Development of a Bifunctional PD-L1-Targeted Sialidase as a Novel Cancer Immunotherapeutic Approach

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 # LB221

Introduction

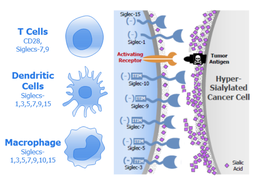
Sialoglycans have emerged as a critical glyco-immune checkpoint that impairs antitumor response by inhibiting innate and adaptive immunity. We have reported previously that Bi-Sialidase – an engineered human sialidase-Fc fusion – potentiates antitumor immune response by cleaving terminal sialic acids from sialoglycans (desialylating) on tumor cells and immune cells. Furthermore, we have shown that a bifunctional tumor-targeted sialidase, a heterodimeric molecule consisting of one chain of sialidase-Fc and a second chain of a HER2-targeting antibody (trastuzumab), leads to more efficient desialylation of tumor cells than the non-targeted Bi-Sialidase and demonstrates antitumor activity in trastuzumab-resistant and low HER2-expressing tumor models.

To evaluate the impact of targeted desialylation of both tumor cells and immune cells, we designed and characterized a bifunctional PD-L1-targeted sialidase in preclinical models, since PD-L1 is expressed on many types of tumor cells and immune cells. Furthermore, the bifunctional PD-L1-targeted sialidase simultaneously inhibits two orthogonal immune checkpoint pathways, immunosuppressive sialoglycans and the PD-1/PD-L1 axis. We generated a humanized anti-human PD-L1 antibody with potency of PD-1/PD-L1 blockade comparable to the existing anti-PD-L1 drugs, atezolizumab and avelumab. Subsequently, we constructed and screened multiple bifunctional PD-L1-targeted sialidases in various configurations and selected a heterodimeric molecule (E-705) as the lead, based on developability and potency.

The PD-L1-Targeted Sialidase (E-705) consists of one chain of sialidase-Fc from the third generation of engineered human sialidase (Neu2) and a second chain of the in-house generated anti-PD-L1 antibody. We further characterized E-705 using various biochemical assays in vitro and tested its antitumor activity in vivo using the transgenic CT26-human PD-L1 mouse tumor model expressing human PD-1 and PD-L1 in replace of the murine counterparts.

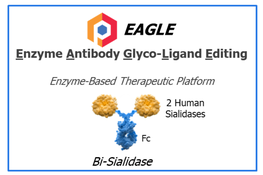
Degradation of Immunosuppressive Sialoglycans Using Human Sialidase-Based *EAGLE* Technology to Treat Cancer

Sialoglycans Suppress Immune Response Through Multiple Immune Receptors



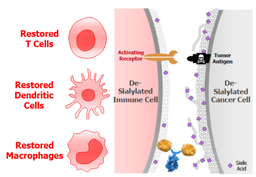
- **Hypersialylation** is so immunosuppressive in cancer because it impacts multiple receptors concurrently
- Blocking an individual Siglec receptor cannot overcome this **redundancy**

Palleon Approach: Sialoglycan Degradation



- **Sialic acid** is the **single common dependency** of this highly redundant immune suppressive axis
- **Sialoglycan Degradation**: Sialidase enzymes remove sialic acids on both immune cell and cancer cell surfaces
- Impact: **Restored Anti-Tumor Immunity**

Desialylation Overcomes Redundancy and Enables Anti-Tumor Immunity



- Modest reduction of sialic acid density releases multiple inhibitory signals simultaneously (Siglecs, CD28 blockade, others)
- Impact: **Robust anti-tumor response** from both innate and adaptive immune cells

Figure 1. Schematic representation of the sialoglycan immune checkpoint axis and the *EAGLE* therapeutic platform. At the immunological synapse, a dense array of various sialoglycans interact with multiple sialic-acid sensing immune receptors, including Siglecs[1-3] (sialic acid-binding Ig-like lectins) and CD28[4] on immune cells, dampening innate and adaptive immune responses. The interactions between sialoglycans and sialic-acid-sensing receptors are "Velcro"-like with overlapping/promiscuous binding preferences and tremendous redundancy, which poses a challenge for therapeutic intervention of this axis because targeting a single receptor or ligand cannot overcome the redundancy of this biology. The engineered human sialidase-based *EAGLE* therapeutic platform overcomes this hurdle by removing terminal sialic acids, the common motif of various sialoglycans, to release sialoglycan-mediated immunosuppression.

Bifunctional and Targeted Sialidase Enables Dual Antitumor Mechanisms and More Efficient Desialylation of Targeted Cells Compared to Non-Targeted Bi-Sialidase

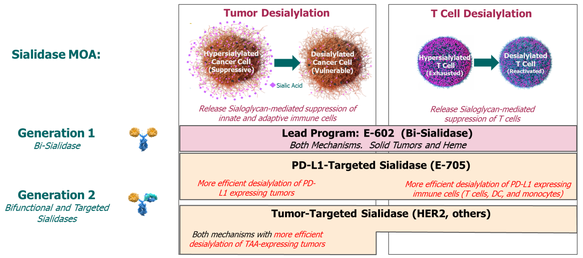


Figure 2. Schematic representation of the primary MoAs of *EAGLE*, including 1) desialylation of tumor cells to release tumor- associated sialoglycan-mediated immunosuppression of innate and adaptive antitumor immunity, 2) desialylation of T cells to enhance sialoglycan-mediated suppression of T cell function. E-602 is a homodimer of the engineered human sialidase Neu2 (v2.0) genetically fused to a human IgG1 Fc region by an IgG1 hinge. Targeted-Sialidases are bifunctional molecules consisting of the sialidase moiety and a tumor-associated antigen (TAA) or an immune cell-targeted (such as PD-1) arm.

Results

Selection of the Bifunctional PD-L1-Targeted Sialidase (E-705) (Lead Identification and Lead Optimization)

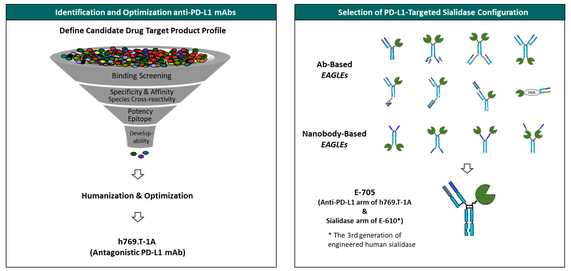


Figure 3. The lead identification flowchart of anti-PD-L1 mAb (left) and schematic representation of various PD-L1-targeted sialidase configurations. The lead (E-705) was selected based on expression yield, stability, sialidase activity, and PD-1/PD-L1 inhibition potency.

E-705 Demonstrated Dual Functions of Cleaving Sialic Acids and Inhibiting PD-1/PD-L1 Interaction

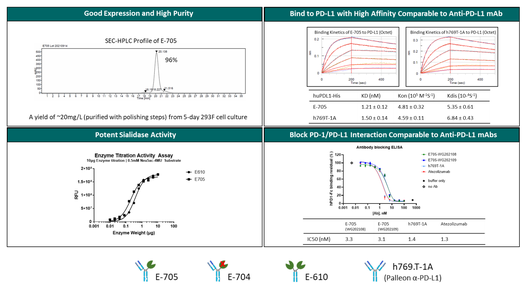


Figure 4. Biochemical and functional characterization of PD-L1-targeted sialidase (E-705). E-705 yielded 20mg/L with 96% purity from a 5-day transient 293F cell expression and a two-step purification of protein A and CHT chromatography. E-705 showed comparable desialylation potency to its parental sialidase-Fc (E-601) toward a fluorescently labeled sialic acid substrate (4-MU-Neu5Ac), confirming that pairing with the anti-PD-L1 arm didn't impact sialidase activity. In addition, E-705 demonstrated binding to PD-L1 and EC50s of inhibiting PD-1/PD-L1 interaction comparable to its parental anti-PD-L1 mAb (h769T-1A) or atezolizumab.

The PD-L1-Targeting Moiety of E-705 Enabled More Efficient Desialylation of PD-L1-Expressing Cells

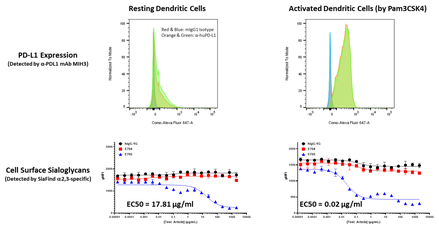


Figure 5. E-705 showed more efficient desialylation of activated DCs with abundant PD-L1 expression than resting DCs with low expression of PD-L1. Monocyte-derived dendritic cells (mDCs) were differentiated from peripheral CD14+ monocytes treated with GM-CSF and IL-4. Activated mDCs were simulated by TLR2/1 agonist Pam3CSK4, a synthetic triacylated lipopeptide. Resting and activated mDCs were treated with E-705, isotype, or silent-sialidase control (E-704), respectively. Activated mDCs showed more than 10-fold higher expression of PD-L1 than that of the resting mDCs. As expected, E-705 demonstrated higher potency (~900-fold) in desialylating activated mDCs (EC50 = 0.02 µg/ml) than resting mDCs (EC50 = 17.81 µg/ml).

E-705 Demonstrated Improved Antitumor Activity than PD-L1 Blockade or Sialidase in Transgenic Syngeneic CT26-hPD-L1 Mouse Tumor Model

MC38-hPD-L1 Transgenic Syngeneic Mouse Tumor Model Expressing Human PD-1 and PD-L1

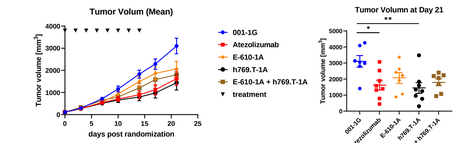


Figure 6. Efficacy study in MC38-hPD-L1 transgenic syngeneic mouse tumor model expressing human PD-1 and PD-L1 in replacement of mouse counterparts. Palleon's anti-PD-L1 mAb (h769.T-1A) showed comparable efficacy to atezolizumab. Furthermore, sialidase (E-610) also demonstrated antitumor activity in this PD-1/PD-L1 biology-focused transgenic mouse tumor model. However, the combination of h769.T-1A and E-610 did not show improved activity versus the single agent, suggesting the MC38 model is not suitable for studying the additive effect of combining desialylation and inhibition of PD1/PD-L1 axis.

CT26-hPD-L1 Transgenic Syngeneic Mouse Tumor Model Expressing Human PD-1 and PD-L1

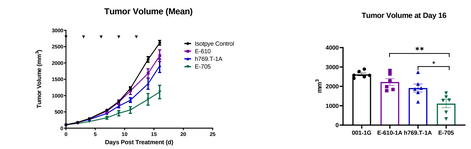


Figure 7. Efficacy study in CT26-hPD-L1 transgeneic mouse tumor model expressing human PD-1 and PD-L1 in replacement of mouse counterparts. E-705 showed improved antitumor activity compared to its parental sialidase (E-610) or anti-PD-L1 mAb (h769.T-1A) alone in the CT26-hPD-L1 transgenic syngeneic mouse tumor model.

Pharmacodynamics Study of E-705-Mediated Immune Modulation in Tumor Microenvironment

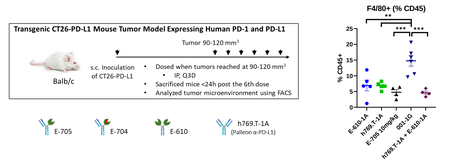


Figure 8. FACS analysis of E-705-mediated immune modulation in the tumor microenvironment. Tumors were harvested within 24 hours post the 6th dose of treatment and subjected to immunophenotyping using flow cytometry. A significant decrease of macrophages in the tumor microenvironment was observed from either desialylation (E-610) or PD-L1 blockade. In addition, E-705 and the combination E-610 and h769.T-1A led to slightly more reduced tumor-infiltrating macrophages compared to the corresponding single-agent treatments.

Conclusions

- Degradation of sialoglycans using human sialidase-based *EAGLE* platform technology can effectively release sialoglycan-mediated immunosuppression of antitumor immune response.
- The PD-L1-Targeted Sialidase (E-705) enabled more efficient desialylation of PD-L1-expressing immune cells and tumor cells and showed improved antitumor activity compared to its parental sialidase or anti-PD-L1 mAb in the CT26-hPD-L1 transgenic syngeneic mouse tumor model.
- The PD-L1-Targeted Sialidase (E-705) offers a novel immunotherapeutic approach of inhibiting two orthogonal checkpoint pathways by cleaving immunosuppressive sialic acids and blocking the PD-1/PD-L1 axis.

References:
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