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 (sialoglycan/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[1-3]. Upon ligation of sialylated glycans to ITIM-containing Siglecs on immune cells, this pathway 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 sialoglycans on tumor cells.

Glyco-Immune Checkpoints Suppress Multiple Steps in Cancer

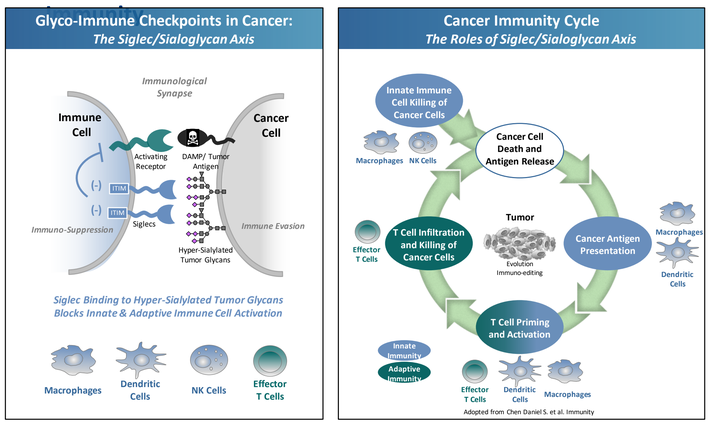

Figure 1. Glyco-immune checkpoints. (Left) Schematic representation of glyco-immune checkpoints axis. At the immunological synapse, Siglecs (sialic acid-binding Ig-like lectins) on immune cells interact with tumor associated sialoglycans and recruit SHP phosphatases, dampening immune responses. Siglecs are expressed on macrophages, monocytes, DC, and NK cells, recognizing a variety of tumor associated glycans which contain a terminal sialic acid. (Right) The role of Siglec/Sialoglycan axis in cancer immunity cycle. The Siglec/Sialoglycan plays roles in cancer cell killing by innate immune cells, cancer antigen presentation, T cell priming and activation, and cancer cell killing by T effector cells. Sialylated glycans, sLe^x and sTn, were reported to associate with poor outcome in gastric and breast cancer patients.

Results

Development of the *EAGLE* Therapeutic Platform (Enzyme-Antibody Glycan-Ligand Editing)

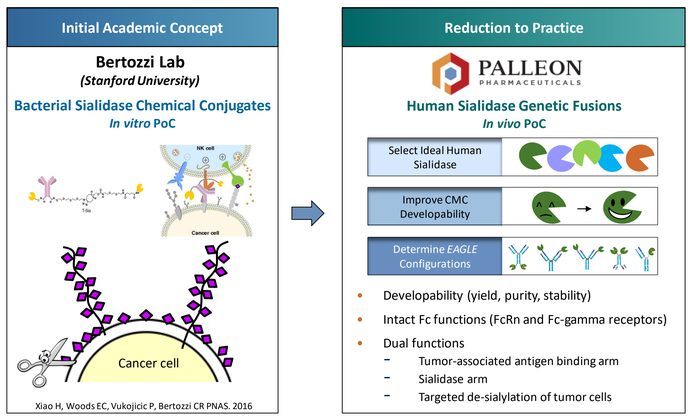

Figure 2. Evolution of *EAGLE* platform. (A) Schematic representation of antibody-sialidase chemical conjugates. Conjugating bacterial sialidase to antibodies which recognize tumor-associated antigens enables targeted removal of sialic acids of sialoglycans on tumor cells[4]. (B) Development of *EAGLE* therapeutic platform.

*EAGLE*s Showed Targeted Cleavage of Terminal Sialic Acids from Her2+ Tumor Cells

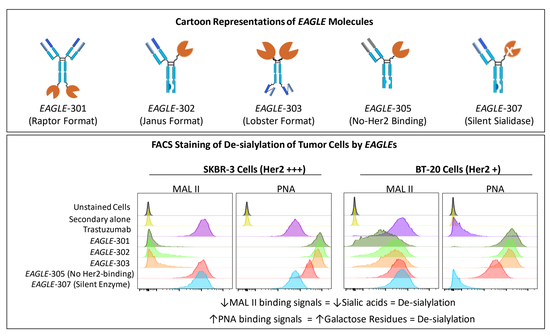

Figure 3. FACS staining of desialylation of tumor cells treated with *EAGLE*s. Compared to the control group of trastuzumab treatment, various *EAGLE*s (301, 302, and 303) decreased sialic acid levels on cancer cells with high or low levels of Her2. The cleavage of sialic acids on tumor cells are specific, since no sialic acid cleavage was observed for the silent enzyme control (*EAGLE*-307) and substantially reduced cleavage for the silent Her2-binding (*EAGLE*-305).

EAGLE Reduced Tumor Cell Surface Sialic Acids Levels *in vivo*

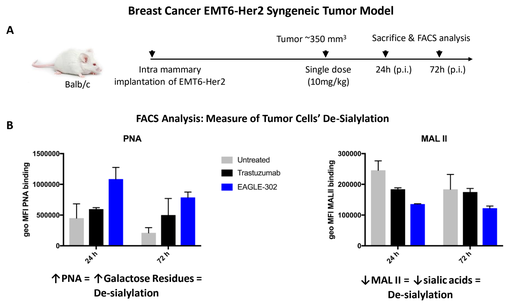

Figure 4. Systematic delivery of *EAGLE* cleaved sialic acids from tumors *in vivo*. (A) Experiment design of *EAGLE* treatment. (B) FACS analysis of surface sialic acid levels of tumors.

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

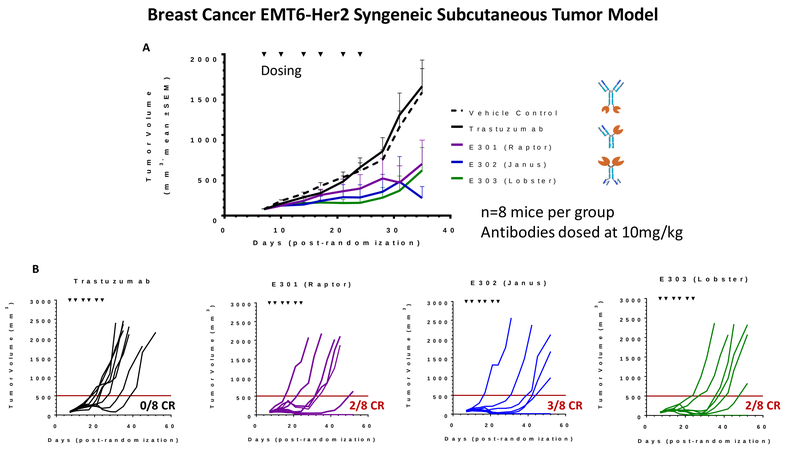

Figure 5. Efficacy studies of *EAGLE*s in syngeneic breast cancer EMT6-Her2 tumor model. Wild type BALB/c mice (n = 8 per group) were injected s.c. EMT6-Her2 cells. When tumor sizes reached about ~120 mm^3, mice were treated with *EAGLE*-301, 302, 303, Trastuzumab, or vehicle control twice a week for 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

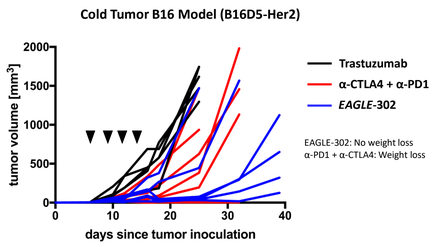

Figure 6. *EAGLE* demonstrated single agent anti-tumor activity in B16D5-Her2 syngeneic tumor model. Wild type C57BL/6 mice (n = 6 per group) were inoculated with B16D5-Her2 cells subcutaneously and treated with *EAGLE*-302, the combination of anti-CTLA4 and anti-PD-1, or Trastuzumab when tumor sizes reached about ~100 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.

EAGLE Showed Sialidase-Dependent Efficacy, Induced Anti-Tumor Immunological Memory, and Increased Immune Cell Infiltration

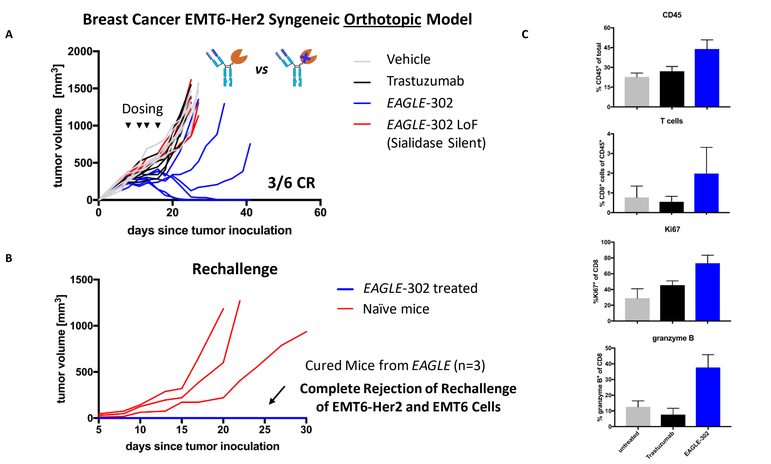

Figure 7. Efficacy studies of *EAGLE* in orthotopic syngeneic breast cancer tumor model. (A) *EAGLE* demonstrated sialidase-dependent anti-tumor activity. Wild type BALB/c mice (n = 6 per group) were inoculated with EMT6-Her2 cells into mammary fat and treated with *EAGLE*-302 and its loss-of function control when tumor sizes reached about ~250 mm^3. *EAGLE*s and trastuzumab were dosed twice a week for 2 weeks at 10mg/kg. (B) Cured mice from *EAGLE* treatment in experiment (A) rejected re-challenge of EMT6-Her2 and EMT6 cells. (C) FACS analysis of tumor infiltrating lymphocytes after *EAGLE* treatment. *EAGLE* increased T cell infiltration and activation.

The Mechanism of Action of *EAGLE* Involves Innate and Adaptive Immunity

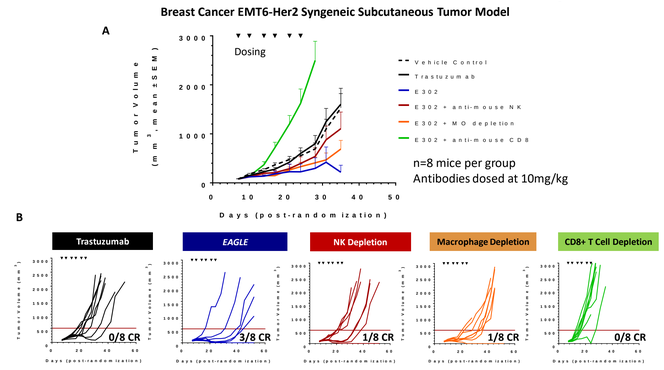

Figure 8. The MoA of *EAGLE* involves NK cells, macrophages, and CD8+ T-cells. Wild type BALB/c mice (n = 8 per group) were injected s.c. EMT6-Her2 cells. When tumor sizes reached about ~120 mm^3, mice were treated on the same days as *EAGLE*-302 with either anti-mouse NK1.1 (10 mg/kg) to deplete natural killer cells, liposomal clodronate (0.5 mg/mouse, three times a week for two weeks) to deplete macrophages, or anti-mouse CD8α (10 mg/kg) to deplete CD8+ T cells. (A) Tumor growth curves of mean tumor sizes of each group. (B) Tumor growth curves of individual mice.

EAGLE Has Significant Combination Efficacy with T-Cell Checkpoint Inhibition

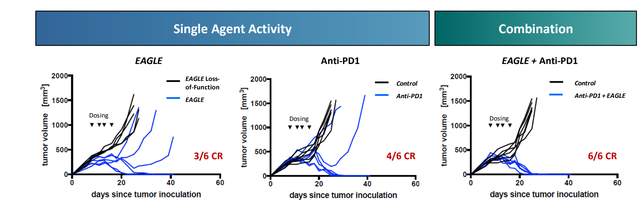

Figure 9. *EAGLE* achieved 100% cures in breast cancer EMT6-Her2 orthotopic model in combination with PD-1/PD-L1 inhibitions. Wild type *BALB/c* mice (n = 6 per group) were inoculated with EMT6-Her2 cells into mammary fat. When tumor sizes reached about ~250 mm3, mice were treated with *EAGLE*-302, anti- PD1 mAb, the combination of *EAGLE*-302 and anti-PD1 mAb, or controls of *EAGLE*-loss-of function and Trastuzumab twice a week for 2 weeks at 10mg/kg.

EAGLE-Her2 Pilot Rat Toxicity Study Showed No Major Events

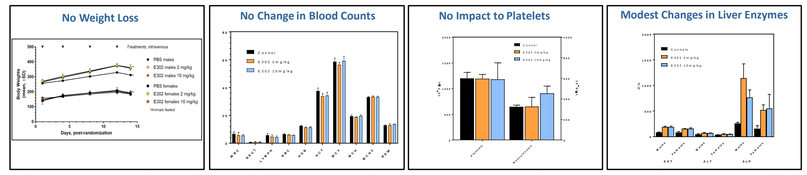

Figure 10. Rat 14-day toxicity study for *EAGLE*-Her2. *EAGLE*-302 was given by intravenous administration on days 1, 4, 8 and 12 to Sprague-Dawley rats. There were no significant changes in body weights, white or red blood cell counts, or histopathological findings.

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

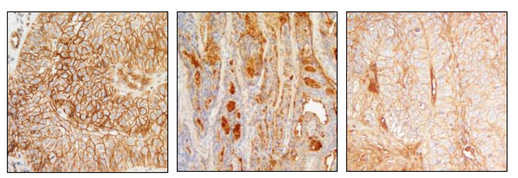

Figure 11. Representative *Hydra* staining of tumor sialoglycan levels in NSCLC, colon cancer, and breast cancer for patient selection.

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 immunological memory
  - Efficacious in cold tumor model
  - 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 sialoglycans
  - Disables immunosuppressive glycan functions within tumor microenvironment
  - Scalable, potential to transform existing tumor targeting mAbs into immune-modulating agents

References

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