A BISPECIFIC ANTIBODY TARGETING CCR8 & CTLA-4: POTENT ANTI-TUMOR EFFICACY WITH BETTER SAFETY BY PREFERENTIALLY ELIMINATING TUMOR-INFLTRATING 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 promote tumor development and progression. 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. 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 CTLA4 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 CTLA4 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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REFERENCES

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.