Cancer therapy has been revolutionized by the development 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 immune therapy, highlighting the need to identify new mechanisms of immune response in cancer and to develop new therapeutic modalities. Recently, the glyco-immune checkpoint axis (sialidase/NSG pathway) has emerged as a novel mechanism of immune regulation involving innate and adaptive immunity and an important mechanism of cancer immune escape. Upon ignition of sialylated glycans to PAMP-containing ligands on immune cells, this pathway play a previously unrecognized role in regulating function of NK cells, 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 of the immune system of anti-cancer T cells, immune cell recruitment, tumor cell infiltration, and development of tumor cell resistance mechanisms. Therefore, the development of human sialidase (EAGLE) therapeutics could represent a novel therapeutic modality, a multi-functional antibody-like molecule enabling EAGLE (Enzyme Antibody DynaFlex Edit) to inhibit the NSG pathway and work in the tumor microenvironment by selectively removing the terminal sialic acids of glycans on tumor cells.

**Glyco-Immune Checkpoints Suppress Innate and Adaptive Immunity**

**Suppression of innate (NSG)-Adaptive Immunity**

**Tumor-Growth Evaluation**

- **Intratumoral sialidase administration**
  - Significantly reduces tumor growth compared with vehicle-treated animals
  - Restores anti-tumor cellular immune responses

**Immune Checkpoints
during Tumor Progression**

- **Sialylation of carbohydrates**
  - Enhances immune checkpoint expression

**EAGLE Transduces Immune Cells**

- **EAGLE administration**
  - Induces reactivation of immune cells
  - Enhances ADCC and CDC activity

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

- **EAGLE technology**
  - Enables targeted delivery of sialidase activity to cancer cells

**Results**

**EAGLE-Secured Targeted Cleavage of Sialic Acids from Her2+ Tumor Cells**

- **In vitro**
  - EAGLE treatment significantly reduces sialylation of Her2+ tumor cells

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

- **Innate immunity**
  - Activates NK cells
  - Enhances ADCC and CDC activity

- **Adaptive immunity**
  - Induces reactivation of immune cells
  - Enhances cellular immune responses

**EAGLE Has Monotherapy Effectiveness Comparable to the Combination of a-PD1 and -CTLA4 in the Cold Tumor Tissue Model**

- **Combination therapy**
  - Shows synergistic antitumor effects

**EAGLE Has Signifcant Combination Effectiveness with T-Cell checkpoint Inhibition**

- **Her2+ Orthotopic Tumor Model**
  - EAGLE in combination with anti-PD1 shows superior antitumor efficacy

**Conclusions**

- **Glyco-immune checkpoints play critical roles in cancer immune evasion**
  - Innate immune response
  - Adaptive immune response

- **EAGLE showed compelling monotherapy efficiency in syngeneic tumor models**
  - Single-agent complete regressions with immunological memory
  - Efficient in solid tumor models

- **Striking activity in combination with PD-1/PD-L1**

- **EAGLE offers new opportunities to treat cancer targeting glyco-immune checkpoints**
  - Conquers the heterogeneity and challenges of tumor glycans
  - Enables immunosuppressive glycan functions within tumor microenvironment
  - Transforms existing tumor targeting modalities into immune-regulating agents