CHARACTERIZATION OF ELA026, A CLINICAL-STAGE MONOCLONAL ANTIBODY THAT RAPIDLY AND POTENTLY DEPLETES MYELOID CELLS AND T LYMPHOCYTES

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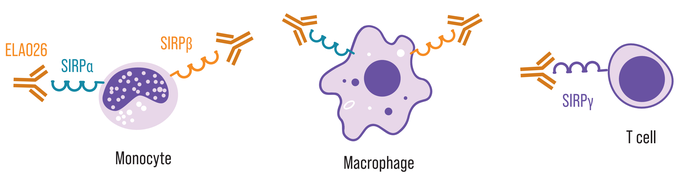
BACKGROUND

Hemophagocytic lymphohistiocytosis (HLH) is a rare and life-threatening syndrome of excessive immune activation. The disease is associated with a massive systemic inflammatory response requiring immediate and aggressive treatment. Secondary HLH (sHLH) is triggered by malignancy, autoimmune disease, or infection and is believed to be caused by uncontrolled activation of immune cells, particularly monocytes/macrophages and CD8 T cells. Currently there are no approved therapies for sHLH.

Signal regulatory proteins (SIRPs) are cell surface receptors expressed on cells of the myeloid lineage and T cells. ELA026 is a fully human, monoclonal immunoglobulin G1 (IgG1) SIRP-directed antibody that binds to and marks for destruction SIRP-expressing cells. By reducing myeloid-derived antigen-presenting cells and interferon gamma-producing CD8 T cells, ELA026 has the potential to halt the initiation and progression of the inflammatory process in sHLH. A proof-of-concept Phase 1b trial for assessing ELA026 efficacy in sHLH is currently underway (ClinicalTrials.gov Identifier: NCT05416307).

ELA026 Takes Advantage of the Restricted Expression of SIRP to Drive Targeted Depletion of Immune Cells



SIRPs are cell surface receptors expressed on myeloid (SIRPα, SIRPβ) and T cells (SIRPγ). ELA026 is a fully human IgG1 monoclonal antibody that targets SIRPα, SIRPβ, and SIRPγ.

Preclinical *in vitro* evaluation shows that ELA026:

- Does not inhibit CD47 binding to SIRPs or SIRP signaling
- Binds with comparable affinity to homologous human and cyno SIRP isoforms
- Binds to same human and cyno immune cells
- Induces antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) similarly across human and cyno monocytes and T cells

Preclinical *in vivo* evaluation of ELA026 that shows:

- Rapid, potent and reversible depletion of circulating monocytes and lymphocytes
- Affected cells come back to predose levels once drug levels drop below 0.1 µg/mL
- Increasing dose leads to increased durability of response
- Safe and well tolerated in 28-day and 6-month GLP toxicology studies

METHODS

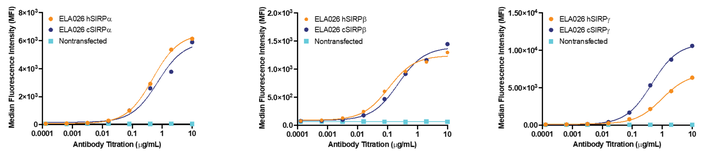
- *Flow cytometry*: Human and cynomolgus monkey SIRP isoforms were transiently expressed on CHO cells separately. ELA026 binding was assessed by flow cytometry. EC50 values were generated using a three-parameter logistic model and are within 3-fold for respective human and cynomolgus monkey isoform pair.
- *ADCC/ADCP assays*: CD14+ monocytes and CD8+ T cells (target cells) were negatively selected via magnetic beads from whole blood of individual human donors and cynomolgus monkeys. CD8+ T cells were stimulated with IL-2. For ADCC, target cells were dyed with CellTracker Green dye, opsonized with ELA026 or isotype control at various concentrations, and incubated with human NK cells (effector cells) at an effector-to-target cell ratio of 2:1. Cell death was quantified by flow cytometry using Zombie Violet dye. For ADCP, a reporter cell line in which FcγRIIa engagement upregulates luciferase activity was used as a surrogate measure to assess ADCP activity. Target cells were opsonized with ELA026 or isotype control at various concentrations and then incubated with the reporter effector cells line at an effector-to-target ratio of 2:1 at 37°C for 6 hrs. ADCP was quantified in relative luminescence units (RLUs).
- *In vivo ELA026 administration in cynomolgus macaques*: ELA026 was administered intravenously (IV) (n=3 monkeys/dose group). Plasma ELA026 concentrations ($ELA026_{plasma}$) were quantified via ELISA. The lower limit of quantification (LLOQ) for $ELA026_{plasma}$ = 0.5 ng/mL (0.0005 µg/mL); concentrations below the limit of quantification (BLQ) are not illustrated.

RESULTS

In vitro

ELA026 Binds Comparably to Human and Cynomolgus Monkey SIRP Isoforms

A



B

SIRP Expression on CHO	EC50 (µg/mL)
Human SIRPα	0.482
Cynomolgus monkey SIRPα	0.707
Human SIRPβ	0.110
Cynomolgus monkey SIRPβ	0.228
Human SIRPγ	0.982
Cynomolgus monkey SIRPγ	0.449

FIGURE 1. ELA026 shows comparable binding to human and cynomolgus monkey SIRP isoforms SIRPα, SIRPβ, and SIRPγ. (A) Flow cytometry binding curves of ELA026 to individual SIRPs expressed on CHO cells compared to nontransfected CHO cells. (B) EC50 values for ELA026 binding are within 3-fold for homologous human and cynomolgus monkey isoforms.

ELA026 Binds to Human and Cynomolgus Monkey Monocytes and T Cells

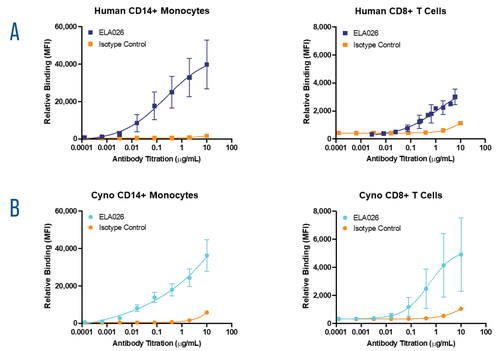


FIGURE 2. *Ex vivo*, ELA026 binds to target primary cells of individual donors, as measured by flow cytometry. (A) ELA026 has comparable binding to primary human and cynomolgus monkey CD14+ monocytes in whole blood (n=6 donors and monkeys). (B) ELA026 binds to isolated primary CD8+ T cells maintained in IL-2 (n=3 human donors and n=6 for monkey donors).

ELA026-Induced *In Vitro* ADCC and ADCP of Human and Cynomolgus Monkey Monocytes and T Cells

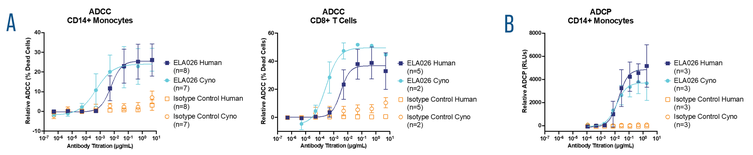


FIGURE 3. *In vitro*, ELA026 induces ADCC and ADCP of primary human and cynomolgus monkey target cells with EC90 < 0.1 µg/mL. CD14+ monocytes and CD8+ T cells were negatively selected via magnetic beads from whole blood of individual donors. CD8+ T cells were stimulated with IL-2. (A) ELA026 induces human CD56+ NK cell-mediated ADCC of monocytes and T cells measured via flow cytometry. (B) ELA026 induces ADCP of monocytes as assessed by FcγRIIa-expressing reporter effector cells in a plate-based bioassay.

In vivo

ELA026 Induces a Rapid, Potent, and Reversible Depletion of Circulating Monocytes and Lymphocytes

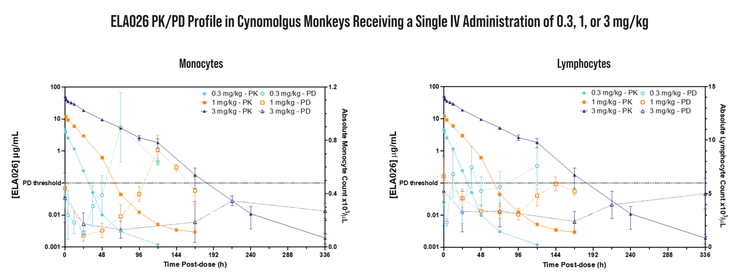
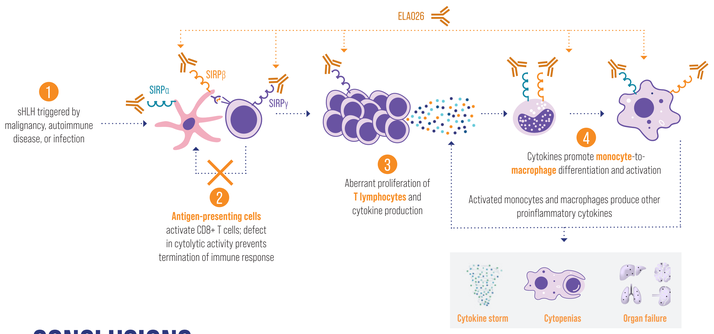


FIGURE 4. Plasma ELA026 concentrations (left y-axis) vs PD effects on cell counts (right y-axis), reported as absolute values (cells/µL) for monocytes and lymphocytes in cynomolgus monkeys receiving a single IV administration of 0.3, 1, or 3 mg/kg ELA026. A PD threshold of ~0.1 µg/mL ELA026 was identified (dashed horizontal line); plasma ELA026 concentrations <0.1 µg/mL were associated with loss of PD effect and return of cell counts towards baseline values.

ELA026 Targets the Principal Cells Responsible for Driving Pathogenesis in sHLH



CONCLUSIONS

- *In vitro*, ELA026 has comparable binding to human and cynomolgus monkey homologous SIRP isoforms
- *In vitro*, ELA026 binds to SIRP proteins on the surface of primary human and cynomolgus monkey monocytes and T cells, inducing potent ADCC and ADCP
- *In vivo* administration of ELA026 in cynomolgus monkeys shows a rapid and potent depletion of SIRP-expressing monocytes and lymphocytes, with a well-defined PK/PD relationship consistent with *in vitro* pharmacological results
- Reversibility of the PD effect was achieved following washout, suggesting that ELA026 treatment is not associated with long-term immunosuppression
- ELA026 was shown to be safe and well tolerated in 28-day and 6-month GLP toxicology studies in cynomolgus macaques and is currently in Phase 1 studies in both healthy volunteers (NCT05556863) and sHLH patients (NCT05416307; ASH 2022 Poster #3730)

Data support clinical development of ELA026 for the treatment of numerous myeloid and T lymphocyte–driven disorders, including sHLH