Supplementary Materials

A Pooled Analysis of Eight Clinical Studies Suggests a Link between Influenza-Like Symptoms and Pharmacodynamics of the Toll-Like Receptor 7 Agonist Vesatolimod

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Supplemental Text 1. Vesatolimod dose strategy across eight clinical studies

For GS-US-243-0101, there were seven VES dose groups (0.3, 1, 2, 4, 6, 8, and 12 mg). Each group included six healthy participants, except the VES 8-mg group (n = 19). The VES dose in the four studies involving PWHBV (n = 394) was capped at 4 mg and included four doses (0.3, 1, 2, and 4 mg). GS-US-283-0102 (N = 43) and GS-US-283-0106 (N = 41) included all four dose groups. GS-US-283-1059 (N = 146) and GS-US-283-1062 (N = 164) included only three dose groups (1, 2, and 4 mg). Participants were balanced between groups in each study. Across these four HBV studies, the numbers of participants who received each VES dose were as follows: 0.3 mg, 21 participants; 1 mg, 124 participants; 2 mg, 127 participants; and 4 mg, 122 participants. The two placebo-controlled studies in PWH (N = 53) were GS-US-382-1450 (seven dose groups, 1–12 mg; N = 36) and GS-US-382-3961 (three dose groups, 4–8 mg; N = 7). For GS-US-382-1450, each VES group contained six participants (including six escalated from 10–12 mg). While GS-US-382-3961 was underway, additional data emerged that suggested higher VES doses than originally planned would be safe and potentially more effective. The initial VES dose of 4 mg was increased to 6 mg and then eventually to 8 mg. Intraparticipant dose escalations within the VES group were performed as follows: two participants received 4 mg only, four participants received 4 and 6 mg, five participants received 6 mg only, three participants received 6 and 8 mg, and three participants received 8 mg only (**Tables S2 and S3**).

Supplemental Text 2. Tukey trend test method

The ordinal scale (1 = placebo; 2 = VES 0.3 mg; 3 = VES 1 mg; 4 = VES 2 mg; 5 = VES 4 mg; 6 = VES 6 mg; 7 = VES 8 mg; 8 = VES 10 mg; and 9 = VES 12 mg) was used to identify the optimal dose combination for predicting flu-like AEI at first visit after VES administration. Similarly, participants with VES 10 or 12 mg were combined (ie, treatment modified for grouped scale: 0 = placebo; 1 = VES 0.3 mg; 1 = VES 1 mg; 1 = VES 2 mg; 1 = VES 4 mg; 1 = VES 6 mg; 1 = VES 8 mg; 2 = VES 10 mg; and 2 = VES 12 mg) to evaluate prognostic and predictive modeling along with the ordinal scale from Tukey's trend test. In the generalized linear mixed-effect model, using the formula shown below, flu-like AEI is treated as a binary outcome and each distinct trail as the random effect. The covariates included age, sex at birth, and race. Post-hoc Tukey trend test was performed by first fitting a linear regression model and then performing statistical analysis for significance, with P values < .05 obtained using a grouped mixed-effect model for probability used as an indication for probability of having a flu-like AEI after the first VES administration.

Table S1 Summary of eight studies included in pooled analysis

Study/ ClinicalTrials.gov ID	Population	Design	Total participants (VES/placebo)	VES administration	VES dose, mg	Endpoints
GS-US-243-0101/ NA	Healthy participants	Phase 1, randomized, double-blind, placebo-controlled study	75 (55/20)	Single oral dose	0.3, 1, 2, 4, 6, 8, 12	Safety, tolerability, PK, and PD of VES
GS-US-283-0102/ NCT01590654	Virologically suppressed chronic HBV	Phase 1, double-blind, randomized, placebo-controlled, single- and multiple-dose–ranging, adaptive study	51 (43/8)	Single dose or 2 doses 1 week apart	0.3, 1, 2, 4	Safety, tolerability, PK, PD, and antiviral activity of VES
GS-US-283-0106/ NCT01590641	Treatment-naive chronic HBV	Phase 1, double-blind, randomized, placebo-controlled, single- and multiple-dose–ranging, adaptive study	49 (41/8)	Single dose or 2 doses 1 week apart	0.3, 1, 2, 4	
GS-US-283-1059/ NCT02166047	Virologically suppressed chronic HBV	Phase 1, double-blind, randomized, placebo-controlled, multiple-dose–ranging, adaptive study	162 (146/16)	Weekly dose for 12 week	1, 2, 4	
GS-US-283-1062/ NCT02579382	Chronic HBV not on treatment	Phase 2, randomized, double-blind, placebo-controlled, multicenter study	192 (164/28)	Weekly dose for 12 week	1, 2, 4	Safety and efficacy of VES in combination with TDF
GS-US-382-1450/ NCT02858401	Virologically suppressed HIV-1	Phase 1b, randomized, blinded, placebo-controlled, dose-escalation study	48 (36/12)	Biweekly dose for 10 week	1, 2, 4, 6, 8, 10, 12	Safety and biological activity of VES
GS-US-382-3961/ NCT03060447	ART-treated controllers with HIV-1	Phase 1b, randomized, double-blind, placebo-controlled study	25 (17/8)	Biweekly dose for 10 week	4, 6, 8	Safety and efficacy of VES
GS-US-420-5372/ NA	Healthy participants	Phase 1 randomized, placebo-controlled, staggered, multiple-ascending–dose study	4 (3/1)	Single dose	8	Safety, tolerability, and PK of EVM in combination with VES

ARV antiretroviral therapy, *EVM* elipovimab, *HBV* hepatitis B virus, *PD* pharmacodynamic, *PK* pharmacokinetic, *TDF* tenofovir disoproxil fumarate, *VES* vesatolimod.

TABLE S2 Vesatolimod dose levels by study

Study	Placebo (N=101)	0.3 mg (N=27)	1 mg (N=136)	2 mg (N=139)	4 mg (N=140)	6 mg (N=24)	8 mg (N=34)	10 mg (N=6)	12 mg (N=12)	All VES (N=505)
GS-US-243-0101	20 (19.8%)	6 (22.2%)	6 (4.4%)	6 (4.3%)	6 (4.3%)	6 (25.0%)	19 (55.9%)	0	6 (50.0%)	55 (10.0%)
GS-US-283-0102	8 (7.9%)	10 (37.0%)	11 (8.1%)	12 (8.6%)	10 (7.1%)	0	0	0	0	43 (8.5%)
GS-US-283-0106	8 (7.9%)	11 (40.7%)	10 (7.4%)	10 (7.2%)	10 (7.1%)	0	0	0	0	41 (8.1%)
GS-US-283-1059	16 (15.8%)	0	50 (36.8%)	49 (35.3%)	47 (33.6%)	0	0	0	0	146 (28.9%)
GS-US-283-1062	28 (27.7%)	0	53 (39.0%)	56 (40.3%)	55 (39.3%)	0	0	0	0	164 (32.5%)
GS-US-382-1450	12 (11.9%)	0	6 (4.4%)	6 (4.3%)	6 (4.3%)	6 (25.0%)	6 (17.6%)	6 (100.0%)	6 (50.0%)	36 (7.1%)
GS-US-382-3961	8 (7.9%)	0	0	0	6 (4.3%)	12 (50.0%)	6 (17.6%)	0	0	17 (3.4%)
GS-US-420-5372	1 (1.0%)	0	0	0	0	0	3 (8.8%)	0	0	3 (0.6%)

Data are n (%).
VES vesatolimod.

TABLE S3 No. of doses administered by study; includes vesatolimod and placebo

Study	VES dose number												
	1 (N=606)	2 (N=472)	3 (N=422)	4 (N=420)	5 (N=369)	6 (N=366)	7 (N=341)	8 (N=340)	9 (N=287)	10 (N=281)	11 (N=234)	12 (N=228)	13 (N=2)
GS-US-243-0101	75 (12.4%)	0	0	0	0	0	0	0	0	0	0	0	0
GS-US-283-0102	51 (8.4%)	24 (5.1%)	0	0	0	0	0	0	0	0	0	0	0
GS-US-283-0106	49 (8.1%)	24 (5.1%)	0	0	0	0	0	0	0	0	0	0	0
GS-US-283-1059	162 (26.7%)	160 (33.9%)	160 (37.9%)	159 (37.9%)	108 (29.3%)	108 (29.5%)	107 (31.4%)	107 (31.5%)	54 (18.8%)	51 (18.1%)	51 (21.8%)	49 (21.5%)	0
GS-US-283-1062	192 (31.7%)	192 (40.7%)	191 (45.3%)	190 (45.2%)	190 (51.5%)	188 (51.4%)	187 (54.8%)	186 (54.7%)	186 (64.8%)	184 (65.5%)	183 (78.2%)	179 (78.5%)	2 (100%)
GS-US-382-1450	48 (7.9%)	48 (10.2%)	47 (11.1%)	47 (11.2%)	47 (12.7%)	47 (12.8%)	24 (7.0%)	24 (7.1%)	24 (8.4%)	23 (8.2%)	0	0	0
GS-US-382-3961	25 (4.1%)	24 (5.1%)	24 (5.7%)	24 (5.7%)	24 (6.5%)	23 (6.3%)	23 (6.7%)	23 (6.8%)	23 (8.0%)	23 (8.2%)	0	0	0
GS-US-420-5372	4 (0.7%)	0	0	0	0	0	0	0	0	0	0	0	0

Data are n (%).
VES vesatolimod.

TABLE S4 Gilead grading scale for severity of adverse events

	Grade 1	Grade 2	Grade 3	Grade 4
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
Nausea	Transient (<24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24–48 hours	Persistent nausea resulting in minimal oral intake for >48 hours OR aggressive rehydration indicated (eg, IV fluids)	Life-threatening consequences (eg, hypotensive shock)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR aggressive rehydration indicated	Life-threatening consequences (eg, hypotensive shock)
Headache	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	Symptoms causing inability to perform basic self-care functions OR hospitalization indicated (other than ER visit) OR headache with significant impairment of alertness or other neurologic function
Arthralgia	Joint pain causing no or minimal interference with usual social and functional activities	Joint pain causing greater than minimal interference with usual social and functional activities	Joint pain causing inability to perform usual social and functional activities	Disabling joint pain causing inability to perform basic self-care functions
Myalgia	Muscle pain causing no or minimal interference with usual social and functional activities	Muscle pain causing greater than minimal interference with usual social and functional activities	Muscle pain causing inability to perform usual social and functional activities	Disabling muscle pain causing inability to perform basic self-care functions
Chills	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	NA
Fatigue Malaise	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	Incapacitating fatigue/malaise symptoms causing inability to perform basic self-care functions
Pyrexia	37.7°C to 38.6°C 99.8°F to 101.5°F	38.7°C to 39.3°C 101.6°F to 102.8°F	39.4°C to 40.5°C 102.9°F to 104.9°F	>40.5°C >104.9°F
Infection (any other than HIV)	Localized, no systemic antimicrobial treatment indicated AND symptoms causing no or minimal interference with usual social and functional activities	Systemic antimicrobial treatment indicated OR symptoms causing greater than minimal interference with usual social and functional activities	Systemic antimicrobial treatment indicated AND symptoms causing inability to perform usual social and functional activities OR operative intervention (other than simple incision and drainage) indicated	Life-threatening consequences (eg, septic shock)

ER emergency room, *IV* intravenous.

TABLE S5 MedDRA preferred terms selected for analysis of influenza-like adverse events and influenza-like adverse events of interest

Flu-like AEs (all grades)	Flu-like AEI
Cytokine release syndrome	Cytokine release syndrome, any grade
Hemophagocytic lymphohistiocytosis	
Cytokine storm	
Capillary leak syndrome	
Pyrexia	Pyrexia, any grade
Chills	Chills, any grade
Headache	Headache, grade ≥2
Fatigue	Fatigue, grade ≥2
Cancer fatigue	
Nausea	
Vomiting	
Systemic inflammatory response syndrome	
Hypotension	
Hypoxia	
Influenza-like illness	Influenza-like illness, any grade
Myalgia	Myalgia, grade ≥2
Influenza	Influenza, any grade
Malaise	Malaise, grade ≥2

AE adverse event, *flu-like AEIs* influenza-like adverse events of interest, *VES* vesatolimod.

TABLE S6 Biomarker assays

Study	Biomarker	Assay	Vendor
GS-US-283-1059	IFNα	Ultrasensitive ELISA	Aushon BioSystems, Billerica, Massachusetts, USA
	IP-10, ITAC, IFNγ, IL-6	Multiplex ELISA	
GS-US-283-1062	IFNα	Ultrasensitive ELISA	Aushon BioSystems
	IP-10, ITAC	Multiplex ELISA	
	$CD69^+$ NK	Flow cytometry (whole blood)	LabCorp, Seattle, Washington, USA
GS-US-382-1450	IFNα	Ultrasensitive Ciraplex cytokine assay	Aushon BioSystems
	IP-10, ITAC, IFNγ, IL-6	Ciraplex cytokine assay	
	CRP	ELISA	University of Pittsburgh, Pittsburgh, Pennsylvania, USA
	$CD69^+$ NK	Flow cytometry assay (whole blood)	LabCorp
GS-US-382-3961	IFNα	Ultrasensitive Ciraplex cytokine assay	Aushon BioSystems
	IP-10, ITAC, IFNγ, IL-6	Ciraplex cytokine assay	
	CRP	ELISA	University of Pittsburgh
GS-US-420-5372	IFNα	Single molecule array (SIMOA) assay	Rules-Based Medicine, Austin, Texas, USA
	IP-10, ITAC, IFNγ, IL-6	Multiplex ELISA	
	$CD69^+$ NK	Flow cytometry assay (whole blood)	LabCorp

Pharmacodynamic data were obtained from all eight studies included in the pooled analysis; however, details on the biomarker assays were only available for five of the eight studies. Serum concentrations of IFNα, ITAC, IP-10, IL-6, IFNγ, and TNFα were quantified using high-sensitivity Ciraplex assays at Aushon BioSystems (GS-US-283-1059, GS-US-283-1062, GS-US-382-1450, and GS-US-382-3961) or Rules-Based Medicine (GS-US-420-5372). An ultrasensitive assay was used to evaluate serum IFNα and IL-1RA concentrations in GS-US-283-1059, GS-US-283-1062, GS-US-382-1450, and GS-US-382-3961 (Aushon) and IFNα concentrations in GS-US-420-5372 (Rules-Based Medicine). Serum CRP concentrations were quantified using ELISA at the University of Pittsburgh (PA, USA) for GS-US-382-1450 and GS-US-382-3961. Additionally, whole blood cell immunophenotyping was analyzed using flow cytometry to evaluate the frequency of activated $CD69^+$ NK cells (LabCorp Drug Development, Indianapolis, Indiana, USA) for GS-US-382-1450, GS-US-283-1062, and GS-US-420-5372.

CRP C-reactive protein, *IFNα* interferon-α, *IL-1RA* interleukin-1 receptor antagonist, *IP-10* interferon-γ-induced protein 10kDa, *ITAC* interferon-inducible T-cell-α chemoattractant, *NK* natural killer.

TABLE S7 Participant demographics

	Healthy volunteers		People with HIV		People with HBV				
	243-0101	420-5372	382-1450	382-3961	283-0102	283-0106	283-1059	283-1062	Total
Age									
N	75	4	48	25	51	49	162	192	606
Mean (SD)	29.8 (8.51)	40.5 (4.20)	45.6 (10.91)	46.5 (11.16)	44.4 (10.39)	38.9 (11.19)	47.9 (9.70)	42.3 (10.14)	42.6 (11.41)
Median	27.0	40.5	47.0	45.0	43.0	37.0	49.5	42.0	43.0
Q1, Q3	23.0, 35.0	37.0, 44.0	39.0, 54.0	37.0, 53.0	37.0, 53.0	30.0, 48.0	41.0, 55.0	35.0, 49.0	34.0, 51.0
Min, max	18.0, 51.0	36.0, 45.0	23.0, 66.0	27.0, 66.0	23.0, 65.0	19.0, 61.0	25.0, 65.0	20.0, 65.0	18.0, 66.0
Sex									
Male	55 (73.3%)	3 (75.0%)	43 (89.6%)	21 (84.0%)	40 (78.4%)	34 (69.4%)	123 (75.9%)	123 (64.1%)	442 (72.9%)
Female	20 (26.7%)	1 (25.0%)	5 (10.4%)	4 (16.0%)	11 (21.6%)	15 (30.6%)	39 (24.1%)	69 (35.9%)	164 (27.1%)
Race									
Asian or Native Hawaiian	0	0	0	0	33 (64.7%)	23 (46.9%)	120 (74.1%)	157 (81.8%)	333 (55.0%)
Black or African American	16 (21.3%)	0	2 (4.2%)	8 (32.0%)	4 (7.8%)	15 (30.6%)	4 (2.5%)	9 (4.7%)	58 (9.6%)
White	58 (77.3%)	4 (100.0%)	46 (95.8%)	17 (68.0%)	12 (23.5%)	6 (12.2%)	37 (22.8%)	25 (13.0%)	205 (33.8%)
Other	1 (1.3%)	0	0	0	2 (3.9%)	5 (10.2%)	1 (0.6%)	1 (0.5%)	10 (1.7%)

HBV hepatitis B virus, *Q* quartile, *SD* standard deviation.

TABLE S8 Summary of influenza-like adverse events; all grades/fasted

Participants, n (%)	Placebo n=101	0.3 mg n=27	1 mg n=136	2 mg n=139	4 mg n=140	6 mg n=24	8 mg n=21	10/12 mg n=12	All VES n=492
Any flu-like AE[a]	31 (31%)	5 (19%)	51 (38%)	46 (33%)	62 (44%)	9 (38%)	13 (62%)	9 (75%)	194 (39%)
Headache	21 (21%)	1 (4%)	23 (17%)	27 (19%)	27 (19%)	2 (8%)	6 (29%)	5 (42%)	91 (19%)
Fatigue	14 (14%)	2 (7%)	20 (15%)	14 (10%)	23 (16%)	3 (13%)	0	3 (25%)	65 (13%)
Pyrexia	3 (3%)	1 (4%)	9 (7%)	8 (6%)	18 (13%)	1 (4%)	3 (14%)	6 (50%)	46 (9%)
Myalgia	5 (5%)	3 (11%)	9 (7%)	7 (5%)	14 (10%)	2 (8%)	4 (19%)	5 (42%)	44 (9%)
Chills	2 (2%)	0	3 (2%)	9 (7%)	13 (9%)	0	4 (19%)	6 (50%)	35 (7%)
Nausea	5 (5%)	0	12 (9%)	7 (5%)	8 (6%)	2 (8%)	3 (14%)	2 (17%)	34 (7%)
Arthralgia	1 (1%)	0	2 (2%)	2 (1%)	12 (9%)	3 (13%)	0	0	19 (4%)
Influenza-like illness	0	0	7 (5%)	1 (1%)	9 (6%)	0	0	0	17 (3%)
Vomiting	1 (1%)	0	3 (2%)	0	6 (4%)	0	1 (5%)	0	10 (2%)
Malaise	1 (1%)	0	0	0	5 (4%)	0	1 (5%)	0	6 (1%)
Influenza	0	0	2 (2%)	0	1 (1%)	0	0	0	3 (1%)
Cytokine release syndrome	0	0	0	0	0	0	1 (5%)	0	1 (<1%)
Hypotension	1 (1%)	0	0	0	1 (1%)	0	0	0	1 (<1%)

Fasted state excluded participants who received VES with a high-fat meal, moderate-fat meal, or 4 h after a high-fat meal. Six participants received placebo with a meal. Eighteen participants were treated with VES 8 mg immediately after a high-fat or moderate-fat meal, or 4 h after a high-fat meal (n=6 per group). Among those, five participants crossed over from the fasted group to the high-fat meal group after a 1-month washout. Four participants in GS-US-382-3961 crossed over from VES 4 mg to 6 mg and three participants in GS-US-382-3961 crossed over from VES 6 mg to 8 mg after a washout. Multiple AEs were counted only once per participant for each preferred term.

AE adverse event, *VES* vesatolimod.

[a]There were no reported AEs of hemophagocytic lymphohistiocytosis, cytokine storm, capillary leak syndrome, cancer fatigue, hypoxia, or systemic inflammatory response syndrome.

TABLE S9 Baseline ITAC, IFNα, IL-6, and TNFα are predictive biomarkers for influenza-like adverse events of interest after first vesatolimod dose

Effect	Treatment scale	Estimate	SE	DF	t value	Pr > |t|
CRP	Linear	0.476	0.347	66	1.37	0.1746
	Ordinal	0.850	0.563	66	1.51	0.1355
	Grouped	2.497	2.179	66	1.15	0.2559
CXCL10 (IP10)	Linear	–0.017	0.140	422	–0.12	0.9009
	Ordinal	–0.024	0.244	422	–0.10	0.9215
	Grouped	0.797	1.111	422	0.72	0.4737
CXCL11 (ITAC)	Linear	–0.225	0.137	473	–1.64	0.1008
	Ordinal	–0.544	0.239	473	–2.27	0.0236
	Grouped	–2.375	1.082	413	–2.20	0.0286
IFNα	Linear	–0.038	0.236	473	–0.16	0.8715
	Ordinal	–0.467	0.317	473	–1.47	0.1419
	Grouped	–3.403	1.215	473	–2.80	0.0053
IFNγ	Linear	–0.154	0.145	281	–1.06	0.2892
	Ordinal	–0.258	0.249	281	–1.04	0.3009
	Grouped	–0.621	1.358	281	–0.46	0.6479
IL-1RA	Linear	0.074	0.236	120	0.31	0.7535
	Ordinal	0.302	0.435	120	0.69	0.4884
	Grouped	–0.183	1.446	120	–0.13	0.8996
IL-6	Linear	–0.295	0.165	281	–1.79	0.0742
	Ordinal	–0.484	0.267	281	–1.81	0.0708
	Grouped	–3.054	1.225	281	–2.49	0.0132
NKCD69	Linear	1.532	0.983	94	1.56	0.1223
	Ordinal	2.306	1.454	94	1.59	0.1161
	Grouped	2.555	3.389	94	0.75	0.4527
	Linear	–0.158	0.144	277	–1.10	0.2718

TNF_{α}	Ordinal	–0.440	0.253	277	–1.74	0.0826
	Grouped	–4.006	1.462	277	–2.74	0.0066

Generalized linear mixed-effect model for probability of having flu-like AE at first visit after VES administration. Grouped by treatment on linear scale, ordinal scale or on scale with VES doses combined. All predictive biomarkers/excluded patients with food effect. Only includes flu-like adverse events of interest. Treatment (linear): 0 = placebo, 0.3 = VES 0.3 mg. 1 = VES 1 mg, 2 = VES 2 mg, 4 = VES 4 mg, 6 = VES 6 mg, 8 = VES 8 mg, 10 = VES 10 mg, 12 = VES 12 mg. Treatment (ordinal), 1 = placebo, 2 = VES 0.3 mg, 3 = VES 1 mg, 4 = VES 2 mg, 5 = VES 4 mg, 6 = VES 6 mg, 7 = VES 8 mg, 8 = VES 10 mg, 9 = VES 12 mg. Treatment (grouped); 0 = placebo, 1 = VES 0.3 mg, 1 = VES 1 mg, 1 = VES 2 mg, 1 = VES 4 mg, 1 = VES 6 mg, 1 = VES 8 mg, 2 = VES 10 mg, 2 = VES 12 mg. Biomarker and treatment interaction effects in generalized linear mixed-effect model with chi-square *P* value < .05, suggesting potential predictive biomarkers.

CRP C-reactive protein, *flu-like AEIs* influenza-like adverse events of interest, *IFNα* interferon-α, *IL-1RA* interleukin-1 receptor antagonist, *IP-10* interferon-γ-induced protein 10kDa, *NK* natural killer, *TNF α* tumor necrosis factor-alpha, *VES* vesatolimod.

TABLE S10 Baseline ITAC, IFNα, IL-6, TNFα, and IFNγ are prognostic biomarkers for influenza-like adverse events of interest after first vesatolimod dose

Effect	Treatment scale	Estimate	SE	DF	t value	Pr > |t|
CRP	Linear	–2.402	1.962	66	–1.22	0.2253
	Ordinal	–4.315	3.144	66	–1.37	0.1745
	Grouped	–1.840	1.849	66	–1.00	0.3231
CXCL10 (IP10)	Linear	1.047	0.741	422	1.41	0.1583
	Ordinal	1.110	1.274	422	0.87	0.3840
	Grouped	0.309	1.200	422	0.26	0.7968
CXCL11 (ITAC)	Linear	1.426	0.660	473	2.16	0.0312
	Ordinal	3.151	1.233	473	2.56	0.0109
	Grouped	2.904	1.173	473	2.48	0.0137
IFNα	Linear	0.573	1.000	473	0.57	0.5667
	Ordinal	2.459	1.443	473	1.70	0.0890
	Grouped	3.469	1.130	473	3.07	0.0023
IFNγ	Linear	1.755	0.844	281	2.08	0.0385
	Ordinal	2.441	1.370	281	1.78	0.0760
	Grouped	1.886	1.457	281	1.29	0.1966
IL-1RA	Linear	–1.463	1.682	120	–0.87	0.3860
	Ordinal	–2.848	2.779	120	–1.02	0.3075
	Grouped	–0.738	1.584	120	–0.47	0.6422
IL-6	Linear	1.070	0.762	281	1.40	0.1614
	Ordinal	2.262	1.293	281	1.75	0.0814
	Grouped	3.069	1.255	281	2.45	0.0151
NKCD69	Linear	–6.122	4.611	94	–1.33	0.1875
	Ordinal	–11.608	8.389	94	–1.38	0.1697
	Grouped	–2.384	3.371	94	–0.71	0.4812
TNF_{α}	Linear	0.970	0.831	277	1.17	0.2443
	Ordinal	2.450	1.380	277	1.78	0.0769

	Grouped	4.531	1.623	277	2.79	0.0056

Generalized linear mixed-effect model for probability of having flu-like AE at first visit after VES administration. Grouped by treatment on linear scale, ordinal scale or on scale with VES doses combined. All predictive biomarkers/excluded patients with food effect. Only includes flu-like AEIs. Treatment (linear): 0 = placebo, 0.3 = VES 0.3 mg. 1 = VES 1 mg, 2 = VES 2 mg, 4 = VES 4 mg, 6 = VES 6 mg, 8 = VES 8 mg, 10 = VES 10 mg, 12 = VES 12 mg. Treatment (ordinal), 1 = placebo, 2 = VES 0.3 mg, 3 = VES 1 mg, 4 = VES 2 mg, 5 = VES 4 mg, 6 = VES 6 mg, 7 = VES 8 mg, 8 = VES 10 mg, 9 = VES 12 mg. Treatment (grouped); 0 = placebo, 1 = VES 0.3 mg, 1 = VES 1 mg, 1 = VES 2 mg, 1 = VES 4 mg, 1 = VES 6 mg, 1 = VES 8 mg, 2 = VES 10 mg, 2 = VES 12 mg. Biomarker and treatment interaction effects in generalized linear mixed-effect model with chi square *P* value < .05, suggesting potential predictive biomarkers. *CRP* C-reactive protein, *flu-like AEIs* influenza-like adverse events of interest, *IFNα* interferon-α, *IL-1RA* interleukin-1 receptor antagonist, *IP-10* interferon-γ-induced protein 10kDa, *ITAC* interferon-inducible T-cell-α chemoattractant, *NK* natural killer, *TNF α* tumor necrosis factor-alpha, *VES* vesatolimod.

TABLE S11 Demographic summary according to rs179008 (TLR7 SNP) genotype

	Genotype for participants with flu-like AEIs			Genotype for all participants from 8 studies		
	A/A	A/T	T/T	A/A	A/T	T/T
Age						
N	81	5	2	393	11	18
Mean (SD)	45.9 (10.61)	47.6 (6.77)	52.5 (0.71)	45 (10.43)	45 (9.19)	43 (9.13)
Median	45.0	49.0	52.5	45.0	44.0	44.0
Q1, Q3	39.0, 56.0	41.0, 50.0	52.0, 53.0	37.0, 53.0	36.0, 50.0	37.0. 51.0
Min, max	21.0, 65.0	41.0, 57.0	52.0, 53.0	20.0, 66.0	34.0, 63.0	27.0, 56.0
Sex						
Female	27 (30.7%)	5 (5.7%)	0	103 (24.4%)	11 (2.6%)	0
Male	54 (61.4%)	0	2 (2.3%)	290 (68.7%)	0	18 (4.3%)
Race						
Asian	46 (52.3%)	0	0	265 (62.8%)	0	0
Black or African American	3 (3.4%)	1 (1.1%)	0	18 (4.3%)	1 (0.2%)	2 (0.5%)
Native Hawaiian or other Pacific Islander	0	0	0	9 (2.1%)	0	0
White	32 (36.4%)	4 (4.5%)	2 (2.3%)	100 (23.7%)	10 (2.4%)	16 (3.8%)
Other	0	0	0	1 (0.2%)	0	0

Flu-like AEIs influenza-like adverse events of interest, *Q* quartile, *SD* standard deviation, *SNP* single-nucleotide polymorphism, *TLR7* Toll-like receptor 7 agonist.

TABLE S12 Demographic summary according to rs3853839 (TLR7 SNP) genotype

	Genotype for participants with flu-like AEIs			Genotype for all participants from 8 studies		
	C/C	C/G	G/G	C/C	C/G	G/G
Age						
N	38	7	43	158	40	224
Mean (SD)	46.8 (10.76)	47.9 (7.31)	45.4 (10.49)	45.5 (10.90)	45.6 (8.90)	44.4 (10.17)
Median	47.0	47.0	44.0	45.0	45.5	44.0
Q1, Q3	40.0, 56.0	43.0, 52.0	39.0, 55.0	37.0, 54.0	39.0, 52.0	36.5, 52.0
Min, max	28.0, 65.0	38.0, 61.0	21.0, 61.0	25.0, 66.0	23.0, 64.0	20.0, 65.0
Sex						
Female	13 (14.8%)	7 (8.0%)	12 (13.6%)	24 (5.7%)	40 (9.5%)	50 (11.8%)
Male	25 (28.4%)	0	31 (35.2%)	134 (31.8%)	0	174 (41.2%)
Race						
Asian	9 (10.2%)	5 (5.7%)	32 (36.4%)	46 (10.9%)	32 (7.6%)	187 (44.3%)
Black or African American	4 (4.5%)	0	0	19 (4.5%)	1 (0.2%)	1 (0.2%)
Native Hawaiian or other Pacific Islander	0	0	0	2 (0.5%)	0	7 (1.7%)
White	25 (28.4%)	2 (2.3%)	11 (12.5%)	91 (21.6%)	6 (1.4%)	29 (6.9%)
Other	0	0	0	0	1 (0.2%)	0

Flu-like AEIs influenza-like adverse events of interest, *Q* quartile, *SD* standard deviation, *SNP* single-nucleotide polymorphism, *TLR7* Toll-like receptor 7 agonist.

FIG S1 Vesatolimod dose levels in participants with and without influenza-like adverse events of interest. Wilcoxon rank-sum test was used to compare the first VES dose level that was given to participants with versus without flu-like AEI. Median and IQR are shown for the first VES dose level.
Flu-like AEIs influenza-like adverse events of interest, *IQR* interquartile range, *VES* vesatolimod.

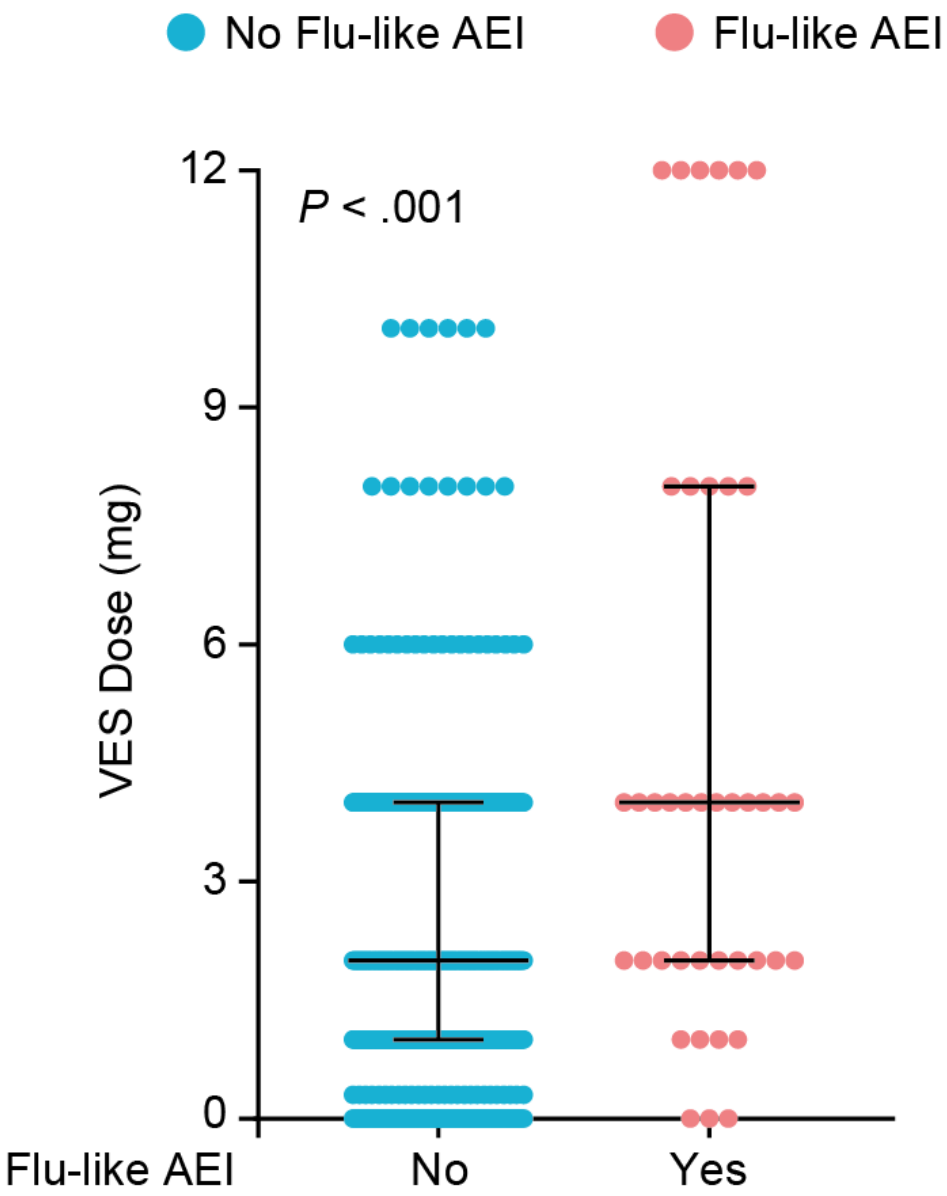

FIG S2 Changes in markers of inflammation associated with CRS in participants with and without influenza-like adverse events of interest 24 hours postdose.
Fold changes from baseline 24 hours after the first dose for inflammatory biomarkers from all eight VES studies were summarized by VES dose (including all doses with data collected). Note: for participants receiving VES, only those with fasted or empty stomach were included for IL-6 and IFNγ. Median, distribution, and IQR are indicated in error bars. Participants presented flu-like AEI are highlighted in red.
CRS cytokine release syndrome, *flu-like AEIs* influenza-like adverse events of interest, *IQR* interquartile range, *VES* vesatolimod.

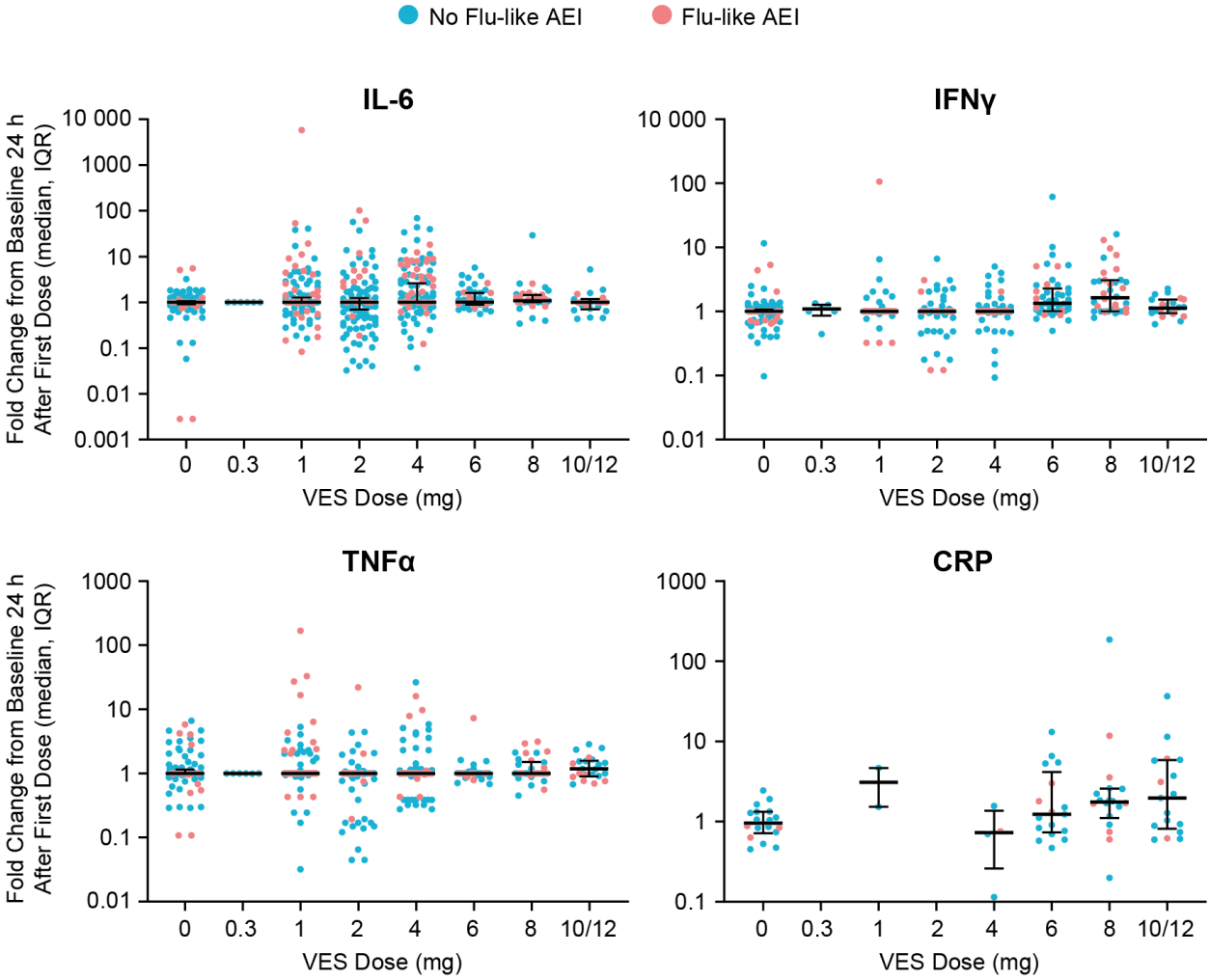

FIG S3 Significant differences in pharmacodynamic and inflammatory biomarkers between participants with and without influenza-like adverse events of interest 24 hours after first dose.
Fold changes from baseline 24 hours after first dose are shown for PD biomarkers (IFNα, ITAC, IL-1RA, and IP-10 concentrations) and inflammatory biomarkers (IL-6 concentration and incidence of $CD69^+$ NK cells) in participants with (red color) and without flu-like AEI (blue color) (includes participants who received VES or placebo). Wilcoxon rank-sum test was used to compare fold changes in each biomarker between participants with and without flu-like AEI.
Flu-like AEIs influenza-like adverse events of interest, *IQR* interquartile range, *PD* pharmacodynamic, *VES* vesatolimod.

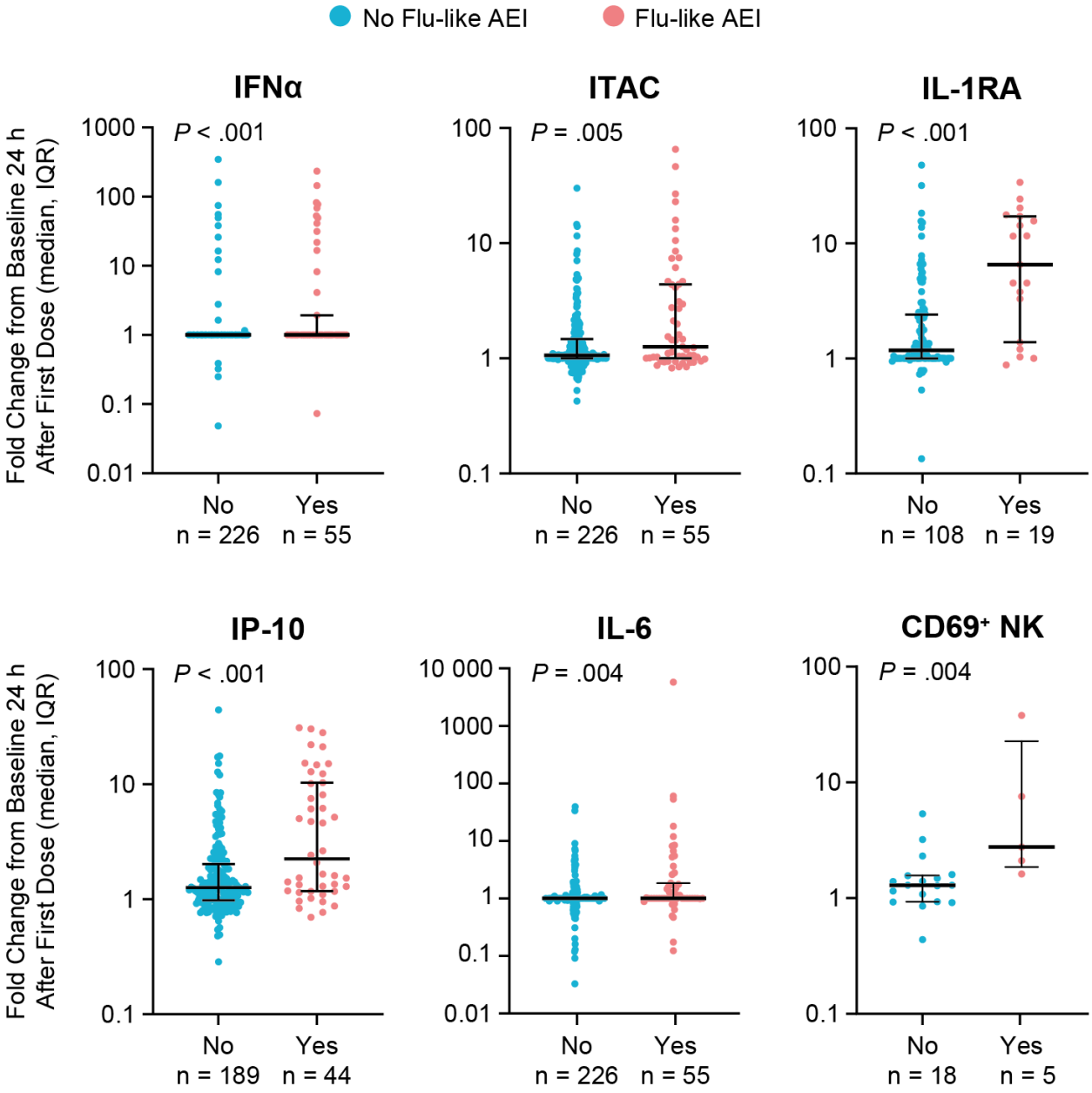

FIG S4 No significant differences in IFNγ, TNFα, CRP, ISG15, MX1, and OAS1 between participants with and without influenza-like adverse events of interest 24 hours after first dose.
Wilcoxon rank-sum test was used to compare fold changes of each biomarker between participants with (red) and without flu-like AEI (blue) (includes participants who received VES or placebo).
BL baseline, *flu-like AEIs* influenza-like adverse events of interest, *IQR* interquartile range, *VES* vesatolimod.