Utility of Humanized Animal Models for In Vivo Evaluation of NK TICA™ Novel Bicycle® Tumor Targeted Immune Cell Agonists® Bicycle TICA™ Designed to Engage NK Cells

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Background

The use of humanized animal models in preclinical evaluation of immunotherapeutics is often dictated by lack of homology between mouse and human target or divergence in the biology of studied immune cell subtypes. A target expressed on NK cells, NKp46 is a key activating receptor contributing to cytolytic function of NK cells. Bicycle® peptides are small (~1.5 kDa), chemically synthetic, structurally constrained bicyclic peptides discovered using phage display. NKp46-binding Bicycles conjugated to a tumor antigen-binding Bicycle® directed human NK cells to kill the tumor cells expressing the target antigen, and we term these molecules NK tumor-targeted immune cell agonists (NK-TICATM). Due to low homology of NKp46 ectodomain between mouse and human (~63% by NCBI BLAST), we evaluated different approaches to animal humanization to establish the most suitable model for in vivo evaluation of NK-TICATM. The main selection criteria included the number of circulating NKp46+ human NK cells as well as expression of activating and inhibitory receptors.

Methods

Distinct approaches to animal humanization were employed: 1) reconstitution of human immune system (HIS) with CD34+ hematopoietic stem cells (HSCs) in NCG mice with hIL-15 hydrodynamic injection (HDI); 2) reconstitution of HIS with CD34+ HSCs in NCG.hIL-15 transgenic mice; 3) infusion of human PBMC-derived NK cells in NCG.hIL-15 transgenic mice. Characterization of NK populations in blood was conducted by multiplex flow cytometry.

Results

Immunophenotyping analysis revealed striking differences in numbers of circulating NKp46+ NK cells between different humanized models. The lowest number was observed in mice engrafted with HSCs with transient expression of hIL-15 and the highest in mice infused with PBMC-derived NK cells in a transgenic model constitutively expressing hIL-15. We also observed expansion of NK cells over time in mice with transgenic expression of hIL-15 but not in mice treated with single hIL-15 HDI. Despite these differences, the proportion of NKp46+ NK cells expressing activation receptor NKG2D or activation marker CD69 was similar between all three study designs. In contrast, expression of NKp30 and NKG2A was different across tested models.

Conclusions

Transgenic mice with constitutive expression of hIL-15 showed the highest numbers of NKp46+ NK cells in blood and a better expression profile of NK activating and inhibitory receptors making it a more suitable model for in vivo evaluation of NK-TICAs™.

Ethics Approval

These studies were approved by the Institutional Animal Care and Use Committee (IACUC). The care and use of animals was conducted in accordance with regulations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).