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Research highlight

CD28 costimulation promotes anti-tumor CD8$^+$ T cell response in myeloid antigen-presenting cell niches.

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Ectopic lymphoid structures found within the tumor stroma such as tertiary lymphoid structures (TLS) are predictive markers of Immune checkpoint blockade (ICB) in solid tumors. In a recent study in *Cancer Cell*, Duraiswamy et al. highlight other immune structures as myeloid antigen-presenting cell (APC) niches in the context of high-grade serous ovarian cancers (HGSOCs) [1]. In these structures, programmed cell death receptor 1 (PD-1)$^+$ tumor-infiltrating T lymphocytes (TIL) receive a full activation program (a license to kill) via the CD28 costimulatory molecule during PD-1 blockade in HGSOC.

Immune checkpoint blockade (ICB) therapy has revolutionized the management of many cancers but results in ovarian cancer remain disappointing. Initial single-agent studies in multiple relapse situations have shown response rates of 10-15%. In addition, phase III studies of more than 1,000 patients with stage III-IV epithelial ovarian cancer treated with surgery as first-line treatment have shown no real benefit from the addition of an anti-programmed death-ligand 1 (PD-L1) mAb in the initial management of this type of cancer [2]. However, ovarian cancer is theoretically an excellent candidate to respond to immunotherapy (PD-L1 expression, lymphocyte infiltrate).

There are probably resistance mechanisms that need to be explored, but there is also the aim to identify biomarkers that will allow the identification of patients who will benefit from these PD-1/PD-L1 treatments. A high density of tumor-infiltrating CD8$^+$ T cells, CD20$^+$ B cells and DC-LAMP$^+$ DCs correlates with prolonged survival in patients with a wide variety of human cancers, including high-grade serous ovarian carcinoma (HGSOC) [3]. Tertiary

Lymphoid Structure (TLS) can be considered as small ectopic lymphoid structures found within the tumor stroma. They harbor a B-cell zone adjacent to a T-cell zone. These TLSs are considered as mature if they contain follicular DCs. TLS is a predictive biomarker of response to ICB therapy in solid tumors [4].

Duraiswamy et al. reported myeloid APC niches on the HGSOC tumor islets and TLS located outside of these tumor islets [1]. These myeloid APC niches are containing DC and macrophages expressing high levels of PD-L1. Interactions between these APCs and $CD8^+$ TILs are detected by using high-resolution tissue based cyclic immunofluorescence (tCyCIF). A large part of CD8+ TILs in the tumor islets are expressing PD-1 and markers induced upon T cell receptor (TCR) triggering such as granzyme-B (GzmB) and nuclear localization of the transcription factor NFATc2. Tumor-associated antigens (TAA)-specific TILs were detected and isolated, then challenged for different TAA peptides such as NY-ESO-1, HER2, or hTERT. PD-1 blockade experiments were performed in these expanded TAA-specific TILs showing that an anti-PD-1 mAb treatment was able *in vitro* to increase T cell proliferation and cytokine (IL-2 and IFNγ) production. Furthermore, upon an adoptive cell transfer into mice bearing autologous patient-derived xenograft (PDX) tumors, a better animal survival was noticed when these expanded TILs are treated with the anti-PD-1 mAb. Altogether $PD\text{-}1^+$ $CD8^+$ TILs were essentially located in myeloid APC niches into tumor islets and these cytotoxic T cells are already activated at the steady state and rejuvenated by PD-1.

As costimulatory like CD28-mediated events are involved in T cell activation during T-APC interactions and as CD28 is required for PD-1 therapies to kill cancer cells and eliminate chronic viral infections [5], CD28 expression has been evaluated in $PD\text{-}1^+$ $CD8^+$ TILs. Half of T cells were expressing CD28 as control $PD1^{NEG}$ $CD8^+$ T cells. However, CD28 surface level is two-times higher in $PD\text{-}1^+$ $CD8^+$ TILs compared to $PD1^{NEG}$ $CD8^+$ T cells. As CD28 ligand, CD86 was expressed in DCs from the tumor islets. By using single-cell RNA/TCR sequencing, the $CD8^+$ TILs exhibiting an exhausted and CD28 costimulatory signature harbor an increased effector fitness upon TAA peptides stimulation. These transcripts data have been compared to protein data from mass cytometry and were showing that the exhausted and CD28 costimulatory signature was corresponding to $CD137^+PD\text{-}1^+CTLA\text{-}4^+CD28^+$ TILs (where CD137/4-1BB is considered as a T-cell activation marker). To verify the role of CD28 in $PD1^+$

$CD8^+$ TILs, T cells were co-cultured or not with APC from the tumor in presence of a predicted CD28 antagonist peptide p2TA (SPMLVAYD; $CD28_{8-15}$). Of note, the p2TA peptide binds to CD28 at least *in vitro* and disrupts the CD28 dimerization necessary for a full activation of this receptor [6]. As reported above, PD-1 treatment induced a $PD\text{-}1^+$ $CD8^+$ TIL proliferation in presence of TAA peptides and APCs. In this context, p2TA peptide blocked this T cell proliferation increase suggesting that CD28 triggering is required for T cell activation in the myeloid APC niches. To further investigate the importance of CD28-CD86 interactions in this process, some animal models have been designed as a syngeneic model of ovarian cancer where C57BL/6 mice were injected i.p. with $Tp53^{-/-}Brca1^{-/-}$ ID8 ovarian cancer cells expressing luciferase. Mice were then treated an anti-PD-1 and a CD28-neutralizing antibody. The tumor growth slowdown induced by PD-1 ICB was abrogated by the CD28 antagonist. To further investigate this point, some CD28 genetic modified mouse strains could be used within the *in vivo* ID8 tumor model [7].

As the more effective ($CD137^+PD\text{-}1^+CTLA\text{-}4^+CD28^+$) TILs were expressing both PD-1 and CTLA-4, there was a rationale to test a combination of PD-1 and CTLA-4 ICB. In both *in vitro* and *in vivo* assays (TIL activation in tumor-digest cultures in response to TAA peptides and ICB; response to ICB in $HLA\text{-}A2^+$ CD34-reconstituted human immune system/NSG mice (HIS-NSG-A2) bearing OVCAR5 tumors), anti-CTLA-4 mAbs amplified response to PD-1 and this amplification was CD28-dependent based on the results with p2TA, CD28 antagonist peptide.

To improve CTLA-4/PD-1 ICB effects on $PD1^+$ $CD8^+$ TIL activation, APCs can be targeted to exacerbate their capacity to prime $CD8^+$ cytotoxic T cell. As the CD40/CD40L interaction leads to activation of DCs (i.e. by increasing expression of CD80/CD86 molecules), CD40 agonist (soluble CD40L) has been included into both *in vitro* and *in vivo* previously described assays. CD40L addition improved the effects of PD-1/CTLA-4 ICB on $PD\text{-}1^+$ $CD8^+$ TILs.

This study was giving several highlights to investigate ICB therapy:

- by repositioning the role of CD28 costimulatory molecules in $CD8^+$ TILs, opening a rationale to test the combination of anti-CTLA-4 / PD-1 antibodies in addition to activate APCs at least in ovarian cancers (Figure 1).

- by paving the way to combination therapies that will costimulate CD28 either directly via an agonist signal or indirectly by the CD40 triggering.
- by better stratifying patients via a detection of immune structures inside the tumor or distant to the tumor islets (myeloid APC niches, TLS). It will be important to develop immunomonitoring platforms to visualize the immune response organization inside the tumor before or during ICB therapy [8].
- by analyzing the behavior of $CD8^+$ TILs in the different immune structures into the tumor microenvironment [9].

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Competing interests

J.A.N. has no competing interests. D.O. is a cofounder and shareholder of Imcheck Therapeutics, Alderaan Biotechnology and Emergence Therapeutics and has research funds from Imcheck Therapeutics, Alderaan Biotechnology, Cellectis and Emergence Therapeutics; all of these are outside of the submitted work.

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Figure 1 legend

Figure 1: The CD28 molecule, a license to kill delivered in myeloid APC niches.

Myeloid APC niches in tumor islets are containing DCs expressing CD86 (B7-2) and PD-L1. These APC are contacting $PD1^+$ $CD28^+$ $CTLA\text{-}4^+$ $CD8^+$ TILs. A full activation of these TILs can be operated by (1) blocking PD-1, (2) CTLA-4 (two immune checkpoints) and furthermore by inducing (3) an APC activation that will increase the expression of CD28 ligands (B7 molecules) at their surface. This strategy focused around the CD28 molecule could optimize ICB response in selected ovarian cancer patients.

TIL, tumor-infiltrating lymphocytes; TCR, T cell receptor; MHC, major histocompatibility complex; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; PD-1, programmed death receptor 1; PD-L1, PD-1 ligand 1; GzmB, granzyme B; PFN, perforin.

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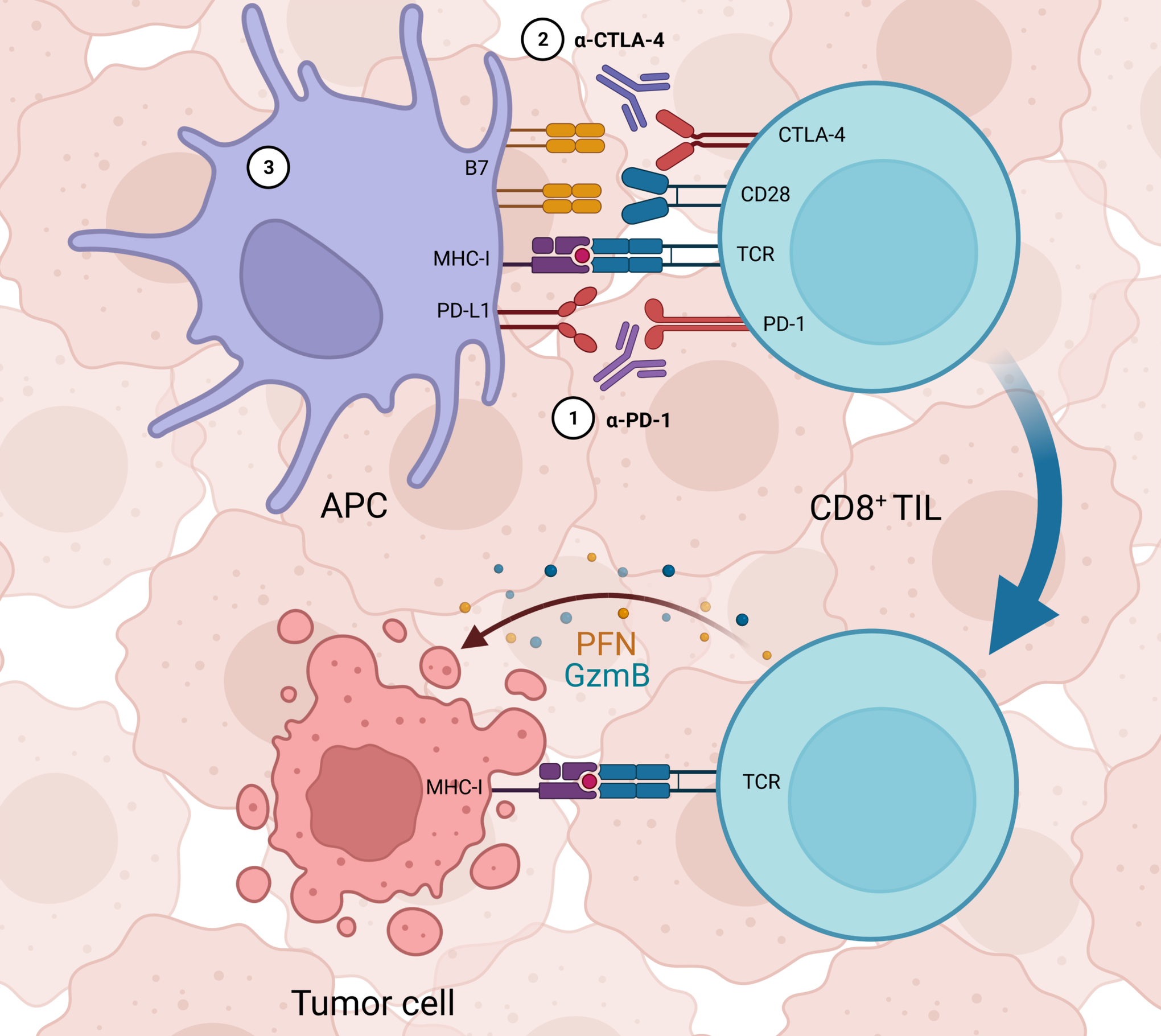
2
α-CTLA-4
3
B7
CTLA-4
CD28
MHC-I
TCR
PD-L1
PD-1
1
α-PD-1
APC
CD8⁺ TIL
PFN
GzmB
MHC-I
TCR
Tumor cell