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

Abstract Code: WEPEA019

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

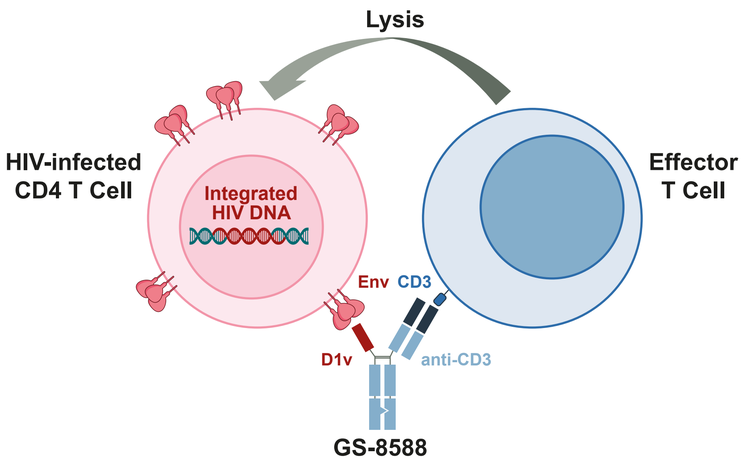
— A functional cure for HIV requires therapeutic interventions that can eliminate reservoirs of integrated proviruses in CD4 T cells

— 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

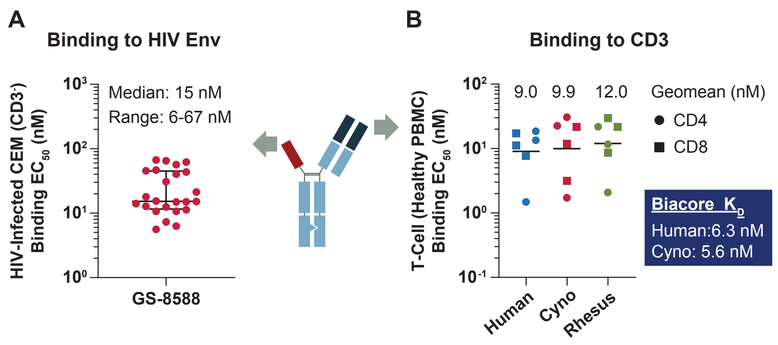
— PK of GS-8588 was evaluated in cynomolgus monkeys at a single 1 mg/kg intravenous dose

— 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



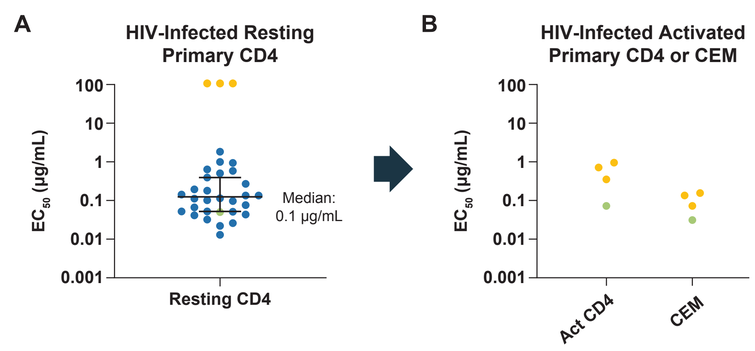
CD×, cluster of differentiation ×; CEM, a T lymphoblast cell line; EC_{50}, half-maximal effective concentration; Env, envelope; Geomean, geometric mean; PBMC, peripheral blood mononuclear cell.

— 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

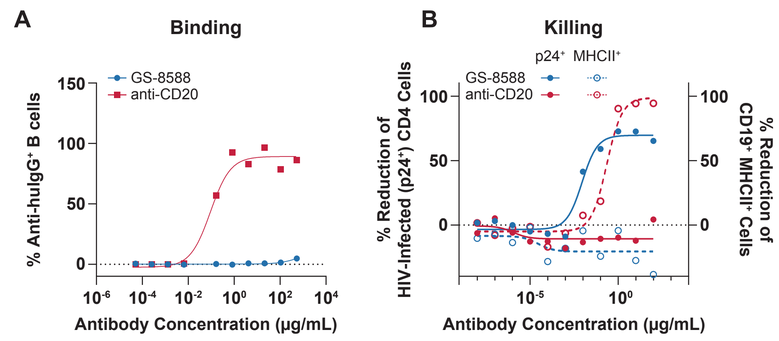


CD×, cluster of differentiation ×; CEM, a T lymphoblast cell line; EC_{50}, half-maximal effective concentration.

— 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

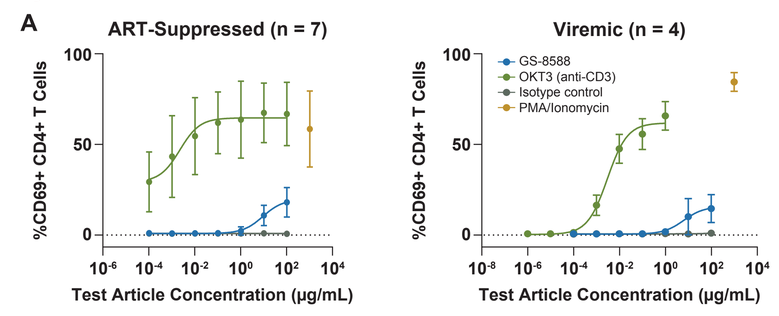


CD×, cluster of differentiation ×; MHCII, major histocompatibility complex class II.

— 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

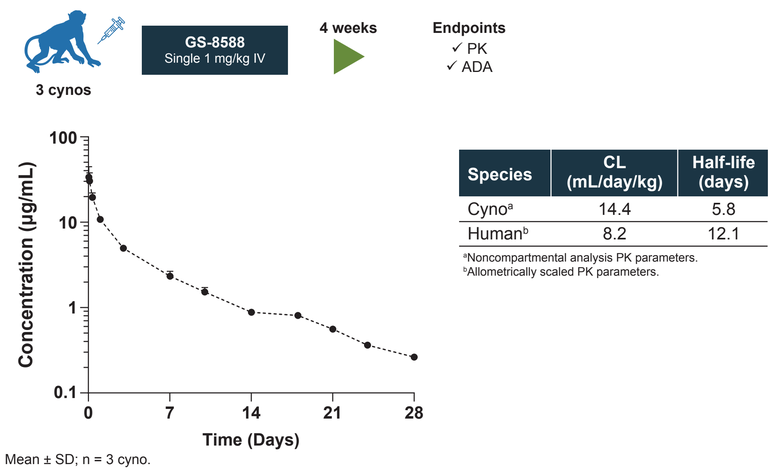
	ART-Suppressed (n = 4)			Viremic (n = 4)		
Cytokine	GS-8588	Isotype Control	PMA/ ionomycin	GS-8588	Isotype Control	PMA/ ionomycin
IFN-γ	7.6	0.5	1756.4	52.0	15.6	6003.7
TNF-α	22.9	5.3	3270.3	9.5	6.9	256.6
IL-6	3.0	2.6	6266.2	35.3	45.0	1157.8
IL-2	3.5	2.8	8814.6	7.8	8.1	2846.9
IL-4	2.9	1.8	22.9	12.4	11.4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

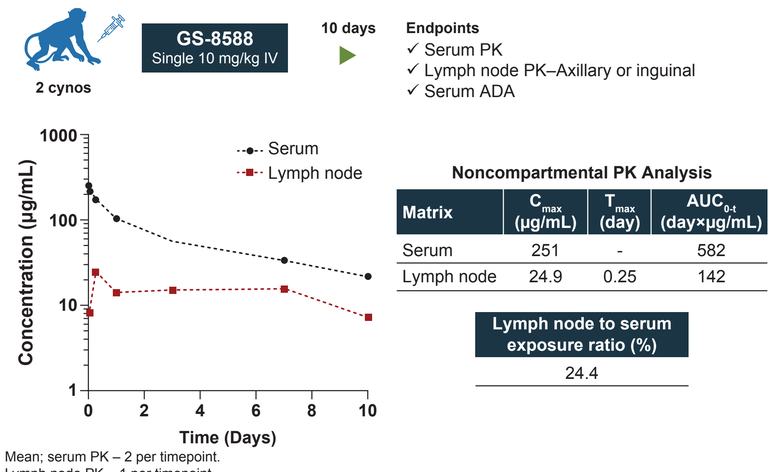
Mean ± SD; n = 3 cyno.

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.

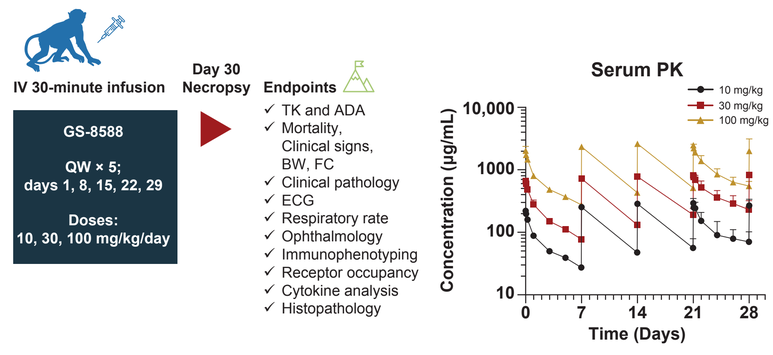
ADA, antidrug antibodies; AUC, area under the curve; C_{max}, maximum plasma concentration; Cyno, cynomolgus; PK, pharmacokinetics; T_{max}, time to maximum concentration.

— 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.

Acknowledgments: We would like to thank all of the GS-8588 team at Gilead in Foster City and Oceanside, CA, USA. This study was funded by Gilead Sciences, Inc. Editing and production assistance were provided by Parexel, and funded by Gilead Sciences, Inc. Some of these data were presented at Science Day, September 21, 2023, Foster City, CA, USA, and at PEGS 2024; May 17; Boston, MA, USA.

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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.