BALB/c-CD3EDG HuGEMM™ Mouse Model for Evaluation of CD3-Targeted Tumor Immunotherapy

Demi X. Liu, Kaixia Lian, Xuefui Yan, Jun Zhou, Jie Lin, Lei Zheng, Xiaoxi Xu, Annie Xiaoyu An, Ludovic Bourre, Jessie JingJing Wang
Crown Bioscience Inc., 16550 West Bernardo Drive, San Diego, CA 92127

Introduction
T cell immune response plays a critical role in cancer immunotherapy. CD3 complex, composed of CD3α, CD3δ, and CD3γ, is an essential component of TCR-CD3 complex mediating TCR signaling. Targeting CD3 complex has therefore become a popular strategy for manipulation of T cell function. Clinically relevant animal models for proper evaluation of human CD3 antibodies are in great demand. Currently, adoptive transfer of human CD3+ T cells or transplantation of human hematopoietic stem/progenitor cells to immunodeficient mice are usually employed. However, these mouse models are not fully immunocompetent and have some limitations. This study evaluated the in vitro CD3EDG functionality and in vivo therapeutic efficacy in BALB/c-CD3EDG knock-in mice in which all the three components of the mouse CD3 complex — CD3ε, CD3δ, and CD3γ — are replaced by their human counterparts, hCD3ε, hCD3δ, and hCD3γ.

Methods
- Humanized CD3EDG mouse model (Hu3EDG) on BALB/c background was generated by replacing the entire mouse CD3EDG with its human counterpart through ES cell-based gene targeting.
- The cell surface expression of hCD3ε and intracellular expression of hCD3δ and hCD3γ were confirmed by FACS on mouse splenocytes and peripheral T cells respectively. Gating was performed on the live cell population.
- CD3+ T cells were isolated from the spleenocytes of WT or homozygous CD3EDG HuGEMM and activated by pre-coated anti-mCD3 or anti-hCD3 antibody respectively. hCD3ε expression in cell culture for 3 days. T cell proliferation was demonstrated by CFSE dilution assay.
- CT26-hEpCAM HuGEMM™ single clone was generated using lentivirus system.
- To validate the TCR functionality of humanized CD3EDG in vivo, the bispecific antibody anti-hCD3/hEpCAM was evaluated in homozygous BALB/c-CD3EDG HuGEMM bearing subcutaneous CT26-hEpCAM tumors. Treatment was initiated when the mean tumor volume reached 100 mm³. Mice were dosed at 1 mg/kg, i.p., QD, 3 on 3 for 4 cycles with anti-hCD3/hEpCAM bispecific.

Results
Balb/c-CD3EDG HuGEMM was successfully generated and the expression of human CD3ε, CD3δ, and CD3γ were confirmed (Figures 1 and 2). The TCR functionality was detected by T cell activation assays using anti-hCD3 and anti-mCD3 antibodies (Figure 3). CT26-hEpCAM grew in BALB/c-CD3EDG mice and hEpCAM antigen was detected in CT26-hEpCAM cell line in vitro and tumor in vivo (Figure 4). In vivo efficacy was demonstrated using a bispecific antibody that simultaneously binds to human CD3 and human tumor associated antigen EpCAM in human CD3EDG knock-in mice engrafted with CT26-HepCAM tumor (Figure 5). The therapeutic efficacy of the bispecific antibody showed significant tumor growth inhibition (32%, p<0.05) compared to control treatment. No significant body weight loss was recorded in any of the animals.

References

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