Development and Engineering of Human Sialidase for Degradation of Immunosuppressive Sialoglycans to Treat Cancer

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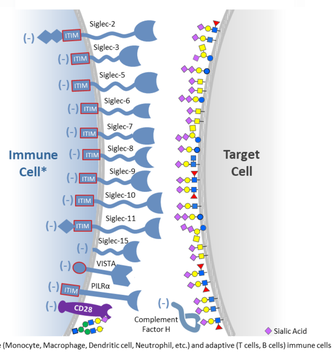
Introduction

Sialoglycans, a type of glycans with a terminal sialic acid, have emerged as a critical glyco-immune checkpoint that impairs antitumor response by inhibiting innate and adaptive immunity. Upregulation of sialoglycans on tumors has been observed for decades and correlates with poor clinical outcomes across many tumor types. Targeted desialylation of tumors has been shown to lead to robust single-agent efficacy in mouse tumor models, using a bifunctional sialidase x antibody molecules consisting of sialidase and a tumor-associated antigen (TAA)-targeting antibody[1,2]. In addition to tumor cells, most immune cells present substantially more abundant sialoglycans than non-hematological healthy cells, which may also contribute to immunosuppression. Therefore, we studied the impact of immune cell desialylation and evaluated the therapeutic potential of a newly developed sialidase-Fc fusion (Bi-Sialidase), which lacks a TAA-targeting moiety and consists of the second generation of engineered human neuraminidase 2 (Neu2v2.0) and human IgG1 Fc region, in preclinical mouse tumor models.

Sialoglycans Suppress Innate and Adaptive Immunity by Engaging Various Sialic-Acid-Sensing Immune Receptors



* Innate (Monocyte, Macrophage, Dendritic cell, Neutrophil, etc.) and adaptive (T cells, B cells) immune cells

Figure 1. Schematic representation of sialoglycan immune checkpoints axis. At the immunological synapse, a dense array of various sialoglycans interact with multiple sialic-acid sensing immune receptors, including Siglecs[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.

Results

Engineering of Human Sialidase for Improved Stability for *EAGLE* Platform Development

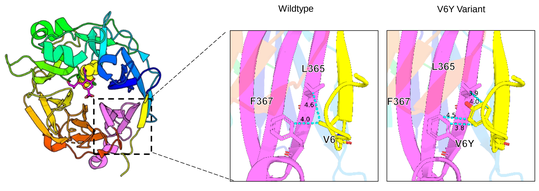


Figure 2. Schematic representation of human sialidase Neu2 with the V6Y mutation for introducing a stabilizing interaction between the N- and C-termini of Neu2. We employed rational design and directed evolution strategies to engineer Neu2 to improve its stability and manufacturability. Based on the 3D structure of Neu2 and sequence homology analysis of the sialidase family, we rationally designed variants and focused libraries targeting the following identified sites of Neu2, including 1) regions to introduce intramolecular interactions to stabilize Neu2 tertiary structure, 2) surface areas to remove potential aggregation-causing patches, and 3) sequence liability motifs prone to aggregation and post-translational modifications. Additionally, we created a randomized mutagenesis library unbiasedly probing the entire Neu2 sequence using error-prone PCRs. The engineering effort led to the discovery of multiple mutations for improving Neu2 expression titer and stability. Here is an example of the mutation of V6Y in the N-terminus of Neu2, which forms stronger hydrophobic interactions with residues L365 and F367 in the C-terminus of Neu2 than wild type Neu2, introducing stabilizing interactions between the N- and C-termini of Neu2.

Table 1. Expression Titers and Enzymatic Activity Characterization of Wild-Type* and Engineered Neu2 Variants

	Expression Titer (14-Day Fed-Batch Cell Culture)	Enzymatic Activity** (14-Day Fed-Batch Cell Culture with Protein A and CHT Purification)	*EAGLE*
Wild-Type Human Sialidase Neu2	**0 g/L**	0%	Not Feasible
Engineered Neu2 v1.0***	**0.1 g/L**	10% Active	Not Feasible
Engineered Neu2 v2.0****	**~2.5 g/L**	100% Active	*EAGLE*'s Building Block

* All four human sialidases (Neu1, 2, 3, and 4) are not developable due to their poor developability profile, including low expression yields (0 mg/L in 14-day fed-batch CHO cell culture and <0.001 g/L in 5-day transient HEK cell culture), high levels of aggregation (~90% aggregates), and poor stability (not stable in 14-day cell culture and the downstream purification processes).
** Neu2 enzymatic activity was characterized using synthetic and natural sialoglycan substrates compared to the wild-type Neu2 produced from the 5-day transient HEK cell culture with a yield of <0.001 g/L.
*** The 1st generation of the engineered Neu2 (Neu2v1.0) demonstrated improved stability but was not robust for building developable *EAGLE* therapeutic molecules.
**** The 2nd generation of the engineered Neu2 (Neu2v2.0) showed a good developability and manufacturability profile with a titer of 2.5 g/L and stable sialidase activity, providing the foundation for developing the *EAGLE* platform.

Discovered a New MoA of *EAGLE*: Desialylation of T Cells Enhances T Cell Immunity

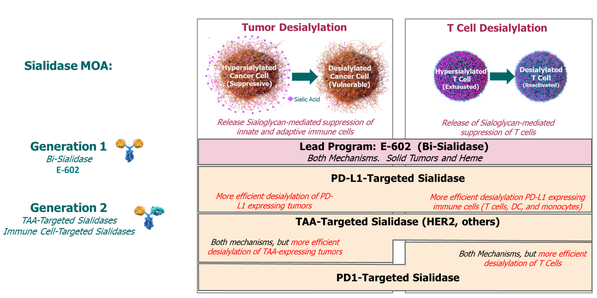
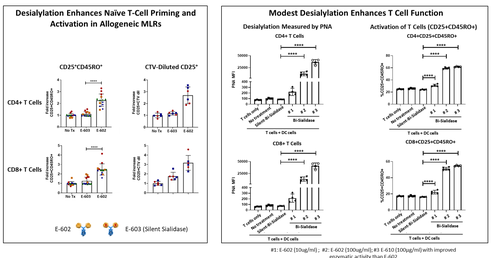


Figure 3. Schematic representation of the primary MoAs of *EAGLE*, including 1) desialylation of tumor cells to release tumor-associated sialoglycan-mediated immunosuppression of innate and adaptive antitumor immunity, 2) desialylation of T cells to enhance sialoglycan-mediated suppression of T cell 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.

Desialylation of T Cells by E-602 Enhances Naïve T Cell Activation in Allogeneic Mixed Lymphocyte Reactions (MLRs)



#1: E-602 (30ug/ml) ; #2: E-602 (300ug/ml); #3 E-610 (300ug/ml) with improved enzymatic activity than E-602

Figure 4. E-602 enhances naïve T cell activation in mixed lymphocyte reactions. The MLR assay was conducted by culturing purified naïve T cells from one donor and DC cells from another unrelated donor. E-602 significantly enhanced the frequency of CD25+CD45RO+ (activated) CD4+ and CD8+ T cells. Additionally, E-602 enhanced the frequency of CTV diluted CD25+ activated/proliferated CD4+ and CD8+ T cells. Statistical analysis was done using a one-way ANOVA test.

Desialylation of T Cells by E-602 Restores Exhausted-like T Cell Function

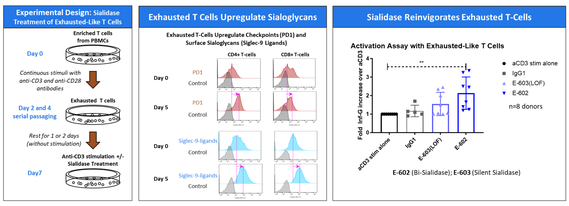


Figure 5. E-602 treatment re-invigorated exhausted-like T cells. Primary T cells were enriched from human PBMCs and serially stimulated with anti-CD3 and anti-CD28 antibodies to yield exhausted-like T cells. T cells were stained for surface sialoglycans by a Siglec-9 reagent-Fc and PD-1 expression on Day 0 and Day 5. Exhausted-like T cells upregulate surface sialoglycans compared to resting T cells, likely as a compensatory mechanism similar to the upregulation of PD-1. Furthermore, E-602 treatment of these exhausted-like T cells restored their function, as shown by the enhanced interferon-gamma (IFN-γ) secretion in eight independent donors.

Desialylation by E-602 Enhances Antigen-Specific T Cell Responses

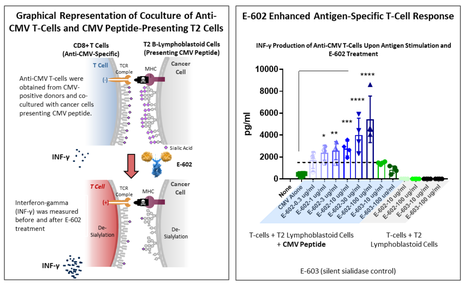


Figure 6. E-602 enhances antigen-specific T-cell responses. The impact of E-602 on antigen-specific T-cell response was tested using the co-culture of cytomegalovirus (CMV)-specific T cells and CMV pp65 peptide-presenting T2 cancer cells. Donor-derived CMV-specific T cells were screened and enriched using the major histocompatibility (MHC) tetramer of CMV pp65 peptide in complex with HLA-A*0201. When loaded with CMV pp65 peptides, T2 cells, which express HLA-A*0201, specifically stimulate these CMV-specific T cells. The co-culture of T cells and T2 cells was conducted with or without stimulation of CMV pp65 peptide. The addition of E 602 in the co-culture system enhanced IFN γ production in response to the CMV pp65 peptide challenge in a dose-dependent manner, while the negative control (E-603 with silent sialidase) had no impact. Data are representative of two independent donors.

Bi-Sialidase Demonstrated Single-Agent Antitumor Activity in Multiple Syngeneic Mouse Tumor Models

Model	Bi-Sialidase Monotherapy *(10mg/kg, every 3-day dosing)*	Anti-PD-1 mAb *(10mg/kg, every 3-day dosing)*
A20 (s.c)	3/8 CR	3/8 CR
MC38 (s.c)	1/8 CR	No efficacy
B16F10 (s.c)	Inhibited tumor growth by 40%	Inhibited tumor growth by 20%
CT26 (s.c)	1/8 CR	1/8 CR, 1/8 PR
EMT6-HER2 (s.c)	4/8 CR	4/6 CR (from a separate study, not a head-to-head comparison)
EL4-CD20 (i.v., dissemination)	Significantly improved survival with anti-CD20 mAb (Ofatumumab) over Ofatumumab alone	We didn't run a head-to-head comparison study. Literature showed PD-1/PD-L1 mAbs had no efficacy in EL4 model.

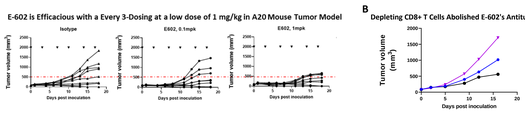
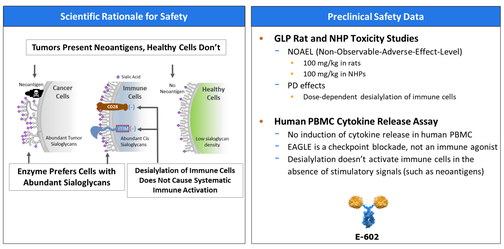


Figure 7. E-602 efficacy studies in A20 and EMT6-HER2 syngeneic mouse tumor models. (A) E-602 demonstrated dose-dependent efficacy in A20 tumor model. (B) Depletion of T cell abolished the antitumor activity of E-602 in EMT6-HER2 model.

Bi-Sialidase (E-602) Has a Wide Safety Margin



Conclusions

- Sialoglycans play an essential role in tumor immune escape, but they are not druggable using conventional drug modalities because of their chemical complexity and heterogeneous nature
- *EAGLE* platform based on the engineered human sialidase Neu2 overcomes the technical hurdles by specific degradation of terminal sialic acids from a diverse of sialoglycans to release sialoglycan-mediated immunosuppression.
- We identified a new MoA of *EAGLE* that desialylation of T cells enhances naïve T cell priming/activation, enhances effector T cell function, and restores exhausted-like T cell functions.
- Bi-Sialidase (E-602) demonstrated single-agent antitumor activity and a wide safety margin in preclinical animal models, offering a novel immunomodulatory approach to enhancing T-cell immunity for cancer treatment.

References:
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