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PD-L1⁺ macrophages suppress T cell-mediated anticancer immunity

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ABSTRACT

Recently, we showed that an autologous DC-based vaccine induces an increase in immunosuppressive PD-L1⁺ tumor-associated macrophages (TAM) both in the tumor and the tumor draining lymph nodes, thereby blunting the efficacy of therapeutic immunization. Only the combination of the DC vaccine with anti-PD-L1 immune checkpoint inhibition, but not the use of antibodies targeting PD-1 alone, was able to set off CD8⁺ cytotoxic T lymphocyte (CTL)-mediated tumor suppression in mice. In sum, we delineated a PD-L1 checkpoint blockade-based strategy to avoid TAM-induced T cell exhaustion during DC vaccine therapy.

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Main text

Poorly immunogenic cancers, characterized by the absence of cytotoxic T lymphocytes (CTLs), fail to respond to current immunotherapies in particular to those encompassing immune checkpoint inhibitor (ICI)-based regimens.¹ Dendritic cell (DC)-based vaccines, which typically consist of patient-derived DCs that are antigen pulsed and matured *ex vivo* before autologous reinfusion, have been developed to prime CD4⁺ and CD8⁺ T cell responses, hence theoretically reinstating anticancer immunity in immunologically cold tumors.^{2,3} Nevertheless, the therapeutic benefit of DC-based vaccines remains limited as their pro-immunogenic potential often is hampered by immunosuppressive mechanisms of the tumor and its microenvironment.^{4–6}

In a recent manuscript published in *Cell Reports Medicine*, we employed bulk RNA-seq to correlate the transcriptome of autologous monocyte-derived DC (moDC)-based vaccines with therapeutic responses in prostate cancer patients.⁷ Additional pseudo-time trajectory analysis and REACTOME pathway enrichment suggested that vaccination efficacy and optimal antigen-directed immunity are associated with increased type I interferon (IFN) responses. To test the capacity of DC-based vaccine optimization, we designed the preclinical DC-based vaccine DCvax-IT that employs bone marrow-derived moDCs pulsed with immunologically cold murine non-small cell lung cancer TC1 cells undergoing TNF-driven apoptosis/necroptosis and were stimulated for maturation with IFN β . DCvax-IT was effective in prophylactic settings and protected mice from tumor challenge while generating immune memory that sufficed to reject TC1 cancers in a second rechallenge. Moreover, *in vivo* immunogenicity of prophylactic DCvax-IT relied on the ability of DCs to sense IFN β as it was absent in a DC vaccine based on *Ifnar1*^{-/-} DC. Similarly, DCvax-IT successfully reduced tumor growth in a curative setup in T cell-infiltrated tumors. However, despite proficient DC

lymph node homing, DCvax-IT failed to inhibit the growth of TC1 tumors in T cell-depleted mice. Further combination with cisplatin-based chemotherapy did not improve the therapeutic efficacy of DCvax-IT against TC1 tumors.⁷

Differential gene expression (DGE) analysis as well as publicly available data indicated that the dominant immune resistance mechanism operating in TC1 tumors is characterized by anti-inflammatory signaling relevant to tumor-associated macrophages (TAMs) and other myeloid cells. Moreover, TC1 tumors exhibited a high abundance of TAMs with an increased M2-to-M1 ratio, and immunophenotyping revealed that TC1 tumors were dominated by immunoregulatory (MHC-II^{LOW}) TAMs expressing significant levels of PD-L1 (PD-L1⁺) (Figure 1a). It is important to note that, in this setting, PD-1 blockade failed to increase T cell recovery, indicating that this phenotype was PD-L1 specific. Mechanistically, the inhibition of TNF-related apoptosis-inducing ligand (TRAIL), but not that of TNF, facilitated T cell recovery in the presence of PD-L1⁺ TAMs, altogether indicating that the TRAIL signaling route is employed by TAMs to blunt T cell responses. Blockage of PD-L1 by monoclonal antibodies markedly reduced the abundance of PD-L1⁺ macrophages via the inhibition of NF- κ B-dependent survival signaling. Accordingly, the combination of DCvax-IT with anti-PD-L1 monoclonal antibodies was highly efficient in controlling the growth of TC1 and LLC tumors. Simultaneous PD-L1 inhibition together with DCvax-IT thus reduced the abundance of concomitantly induced PD-L1⁺ TAMs and lymph node associated macrophages (LAMs) and allowed the vaccine to effectively trigger T cell responses. Moreover, the co-enrichment of PD-L1 (CD274) and TAM gene signatures was identified as a negative prognosticator associated with an increased hazard ratio and poor clinical responses to anti-PD-L1 immune checkpoint blockade across a variety of human cancers. Consequently, we analyzed tumor tissue from

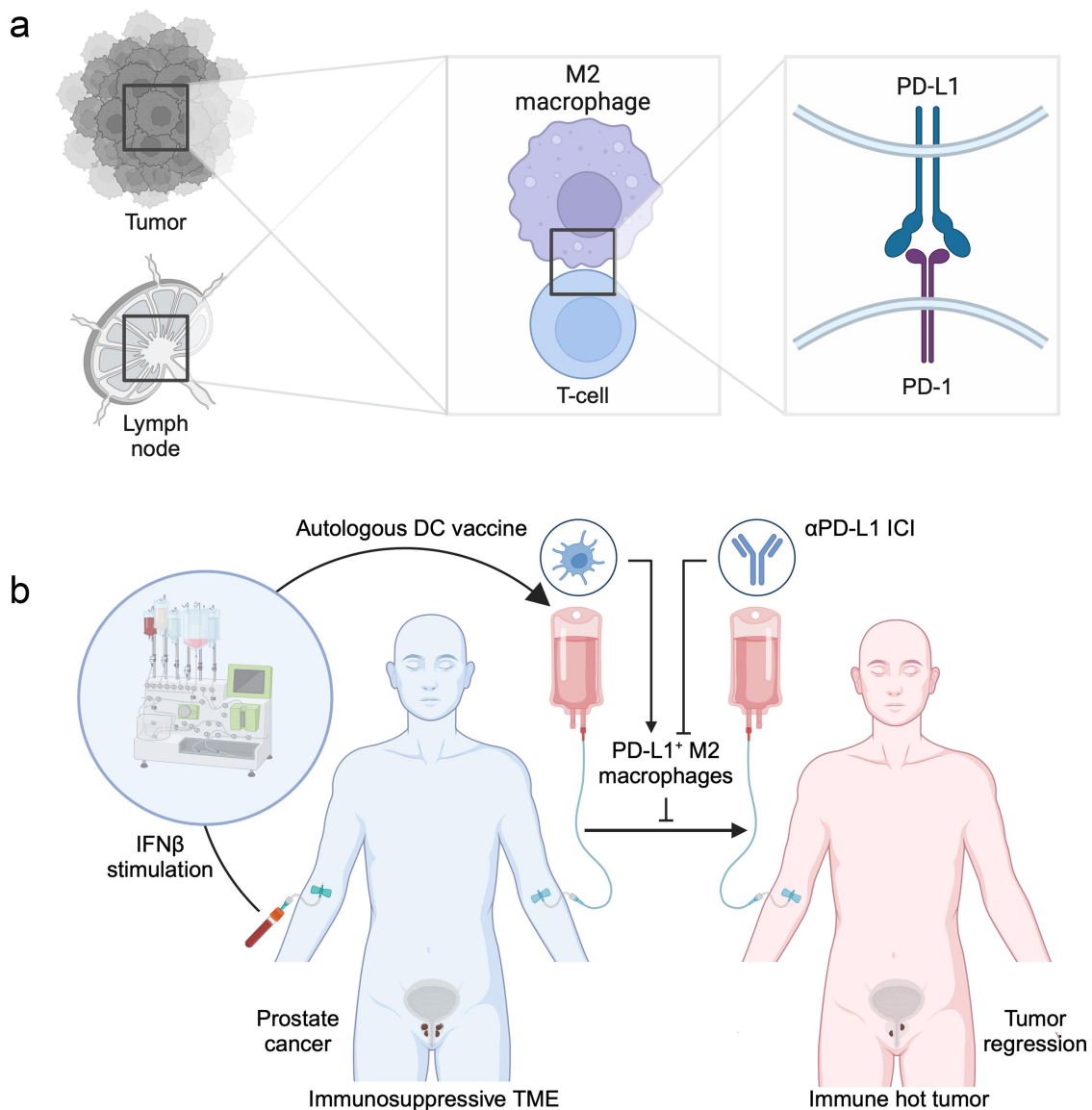


Figure 1

Figure 1. (a) DC vaccine induces an immunosuppressive macrophage niche in the lymph nodes and tumor. The preclinical dendritic cell (DC) vaccine “DCvax-IT” consists of bone-marrow-derived moDCs pulsed with murine non-small cell lung cancer TC1 cells undergoing TNF-driven apoptosis/necroptosis. Despite IFN β stimulation for DC maturation, DCvax-IT lacked therapeutic efficacy in tumor bearing mice due to an increase in immunosuppressive PD-L1⁺M2 lymph node associated macrophages (LAMs) and preexisting PD-L1⁺ TAMs that altogether led to the inhibition of T cell mediated anticancer immune responses. The DC vaccine thus created a PD-L1⁺ macrophage niche fostering immune resistance. (b) Combination therapy consisting of DC-based vaccination plus anti-PD-L1 monoclonal antibody-mediated immune checkpoint inhibition (ICI) could deplete immunosuppressive macrophages, thus overcoming immune resistance and facilitating anticancer immunity.

glioblastoma (GBM) patients that were enrolled in the GlioVax trial (GBM patients receiving tumor lysate-loaded DCs in combination with radio- and chemotherapy) and confirmed that lymphocyte-suppressive PD-L1⁺ TAMs are enriched in this setting.⁷

Despite optimization by IFN β stimulation, the therapeutic efficacy of DCvax-IT was suboptimal, as it increased the abundance of immunosuppressive PD-L1⁺ M2 LAMs via type I IFN responses and further facilitated the surge of preexisting PD-L1⁺ TAMs, thus creating a PD-L1⁺ macrophage niche fostering

DCvax-IT immune resistance (Figure 1a). The combination of DCvax-IT plus PD-L1 blockade was able to neutralize this immunosuppressive niche to facilitate T cell-driven anticancer immunity (Figure 1b). Accumulating preclinical evidence indicates that the efficacy of autologous cell therapies such as DC vaccines but also CAR-T cell therapies could be further improved by concomitant PD-L1-targeted immune checkpoint inhibition to counteract the establishment of an immunosuppressive macrophage niche.⁸

Adaptive immunity and T cell activation are associated with favorable disease outcomes and responses to DC-mediated vaccination in patients with prostate and lung cancer.⁹ Nonetheless, in a recent clinical trial enrolling women with epithelial ovarian carcinoma (EOC), an autologous DC vaccine was more efficient in patients with low tumor mutational burden and limited tumor infiltration by CD8⁺ T-cells than in patients with highly infiltrated EOCs, indicating that, in this setting, DC-based vaccination was able to jumpstart clinically relevant anticancer immune responses.¹⁰

It is tempting to speculate, yet needs to be formally proven, that the use of DC-based vaccines encoding multiple patient-specific tumor neoantigens alone or in combination with ICI will further improve clinical efficacy and elevate this therapeutic approach to standard of care. Future prospective clinical trials in cancer patients need to explore optimal combination regimens and therapeutic schedules that avoid the establishment of an immunosuppressive macrophage niche and hence facilitate the optimal (re)activation of CTLs.

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Author Contributions

PL and LZ summarized data, designed display items and edited the manuscript, OK and GK wrote the manuscript.

Data availability statement

All data that led to the conclusions in this manuscript have been referenced and all sources have been described.

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