A Novel Therapeutic Modality of Inhibiting the Glyco-Immune Checkpoint Axis to Treat Cancer

 # LB-109

Lizhi Cao[1], Adam Petrone[1], Wayne Gatlin[1], Jenny Che[1], Abhishek Das[1], Robert LeBlanc[1], Zakir Siddiquee[1], Sujata Nerle[1], Michal Stanczak[2], Michele Mayo[1], Lihui Xu[1], Karl Normington[1], Jeff Brown[1], Wei Yao[1], Carolyn Bertozzi[3], James Broderick[1], Heinz Läubli[2], Li Peng[1]

[1]Palleon Pharmaceuticals, Waltham, MA, USA, [2] Cancer Immunology Laboratory, University Hospital Basel, Switzerland, [3] Department of Chemistry, Stanford University, Stanford, CA, USA

Introduction

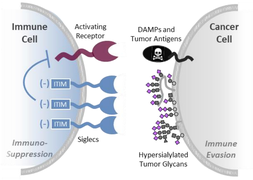
Cancer therapy has been revolutionized by the recent developments of immune-checkpoint inhibitors to harness the power of the immune system in fighting cancer. However, the majority of patients fail to have durable responses or become resistant to immuno-oncology drugs, highlighting the need to identify 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 Innate and Adaptive Immunity

Glyco-Immune Checkpoints in Cancer: *The Siglec/Sialoglycan Axis*

Glyco-Immune Checkpoints:

- Glycan-sensing receptors that block immune activation; play healthy role in self vs non-self discrimination
- Major receptor families: Siglecs (10 unique to human), some CTLs
- Broad expression across various innate and adaptive immune cells
- Exploited by cancer for immune evasion



Tumor Glycan Evolution:

- Tumors evolve and cloak themselves with functionally immunosuppressive glycan patterns
- Several common tumor glycans: ST, STn, SLe^a, SLe^a, GM2, GD3, PSA
- Recurrent pattern: **Hypersialylation**
- On tumor cell surface glyco-proteins, mucins, and glycolipids

Suppression of Innate AND Adaptive Immunity

→ **Potential to address resistant patient populations**

Tumors are Defined by Glyco-Code

→ **Potential for prospective patient stratification**

Figure 1. 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. The Siglec/Sialoglycan plays important roles in cancer cell killing by NK cells and macrophages, cancer antigen presentation, T cell priming and activation, and cancer cell killing by T effector cells.

Results

Development of *EAGLE* (Enzyme-Antibody Glyco-Ligand Editing) Platform

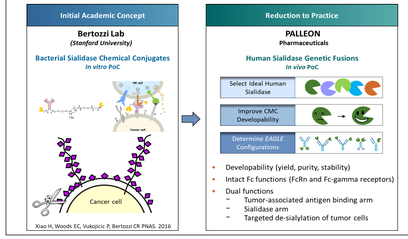
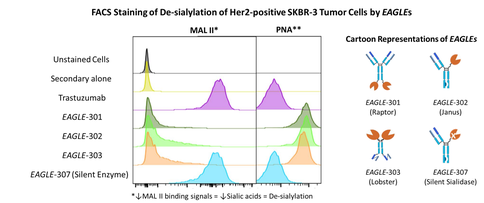
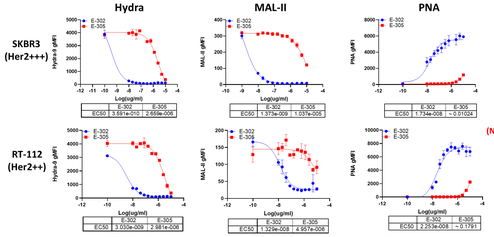


Figure 2. Evolution of *EAGLE* platform. (Left) Schematic representation of antibody-sialidase chemical conjugates using bacterial sialidase. Conjugating bacterial sialidase to antibodies which recognize tumor-associated antigens enables targeted removal of sialic acids of sialoglycans on tumor cells[4]. (Right) Development of *EAGLE* platform. *EAGLE* is a bi-functional antibody-sialidase fusion protein consisting of an engineered human sialidase with improved developability and enzyme activity.

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





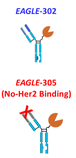


Figure 3. FACS staining of desialylation of tumor cells treated with *EAGLE*s. (Top) Compared to the control group of trastuzumab treatment, various *EAGLE*s (301, 302, and 303) decreased sialic acid levels on Her2-positive cancer cells. (Bottom) Her2-targeting moiety of *EAGLE* enabled more efficient de-sialylation of tumor cells. *EAGLE*-302 with a trastuzumab arm demonstrated 1,000 – 10,000 folds higher potency than non-targeting *EAGLE*-305 consisting of a dummy arm.

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

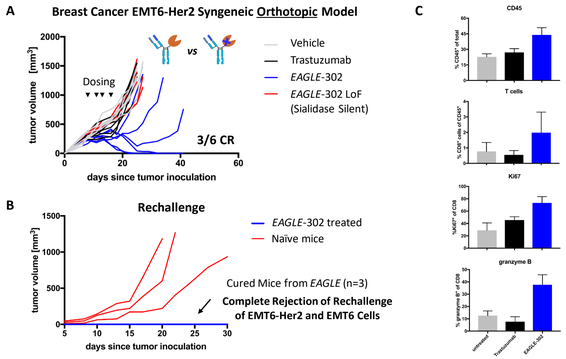


Figure 4. Efficacy studies of *EAGLE* in orthotopic syngeneic breast cancer tumor model. (A) *EAGLE* demonstrated sialidase-dependent anti-tumor activity. Wild type BALB/c mice (n = 6 per group) were inoculated with EMT6-Her2 cells into mammary fat and treated with *EAGLE*-302 and its loss-of function control when tumor sizes reached about ~250 mm³. *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 5. 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 mm³, 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 Monotherapy Efficacy Comparable to the Combination of α-PD1 and α-CTLA4 in the "Cold" Tumor B16 Model

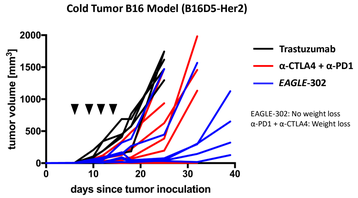


Figure 6. *EAGLE* demonstrated single agent anti-tumor activity in B16D5-Her2 syngeneic tumor model. Wild type C57BL/6 mice (n = 6 per group) were inoculated with B16D5-Her2 cells subcutaneously and treated with *EAGLE*-302, the combination of anti-CTLA4 and anti-PD-1, or Trastuzumab when tumor sizes 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 Has Significant Combination Efficacy with T-Cell Checkpoint Inhibition

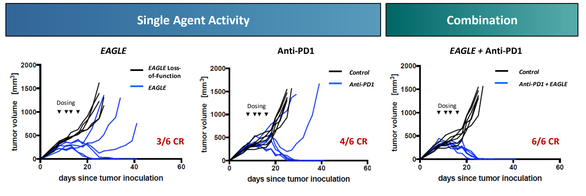
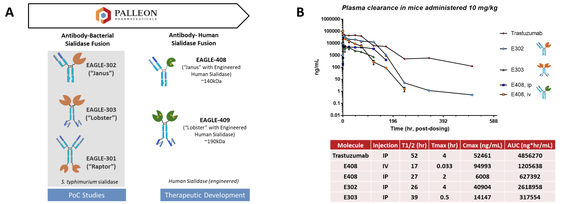


Figure 7. *EAGLE* achieved 100% cures in breast cancer EMT6-Her2 orthotopic model in combination with PD-1/PD-L1 inhibitions. Wild type *BALB/c* mice (n = 6 per group) were inoculated with EMT6-Her2 cells into mammary fat. When tumor sizes reached about ~250 mm3, mice were treated with *EAGLE*-302, anti- 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*s with Engineered Human Salidase



Molecule	Injection	T1/2 (hr)	Tmax (hr)	Cmax (ng/mL)	AUC (ng*hr/mL)
Trastuzumab	IP	52	4	52461	4856270
E408	IV	17	0.033	94993	1205638
E408	IP	27	2	6008	627392
E302	IP	26	4	40904	2618958
E303	IP	39	0.5	14147	317554

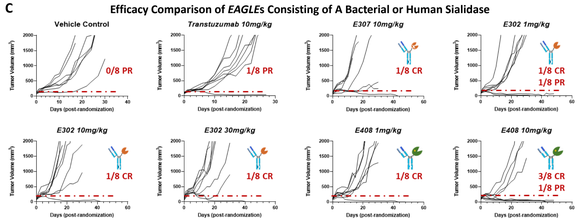


Figure 8. Development of human salidase-based *EAGLE*s. (A) Evolution of *EAGLE* platform. *In vitro* and *in vivo* PoC studies were conducted using the bacterial salidase. The therapeutic *EAGLE*s were developed using a human salidase with improved developability and enzymatic activities through protein engineering. (B) PK profiles of multiple *EAGLE*s in mice. *EAGLE*s demonstrated bispecific antibody-like PK profiles. (C) Efficacy comparison of *EAGLE*s consisting of a bacterial or human salidase in breast cancer EMT6-Her2 syngeneic subcutaneous tumor model. *EAGLE*s with engineered human sialidase (E-408) showed comparable or even superior efficacy than *EAGLE*s of bacterial sialidase (E-302).

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

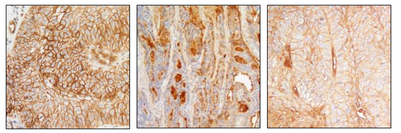


Figure 9. Representative *Hydra* staining of tumor sialoglycan levels in NSCLC, colon cancer, and breast cancer for patient selection.

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
  - Transform existing tumor targeting mAbs into immune-modulating agents

References

1. Crocker PR, Varki A. Siglecs in the immune system. Immunology. 2001;103:137-45.
2. Adams O.J., et al, Targeting sialic acid-Siglec interactions to reverse immune suppression in cancer, Glycobiology, 2018; 28:640-7
3. Stanczak M., et al., Self-associated molecular patterns mediated cancer immune evasion by engaging Siglecs on T cells, J Clin Invest, 2018, 128: 4912–4923
4. Xiao H., et al, Glycocalyx editing for cancer immunotherapy, PNAS 2016, 113:10304-10309