SUPPLEMENTARY MATERIAL

A first-in-human phase 1 study of the PD-1 inhibitor, retifanlimab (INCMGA00012), in patients with advanced solid tumors (POD1UM-101)

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SUPPLEMENTARY METHODS

Patients

Prior systemic therapy for non-small cell lung cancer (squamous cell carcinoma or adenocarcinoma) must have included at least a platinum-based regimen, and patients with known targetable aberrations (eg, *EGFR* mutation, *ALK* fusion, or *ROS1* rearrangement) should have received all approved therapy known to confer clinical benefit prior to enrollment. Enrollment in the sarcoma cohort was restricted to undifferentiated pleomorphic sarcoma, including malignant fibrous histiocytoma, de-differentiated or poorly differentiated liposarcoma, synovial sarcoma, or rhabdomyosarcoma.

Study endpoints and assessments

Maximum serum drug concentration, trough concentration, and time to maximum serum drug concentration values were directly derived from observed serum concentration data; area under the concentration-time curve was calculated using the linear trapezoidal rule for increasing concentrations and the log-trapezoidal rule for decreasing concentrations; clearance was calculated as dose/area under the concentration-time curve from time 0 to infinity; and terminal half-life was calculated as $\log(2)/\lambda_z$.

Pharmacokinetic and pharmacodynamic assessments

Retifanlimab serum concentration was measured by an electrochemiluminescence (ECL) immunoassay using ECL-MSD (Meso Scale Diagnostics, Rockville, Maryland, USA), and analyzed by standard noncompartmental pharmacokinetic methods using Phoenix WinNonlin v8.0 (Certara USA Inc, Princeton, New Jersey, USA). Serum concentration data were summarized by treatment cohort and planned pharmacokinetic timepoints (Supplementary methods).

Anti-retifanlimab antibodies were measured using a bridging enzyme-linked immunosorbent assay (Syneos Health, Raleigh, North Carolina, USA). PD-L1 expression was determined centrally and retrospectively on formalin-fixed, paraffin-embedded baseline tumor biopsy specimens (archival, fresh, or a combination) where available by immunohistochemistry (IHC) using the PD-L1 IHC 22C3 pharmDx (Agilent, Carpinteria, California, USA).

RNA was extracted from formalin-fixed, paraffin-embedded baseline tumor biopsy specimens and assessed for an inflamed gene signature on the NanoString nCounter platform (NanoString Technologies Inc., Seattle, Washington, USA). Direct digital detection of a custom panel of 680 tumor-related and immune-related mRNA molecules of interest was performed using target-specific, color-coded probe pairs.

T-cell proliferation in peripheral blood mononuclear cells was measured using fluorescence-activated cell sorting. Whole blood samples were collected at selected time points following retifanlimab treatment, and immune cell phenotyping was performed using antibodies for CD45, CD3, and Ki67. Single cells in fluorescence-activated cell sorting analyses were successively gated on $CD45^+$ cells (leukocytes), $CD3^+$ cells (T cells), $CD4^+$, $CD8^+$, $CD25^+$, $FoxP3^+$, and $Ki67^+$ (proliferating) cells.

SUPPLEMENTARY TABLES

Supplementary Table 1 Definition of dose-limiting toxicity (first 4-week cycle of retifanlimab administration)

Hematologic toxicity • Grade 4 neutropenia lasting >5 days • Grade ≥3 febrile neutropenia lasting >48 hours, or associated with hemodynamic compromise or objective evidence of infection • Grade 4 thrombocytopenia (irrespective of duration) • Grade 3 thrombocytopenia associated with clinically significant bleeding • Grade ≥3 hemolysis • The following events are EXCLUDED from definition of hematologic dose-limiting toxicity: – Grade ≥3 lymphopenia – Grade 3 anemia not associated with other clinically significant complications
Nonhematologic toxicity • Any grade ≥3 nonhematologic toxicity EXCEPT the following: – Grade 3 • electrolyte abnormality (lasts <72 hours and responds to medical intervention) • fatigue (lasts <7 days) • fever (lasts <72 hours and not associated with hemodynamic compromise) • infusion-related reaction or cytokine release syndrome (lasts <12 hours and responds to medical intervention) • nausea or vomiting (lasts <72 hours and responds to medical intervention) • amylase and/or lipase elevation not associated with either clinical or radiographic evidence suggestive of pancreatitis • gastrointestinal adverse events (resolve to grade <1 within 14 days with medical therapy) • inflammatory reaction attributed to a local antitumor response that resolves to grade ≤2 within 7 days – Grade 3 or 4 endocrinopathy adequately controlled with hormone supplementation

- Adverse events (grade ≥2) that are prolonged inordinately (based upon medical judgment of investigator), and/or lead to permanent discontinuation of retifanlimab due to patient intolerance
- Eye pain or visual acuity reduction (grade ≥2) that does not respond to topical therapy and does not improve to grade 1 within 14 days of topical therapy initiation, or that requires systemic treatment

Hepatic nonhematologic toxicity

• Any elevation of one or more transaminases >8 × institutional upper limit of normal (ULN) irrespective of duration

• Any grade 3 elevation of one or more transaminases >5.0–8.0 × ULN that does not resolve to grade 2 (ie, >3.0–5.0 × ULN) within 7 days and grade 1 (ie, >ULN–3.0 × ULN) within 14 days

• Grade 3 elevation of total bilirubin that is >5 × ULN (irrespective of duration)

• Any grade 3 elevation of total bilirubin >3.0–5.0 × ULN that does not resolve to grade 2 (ie, >1.5–3.0 × ULN) within 7 days and grade 1 (ie, >ULN–1.5 × ULN) within 14 days

• Any event meeting the criteria for Hy's law as follows (all three features):

– Aspartate aminotransferase and/or alanine aminotransferase >3 × ULN

– Concurrent elevation of total bilirubin >2 × ULN without initial evidence of cholestasis

– No alternative etiology can be identified

Supplementary Table 2 Tumor types in patients in the retifanlimab dose-escalation and tumor-specific cohorts

Tumor type	***n* (%)**
Dose escalation cohort	***n*=37**
Endometrial cancer	6 (16.2)
Breast cancer	4 (10.8)
Colorectal cancer	4 (10.8)
Ovarian cancer	4 (10.8)
Sarcoma	4 (10.8)
Cervical cancer	2 (5.4)
Gastric or gastroesophageal junction cancer	2 (5.4)
Pancreatic cancer	2 (5.4)
Bladder cancer	1 (2.7)
Cholangiocarcinoma	1 (2.7)
Esophageal cancer	1 (2.7)
High grade neuroendocrine carcinoma of ascending colon	1 (2.7)
Melanoma	1 (2.7)
Mesothelioma	1 (2.7)
Non-small cell lung cancer	1 (2.7)
Prostate cancer	1 (2.7)
Renal cell cancer	1 (2.7)
NSCLC cohort	***n*=35**
Squamous	18 (51%)
Nonsquamous adenocarcinoma	14 (40%)
Nonsquamous large cell	1 (3%)
Not reported	2 (6%)
Endometrial cancer cohort	***n*=29**
Adenocarcinoma	26 (90%)
Not reported	3 (10%)
Cervical cancer cohort	***n*=35**
Squamous cell carcinoma	23 (66%)
Adenocarcinoma	11 (31%)

Not reported	1 (3%)
Soft tissue sarcoma cohort	***n*=35**
Synovial sarcoma	14 (40%)
Dedifferentiated or poorly differentiated	9 (26%)
Undifferentiated pleomorphic	6 (17%)
Rhabdomyosarcoma	3 (9%)
Not reported	3 (9%)

NSCLC, non-small cell lung cancer.

Supplementary Table 3 Any-grade TRAEs by MedDRA preferred term occurring in ≥2 patients in any retifanlimab dosing cohort (safety evaluable population)

	Retifanlimab dosing				
MedDRA preferred term, n (%)	**Dose escalation (*n*=37)**	**3 mg/kg Q2W (*n*=134)**	**375 mg Q3W (*n*=15)**	**500 mg Q4W (*n*=15)**	**750 mg Q4W (*n*=15)**
Any TRAE	26 (70)	76 (57)	9 (60)	9 (60)	7 (47)
Fatigue	11 (30)	11 (8)	2 (13)	4 (27)	1 (7)
Nausea	5 (13.5)	7 (5)	0	0	2 (13)
Hypothyroidism	4 (11)	11 (8)	0	1 (7)	3 (20)
Pruritus	4 (11)	4 (3)	1 (7)	1 (7)	1 (7)
Tumor flare	4 (11)	1 (1)	2 (13)	0	1 (7)
Influenza-like illness	3 (8)	2 (1.5)	2 (13)	1 (7)	0
Lipase increased	3 (8)	2 (1.5)	1 (7)	0	0
Maculopapular rash	3 (8)	3 (2)	0	0	0
Diarrhea	2 (5)	9 (7)	0	0	0
Hyperthyroidism	2 (5)	8 (6)	0	0	2 (13)
Lymphopenia	2 (5)	2 (1.5)	0	1 (7)	0
Peripheral neuropathy	2 (5)	1 (1)	0	0	0
Rash	2 (5)	8 (6)	0	1 (7)	0
Tumor pain	2 (5)	0	0	2 (13)	0
Asthenia	0	5 (4)	0	0	0
Decreased appetite	0	4 (3)	0	1 (7)	0
Alanine aminotransferase increased	0	5 (4)	0	0	1 (7)

Anemia	0	3 (2)	0	0	1 (7)
Aspartate aminotransferase increased	0	3 (2)	1 (7)	0	1 (7)
Infusion-related reaction	1 (3)	4 (3)	0	0	1 (7)
Arthralgia	0	1 (1)	0	1 (7)	0
Colitis	0	5 (4)	0	0	0
Dry mouth	0	2 (1.5)	1 (7)	1 (7)	0

MedDRA, Medical Dictionary for Regulatory Activities; Q2W, every 2 weeks; Q3W, every 3 weeks; Q4W, every 4 weeks; TRAE, treatment-related adverse event.

Supplementary Table 4 Programmatically-defined irAEs occurring in >2% of patients in any dose-escalation, tumor-specific, or flat-dose cohorts (safety evaluable population)

MedDRA preferred term, *n* (%)	Retifanlimab dose-escalation cohort (*n* = 37)	Tumor-specific cohorts (3 mg/kg Q2W retifanlimab)				Flat-dose retifanlimab cohorts		
		NSCLC (*n* = 35)	Endometrial cancer (*n* = 29)	Cervical cancer (*n* = 35)	Soft tissue sarcoma (*n* = 35)	375 mg Q3W (*n* = 15)	500 mg Q4W (*n* = 15)	750 mg Q4W (*n* = 15)
Any irAE	7 (19)	12 (34)	3 (10)	19 (54)	6 (17)	0	2 (13)	4 (27)
Hypothyroidism	4 (11)	5 (14)	1 (3)	6 (17)	1 (3)	0	1 (7)	3 (20)
Hyperthyroidism	2 (5)	2 (6)	0	4 (11)	4 (11)	0	0	2 (13)
Autoimmune hepatitis	0	1 (3)	0	0	0	0	0	0
Colitis	0	1 (3)	1 (3)	3 (9)	0	0	0	0
Myocarditis	0	1 (3)	0	0	0	0	0	0
Nephritis	0	1 (3)	0	4 (11)	0	0	0	0
Pruritus	0	1 (3)	0	0	0	0	0	0
Rash	1 (3)	1 (3)	0	0	1 (3)	0	0	0
Type 1 diabetes mellitus	0	1 (3)	0	0	0	0	0	0
Polyarthritis	0	0	1 (3)	0	0	0	0	0
Adrenal insufficiency	0	0	0	1 (3)	0	0	0	0
Hepatitis	0	0	0	1 (3)	0	0	0	0
Pneumonitis	0	0	0	1 (3)	0	0	0	1 (7)
Rash erythematous	0	0	0	1 (3)	0	0	0	0

Rash maculopapular	0	0	0	1 (3)	0	0	0	0
Rash pruritic	0	0	0	1 (3)	0	0	0	0
Toxic skin eruption	0	0	0	1 (3)	0	0	0	0
Acute kidney injury	0	0	0	0	1 (3)	0	0	0
Iritis	0	0	0	0	0	0	1 (7)	0
Thyroiditis	1 (3)	0	0	0	0	0	0	1 (7)

irAE, immune-related adverse event; MedDRA, Medical Dictionary for Regulatory Activities; NSCLC, non-small cell lung cancer; Q2W, every 2 weeks; Q3W, every 3 weeks; Q4W, every 4 weeks.

Supplementary Table 5 Summary of retifanlimab pharmacokinetic parameters for body weight–based doses

		First dose							Steady-state	
Dose	***N***	**C_{max}, mg/L**	**T_{max}, h**	**$t_{½}$, day**	**AUC_t, mg/L•day**	**AUC_∞, mg/L•day**	**CL, L/day**	**V_z, L**	**C_{trough}, mg/L**	**$C_{max,ss}$, mg/L**
1 mg/kg Q2W	3	16.5±4.94 (15.9, 34.6)	1.1 (1.0–1.2)	7.97±1.80 (7.8, 23.0)	93.6±21.4 (92, 22.5)	135±43.2 (131, 30.6)	0.442±0.0297 (0.441, 6.6)	5.03±0.857 (4.99, 16.9)	–	–
3 mg/kg Q2W*	127	63.3±20.5 (60.5, 29.9)	1.2 (0.92–24.0)	7.64±2.14 (7.4, 27.2)	372.0±98.4 (359, 28.2)	537±173 (510, 34.2)	0.447±0.149 (0.426, 32.2)	4.70±1.38 (4.53, 27.6)	44.0±18.8 (39.1, 59.8)	106±34.0 (100, 33.6)
3 mg/kg Q4W	9	67.9±13.5 (66.6, 21.4)	1.2 (1.0–7.0)	12.70±4.79 (11.6, 53.1)	555±139 (539, 26.9)	719±218 (685, 34.9)	0.415±0.135 (0.397, 32.0)	7.12±2.61 (6.66, 41.6)	18.1	77.3
10 mg/kg Q2W	7	208±55.2 (201.0, 27.0)	1.1 (1.1–1.3)	9.35±2.27 (9.1, 23.6)	1140±300 (1110, 24.2)	1740±576 (1680, 29.9)	0.453±0.119 (0.437, 30.9)	5.98±1.70 (5.76, 30.6)	–	–
10 mg/kg Q4W	5	225±41.7 (223.0, 17.4)	1.2 (1.0–7.0)	15.60±5.82 (14.8, 37.4)	1920±308 (1900, 17.3)	2640±654 (2570, 25.8)	0.336±0.169 (0.304, 53.9)	7.07±3.36 (6.46, 49.7)	70.6, 51.5	285, 246

Data are mean±standard deviation (geometric mean, CV%), with exception of T_{max} as median (range).

*Steady-state, n=46.

AUC_t, area under the concentration-time curve; AUC_∞, area under the concentration-time curve from time 0 to infinity; CL, clearance; C_{max}, maximum serum drug concentration; $C_{max,ss}$, maximum serum drug concentration at steady-state; C_{trough}, trough concentration; CV, coefficient of variation; Q2W, every 2 weeks; Q4W, every 4 weeks; $t_{½}$, terminal half-life; T_{max}, time to maximum serum drug concentration; V_z, apparent volume of distribution during terminal phase.

Supplementary Table 6 Summary of retifanlimab pharmacokinetic parameters for flat doses

		First dose							Steady-state	
Dose	***N***	**C_{max}, mg/L**	**T_{max}, h**	**$t_{½}$, day**	**AUC_t, mg/L•day**	**AUC_∞, mg/L•day**	**CL, L/day**	**V_z, L**	**C_{trough}, mg/L**	**$C_{max,ss}$, mg/L**
375 mg Q3W*	15	114±32.7	1.2	12.9±3.72	786±238	1170±410	0.366±0.151	6.26±1.41	43.0±15.0	175±32.5
		(110, 30.4)	(1.0–7.0)	(12.4, 28.9)	(752, 31.6)	(1100, 39.2)	(0.341, 39.2)	(6.09, 25.0)	(41.3, 31.5)	(172, 18.7)
500 mg Q4W†	40	168±51.6	1.2	16.1±8.73	1370±437	1940±795	0.302±0.132	6.34±2.49	58.7±26.8	219±65.7
		(159, 35.0)	(1.0–7.3)	(14.7, 42.5)	(1300, 33.8)	(1800, 42.0)	(0.279, 42.0)	(5.90, 40.1)	(52.9, 51.8)	(203, 52.6)
750 mg Q4W‡	13	215±66.5	1.2	17.6±5.21	1830±532	2600±741	0.316±0.115	7.59±2.06	37.5±8.7	264±18.5
		(206, 29.5)	(1.0–22)	(16.9, 31.0)	(1760, 29.3)	(2490, 31.8)	(0.301, 31.8)	(7.35, 27.3)	(36.8, 25.7)	(264, 7.0)

Data are mean±standard deviation (geometric mean, CV%), with exception of T_{max} as median (range).

*Steady-state, n=4.

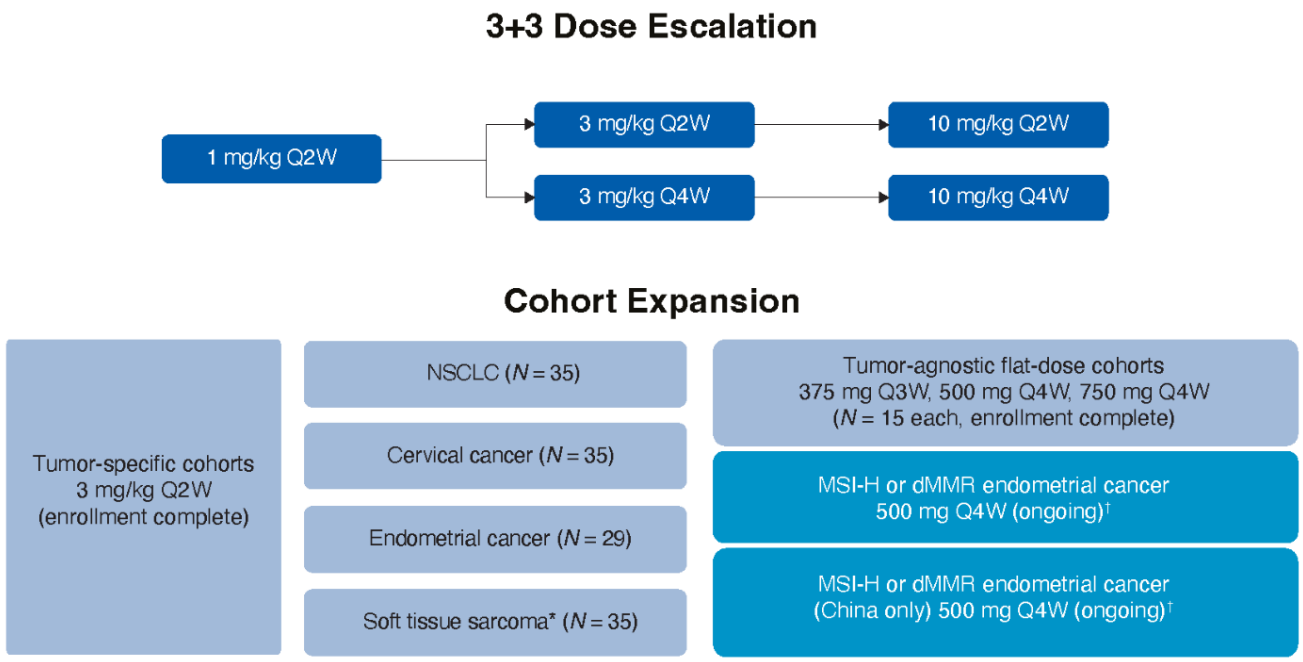
†Steady-state, n=15.

‡Steady-state, n=3.

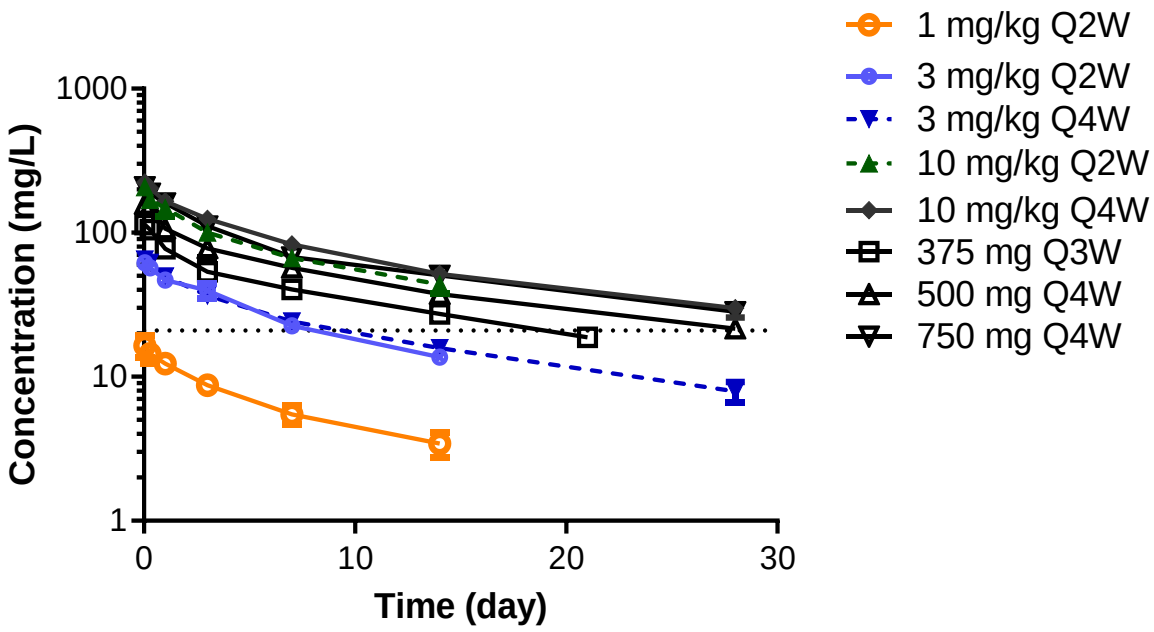
AUC_t, area under the concentration-time curve; AUC_∞, area under the concentration-time curve from time 0 to infinity; CL, clearance; C_{max}, maximum serum drug concentration; $C_{max,ss}$, maximum serum drug concentration at steady-state; C_{trough}, trough concentration; CV, coefficient of variation; Q2W, every 2 weeks; Q4W, every 4 weeks; $t_{½}$, terminal half-life; T_{max}, time to maximum serum drug concentration; V_z, apparent volume of distribution during terminal phase.

SUPPLEMENTARY FIGURES

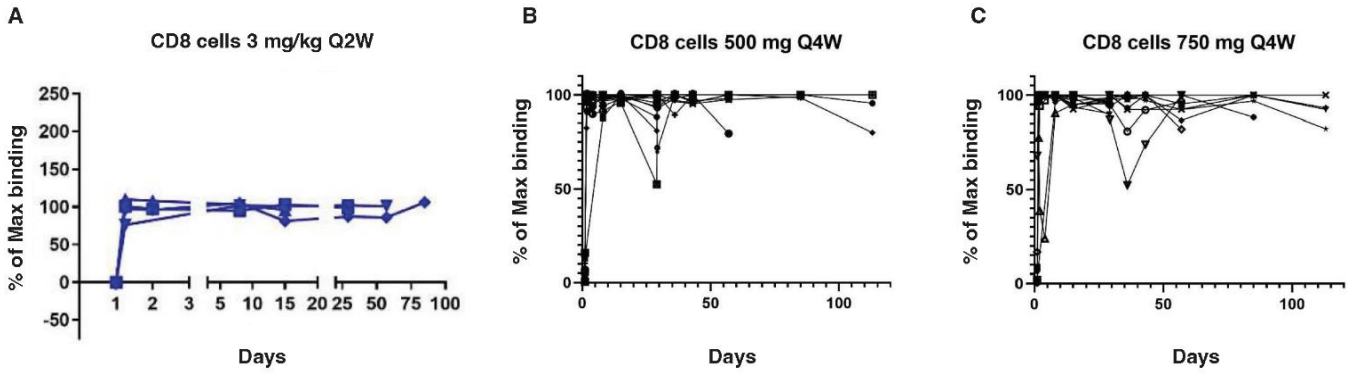
Supplementary Figure 1 Study design. *The following histologies of soft tissue sarcoma were allowed: undifferentiated pleomorphic sarcoma (including malignant fibrous histiocytoma), de-differentiated or poorly differentiated liposarcoma, synovial sarcoma, or rhabdomyosarcoma. †Additional cohorts in deficient mismatch repair endometrial cancer are ongoing to further characterize the safety and clinical activity of 500-mg Q4W flat dosing and will be reported separately. dMMR, deficient mismatch repair; MSI-H, microsatellite instability-high; NSCLC, non-small cell lung cancer; Q2W, every 2 weeks; Q3W, every 3 weeks; Q4W, every 4 weeks.



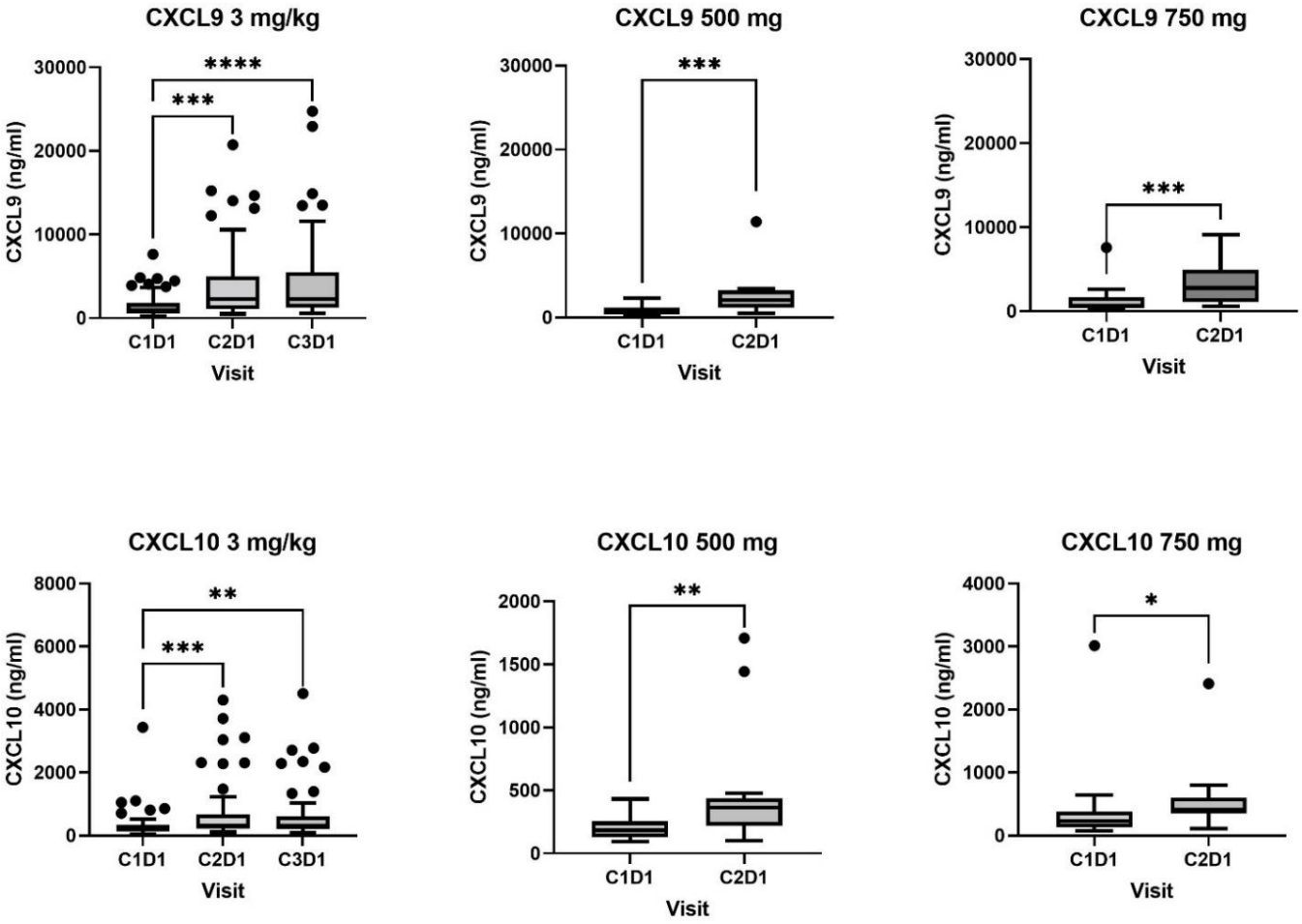
Supplementary Figure 2 Retifanlimab serum concentration-time profile (mean±standard error) after first dose in patients with body weight–based doses and flat doses. Q2W, every 2 weeks; Q3W, every 3 weeks; Q4W, every 4 weeks.



Supplementary Figure 3 Retifanlimab occupancy of programmed death protein-1 (PD-1) receptor on peripheral blood CD8 T cells with weight-based dosing: (A) 3 mg/kg every 2 weeks (Q2W; n=5), and flat dosing (B) 500 mg every 4 weeks (Q4W; n=15) and (C) 750 mg Q4W (n=15). Percentage of maximal retifanlimab binding over time was evaluated by flow cytometry using anti-IgG4Fc antibody.



Supplementary Figure 4 (A) C-X-C motif chemokine ligand 9 (CXCL9) (top panel) and (B) CXCL10 (bottom panel) serum levels in patients with 3-mg/kg every-2-week (Q2W) weight-based dosing, 500-mg every-4-week (Q4W) flat dosing, and 750-mg Q4W flat dosing. Levels of CXCL9 and CXCL10 were measured by immunoassays in serum samples in weight-based dosing (baseline pretreatment cycle 1 day 1 [C1D1], and on-treatment cycle 2 day 1 [C2D1] and cycle 3 day 1 [C3D1]) and flat-dosing cohorts (baseline C1D1 and on-treatment C2D1). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 by analysis of variance mixed-effects analysis (3-mg/kg dosing) or by Wilcoxon matched pairs test (500-mg and 750-mg flat doses) with each time point compared with C1D1.



Supplementary Figure 5 Association of inflamed RNA signature with tumor response following retifanlimab administration. Baseline tumor biopsies from patients enrolled in tumor-specific cohorts (non-small cell lung cancer, endometrial cancer, cervical cancer, soft tissue sarcoma) had RNAseq and gene set enrichment analysis, to derive the enrichment score for inflamed RNA signature. BOR, best objective response; CPR, confirmed partial response; NA, response data not available; PD, progressive disease; PR1, unconfirmed partial response; SD, stable disease.