Assessment of the Safety, Pharmacokinetics, and Pharmacodynamics of a First-in-class Cancer Drug Candidate E-602, a Sialoglycan Degrader, in Non-Human Primates

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 # LB203

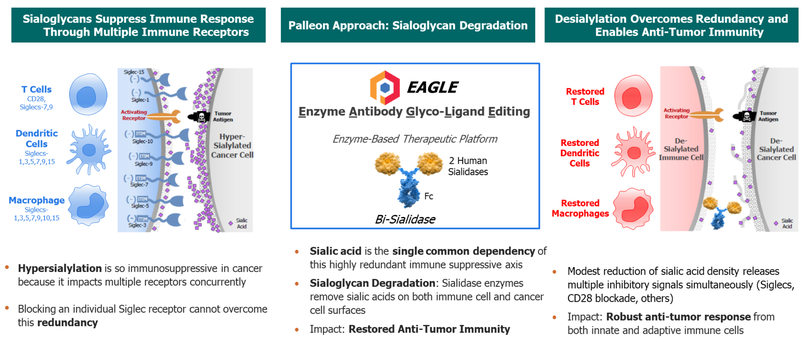
Introduction

Sialoglycans, which are glycans that terminate with a sialic acid, have emerged as a critical glyco-immune checkpoint that impairs antitumor response by inhibiting innate and adaptive immunity. Hypersialylation of both T cells and tumor cells suppresses many facets of antitumor immune responses. To release sialoglycan-mediated immunosuppression and potentiate antitumor immunity, we have engineered human sialidase Neu2 and developed a Neu2-based EAGLE platform, which includes the non-targeted sialidase (Bi-Sialidase, E-602) and various targeted sialidases.

E-602 is a novel, first-in-class, engineered human sialidase (Neu2) Fc fusion developed for cancer treatment that enhances antitumor immunity by desialylating immunosuppressive sialoglycans. We have shown previously that E-602 cleaves off terminal sialic acids from surface sialoglycans on tumor cells and immune cells, restores the immune function of exhausted-like T cells, and enhances dendritic cell priming and activation of naïve T cells. E-602 has also demonstrated antitumor activity as a single agent in multiple syngeneic mouse tumor models.

To support clinical development, we conducted a Good Laboratory Practice (GLP) one-month repeat-dose toxicity study to evaluate E-602's pharmacokinetic (PK) parameters, pharmacodynamic (PD) effects, and safety profiles in cynomolgus monkeys via weekly intravenous infusion of E-602 at 5, 30, 100, and 200 mg/kg for five doses.

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



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

Experimental Design

Group	Test Material	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg) [a]	Terminal (Day 32) M	Terminal (Day 32) F	Recovery [b] (Day 60) M	Recovery [b] (Day 60) F
1	Control	0	0	20	3	3	2	2
2	E-602	5	0.25	20	3	3	-	-
3	E-602	30	1.5	20	3	3	-	-
4	E-602	100	5	20	3	3	-	-
5	E-602	200	10	20	3	3	2	2

[a] Individual dose volume will be calculated based on the most recent body weight.
[b] Recovery animals (2/sex from group 1 and 5) will complete 28 days of observation following the terminal necropsy (31 days from last dose).

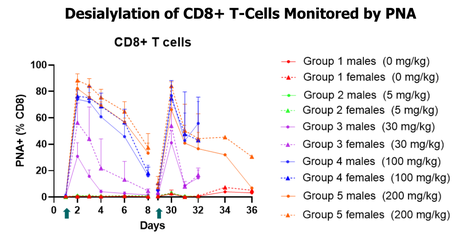
A 32-Day Repeat Dose GLP Toxicity Study of E-602 Administered via Weekly Intravenous Infusion to Cynomolgus Monkeys

Results

Summary of E-602 GLP One-Month NHP Toxicity Study Results

	Procedure Details	Results
Experimental and Clinical Observations	Clinical observations, food evaluation, body weight, ophthalmology examinations, electrocardiography parameters, respiratory rates, neurological assessments	▪ All animals survived ▪ No impact on body weight or food consumption ▪ Sporadic incidences of flushed skin predominately in the high dose groups (200 mg/kg), which may relate to infusion
Clinical Pathology	▪ Hematology ▪ Serum chemistry ▪ Coagulation ▪ Urinalysis	▪ Minimally decreased platelet counts in males administered 200mg/kg ▪ Mildly to moderately increased monocyte counts in animals administered 200mg/kg ▪ Minimally or mildly decreased albumin and mildly increased globulin in males administered ≥100 mg/kg and females administered 200 mg/kg ▪ No histopathology correlations were noted for these clinical findings
Necropsy and Histopathology	Collected tissues were processed to paraffin block, sectioned, stained with H&E, and examined microscopically	▪ No test article-related findings were observed in the gross pathology, organ weights, or histopathology examination
Cytokine Analysis	Analyzed for C3a complement, IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12/IL-23p40, MCP-1, and TNF-α	▪ Dose-dependent transient increase of C3a, no histopathology correlation ▪ No meaningful changes to the cytokines or chemokines monitored
Toxicokinetics (TK)	Blood samples were collected prior to and at various timepoints post dose on Days 1 and 29	▪ E-602 showed terminal half-life (t1/2) of 10-30 hours with dose linearity from 30 mg/kg to 200 mg/kg
Anti-Drug Antibody (ADA) Analysis	Blood samples were collected on Day -11, predose and 96 hours post dose on Day 22, and on Day 60	▪ All animals administered ≥ 30 mg/kg, and three out of six animals administered 5 mg/kg, were positive for ADA predose on Day 22 ▪ Neither E-602 peak nor total exposure appeared to be affected by ADA, as Cmax and AUCtau increased with dose and the increases were dose proportional from 30 mg/kg to 200 mg/kg
NOAEL (Non-Observable-Adverse-Effect-Level)		▪ **100 mg/kg**

Pharmacodynamic Effect of Desialylation of CD8+ T-Cells by E-602



Dosed at day 1 and 29 as shown by arrows

Figure 2. E-602-mediated desialylation of CD8+ T cells monitored by PNA using FACS. Desialylation leads to increased PNA binding, since PNA binds to galactoses exposed upon sialic acid cleavage. Dose-dependent pharmacodynamic effect of desialylation of CD8+ T cells was observed. E-602's PD effect of desialylation of CD8+ T cells lasted for >=7 days, longer than its PK half-life (<= 1 day), consistent with E-602's enzymatic MoA.

Dose Conversion Between Human and Monkeys Based on FDA's Guidance (mg/kg)		
Monkey	30	100
Human	9.7	33.3

Desialylation Doesn't Induce Systematic Immune Activation and Cytokine Release in Human PBMC Assays

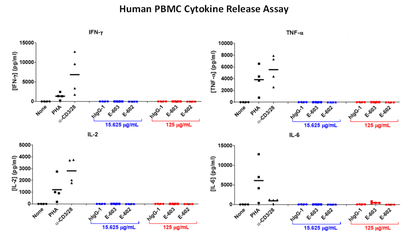
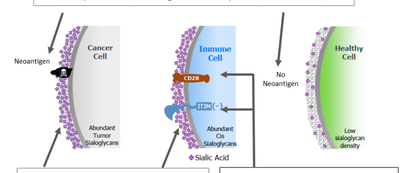


Figure 3. Human PBMC cytokine release assay. IFN-γ, IL-2, TNF-α, IL-6, IL-10, IL-5, IL-13, IL-9, IL-17A, IL-17F, IL-4, and IL-22 were measured by Biolegend's LEGENDplex HU Th Cytokine Panel (12-plex) via manufacturer's standard protocol. Representative cytokines, IFN-γ, IL-2, TNF-α, and IL-6 were shown here. E-603 is a silent enzyme control. E-602 did not cause any cytokine secretion in the soluble phase format. In the solid phase format, E-602 did not cause any enhanced cytokine release compared to the isotype control of human IgG1 (data not shown).

Scientific Rationale for E-602 Wide Safety Margin

Tumors Present Neoantigens, Healthy Cells Don't
- Desialylation releases sialoglycan-mediated immune suppression
- Healthy cells lack neoantigens, so are spared immune attack



Enzyme is More Potent Toward Cells with Abundant Sialoglycans
- E-602 has a high Km (1mM)
- Enzymes with high Km require high concentrations of substrate to achieve maximum rate of reaction (Vmax)

Desialylation of Immune Cells Does Not Cause Systematic Immune Activation
Cleavage of sialoglycans functions as an immune checkpoint blockade, but not an immune agonist.

Conclusions

- A 32-Day repeat dose GLP NHP toxicity study of E-602 demonstrated that E-602 is well tolerated with the NOAEL (No-Observed-Adverse-Effect-Level) determined to be 100 mg/kg.
- E-602 showed a terminal half-life of 10-30 hours with dose linearity from 30 to 200 mg/kg and a dose-dependent sustained pharmacodynamic effect of desialylation of CD8+ T cells up to 7 days, consistent with E-602's enzymatic MoA.
- E-602 does not induce systematic immune activation and cytokine release in human PBMC assays, consistent with its checkpoint blockade MoA of cleaving immunosuppressive sialoglycans.