Immune-Targeted Glucocorticoid ADC for the Treatment of Autoimmune and Inflammatory Diseases

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INTRODUCTION

Glucocorticoids (GCs) are the most efficacious anti-inflammatory drugs rheumatologists have available for patients. Unfortunately, prolonged systemic GC exposure leads to unacceptable toxicities. Targeted delivery of GCs to immune cells has the potential to minimize exposures to other organ systems, thus preserving the clinical efficacy of GCs while mitigating systemic toxicities which preclude their broader, prolonged use.

AIMS

We designed LFD-200 as an antibody drug conjugate (ADC) that minimizes systemic toxicities by delivering a potent GC payload via a linked antibody (Fig. 1A). LFD-200 selectively targets V-domain Ig Suppressor of T cell Activation (VISTA), a cell surface protein mainly expressed on myeloid and lymphoid cells. We conducted non-clinical studies in mice and non-human primates (NHPs) to evaluate if delivering a GC payload directly to immune cells can achieve GC efficacy while limiting systemic toxicity.

METHODS

- **In vitro studies:** VISTA expression was measured by flow cytometry in lipopolysaccharide (LPS) activated (1mg/ml) human whole blood (Fig.1B). LFD-200 internalization rate testing was done by first binding the monoclonal antibody (mAb) or ADC to human monocytes and then measuring intracellular signal by flow cytometry over time (Fig.1C).
- **NHP (cynomolgus macaque) studies:** LFD-200 was administered intravenously (IV) in Studies 1-2 and subcutaneously (SC) in Studies 3-4. **Study 1** (Fig.1D): NHPs (4 Males (M)) were injected with 15 mg/kg LFD-200 and blood was collected for serum PK via ELISA. **Study 2** (Fig.2): NHPs (2-3 Females (F)/group) were dosed with 10 mg/kg LFD-200 or 2 mg/kg dexamethasone (Dex). Tissues were collected at 24 hr or 7 days post dosing and tissue exposures were measured by mass spectrometry (LC-MS-MS). **Study 3** (Fig.4A): NHPs (4M/group) received one dose of vehicle or LFD-200 at 2, 5 and 20 mg/kg; a positive control was included by injecting vehicle-treated animals with 2 doses of Dex (3 mg/kg) on day 6 and 7. **Study 4** (Fig.4B): NHPs (2M,2F/group) received 3 doses of LFD-200 (50, 250 mg/kg) or vehicle control every other week x 4 weeks (toxicity study).
- **Mouse studies** - **Study 5** (Fig.3): Mice were injected SC on day 0 with 10 mg/kg LFD-200 mouse surrogate, which uses the same GC payload with a mAb targeting mouse VISTA. **Study 6** – Toxicity study (Fig.4C-D): CD-1 mice (10F/group) were injected weekly intraperitoneally (IP) with 10 mg/kg LFD-200 mouse surrogate or 5 mg/kg/day Dex (dosed in water then, after 2 weeks, IP 5 days/week) for up to 16 weeks. **Study 7** (Fig.5) T cell transfer colitis model: naïve CD4 T cells from human VISTA knock-in mice were transferred in Rag1 knock-out mice; all treatments (Dex 0.2 or 2 mg/kg; LFD-200 or LFD-200 mAb at 10 mg/kg) were administered IP weekly (day 0 to 61).
- Changes in circulating cortisol/corticosterone were measured via ELISA or LC-MS-MS in mice and NHPs (Studies 3, 4 and 6). Weights and metabolic markers were also measured in mice (Study 6).
- Immunohistochemistry (IHC) & immunofluorescence (IF) were conducted on formalin fixed paraffin embedded tissue samples using a proprietary anti-payload antibody in Study 5 (Fig.3).

RESULTS

Anti-VISTA mAb rapidly delivers GC payload directly to immune cells and avoids prolonged serum exposure (Figure 1)

- VISTA is highly expressed on myeloid cells, T cells, and plasma B cells with no difference between activated (Fig.1B) or naïve (data not shown) and rapidly internalizes the ADC (Fig.1C)
- The Fc-silent anti-VISTA mAb has no pharmacology/function beyond payload delivery (data not shown)
- Robust uptake leads to short serum PK ($t_{1/2}$ ~14 hr; Fig.1D), greatly limiting ADC uptake by off-target tissues (Fig.2)

LFD-200 achieves sustained GC exposure in immune tissues that persists for ≥7 days in NHPs (Figure 2)

- Biodistribution data show GC payload accumulation primarily in immune tissues, which persists for at least 7 days post dosing
- In comparison, Dex shows negligible drug concentrations in most tissues at 24 hours post dose

LFD-200 delivers GC to immune cells and persists for ≥8 days after a single dose in mice (Figure 3)

- IHC/IF studies show the presence of drug in immune tissues at least 8 days post dosing (Fig.3A) and demonstrate targeted payload delivery to T cells, myeloid cells, & high endothelial venules in lymph nodes and spleen (Fig.3B)

LFD-200 treatment showed no evidence of systemic GC toxicity after dosing for up to 4 months (Figure 4)

- After up to 3 high doses (≤250 mg/kg) over ≤1 month in NHPs, LFD-200 does not impact cortisol levels (Fig.4A-B)
- After extended treatment (16 weeks) in mice at an efficacious dose (10 mg/kg), no off-target toxicity is seen as demonstrated by the absence of impact on corticosterone (Fig.4C), weight (Fig.4D), or glucose/lipid metabolism and liver function (data not shown)
- By contrast, Dex shows immediate, profound reductions in cortisol levels in NHPs and mice (Fig.4A, 4C) as well as increased body weight (Fig.4D), insulin, LDL cholesterol, and ALT (data not shown)

LFD-200 treatment achieved similar efficacy vs. GC in mouse models of disease (Figure 5)

- LFD-200 prevents colitis development with similar efficacy to Dex at efficacious doses (10 mg/kg vs. 2 mg/kg, respectively) and disease control is observed up to 3 weeks post treatment
- Similar efficacy vs. GC was seen in other disease models including diabetes, rheumatoid arthritis, and asthma (data not shown)

CONCLUSIONS

Non-clinical experiments in mice and NHPs indicate that LFD-200 rapidly and selectively delivers a potent GC payload to immune tissues and persists for ≥7 days with no systemic toxicity observed even at very high doses. Additionally, LFD-200 showed similar efficacy vs. GC in multiple mouse models of disease. The successful targeting of GCs to the immune system with the sparing of non-immune tissues may offer a new way to treat autoimmune and inflammatory conditions across broad medical disciplines including rheumatology, gastroenterology, pulmonology, and dermatology. LFD-200 is planned to enter the clinic in 2025 for evaluation in healthy volunteers followed by patients with an autoimmune disease.

Figure 1: LFD-200 is a human VISTA antibody-glucocorticoid conjugate that is rapidly internalized in immune cells resulting in short serum PK : *A) Schematic of LFD-200 (drug antibody ratio (DAR) of 8); B) VISTA expression on activated human whole blood (expression on plasma cells and endothelial cells not shown; mono=monocytes, neutro=neutrophils, Treg=regulatory T cells); C) LFD-200 internalization rate; D) LFD-200 serum PK (NHP Study 1) shows a serum half life of ~14 hrs.*

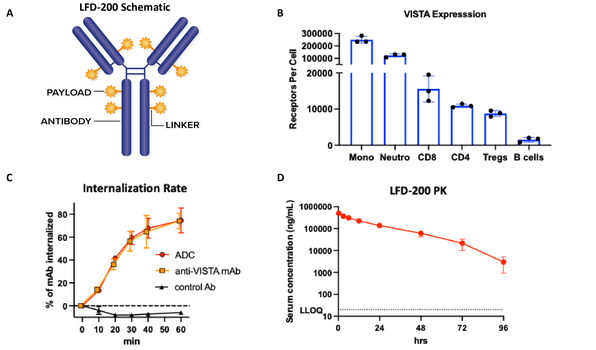


Figure 2: LFD-200 payload predominates in immune tissues after single dosing in NHPs. *Following one dose of LFD-200 at 10 mg/kg, payload is detected in immune tissues up to day 7 post dosing (upper graph). In contrast, Dex levels 24 hr post dosing (2 mg/kg) were below detection levels in most tissues, including immune tissues (lower graph). Note that comparable efficacious doses for LFD-200 & Dex were used.*

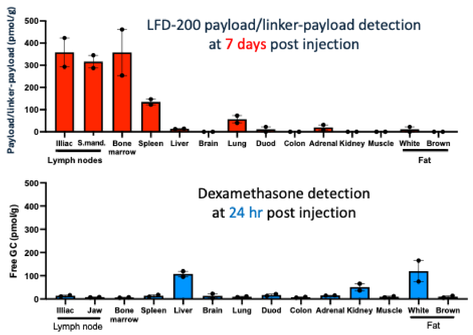


Figure 3: Following targeted delivery, LFD-200 payload shows long intracellular exposure in selected immune cells within secondary lymphoid organs. *Mouse lymph nodes and spleen were collected on days 1, 4, and 8 post dosing. A) IHC on lymph nodes (brown: anti-payload antibody stain, blue: hematoxylin counterstain); B) Immune cell identification by IF co-staining with specific markers on day 1 post dosing.*

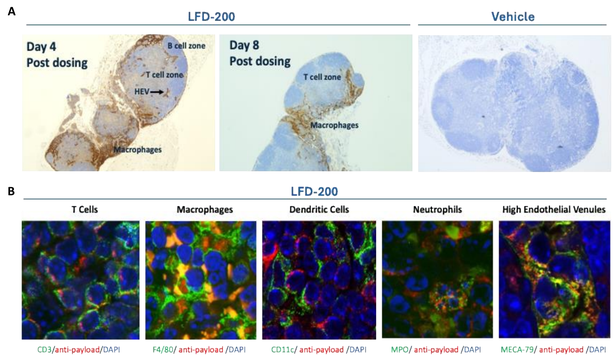
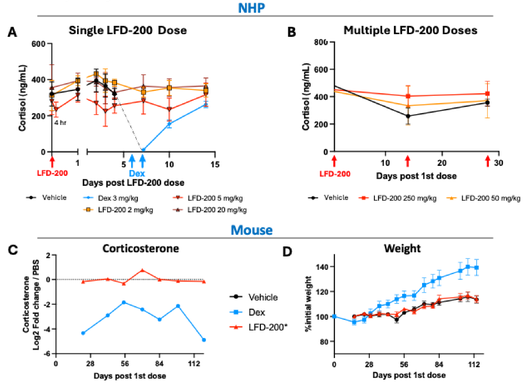


Figure 4: No Glucocorticoid Toxicity After Extended Treatment with LFD-200. *In NHPs, GC toxicity as measured by serum cortisol was not observed after a single (A) or multiple (B) doses of LFD-200 or vehicle control (in contrast to Dex). In CD-1 mice, no GC toxicity was observed as measured by corticosterone levels (C) and body weight (D) after up to 16 weeks of dosing with LFD-200 or vehicle (in contrast to Dex).*



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