A Novel Immunomodulatory Strategy of Targeting Glyco-Immune Checkpoints with EAGLE Technology

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Introduction
Cancer therapy has been revolutionized by the recent developments of immune-checkpoint inhibitors to harness the power of the immune system in fighting cancer. However, the majority of patients fail to have durable responses or become resistant to immuno-oncology drugs, highlighting the need to identify new mechanisms of immune evasion in cancer and to develop new therapeutic modalities. Recently, the glyco-immune checkpoint axis (sialylglycan/Siglec pathway) has emerged as a novel mechanism of immune regulation involving both innate and adaptive immunity and an important mechanism of cancer immune escape. Upon ligandation of sialylated glycans to ITIM-containing Siglecs on immune cells, this path plays a previously unrecognized role in regulating functions of NK cell, macrophages, dendritic cells, monocytes and T-cells in the tumor microenvironment. It suppresses multiple facets of anti-cancer immunity, including cancer antigen release, cancer antigen presentation, priming and activation of anti-cancer T cell immunity, which may represent a novel mechanism of resistance to current immunotherapy. Here, we described a novel therapeutic modality, a multi-functional antibody like molecule named EAGLE (Enzyme-Antibody Glyco-Ligand Editing), to inhibit the glyco-immune checkpoint axis in the tumor microenvironment by selectively removing the terminal sialic acids of sialylglycans on tumor cells.

Glyco-Immune Checkpoints Suppress Multiple Steps in Cancer

EAGLE Reduced Tumor Cell Surface Sialic Acids Levels in vivo

**Figure 4.** Systematic delivery of EAGLE cleaved sialic acids from tumors in vivo. (A) Experiment design of EAGLE treatment. (B) FACS analysis of surface sialic acid levels of tumors.

Multiple EAGLE Formats Achieved Single Agent Complete Regressions in Preclinical Tumor Models

**Figure 5.** Efficacy studies of EAGLEs in syngeneic breast cancer EM6-Her2 tumor model. Wild type BALB/c mice (n = 8 per group) were injected s.c. EM6-Her2 cells. When tumor sizes reached about ~120 mm³, mice were treated with EAGLE-301, 302, 303, Trastuzumab, or vehicle control twice a week for 3 weeks at 10mg/kg. The tumor growth was measured twice a week. (A) Tumor growth curves of mean tumor sizes of each group. (B) Tumor growth curves of individual mice.

EAGLE Has Monotherapy Efficacy Comparable to the Combination of α-PD1 and α-CTLA4 in the “Cold” Tumor B16 Model

**Figure 6.** EAGLE demonstrated single agent anti-tumor activity in B16OS-Hez2 syngeneic tumor model. Wild type C57Bl/6 mice (n = 6 per group) were inoculated with B16OS-Hez2 cells subcutaneously and treated with EAGLE-302, the combination of anti-CTLA4 and anti-PD-1, or Trastuzumab when tumor sizes reached about ~250 mm³. All antibodies were dosed twice a week for 2 weeks at 10mg/kg. Each line represented a tumor growth curve of an individual mouse.

The Mechanism of Action of EAGLE Involves Innate and Adaptive Immunity

**Figure 8.** The MoA of EAGLE involves NK cells, macrophages, and CD8+ T-cells. Wild type BALB/c mice (n = 8 per group) were inoculated with EM6-Her2 cells into mammary fat. When tumor sizes reached about ~250 mm³, mice were treated with EAGLE-302, anti-PD-1 mAb, the combination of EAGLE-302 and anti-PD-1 mAb, or controls of EAGLE loss-of-function and Trastuzumab twice a week for 2 weeks at 10mg/kg.

EAGLE-Her2 Pilot Tumor Toxicity Study Showed No Major Events

**Figure 10.** Flat 14-day toxicity study for EAGLE-Her2. EAGLE-302 was given by intravenous administration on days 1, 8, and 15 to Sprague-Dawley rats. There were no significant changes in body weights, white or red blood cell counts, or histopathological findings.

Proprietary Hydra Biomarker Assays to Detect Tumor Glyco-Codes in Patients

**Figure 11.** Representative Hydra staining of tumor sialylglycan levels in NSCLC, colon cancer, and breast cancer for patient selection.

Conclusions

- Glyco-immune checkpoints play critical roles in cancer immune evasion
- Innate immune response
- Adaptive immune response
- EAGLE showed compelling monotherapy efficacy in syngeneic tumor models
  - Single agent complete regressions with immunomodulatory mechanisms
  - Efficacious in cold tumor models
  - Striking activity in combination with PD-1/PD-L1
- EAGLE offers new opportunities to treat cancer targeting glyco-immune checkpoints
  - Overcomes the heterogeneity challenges of tumor sialylglycans
  - Disables immunosuppressive glycan functions within tumor microenvironment
  - Scalable, potential to transform existing tumor targeting mAbs into immune-modulating agents

References