Nature Reviews Clinical Oncology published online 29 April 2014; doi:10.1038/nrclinonc.2013.208-c1

Regulatory T cells—an important target for cancer immunotherapy

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A recent article by Drake *et al.* (Breathing new life into immunotherapy: review of melanoma, lung and kidney cancer. *Nat. Rev. Clin. Oncol.* **11**, 24–37 [2014])[1] reviewed how monoclonal antibodies against the immune checkpoint molecules cytotoxic T-lymphocyte antigen-4 (CTLA-4), programmed death-1 (PD-1) and PD-ligand 1 (PD-L1) are effective in mediating tumour shrinkage in several cancer types. Drake *et al.*[1] also highlighted ongoing phase III clinical trials and discussed the increased antitumour activity of the combinations of these antibodies compared with blocking either checkpoint alone. However, we would like to note that regulatory T cells (T_{REG}) might represent another important immunological checkpoint to target in cancer immunotherapy.[2,3] In fact, T_{REG} that express CD4, CD25 and the forkhead protein 3 (FoxP3) can inhibit the antitumour immune response, thereby limiting the power of cancer immunotherapies.[2]

Although Drake *et al.*[1] did not discuss the effects of CTLA-4 and PD-1 or PD-L1 blockade on $CD4^+CD25^+FoxP3^+$ T_{REG}, this needs to be considered. PD-1 blockade negatively regulates intracellular FoxP3 expression in T_{REG}.[4,5] Specifically, Wang *et al.*[4] showed that PD-1 blockade leads to the down-regulation of intracellular FoxP3 expression in T_{REG} of patients with melanoma, suggesting that PD-1 is implicated in the regulation of T_{REG} function. Furthermore, Sharma *et al.*[5] demonstrated that the ability of T_{REG} to suppress target T-cell proliferation is abrogated by PD-1 and PD-L1 antibodies. The effect of CTLA-4 blockade on T_{REG} is less clear.[6,7] CTLA-4 is constitutively expressed on CD4+ T_{REG}[8,9] and Wing *et al.*[8] reported that T_{REG}-specific CTLA-4 deficiency impaired *in vivo* and *in vitro* suppressive function of T_{REG}, and also produced potent tumour immunity. Regarding the effect of CTLA-4 blockade on T_{REG}, Kavanagh *et al.*[6] showed that treatment with CTLA-4 antibodies in patients with metastatic prostate cancer induces an increase in the number of activated effector $CD4^+$ T cells and $CD4^+CD25^+FoxP3^+$ T_{REG}, suggesting that CTLA-4 antibodies enhance antitumour immunity by the activation of effector T cells rather than by depleting $CD4^+CD25^+FoxP3^+$ T_{REG} *in vivo*.[6] Conversely, Simpson *et al.*[7] reported that treatment with an anti-CTLA-4 antibody induces a selective depletion of T_{REG} within the tumour lesions in a mouse model of melanoma.

On the basis of the different effects that immune checkpoint blockade can have on T_{REG} function, it is possible that circumventing the activity of T_{REG} might represent an important step to overcome some of the obstacles that, so far, have prevented the complete exploitation of the immunotherapy potential for the successful treatments of many cancers, including melanoma.[3] A substantial number of T_{REG}, in tumour tissues and peripheral blood specifically express C-C chemokine receptor 4 (CCR4), therefore, treatment with anti-CCR4 monoclonal antibody can evoke and augment antitumour immunity in patients with melanoma by selectively depleting or inhibiting T_{REG} from the tumour tissue.[10,11] The combinations of CTLA-4 and PD-1 or PD-L1 blockade showed an increased antitumour immunity compared with using each antibody alone;[1] however, an alternative combination strategy could include both immune checkpoint blocking antibodies and T_{REG}-depleting molecules. For example, depletion of T_{REG} by intraperitoneal administration of interleukin-2 diphtheria toxin followed by sequential PD-1 or PD-L1 blockade showed superior efficacy for eradication of acute myeloid leukaemia in a mouse model than did PD-1 or PD-L1 blockade alone.[12] Furthermore, Goding *et al.*[13] demonstrated that either blockade of the PD-1 pathway with anti-PD-L1 antibodies or depletion of tumour-specific T_{REG} alone did not prevent tumour recurrence in a mouse model of melanoma. However, the combination of PD-L1 blockade and intumour T_{REG} depletion via administration of interleukin-2 diphtheria toxin effectively mediated melanoma regression.[13] These results indicate that primary and relapsing cancer might have different characteristics and the use of combined immunotherapy approaches that specifically target T_{REG} cells, could be required for highly resistant recurrent disease.

Overall, we believe that the effect of immune checkpoint blockade (via CTLA-4, PD-1 or PD-L1) on T_{REG} should be considered when evaluating the efficacy of cancer-immunotherapy and that the available data clearly warrant clinical studies of combined immune checkpoint blockade and a T_{REG}-targeting strategy in primary and relapsed cancers.

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Competing interests
The authors declare no competing interests.

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