**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 check point 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 (sialyl/alkylation/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 ligation of sialylated glycans to ITIM-containing Siglec on immune cells, this pathway plays a previously unrecognized role in regulating functions of NK, macrophages, dendritic cells, monocytes, and T cells in the tumor microenvironment. It suppresses multiple facets of anti-cancer immunity, by inducing in 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 describe a novel therapeutic modality, a multi-functional antibody-like molecule named EAGLE (Enzyme-Immune Glyco-Ligand Editing), to inhibit the glyco-immune checkpoint axis and further enhance the tumor immunosuppression by selectively removing the terminal sialic acids of sialylglycans on tumor cells.

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### Glyco-Immune Checkpoints Suppress Multiple Steps in Cancer Immunity

- **Adaptive Immunity**
  - **Immune Tolerance**
  - **Innate Immune System**
  - **Adaptive Immune Response**
  - **Immune Checkpoints**
- **Innate Immune Response**
- **Adaptive Immune Response**

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### Results

**Development of the EAGLE Therapeutic Platform**

(Enzyme-Immune Glycan-Ligand Editing)

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### EAGLE Has Significant Combination Efficacy with T-Cell Checkpoint Inhibition

- **Breast Cancer EMT6-Her2 Orthotopic Model**
  - Single Agent Activity
  - Combination

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### Conclusions

- **EAGLE** shows promising efficacy in preclinical models.
- **EAGLE** has the potential to enhance the efficacy of current immunotherapies.
- **EAGLE** holds promise for the treatment of various cancer types.

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**Figure 1**: Glyco-immune checkpoints. (Left) Schematic representation of glyco-immune checkpoints axis. At the immunological synapse, Sialyl (sialic acid binding or lectin) on immune cells interacts with tumor associated sialyllectigs and recruit SHP phosphatases, dampening immune responses. Sialylated epitopes are recognized by macrophages, monocytes, DC, and NK cells, recognizing a variety of tumor-associated antigens which contain terminal sialic acids (Right, the role of Sialytransferase was in cancer immunity cycle. The Sialytransferase plays roles in cancer cell killing by innate immune cells, cancer antigen presentation, T cell priming and activation, and cancer cell killing by effector cells. Glycolectins, sialic acid, and/or sialidases are associated with poor outcomes in gastric and breast cancer patients.

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**Figure 2**: EAGLE has monotherapy efficacy comparable to the combination of a-PD1 and a CTLA4 in the “Cold” Tumor B16 Model.

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**Figure 3**: FACS staining of depleting tumor cells treated with EAGLE. Compared to the control group of treatment with either anti-PD1 or anti-CTLA4, all antibodies were dosed twice a week for 2 weeks at 10mg/kg. Each line represented a tumor growth curve of an individual mouse.

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**Figure 4**: EAGLE demonstrates single agent anti-tumor activity in EMT6-Her2 syngeneic tumor models. Wild-type EMT6-Her2 tumor inoculated with EAGLE-302 cells 1 week post-implantation 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.

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**Figure 5**: EAGLE showed targeted cleavage of terminal sialic acids from Her2+ Tumor Cells.

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**Figure 6**: Systematic delivery of EAGLE showed sialic acids from tumors in vivo. (A) Experiment Design of EAGLE treatment. (B) FACS analysis of surface sialic acid levels of tumor.

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**Figure 7**: Multiple EAGLE Formats Achieved Single Agent Complete Response in Preclinical Tumor Models.

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**Figure 8**: EAGLE Reduced Tumor Cell Surface Sialic Acid Levels In Vivo.

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**Figure 9**: EAGLE demonstrated single agent anti-tumor activity in EMT6-Her2 syngeneic tumor models. On the day 7 of treatment, all groups were dosed twice a week with EAGLE-302 cells and treated with EAGLE-302 and anti-PD-1 or anti-CTLA4, 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.

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**Figure 10**: EAGLE-302 shows improved efficacy compared to the combination of PD-1 and PD-L1.

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**Figure 11**: Proprietary Hydro Biomarker Assays to Detect Tumor Glyco-Codes in Patients.

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**Figure 12**: The Mechanism of Action of EAGLE Involves Innate and Adaptive Immune Response.

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**Figure 13**: Representative Hydro Biomarker Assays to Detect Tumor Glyco-Codes in Patients.

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**Figure 14**: EAGLE treatment of breast cancer EMT6-Her2 orthotopic model in combination with PD-1/2/3 inhibition.

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**Figure 15**: EAGLE-302 achieved 100% cures in breast cancer EMT6-Her2 orthotopic model in combination with PD-1/2/3 inhibition.

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**Figure 16**: In preclinical models, EAGLE reduced tumor cell surface sialic acid levels and dampened immune responses. Sialidase arm of EAGLE Molecules plays an important role in cancer immunity, by inducing 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 describe a novel therapeutic modality, a multi-functional antibody-like molecule named EAGLE (Enzyme-Immune Glyco-Ligand Editing), to inhibit the glyco-immune checkpoint axis and further enhance the tumor immunosuppression by selectively removing the terminal sialic acids of sialylglycans on tumor cells.

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**Figure 17**: EAGLE has significant combination efficacy with T-cell checkpoint inhibition.

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**Figure 18**: EAGLE-302 shows improved efficacy compared to the combination of PD-1 and PD-L1.

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**Figure 19**: Proprietary Hydro Biomarker Assays to Detect Tumor Glyco-Codes in Patients.

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**Figure 20**: The Mechanism of Action of EAGLE Involves Innate and Adaptive Immune Response.

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**Figure 21**: Representative Hydro Biomarker Assays to Detect Tumor Glyco-Codes in Patients.

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**Figure 22**: EAGLE treatment of breast cancer EMT6-Her2 orthotopic model in combination with PD-1/2/3 inhibition.

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**Figure 23**: In preclinical models, EAGLE reduced tumor cell surface sialic acid levels and dampened immune responses. Sialidase arm of EAGLE Molecules plays an important role in cancer immunity, by inducing 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 describe a novel therapeutic modality, a multi-functional antibody-like molecule named EAGLE (Enzyme-Immune Glyco-Ligand Editing), to inhibit the glyco-immune checkpoint axis and further enhance the tumor immunosuppression by selectively removing the terminal sialic acids of sialylglycans on tumor cells.

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**Figure 24**: EAGLE has significant combination efficacy with T-cell checkpoint inhibition.

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**Figure 25**: EAGLE-302 shows improved efficacy compared to the combination of PD-1 and PD-L1.

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**Figure 26**: Proprietary Hydro Biomarker Assays to Detect Tumor Glyco-Codes in Patients.

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**Figure 27**: The Mechanism of Action of EAGLE Involves Innate and Adaptive Immune Response.

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**Figure 28**: Representative Hydro Biomarker Assays to Detect Tumor Glyco-Codes in Patients.

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**Figure 29**: EAGLE treatment of breast cancer EMT6-Her2 orthotopic model in combination with PD-1/2/3 inhibition.

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**Figure 30**: In preclinical models, EAGLE reduced tumor cell surface sialic acid levels and dampened immune responses. Sialidase arm of EAGLE Molecules plays an important role in cancer immunity, by inducing 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 describe a novel therapeutic modality, a multi-functional antibody-like molecule named EAGLE (Enzyme-Immune Glyco-Ligand Editing), to inhibit the glyco-immune checkpoint axis and further enhance the tumor immunosuppression by selectively removing the terminal sialic acids of sialylglycans on tumor cells.