Effects of RN168 on immune cells. The changes in white blood cell counts (WBC) and T, B, and NK cells are shown in **Table 1, Figure 3, Supplemental Figure 2**. Total WBC and total lymphocyte counts were compared with the baseline levels throughout the study. The WBC counts declined within the first week of drug administration but remained in the normal range in all but 1 subject who was in the 3 mg/kg group (**Table 1, Supplemental Figure 2A**).

When the pooled data were analyzed with a mixed model with repeated measures with fixed effects for baseline levels, there was a significant decline in the naive CD4+ but not CD8+ naive T cells (P<0.01, p=0.07, respectively) (**Supplemental Table 3, Figure 3A, B**). There was also a significant decline in CD4+ effector memory and central memory T-cell populations compared with the placebo cohort (**Figure 3C, E**) (CD4 effector memory, *P* = 0.001, CD4 central memory, *P* = 0.0007). Although CD8+ effector memory and central memory cells were also decreased (*P* = 0.012, 0.017 respectively), there was greater variability compared with the CD4+ T cells (**Figure 3D, F**). These described changes in T-cell subsets were seen at all doses of the drug except for 1 mg/kg Q2wk in which only the effector memory CD4+ cells showed a significant decline over time (*P* = 0.003).

There was a general increase in the frequency of CD4+ and CD8+PD-1+ T cells at day 85, but the changes in the PD-1+ cells did not reach statistical significance (**Supplemental Figure 3**, (mixed model with pooled data with fixed effects for baseline values, *P* = 0.11 for both).

The absolute number of Tregs declined at all doses greater than 1 mg/kg (**Figure 4A**). The ratio of Tregs to CD4+ and CD8+ effector memory T cells was increased at all doses, reaching statistical significance at the 6 mg/kg Q1wk dose for CD4+ effector memory T cells (*P* = 0.04) (**Figure 4B , C**) (mixed model with repeated measures with fixed effects for baseline levels). These ratio effects were attributed to enhanced effects of RN168 on reducing effector T

cells (**Figure 3**) than Tregs. The relative number of NK cells increased at doses above 1 mg/kg but we did not observe changes in B cells **(Supplemental Figures 2D and E).**

Effects of RN168 on the transcriptome of T cells. Total RNA was isolated from CD4+ and CD8+ T cells magnetically separated from previously frozen peripheral blood mononuclear cells (PBMC). Whole transcriptome analysis of CD4+ and CD8+ PBMC T cells was performed using RNA-seq and the differences in gene expression were compared between day 85 and baseline. In CD8+ T cells there were 60- downregulated and 1 upregulated protein-coding genes that showed a statistically significant change (paired test from DEseq2, $P < 0.05$) after correction for multiple comparisons. In the CD8+ cells, a pathway analysis identified reduced expression of genes involved in T cell activation (*CD40L*), differentiation (*LTK, KLRB1*), and effector function (*IL-12, GZMK*) (**Figure 5C, D**). In the CD4+ T cells there were 60- downregulated and 3-upregulated protein-coding genes (**Figure 5A, B**). Genes down-regulated in CD4+ T cells included those associated with T-cell trafficking and differentiation (*CCR4, CCR6, Tbet, CXCR3*). There was limited overlap in the genes down-regulated in CD4 and CD8 T-cell subsets suggesting differential effects of RN168 on each cell population.

To further detail the PD effects of RN168 on T-cell gene expression profiles, the relationship between serum RN168 exposure expressed as the average serum levels (Cavg), and gene expression change between day 85 and baseline in CD8+ T cells was examined. Using this dose-response analysis, there were 220 genes downregulated and 80 upregulated ($P < 0.01$). **Supplemental Table 4** shows a listing of the changes in the top 50 differentially expressed genes in CD8+ T cells associated with exposure to RN168. The strongest relationships were between the reduced expression of suppressor of cytokine signaling-2 (SOCS2), Bcl-2, and increased expression of cyclin dependent kinase inhibitor-2A interacting protein (CDKN2AIP), a protein that regulates DNA damage response (**Figure 6A**). Members of

the IL7R signaling pathway were specifically analyzed (**Supplemental Table 5**, **Figure 6B**). The expression of IL2RA (CD25, correlation between expression change and Cavg by linear regression, *P* = 0.008) and the common γ chain (*P* = 0.0053), and other transcripts associated with signaling (MAP2K2, STAT5B) showed reduced expression at higher Cavg. The *IL7R* gene itself showed increased gene expression at higher Cavg (CD127, *P* = 0.024) (**Figure 6C**).

To confirm our transcriptome findings, we analyzed the expression of one of the affected genes, Bcl-2 in CD3+ T cells, by flow cytometry. The protein expression was reduced overall in both CD4+ (molecules of equivalent soluble fluorochrome; pooled RN168 vs placebo: mixed model with pooled data with repeated measures and fixed effects for baseline values, *P* = 0.0014; **Figure 7A**) and CD8+ (*P* = 0.008) T cells (**Figure 7B**). The levels were reduced at day 85 (*P* = 0.0009 and 0.02) and returned to baseline values approximately 30 days after the last dose (CD8+ not shown).

Table 3 can be deleted. It is a duplicate of Supplemental Table 5 which is called out in the text. I made a modification to Supplemental Table 5 to include the statistical test.

Supplemental Material

Supplemental Methods

Complete inclusion/exclusion criteria. Adult patients diagnosed with type 1 diabetes (T1D) within 2 years of study entry and with at least 1 T1D-related autoantibody without other significant medical conditions or use of other immune modulatory therapy were enrolled. Additional inclusion criteria were peak stimulated C-peptide levels ≥0.15 ng/mL (≥0.05 nmol/L) measured during mixed meal tolerance test before randomization, body mass index 18.5 to 32 kg/m^2, and total body weight 40 to 120 kg (inclusive).

Exclusion criteria included ≥2 hospitalizations for diabetic ketoacidosis within 90 days of randomization; severe hypoglycemia within 60 days of randomization; history of organ transplant including islet-cell transplant; febrile illness within 24 hours of randomization; and evidence or history of diabetic complications involving significant end-organ damage (eg, proliferative retinopathy, macular edema, diabetic nephropathy, severe neuropathy, neuropathic ulcers). Any acute infections had to be resolved before study treatment was initiated. Potential subjects with any significant hematologic, renal, other endocrine, pulmonary, gastrointestinal, cardiovascular, hepatic, psychiatric, neurologic, or allergic disease, and any severe acute/chronic condition that might increase risk associated with study participation or interfere with interpretation of study results were also excluded, as were those with a history of cancer, other than cutaneous basal cell or squamous cell cancer cured by excision, within 5 years before the study. Additional exclusion criteria included a history of any clinical significant cardiovascular or cerebrovascular event or procedure within 1 year of randomization; current New York Heart Association class III or IV congestive heart failure; or poorly controlled hypertension (systolic blood pressure [BP] >160 mm Hg or diastolic BP >90 mm Hg), and donation of ≥550 mL of blood within 56 days of randomization or intent to donate blood during the study period. Persons with a history of alcoholism or drug addiction, use of recreational

drugs within 12 months of screening, or positive urine drug screen for substances of abuse at screening were prohibited from enrolling. Women who were pregnant or lactating were excluded, and fertile men and women had to agree to use 2 highly effective methods of contraception during the study. Ongoing use of diabetes medications other than insulin, medications that affect either the course of T1D or immunologic status, or systemic glucocorticoids, as well as treatment with any monoclonal antibody within the longer of 6 months or 5 half-lives, or receipt of any other investigational drug within 30 days or 5 half-lives were also grounds for exclusion. Additional exclusion criteria included history of allergy/anaphylactic reaction to any monoclonal antibodies or their components, sensitivity to heparin or any of the components of the study drug, or heparin-induced thrombocytopenia, as well as any vaccination other than influenza virus vaccine in the 4 weeks prior to randomization. Potential subjects could not enroll if they were positive test for TB, HIV, or hepatitis B or C; had hemoglobin <12 g/dL in men or <11 g/dL in women, direct bilirubin >ULN, ALT or AST ≥2xULN, serum creatinine >1.5xULN, TSH >ULN or <LLN, HbA1c >9%, or other abnormal lab value of clinical significance; or a clinically significant ECG abnormality.

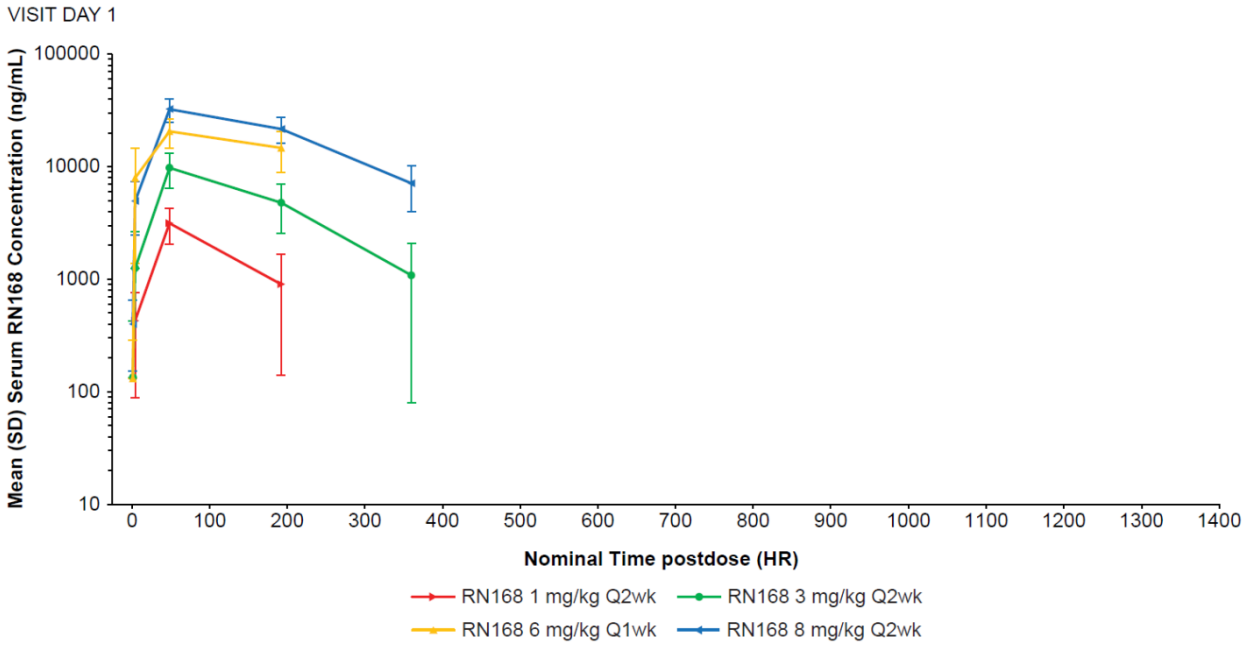
Persons who were currently participating or who anticipated participation in another clinical trial were excluded.

Pharmacokinetic sampling. Blood samples for pharmacokinetic (PK) analysis were collected at baseline, 1 and 4 hours postdose on day 1, 48 hours postdose on day 3; days 8, 15, 29, 43, and 57; day 71 (at hours at 0, 1, and 4 hours postdose in the first 3 cohorts; just once in the fourth cohort); 48 hours postdose on day 73 (cohorts 1–3 only); day 78 (just once in cohorts 1–3; at hours 0, 1, and 4 in cohort 4); and day 85; and then during the follow-up period on days 92, 99, 113, and 127. RN168 concentrations were analyzed at ICON Development Solutions, LLC (Whitesboro, NY, USA) using a validated sensitive and specific ELISA with a between-day accuracy (% relative error) ranging from 0.444% to 20.0% and assay precision (between-day % coefficient of variation) ≤9.81%.

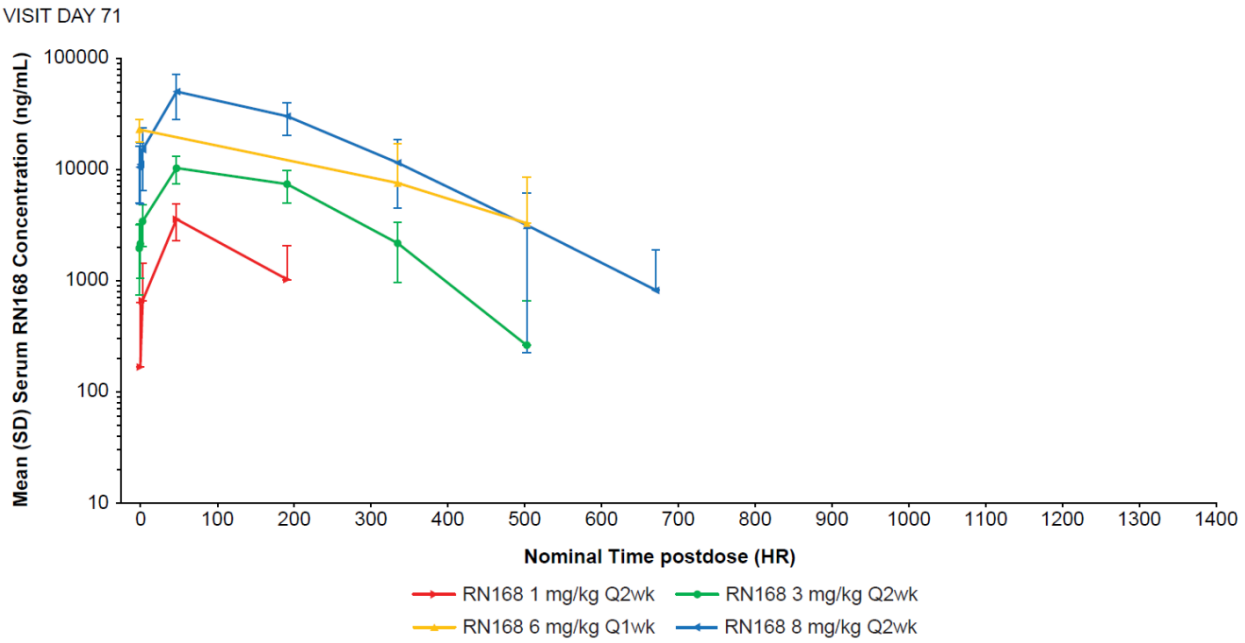
PK parameters for subjects positive for antidrug antibodies were analyzed separately to assess the effects of the antibodies on RN168 concentrations; if there was no effect on concentration versus time profile, these subjects were included in the overall descriptive statistics of concentrations and PK parameters.

Supplemental Figure 1. Median (SD) serum RN168 concentration-time profiles in adults with type 1 diabetes Serum RN168 concentrations were summarized descriptively by dose and nominal PK sampling time. (See Supplemental Table 2.)

A After the first dose

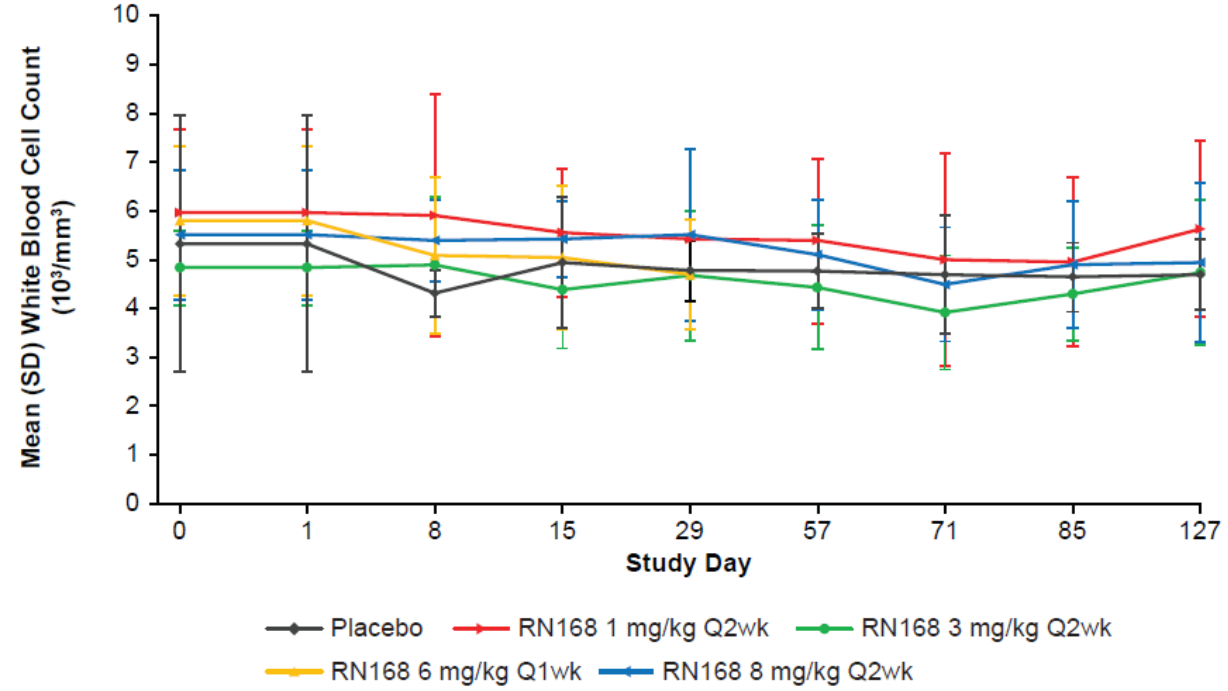


B After multiple doses

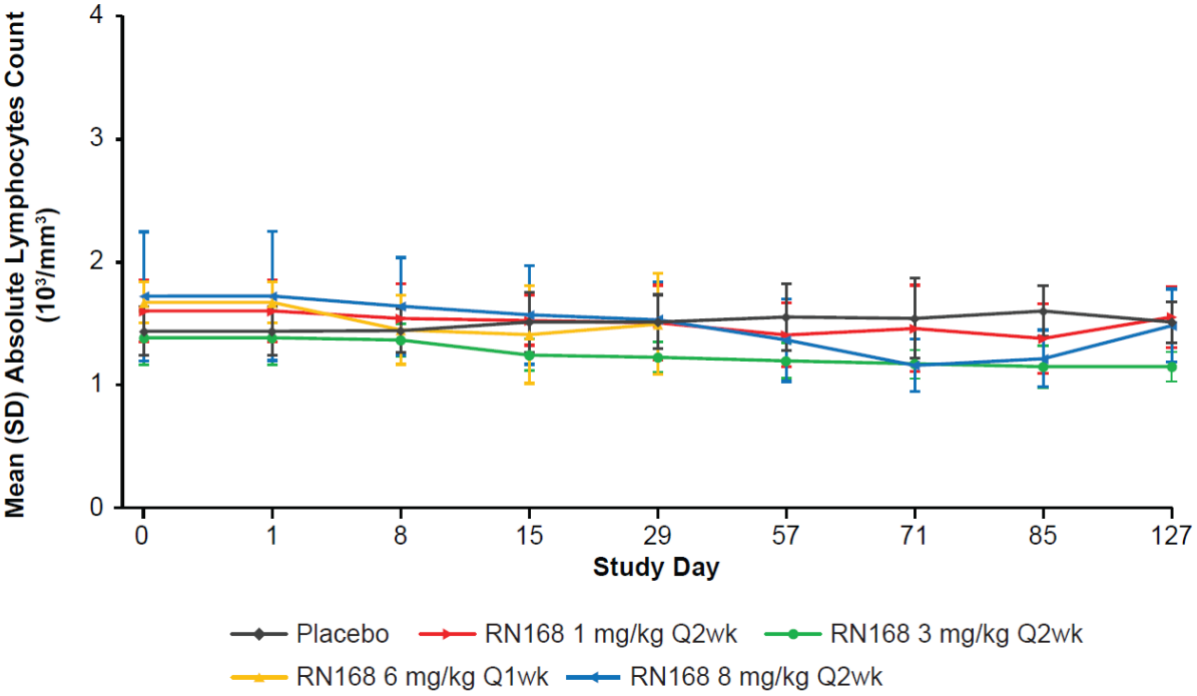


Supplemental Figure 2. Effects of RN168 on mean (SD) immune cells. (A) white blood cell count, (B) absolute lymphocytes count, (C) percent CD3+ T Cells, (D) percent PCD19+ B cells, (D) percent CD16+56+ natural killer cells as percentage of total lymphocytes sampled, during treatment with RN168.

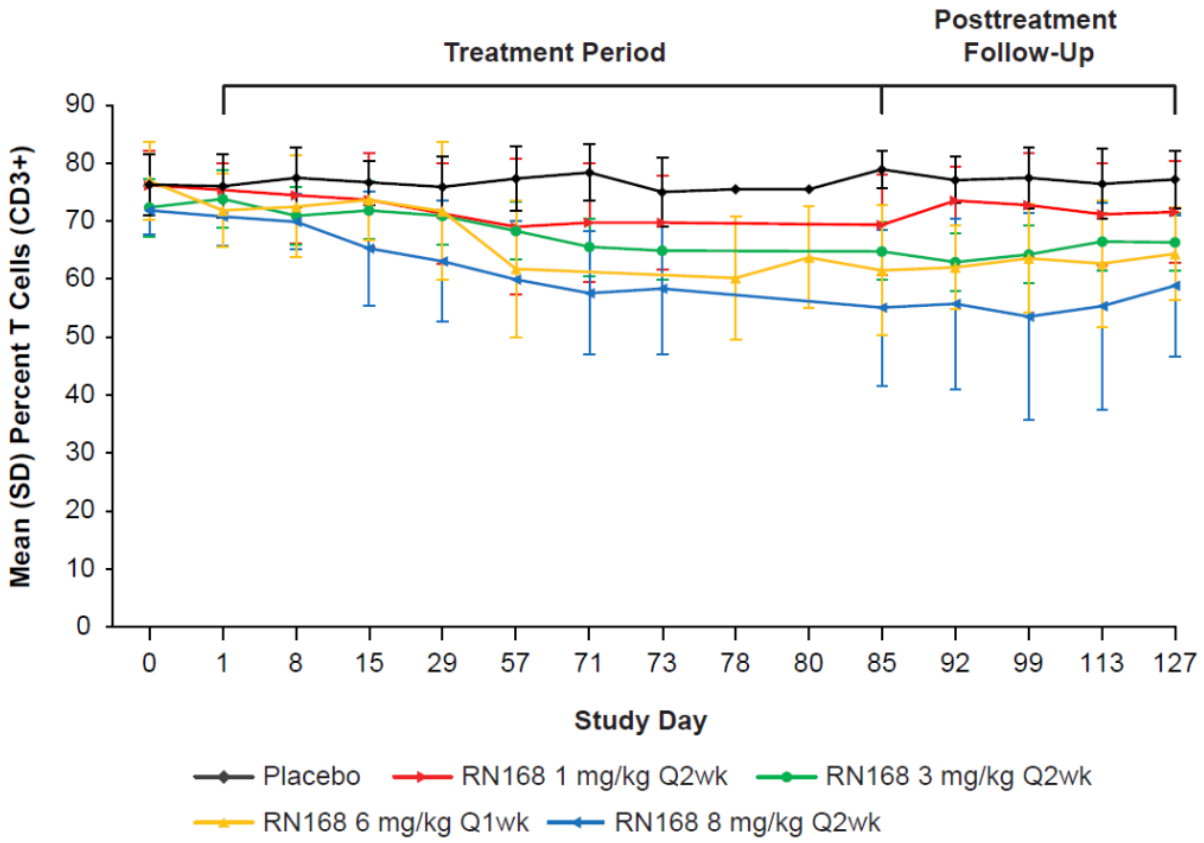
A White blood cell count



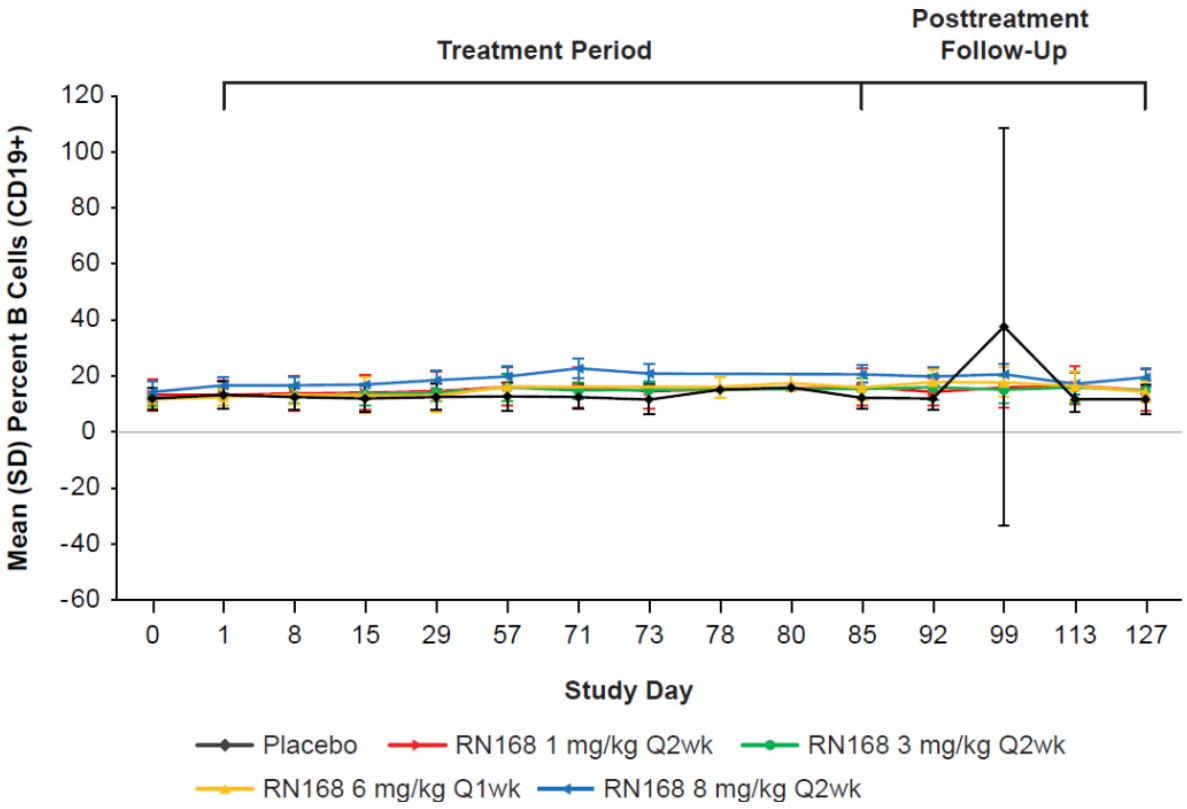
B Absolute lymphocytes count



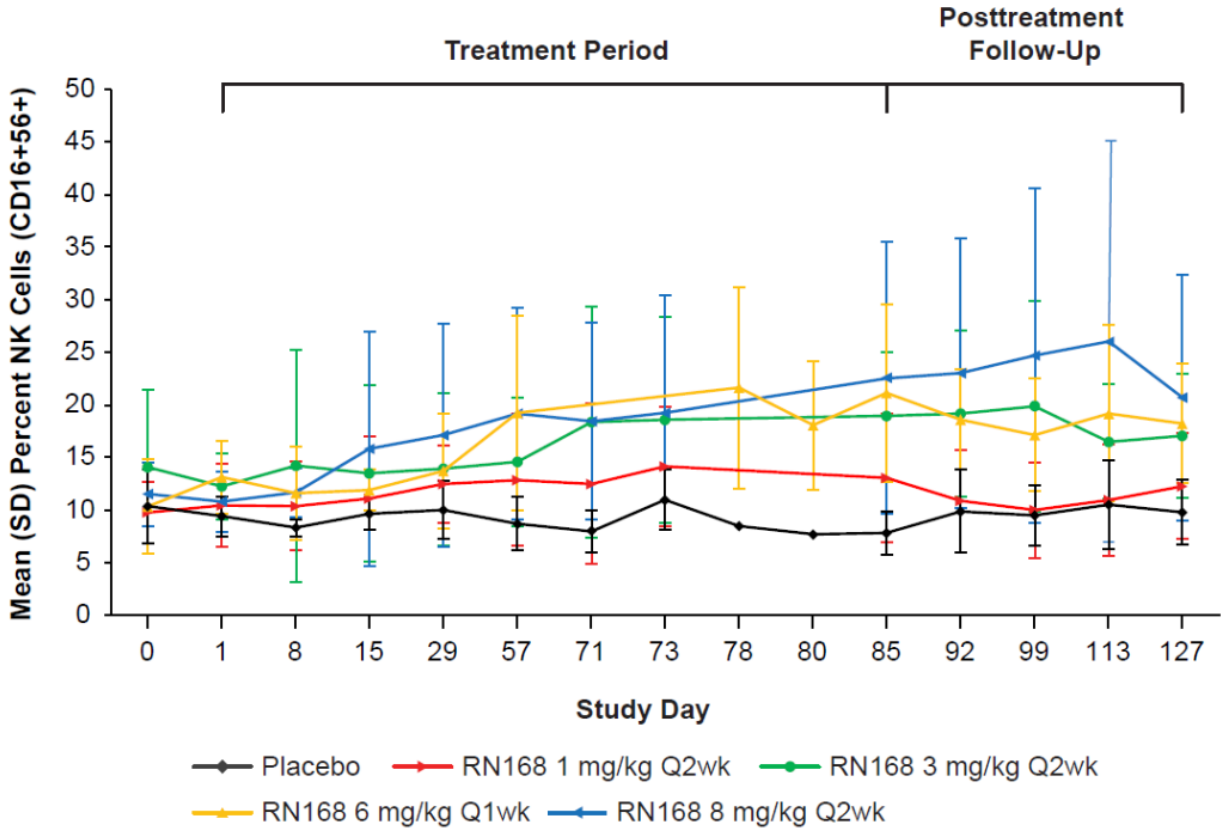
C CD3+ T Cells


Treatment Period
Posttreatment Follow-Up
Mean (SD) Percent T Cells (CD3+)
90
80
70
60
50
40
30
20
10
0
0
1
8
15
29
57
71
73
78
80
85
92
99
113
127
Study Day
Placebo
RN168 1 mg/kg Q2wk
RN168 3 mg/kg Q2wk
RN168 6 mg/kg Q1wk
RN168 8 mg/kg Q2wk

D CD16+56+ natural killer cells as percentage of total lymphocytes sampled

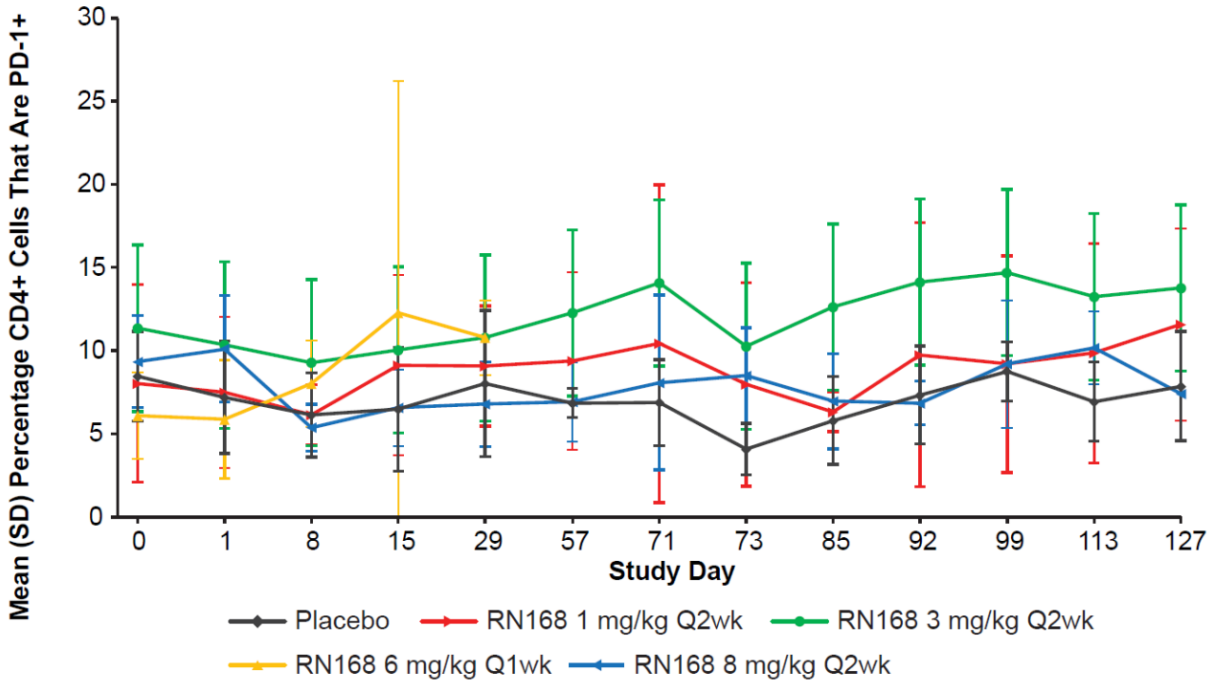


E CD16+56+ natural killer cells as percentage of total lymphocytes sampled

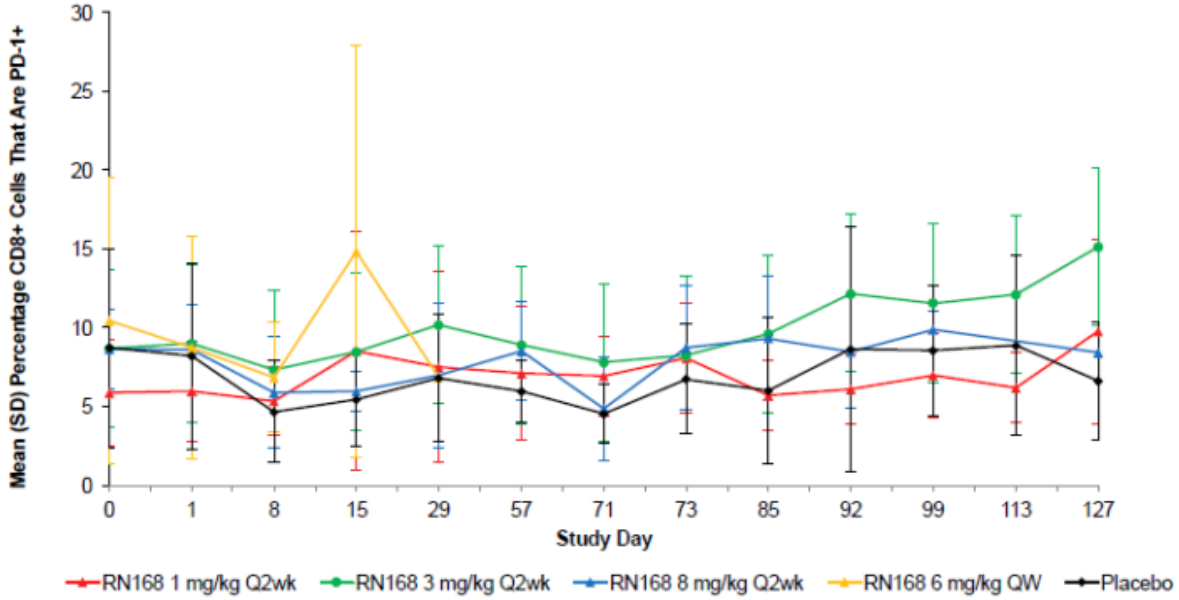


Supplemental Figure 3. Effects of RN168 on PD-1+ T cells.

A CD4+PD-1+ Cells

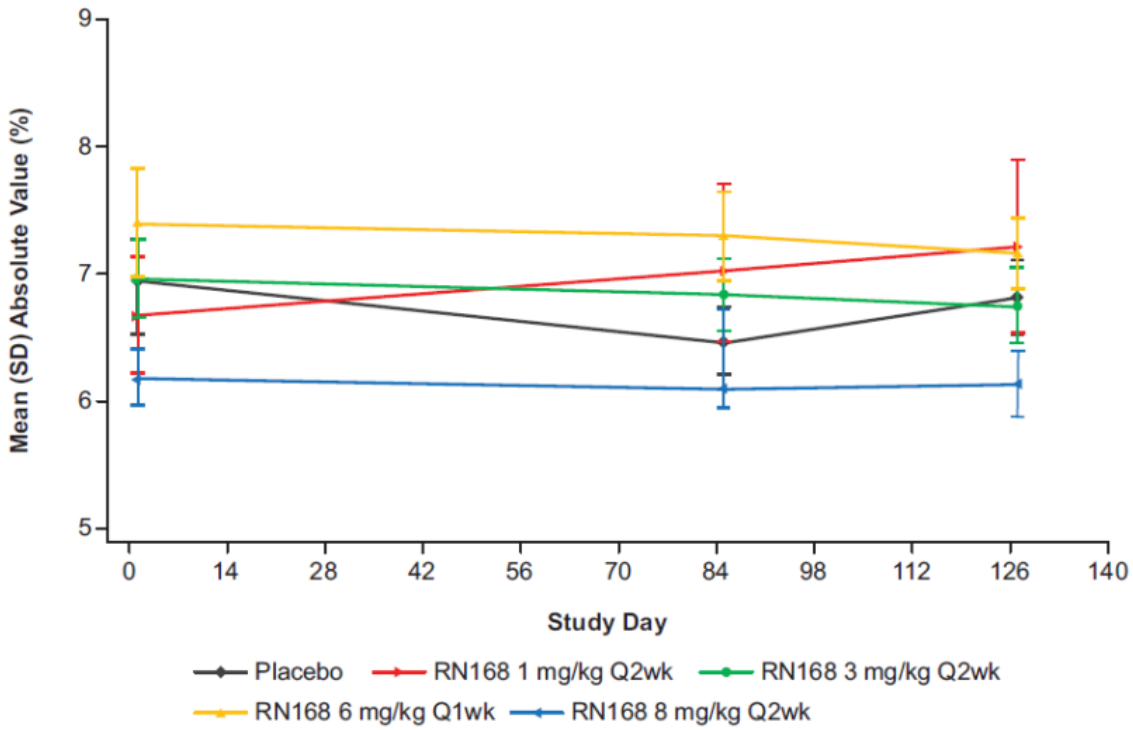


B CD8+PD-1+ Cells

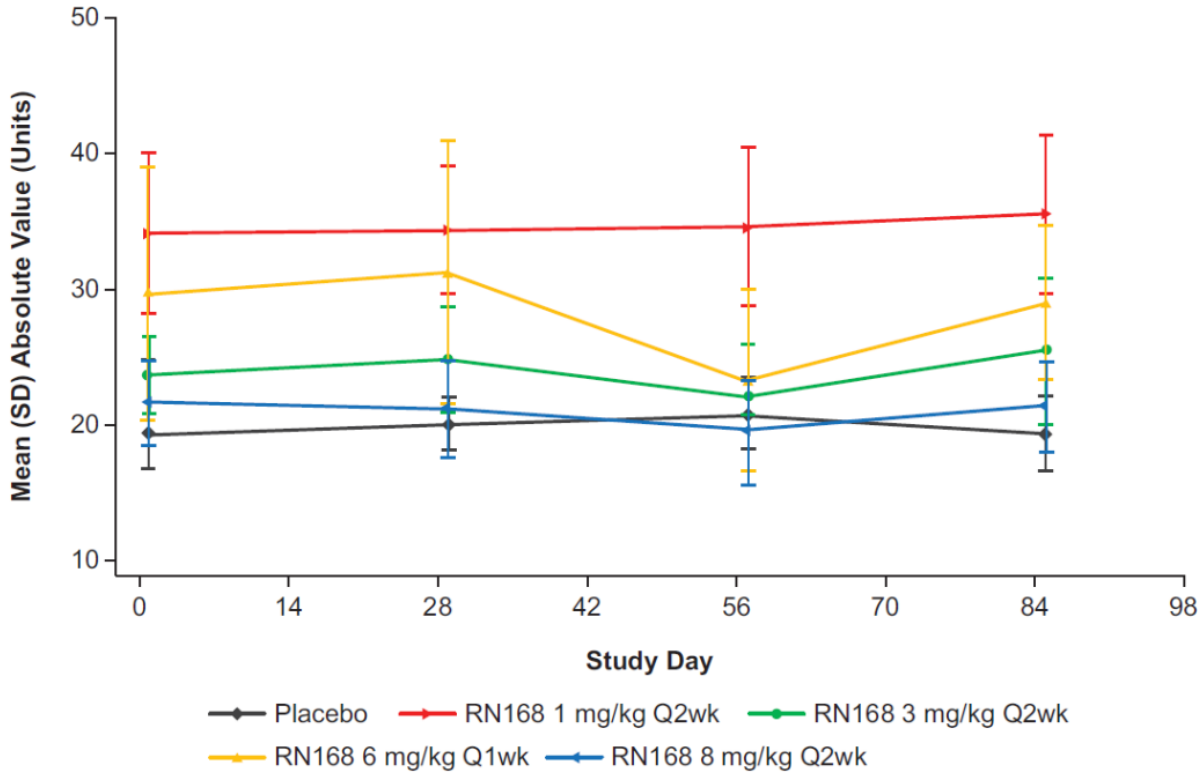


Supplemental Figure 4. Effects of RN168 on Hemoglobin A1c and insulin use over time.

A Hemoglobin A1c



B Insulin use



Supplemental Table 1. Subject demographics

Parameter	RN168 1 mg/kg Q2wk (N=8)	RN168 3 mg/kg Q2wk (N=9)	RN168 8 mg/kg Q2wk (N=8)	RN168 6 mg/kg QW (N=5)	Placebo (N=7)
Sex, n					
Male	5	3	4	5	5
Female	3	6	4	0	2
Age, years					
Mean (SD)	25.6 (8.5)	37.8 (15.8)	30.8 (7.7)	29.0 (12.3)	33.9 (11.1)
Range	19–44	20–59	20–42	18–49	19–53
Race, n					
White	8	7	8	5	7
Black	0	1	0	0	0
Other	0	1	0	0	0
Ethnicity, n					
Hispanic/Latino	0	0	1	0	0
Not Hispanic/Latino	8	9	7	5	7
BMI, kg/m^2					
Mean (SD)	25.1 (3.6)	24.3 (2.7)	21.8 (2.2)	24.9 (2.6)	24.2 (2.1)
Range	19.8–30.6	18.7–27.3	19.2–25.0	22.1–27.5	19.7–25.8

BMI, body mass index; QW, once weekly; Q2wk, once every 2 weeks; SD, standard deviation.

Supplemental Table 2. Summary of serum RN168 pharmacokinetic parameter values[A] following single and multiple (Q2wk or QW) subcutaneous doses. Pharmacokinetic parameters assessed included maximum concentration (C_{max}), time to C_{max} (T_{max}), area under the concentration-time curve from time 0 to time tau (AUC_τ; the dosing interval, where τ=168 h for weekly dosing and 336 h for dosing once every 2 weeks), average concentration over the dosing interval (C_{av}), lowest concentration (C_{min}), terminal half-life ($T_{1/2}$), apparent clearance (CL/F), apparent volume of distribution (V_z/F), observed accumulation ratio (R_{ac}), and observed accumulation ratio for C_{max} ($R_{ac,Cmax}$).

Parameter	RN168 1 mg/kg Q2wk	RN168 3 mg/kg Q2wk	RN168 8 mg/kg Q2wk	RN168 6 mg/kg Q1wk
Single SC dose (day 1)				
N	8	8	8	5
AUC_τ, ng•h/mL	384,900 (43)[B]	1,323,000 (43)	5,805,000 (28)	2,570,000 (31)
C_{max}, ng/mL	2114 (83)	8512 (42)	31,610 (27)	20890 (29)
T_{max}, h	48.8 (45.9–169)	48.9 (47.4–96.8)	51.8 (45.5–71.8)	48.1 (46.2–73.0)
C_{av}, ng/mL	1145 (43)[B]	3933 (43)	17,300 (28)	15,300 (31)
Multiple SC doses (day 71)				
N, n	8, 1	8, 5	8, 7	2, 2
AUC_τ, ng•h/mL	426,700 (58)[C]	2,029,000 (29)	7,869,000 (42)	NC[E]
C_{max}, ng/mL	2612 (89)	10,600 (22)	39,870 (34)	NC[E]
T_{max}, h	48.9 (22.5–72.1)	60.2 (45.8–143)	86.3 (47.5–146)	NC[E]
C_{av}, ng/mL	1270 (58)[c]	6,037 (29)	23,420 (42)	NC[E]
CL/F, mL/h/kg	2.340 (58)	1.477 (29)	1.0 (42)	NC[E]
V_z/F, mL/kg	NR[D]	141.2 (18)	129.5 (18)	NC[E]
C_{min}, ng/mL	29.0 (283)	1,745 (57)	9,547 (67)	NC[E]
$T_{½}$ h	NR[D]	64.6 (7.3)	85.5 (23.5)	NR[E]
R_{ac}	1.115 (48)[C]	1.5 (28)	1.4 (19)	NC[E]
R_{ac},C_{max}	1.235 (66)	1.2 (25)	1.3 (21)	NC[E]

[A]Geometric mean (geometric percent coefficient of variation [%CV]) for all except median (range) for T_{max}, arithmetic mean (SD) for $T_{1/2}$, and arithmetic mean (%CV) for C_{min}.
[B]3 subjects had reportable AUC_τ and C_{av} in this group.
[C]6 subjects had reportable AUC_τ and C_{av} and 3 subjects had reportable R_{ac} in this group.
[D]Parameters not calculated because the first post-dose measurement was 168 hours (τ).
[E]Parameters not reported because fewer than 3 subjects had reportable values.

$AUC_{\tau,}$ area under the concentration-time curve from time 0 to time tau; C_{av}, average concentration over the dosing interval; CL/F, apparent clearance; C_{max} , maximum concentration; C_{min}, lowest concentration; QW, once weekly; Q2wk, once every 2 weeks; R_{ac}, observed accumulation ratio; SC, subcutaneous; T_{max}, time to maximum concentration; $R_{ac,Cmax}$, observed accumulation ratio for C_{max}; V_z/F, apparent volume of distribution.

Supplemental Table 3. RN168 treatment-related effects on depletion of memory and naive T cells (mixed model with pooled data with fixed effects for baseline values,)

Cell subset (cells/µl)	**Difference in LS means (RN168 vs placebo)**	**Standard error**	**90% CI**	***P* value**
CD4+ central memory	-157.7	40.3	-227, -88.5	0.0007
CD8+ central memory	-26.6	10.2	-44.2, -9	0.0168
CD4+ effector memory	62.9	16.6	-91.5, -34.4	0.001
CD8+ effector memory	-37.7	13.6	-61.2, -14.2	0.0124
CD4+ naive	-135.9	48	-218.3, -53.5	0.0097
CD8+ naive	-48.1	25.6	-92.1, -4	0.075

Supplemental Table 4. Association of IL2RG expression with RN168 C_{avg} in CD8+ T cells.

Gene Name	t-statistic*	*P* value
SOCS2	-6.03	2.32 E-05
BCL2	-5.58	5.29 E-05
TMEM251	-5.38	7.63 E-05
AC092580.4	-5.20	1.08 E-04
TRIM52-AS1	5.08	1.36 E-04
PSMA8	-4.93	1.82 E-04
DNASE1L1	-4.85	2.11 E-04
CDKN2AIP	4.80	2.34 E-04
RPL10P3	4.61	3.39 E-04
SIGMAR1	-4.57	3.65 E-04
CCDC107	-4.55	3.83 E-04
SPATS2L	-4.37	5.44 E-04
C12orf61	-4.36	5.60 E-04
FOXJ3	4.31	6.14 E-04
RP11-1094M14.8	-4.30	6.33 E-04
KCNK12	-4.28	6.55 E-04
TMEM102	-4.27	6.75 E-04
PRPF4	4.26	6.81 E-04
ZNF395	4.25	7.02 E-04
DTD2	-4.24	7.09 E-04
ZNF883	-4.23	7.27 E-04
HOMER1	-4.21	7.56 E-04
ARRDC3	4.20	7.69 E-04
FDXR	-4.16	8.33 E-04
MID2	4.15	8.47 E-04
B3GALNT2	-4.15	8.54 E-04
CLYBL	-4.14	8.65 E-04
RP11-137L10.6	-4.11	9.20 E-04
SLC4A10	-4.07	1.01 E-03

*t statistic associated with exposure from the ANCOVA

Supplemental Table 5. Dose-response analysis of RN168 gene expression change between day 85 and baseline in CD8+ T cells – correlation of IL7R pathway genes

Gene Name	t-statistic**	*P* value
IL2RG	-3.25	5.34E-03
IL2RA	-3.06	7.97E-03
AKT1	-2.99	9.17E-03
IL7R	2.51	2.38E-02
MAP2K2	-2.34	3.33E-02
CDKN1B	2.27	3.84E-02

** t statistic associated with exposure from ANCOVA with correction for the baseline value (not adjusted for multiple comparisons)

Supplemental Table 6. Incidence of all-causality TEAEs occurring in ≥2 subjects

	TEAE Incidence, n (%)				
AE, MedDRA Version 19.1 Preferred Term	**RN168 1 mg/kg Q2wk (n=8)**	**RN168 3 mg/kg Q2wk (n=9)**	**RN168 8 mg/kg Q2wk (n=8)**	**RN168 6 mg/kg QW (n=5)**	**Placebo (n=7)**
Hypoglycemia	4 (50.0)	5 (55.6)	3 (37.5)	1 (20.0)	4 (57.1)
Headache	4 (50.0)	4 (44.4)	3 (37.5)	0	0
Nasopharyngitis	1 (12.5)	1 (11.1)	3 (37.5)	2 (40.0)	3 (42.9)
Lymphadenopathy	1 (12.5)	1 (11.1)	1 (12.5)	1 (20.0)	1 (14.3)
Nausea	2 (25.0)	2 (22.2)	0	1 (20.0)	0
Oropharyngeal pain	2 (25.0)	1 (11.1)	1 (12.5)	1 (20.0)	0
Cough	2 (25.0)	0	1 (12.5)	1 (20.0)	0
Diarrhea	1 (12.5)	0	1 (12.5)	1 (20.0)	1 (14.3)
Fatigue	1 (12.5)	0	1 (12.5)	0	2 (28.6)
Injection site pain	0	0	2 (25.0)	1 (20.0)	1 (14.3)
Lymphocytes decreased	1 (12.5)	0	1 (12.5)	2 (40.0)	0
Vomiting	2 (25.0)	1 (11.1)	0	0	1 (14.3)
Injection site bruising	1 (12.5)	1 (11.1)	0	1 (20.0)	0
Injection site erythema	0	0	1 (12.5)	1 (20.0)	1 (14.3)
Rash	1 (12.5)	0	0	0	2 (28.6)
URTI	2 (25.0)	0	0	0	1 (14.3)
Viral infection	1 (12.5)	0	1 (12.5)	1 (20.0)	0
WBC decreased	1 (12.5)	0	0	1 (20.0)	1 (14.3)
Abdominal distension	0	1 (11.1)	0	0	1 (14.3)
Abdominal pain	1 (12.5)	1 (11.1)	0	0	0
Back pain	0	2 (22.2)	0	0	0
Contusion	0	0	0	0	2 (28.6)
Dermatitis contact	0	1 (11.1)	0	0	1 (14.3)
Dysmenorrhea	1 (12.5)	1 (11.1)	0	0	0
Hyperhidrosis	1 (12.5)	0	0	0	1 (14.3)
Injection site pruritus	0	0	1 (12.5)	0	1 (14.3)
Lethargy	0	1 (11.1)	0	1 (20.0)	0
Neutrophils decreased	0	0	0	1 (20.0)	1 (14.3)
Pain	2 (25.0)	0	0	0	0
Productive cough	0	0	1 (12.5)	0	1 (14.3)
Rhinitis	0	0	1 (12.5)	1 (20.0)	0
Seasonal allergy	2 (25.0)	0	0	0	0

MedDRA, Medical Dictionary for Regulatory Activities; Q2wk, every other week; QW, weekly; TEAE, treatment-emergent adverse event; URTI, upper respiratory tract infection; WBC, white blood cell count.

Supplemental Acknowledgments

RN168 Working Group List

- Pearl Amos
- Marzieh Golmakani
- Barry Gumbiner
- Bart Jessen
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- Lisa Portale
- Carrie Taylor
- John Teeter
  Jason Williams

All are employees of Pfizer Inc.

16.1.3.1 LIST OF INDEPENDENT ETHICS COMMITTEE (IEC) OR INSTITUTIONAL REVIEW BOARD (IRB)

UNITED STATES

Study Site Number	Independent Ethics Committee or Institutional Review Board Address(es)
1001	Committee on Human Research 3333 California St, UCSF Ofc of Research, Ste 315, Box 0962 San Francisco, CA 94118 UNITED STATES
1002	University of Chicago Institutional Review Board McGiffert Hall 5751 S Woodlawn Ave, 2nd Fl Chicago, IL 60637 UNITED STATES
1003	University of Minnesota Institutional Review Board MMC 820, 420 Delaware St S E Minneapolis, MN 55455 UNITED STATES
1004	Washington University Institutional Review Board 660 S Euclid Ave, Campus Box 8089 St. Louis, MO 63110 UNITED STATES
1005	VA San Diego Healthcare System Institutional Review Board Department 151 3350 La Jolla Village Dr San Diego, CA 92161 UNITED STATES
1008	Sterling IRB 6300 Powers Ferry Rd, Ste 600-351 Atlanta, GA 30339 UNITED STATES
1009	Colorado Multiple Institutional Review Board Central Services and Administration PO Box 6508, 13001 E 17th Place, Mail Stop F-490 Aurora, CO 80045 UNITED STATES
1011	Yale University School of Medicine 55 College St, Human Investigation Committee New Haven, CT 06510 UNITED STATES
1013	Committee on Human Studies Joslin Diabetes Center 1 joslin Place Boston, MA 02215 UNITED STATES

Study Site Number	Independent Ethics Committee or Institutional Review Board Address(es)
1014	Committee for the Protection of Human Subjects in Research 55 Lake Ave N, AC7, University of Massachusetts Medical School Worcester, MA 01655 UNITED STATES
1015	Copernicus Group Independent Review Board One Triangle Dr, Ste 100 Durham, NC 27713 UNITED STATES