Commentary Title: CTLA-4 – checkpoints beyond the membrane

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Commentary on article: Kennedy et al (2023) Soluble CTLA-4 attenuates T-cell activation and modulates anti-tumour immunity.

Commentary

In 2018 the Nobel Prize in Physiology or Medicine was awarded to Professors James Allison and Tasuku Honjo for their pioneering research on CTLA-4 and PD-1. The discovery that checkpoint receptors CTLA-4 and PD-1 regulate anti-tumour T-cell immunity has revolutionised the field of cancer therapy since ipilimumab, a monoclonal antibody (mAb) specific for CTLA-4, was first shown to prolong survival in patients with difficult-to-treat metastatic melanoma over 10 years ago. While the assumption has been that CTLA-4-targeting mAb target the membrane-bound form of CTLA-4, an understudied soluble form of CTLA-4 (sCTLA-4) has also been described. In this issue of Molecular Therapy, Kennedy et al comprehensively demonstrate that sCTLA-4 alone confers a growth advantage to tumour cells and importantly, a monoclonal antibody specific for sCTLA-4, and not membrane (m)CTLA-4, proved an effective therapeutic agent in vivo [1]. The authors provide further insights into the cellular mechanisms by which sCTLA-4, and anti-sCTLA-4 alter the tumour microenvironment (TME). These findings challenge long-held assumptions about the mechanism(s) of action of anti-CTLA-4 mAb and provide a basis for considering the clinical potential of sCTLA-4 targeting mAb.

CTLA-4 is a dimeric receptor upregulated to moderate levels on effector T cells and constitutively highly expressed on regulatory T cells (Tregs). Professional antigen presenting cells, via their expression of CD80 and CD86 (also known as a B7-1 and B7-2 respectively), are able to bind with relatively high affinity to CTLA-4 and with lower affinity to the classical T-cell co-stimulatory receptor CD28. CTLA-4 is therefore considered a competitive inhibitor of T-cell activation, acting in opposition to CD28. Mab-mediated blockade of CTLA-4 may therefore increase the bioavailability of CD80/86 for co-stimulation through CD28 by sequestering CTLA-4 as well as reducing CTLA-4-mediated transendocytosis of CD80/86 [2]. Some anti-CTLA-4 mAb have a complementary mode of action by depletion of CTLA-4^{hi} Tregs via an Fc-dependent mechanism, although the importance of Treg depletion after ipilimumab treatment in humans remains controversial [3, 4]. A recent study has also

shown an anti-CTLA-4 mAb, to drive a Fc-dependent increase in inflammatory myeloid populations in the TME [5]. The relative dominance of these mechanisms downstream of anti-CTLA-4 mAb is influenced by several factors including the isotype of the relevant antibody with some (e.g. human IgG1, ipilimumab) predicted to be superior at depletion, and others (e.g. human IgG2) more adept at promoting effector function [4]. For optimal efficacy, animal models indicate that both promotion of effector cells and depletion of Treg cells are required [6].

A commonality of these studies is lack of consideration of the soluble form of CTLA-4, despite sCTLA-4-encoding transcripts detected in human lung adenocarcinoma and melanoma, albeit at lower frequencies than full length CTLA-4 transcripts (Kennedy et al, this issue), and prior evidence that circulating sCTLA-4 correlates with cancer progression [7]. SCTLA-4 lacks the transmembrane and intracellular domains present in mCTLA-4 and instead incorporates a novel C-terminal sequence of 22 residues, providing a unique target for the generation of sCTLA-4-specific mAb [8]. Importantly the B7-binding domain is identical in both isoforms implying that sCTLA-4 has similar ability to compete with CD28 as mCTLA-4. Kennedy et al generated cell lines expressing sCTLA-4 and showed that sCTLA-4 adopted a dimeric structure, akin to mCTLA-4, further suggestive of similar B7-binding stoichiometry by the two isoforms. Importantly, sCTLA-4 expression conferred a significant growth/survival advantage apparent only in cells grown in the presence of PBMCs or in immune-competent mice, and thereby providing clear evidence of an immune-modulatory influence of sCTLA-4. Furthermore, sCTLA-4 expressing cells (but not empty vector controls) inhibited T-cell proliferation and were resistant to cell death when cultured in the presence of NK-cell depleted PBMCs. Taken together, these data strongly implicate the T-cell compartment as a significant target for sCTLA-4-mediated modulation.

To gain further insight into the mechanism(s) through which sCTLA-4 influences the TME, the authors used mass cytometry to compare the TME in mice seeded with sCTLA-4-transduced or control MCA-205 fibrosarcoma. Perhaps surprisingly, the most striking difference was not in a T-cell population but instead was a substantial sCTLA-4-associated increase in an 'undetermined' cell type (expressing amongst other markers CD44, Foxp3, F4/80 and CTLA-4) with a corresponding decrease in macrophage and Ly6C^{hi} monocyte populations. The nature of this undetermined cell population is intriguing and requires further characterisation; while tumour-associated F4/80+Foxp3+ macrophages have been described [9] and are associated with high efferocytic activity in stroke [10], the significance of such cells in a TME is not clear. Targeted analysis of the relatively small (<5%) population of CD8+ tumour infiltrating cells revealed a relative decrease in CD69+ cells in the sCTLA-4+ TME but no consistent changes in Treg/effector cell ratios. Subsequent experiments made use of a sCTLA-4-specific mAb (JMW-3B3) [8], to show that sCTLA-4 targeting alone is sufficient to slow the growth of colorectal native MC38 tumour in vivo. The anti-sCTLA-4 mAb used in this study was of mIgG1 isotype, predicted to have blocking/effector-promoting rather than depleting activity. This seems a logical choice given that sCTLA-4 is unlikely to be Treg associated and therefore it is difficult to envision how a cell-depleting anti-sCTLA-4 mAb might be of benefit (Figure 1). Indeed, Kennedy et al, did not observe changes in Treg frequency in the TME after anti-sCTLA-4 treatment. Cells enriched in the TME after anti-sCTLA-4 treatment were broadly the reciprocal of those enriched in sCTLA-4-secreting tumours, with decreased frequency of an 'undetermined' myeloid-like population (expressing F4/80, Foxp3 and CTLA-4) and enrichment of 'effector' like (granzyme B^{hi}, perforin^{hi}) CD8+ T cells. The extent to which these populations (CD8+ T cells, or 'undetermined') contribute directly to the impairment of tumour growth remains to be seen. Of interest, others have shown that a mIgG1 anti-CTLA-4 mAb (presumably targeting both soluble and membrane CTLA-4) given to MC38-bearing mice fails to confer protection, and no significant differences were observed in the TME with or without anti-CTLA-4 treatment [5]. However, tumour protection and significant modifications to the myeloid compartment were observed after administration of a mIgG2a isotype

anti-CTLA-4 in the same model [5]. Future studies comparing the TME after anti-CTLA-4 and anti-sCTLA-4 mAb treatment will be informative to identify common mechanisms of action. Important insights may also be gained from study of the tumour draining lymph nodes (TDLN) after anti-sCTLA-4 treatment given the high frequencies of CD80/86-expressing cells and CTLA-4^{hi} Tregs at this site [5].

This important study by Kennedy et al, highlights a previously unappreciated role for sCTLA-4 in suppressing tumour immunity and may herald a new subclass of soluble-specific check-point inhibitors for clinical exploitation. Given the significant immune related adverse events suffered by patients treated with existing anti-CTLA-4 mAb [2], and absence of detectable peripheral immune activation in mice given anti-sCTLA-4 (Kennedy et al, this issue), the possibility that anti-sCTLA-4 mAb might allow decoupling of anti-tumour immunity from significant side effects offers additional grounds for optimism.

Declaration of Interest

The author declares no competing interests.

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Figure 1. Mechanisms through which anti-CTLA-4 mAb may influence T-cell activation

Conventional T (Tcon) and T regulatory cells (Treg) express CTLA-4. While much CTLA-4 is intracellular, for simplicity, mCTLA-4 is shown here only on the cell surface. (A) In homeostasis, bioavailability of CD80 and CD86 is limited by the presence of mCTLA-4 which mediates CD80/86 internalisation through transendocytosis (not shown). A reasonable assumption is that sCTLA-4 also precludes binding of CD80/86 to CD28 by steric hindrance. (B) Anti-CTLA-4 mAbs increase availability of CD80/86 to provide co-stimulatory signals through CD28 on Tcon, and/or remove the suppressive influence of Tregs by direct cytotoxicity, antibody dependent cellular cytotoxicity or by blockade of CTLA-4 on Tregs. Anti-CTLA-4 mAb would also be predicted to bind sCTLA-4. (C) Anti-sCTLA-4 mAbs would not be predicted to exert direct influence on Tregs but may increase bioavailability of CD80/86 for Tcon co-stimulation. A further intriguing possibility is that anti-sCTLA-4 mAb (and indeed anti-CTLA-4 mAb) may cross-link CD80/86-bound sCTLA-4 to promote reverse signalling through CD80/86 thereby increasing APC activation, although this remains speculation.