Supplemental Tables/Figures

Supplemental Table 1. Patient disposition of the treated population

Patient and treatment status, n (%)	**Part 1 GSK3174998 (n=45)**	**Part 2 GSK3174998 + pembrolizumab (n=96)[a]**
Discontinued treatment	45 (100)	96 (100)
Reasons for treatment discontinuation		
Disease progression	40 (89)	76 (79)
Adverse event	1 (2)[c]	3 (3)
Physician decision	3 (7)	7 (7)
Patient withdrawal	1 (2)	4 (4)
Completed maximum 2-year duration	0 (0)	6 (6)
Completed study, n (%)	31 (69)	60 (63)
Withdrawn from study, n (%)	14 (31)	36 (38)
Reasons for study withdrawal, n (%)		
Study terminated by sponsor[b]	1 (2)	19 (20)
Lost to follow-up	1 (2)	0
Physician decision	4 (9)	1 (1)
Patient withdrawal	8 (18)	10 (10)
Investigator site closed	0	6 (6)

[a] Due to rounding, percentages may not add up to 100%.

[b] At the time of study termination, all patients had discontinued treatment and were in study follow-up for disease status and survival.

[c] Not treatment related.

Supplemental Table 2. Plasma pharmacokinetics of GSK3174998 and pembrolizumab following a single infusion.

	Part 1 GSK3174998						**Part 2 GSK3174998**						**Part 2 pembrolizumab**			
Dose in mg/kg	**Mean AUC_{0-t} in day*ug/mL (CV%)**	**C_{max} in µg/mL (CV%)**	**Median t_{max} in days (range)**	**CL in mL/day (CV%)**	**$t_{1/2}$ in day (CV%)**	**VSS in mL (CV%)**	**Mean AUC_{0-t} in day*ug/mL (CV%)**	**C_{max} in µg/mL (CV%)**	**Median t_{max} in days (range)**	**CL in mL/day (CV%)**	**$t_{1/2}$ in day (CV%)**	**VSS in mL (CV%)**	**Mean AUC_{0-t} in day*ug/mL (CV%)**	**C_{max} in µg/mL (CV%)**	**Median t_{max} in days (range)**	**$t_{1/2}$ in day (CV%)**
0.003	-	0.0444 (-)	0.0410 (0.0410, 0.0410)	-	-	-	-	0.0532 (37.3)	0.0458 (0.0285, 0.181)	-	-	-	402 (43.3)	51.8 (48.5)	0.0229 (0.0208, 0.0417)	18.2 (16.3)
0.01	-	0.181 (-)	0.0340 (0.0340, 0.0340)	-	-	-	-	0.251 (23.6)	0.0417 (0.0174, 0.176)	-	-	-	561 (34.2)	41.0 (-)	0.0479 (0.0479, 0.0479)	13.2 (58.5)
0.03	2.94 (56.6)	0.841 (43.1)	0.0424 (0.0403, 0.213)	838 (38.6)	2.49 (29.1)	3040 (31.4)	4.23 (77.9)	0.675 (95.1)	0.0417 (0.0229, 0.188)	251 (218)	3.81 (61.6)	1450 (85.6)	616 (43.7)	79.0 (34.8)	0.0431 (0.0250, 0.0521)	13.8 (35.4)
0.1	11.3 (57.8)	2.81 (35.5)	0.0458 (0.0364, 0.190)	677 (53.2)	2.99 (31.6)	3080 (36.1)	11.6 (42.4)	2.45 (23.4)	0.0438 (0.0417, 0.188)	591 (57.7)	4.02 (41.9)	3410 (25.4)	582 (34.1)	57.0 (52.7)	0.0420 (0.0208, 0.0451)	14.2 (25.3)
0.3	39.4 (17.5)	6.00 (24.0)	0.0493 (0.0417, 0.191)	495 (36.8)	8.45 (57.0)	5210 (36.2)	40.9 (34.2)	6.35 (30.8)	0.0417 (0.0250, 0.188)	507 (29.1)	6.90 (33.2)	4950 (31.3)	528 (33.4)	60.2 (36.2)	0.0417 (0.0188, 0.0479)	14.9 (40.1)

1.0	175 (33.2)	22.7 (26.0)	0.0417 (0.0313, 0.0444)	334 (42.3)	8.91 (16.3)	4400 (31.2)	175 (32.6)	23.8 (18.9)	0.0403 (0.0236, 0.271)	340 (34.3)	11.5 (73.6)	4570 (30.9)	640 (44.8)	72.7 (34.2)	0.0390 (0.0229, 0.0486)	16.8 (33.7)
3.0	631 (23.7)	75.8 (22.9)	0.0458 (0.0368, 0.0528)	253 (16.6)	12.3 (20.4)	4300 (13.1)	605 (29.2)	71.4 (24.1)	0.0493 (0.0347, 0.194)	249 (27.8)	14.7 (29.0)	4630 (23.2)	657 (22.5)	64.7 (22.0)	0.0434 (0.0257, 0.0583)	17.6 (25.5)
10.0	2130 (30.4)	276 (10.7)	0.0497 (0.0486, 0.0576)	213 (19.1)	15.8 (27.8)	3890 (50.6)	1470 (20.3)	191 (16.0)	0.0656 (0.0417, 0.165)	347 (8.3)	10.1 (35.9)	5000 (31.6)	704 (27.1)	63.7 (25.1)	0.0424 (0.0417, 0.0451)	13.0 (58.7)
0.3 Melanoma	-	-	-	-	-	-	38.8 (9.6)	6.92 (19.1)	0.0483 (0.0306, 0.0535)	564 (20.8)	6.20 (45.9)	4980 (42.0)	471 (31.5)	56.0 (19.6)	0.0417 (0.0278, 0.0493)	14.2 (26.4)
0.3 STS	-	-	-	-	-	-	38.2 (43.4)	5.72 (17.4)	0.0521 (0.0243, 0.192)	448 (52.8)	7.65 (51.4)	4800 (29.1)	547 (29.1)	65.3 (21.1)	0.0306 (0.0201, 0.0451)	18.4 (43.7)
0.3 NSCLC	-	-	-	-	-	-	46.5 (21.9)	7.60 (8.7)	0.0208 (0.0208, 0.0972)	492 (11.3)	5.82 (34.2)	3980 (19.7)	487 (-)	64.6 (-)	0.0347 (0.0347, 0.0347)	15.4 (-)

AUC, area under the curve; CL, drug clearance; C_{max}, maximum serum concentration; CV, coefficient of variation; NSCLC, non-small cell lung cancer; STS, soft tissue sarcoma; $t_{1/2}$, half-life; t_{max}, time to maximum plasma concentration; VSS, volume of distribution at steady state.

Supplemental Table 3. Summary of investigator-confirmed best responses.

Best response assessed via irRECIST, n (%)	**Part 1 GSK3174998 (n=45)**	**Part 2 GSK3174998 + pembrolizumab (n=96)**	
		a. Dose Escalation (n=74)	**b. Dose Expansion (n=22)**
ORR (CR + PR)	0	6 (8)	0
DCR (CR + PR + SD≥24 weeks)	4 (9)	21 (28)	3 (14)
DCR (CR + PR + SD≥12 weeks)	8 (18)	26 (35)	5 (23)
CR	0	2	0
PR	0	4	0
SD≥24 weeks	4	15	3
SD≥12 weeks	8	20	5
CPD	6	6	3
NE	4	8	4
NA	22	28	10
Missing/unknown	5	6	0
Best response assessed via RECIST, n (%)			
ORR (CR + PR)	0	6 (8)	0

DCR (CR + PR + SD≥24 weeks) DCR (CR + PR + SD≥12 weeks)	4 (9) 7 (16)	20 (27) 24 (32)	3 (14) 4 (18)
CR	0	2	0
PR	0	4	0
SD≥24 weeks SD≥12 weeks	4 7	14 18	3 4
PD	31	37	16
NE	2	7	2
Missing/unknown	5	6	0

CPD, confirmed progressive disease; CR, complete response, DCR, disease control rate; irRECIST, immune-related Response Evaluation Criteria in Solid Tumors; NA, not applicable; NE, not evaluable; ORR, overall response rate; PD, progressive disease; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD stable disease.

Supplemental Table 4. Plasma cytokine analysis.

A.

Part 1 GSK3174998 monotherapy (n=44)			**Part 2 GSK3174998 + pembrolizumab (n=62)**		
Cytokine	**p-value**	**direction**	**Cytokine**	**p-value**	**direction**
MIP-1α	**0.0001**	↑	**MIP-1α**	**7.86E-10**	↑
MIP-1β	**8.71E-09**	↑	**MIP-1β**	**9.69E-11**	↑
MIP-3β (CCL19)	0.2529	-	**MIP-3β (CCL19)**	**1.99E-07**	↑
IL-10	0.4646	-	**IL-10**	**1.17E-09**	↑
IP-10	**7.66E-09**	↑	**IP-10**	**1.28E-09**	↑
Soluble VCAM1	**8.57E-06**	↑	**Soluble VCAM1**	**7.38E-09**	↑
IL-6	0.8820	-	**IL-6**	**8.75E-09**	↑
IFN-γ	0.0489	-	**IFN-γ**	**2.81E-07**	↑
soluble ICAM1	0.2112	-	**Soluble ICAM1**	**7.39E-06**	↑
MCP-1	**0.0063**	↑	**MCP-1**	**0.0002**	↑
Soluble VEGFR1	**0.0002**	↑	Soluble VEGFR1	0.0231	-
G-CSF	0.0634	-	**G-CSF**	**0.0006**	↑
IL-5	0.8968	-	**IL-5**	**0.0008**	↑

B.

	Part 1 GSK3174998 monotherapy				**Part 2 GSK3174998 + pembrolizumab**			
Cytokine	**Median values (pg/mL)**		**Median Ratio (24h:BL)**	**Min - Max ratio (24h:BL)**	**Median values (pg/mL)**		**Median Ratio (24h:BL)**	**Min - Max ratio (24h:BL)**
	Baseline (BL)	**24-h post-dose**			**Baseline (BL)**	**24-h post-dose**		
MIP-1α	**11.77**	**14.39**	**1.28**	**0.84 - 5.72**	**15.91**	**21.58**	**1.52**	**0.75 - 2.79**
MIP-1β	**131.14**	**188.52**	**1.46**	**0.81 - 6.50**	**142.57**	**247.04**	**1.54**	**0.69 - 4.60**
MIP-3β (CCL19)	155.69	142.82	0.97	0.66 - 1.91	**117.05**	**193.37**	**1.68**	**0.17 - 8.89**
IL-10	1.50	1.64	1.05	0.61 - 10.72	**2.36**	**3.51**	**1.44**	**0.86 - 3.61**

IP-10	**224.58**	**509.25**	**1.64**	**0.85 - 21.04**	**262.37**	**589.09**	**1.99**	**0.14 - 12.94**
Soluble VCAM1	**1581910.88**	**1917223.09**	**1.15**	**0.83 - 1.64**	**1880487.19**	**212694.07**	**1.15**	**0.69 - 1.80**
IL-6	4.23	4.03	1.04	0.21 - 2.97	**5.87**	**8.78**	**1.38**	**0.54 - 4.58**
IFN-γ	0.13	0.13	1.08	0.18 - 1.96	**0.45**	**0.82**	**1.21**	**0.60 - 6.63**
Soluble ICAM1	474957.86	430597.25	1.04	0.66 - 1.42	**427206.03**	**508821.01**	**1.07**	**0.85 - 1.40**
MCP-1	**659.61**	**691.34**	**1.15**	**0.60 - 2.70**	**643.92**	**744.86**	**1.18**	**0.19 - 6.09**
Soluble VEGFR1	**66.43**	**73.38**	**1.17**	**0.74 - 11.85**	62.16	69.90	1.13	0.06 - 11.73
G-CSF	16.29	18.52	1.06	0.77 - 1.68	**18.32**	**21.38**	**1.11**	**0.66 - 4.23**
IL-5	2.97	4.44	1.01	0.61 - 2.06	**2.07**	**1.57**	**1.05**	**0.63 - 18.46**

Supplemental Table 4. Plasma cytokine analysis. **A**) Nonparametric analysis comparing changes in plasma cytokine levels from baseline to 24 hours post-treatment, identified statistically significant ($p<0.005$) increases of 13 plasma cytokines (**bold**). **B**) Median concentrations (pg/mL) of the 13 identified plasma cytokines (**bold**) with statistically significant increases ($p<0.005$) are summarized for the baseline and 24-hour plasma samples from patients treated in Part 1 and Part 2 of the trial across the 0.003-10 mg/kg dose range of GSK3174998. While statistically significant, the changes are moderate in magnitude (up to ≈2-fold) as indicated by the median 24-hour post-treatment to baseline ratios (fold-change).

Supplemental Table 5. Industry-sponsored trials of OX40 agonists in patients with advanced solid tumors[a]

CT.gov identifier	Phase	N	Sponsor	Dose of anti–OX40 agent (IgG subtype)	Dose of combination agent (type)	Clinical Activity	DLTs	Grade ≥3 TRAEs, %
NCT02528357 (ENGAGE-1)	1	138[b]	GSK	0.003-10 mg/kg GSK3174998 (IgG1)	200 mg pembro (anti–PD-1 mAb)	irRECIST Mono (n=45): 1 unPR Combo pembro (n=96): 2C R + 4 PR	Mono: 0 Combo pembro: 2 DLTs (G3 non-malignant pleural effusion and G1 myocarditis with G3 increased troponin)	Mono: 3/45 (7%) Combo pembro: 8/96 (8%)
NCT02737475[29]	1/2a	165	BMS	20-320 mg BMS986178 (IgG1)	± 240-480 mg nivo (anti–PD-1 mAb) ± 1-3 mg/kg ipi (anti–CTLA-4 mAb)	RECIST v1.1 Mono (n=20): no response Combo nivo (n=79): 1 CR, 5 PR Combo ipi (n=41): no response Combo nivo/ipi (n=23): 3 PR	Mono: 0 Combo nivo: 0 Combo ipi: 0 Combo nivo/ipi: NR	Mono: 1/20 (5%) Combo nivo: 6/79 (8%) Combo ipi: 2/6 (33%) Combo nivo/ipi: 3/23 (13%)
NCT02318394[30]	1	55	AZ	0.03-10 mg/kg MEDI0562 (IgG1)	-	irRECIST Mono (n=55): 2 PR	Mono: 1 (G3 diarrhea)[c]	Mono: 8/55 (14%)
NCT02315066[20,31,39]	1	94	Pfizer	0.01-10 mg/kg ivuxolimab (IgG2)	20-100 mg uto (anti–4-1BB mAb)	RECIST v1.1 Mono (n=52): 3 PR Combo uto (n=87): 3 PR	Mono: 0 Combo uto: 0	Mono: 1/52 (2%) Combo uto: 5/30 (17%)
NCT02219724[32]	1	174	Roche	0.2-1200 mg MOXR0916 (IgG1)	-	RECIST v1.1 Mono (n=174): 1 uPR, 1 PR	Mono: 0	Mono: 7/172 (4%)
NCT02410512[40,41]	1b	298	Roche	0.8-1200 mg MOXR0916 (IgG1)	1200 mg atezo (anti–PD-L1 mAb) 15 mg/kg bev (anti-VEGF mAb)	RECIST v1.1 Combo atezo +/- bev: (n=298): 1 CR, 11 PR	Combo atezo ± bev: 0	Combo Atezo +/- bev: not reported

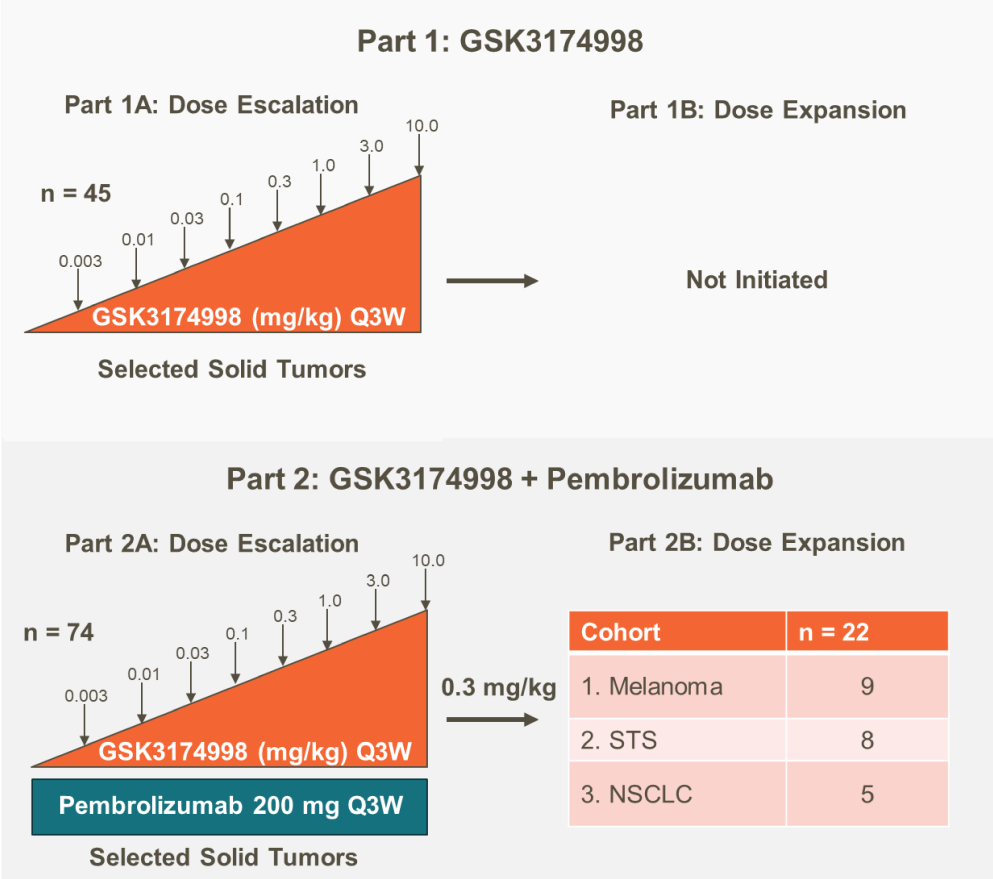
[a] In the absence of head-to-head studies, comparisons of efficacy or safety are not implied and should not be inferred from this data.

[b] 3 patients crossed over from the monotherapy part to the combination therapy part.

[c] G3 diarrhea was observed during the expansion of dose escalation. No DLTs were observed in the dose escalation part of the study.

Atezo, atezolizumab; AZ, AstraZeneca; bev, bevacizumab; CR, complete response; CTLA-4, cytotoxic T lymphocyte associated protein 4; IgG, immunoglobulin; ipi, ipilimumab; irRECIST, immune related Response Evaluation Criteria for Solid Tumors; mAb, monoclonal antibody; nivo, nivolumab; NR, not reported; ORR, overall response rate; PD-1, programmed cell death 1 protein; PD-L1, programmed cell death 1 ligand; pembro, pembrolizumab; PR, partial response; RCC, renal cell carcinoma; uPR, unconfirmed partial response; uto, utomilumab.

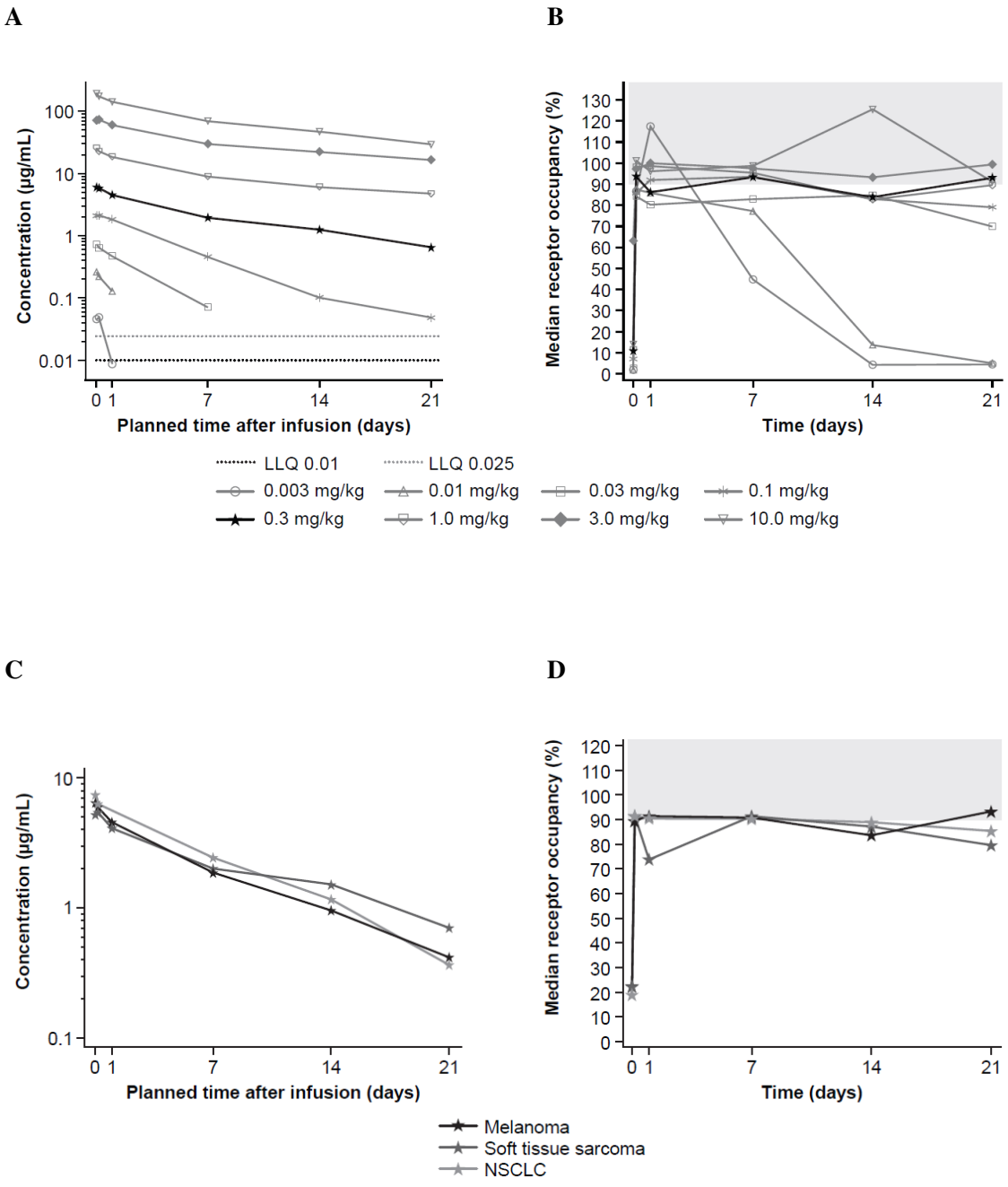
Supplemental Figure 1.

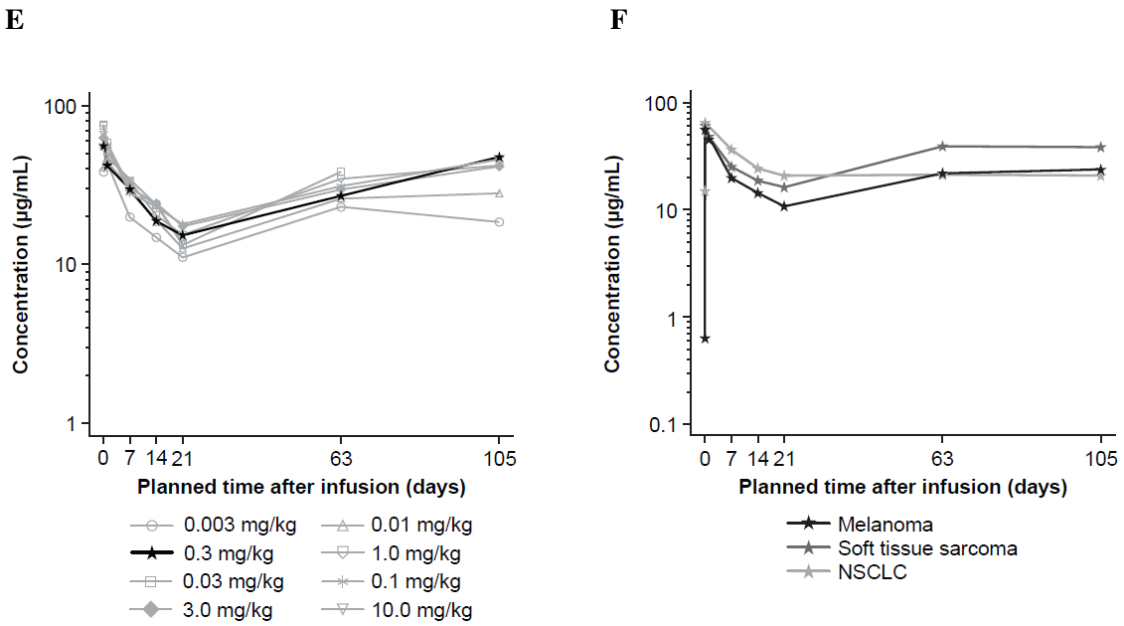


Supplemental Figure 1. ENGAGE-1 (NCT02528357) study design. This study composed of two parts: GSK3174998 monotherapy (Part 1) and GSK3174998 + pembrolizumab (Part 2). Each part consisted of two phases: a dose-escalation and a dose-expansion phase. In the dose-escalation phase, patients were given GSK3174998 by IV infusion in doses ranging from 0.003 mg/kg to 10 mg/kg every 3 weeks and 1 to 2 hours prior to IV infusion with pembrolizumab (Part 2 only). The dose-expansion phase of Part 1 was not initiated. In Part 2, patients with melanoma, STS (dedifferentiated liposarcoma), and NSCLC were enrolled in expansion cohorts once the dose of 0.3 mg/kg of GSK3174998 was determined suitable for expansion. Crossover

from Part 1 to Part 2 was allowed; 3 patients crossed over. IV, intravenous; NSCLC, non-small cell lung cancer; Q3W, every 3 weeks; STS, soft tissue sarcoma.

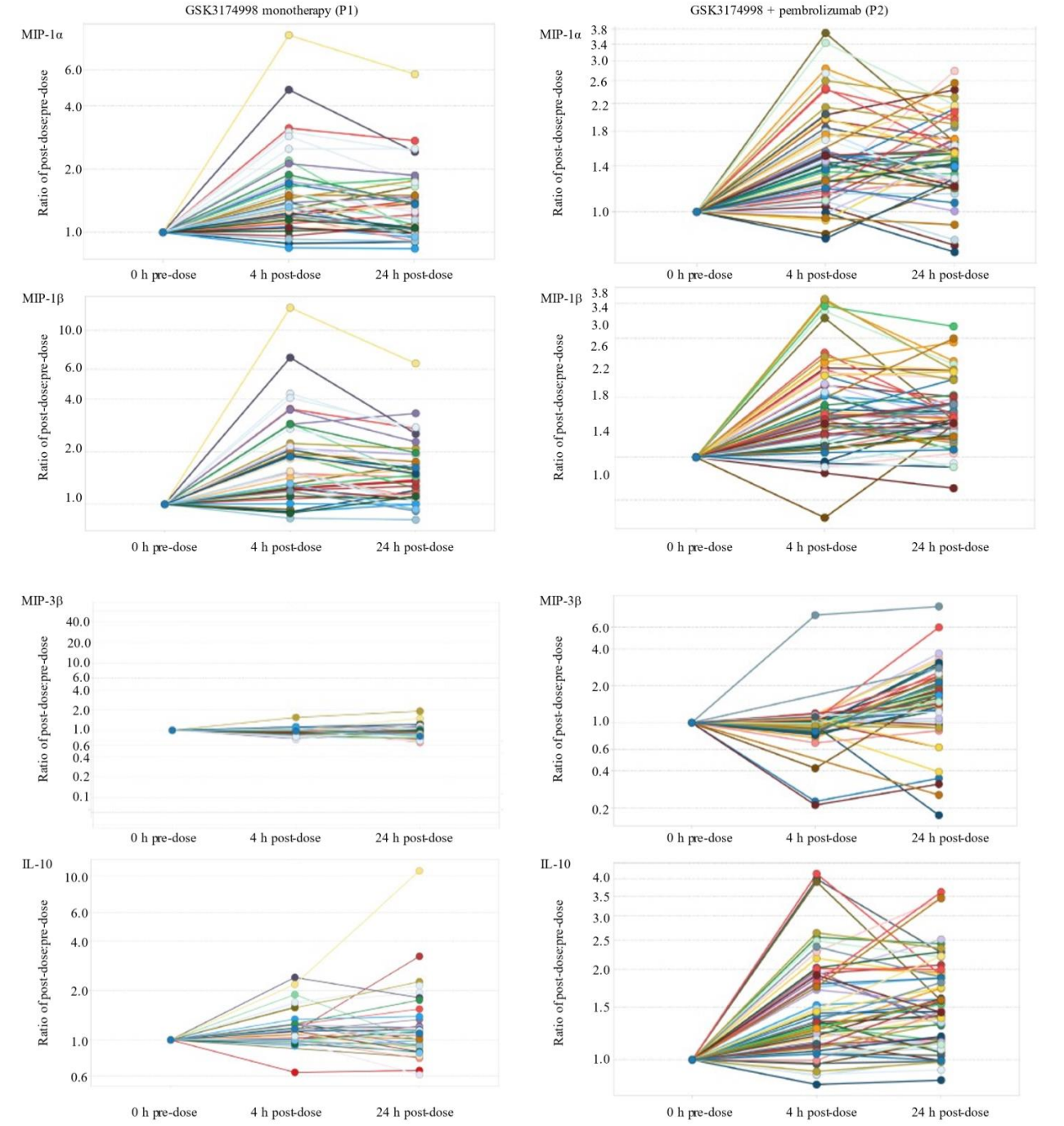
Supplemental Figure 2.

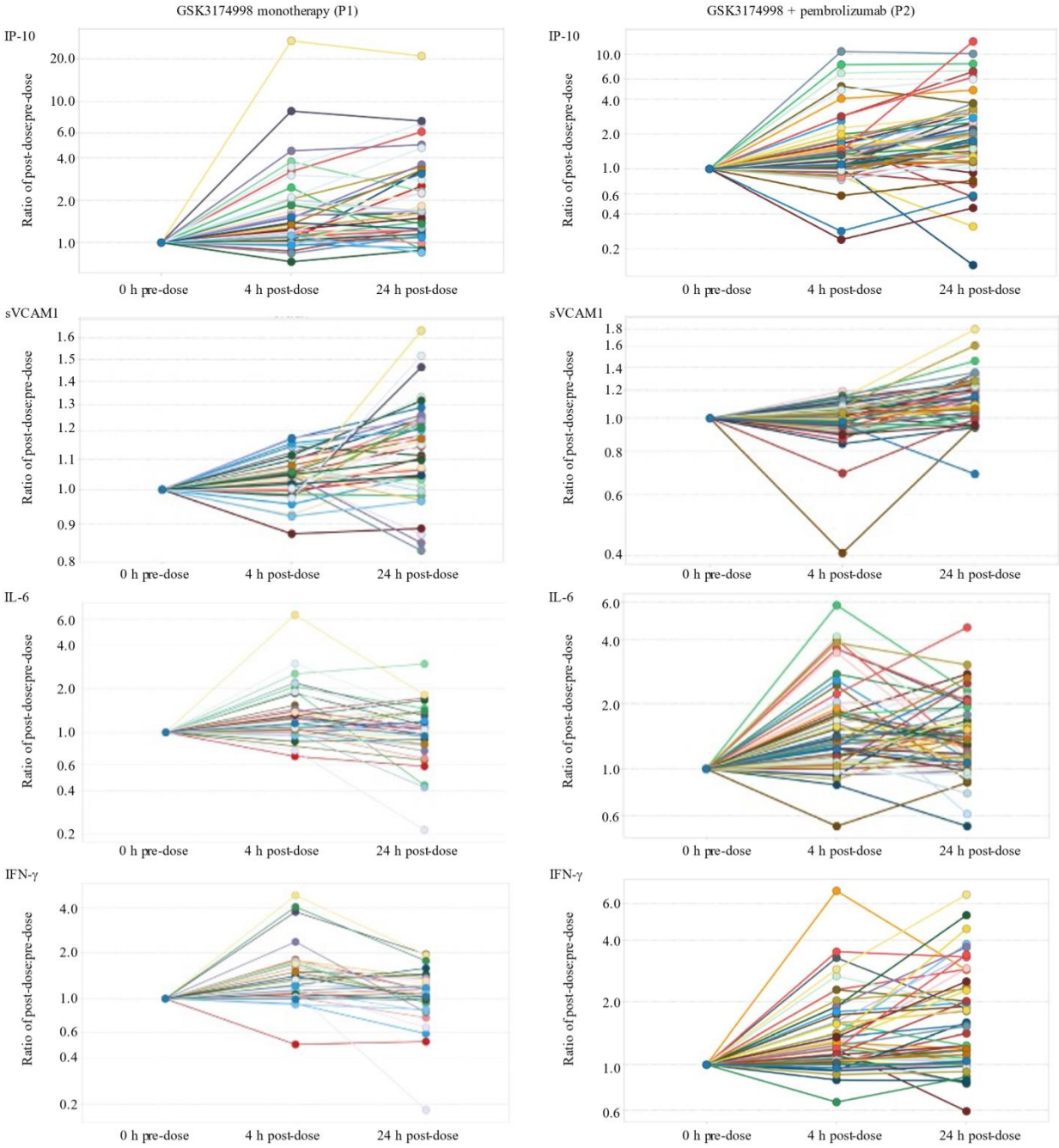


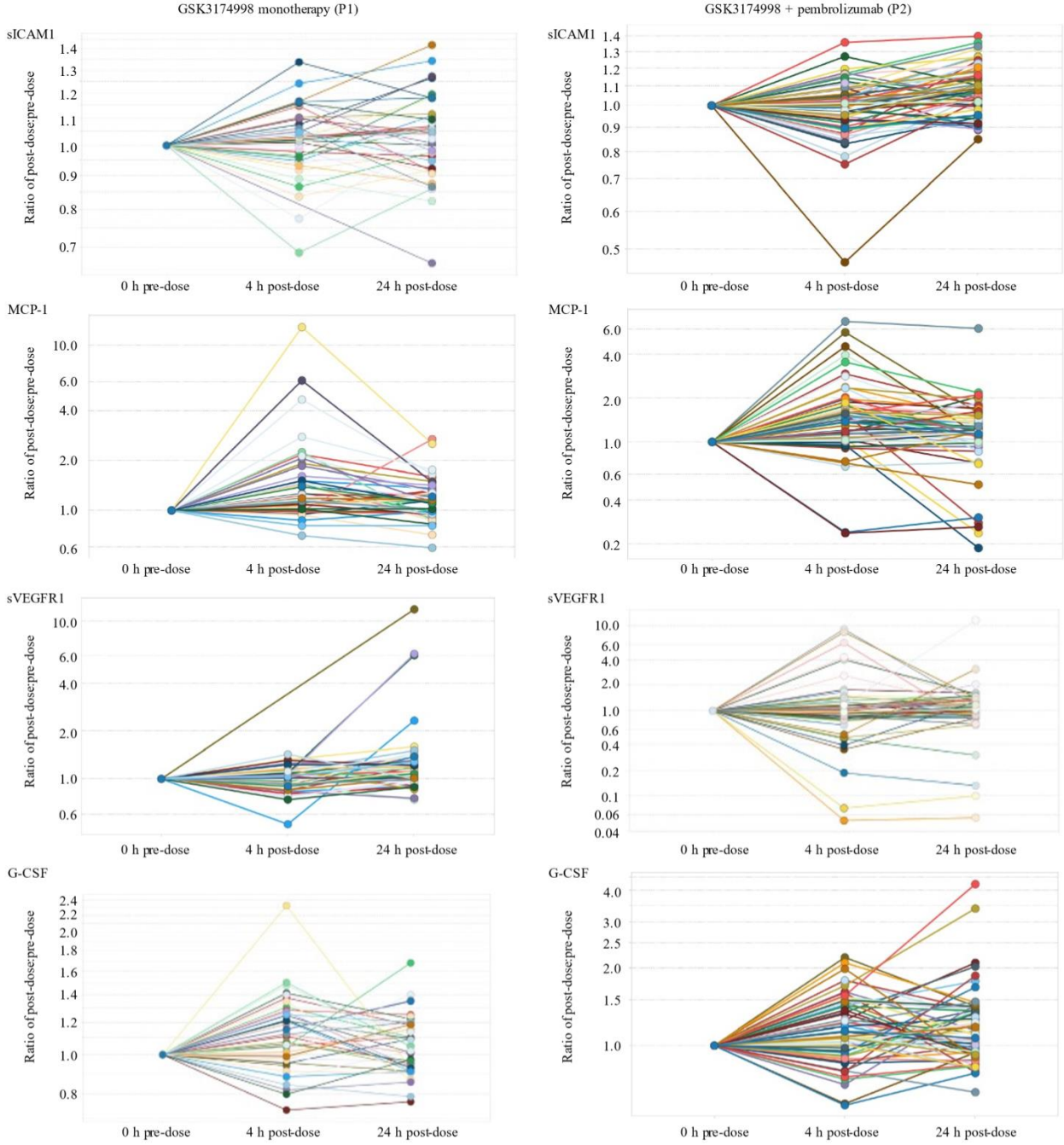


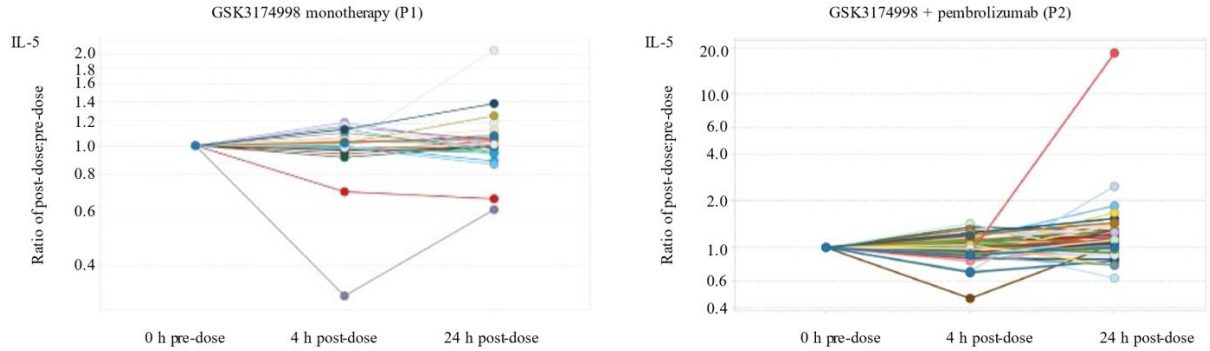
Supplemental Figure 2. Pharmacokinetics and RO of GSK3174998 during the first dosing cycle of GSK3174998 + pembrolizumab (**A**) median plasma concentration-time profiles of GSK3174998 for cycle 1 displayed on a semilogarithmic scale in Part 2a dose escalation; (**B**) median RO of GSK3174998 (%) over time in Part 2a dose escalation; (**C**) median plasma concentration-time profiles of GSK3174998 for cycle 1 displayed on a semilogarithmic scale in Part 2b dose expansion; (**D**) median RO of GSK3174998 (%) over time in Part 2b dose expansion; (**E**) serum concentrations of pembrolizumab over time for cycle 1 in Part 2a dose escalation; (**F**) serum concentrations of pembrolizumab over time for cycle 1 in Part 2b dose expansion. LLQ, lower limit of quantification; NSCLC, non-small cell lung cancer; RO, receptor occupancy.

Supplemental Figure 3.



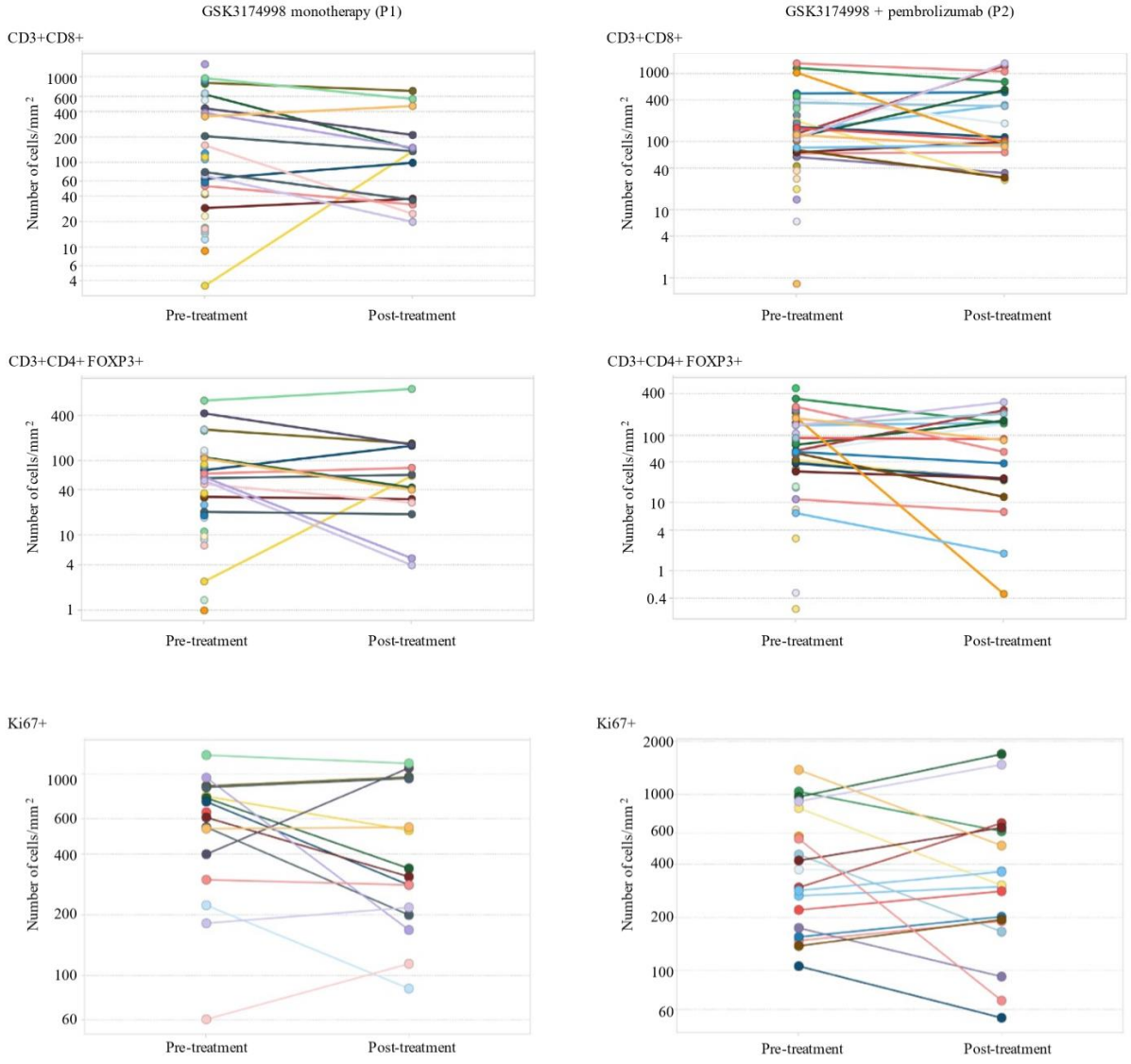

GSK3174998 monotherapy (P1)
GSK3174998 + pembrolizumab (P2)
IP-10
sVCAM1
IL-6
IFN-γ
Ratio of post-dose:pre-dose
0 h pre-dose
4 h post-dose
24 h post-dose


GSK3174998 monotherapy (P1)
GSK3174998 + pembrolizumab (P2)
sICAM1
MCP-1
sVEGFR1
G-CSF
Ratio of post-dose:pre-dose
0 h pre-dose
4 h post-dose
24 h post-dose



Supplemental Figure 3: Plasma cytokine analysis by flow cytometry. Plasma samples were collected at pre-dose, 4 hours post-dose, and 24 hours post-dose. Each color line represents an individual patient, illustrating the variability in cytokine changes.

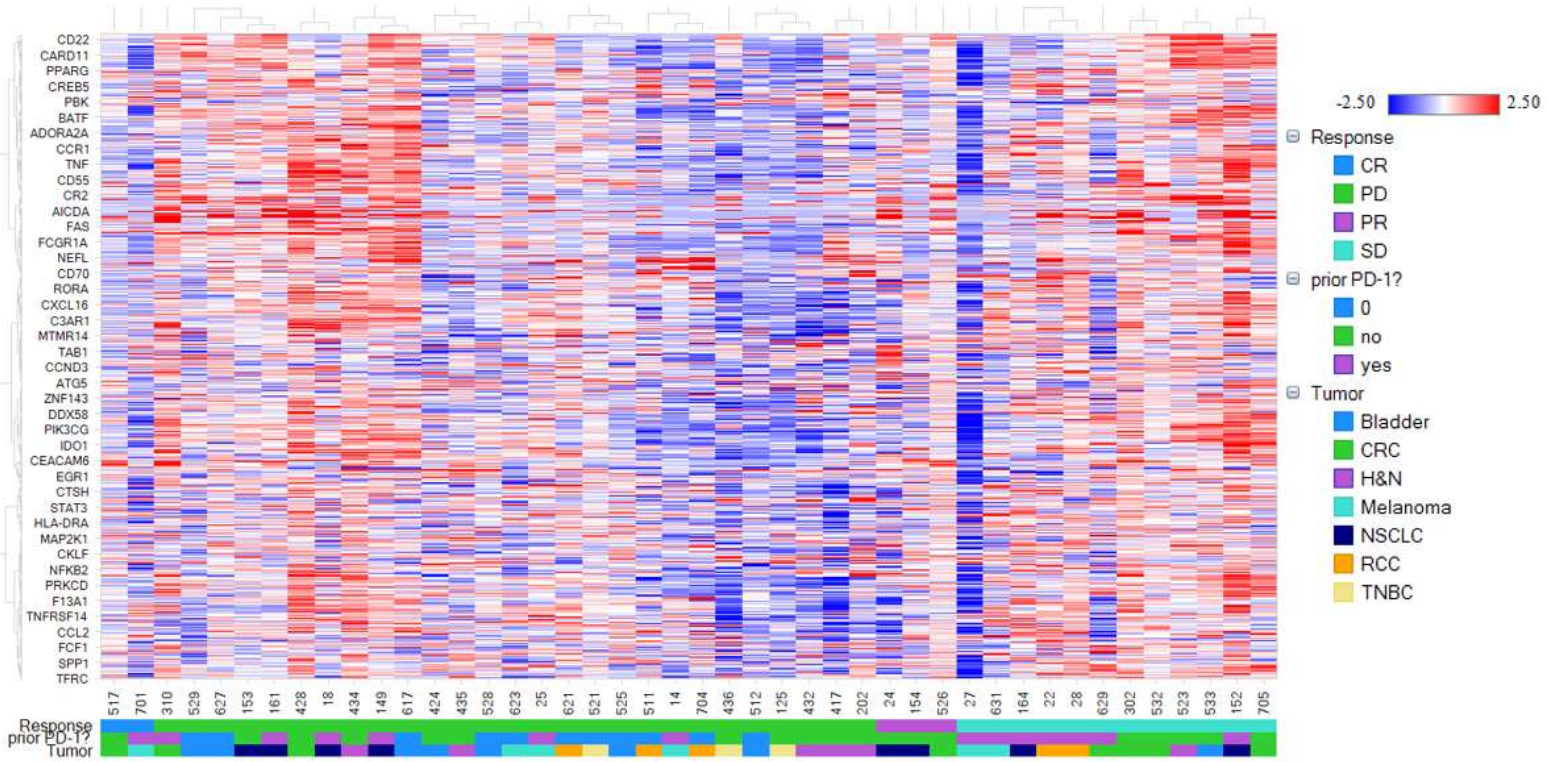
Supplemental Figure 4.



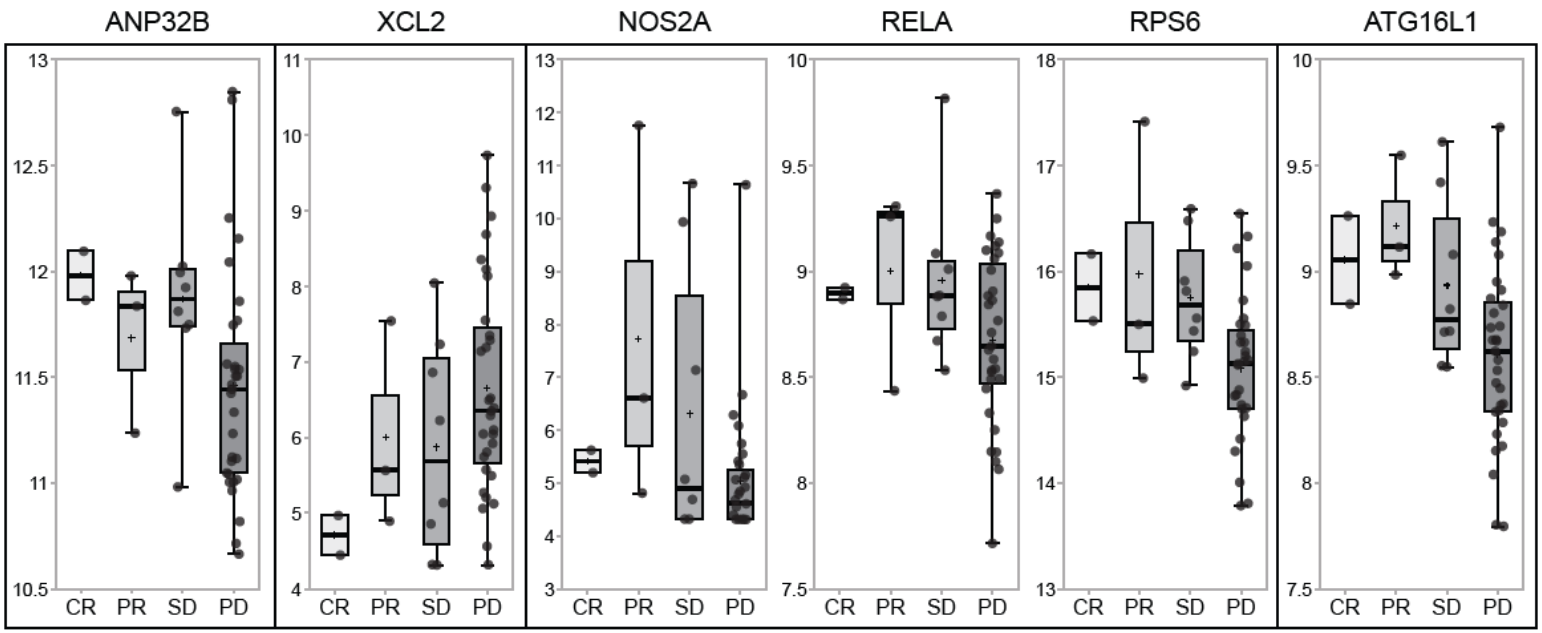
Supplemental Figure 4: MultiOmyx immunofluorescence data for the CD8+CD3+, Foxp3+CD4+, and non-tumor (PanCKneg) Ki67+ T cell populations pre- and post-treatment are shown for GSK3174998 monotherapy (P1) and GSK3174998 + pembrolizumab (P2). Paired fresh tumor tissue biopsies were obtained at baseline and week 6 for 34 patients. Each color line represents an individual patient.

Supplemental Figure 5.

A.



B.



Supplemental Figure 5. Nanostring immune profiling data. **A**) Fresh paired tumor biopsies (baseline and week 6) from 26 patients plus baseline archival tissue samples from 48 patients were investigated using the NanoString PanCancer Immune Profiling panel (730 genes plus 40 housekeeping genes). The assay was run on the nCounter Analysis System (Nanostring Technologies, Inc.). Unsupervised hierarchical clustering revealed 15 statistically significant genes associated with response or disease control: complete response (CR), partial response (PR), stable disease (SD) versus progressive disease (PD). **B**) Six of these genes (*ANP32B, XCL2,*

NOS2A, RELA, RPS6, and ATG16L1) were up- or downregulated >2-fold. From left to right within each panel of the figure, data are shown for CR, PR, SD, and PD; each dot represents an individual patient. CRC, colorectal cancer; H&N, head and neck; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma; TNBC, triple-negative breast cancer.