17 HUMANIZED NKP46 MOUSE MODELS FOR TESTING NOVEL NK CELL-BASED IMMUNOTHERAPIES

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Background NKP46 is a key activating receptor on the surface of human NK cells that can trigger NK-mediated killing activity. While stimulation of NKP46 activity is a promising therapeutic strategy, evaluating novel anti-NKP46-targeting drugs *in vivo* is challenging due to differences between murine and human NKP46 protein. Using gene editing technology, we generated humanized NKP46 alleles on the C57BL/6, BALB/c, and CB-17 SCID backgrounds via genomic knock-in, for the purpose of evaluating novel NKP46 modulators in the context of various human or murine tumor cell lines.

Methods Using flow cytometry, we evaluated the expression of murine and human NKP46+ cell populations in each strain of humanized NKP46 (hNKP46) mice compared with the NK cell markers NK1.1 (C57BL/6) and CD49b (BALB/c and CB-17 SCID). In addition, the overall distribution of leukocytes was examined in homozygous hNKP46 mice on the C57BL/6 background. As a proof-of-concept study, MC38 tumor efficacy studies were performed: $5x10^5$ MC38 cells were subcutaneously implanted in hNKP46-expressing C57BL/6 mice. When the tumor volume reached 100 mm^3, mice were grouped and intraperitoneally injected with three different anti-human NKP46 antibodies (6 mg/kg) twice a week. Tumor volume and body weight were monitored until the endpoint at 20 days.

Results In homozygous hNKP46 C57BL/6 mice, human NKP46 was co-expressed with the murine NK marker NK1.1. In homozygous hNKP46 BALB/c and CB-17 SCID mice, NKP46 was co-expressed with CD49b and not with CD19 or CD3. Flow cytometry assessments using other lineage markers confirmed that humanization of NKP46 on the C57BL/6 background did not alter the proportion of lymphoid or myeloid cell populations. Finally, tumor efficacy studies demonstrate that treatment with selected anti-human NKP46 antibodies reduced MC38 tumor growth in homozygous hNKP46 mice compared to PBS-injected controls.

Conclusions Humanized NKP46 mice on the C57 BL/6 and BALB/c backgrounds are a promising tool to evaluate the role of anti-human NKP46 antibodies in preventing growth of syngeneic tumor cell lines. Humanized NKP46 mice on the CB-17 SCID background can also be used to assess the role of NKP46 modulators in killing human tumor cells.

Ethics Approval All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Biocytogen Beijing Co., Ltd.

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