SUPPLEMENTAL MATERIALS

Pharmacokinetics and Pharmacodynamic Effects of Nemvaleukin Alfa, a Selective Agonist of the Intermediate-Affinity IL-2 Receptor, in Cynomolgus Monkeys

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Supplemental Methods

The pharmacokinetic assay was validated by evaluating its precision and accuracy. Precision was expressed as the percentage of coefficient of variation (%CV) of each quality control (QC) concentration. The mean inter-assay (between run) precision (%CV) for the low QC, medium QC, and high QC ranged from 8.0% to 12.8% and achieved the targeted acceptance criterion of %CV <20%. The mean inter-assay precision (%CV) for the lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) ranged from 7.4% to 15.1% and achieved the targeted acceptance criterion of %CV <25%. The mean intra-assay (within run) precision (%CV) for the low QC, medium QC, and high QC ranged from 0.4% to 6.4% and achieved the targeted acceptance criterion of %CV <20%. The mean inter-assay precision (%CV) for the LLOQ and ULOQ ranged from 0.8% to 6.6% and achieved the targeted acceptance criterion of %CV <25%. Accuracy was assessed as the percentage difference from the theoretical analyte concentration (%bias). The mean inter-assay accuracy (%bias) for the low QC, medium QC, and high QC ranged from 7.9% to 10.8% and achieved the targeted acceptance criterion of %bias of ±20%. The mean inter-assay accuracy (%bias) for LLOQ and ULOQ ranged from 0.8% to 12.3% and achieved the targeted acceptance criterion of %bias of ±25%. The mean intra-assay accuracy (%bias) for the low QC, medium QC, and high QC ranged from –5.9% to 24.9% and achieved the targeted acceptance criterion of %bias of ±20% for medium and high QC and for four of six runs (67%) at low QC. The mean intra-assay accuracy (%bias) for the LLOQ and ULOQ ranged from –8.7 to 28.4% and achieved the targeted acceptance criterion of %bias of ±25% for 67% of the runs.

The total error (TE) represents the overall error that may occur in a test result due to both the imprecision and inaccuracy of the measurement procedure. The mean TE for the low QC, medium QC, and high QC ranged from 15.9% to 23.6% and achieved the targeted acceptance criterion of TE <30%. The mean TE for the LLOQ and ULOQ ranged from 8.2% to 27.4% and achieved the targeted acceptance criterion of TE <40%.

Supplemental Table 1

Antibodies and fluorescent dyes used for determination of pSTAT5 levels in white blood cells from cynomolgus monkeys by flow cytometry

Antibodies/Dyes	Vendor
Anti-human CD3 V500 (clone: SP34-2)	BD Biosciences
Anti-human CD4 PE-Cy7 (clone: L200)	BD Biosciences
Anti-human CD8 APC (clone: SK1)	BioLegend
Anti-human CD14 PE-Texas Red (clone: TuK4)	Life Technologies
Anti-human CD16 BV605 (clone: 3G8)	BD Biosciences
Anti-human CD25 BV421 (clone: M-A251)	BD Biosciences
Anti-human CD28 PE (clone: 10F3)	BD Biosciences
Anti-human CD45RA APC-H7 (clone: 5H9)	BD Biosciences
Anti-human CD56 PE (clone: MY31)	BD Biosciences
Anti-human CD95 BV421 (clone: DX2)	BD Biosciences
Anti-STAT5 (pY694) Alexa Fluor 488 (clone: 47)	BD Biosciences
Anti-human FOXP3 Alexa Fluor 647 (clone: 259D)	BioLegend
Zombie NIR Fixable Dye Kit	BioLegend
Zombie Red Fixable Dye Kit	BioLegend

Supplemental Table 2

Markers used to identify immune cell subtypes for pSTAT5 analyses from cynomolgus monkeys

Cynomolgus Monkey Immune Cell Population	Gated Biomarkers
$CD4^+$ T_{regs}	$CD14^-$ $CD3^+$ $CD4^+$ $CD25^{high}$ $FoxP3^+$
NK cells	$CD14^-$ $CD3^-$ $CD16^+$
Naïve $CD8^+$ T cells	$CD14^-$ $CD3^+$ $CD8^+$ $CD28^+$ $CD45RA^+$ $CD95^-$
Central/Transitional memory $CD8^+$ T cells	$CD14^-$ $CD3^+$ $CD8^+$ $CD28^+$ $CD45RA^-$ $CD95^+$
Effector memory $CD8^+$ T cells	$CD14^-$ $CD3^+$ $CD8^+$ $CD28^-$ $CD45RA^-$ CD95+
Terminal effector $CD8^+$ T cells	$CD14^-$ $CD3^+$ $CD8^+$ $CD28^-$ $CD45RA^+$ $CD95^+$

Supplemental Table 3

Antibodies and fluorescent dyes used for phenotyping of white blood cells by flow cytometry in peripheral blood of cynomolgus monkeys

Antibodies/Dyes	Vendor
Zombie UV Dye	BioLegend
CD127 PE-Vio770 (MB15-18C9)	Miltenyi Biotec
ICOS PerCP Cy5.5 (C398.4A)	BioLegend
Armenian Hamster IgG PerCP Cy5.5	BioLegend
CD56 PE (NCAM 16.2)	BD Biosciences
Ki-67 Alexa 488 (B56)	BD Biosciences
Mouse IgG1,k alexa 488 (MOPC-21)	BD Biosciences
CD14 APC-H7 (M5E2)	BD Biosciences
CD4 alexa 700 (L200)	BD Biosciences
FOXP3 alexa 647 (259D)	BioLegend
Mouse IgG1, k alexa 647 (MOPC-21)	BioLegend
CD16 BV605 (3G8)	BD Biosciences
CD69 BV510 (FN50)	BioLegend
Mouse IgG1, k BV510 (MOPC-21)	BioLegend
CD25 BV421 (M-A251)	BD Biosciences
Mouse IgG1, k BV421 (X40)	BD Biosciences
CD20 BUV737 (2H7)	BD Biosciences
CD3 BUV395 (SP34-2)	BD Biosciences
CCR7 PE-Cy7 (G043H7)	BioLegend

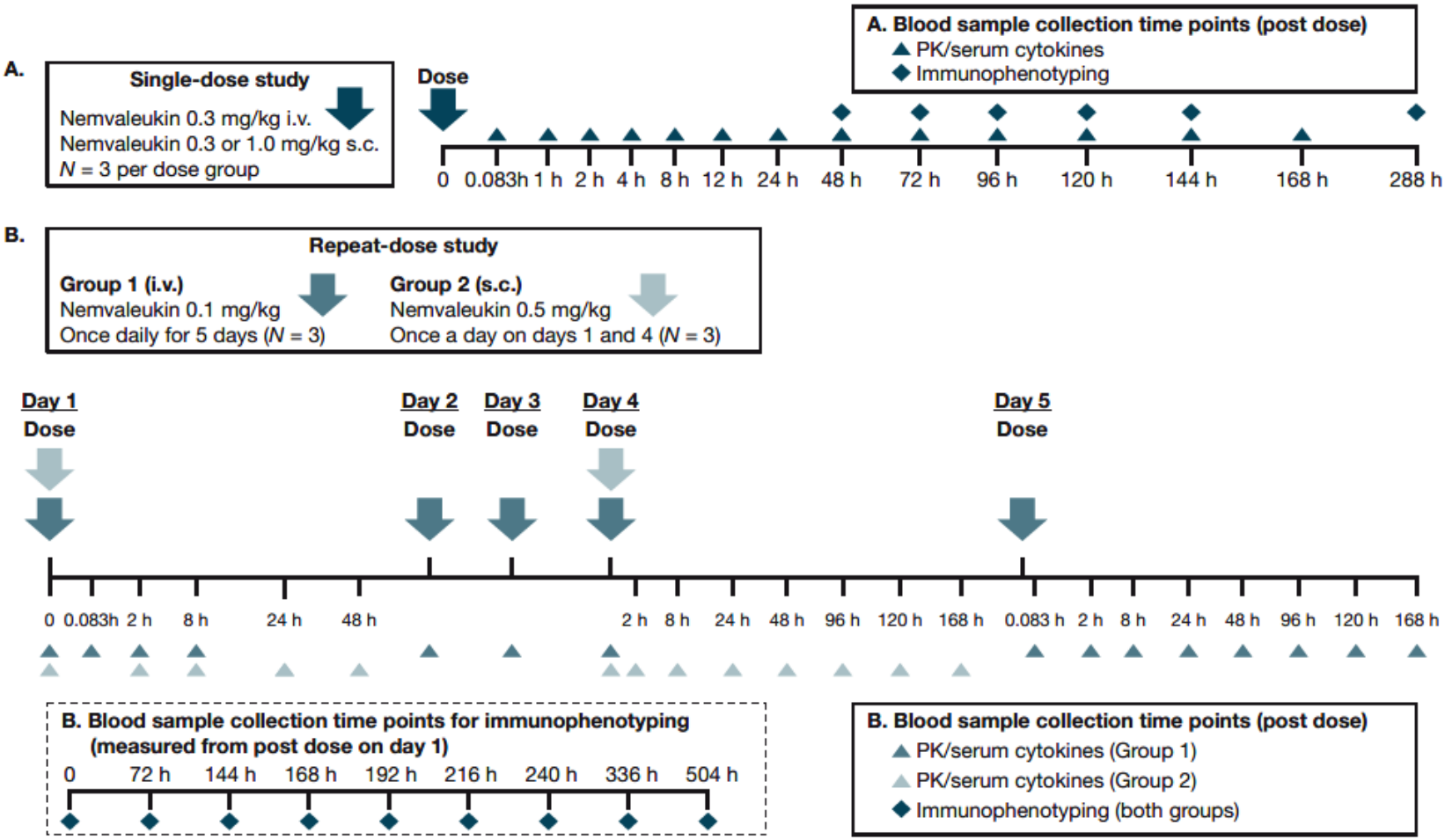
CD4 PerCP Cy5.5 (L200)	BD Biosciences
CD28 PE (10F3)	ThermoFisher
CD45RA APC-H7 (5H9)	BD Biosciences
CD14 alexa 700 (M5E2)	BD Biosciences
Mouse IgG2b APC (IS6-11E5.11)	Miltenyi Biotec
CD95 BV605 (DX2)	BioLegend
CD8 BV510 (SK1)	BioLegend
Armenian Hamster IgG BV421	BioLegend

Supplemental Table 4

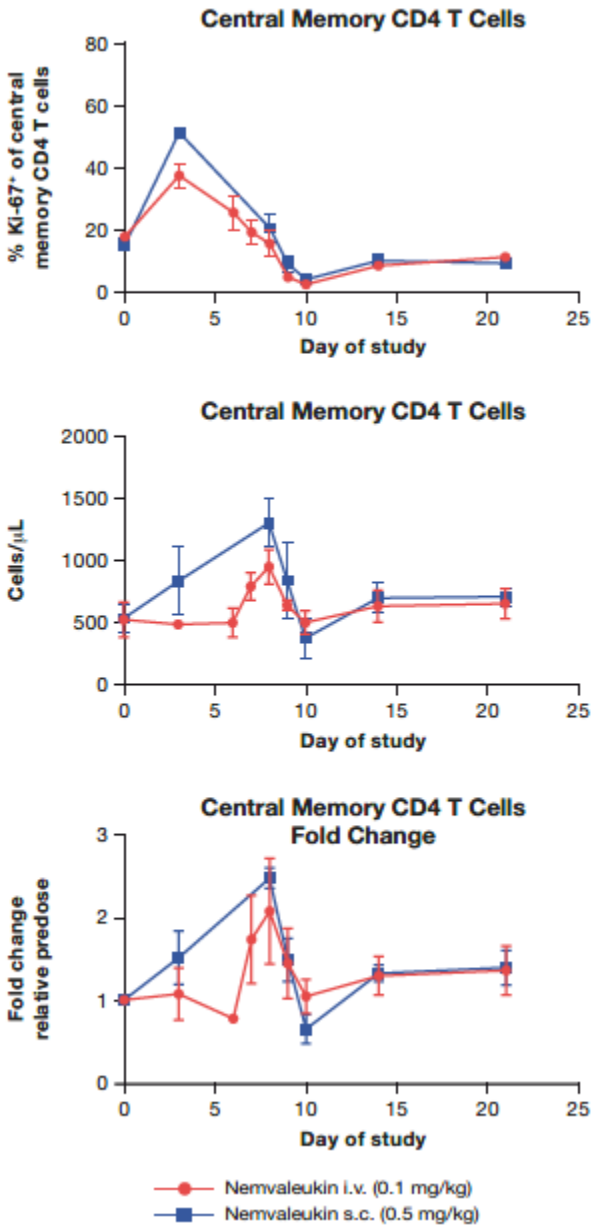
Markers used to identify immune cell subsets in the peripheral blood of cynomolgus monkeys

Immune Cell Population	Positively Gated Biomarkers	Negatively Gated Biomarkers
NK cells	$CD56^+$	$CD14^-$ $CD3^-$
Naïve $CD8^+$ T cells	$CD3^+$ $CD8^+$ $CD45RA^+$	$CD14^-$ $CD4^-$ $CD95^-$
Central memory $CD8^+$ T cells	$CD3^+$ $CD8^+$ $CD95^+$ $CD28^+$ $CCR7^+$	$CD14^-$ $CD4^-$ $CD45RA^-$
Effector memory $CD8^+$ T cells	$CD3^+$ $CD8^+$ $CD95^+$	$CD14^-$ $CD4^-$ $CD28^-$ $CCR7^-$ $CD45RA^-$
Terminal effector $CD8^+$ T cells	$CD3^+$ $CD8^+$ $CD95^+$ $CD45RA^+$	$CD14^-$ $CD4^-$ $CD28^-$ $CCR7^-$
Central memory $CD4^+$ T cells	$CD3^+$ $CD4^+$ $CD95^+$ $CD28^+$ $CCR7^+$	$CD14^-$ $CD8^-$ $CD45RA^-$
$CD4^+$ T_{regs}	$CD3^+$ $CD4^+$ $CD25^+$ $FoxP3^+$	$CD14^-$ $CD127^{-/low}$

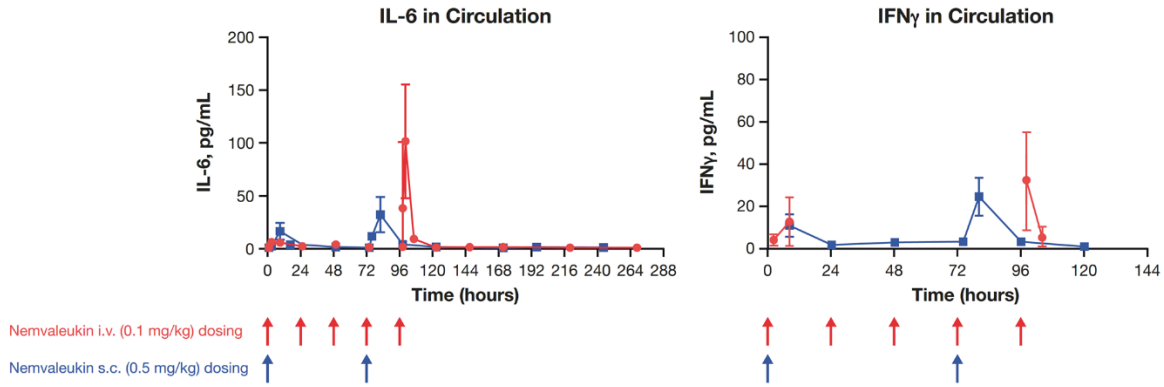
Supplemental Fig. 1. Study design and dosing schedules for the (A) single- and (B) repeat-dose studies. Blood samples taken prior to dosing on each day of dose administration.



Supplemental Fig. 2. Effect of repeated i.v. (0.1 mg/kg for 5 consecutive days) or s.c. (0.5 mg/kg on days 1 and 4) administration of nemvaleukin on central memory $CD4^+$ T cells and the proportion of cells expressing the cell proliferation marker Ki-67. Data shown are the mean ± standard error of the mean with *N* = 3 per treatment group.



Supplemental Fig. 3. Effect of repeated i.v. (0.1 mg/kg for 5 consecutive days) or s.c. (0.5 mg/kg on days 1 and 4) administration of nemvaleukin on IL-6 and IFNɣ levels. *N* = 3 per treatment group.



Dosing regimen	IL-6		IFNγ	
	C_{max} (pg/mL), mean ± SD (range)	T_{max} (h)	C_{max} (pg/mL), mean ± SD (range)	T_{max} (h)
Nemvaleukin i.v. (0.1 mg/kg) q.d. ×5 (on days 1–5)	102 ± 54 (42–148)	98	33 ± 24 (6–52)	98
Nemvaleukin s.c. (0.5 mg/kg) (on days 1 and 4)	32 ± 17 (13–42)	90	25 ± 15 (10–41)	90