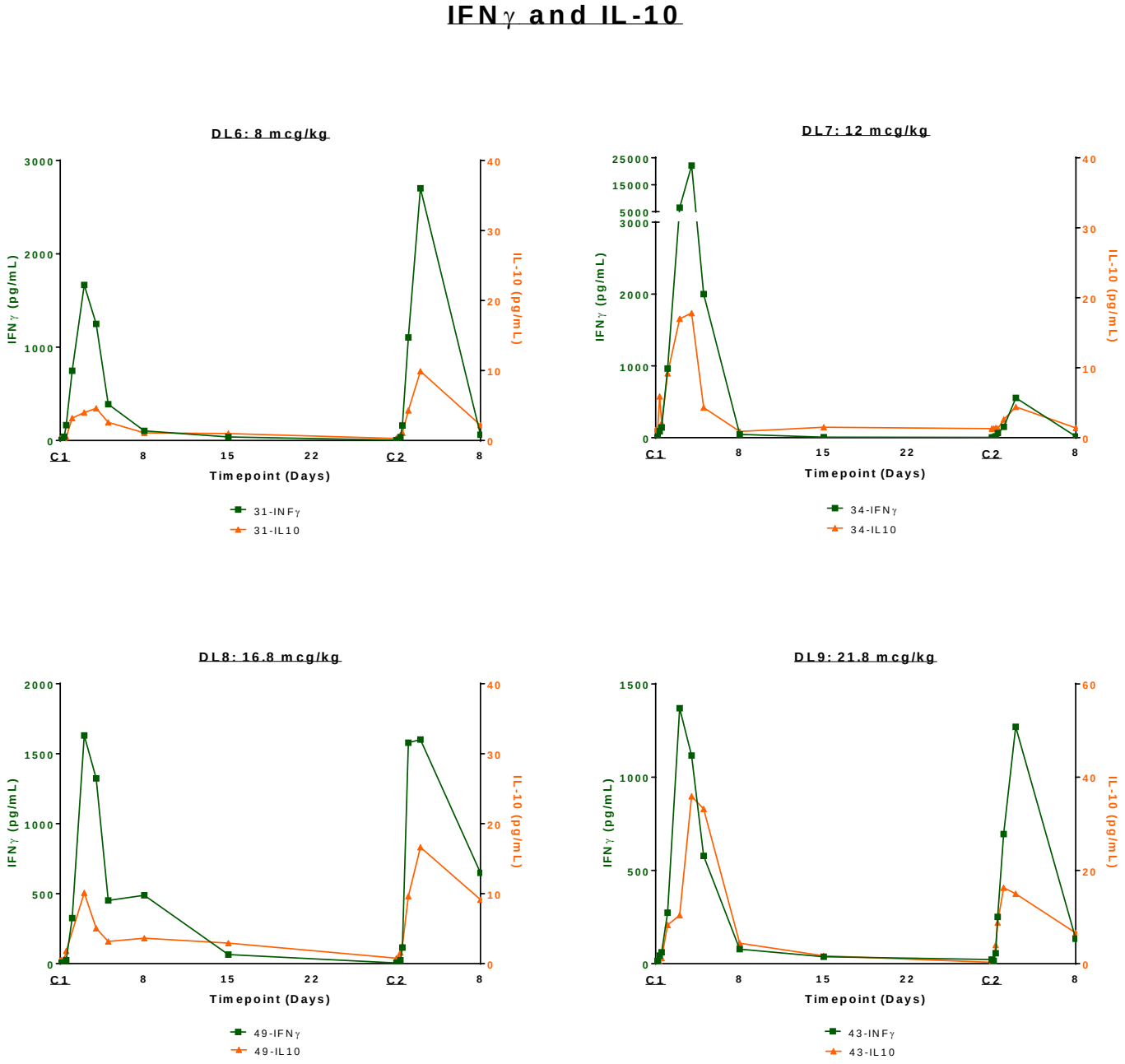
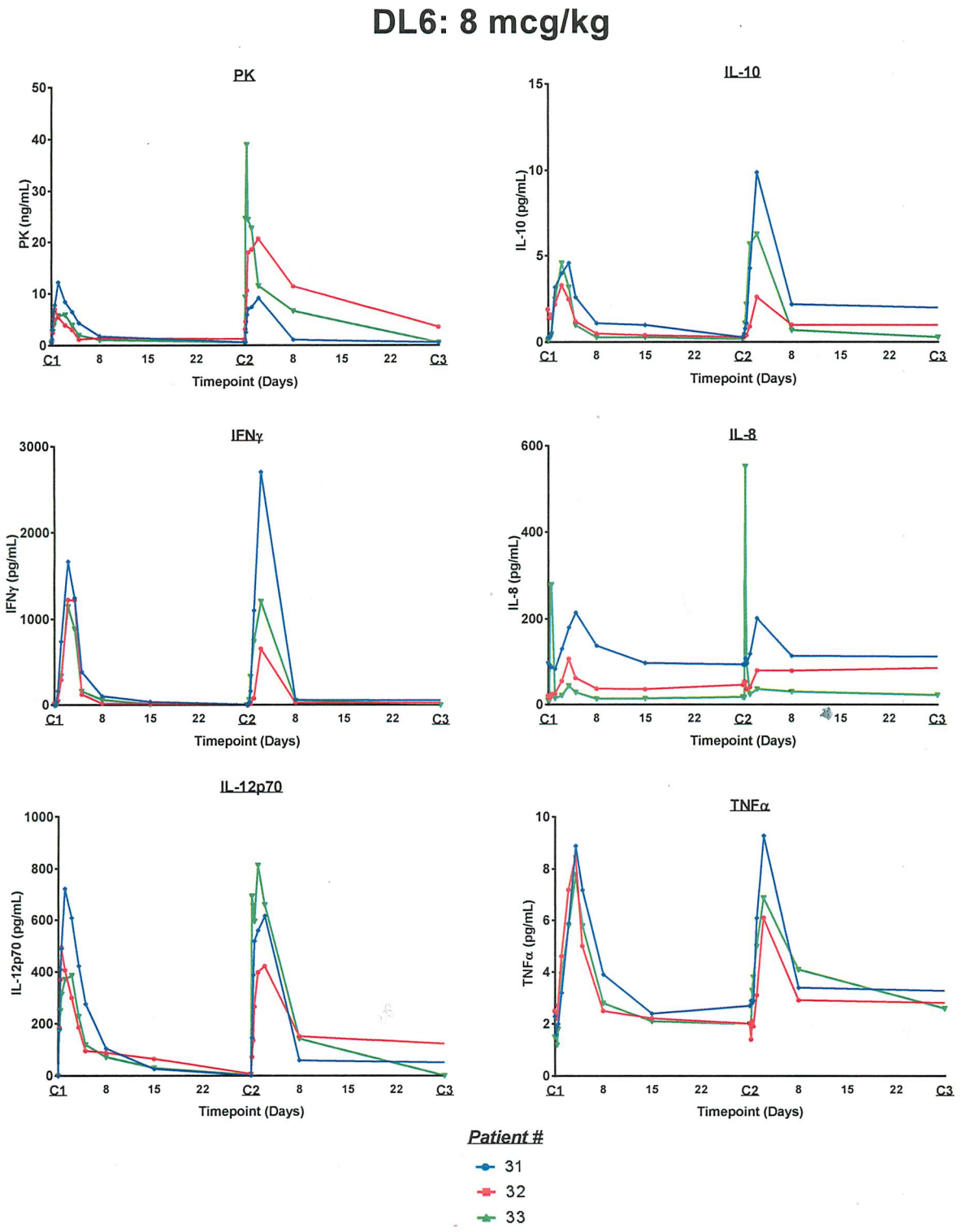
Supplementary Figure 1



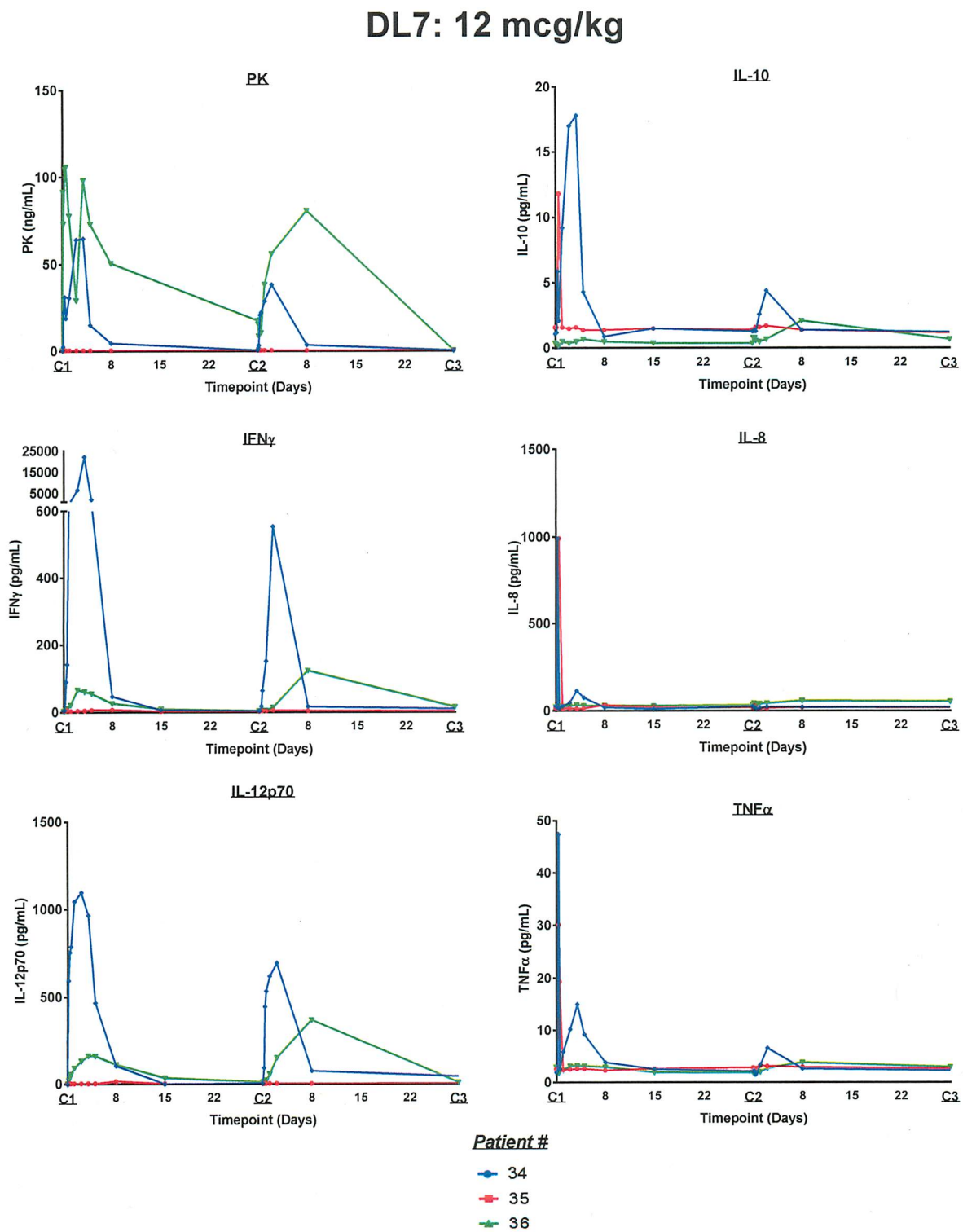
Supplementary Figure 1 demonstrates a time-dependent rise in IFN-γ after administration of NHS-IL12, and an associated rise of IL-10 in a single patient at each of dose levels 6, 7, 8, and 9.

Supplementary Figure 2



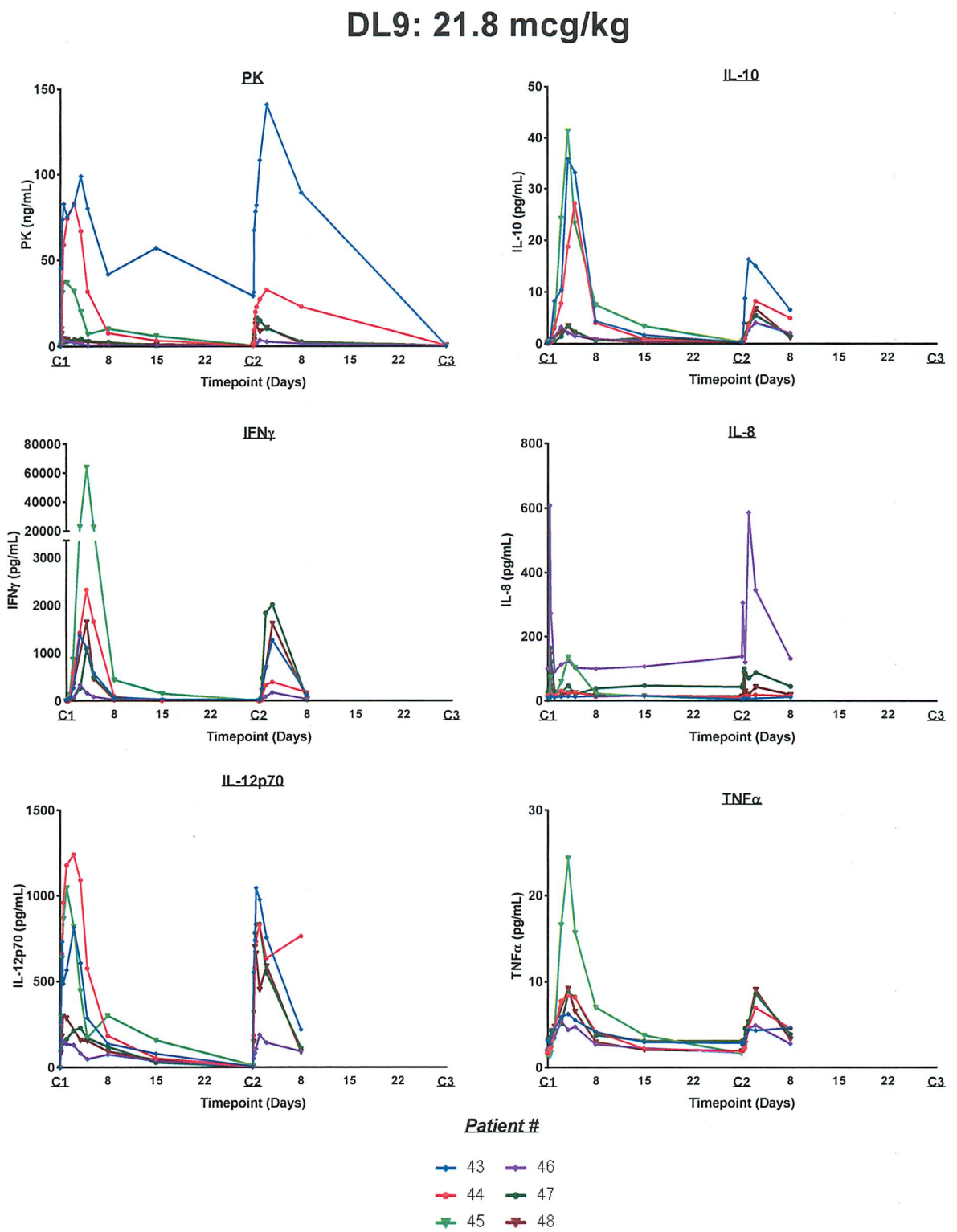
Supplementary Figure 2 shows the pharmacokinetic and pharmacodynamic results for dose level 6 of NHS-IL12.

Supplementary Figure 3



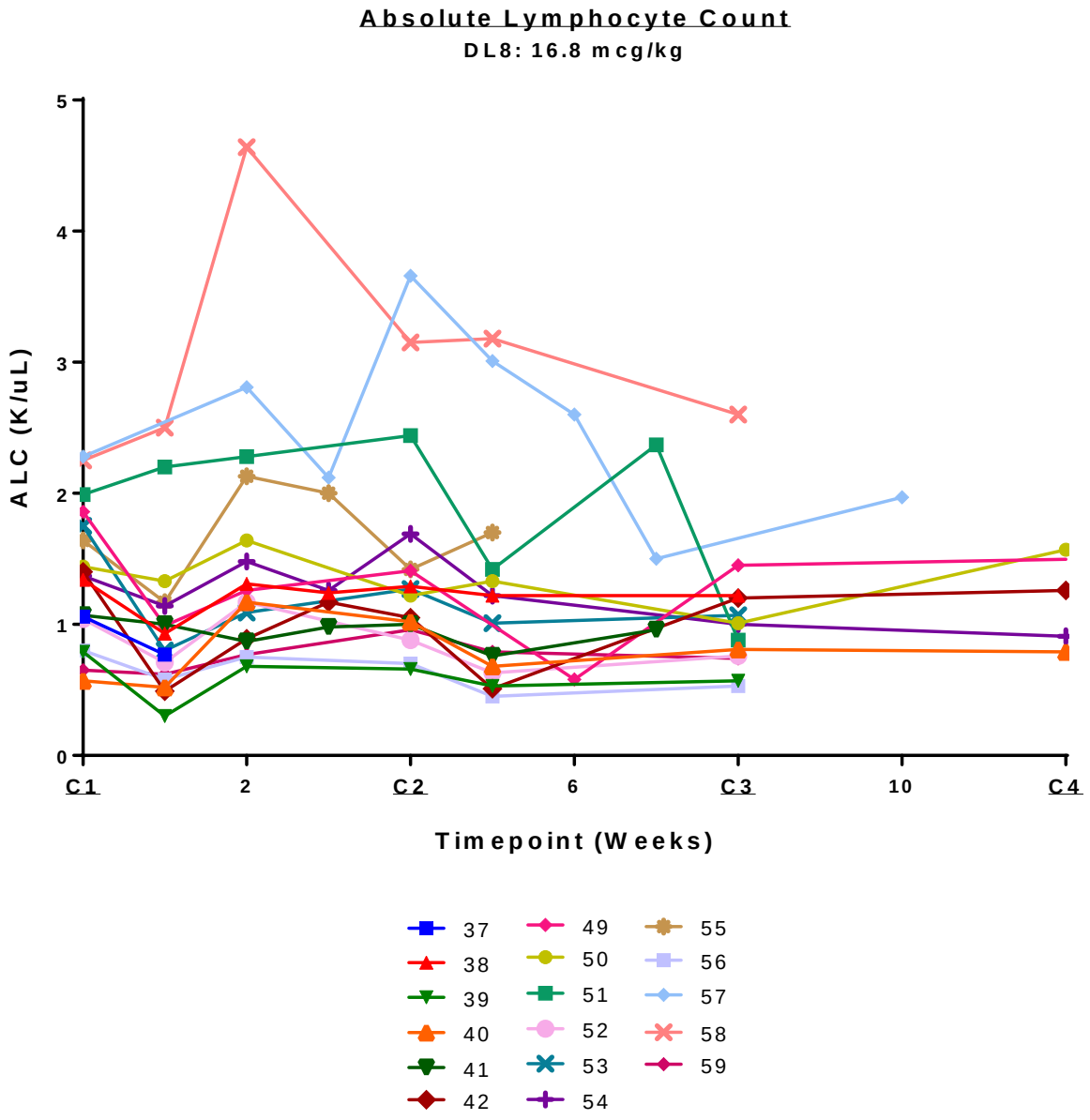
Supplementary Figure 3 shows the pharmacokinetic and pharmacodynamic results for dose level 7 of NHS-IL12.

Supplementary Figure 4



Supplementary Figure 4 shows the pharmacokinetic and pharmacodynamic results for dose level 9 of NHS-IL12.

Supplementary Figure 5



Supplementary Figure 5 shows the change in absolute lymphocyte count (ALC) for patients treated on dose level 8. A number of patients had a notable decrease in their ALC, all of which returned to baseline levels before the second cycle.

Supplementary Table 1.

A: Individual pharmacokinetics (PK) parameters for NHS-IL12 and descriptive statistics by treatment group after single dose on day 1

	$t_{1/2}$ (h)	C_{max} (ng/mL)	$AUC_{0\text{-}168h}$ (h*ng/mL)	$AUC_{0\text{-}\infty}$ (h*ng/mL)
0.5 µg/kg				
(n = 1)	NC	9.83	24.78	NC
mean	NC	9.83	24.78	NC
median				
1 µg/kg				
(n = 2)	NC	1.63	80.92	NC
mean	NC	1.63	80.92	NC
median				
2 µg/kg	(n = 4)	(n = 5)	(n = 5)	(n = 4)
(n = 5)	84.72	9.84	1034.84	2885.44
mean	49.29	3.65	209.96	243.97
median				
4 µg/kg	(n = 5)	(n = 7)	(n = 7)	(n = 5)
(n = 7)	109.13	3.27	250.59	444.27
mean	84.19	2.24	212.72	465.36
median				
8 µg/kg				
(n = 6)	184.61	6.69	605.05	1188.22
mean	131.25	6.42	491.38	945.19
median				
12 µg/kg	(n = 3)	(n = 4)	(n = 4)	(n = 3)
(n = 4)	211.30	44.80	4217.18	12115.81
mean	191.19	34.56	2565.61	929.53
median				
16.8 µg/kg	(n = 11)	(n = 13)	(n = 6)	(n = 11)
(n = 13)	379.16	24.16	2049.71	3152.197
mean	163.68	8.99	1211.03	1796.47
median				
21.8 µg/kg	200.84	39.11	3944.71	12200.80
(n = 6)	144.95	22.27	1817.03	3254.14
mean				
median				

B: Individual PK parameters for NHS-IL12 and descriptive statistics by treatment group after multiple doses on day 29

	$t_{1/2}$ (h)	C_{max} (ng/mL)	$AUC_{0\text{-}168h}$ (h*ng/mL)	$AUC_{0\text{-}\infty}$ (h*ng/mL)

2 µg/kg (n = 2) mean median	(n = 1) 1307.33 1307.33	(n = 2) 3.13 3.13	(n = 2) 220.04 220.04	(n = 1) 2101.45 2101.45
4 µg/kg (n = 3) mean median	(n = 1) 42.52 42.52	(n = 3) 9.40 5.60	(n = 3) 723.92 680.21	(n = 1) 1407.66 1407.66
8 µg/kg (n = 3) mean median	(n = 1) 86.61 86.61	(n = 3) 22.90 20.64	(n = 3) 1824.96 2033.81	(n = 1) 2807.26 2807.26
12 µg/kg (n = 2) mean median	 NC NC	(n = 2) 59.54 59.54	(n = 2) 6407.76 6407.76	 NC NC
16.8 µg/kg (n = 12) mean median	(n = 6) 207.65 113.58	(n = 12) 29.93 11.80	(n = 12) 2650.99 1266.24	(n = 6) 2657.04 1920.55
21.8 µg/kg (n = 5) mean median	(n = 2) 102.24 102.24	(n = 5) 41.40 16.88	(n = 5) 5140.01 1292.68	(n = 2) 1633.16 1633.16

NC, not calculable

Supplementary Table 2. Frequencies of nine standard immune cell subsets pre-treatment vs. post–2 cycles of NHS-IL12 for 13 subjects at dose level 8 (16.8 μg/kg)

Immune cell subset	Day 1 (Pre-treatment)	Day 57 (Post–2 cycles)	*P*-value	Adjusted *P*-value
CD4	15.9 (10.4-25.8)	18.0 (10.3-30.4)	0.79	ns
CD8	13.7 (11.5-22.0)	13.2 (9.2-29.8)	0.64	ns
Tregs	0.4 (0.2-0.5)	0.4 (0.2-0.5)	>0.99	ns
NK	7.4 (4.8-9.3)	9.0 (3.6-13.8)	0.27	ns
NKT	1.7 (0.8-5.3)	2.8 (1.1-6.2)	0.0017	0.007
cDC	0.9 (0.7-2.0)	0.8 (0.7-1.9)	0.27	ns
pDC	0.3 (0.2-0.4)	0.3 (0.1-0.4)	0.38	ns
B cells	6.9 (2.9-12.6)	5.7 (3.0-10.6)	0.64	ns
MDSC	13.7 (3.9-22.0)	7.5 (1.8-19.4)	0.68	ns

Flow cytometry was performed to evaluate 123 discrete immune cell subsets, including nine standard subsets (shown) and 114 refined subsets, as described in the Methods section. Values shown are the frequencies out of all PBMC, with median and interquartile range (IQR).

Tregs, regulatory T cells
NK, natural killer cells
NKT, natural killer T cells
cDC, conventional dendritic cells
pDC, plasmacytoid dendritic cells
MDSC, myeloid-derived suppressor cells
ns, not significant

Supplementary Table 3: Toxicity trends through Cycle 4 in the multi-dose patients

	All Events/Cycle (% of Pts)		
Cycle (n)	**Grade 1**	**Grade 2**	**Grade 3**
1 (37)	3.5 (81.1)	1.3 (64.9)	0.4 (27.0)
2 (32)	2 (71.9)	0.6 (46.9)	0.03 (3.1)
3 (18)	0.9 (55.6)	0.3 (22.2)	0.06 (5.6)
4 (12)	0.4 (41.7)	0.3 (33.3)	0 (0)
	Physical Events/Cycle (% of Pts)		
Cycle (n)	**Grade 1**	**Grade 2**	**Grade 3**
1 (37)	1.2 (70.3)	0.2 (13.5)	0. 05 (5.4)
2 (32)	0.7 (50)	0.09 (9.4)	0 (0)
3 (18)	0.4 (27.8)	0 (0)	0 (0)
4 (12)	0.3 (25)	0.2 (16.7)	0 (0)

The “Cycle” column indicates the number of subjects who completed each number of cycles. The data is shown as # events/cycle (% of patients experiencing toxicity). The upper table includes all adverse events (AEs) with an attribution of 3+, and the lower table shows only physical AEs such as fever or fatigue (no labs). Both events/cycle and % of patients experiencing AEs decreased between Cycle 1 and Cycle 2.