10 ENHANCING THE THERAPEUTIC RESPONSES OF ANTI-HUMAN CCR8 MABS UTILIZING THE FULLY HUMANIZED CCR8 KNOCKIN MICE

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Background Depletion of tumor-resident CCR8$^+$ Treg cells has been demonstrated remarkable antitumor effects. A few hCCR8-targeted therapeutic antibodies have been developed and are now undergone clinical testing in patients with advanced or metastatic solid tumors. However, the challenge remains in preclinical evaluation for human-specific CCR8 mAbs in immunocompetent system.[1–3] Therefore, we developed the first-generation chimeric h/m CCR8 knockin mouse model, i.e., hCCR8 (v1) for in vivo test of anti-hCCR8 mAbs. Whereas, we found that MC38 syngeneic tumors displayed no responses to anti-hCCR8 blockade in hCCR8 (v1) mice, which has been a 'hot' tumor model to ICIs or Treg-depletion. We guess that the conformational change of the 7-transmembrane GPCR does matter, because hCCR8 (v1) mice express chimeric h/mCCR8 proteins that only aa 1–280 are humanized and aa 281–355 are still mouse sequence.[4] Hence, we developed the second-generation hCCR8 knockin mouse model, i.e., hCCR8 (v2) for this, in which only full-length of human CCR8 proteins are expressed for homozygous hCCR8 (v2). Here we demonstrated that hCCR8 (v2) mice have higher level of hCCR8 protein expression on the activated CD4$^+$ and CD8$^+$ T cells as well as Treg cells. Intriguingly, we observed distinct antitumor effects in hCCR8 (v2) mice engrafted with MC38 tumors treated with anti-hCCR8 at both low and high dose.

Methods The hCCR8 (v2) knockin mice was established via CRISPR/Cas9 engineering that the entire coding sequence of mouse CCR8 is replaced by full-length human CCR8 in C57BL/6 mice. The expression of full-length hCCR8 protein in hCCR8 (v2) knockin mice was validated by FACS. Then, the binding and EC50 of BMS-986340 and anti-hCCR8 (BD, clone 433H) was determined by FACS using the activated splenocytes from hCCR8 (v2) knockin mice by anti-mCD3/anti-mCD28.2/mIL-2. Finally, hCCR8 (v2) knockin mice were engrafted with MC38 syngeneic tumors and treated with anti-hCCR8 (BMS-986340) via *i.p.* injection to evaluate tumor growth inhibition (TGI) of anti-hCCR8 mAbs *in vivo*.

Results Anti-hCCR8 mAb led to ~38% TGI at 2 mg/kg and ~50% TGI at 10 mg/kg in MC38 tumor model, respectively. We observed the tumor-resident CCR8$^+$ Treg populations decreased significantly and CD4$^+$/CD8$^+$ T cells proliferation as well as PD-1 upregulation in CD4$^+$/CD8$^+$ T cells 24h post 6th dose.

Conclusions The hCCR8(v2) mouse model is a more suitable preclinical humanized model distinguished with the previous hCCR8(v1) mouse model, enabling the in vivo evaluation of various human-specific CCR8 mAbs alone or in combination with immune checkpoint inhibitors like PD-1 and CTLA-4.

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Ethics Approval All studies were conducted following an approved IACUC protocol (SMOC-IACUC NO. 2022–0049). Although this study was not conducted in accordance with the FDA Good Laboratory Practice regulations, 21 CFR Part 58, all experimental data management and reporting procedures were in strict accordance with applicable Shanghai Model Organisms Center, Inc. Guidelines. Guidelines and Standard Operating Procedures. The methods and results in this study accurately reflect the raw data generated during the execution of the study.

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