

## PD-L1 immune suppression in cancer: Tumor cells or host cells?

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### ABSTRACT

Four recent publications reported the role of PD-L1 expression on host versus malignant cells within the tumor for PD-1/PD-L1 checkpoint blockade therapy. All four research groups harmoniously report: PD-L1 expressed by both host as well as tumor cells are capable of suppressing T cell functions. Thus, checkpoint therapy can be effective, if malignant cells do not express PD-L1.

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Within a time window of 2 mo in early 2017, four independent research groups published papers in high impact journals advocating a similar message using gene silencing technologies *in vivo*. All groups investigated the role of PD-L1 expression on different cell types within the tumor-microenvironment, in terms of T cell inhibition and/or immune-checkpoint blockade therapy (Fig. 1). Not only is this a tribute to the importance of the PD-1/PD-L1 axis in tumor immunology, but also illustrates the speed at which the oncoimmunology field is progressing.

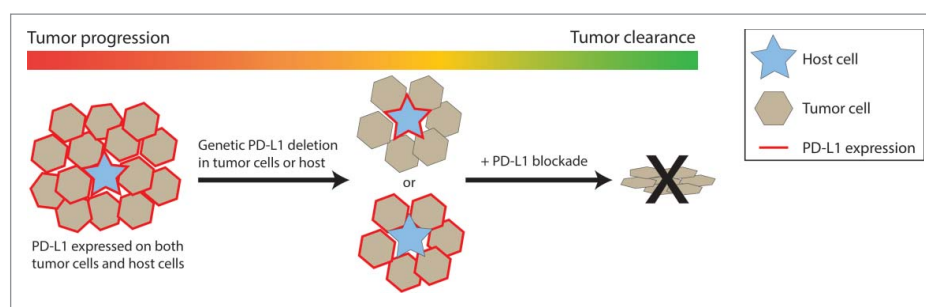
In Cancer Immunology Research, Noguchi *et al.* used two variants of an MCA-induced mouse tumor model that either grow progressively or regress spontaneously.<sup>1</sup> By overexpressing or knocking out PD-L1, using CRISPR-Cas9, they described how PD-L1 on tumor cells determines whether tumors progress or regress, by inhibiting the antitumor T cell response. Tumors that were otherwise too immunogenic to establish, were growing out rapidly by overexpression of PD-L1. Conversely, tumors growing out in immune competent mice were spontaneously eradicated or slowed in their tumor-outgrowth when PD-L1 was knocked out from tumor cells. In all these cases, blocking PD-L1 with a therapeutic antibody had additional effect, indicating a role of PD-L1 on stromal cells.

In Nature Communications, Lau *et al.* used both PD-L1<sup>-/-</sup> tumor cells, generated with CRISPR-Cas9, and a newly generated PD-L1 knockout mouse as a host, to study T cell inhibition by PD-L1 in two commonly used mouse tumor models, MC38 and CT26.<sup>2</sup> PD-L1 knockout variants of these tumors spontaneously regress or grow slower than WT counterparts, while therapeutic PD-L1 blockade further extends survival, indicating an additive role of PD-L1 on both tumor and host cells. Gene expression analysis showed the strongest enrichment for T cell immunity-related genes when PD-L1 was lacking on both tumor cells and host cells. The authors describe several alternative immune escape mechanisms in outgrowing PD-L1

knockout tumors, including reduced MHC-I expression and increased PD-L2 expression.

In the Journal of Experimental Medicine, Juneja *et al.* emphasize that while both tumor and host cell PD-L1 expression can play a crucial role in T cell suppression and response to blockade therapy, their exact relative contribution is context-dependent, as it differed per tumor model in their experiments.<sup>3</sup> The authors used three different mouse tumor models in host mice deficient for either PD-1 or for both PD-L1 and PD-L2, as compared with WT hosts. Whereas B16 and BRAF.PTEN tumors grew slower in both PD-1 deficient mice and PD-L1 deficient mice, MC38 tumors only benefited from the absence of host PD-1, indicating no relevant role for host PD-L1 in MC38 tumor growth. The strong effect of therapeutic PD-L1 blockade was therefore largely dependent on tumor cell-expressed PD-L1, and could be mimicked by knocking out PD-L1 selectively on tumor cells. The authors further argue that PD-1 expression on T cells in tumors or tumor-draining lymph nodes may well reflect recent activation and not necessarily a dysfunctional state, especially when PD-1 ligands are lacking or blocked at the target site.

Finally, in OncoImmunology, we have described a non-redundant role of PD-L1 expression on tumor cells and host cells.<sup>4</sup> Using CRISPR-Cas9 technology, we created PD-L1 knockout variants of MC38 and CT26, the two most widely used pre-clinical tumor models in tumor immunotherapy research, which both grew out more slowly than WT tumors. In straight-forward experiments, we show that blocking PD-L1 or PD-1 with therapeutic antibodies still has tumor-eradicating effects on these tumors, indicating an additional role for PD-L1 on immune infiltrating cells within the tumor microenvironment. T cell depletion studies emphasized the crucial role of CD8 T cells in the antitumor effects of both the lack of PD-L1 on tumor cells and of blocking antibody therapy.



**Figure 1.** PD-L1 expression on tumor cells and host cells jointly support tumor outgrowth. *Left:* in a typical immunogenic tumor, both tumor cells and intratumoral host cells express PD-L1, resulting in progressive tumor growth. *Middle:* experimental genetic deletion of PD-L1 on tumor cells or the host results in reduced tumor growth, mediated by CD8 T cells. *Right:* complete removal of PD-L1 expression by addition of blocking antibodies results in tumor eradication.

Each study reveals, from a different angle, that PD-L1 on tumor and host cells is involved in suppressing the antitumor T cell response. All manuscripts show that in immunocompetent mice, tumor cells grow out slower or regress spontaneously when PD-L1 is genetically knocked out, an effect mediated by T-cell responses (Fig. 1). There was, however, a minor discrepancy between the studies. Juneja *et al.* concluded that PD-L1 expression on MC38 tumor cells was fully responsible for inhibiting antitumor T cell responses, with no additional role for PD-L1 on host cells, whereas the papers of Lau and ourselves both showed that PD-L1 blocking antibody therapy of MC38 PD-L1 knockout tumors still gives a therapeutic response. These two conclusions were based on slightly different experimental setups, which may explain the differences. In Lau *et al.* and our paper, the role of host PD-L1 expression was shown by therapeutic PD-L1 blockade in WT mice-bearing PD-L1 knockout MC38 tumors. Although in Juneja's study, outgrowth of untreated PD-L1 knockout MC38 tumors was much more hampered compared with the other studies, an additional role of PD-L1 on host cells cannot be fully excluded, since they did not treat these mice with PD-L1 blocking antibody.

The knowledge gained by the four studies contributes greatly to our understanding of tumor immunology. By investigating the topic from different angles and using various techniques and pre-clinical models, the four studies complement and validate each other. The combined outcomes signify an important biomarker for the use of PD-1 and PD-L1 blocking antibody therapeutics. Expression of PD-L1 within the tumor, but not necessarily on tumor cells, is sufficient for a therapeutic effect of PD-1/PD-L1 blocking

antibodies, meaning that absence of PD-L1 expression on tumor cells does not disqualify patients for treatment.

### Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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