumors expressed surface PD-1 (which correlated with the time after engraftment and the tumor volume), whereas no circulating monocytes or splenic macrophages expressed detectable levels of PD-1. These PD-1 positive TAMs expressed an M2-like surface profile, and these findings were confirmed in human colon cancer specimens. The authors also found, by using a bone marrow transplantation mice model, that the time-dependent increase in PD-1 positive TAMs is mainly attributed to bone marrow-derived macrophages homing to the inflammatory tumor microenvironment, rather than from tissue-resident macrophages differentiating into PD-1 positive TAMs. *Ex vivo* phagocytosis assay with GFP⁺ *Staphylococcus aureus* bioparticles and *in vivo* experiments with a mice model lacking an adaptive immune system revealed that PD-1 positive TAMs showed reduced degree of phagocytosis, compared to their PD-1 negative counterparts. The *in vivo* model also showed that the phagocytic ability of PD-1 positive TAMs further decreased if co-existent tumor cells expressed PD-L1. These results led the authors to perform experiments to treat mice (which also lack an adaptive immune system) with PD-L1 positive tumors by anti-PD-1, anti-PD-L1, or combination of anti-PD-L1 and anti-CD47 agents. As they expected, these immunotherapies showed efficacy over PBS control in terms of tumor volume and survival of mice. This novel finding may enhance our knowledge about the roles of the PD-1/PD-L1 pathway in the tumor immune microenvironment involving tumor cells, cytotoxic T cells, and TAMs (Figure 2).

The first question for this study is whether or not these results can be applied to NSCLC patients. Since these experiments utilized mouse models with subcutaneous injection of cancer cells, they may not have site-specific features (such as colon-specific or lung-specific) of the tumor immune microenvironment. In addition, a recent large scale comprehensive genomic study reported that the significantly mutated genes in lung adenocarcinomas were most similar to those in glioblastoma and colorectal cancer (19). Therefore, I propose that the roles of PD-1 positive TAMs, and the effects of anti-PD-1/anti-PD-L1 drugs on these TAMs in NSCLC patients are worthy of investigation in future studies, at least in lung adenocarcinoma patients.

Among lung adenocarcinoma patients, we now know that lung cancers with epidermal growth factor receptor (*EGFR*) activating mutations respond poorly to anti-PD-1/anti-PD-L1 drugs compared with lung cancers with wild-type *EGFR* (20,21). Lower immunogenicity due to lower mutation burden

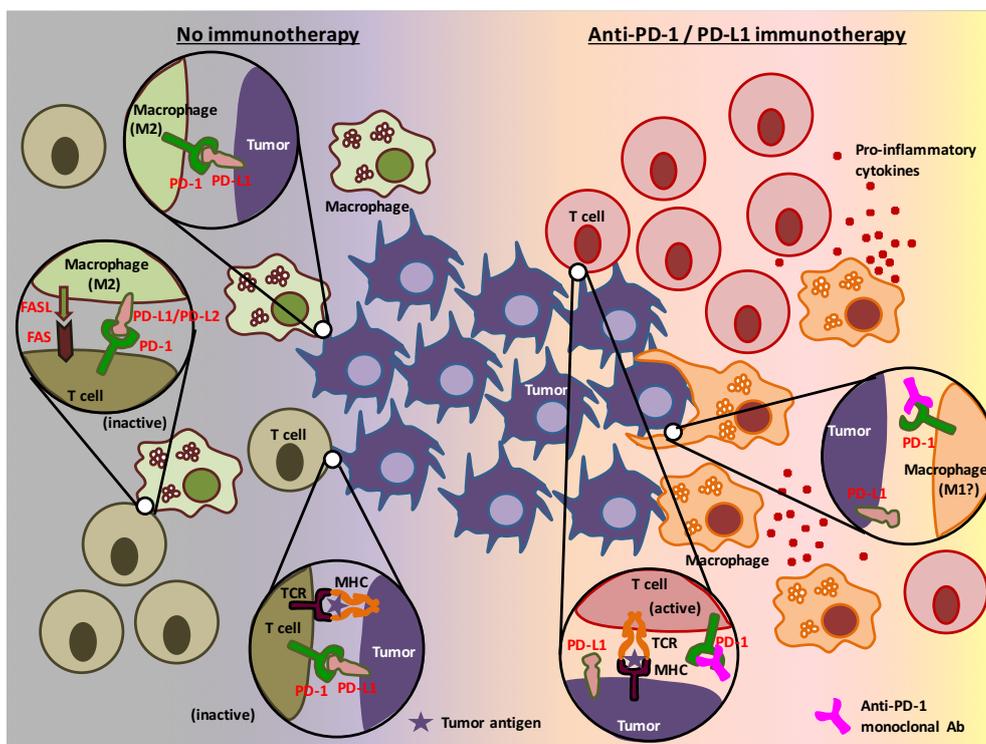


Figure 2 Model of action of anti-PD-1/PD-L1 immunotherapy focusing on tumor cells, cytotoxic T cells, and tumor associated macrophages (TAMs). PD-1 is expressed not only in T cells, but also in TAMs, leading these cells to become inactivated upon binding to PD-L1 expressed on tumor cells. TAMs (M2 TAMs) also express PD-L1 and the ligand for the death receptor FAS that inactivates T cells and triggers caspase-dependent cell death in T cells, respectively (left). Treatment with an anti-PD-1 or anti-PD-L1 monoclonal antibody drug not only activates cytotoxic T cells, but also affects TAMs so that they increase phagocytic potency against tumor cells (right).

[higher mutation burden is one of potential predictive markers for higher efficacy of anti-PD1/anti-PD-L1 drugs in lung cancers (22)] in lung cancers with *EGFR* mutations may be one of explanations for these clinical observations. However, it is of note that a recent study that analyzed the correlation between intra-tumoral immune cell densities and genetic alterations in lung adenocarcinomas found that intra-tumoral macrophage density was significantly lower in tumors with *EGFR* mutations compared with those with wild-type *EGFR* (23). In this study, the density of neutrophils was also lower in lung cancers with *EGFR* mutations, while the densities of CD8 positive T-cells (cytotoxic T cells) and mature dendritic cells were identical. It is possible that lower density of intra-tumoral macrophages in lung cancers with *EGFR* mutations is one of additional mechanisms behind the lower efficacy of current immunotherapies in lung cancer patients with *EGFR* mutations.

The mechanisms of action of small molecule molecular targeted agents (e.g., *EGFR* tyrosine kinase inhibitors) are

simple, since these drugs target molecular aberration(s) found only in tumor cells. In contrast, the target molecules of immunotherapies, i.e., immune checkpoint molecules, are shared by multiple types of immune cells/non-cancerous cells/tumor cells, and these cells interact with each other. Understanding the complexity of the tumor immune microenvironment may be essential to optimize and to personalize immunotherapies, as well as to precisely predict their efficacies and/or adverse events.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

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