EVALUATION OF EFFICACY AND TOXICITY OF HUMAN CD40 AGONISTIC ANTIBODIES IN C57BL/6-HCD40/HFcγRIIB HuGEMM™

Demi X Liu1, Xiaoxia Lian2, Xuefei Yan2, Rong Wang2, Jie Lin3, Xinhe Feng2, Xiaolong Tu1, Chengcheng Wang2, Lei Zheng2, Xiaoxi Xu2, Annie An2, Ludovic Bourre2, Jessie Wang2, 1Crown Bioscience, Beijing, China; 2Crown Bioscience Inc., San Diego, CA, USA

Background CD40 is a popular target for tumor immunotherapy. However, the clinical development of CD40 agonistic antibodies has been severely hampered by the frequent association of immune-related adverse events (irAE). Engagement of FcγRIIB by the Fc portion of agonistic anti-CD40 was reported as critical for its antitumor efficacy and related irAE in both mouse experiments and clinical trials. To evaluate both the efficacy and the irAE of anti-CD40 agonists in preclinical studies, we established a HuGEMM™ mouse model in which both human CD40 and FcγRIIB were knock-in in C57BL/6 background (C57BL/6-hCD40/hFcγRIIB) mice.

Methods Homozygous C57BL/6-hCD40/hFcγRIIB HuGEMM™ were bred from CRISPR/Cas9-mediated gene editing of human CD40 and FcγRIIB gene single knock-in mice. The cell surface expression of hCD40 and hFcγRIIB on mouse B cells and B cell activation by various agonistic anti-hCD40 antibodies were confirmed by FACS. To evaluate the antibody efficacy and irAE, homozygous C57BL/6-CD40/FcγRIIB HuGEMM™ were subcutaneously inoculated with MC38 tumor cells. AST and ALT levels were measured by biochemical analysis. Serum TNF-α, IL-12p70, IL-6 and IFN-γ were measured by ELISA. Immune cell infiltration in the liver and tumor was analyzed by immunohistochemistry (IHC) and/or FACS.

Results C57BL/6-CD40/FcγRIIB HuGEMM™ was successfully generated with confirmed expression of human CD40 and FcγRIIB. FcγRIIB engagement enhanced the agonistic activity of the anti-hCD40 antibody as detected by an in vitro B cell activation assay. Human IgG1 isotype anti-hCD40 agonist with hFcγRIIB binding capability (APX005M-S267E) significantly promoted B cell activation compared to its analogue without hFcγRIIB binding capability (APX005M-N297A). Interestingly, a hIgG2a isotype hCD40 antibody, Selicrelumab, which cannot bind hFcγRIIB, also activated the humanized B cells, even at a higher level, suggesting robust hFcγRIIB-independent hCD40 activation. In vivo efficacy and irAE of these agonistic anti-hCD40 antibodies demonstrated that anti-hCD40 antibody with FcγRIIB binding capability significantly inhibited tumor growth and promoted CD8+ T cell infiltration of tumors, compared to the control anti-hCD40 antibody without hFcγRIIB binding capability. In addition, Selicrelumab showed a highly efficient anti-tumor effect with increased T cell infiltration in tumors compared with APX005M-hIgG1(S267E). No significant body weight loss was observed in any of these animals. However, FcγRIIB engagement exacerbated the irAE response with enhanced liver damage, pro-inflammatory cytokine secretion, and immune cell infiltration into livers.

Conclusions This study revealed that human CD40 agonistic antibodies evaluated in C57BL/6-CD40/FcγRIIB HuGEMM™ mice reproduce the efficacy and toxicity phenotypes observed in clinic, demonstrating the improved clinical relevance of this HuGEMM™ model to evaluate future biologic molecules targeting CD40.

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