A phase 1/2 dose escalation/expansion study evaluating the safety, pharmacokinetics, pharmacodynamics, and antitumor activity of E-602, a bi-sialidase fusion protein, in advanced cancer (GLIMMER-01)

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BACKGROUND

- Sialoglycans are immunosuppressive in cancer, associated with poorer outcomes across numerous tumor indications, and have emerged as a critical glyco-immune checkpoint [1,2].
- E-602 is an engineered human sialidase (neuraminidase, Neu2) fused to a human IgG1 Fc region by an IgG1 hinge.
- Sialidase moieties of E-602 cleave terminal sialic acid residues from sialoglycans on diverse immune cell subsets and tumor cells. Cleavage of the terminal sialic acid releases sialoglycan-mediated immunosuppression without causing systemic immune activation.
- Sialoglycans interact with multiple receptors, including Siglecs (sialic acid-binding Ig-like lectins) and CD28 on the surface of immune cells. Interactions between sialoglycans and their receptors can be immunosuppressive and dampen immune activation.
- In pre-clinical studies, sialidase-mediated cleavage of terminal sialic acids improves antitumor immunity by restoring the immune function of exhausted-like T cells and enhancing dendritic cell priming and naïve T cell activation [3].
- In multiple syngeneic mouse tumor models, sialidase treatment has demonstrated antitumor activity as monotherapy [3] and additive antitumor activity when combined with anti-PD-1 and anti-PD-L1 blockade.
- E-602 is projected to have a wide safety margin as demonstrated by a GLP one-month repeat dose toxicology study. In addition, E-602 is not an immune agonist and does not stimulate cytokine activation in an *in vitro* PBMC cytokine release asssay [3,4].
- In humans, E-602 desialylation of tumor cells and immune cells is expected to have antitumor activity as monotherapy and in combination with an anti-PD-1 agent.

METHODS

- GLIMMER-01 (**Gl**ycan Mediated **Imm**un**e R**egulation) is an ongoing Phase 1/2, first-in-human, open label, dose escalation and expansion study of E-602 administered as monotherapy and in combination with an anti-PD-1 agent to evaluate the safety, pharmacokinetics, pharmacodynamics and antitumor activity in participants with advanced cancers (**PAL-E602-001 ClinicalTrials.gov Identifier:** NCT05259696).
- Serial blood and tumor biopsy samples will be assessed for pharmacodynamic effects of E-602 including evaluation of immune and tumor cell desialylation in both the periphery and tumor microenvironment to measure the on-target effects of E-602.

Phase 1:

- Five (5) planned dose escalation cohorts of E-602 monotherapy and 2 planned dose escalation cohorts of E-602 in combination with an anti-PD-1 agent
- Modified 3+3 study design will evaluate the safety of the dose regimens and will identify the maximum tolerated dose and/or recommended Phase 2 dose
- Additional participants (backfill) may be enrolled to obtain additional safety, pharmacokinetic and pharmacodynamic data

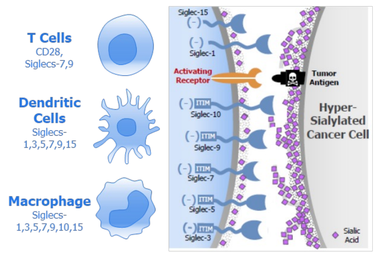
Phase 2:

- Will include up to 3 disease indications, evaluating E-602 as monotherapy and/or in combination with an anti-PD-1 agent utilizing a Simon's minimax 2-stage design

- **Ethics approval:** The study is approved by the Advarra Institutional Review Board, approval number Pro00058627 and participants provided informed consent to participate in the study.

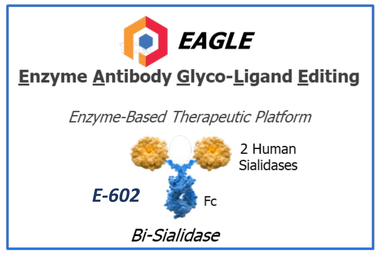
Mechanism of Action of E-602

Sialoglycans Suppress Immune Responses Through Multiple Immune Receptors



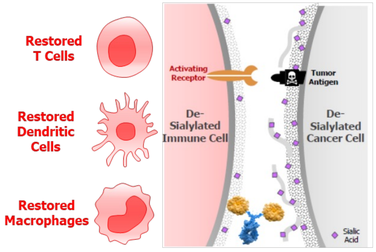
- **Hypersialylation** is immunosuppressive in cancer because it impacts multiple receptors concurrently
- Blocking an individual Siglec receptor cannot overcome this **redundancy**

E-602 Bi-Sialidase Degrades Sialoglycans



- **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

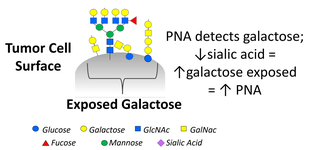
Schematic representation of the sialoglycan immune checkpoint axis and the *EAGLE* therapeutic platform. At the immunological synapse, a dense array of various sialoglycans interact with multiple sialic-acid sensing immune receptors, including Siglecs and CD28 on immune cells, dampening innate and adaptive 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 all sialoglycans, to release sialoglycan-mediated immunosuppression.

Measuring Sialylation

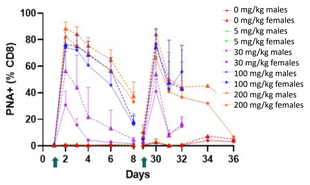
Measuring Desialylation of Peripheral Immune Cells

Peanut Agglutinin (PNA)

- PNA binds **galactose on O-linked glycans** (a fraction of desialylated glycans)
- Increased PNA signal is a sensitive measure of desialylation
- Convenient for monitoring immune cell desialylation as low base level staining on immune cells



E-602 Dose-dependent Desialylation of CD8+ T cells in Monkeys



Dosed at days 1 and 29 as shown by arrows

Dose Conversion Between Human and Monkeys Based on FDA's Guidance (mg/kg)

Monkey	30	100
Human	9.7	33.3

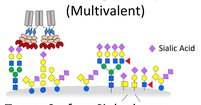
E-602-mediated desialylation of CD8+ T cells monitored by PNA using FACS. Dose-dependent desialylation of CD8+ T cells was observed and desialylation of CD8+ T cells was maintained for >=7 days, longer than its PK half-life (<= 1 day). Figure adapted from Cao, L. et al. [4].

Measuring Sialoglycan Modification in Tumor

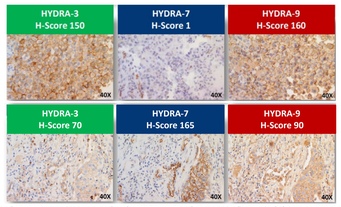
HYDRA

- HYDRA is a set of proprietary reagents capable of detecting **functional Siglec ligands** on cell surfaces
- HYDRA is a multimeric construct of Siglec carbohydrate recognition domains (CRDs) fused together genetically

HYDRA: IHC Reagents based on hexameric Siglec receptors (Multivalent)

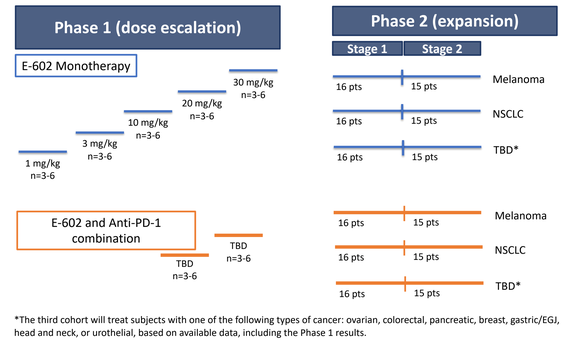


Tumor Surface Sialoglycans



Representative images from melanoma and NSCLC tumor cases stained with HYDRA-3, -7 and -9 reagents by IHC and shown at 40X magnification. Histo-scores (H-scores) provided and are the sum of the products of percentage tumor cell staining at each intensity level (0, 1+, 2+ and 3+) and thus range from 0 – 300. Figure adapted from Prendergast, J. et al. [5].

STUDY DESIGN



*The third cohort will treat subjects with one of the following types of cancer: ovarian, colorectal, pancreatic, breast, gastric/EGJ, head and neck, or urothelial, based on available data, including the Phase 1 results.

STUDY OBJECTIVES

	Phase 1 Dose Escalation	Phase 2 Expansion
Primary (monotherapy and combined with anti-PD-1)	• To evaluate the safety and tolerability of E-602 • To determine the MTD and/or RP2D of E-602	• To evaluate the preliminary antitumor activity of E-602 in participants with advanced cancers
Secondary (monotherapy and combined with anti-PD-1)	• To assess the PK and immunogenicity of E-602 • To evaluate preliminary antitumor activity of E-602	• To evaluate the safety and tolerability of E-602 • To assess the PK and immunogenicity of E-602
Exploratory	• To evaluate exploratory pharmacodynamic biomarkers of E-602 activity as monotherapy and in combination with anti-PD-1	

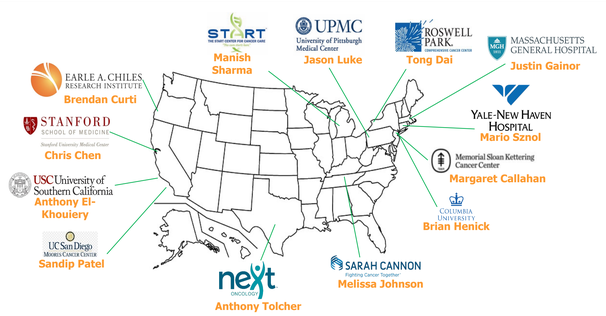
ELIGIBILITY

ELIGIBLE TUMOR INDICATIONS

Patients must have advanced or metastatic cancer in tumor types that have greater sialylation and have failed prior standard therapies.

PD-(L)1 Resistant	IO Non-Responsive
• Stage III or IV cutaneous or mucosal **melanoma** • Stage IV **NSCLC** without known driver mutations • MSI-H or dMMR **colorectal** or **gastric/EGJ** cancers • **Triple-negative breast** cancer • **Head and neck** cancer • **Urothelial** cancer	• Advanced **ovarian** cancer (epithelial, primary peritoneal, or fallopian tube) • Non-MSI-H or dMMR **colorectal or gastric/EGJ** cancers • Metastatic **pancreatic** adenocarcinoma • Advanced/metastatic **breast cancer other than TNBC**

STUDY SITES



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