809 A BISPECIFIC ANTIBODY TARGETING CCR8 & CTLA-4: POTENT ANTI-TUMOR EFFICACY WITH BETTER SAFETY BY PREFERENTIALLY ELIMINATING TUMOR-INFILTRATING REGULATORY T CELLS

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Background Regulatory T (Treg) cells play a critical role in maintaining homeostasis and self-tolerance. In tumor immunology, Tregs hamper anti-tumor immune responses and thus promotes tumor development and progression.[1] Many Treg-targeting therapies are currently under clinical investigation. However, due to difficulty in selectively targeting tumor-infiltrating Treg cells, many of these agents have shown limited efficacy and/or treatment-limiting systemic toxicity. CCR8, a chemokine receptor, has recently been identified to mainly be expressed on tumor associated Tregs but other effector T cell populations also express CCR8, raising the uncertainty of universally depleting CCR8-positive cells.[2] To increase the specificity of targeting tumor Tregs, CTLA-4 was selected as a target pair with CCR8 for the generation of an IgG-like bispecific antibody.

Methods Anti-CCR8 x CTLA-4 bispecifics were generated in a 1+1 format and screened from multiple anti-CCR8 and anti-CTLA-4 binding arms with a wide range of affinities. CCR8 and CTLA-4 single-positive cells and CCR8/CTLA-4 double-positive cells were generated to screen for bispecifics with preferential binding and killing towards double-positive cells over single-positive cells *in vitro*. Lead candidates were generated to evaluate anti-tumor efficacy as a proof-of-concept study in human-CCR8/CTLA-4 double knock-in mice, inoculated with CT-26 tumor cells. An anti-CCR8 x CTLA-4 surrogate antibody was also generated to evaluate immunotoxicity in human-CLTA-4 knock-in mice.

Results Our lead anti-CCR8 x CTLA-4 bispecific showed strong binding to CCR8/CTLA-4 double-positive cells but weak to minimal binding to either CCR8 or CTLA-4 single-positive cells. This binding profile translated to strong ADCC activity towards double-positive cells but much weaker activity towards CCR8 or CLTA4 single-positive cells. Accordingly, anti-CCR8 x CTLA-4 bispecifics showed superior anti-tumor efficacy *in vivo*, with similar or better tumor growth inhibition compared to the corresponding monospecific agents. Importantly, the bispecifics showed limited depletion of surface CLTA4 and weak blocking activity against CTLA-4 single-positive cells, which have been proposed to be contributing factors of immunotoxicity in the clinic when targeting CTLA-4. In agreement with this concept, a CCR8 x CTLA-4 surrogate bispecific did not induce immunotoxicity in a mouse model in combination with anti-PD1.

Conclusions We have successfully generated an anti-CCR8 x CTLA-4 bispecific that preferentially eliminates CCR8/CTLA-4 double-positive tumor-infiltrating Treg cells with minimized interference towards single-positive cells. This achieves superior anti-tumor activity than the corresponding monospecific agents, with lower immunotoxicity, and may thus function as a safer, more specific, and highly effective tumor-infiltrating Treg-depleting agent.

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Ethics Approval All mice were maintained under specified pathogen-free conditions, and all studies were approved by the Animal Care and Use Committee of HUST-Suzhou Institute for Brainsmatics.

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