# GS-8588, A Novel Envelope-Targeting Bispecific T-Cell Engager for HIV Cure

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GS-8588 study

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## Conclusions

- GS-8588 is a novel bispecific T-cell engager (TCE) that mediates potent, broad, and specific killing of cluster of differentiation 4 (CD4) T cells infected with a diverse panel of HIV clinical isolates in vitro
- GS-8588 induces low-level T-cell activation and cytokine secretion when incubated with peripheral blood mononuclear cells (PBMCs) from people with HIV (PWH) ex vivo
- GS-8588 exhibits IgG-like pharmacokinetics (PK), with ~25% lymph node to serum exposure ratio in a nonhuman primate (NHP) model
- No adverse findings were observed in good laboratory practice (GLP) cynomolgus toxicology studies when GS-8588 was dosed to 100 mg/kg
- These results support the clinical evaluation of GS-8588 as a therapeutic candidate for the elimination of latent HIV-infected cells in PWH

## Plain Language Summary

- Current antiretroviral therapy (ART) helps PWH by stopping the virus from replicating in the body. However, it cannot completely cure the disease because there are some HIV-infected cells that are hidden in the body, and these cells can cause the virus to come back if ART is stopped[1]
- GS-8588 is a novel antibody-like protein designed to recruit one's own immune cells to kill HIV-infected cells
- In this study, we examined how well GS-8588 can destroy HIV-infected cells in laboratory cell systems. We also studied how the body absorbs and processes GS-8588, as well as its safety, using cynomolgus monkeys as a model
- We showed that GS-8588 kills HIV-infected cells effectively, and it is well tolerated by cynomolgus monkeys at doses higher than the predicted dose that would be used to treat people
- GS-8588 is currently being evaluated in a phase 1 clinical trial in PWH who have the virus under control with ART

## Introduction

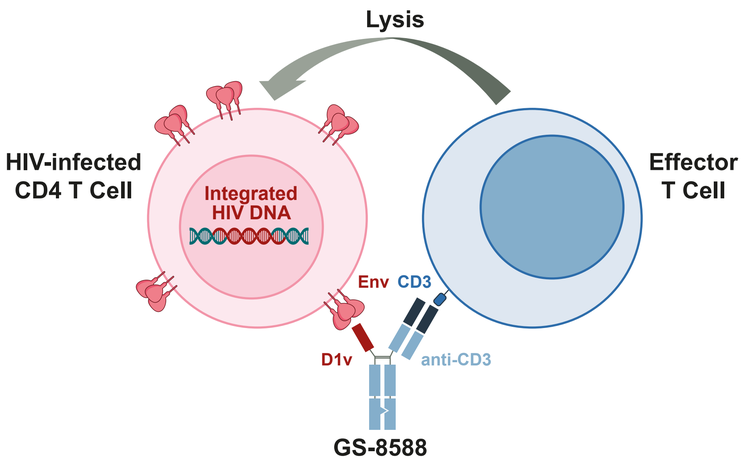
— A functional cure for HIV requires therapeutic interventions that can eliminate reservoirs of integrated proviruses in CD4 T cells

— GS-8588 is a novel bispecific TCE designed to redirect polyclonal effector T cells to kill HIV-infected, envelope (Env)-expressing CD4 T cells (**Figure 1**)

— GS-8588 consists of an engineered CD4 domain 1 variant (D1v) that exhibits improved Env-targeting potency and developability profile,[2] a humanized anti-CD3 Fab, and an effector-silent human IgG1 hetero-Fc

— Here, we characterize GS-8588 for its binding and killing activities in vitro, T-cell activation and cytokine release ex vivo, and PK and toxicology profiles in NHP

### Figure 1. GS-8588 Mechanism of Action




CD×, cluster of differentiation ×; D1v, domain 1 variant.

## Methods

— Binding to Env or CD3 was evaluated in vitro by incubating GS-8588 dilution series with HIV-infected CEM (a T-lymphoblast cell line) or PBMCs from healthy donors, respectively

— Specific killing was evaluated in vitro by incubating GS-8588 dilution series with HIV-infected target cells (resting or activated primary CD4 T cells, or CEM cells) and PBMC effector cells; off-target killing of major histocompatibility complex class II (MHCII)–expressing B cells was assessed in the same assay

— T-cell activation and cytokine secretion were monitored ex vivo in GS-8588–treated PBMC samples derived from PWH

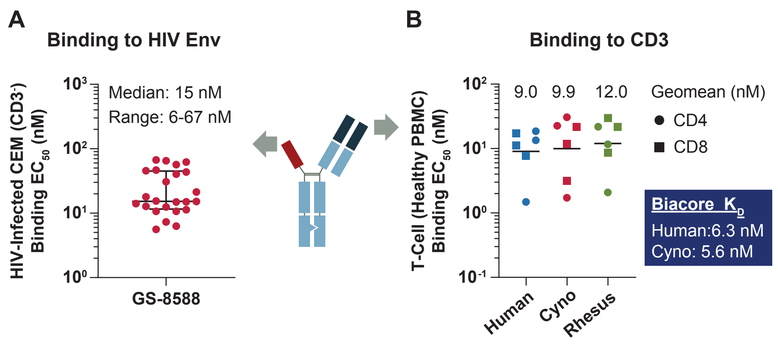
— PK of GS-8588 was evaluated in cynomolgus monkeys at a single 1 mg/kg intravenous dose

— Lymph node to serum exposure ratio of GS-8588 was evaluated in cynomolgus monkeys at a single 10 mg/kg intravenous dose

— Safety and tolerability of GS-8588 were evaluated in a cynomolgus monkey GLP toxicology study at 5 weekly doses up to 100 mg/kg/week

## Results

### Figure 2. GS-8588 Binds to Cell Surface Env and CD3



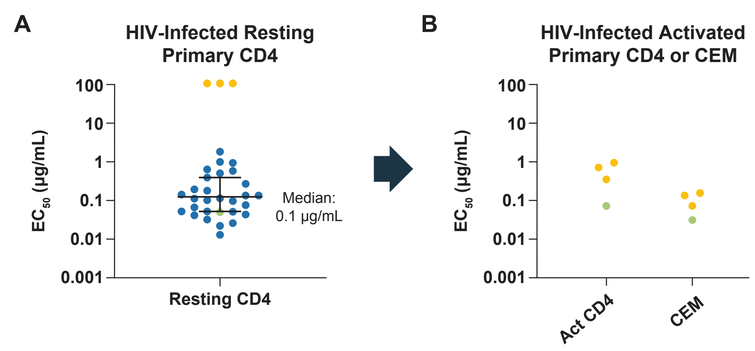

CD×, cluster of differentiation ×; CEM, a T lymphoblast cell line; $EC_{50}$, half-maximal effective concentration; Env, envelope; Geomean, geometric mean; PBMC, peripheral blood mononuclear cell.

— GS-8588 binds to HIV-infected CEM cells (CD3-negative) with half-maximal effective concentration ($EC_{50}$) values ranging from 6 to 67 nM across 24 clade B clinical isolates (**Figure 2A**)

— GS-8588 binds to healthy CD4 and CD8 T cells from human, cynomolgus, and rhesus monkeys with similar potency (**Figure 2B**)

## Results

### Figure 3. GS-8588 Mediates Potent and Broad Killing of HIV-Infected CD4 Cells

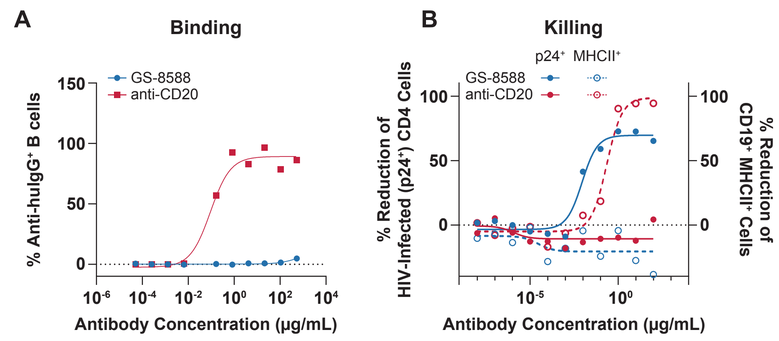



CD×, cluster of differentiation ×; CEM, a T lymphoblast cell line; $EC_{50}$, half-maximal effective concentration.

— GS-8588 mediated killing of resting primary CD4 T cells infected with 29 of the 32 HIV-1 isolates tested (median $EC_{50}$, 0.1 µg/mL) (**Figure 3A**)

— For the 3 remaining isolates, GS-8588-mediated killing was observed in activated PBMC-based and CEM-based killing assays (**Figure 3B**)

### Figure 4. GS-8588 Exhibits No Detectable Binding or Killing of MHCII-Expressing B Cells

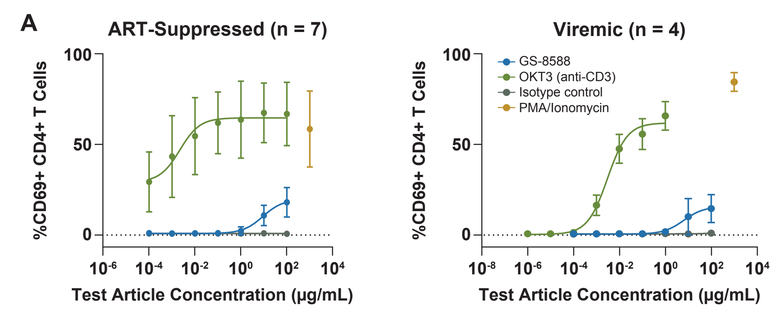



CD×, cluster of differentiation ×; MHCII, major histocompatibility complex class II.

— GS-8588 exhibits no detectable binding to B cells (**Figure 4A**)

— GS-8588 mediated killing of HIV-infected CD4 cells, and not $CD19^+$ $MHCII^+$ B cells, in the same assay well (**Figure 4B**)

### Figure 5. GS-8588 Mediates Low-Level T-Cell Activation and Cytokine Release Ex Vivo




B

**Mean Maximum Signals[a] (pg/mL) of Cytokines in Culture Supernatant of PBMCs Derived From PWH**

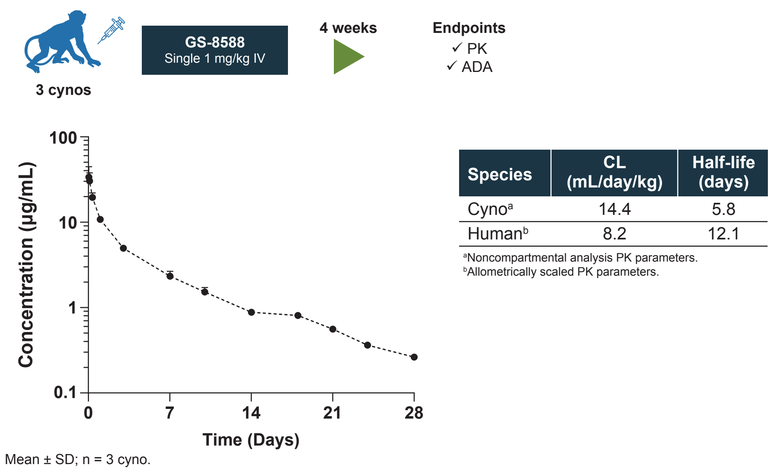
| | ART-Suppressed (n = 4) | | | Viremic (n = 4) | | |
|---|---|---|---|---|---|---|
| Cytokine | GS-8588 | Isotype Control | PMA/ ionomycin | GS-8588 | Isotype Control | PMA/ ionomycin |
| IFN-γ | 7.6 | 0.5 | 1756.4 | 52.0 | 15.6 | 6003.7 |
| TNF-α | 22.9 | 5.3 | 3270.3 | 9.5 | 6.9 | 256.6 |
| IL-6 | 3.0 | 2.6 | 6266.2 | 35.3 | 45.0 | 1157.8 |
| IL-2 | 3.5 | 2.8 | 8814.6 | 7.8 | 8.1 | 2846.9 |
| IL-4 | 2.9 | 1.8 | 22.9 | 12.4 | 11.4 | 21.7 |
| IL-10 | 0.7 | 0.3 | 2.9 | 12.3 | 11.9 | 18.0 |

[a]Mean concentration of cytokines measured for supernatants from PBMCs treated with 100 µg/mL of GS-8588, isotype control (GS-832677), and 50 ng/mL and 0.5 µg/mL of PMA/ionomycin combination.
ART, antiretroviral therapy; CD×, cluster of differentiation ×; IFN, interferon; IL, interleukin; PMA, phorbol myristate acetate; PBMC, peripheral blood mononuclear cell; PMA, phorbol myristate acetate; PWH, people with HIV; TNF, tumor necrosis factor.

— GS-8588 induces low-level CD69 upregulation (geometric mean $EC_{10}$ ≥ 10.3 µg/mL) when co-incubated with PBMCs derived from ART-suppressed (n = 7) and viremic (n = 4) PWH; $EC_{10}$ is defined as GS-8588 concentration corresponding to 10% CD69+ T cells (**Figure 5A**)

— GS-8588 induces low-level cytokine production (mean maximum signals ≤ 52 pg/mL) in the supernatant (**Figure 5B**)

### Figure 6. GS-8588 Exhibits IgG-Like Serum PK in NHP Model




| Species | CL (mL/day/kg) | Half-life (days) |
|---|---|---|
| Cyno[a] | 14.4 | 5.8 |
| Human[b] | 8.2 | 12.1 |

[a]Noncompartmental analysis PK parameters.
[b]Allometrically scaled PK parameters.

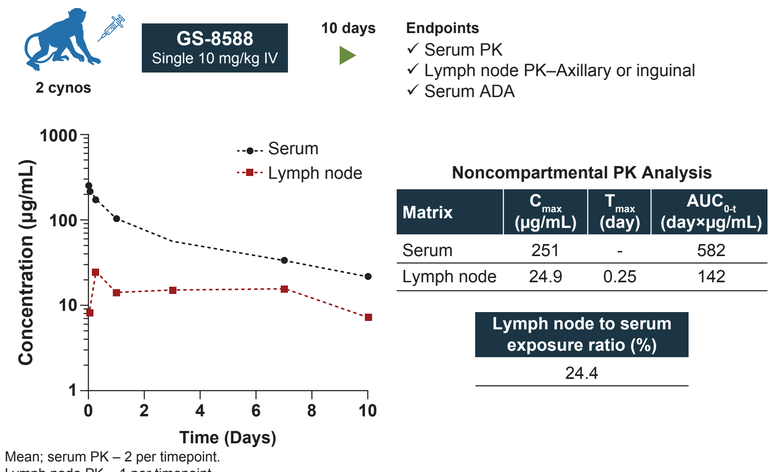
Mean ± SD; n = 3 cyno.

ADA, antidrug antibodies; CL, clearance; Cyno, cynomolgus; NHP, nonhuman primate; PK, pharmacokinetics.

— GS-8588 exhibits a typical biphasic serum PK profile, with a projected ~12-day half-life in humans (**Figure 6**)

— Antidrug antibodies (ADAs) were detected and had minimal impact on PK in 2 of 3 cynomolgus at ≥ 24 days

### Figure 7. GS-8588 Lymph Node to Serum Exposure Ratio Is ~25%




**Noncompartmental PK Analysis**

| Matrix | $C_{max}$ (µg/mL) | $T_{max}$ (day) | $AUC_{0-t}$ (day×µg/mL) |
|---|---|---|---|
| Serum | 251 | - | 582 |
| Lymph node | 24.9 | 0.25 | 142 |

| Lymph node to serum exposure ratio (%) |
|---|
| 24.4 |

Mean; serum PK – 2 per timepoint.
Lymph node PK – 1 per timepoint.

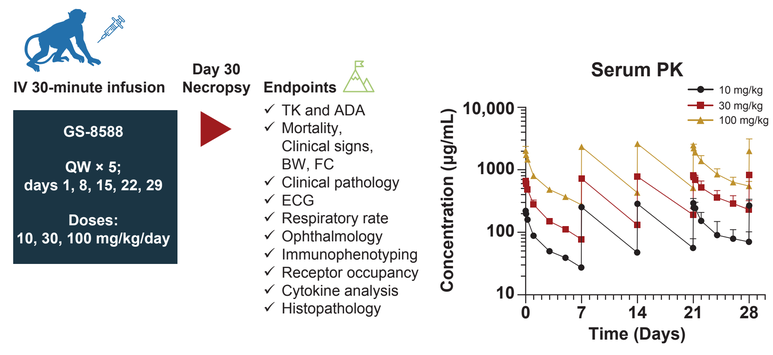
ADA, antidrug antibodies; AUC, area under the curve; $C_{max}$, maximum plasma concentration; Cyno, cynomolgus; PK, pharmacokinetics; $T_{max}$, time to maximum concentration.

— Lymphoid tissues are the predominant sites of HIV reservoirs[3]

— Based on $AUC_{0-t}$, a lymph node to serum exposure ratio of ~25% was calculated (**Figure 7**)

— This exposure ratio and in vitro killing data were used to estimate the minimal efficacious dose

### Figure 8. No Adverse Findings in GLP Cynomolgus Toxicology Study up to 100 mg/kg




ADA, antidrug antibodies; BW, body weight; FC, food consumption; GLP, good laboratory practice; PK, pharmacokinetics; TK, toxicokinetics.

— GS-8588 was well tolerated at all dose levels with no mortality or adverse findings (**Figure 8**)

— Dose-proportional serum PK was observed (**Figure 8**)

— Estimated margins were > 100-fold, based on initially projected minimally efficacious dose

**References:** **1.** Chun TW, et al. *Nat Immunol*. 2015;16:584-9. **2.** Chen W, et al. *J Virol*. 2014;88:1125-39. **3.** Banga R, et al. *Curr Opin HIV AIDS*. 2024;19:116-23.

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**Disclosures:** All authors were employed at Gilead Sciences when this work was conducted.

**Abbreviations:** ADA, antidrug antibody; ART, antiretroviral therapy; AUC, area under the curve; CD×, cluster of differentiation ×; CEM, a T lymphoblast cell line; $C_{max}$, maximum plasma concentration; Cyno, cynomolgus; D1v, domain 1 variant; $EC_{50}$, half-maximal effective concentration; Env, envelope; Fab, fragment antigen-binding; FC, food consumption; GLP, good laboratory practice; IFN, interferon; IL, interleukin; MHCII, major histocompatibility complex class II; NHP, nonhuman primate; PBMC, peripheral blood mononuclear cell; PK, pharmacokinetics; PMA, phorbol myristate acetate; PWH, people with HIV; TCE, T-cell engager; TK, toxicokinetics; $T_{max}$, time to maximum concentration; TNF, tumor necrosis factor.