Introduction

The glyco-immune checkpoint (Siglec/Siglax) axis has recently emerged as a novel mechanism of cancer immune evasion. We have previously described an antibody-based fusion platform technology named EAGLE (EAGLE Anti-Glycan Ligand Editing), using a bacterial platform for the construction of recombinant antibodies for glycan-targeting therapy. Using this technology, we have created a novel antibody-based approach to target and block the Siglec/Sialoglycan axis (Siglax) in human tumors. This manuscript describes optimization and targeted use of a humanized antibody to overcome its low expression and poor stability issues. We confirmed the antitumor activity of the humanized antibody (EAGLE mAb) and identified preclinical and nonclinical pharmacodynamic (PD) biomarkers to EAGLE mAb treatment in preclinical tumor models. EAGLE mAb with engineered human siglax has an efficacy in immunomodulatory model mimicking mouse tumor immunotherapy by releasing immunosuppression of Siglec/Sialglycan axis in the tumor microenvironment.

Glyco-Immune Checkpoints Suppress Innate and Adaptive Immunity

Results

Construction of EAGLES in Various Configurations (Enzyme-Activity Glyco-Ligand Editing)

Desialylation of Tumor Cells and Immunomodulation by EAGLE

Engineering of Human Siglax for Improved Developability for EAGLE Platform Development

Figure 1. Schematic representation of glyco-immune checkpoint. At the immunological synapse, Siglecs on antigen-presenting cells (APCs) or tumor cells activate and recruit antitumor effector immune cells, causing a variety of tumor-associated antigens which contain a terminal sialic acid. The Siglec/Sialoglycan axis plays important roles in cancer cells binding by T cells and macrophage, cancer antigen presentation, T cell priming and activation, and cancer killing by T effector cells.

Figure 2. Construction and characterization of EAGLEs in various configurations. "Tumor" configuration has the highest expression yield. A) Configuration of EAGLE mAb molecules. The molecular weight of recombinant EAGLE miC molecules in SDS-PAGE was 37.5 kDa. B) Configuration of EAGLE miC molecules. C) Human antibody binding to EAGLE miC molecule compared with Trastuzumab. D) Siglec-surface enzyme activity of EAGLE miC molecules.

Figure 3. EAGLE-HRE/HER2 with engineered human siglax as a therapeutic drug candidate. A) EAGLE-HRE/HER2 interacts with the HER2 receptor (771) on human tumor cells. Tumor growth was measured twice a week. Substrate specificity of Neu2 and mutant 434 towards Sia (except for a mild transient elevation of ALP only observed in rats), and histology analysis at tumor - targeting arm, in rats and cynomolgus monkeys. EAGLE-Sialglycan is tolerated up to 100 mg/kg in rats and cynomolgus monkeys. A) 14-day non-toxicity studies, two groups of rats (100 mg/kg, q.d. × 14) and cynomolgus monkeys (10 mg/kg, q.d. × 14). No significant toxicity findings in rat and monkey toxicity studies (1 in the assessment, clinical chemistry, hematology, organ histology, and organ histology analysis. Observation of pharmacodynamic effects of desialylation of immune cells. EAGLE doesn’t cause cytokine release in human primary lymphocytes


Conclusions

EAGLE demonstrates a wide margin of safety — the safety of the EAGLE platform was evaluated by testing on EAGLE Siglax molecule, a humanized Siglax-Fc fusion with a tumor-associated antigen targeting arm, in rats and cynomolgus monkeys - EAGLE-Sialglycan is tolerated up to 100 mg/kg in rats and cynomolgus monkeys. A) 14-day non-toxicity studies, two groups of rats (100 mg/kg, q.d. × 14) and cynomolgus monkeys (10 mg/kg, q.d. × 14). No significant toxicity findings in rat and monkey toxicity studies (1 in the assessment, clinical chemistry, hematology, organ histology, and organ histology analysis. Observation of pharmacodynamic effects of desialylation of immune cells. EAGLE doesn’t cause cytokine release in human primary lymphocytes

EAGLE demonstrates immunomodulation and compelling monotherapy efficacy in preclinical tumor models, a wide margin of safety in rats and monkeys, and enhancement of anti-cancer immune responses of NK cells, macrophages, DCs, and T cells in human cell systems mimicking tumor-mediated immuno-suppression

EAGLE demonstrates potent antitumor activity in xenograft tumor models in mice and monkeys with immunological memory — No observable adverse effect level (NOAEL) > 150 mg/kg (two doses in monkeys) with NOAEL effects — Potentiation of innate immunity and T cell activation

EAGLE offers new opportunities to treat cancer targeting Siglec/Sialglycan axis — Overcomes the heterogeneous strategies of tumor siglax

Double-targeting strategies promise to enhance tumor immunoevasion

Transformation existing tumor targeting into innate-immune stimulating agents