Today’s understanding of immune defenses against cancer is a result of long and winding roads in basic and clinical research. Although the immune system is not specifically designed to destroy cancer, evolution has equipped mammals with strong immune strategies against infections. Antiviral immunity has taught many lessons to tumor immunologists in the past decades and remains today a reference in the study of immune responses to cancer. Acute viral infections trigger robust immune responses that rapidly destroy infected cells and prevent the potentially fatal spread of virus throughout the body. These antiviral defense mechanisms strongly rely on CD8+ cytotoxic T lymphocytes (CTL), which eliminate infected cells in two phases: activation upon encounter with professional antigen presenting cells (dendritic cells) mainly in lymphatic tissues, followed by specific killing of virally infected cells in peripheral tissues. During the activation phase, CTLs recognize viral antigen in conjunction with MHC class I (MHC-I) molecules presented by dendritic cells (DC). Additionally, DCs express co-stimulatory signals on their surface (e.g., B7.1, B7.2, and 4-1BB ligand) and secrete soluble factors such as cytokines (e.g., IL12). Following these three essential signals provided by DCs (antigen–MHC complex, surface co-stimulatory molecules and cytokines), antigen-specific CTLs proliferate, differentiate to cytotoxic effector cells, and spread to diseased tissues. During the second (“effector”) phase, activated CTLs recognize viral antigen–MHC-I complexes on the surface of infected host cells and exert effector functions that target these cells for destruction. Efficiently stimulated CTLs upregulate stimulatory receptors (e.g., CD137, also called 4-1BB) that help sustain CTL activation when triggered by appropriate ligands. In recent years, immunologists additionally discovered the existence of inhibitory receptors and ligands (e.g., CTLA-4, PD-1/PD-L1, TIM-3, and LAG-3) that contribute to the natural contention of CTL immune responses.

In contrast to the exponential increase in CTLs during early phases of viral infections, anticancer CTL responses tend to be absent or weak. This is mainly a consequence of the intricate mechanisms of tumors to escape immune control (1). First, the uncontrolled proliferation of tumor cells supports the selection of mutants that can escape CTL responses. Additionally, tumors tend to be poorly immunogenic, resulting in weak immune responses and low CTL activation. Finally, the few CTLs that manage to acquire adequate activation signals face the hostility of the tumor microenvironment, with its complex network of immunosuppressive processes driven by soluble factors (e.g., IL10, TGFβ, VEGF, IDO, and gangliosides) and cell-surface ligands (e.g., PD-1 ligand), resulting in profound CTL inhibition.

In comparing CTL responses to viruses and tumors, it became apparent that CTLs could strongly benefit from external support to acquire and sustain appropriate activation signals in the context of cancer. Together with the discovery of inhibitory receptors on CTLs, therapeutic development has been mainly directed toward using monoclonal antibodies (mAb) that target these receptors or their ligands, with the aim of enhancing and sustaining immune responses that would otherwise be hindered. This therapy, called “checkpoint blockade,” can achieve substantial clinical benefit and constitutes the current best immunotherapeutic option for an increasing number of patients with solid tumors. The anti–CTLA-4 antibody ipilimumab was the first to obtain FDA approval in 2011 after initial clinical trials indicated therapeutic effects against melanoma. Since then, the use of anti–PD-1/PD-L1 antibodies has progressed rapidly, based on promising clinical results against the most frequently occurring carcinomas (2, 3).

Checkpoint blockade represents a revolution in cancer therapy. However, although this treatment often leads to disease regression or long-term stabilization, still too many patients experience cancer progression. Thus, the pressure for more efficient anticancer therapies has rendered combination therapy increasingly attractive. Not only are immunologists now incorporating several “checkpoint blockers” in therapeutic protocols, they are also turning to the manipulation of
older concepts, including co-stimulatory receptors and antigen presentation. Accordingly, with the intention to amplify CTL activation, co-stimulatory receptors such as CD137 are now being targeted by agonistic antibodies in growing numbers of clinical trials (2, 4).

Therapeutic options directed at increasing the immunogenicity of tumor antigens are also currently being explored. Similar to most other cells of the body, tumor cells alone are inefficient at activating CTLs, essentially because they lack appropriate co-stimulatory surface receptors and cytokines. Therefore, the activation of antitumor CTLs relies mainly on presentation of tumor antigens and stimulation by DCs.

Antigens that are acquired from extracellular compartments are taken up, processed, and presented on MHC-II molecules to CD4+ T helper cells, but these antigens do not reach the MHC-I antigen presentation pathway and can therefore not be presented on MHC-I to CTLs (the CD8+ T-cells) in most cell types. The antigens that are presented by MHC-I are generally derived from endogenous synthesis that occurs via the classic MHC-I antigen presentation pathway, ensuring efficient presentation of cell internal antigens, highly relevant in infected cells. Because tumor cells are not infectious, tumor antigens are almost exclusively presented by the tumor cells themselves, which are however poorly stimulatory for T cells. Fortunately there exist specialized subsets of DCs that can channel antigens picked up from the outside, e.g., from tumor cells, into the MHC-I presentation pathway, a process that is called cross-presentation (5). Two main cross-presenting DC subsets have been identified in mice: the resident CD8α+ CLEC9A/DNGR-1+ XCR1+ CD11c+ DCs, located in secondary lymphoid organs, and the migratory CD103+ CLEC9A/DNGR-1+ XCR1+ CD11c+ DCs, located in peripheral tissues. The differentiation and functional properties including IL12 production and cross-presenting faculties of both these subsets are dependent on the basic leucine zipper transcription factor ATF-like 3 (BATF3). Importantly, BATF3 expression is highly specific to known cross-presenting DCs, and absent or low in other immune cells and non-immune tissues. Thus, BATF3-deficient mice selectively lack immune defense mechanisms relying on cross-presentation by DCs, leading to impaired CTL responses against tumors, and also against pathogens that do not infect DCs (6).

In the context of current efforts to optimize tumor antigen recognition by CTLs in cancer therapy, Sánchez-Paulete and colleagues demonstrate in this issue (7) that BATF3-dependent cells are required for successful immunotherapy with inhibitory (anti–PD-1) or stimulatory (anti–CD137) mAbs (Fig. 1). The increased efficiency of immunotherapy in the presence of endogenous cross-presenting cells is shown in two mouse models of cancer (MC38 colon cancer and B16 melanoma) that are known to be differentially sensitive to checkpoint blockade therapy. Surprisingly, the endogenous repertoire of cross-presenting cells in tumors of wild-type (WT) mice does not delay progression of disease when compared with tumors of BATF3-deficient mice; rather, the beneficial role of these cells becomes evident in the presence of a CTL response promoted by immunotherapies. Successful CTL activation by BATF3-dependent DCs directly relies on cross-presentation and not on IL12, a stimulatory cytokine produced at high levels by these cells. Compared with WT counterparts, tumor antigen-specific CTLs from BATF3-deficient tumors expressed significantly lower levels of PD-1 and CD137, the direct targets of therapeutic mAbs currently tested in many clinical trials. The benefits of BATF3-dependent DCs are further underlined by the delay in tumor growth observed in WT mice after administration of sFLT3L and poly-ICLC, molecules destined to promote differentiation and activation of cross-presenting cells. When administered in immunotherapeutic protocols together with anti–PD-1 or anti–CD137, these molecules mediated a synergic effect in up to one third of the mice showing long-term survival to disease. Thus, the data demonstrate a fundamental role for professional antigen-presenting cells in providing antigen specificity to antitumor CTL responses, and highlight that

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**Figure 1.** BATF3-expressing professional antigen-presenting cells (DC) are able to cross-present tumor antigen (TA) by channeling material taken up from their environment into the MHC-I presentation pathway, a process called cross-presentation. Cross-presented tumor antigens are recognized by CD8+ CTLs via their tumor antigen specific T-cell receptors (TCR). CTL activation can be enhanced with monoclonal antibodies (mAb) that stimulate activatory receptors (e.g., αCD137 mAb) and/or block inhibitory receptors (e.g., αPD-1 mAb). These treatments induce potent CTL responses capable of destroying cancer cells, resulting in tumor regression and/or disease stabilization.
immunotherapy based solely on mAbs could highly benefit from additional means to promote the presence and the function of cross-presenting cells.

Cross-presentation is an inefficient process and remains a major bottleneck for the mounting of anticancer CTL responses. Several aspects should be considered when designing immunotherapeutic protocols to promote this process. Although DCs appear to be the main cross-presenting cells in vivo (5), stromal cells in tumors are also capable of cross-presentation and enhance tumor destruction by CTLs (8). Thus, characterizing the contribution and mechanisms of stromal cell cross-presentation in anticancer immune responses will help tailor therapies aimed at promoting this pathway. DC biology can be targeted in the form of either vaccines, consisting of DCs activated ex vivo and programmed to present defined tumor antigens, or molecules known to stimulate DC survival and function directly in vivo (9). In both strategies, the proper activation of DCs remains indispensable, as CTL responses can be downregulated by tolerizing signals of incompletely activated DCs. Sánchez-Paulete and colleagues show that the combination of sFLT3L and poly-ICLC can overcome resistance to treatment with mAbs, arguing in favor of this cross-presenting DC-stimulating cocktail for clinical development. DC vaccines tailored to cross-presentation should favor antigens with high affinity for MHC-I (8). Additionally, common markers of cross-presenting DCs in mice and humans, such as the chemokine receptor XCR1 and CLEC9A/DNGR1, could be targeted with fusion proteins of their ligands and tumor antigens or DC-stimulating agents. A recent study already shows promising induction of protective CTL responses with this approach (10).

In the future, a better understanding of the underlying immune mechanisms will help in mobilizing cross-presenting cells and antitumor CTL responses. It will be crucial to elucidate the role of individual cross-presenting cells in mounting endogenous immune responses against tumors and in modulating distinct stimulatory and inhibitory signals. Research will likely continue to promote clinical benefits of individual immunotherapeutic drugs and of novel combination therapies, providing promising new options to treat cancer patients.

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No potential conflicts of interest were disclosed.

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