

# Novel regulators of PD-L1 expression in cancer: CMTM6 and CMTM4—a new avenue to enhance the therapeutic benefits of immune checkpoint inhibitors

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During the past several decades, the efforts of cancer immunotherapy have been focused on identifying specific tumor antigens to augment antitumor immunity. However, this approach has been constantly challenged by the complexity and diversity of gene mutations and protein modification present in human cancers as well as a complex tumor microenvironment, resulting in limited or failed therapeutic success (1-3). Recently discovered immune checkpoint pathways, including programmed death receptor 1/programmed death ligand 1 (PD-1/PD-L1) signaling pathway, were shown to protect host from overactive T-effector cells not only in cancer but also during microbial infections (4,5). PD-1 is preferentially expressed on activated T-cells with particularly high expression in the population of tumor-infiltrating T-cells, while PD-L1, one of its key ligands, has been found to be overexpressed (activated) in various solid malignancies, most notably in malignant melanoma, non-small cell lung carcinoma (NSCLC), renal cell carcinoma, advanced urothelial carcinoma and various solid cancers exhibiting high mutational load and/or microsatellite instability (MSI-H) (2,6,7). PD-L1 activation has also been described in hematologic malignancies, such as multiple myeloma, both non-Hodgkin and Hodgkin lymphomas (most notable in classical Hodgkin lymphoma), some leukemias as well as systemic histiocytoses (3,8-10).

Development of therapeutic antibodies that inhibit PD-1/PD-L1 pathway (immune checkpoint inhibitors) seems to be a promising tool in terms of clinical benefits and overall patients' survival (11). However, cancer cells frequently manipulate PD-1/PD-L1 axis to evade immune surveillance (3,12). Therefore, only a subset of patients respond to these agents and fewer still achieve a durable response (11).

Among the well-known mechanisms that lead to unresponsiveness to anti-PD1/PD-L1 therapy are loss-of-function mutations in Janus kinase 1/2 (*JAK1/2*) genes (13). A key functional result from somatic *JAK1/2* mutations in cancer cells is the inability to respond to interferon gamma ( $INF-\gamma$ ) by overexpressing PD-L1 and many other interferon-stimulated genes (e.g., *IFNGR1*, *TYK2*, *STAT1*, *Stat3*, *IRF1*) (13). Patients with such burden would be unlikely to respond to PD-1/PD-L1 blockade therapy (14,15).

Other pathways and regulators have also been found to upregulate PD-L1 in cancer cells including PTEN-PI3K-AKT pathway, ALK, STAT3, HDAC6, interleukins 4 and 10, vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor (G-CSF) and bacterial IF- $\gamma$  lipopolysaccharide (3). However,  $INF-\gamma$  appears to be a key regulator (16,17).

Understanding the mechanisms contributing to an

effective response and resistance are of utmost importance to optimize treatment with immune checkpoint inhibitors (11,18). In this context, two potential pathways (CMTM6 and CMTM4), considered as PD-L1 regulators, have been recently discovered in maintaining antitumor immunity (19,20).

Burr *et al.* and Mezzadra *et al.* independently identified CKLF-like MARVEL transmembrane domain containing protein 6 (CMTM6) using CRISPR-Cas9 deletion library screen (19,20). CMTM6 is a ubiquitously expressed protein with previously unknown function; it appears to play a role in limiting antitumor immunity. CMTM6 belongs to a family of proteins, primarily encoded by two distinct gene clusters located on chromosome 16 (CMTM1–4) and chromosome 3 (CMTM6–8). MARVEL domain proteins have been implicated in regulating trafficking of transmembrane and secretory proteins (19). Depletion of CMTM6 led to a marked reduction of PD-L1 protein level, but not its transcriptional levels. Levels of MHC I and PD-L2 were not significantly affected by CMTM6 inhibition, indicating that although CMTM6 regulated PD-L1 at the protein level, it is not a general regulator of protein translation or stability (20). Interestingly, most common PD-L1 up-regulator INF- $\gamma$  was not correlated with CMTM6 pathway.

A close family member CMTM4 with 55% homology to CMTM6 was identified to share this function in CMTM6-deficient HAP1 cells, but not any other CMTM member. CMTM4 is considered as a back-up regulator of PD-L1 expression (20).

Among all proteins upon cell surface, only small number of high-confidence proteins are interacting with CMTM6. PD-L1 was one of the top ranked. CMTM6 is established as a new regulatory target to specifically vary the cell surface expression of this critical immune checkpoint molecule. Although CMTM6 is found within cytosol and plasma membrane, it is not required for the trafficking of PD-L1 from the endoplasmic reticulum to the cell surface, but is required for stable expression of PD-L1 at the cell membrane (19,20). CMTM6-deficient tumor cells were also more susceptible to antigen-specific cytotoxic T-lymphocytes *in vitro* and were associated with longer survival in a murine transplantable melanoma model.

Another interesting observation from these studies is that CMTM6 protects PD-L1 from ubiquitination and helps it be recycled and evade lysosomal degradation (19). Also, STUB1, an E3 ubiquitin ligase, has been identified as a negative regulator of PD-L1. Deletion of STUB1 resulted

in a stronger increase in PD-L1 levels in CMTM6-deficient than in CMTM6-proficient cells (20).

Although several questions are yet to be addressed (e.g., how to translate these findings into therapeutics, potential adverse side effects), CMTM6 is surely an important player in the tumor microenvironment and antitumor immunity. CMTM6 was found to be a key regulator of T lymphocyte-mediated anti-tumor immunity, in diseases that respond to immune checkpoint inhibitors through modulation of the PD-L1 expression *ex vivo* (in humans) and *in vivo* (mouse), respectively (19). Further basic and translational studies should confirm these findings and enable novel approaches to enhance the effectiveness and clinical benefits of the immune checkpoint inhibitors in humans.

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### Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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