Supplemental material

Vaishampayan UN, et al. Nemvaleukin alfa, a modified interleukin-2 cytokine, as monotherapy and with pembrolizumab in patients with advanced solid tumors (ARTISTRY-1)

This supplemental material has been provided by the authors to give readers additional information about their work.

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List of sites and investigators*

Investigator name	Country	Site ID
Underhill, Craig	Australia	240101
Van der Westhuizen, Andre	Australia	240102
Ganju, Vinod	Australia	240103
Debruyne, Philip	Belgium	340101
Machiels, Jean-Pascal	Belgium	340102
Canon, Jean-Luc	Belgium	340104
Rottey, Sylvie	Belgium	340105
Spreafico, Anna	Canada	124107
Gilbert, Lucy	Canada	124108
Chu, Quincy Siu	Canada	124109
Hirte, Holger (Hal)	Canada	124110
Dumas, Olivier	Canada	124111
Miller, Wilson	Canada	124112
Galffy, Gabriella	Hungary	440101
Geczi, Lajos	Hungary	440102
Rubovszky, Gabor	Hungary	440103
Muller, Veronica	Hungary	440104
Kalinka, Ewa	Poland	540101
Tomczak, Piotr	Poland	540102
Hong, Jungyong	South Korea	640101
Lee, Keun-Wook	South Korea	640102
Kang, Jin-Hyoung	South Korea	640103
Shin, Sang Joon	South Korea	640105
Lee, Hyo Jin	South Korea	640106
Choi, Yoon Ji	South Korea	640107
Moreno-Vega, Alberto	Spain	740101
Cervantes Ruiperez, Andres	Spain	740102
Victoria, Ivan	Spain	740103
Grande Pulido, Enrique	Spain	740104
Pinto Marin, Alvaro	Spain	740105
Longo, Federico	Spain	740106
Manzano, Arancha	Spain	740107
Calvo, Emiliano	Spain	740108
Luis, Paz-Arez Rodrigurez	Spain	740111
McDermott, David	USA	840101
Ernstoff, Marc	USA	840102
Kauffman, Howard	USA	840103
Shepard, Dale	USA	840104
Winer, Ira	USA	840105
Velcheti, Vamsidhar	USA	840106

Bruno, Deborah	USA	840108
Muzaffar, Jameel	USA	840109
Chaudhry, Arvind	USA	840110
Rosen, Seth	USA	840111
Chauhan, Aman	USA	840112
Spira, Alex	USA	840113
Rocha-Lima, Caio Max	USA	840114
Strauss, James	USA	840115
Grewal, Jaspreet	USA	840116
Lewis, Karl	USA	840117
Frank, Richard	USA	840119

*Includes all sites where ARTISTRY-1 was conducted.

SUPPLEMENTAL METHODS

Part C cohorts

The seven predefined cohorts in Part C were cohort 1 (PD-[L]1 inhibitor unapproved), cohort 2 (PD-[L]1 inhibitor approved and pretreated), cohort 3 (PD-[L]1 inhibitor approved and treatment naive), cohort 4 (monotherapy rollover), cohort 5 (melanoma), cohort 6 (non–small-cell lung cancer), and cohort 7 (squamous cell carcinoma of the head and neck). PD-(L)1 inhibitor–approved/–unapproved status was based on US Food and Drug Administration prescribing information at the time of the study design and enrollment.

Inclusion criteria for Parts A, B, and C

In Part A, all patients had the diagnosis of an advanced solid tumor that was refractory or intolerant to established therapies. In Parts B and C, all patients had an advanced solid tumor and have had the minimum prior lines of therapy as defined by the specific cohort into which the patient was enrolled. Treatment with prior immunotherapy was permitted with the exception of patients enrolled into cohort 3, the PD-1/L1 inhibitor–approved tumor types (PD-1/L1 treatment naive) cohort; cohort 5, the melanoma cohort; and cohort 7, the squamous cell carcinoma of the head and neck cohort, who were not permitted to have received prior anti-PD-1/L1 therapy. In Part C, patients must have had completed the last dose of any broad-spectrum antibiotic at least 30 days prior to first dose (cycle 1 day 1).

In Parts B and C, patients must have had at least one lesion that qualified as a target lesion according to the Response Evaluation Criteria In Solid Tumors (RECIST) v1.1 and must have provided archival tumor tissue biopsy sample(s), if available, within 30 days of study enrollment. All patients must have had an estimated life expectancy of ≥3 months; must have had adequate hematological reserve, hepatic function, and renal function; must have been recovered from the effects of any previous chemotherapy, immunotherapy, other prior systemic anticancer therapy, radiotherapy, or surgery; and must have waited at least 5 half-lives or 4 weeks (whichever was

shorter) following prior investigational therapy before enrollment into the study, or 4 weeks if the half-life of the investigational agent was not known.

Exclusion criteria for Parts A, B, and C

Patients were excluded if they had active or symptomatic central nervous system metastases unless the metastases had been treated and the patient was neurologically stable, or known hypersensitivity to any components of nemvaleukin, or required pharmacologic doses of corticosteroids (>10 mg of prednisone daily or equivalent); had developed grade ≥3 autoimmune disorders (eg, pneumonitis, nephritis, neuropathy) while receiving prior immunotherapy; had HIV, active tuberculosis or a known history of tuberculosis, or active hepatitis B or C; had known hypersensitivity to any components of pembrolizumab (Part C only); had active autoimmune disease requiring systemic treatment within the prior 3 months or documented history of clinically severe autoimmune disease that has required systemic steroids and/or immunosuppressive agents; had previous interleukin (IL)-2-based or IL-15-based cytokine therapy, systemic immunomodulatory agents within 28 days prior to cycle 1 day 1, or radiotherapy within the last 4 weeks before start of study treatment administration; or had previous solid organ and/or non-autologous hematopoietic stem cell or bone marrow transplant. Patients were also excluded if they had a second malignancy within the previous 3 years except those with an adequately treated basal cell or squamous cell skin cancer, carcinoma in situ of the cervix, prostate cancer Gleason score <6 with undetectable prostate-specific antigen over the previous 12 months, or ductal breast carcinoma in situ with full surgical resection.

Part A procedures

In Part A, nemvaleukin was administered in an inpatient setting at a medical facility, with access to medical support measures for the first two treatment cycles; in the absence of dose-limiting toxicities, subsequent treatment cycles occurred in the outpatient setting. The starting dose of

0.1 µg/kg/d was selected on the basis of the minimal anticipated biological effect level. If two or more patients experienced dose-limiting toxicities at a dose level, no further dose escalations would occur. The maximum tolerated dose was defined as the dose level immediately below that for which two or more of six evaluable patients experienced dose-limiting toxicities. Adverse events in the absence of dose-limiting toxicity criteria, vital signs, clinical and laboratory assessments, and electrocardiogram parameters during the dose-limiting toxicity observation period were monitored.

Dose-limiting toxicities included the following events that could be related to nemvaleukin:

- Grade 4 neutropenia that did not recover to grade 2 (≥1000 cells/µL) within 15 days of the start of the cycle or that requires an urgent intervention.
- Febrile neutropenia (absolute neutrophil count <1000 cells/µL with temperature >38.3°C) that persisted for >48 hours or required an urgent intervention or was associated with clinically significant infection.
- Grade 4 thrombocytopenia that did not recover to grade ≤2 within 15 days of the start of the treatment cycle or thrombocytopenia equivalent to a platelet count <30,000 with clinically significant bleeding.
- Any grade 3 cardiac or central nervous system toxicity.
- Liver transaminase elevation higher than 8 × the upper limit of normal or total bilirubin higher than 6 × the upper limit of normal that that did not recover to grade ≤2 or baseline in ≤1 week.
- Grade 4 hypoalbuminemia.
- Fever >40°C (>104°F) sustained for >24 hours.
- Hypotension that required the use of pressors (eg, phenylephrine or dopamine) or prolonged hospitalization (>48 hours) for hypotension requiring medical intervention.

- Grade 3 or higher electrolyte abnormalities that did not recover to grade ≤1 in <48 hours following medical management.
- Increase in amylase or lipase that was asymptomatic grade 4, or asymptomatic grade 3 elevation that did not resolve in <14 days, or >3 × the upper limit of normal with acute severe abdominal pain.
- Grade 3 or higher nausea, vomiting, or diarrhea lasting longer than 48 hours despite maximum supportive care.
- Any other grade 4 non-hematologic toxicity or any other grade 3 non-hematologic toxicity that did not resolve to grade ≤2 within 96 hours, except fatigue or anorexia.
- Any other toxicity or AE that resulted in patient removal from the study or discontinuation of dosing by the investigator.

Part B and C procedures

For Parts B and C, nemvaleukin intravenous infusion could be administered in an outpatient setting. In Part C, nemvaleukin must have been administered 60 (±30) min after the completion of pembrolizumab infusion. Pembrolizumab was administered as an intravenous infusion over 30 min in a dose of 200 mg every 3 weeks, for up to 2 years as long as patients were deriving clinical benefit.

A serious adverse event was defined as an adverse event occurring at any dose and regardless of causality that resulted in death or posed immediate risk of death from the reaction, requiring inpatient hospitalization or prolongation of existing hospitalization, or resulting in persistent and significant disability/incapacity or a congenital anomaly/birth defect. The relationship of adverse events to study drug(s) was assessed by the investigator. In Part C, immune-mediated adverse events considered related to pembrolizumab by the investigator were managed according to the pembrolizumab prescribing information.

If due to an ongoing adverse event, a treatment cycle could be delayed by 7 calendar days (up to day 21 [cycle 1] or day 28 [cycle 2 and beyond]) until the patient has recovered from the AE. Patients could continue treatment until evidence of progressive disease, intolerance to nemvaleukin, removal by the investigator, withdrawal of consent, or any other criteria for study removal. In Part C, patients who discontinued one drug (eg, due to unacceptable toxicity that could not be managed by dose modification) had to discontinue the entire study treatment. Tumor imaging scans were conducted every 9 weeks during the follow-up assessment period until study discontinuation or initiation of new therapy. In addition, a central review might be assessed for Part B cohorts and cohorts 5, 6, and 7 in Part C.

Premedication

To reduce the potential for infusion-related fever or chills, premedication with an antipyretic agent (nonsteroidal anti-inflammatory drugs and acetaminophen) was required at least 15 min prior to nemvaleukin administration and until at least 12 h after the last dose of nemvaleukin unless the investigator had a rationale to withhold it from the patient.

Pharmacokinetics and pharmacodynamics study populations and procedures

Pharmacokinetic samples were collected on days 1 and 5 of cycle 1 and cycle 2 at pre-dose, at the completion of the infusion, and at 1, 2, 4, 8, and 16 h after the start of the infusion. Additional samples were collected at pre-dose on days 2 through 4 and on day 6 (24 hours post day 5 dosing), day 8 (cycle 1 and cycle 2), and day 15 (cycle 2 only). For cycle 3 and beyond, all pharmacokinetic samples were collected at pre-dose and at the end of the infusion on days 1, 3, and 5. The pharmacokinetics population included all patients who received at least one dose of nemvaleukin and had at least one measurable serum concentration of nemvaleukin at any scheduled pharmacokinetic time point.

Samples for pharmacodynamics analysis were collected on days 1 to 5 and 8 for cycle 1, days 1 to 5, 8, and 15 for cycle 2, days 1, 3, and 15 for cycles 3 and 4, and days 1 and 15 for subsequent cycles. The pharmacodynamics population included all patients who received at least one dose of nemvaleukin and had at least one available postbaseline pharmacodynamics measurement.

A validated electrochemiluminescence method using the Meso Scale Discovery platform was used for the quantitation of nemvaleukin in human serum. Non-compartmental pharmacokinetic analyses were performed to estimate the pharmacokinetic parameters for nemvaleukin. Pharmacokinetic and pharmacodynamic parameters were calculated by model independent procedures using Phoenix® WinNonlin® v.8.3 or R software version 3.4.2. Tables and figures were generated using R software version 3.4.2, Phoenix® WinNonlin® v.8.3 and third-party reporting tools, including Microsoft® Office 365. Parameters were computed with actual elapsed times post dose. Flow cytometry was used to assess pharmacodynamic effects by measuring circulating $CD8^+$ T cells, $FoxP3^+$ regulatory T cells, and natural killer cells in peripheral blood samples from each patient at predetermined time points.

Secondary outcomes for Parts A, B, and C

The secondary objectives in Part A were to characterize the clinical pharmacokinetic profile, pharmacodynamic effects, and antitumor activity for nemvaleukin monotherapy. The secondary objectives in Parts B and C were to characterize the clinical pharmacokinetic profile and pharmacodynamic effects, and to evaluate the duration of response (DOR), durable response rate (DRR), and time to response for patients treated with nemvaleukin alone (Part B) or in combination with pembrolizumab (Part C).

The secondary endpoints were serum concentrations of nemvaleukin and descriptive pharmacokinetic parameters, presence of anti-nemvaleukin antibodies in serum, numbers of circulating $CD8^+$ T cells, regulatory T cells, and natural killer cells in peripheral blood, serum

concentrations of IL-6 and other cytokines, disease control rate (DCR), and DOR based on RECIST guidelines. DCR was the percentage of patients who had stable disease (at cycle 4 or later for confirmed response) or had achieved a response. Secondary endpoints for Part B and Part C cohorts 5, 6, and 7 also included DRR and progression-free survival based on RECIST.

Statistical analyses

In general, all data were reported using summary statistics (n, mean, standard deviation, median, minimum, and maximum values for continuous variables and number and percentage of patients in each category for categorical variables). Dose proportionality and additional pharmacokinetic analyses were performed as appropriate. Pharmacodynamic data were also summarized descriptively. Where possible, the relationship between serum pharmacokinetic parameters or concentration of nemvaleukin and pharmacodynamic responses were evaluated by correlation analysis.

Supplemental table 1 Summary of treatment exposure in Part A (nemvaleukin monotherapy dose escalation), Part B (nemvaleukin monotherapy dose expansion), and Part C (nemvaleukin plus pembrolizumab) in patients with advanced solid tumors

Patients	**Part A** **n=46**	**Part B** **n=74**	**Part C** **n=166**
Treatment duration, median (range), weeks*	7.6 (1.4–42.1)	18.1 (0.3–178.1)	12.4 (0.7–220.1)
Number of cycles, median (range)	2 (1–12)	6 (1–52)	4 (1–71)
Exposure duration, median (range), weeks†	3.7 (0.4–38.7)	14.7 (0.1–174.4)	9.9 (0.3–218.7)
Relative dose intensity, %, median (range)	99.8 (43–139)	97.7 (46–110)	93.9 (31–168)
Adverse events leading to dose interruptions, no. (%)	13 (28.3)	37 (50.0)	81 (48.8)
Adverse events leading to dose reductions, no. (%)	2 (4.3)	6 (8.1)	3 (2.2)

*Treatment duration (week) = (last cycle end date – date of cycle 1 day 1 + 1)/7.

†Duration of exposure (week) = (last dose date – first dose date +1)/7.

Supplemental table 2 Summary of treatment-emergent adverse events in Part A (safety population)

	Nemvaleukin dose (µg/kg)							
	0.1	**0.3**	**1**	**3**	**6**	**8**	**10**	**Total**
	n=5	**n=4**	**n=7**	**n=8**	**n=12**	**n=3**	**n=7**	**N=46**
Summary								
Any event	5 (100)	4 (100)	7 (100)	8 (100)	12 (100)	3 (100)	7 (100)	46 (100)
Serious events	2 (40)	0	2 (29)	3 (38)	5 (42)	0	5 (71)	17 (37)
Grade ≥3 events	3 (60)	1 (25)	3 (43)	6 (75)	9 (75)	2 (67)	6 (86)	30 (65)
Any treatment-related event	3 (60)	3 (75)	7 (100)	8 (100)	12 (100)	3 (100)	7 (100)	43 (93)
Grade 1 or 2 treatment-related events	3 (60)	3 (75)	7 (100)	8 (100)	12 (100)	3 (100)	7 (100)	43 (93)
Grade ≥3 treatment-related events	0	0	0	5 (63)	6 (50)	2 (67)	4 (57)	17 (37)

	Nemvaleukin dose (µg/kg)							
	0.1	**0.3**	**1**	**3**	**6**	**8**	**10**	**Total**
	n=5	**n=4**	**n=7**	**n=8**	**n=12**	**n=3**	**n=7**	**N=46**
Events leading to discontinuation	1 (20)	0	1 (14)	4 (50)	1 (8)	1 (33)	2 (29)	10 (22)
Events leading to death*	0	0	0	0	1 (8)	0	1 (14)	2 (4)
Most common treatment-emergent adverse events, reported in ≥20% of overall patients								
Pyrexia	0	2 (50)	5 (71)	8 (100)	12 (100)	2 (67)	3 (43)	32 (70)
Chills	0	2 (50)	5 (71)	8 (100)	11 (92)	3 (100)	2 (29)	31 (67)
Fatigue	4 (80)	1 (25)	2 (29)	3 (38)	4 (33)	2 (67)	3 (43)	19 (41)
Vomiting	2 (40)	2 (50)	2 (29)	4 (50)	5 (42)	3 (100)	0	18 (39)
Hypotension	0	1 (25)	0	3 (38)	7 (58)	1 (33)	4 (57)	16 (35)
Nausea	2 (40)	1 (25)	1 (14)	4 (50)	5 (42)	3 (100)	0	16 (35)
Anemia	1 (20)	0	3 (43)	3 (38)	1 (8)	2 (67)	5 (71)	15 (33)
Constipation	2 (40)	1 (25)	2 (29)	4 (50)	1 (8)	3 (100)	1 (14)	14 (30)
Dyspnea	2 (40)	1 (25)	1 (14)	3 (38)	2 (17)	1 (33)	2 (29)	12 (26)
Abdominal pain	1 (20)	0	2 (29)	2 (25)	1 (8)	3 (100)	2 (29)	11 (24)

	Nemvaleukin dose (µg/kg)							
	0.1	**0.3**	**1**	**3**	**6**	**8**	**10**	**Total**
	n=5	**n=4**	**n=7**	**n=8**	**n=12**	**n=3**	**n=7**	**N=46**
Decreased appetite	1 (20)	1 (25)	1 (14)	3 (38)	1 (8)	1 (33)	2 (29)	10 (22)
Headache	0	1 (25)	1 (14)	2 (25)	3 (25)	2 (67)	1 (14)	10 (22)
Neutropenia	0	0	0	2 (25)	2 (17)	2 (67)	4 (57)	10 (22)
Blood creatinine increased	0	0	3 (43)	4 (50)	1 (8)	1 (33)	0	9 (20)
Hypertension	1 (20)	1 (25)	1 (14)	2 (25)	2 (17)	2 (67)	0	9 (20)
Grade ≥3 treatment-related treatment-emergent adverse events								
Neutropenia	0	0	0	2 (25)	2 (17)	1 (33)	0	5 (11)
Anemia	0	0	0	0	1 (8)	0	1 (14)	2 (4)
Hyperbilirubinemia	0	0	0	1 (13)	0	0	1 (14)	2 (4)
Pyrexia	0	0	0	0	2 (17)	0	0	2 (4)
Fatigue	0	0	0	0	0	1 (33)	0	1 (2)
Diarrhea	0	0	0	1 (13)	0	0	0	1 (2)
Hypoalbuminemia	0	0	0	1 (13)	0	0	0	1 (2)

	Nemvaleukin dose (µg/kg)							
	0.1 **n=5**	**0.3** **n=4**	**1** **n=7**	**3** **n=8**	**6** **n=12**	**8** **n=3**	**10** **n=7**	**Total** **N=46**
Increased aspartate aminotransferase	0	0	0	0	1 (8)	0	0	1 (2)
Increased blood alkaline phosphatase	0	0	0	0	0	0	1 (14)	1 (2)
Acute kidney injury	0	0	0	0	0	0	1 (14)	1 (2)
Cholangitis	0	0	0	1 (13)	0	0	0	1 (2)
Febrile neutropenia	0	0	0	1 (13)	0	0	0	1 (2)
Lymphopenia	0	0	0	1 (13)	0	0	0	1 (2)
Increased gamma glutamyltransferase	0	0	0	0	0	0	1 (14)	1 (2)

Data as of 27 March 2023. Data are presented as number of patients (%).

*One patient in the 6 µg/kg group experienced a pneumonia aspiration with fatal outcome and another patient in the 10 µg/kg group experienced sepsis with fatal outcome (both events assessed as not related to nemvaleukin by the investigator). The safety population included all patients who received nemvaleukin. Adverse events coded using Medical Dictionary for Regulatory Activities (MedDRA) version 25.0.

The toxicity severity of adverse events was graded using National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03 and version 5.0.

Supplemental table 3 Serum pharmacokinetic parameters of nemvaleukin following the first intravenous infusion in cycle 1 day 1 in part A in patients with advanced solid tumors

	Nemvaleukin dose µg/kg/d						
	0.1 **n=5**	**0.3** **n=4**	**1** **n=6**	**3** **n=8**	**6** **n=12**	**8** **n=3**	**10** **n=6**
AUC_{0-24} (h*ng/mL)*	NC	21.90 (21.9)‡	82.40 (37.40)§	327.00 (20.10)‖	585.00 (28.9)	668.00 (31.30)	771.00 (39.90)
AUC_{last} (h*ng/mL)*	3.33 (63.7)	17.70 (33.3)	70.40 (40.90)	286.00 (44.90)	578.00 (29.6)	649.00 (36.00)	764.00 (39.00)
C_{max} (ng/mL)*	2.24 (47.1)	6.84 (22.0)	21.10 (25.40)	64.90 (46.30)	118.00 (30.0)[f]	157.00 (36.10)	159.00 (20.30)
t_{max} (h)†	0.60 (0.57, 0.83)	0.63 (0.52, 0.73)	0.55 (0.50, 0.78)	0.57 (0.50, 0.75)	0.57 (0.50, 0.70)¶	0.57 (0.53, 0.57)	0.51 (0.50, 0.62)
t_{last} (h)†	2.10 (1.92, 4.58)	7.81 (3.97, 7.98)	8.02 (4.23, 23.90)	22.50 (4.02, 23.83)	22.71 (10.10, 24.00)	22.53 (8.25, 22.58)	22.88 (21.45, 25.25)

*AUCs and C_{max} are expressed as arithmetic mean (CV%).

†Time expressed as median (min, max).

‡n=3.

§n=4.

‖n=7.

¶n=11.

NC, not calculated (unreliable terminal phase characterization); AUC_{0-24}, area under the concentration-time curve from time zero to 24 hours post dose; AUC_{last}, AUC from time zero to the last quantifiable concentration; C_{max}, maximum concentration observed at the end of infusion; t_{max}, time to C_{max}; t_{last}, time to last quantifiable concentration.

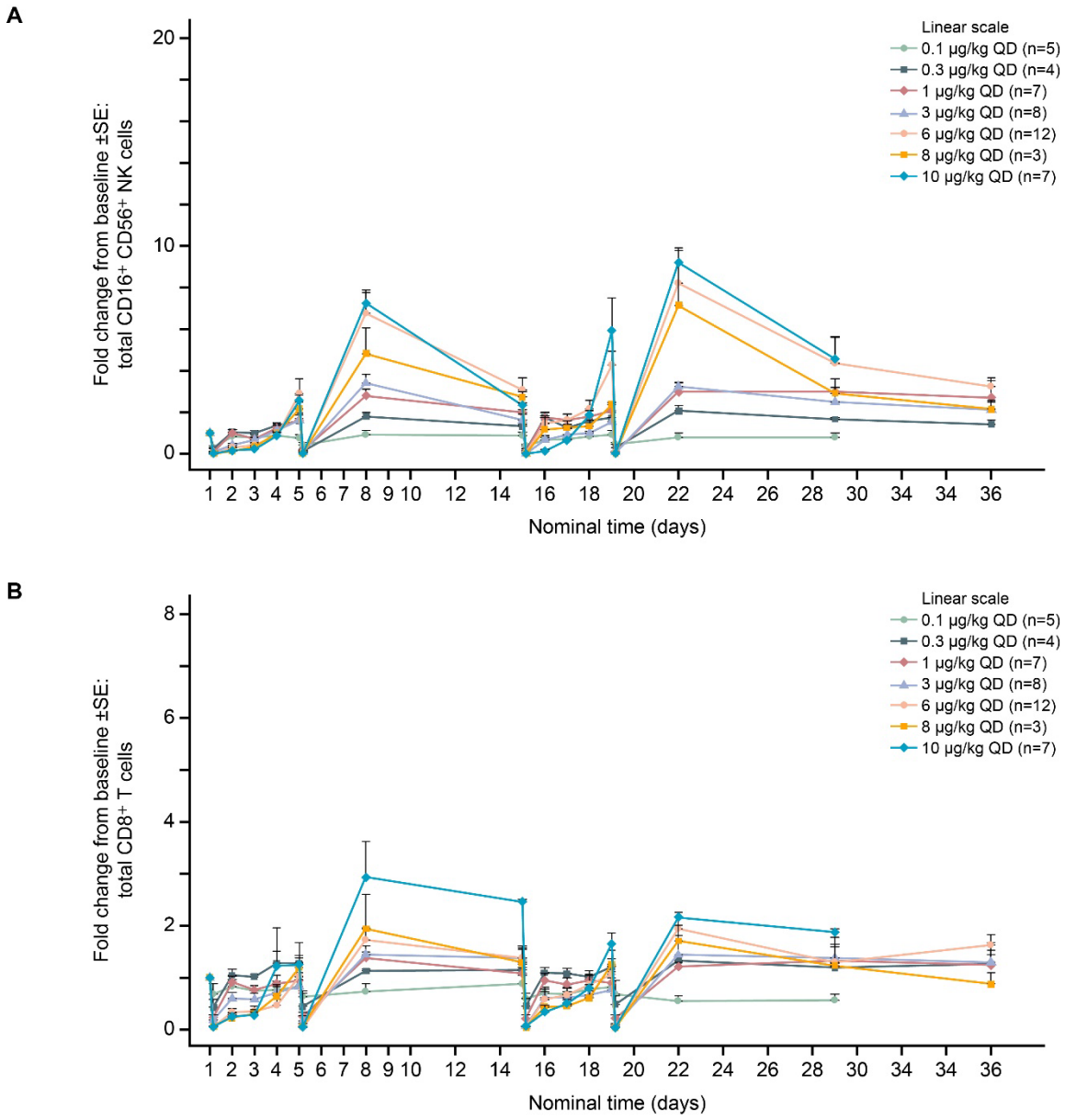
Supplemental table 4 Maximum fold change from baseline in immune cell expansion during cycles 1 and 2

	NK ($CD16^+/56^+$) **n=61**	**$CD8^+$ T cells** **n=61**	**T_{regs}** **n=54**
F_{max}, mean (±SE)	6.52 (±0.41)	2.53 (±0.14)	2.57 (±0.38)
Range	1.99–16.23	0.87–6.55	0.89–18.82

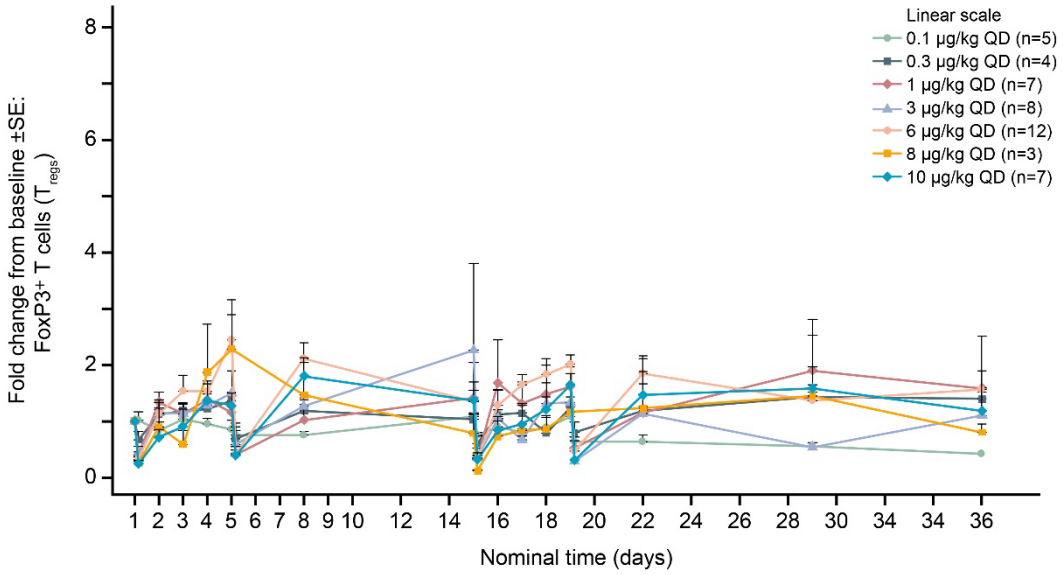
Analysis was conducted in antitumor evaluable population.

F_{max}, maximum fold change; NK, natural killer; SE, standard error; T_{regs}, regulatory T cells.

Supplemental figure 1 Dose-dependent pharmacodynamic changes following nemvaleukin administration in patients with advanced solid tumors in Part A. Fold change from baseline in (A) natural killer (NK) ($CD16^+CD56^+$) cells, (B) $CD8^+$ T cells, and (C) regulatory T cells (T_{regs}).



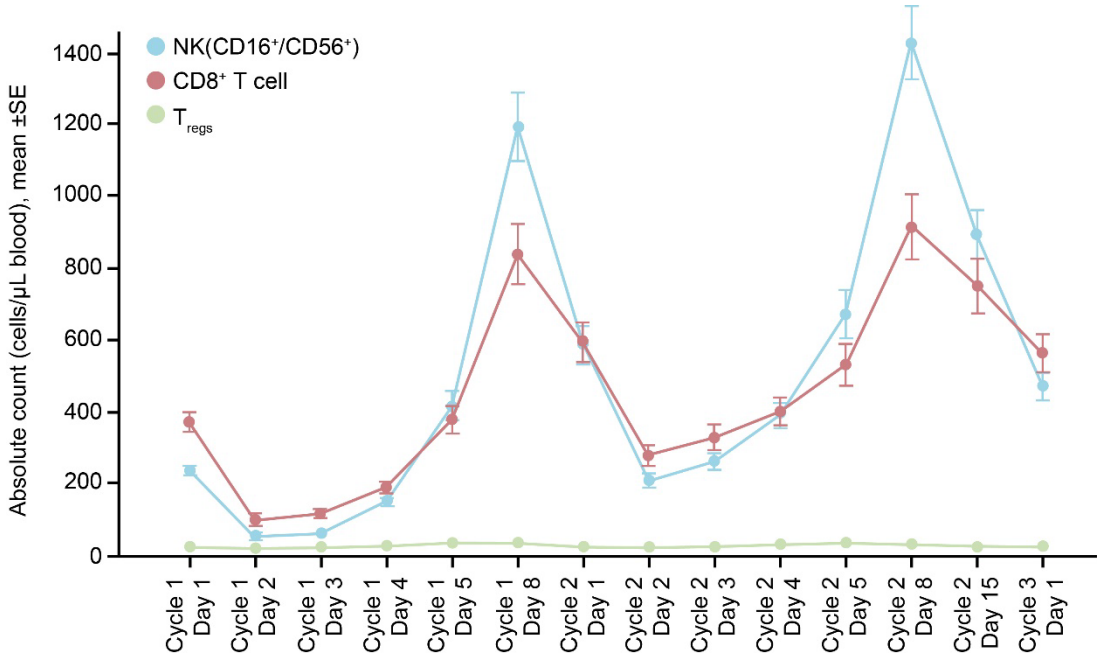
C



Whole blood total cells fold change from baseline profiles following the administration of nemvaleukin intravenous infusions in cycles 1 and 2. Data are presented as mean ± standard error (SE).

QD, daily.

Supplemental figure 2 Clinical pharmacodynamic effects of nemvaleukin monotherapy in whole blood in patients with advanced melanoma or renal cell carcinoma in Part B.



Absolute immune cell expansion (cells/µL) during cycles 1–2. Data are presented as mean ± standard error (SE).