Mechanistic Elucidation and Therapeutic Improvement of Anti-CTLA-4 Therapies

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Abstract

Immunotherapies have completely shifted the way in which we think about cancer treatment. One of the most successful classes of cancer immunotherapies is checkpoint blockade, which consists of monoclonal antibodies that target inhibitory immune receptors. Antagonism of these checkpoint molecules blocks their ability to perform their immunosuppressive functions, enhancing the overall endogenous antitumor immune response. The first FDA-approved checkpoint inhibitor was an anti-CTLA-4 antibody, ipilimumab, which has successfully elicited durable tumor regression in the clinic. However, long-term benefit is limited to a subset of patients for select cancer indications.

The incomplete understanding of the anti-CTLA-4 antibody mechanism of action has hindered efforts at improvement, with conflicting hypotheses proposing either antagonism of the CTLA-4:B7 axis or Fc effector-mediated regulatory T cell (Treg) depletion governing efficacy. Here we report the engineering of a non-antagonistic CTLA-4 binding domain (b1s1e2) that depletes intratumoral Tregs as an Fc fusion. Comparison of b1s1e2-Fc to 9d9, an antagonistic anti-CTLA-4 antibody, allowed for determination of the separate contributions of CTLA-4 antagonism and Treg depletion to efficacy. Despite equivalent levels of intratumoral Treg depletion, 9d9 achieved more long-term cures than b1s1e2-Fc in MC38 tumors, demonstrating that CTLA-4 antagonism provided additional survival benefit. Consistent with prior reports that CTLA-4 antagonism enhances priming, treatment with 9d9, but not b1s1e2-Fc, increased the percentage of activated T cells in the tumor-draining lymph node (tdLN). Treg depletion with both constructs was restricted to the tumor due to insufficient surface CTLA-4 expression on Tregs in other compartments to elicit Fc effector-mediated Treg depletion.

Through intratumoral administration of diphtheria toxin (DT) in Foxp3-DTR mice, we show that depletion of both intratumoral and intranodal Tregs provided even greater survival benefit than 9d9, consistent with Treg-mediated restraint of priming in the tdLN. Lastly, we engineered a CTLA-4-targeted enzyme fusion as a potentially translatable approach for combined intratumoral and intranodal Treg depletion. Preliminary data suggest that CTLA-4 targeting increases local Treg death as a result of proximal enzymatic activity, but further characterization remains to be done. Overall, our data demonstrate that anti-CTLA-4 therapies require both CTLA-4 antagonism and intratumoral Treg depletion for maximum efficacy - but that future therapies capable of depleting intranodal Tregs could show superior efficacy, even in the absence of CTLA-4 antagonism.