



Selective expansion of regulatory T cells by NKTR-358 in healthy volunteers and patients with systemic lupus erythematosus

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ABSTRACT

Objective: To evaluate NKTR-358, a polyethylene glycol-interleukin-2 conjugate composition designed to selectively induce regulatory T cells (Tregs), in first-in-human studies.

Methods: Healthy volunteers and patients with systemic lupus erythematosus (SLE) received single- or multiple-dose (biweekly) NKTR-358 or placebo in two sequential, randomized, phase 1 studies (single ascending dose [SAD; NCT04133116] and multiple ascending dose [MAD; NCT03556007]). Primary objectives were safety and tolerability; secondary objectives included pharmacokinetics (PK) and immune effects of NKTR-358; exploratory objectives included effects on SLE disease activity.

Results: There were eight ascending dose cohorts in the SAD study (0.3–28.0 µg/kg; n = 76; placebo: n = 24) and four in the MAD study (3–24.0 µg/kg; n = 36; placebo: n = 12). Most adverse events (AEs) were grade 1–2 injection-site reactions, with no treatment-related serious or severe AEs, or deaths. PK data showed dose proportionality and prolonged exposure (mean half-life: 7.4–12.9 days). Dose-dependent, selective, and sustained increases in percentages and absolute numbers of total CD4⁺ Tregs and CD25^{bright} Tregs were observed, with no significant changes in conventional CD4⁺ and CD8⁺ T cells, and low-level increases in natural killer cells. At the highest doses tested, administration of NKTR-358 resulted in a 12–17-fold increase in CD25^{bright} Tregs over baseline that was sustained for 20–30 days.

Conclusion: NKTR-358 was well tolerated, had a suitable PK profile for biweekly dosing, and led to marked and selective dose-dependent increases in CD25^{bright} Tregs, with no significant changes in conventional T cells. These results provide strong support for further testing in SLE and other inflammatory diseases.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with multi-organ involvement and diverse clinical manifestations. Current treatment options primarily include corticosteroids and immunosuppressants, which have been shown to provide partial clinical benefit but are associated with substantial toxicity and morbidity. Recent treatment advances have been limited, and the focus of most therapeutics in

development has been on inhibition of specific immune pathways potentially involved in disease pathogenesis. There remains a high unmet need for effective and safer treatment options [1,2].

SLE is characterized by a loss of immune self-tolerance, the production of pathogenic autoantibodies, and the accrual of tissue damage [1,3]. Impaired interleukin-2 (IL-2) production and dysfunction of regulatory T cells (Tregs) have been identified as key immunologic defects leading to the breakdown of immune self-tolerance in SLE [4,5]. IL-2 is a cytokine that has an essential role in preserving immune homeostasis

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Abbreviations			
AEs	adverse events	PD	pharmacodynamics
AUC	area under the curve	PEG	polyethylene glycol
BMI	body mass index	PK	pharmacokinetics
CLASI-A	cutaneous lupus erythematosus disease area and severity index-activity	rhIL-2	recombinant human IL-2
C _{max}	maximum observed concentration	SAD	single ascending dose
IL-2	interleukin-2	SD	standard deviation
IL-5	interleukin-5	SEM	standard error of the mean
MAD	multiple ascending dose	SLE	systemic lupus erythematosus
MedDRA	Medical Dictionary for Regulatory Activities	SLEDAI	SLE disease activity index
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events	Tcons	conventional T cell subsets
NK	natural killer	TEAE	treatment-emergent adverse event
		Teffs	T effector cells
		Tregs	regulatory T cells
		TSDR	Treg-specific demethylation region

and maintaining a balance between Tregs and T effector cells (Teffs). At higher levels, IL-2 stimulates the proliferation, differentiation, and function of Teffs and natural killer (NK) cells, which has resulted in it being used therapeutically at high doses to promote antitumor immune responses [5,6]. Conversely, another important role for IL-2 is to expand and stimulate Tregs, which control immune system over-activation and prevent autoimmunity [5,7]. The contrasting effects of IL-2 on T cell subsets are due to differences in IL-2 receptor biology between these T cell subsets. Tregs are induced at low IL-2 concentrations due to constitutive expression of the high-affinity IL-2R $\alpha\beta\gamma$ complex, whereas Teffs and NK cells mostly express the intermediate-affinity IL-2R $\beta\gamma$ chains that require higher IL-2 concentrations for activation [5,8,9]. In light of these differential effects, low-dose recombinant human IL-2 (rhIL-2) therapy has been evaluated for its ability to selectively induce Tregs and thereby treat autoimmune diseases, including SLE [4,10–13]. Despite promising early results, low-dose rhIL-2 treatment is limited by a narrow therapeutic window for Treg selectivity and a short half-life, necessitating frequent administration [14].

NKTR-358 (also known as LY3471851) is a composition of polyethylene glycol (PEG) conjugates of rhIL-2 in which the rhIL-2 polypeptide (the same amino acid sequence as aldesleukin) has been stably covalently attached to PEG chains [15]. NKTR-358 has reduced binding affinity for IL-2R β compared with rhIL-2, resulting in a different binding selectivity for cells expressing IL-2R β versus IL-2R α [15]. This design property renders Tregs with high-affinity (IL-2R $\alpha\beta\gamma$) receptors more sensitive than Teffs to activation by NKTR-358. In preclinical models, NKTR-358 demonstrated a markedly prolonged half-life compared with rhIL-2 and a sustained and selective proliferation and activation of Tregs with minimal effects on Teffs and other conventional (non-Treg) CD4⁺ and CD8⁺ T cell subsets (Tcons) [15]. Furthermore, repeated dosing of NKTR-358 in cynomolgus monkeys led to cyclical increases in Tregs with no loss of activity over the 6-month treatment period. NKTR-358 administration also suppressed lupus-like disease in a murine model of SLE [15].

Here, we report the results of two randomized, phase 1 studies – a single ascending dose (SAD) study in healthy volunteers and a multiple ascending dose (MAD) study in patients with SLE – each assessing the safety, tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of NKTR-358 versus placebo.

2. Material & methods

2.1. Study designs and treatment

The SAD study (NCT04133116) was a first-in-human, randomized, double-blind, placebo-controlled, phase 1 study in healthy volunteers conducted in a single center in the US. The study enrolled 100

participants in eight ascending dose cohorts. Each cohort included nine participants randomized to NKTR-358 and three to placebo. Participants received a single dose of NKTR-358 or placebo subcutaneously on Day 1 of each dosing cohort, with a starting dose of 0.3 $\mu\text{g}/\text{kg}$. A sentinel dosing approach was applied at each dose level whereby two participants were randomized to receive NKTR-358 or placebo and monitored for possible side effects for at least 7 days before the remainder of the cohort was randomized. Dose escalation was informed by safety and tolerability, cytokine levels, the expected NKTR-358 exposure based on PK simulations, as well as Treg and Tcon responses. Each cohort was followed for 50 days. A protocol amendment allowed four additional participants to be enrolled to receive open-label NKTR-358 at 20 $\mu\text{g}/\text{kg}$ to evaluate additional PD parameters following safety review.

The MAD study (NCT03556007) was a randomized, double-blind, placebo-controlled, phase 1 study in patients with SLE conducted at 11 centers in the US. The study randomized 48 patients to four sequential ascending dose cohorts, with nine patients receiving NKTR-358 and three receiving placebo per cohort. Patients received three doses of NKTR-358 or placebo subcutaneously on a biweekly schedule, with doses given on Days 1, 15, and 29 at a starting dose of 3 $\mu\text{g}/\text{kg}$. Subsequent NKTR-358 dose levels were based on safety findings, as well as Treg, Tcon, and NK cell responses, cytokine levels, and available PK data. Patients were followed for 50 days after the last dose.

Study drug assignments in both studies occurred in a double-blinded fashion (unless otherwise stated) in accordance with a computer-generated randomization scheme prepared by the study sponsor, with participants, investigators, and study site personnel blinded to treatment assignment.

The studies were conducted in accordance with the Declaration of Helsinki, Good Clinical Practice guidelines, and applicable local laws. Approvals were obtained from the appropriate institutional review boards. All participants provided written informed consent prior to study entry.

2.2. Study populations

The SAD study enrolled healthy adults aged 18–55 years. Participants were excluded if they had previous or concurrent immune-mediated disease, other relevant medical conditions, or were taking confounding medications. Additional eligibility criteria are provided in the Supplementary Materials and Methods.

In the MAD study, eligible patients had been diagnosed with SLE for at least 6 months, meeting at least four of the 11 American College of Rheumatology criteria for SLE (at least one of which must have been: positive antinuclear antibody test titer of $\geq 1:80$ at screening; above normal anti-double-stranded DNA antibodies at screening; or above normal anti-Smith antibody at screening). Patients were required to

have minimal-to-moderate SLE disease activity, and to be on a stable dose of concomitant medications for ≥ 8 weeks prior to study start. Patients with active lupus nephritis or central nervous system disease were excluded. If a patient was taking prednisone, the dose had to be ≤ 10 mg/day for a minimum of 8 weeks prior to screening and stable for a minimum of 2 weeks. Additional eligibility criteria are provided in the Supplementary Materials and Methods.

2.3. Outcomes

The primary objective was to evaluate the safety and tolerability of NKTR-358 administered as a single subcutaneous dose in healthy volunteers (SAD study) and as multiple doses in patients with SLE (MAD study). Secondary objectives were to characterize the PK of NKTR-358 following single and multiple doses, as well as the effects of NKTR-358 on the time course and extent of changes in the number and/or activity of circulating Tregs and Treg subpopulations, Tcon subsets, and major non-T cell subsets. Safety laboratories, blood cytokine levels, changes in gene expression, and anti-drug antibodies were also quantified. Disease activity was assessed as an exploratory endpoint in the MAD study, as measured by the SLE disease activity index (SLEDAI), cutaneous lupus erythematosus disease area and severity index-activity (CLASI-A), and joint counts.

2.4. Assessments

Safety and tolerability were assessed by monitoring adverse events (AEs), vital signs, physical examination, electrocardiography, and laboratory findings. AEs were collected throughout the study until completion (50 days after last dose) or at early termination visits. AEs were coded based on Medical Dictionary for Regulatory Activities (MedDRA) version 20.0 in the SAD study and version 21.0 in the MAD study and assessed for severity using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03.

Serum samples collected at pre-dose, during the study, and at the final study visit or early termination visit were used for the immunogenicity (anti-drug antibodies) testing.

Plasma PK sampling schedules, assays, and PK parameter calculation methods are summarized in the Supplementary Materials and Methods.

Immunophenotyping was performed by multicolor flow cytometry to quantify immune cell subsets using whole blood collected at pre-dose and throughout the study, until completion (50 days after the last dose) or the early termination visit. PD parameters included the absolute numbers and percentages of Tregs, Tcons, and NK cells, and percentage and expression level of functional markers of Treg activation (Ki67, ICOS, Helios, and CTLA4). Total Tregs were identified as $CD4^+FoxP3^+CD25^+$ cells; $CD25^{bright}$ Tregs have been previously identified as a Treg subpopulation with high activity and suppressive capacity [16] and were identified as the $CD4^+FoxP3^+CD25^+$ subpopulation with the highest CD25 expression (see Supplementary Fig. 1 and Supplementary Materials and Methods). $CD4^+$ T cells were $CD3^+CD4^+$ Tcons; $CD8^+$ T cells were $CD3^+CD8^+$ Tcons; and total NK cells were $CD3^-CD56^+$ cells, with NK^{bright} ($CD3^-CD56^{+++}CD16^-$ cells), and NK^{dim} ($CD3^-CD56^+CD16^+$) subsets also identified in the MAD study (see Supplementary Materials and Methods).

The methylation status of the Treg-specific demethylation region (TSDR) of the *FoxP3* gene was evaluated using the Epiontis ID quantitative polymerase chain reaction-based assay using whole blood collected at multiple time points from pre-dose through Day 50 post-dose [17].

2.5. Statistical analysis

All analyses were descriptive in nature and were performed using SAS[®] Version 9.4 or later. No formal sample size calculations were

performed. All participants who received at least one dose of study drug (NKTR-358 or placebo) were included in the safety analysis. Those who also had adequate data were included in the PK and PD analyses.

The relationship between Tregs identified by flow cytometry and by epigenetic analysis was assessed using Spearman's correlation coefficient using GraphPad Prism software v8.4.2 (GraphPad; San Diego, CA).

3. Results

3.1. Disposition and baseline characteristics

In the SAD study, 100 participants were enrolled and randomized to receive a single dose of NKTR-358 in eight ascending dose cohorts ranging from 0.3 $\mu\text{g}/\text{kg}$ to 28.0 $\mu\text{g}/\text{kg}$ ($n = 76$) or placebo ($n = 24$; Supplementary Fig. 2A). Seven participants who received NKTR-358 and two who received placebo discontinued treatment either because they were lost to follow-up ($n = 6$) or withdrew from the study ($n = 3$). None of the participants discontinued due to an AE. All participants were included in the safety and PD analyses, and 70 of 76 participants who received NKTR-358 had adequate data points for the PK analysis. All participants received the full dose of study drug except one who was randomized to receive 13.5 $\mu\text{g}/\text{kg}$ but only received 6.75 $\mu\text{g}/\text{kg}$ due to syringe malfunction. Dose escalation was stopped based on a non-clinical no observed adverse effect exposure level ceiling that was not to be exceeded.

The MAD study enrolled 48 patients with SLE with minimal-to-moderate disease activity who were randomized to receive NKTR-358 in four ascending dose cohorts ranging from 3 $\mu\text{g}/\text{kg}$ to 24 $\mu\text{g}/\text{kg}$ ($n = 36$) or placebo ($n = 12$; Supplementary Fig. 2B). Three patients who received NKTR-358 discontinued treatment because they withdrew consent ($n = 2$) or because of an AE ($n = 1$). One patient who withdrew consent for treatment also withdrew from the study. All patients received at least one dose of NKTR-358 or placebo and were included in the safety and PD analyses, while one patient of the 36 who received NKTR-358 had no measurable drug concentration and was excluded from the PK analysis.

Within each study, no meaningful differences were observed between the NKTR-358 and placebo groups with respect to demographics and baseline characteristics (Table 1). Across studies, more participants were female in the MAD study (96%) versus the SAD study (40%), reflecting the SLE disease population.

3.2. Safety

Overall, 83% and 97% of participants who received single and multiple doses of NKTR-358 experienced a treatment-emergent AE (TEAE) versus 25% and 58% with placebo, respectively (Table 2). All TEAEs, except one in each study, were mild or moderate (grades 1–2) in severity. In participants receiving NKTR-358, TEAEs were primarily injection site reactions, most frequently injection site erythema. Injection site reactions usually began 1–2 days after injection. One participant who received a single dose of NKTR-358 at 28 $\mu\text{g}/\text{kg}$ developed grade 1 pyrexia, decreased appetite, nausea, vomiting, diarrhea, tachycardia, and myalgia that were attributed to elevated cytokine levels (cytokine-release syndrome). Clinical manifestations started 2 days after administration of drug and resolved without treatment within 4 days. One patient in the MAD study (NKTR-358 at 24 $\mu\text{g}/\text{kg}$) had two treatment-related TEAEs of grade 1 flu-like symptoms following the second and third infusion, which resolved without treatment after 2 days. Although clinical manifestations were labeled as cytokine-release syndrome by the investigator, no clinically relevant hematology, chemistry, or elevated cytokine values were associated with either of the two episodes. Two serious TEAEs were reported following treatment with NKTR-358 (grade 4 attempted suicide at Day 45 in the SAD study and grade 3 migraine on Day 50 in the MAD study), neither of which were considered related to study drug. One patient had treatment

Table 1
Demographics and baseline disease characteristics.

	SAD (healthy volunteers)		MAD (patients with SLE)	
	NKTR-358 (n = 76)	Placebo (n = 24)	NKTR-358 (n = 36)	Placebo (n = 12)
Age, years, mean (SD)	33.3 (9.6)	34.0 (13.0)	47.2 (12.5)	47.8 (8.3)
Sex, n (%)				
Male	48 (63)	12 (50)	2 (6)	0
Female	28 (37)	12 (50)	34 (94)	12 (100)
Race, n (%)				
White	40 (53)	12 (50)	24 (67)	8 (67)
Black or African American	33 (43)	11 (46)	10 (28)	2 (17)
Asian	0	1 (4)	1 (3)	1 (8)
Multiple races	3 (4)	0	0	1 (8)
Not reported	0	0	1 (3)	0
BMI, kg/m ² , mean (SD)	26.1 (3.7)	26.6 (2.9)	26.9 (3.0)	26.7 (4.6)
Concomitant medication, n (%)	26 (34)	8 (33)	36 (100)	12 (100)
Medication of interest				
Hydroxychloroquine	0	0	12 (33)	4 (33)
Hydroxychloroquine sulfate	0	0	12 (33)	2 (17)
Prednisone	0	0	11 (31)	1 (8)
Azathioprine	0	0	4 (11)	0
Methotrexate	0	0	2 (6)	0
Mycophenolate	0	0	1 (3)	2 (17)
Duration of SLE, months, mean (SD)	NA	NA	9.5 (8.9)	14.3 (9.7)
SLEDAI-2K score, mean (SD, range)	NA	NA	6.0 (2.8, 0–10)	5.2 (2.2, 2–10)
CLASI-A activity score, mean (SD, range)	NA	NA	4.1 (4.7, 0–22)	2.7 (3.2, 0–9)
Joint counts, mean (SD, range)				
Swelling	NA	NA	6.0 (6.9, 0–24)	2.8 (4.5, 0–13)
Tenderness	NA	NA	10.1 (9.6, 0–28)	6.8 (3.9, 1–13)

Abbreviations: BMI: body mass index; CLASI-A: cutaneous lupus erythematosus disease area and severity index–activity; MAD: multiple ascending dose; NA: not applicable; SAD: single ascending dose; SD: standard deviation; SLE: systemic lupus erythematosus; SLEDAI: SLE disease activity index.

(NKTR-358 at 24 µg/kg) discontinued due to moderate hyper-eosinophilia in the MAD study. Increased eosinophil levels resolved without treatment, and there were no associated clinical manifestations. No deaths were reported in either study.

In the SAD study, no clinically meaningful changes from baseline were observed for clinical laboratory values, and no laboratory abnormality was reported as a TEAE. In the MAD study, the only observed laboratory abnormalities were shifts from normal baseline to high post-baseline eosinophil values across NKTR-358 dose cohorts; three patients experienced hyper-eosinophilia (eosinophil count $>1.5 \times 10^9$ cells/L) at the 12 or 24 µg/kg doses. All events were mild or moderate in severity, and each of these events started after the second dose of NKTR-358 on Day 21 or 22, was not associated with clinical manifestations, and resolved after the last dose of study drug. No clinically significant vital sign or electrocardiogram abnormalities were observed in either study. No NKTR-358 anti-drug antibodies were detected at any dose or any time point in either study.

3.3. Pharmacokinetics

NKTR-358 showed approximately linear PK, with maximum observed concentration (C_{max}) and area under the curve (AUC) increasing proportionally with dose (Fig. 1). NKTR-358 reached maximum plasma concentrations approximately 5–7 days post-dose, and subsequently declined with a mean half-life of 7.4–12.9 days (Supplementary Tables 1 and 2).

3.4. Pharmacodynamics

At doses ≥ 3 µg/kg, NKTR-358 led to a dose-dependent and sustained increase in the absolute numbers and percentages of total Tregs ($CD4^+FoxP3^+CD25^+$ cells) (Supplementary Fig. 3A and B) and $CD25^{bright}$ Tregs ($CD4^+FoxP3^+CD25^{bright}$ cells) in both healthy volunteers and patients with SLE (Fig. 2A–D). While there was greater variability in baseline absolute numbers of Tregs in patients with SLE compared to healthy volunteers, the dose-dependent increase in the frequency of $CD25^{bright}$ Tregs as a percentage of $CD4^+$ T cells was clearly

observed in both patients with SLE and healthy participants. At the highest doses tested in both studies, there was a maximum 17-fold (SAD) and 12-fold (MAD) mean peak increase over baseline in the numbers of $CD25^{bright}$ Tregs, and levels peaked around Day 10 and remained above baseline for 20–30 days following administration of the last dose. In the SLE population, the magnitude of the response appeared modestly attenuated in several patients with repeat dosing at 24 µg/kg, but the mean $CD25^{bright}$ Treg population remained above baseline levels through approximately Day 60. Importantly, following the first dose of NKTR-358, a similar dose-dependent induction of $CD25^{bright}$ Tregs was seen in both healthy volunteers and patients with SLE (Fig. 2E). Disease activity and medications did not have significant impacts on the observed response, including the degree of Treg induction (data not shown).

Using the methylation status of the *FoxP3* gene as an additional method for identifying NKTR-358-induced Tregs, we observed a significant correlation between NKTR-358-induced Tregs identified by flow cytometry ($CD4^+FoxP3^+CD25^+$ cells) and by epigenetic analysis (percentage demethylation of *FoxP3* TSDR) after treatment with 28 µg/kg NKTR-358 (SAD) and 24 µg/kg NKTR-358 (MAD) (Supplementary Fig. 4A and B).

Several approaches were used to evaluate the activity of the NKTR-358-induced Tregs. NKTR-358 led to a dose-dependent increase in the percentage of $CD25^{bright}$ Tregs expressing Ki67 – a marker of proliferation and, therefore, of Treg activation – at doses ≥ 12 µg/kg in both studies (Supplementary Fig. 5A). A maximum of 6-fold (SAD) and 5-fold (MAD) mean peak increases over baseline in $Ki67^+CD25^{bright}$ Tregs were observed. Similarly, a sustained induction of markers associated with suppressive Treg activity, including Helios and CTLA4, was observed in the higher dose groups, with similar magnitudes of increase in both studies (Supplementary Fig. 5B and 5C). The percentage of ICOS⁺ total Tregs, which also has an important role in Treg suppressive function, increased in response to NKTR-358 in the SAD study (Supplementary Fig. 5D), but the effect was more difficult to determine in the MAD study due to increased variability across patients (data not shown).

No meaningful changes from baseline in the number of Tcon cell populations ($CD4^+$, $CD8^+$) were observed at any of the dose levels tested

Table 2

Summary of treatment-emergent adverse events in the single ascending dose and multiple ascending dose studies.

n (%)	SAD (healthy volunteers)		MAD (patients with SLE)	
	NKTR-358 (n = 76)	Placebo (n = 24)	NKTR-358 (n = 36)	Placebo (n = 12)
Any TEAEs	63 (83)	6 (25)	35 (97)	7 (58)
Grade 3–4	1 (1) ^a	0	1 (3) ^b	0
Grade 5	0	0	0	0
Any treatment-related TEAEs	55 (72)	2 (8)	33 (92)	1 (8)
Serious TEAEs	1 (1) ^a	0	1 (3) ^b	0
TEAEs leading to discontinuation	0	0	1 (3) ^c	0
Injection site-related TEAEs				
Injection site erythema	50 (66)	0	28 (78)	0
Injection site pain	34 (45)	0	8 (22)	0
Injection site swelling	17 (22)	0	2 (6)	0
Injection site pruritus	11 (14)	0	22 (61)	0
Injection site induration	5 (7)	0	2 (6)	0
Injection site reaction	0	0	14 (39)	0
Injection site warmth	0	0	5 (14)	0
Injection site edema	0	0	3 (8)	0
Most common TEAEs				
Headache	9 (12)	5 (21)	1 (3)	0
Back pain	4 (5)	1 (4)	0	1 (8)
Urinary tract infection	1 (1)	0	4 (11)	2 (17)
Nasopharyngitis	0	0	3 (8)	0
Eosinophilia	0	0	3 (8)	0
Insomnia	0	0	3 (8)	0
Fatigue	0	0	2 (6)	0
Maculo-papular rash	0	0	2 (6)	0
Sinusitis	0	0	2 (6)	0

Abbreviations: MAD: multiple ascending dose; SAD: single ascending dose; SLE: systemic lupus erythematosus; TEAE: treatment-emergent adverse event.

^a Grade 4 attempted suicide not considered related to study drug.^b Grade 3 migraine not considered related to study drug.^c Treatment-related grade 2 eosinophilia.

(Fig. 3A and B). Due to the relatively small numbers of total Tregs as compared to the total population of CD4⁺ T cells, there was no difference when CD4⁺ Tcons were analyzed with CD4⁺FoxP3⁺CD25⁺ Tregs excluded from the CD4⁺ population or from the total CD4⁺ population. Low-level increases in total CD56⁺ NK cells were observed at the higher dose levels tested in both studies (Fig. 3C). In the MAD study, the observed increase in absolute number of NK cells was driven largely by two patients whose values were notably higher than the other patients in the cohort (Fig. 3D). The CD56^{bright} population was more sensitive to NKTR-358 than the CD56^{dim} population, showing greater induction following NKTR-358 administration, with the ratio of CD56^{bright} to CD56^{dim} NK cells increased 16-fold over pre-dose at 24 μg/kg (Supplementary Fig. 6).

NKTR-358 administration in patients with SLE resulted in selective expansion of Tregs to levels similar to healthy volunteers in the SAD study, and maintenance of this selectivity after multiple administrations.

At 24 μg/kg, there was a 12-fold increase from baseline in the mean peak CD25^{bright} Tregs:Tcon ratio after the first administration and a 7-fold increase after the third administration (data available for six patients only; Supplementary Fig. 7).

3.5. SLE disease activity

In the MAD study in patients with SLE, exploratory analyses examined disease activity as measured by changes in SLEDAI, CLASI-A score, and joint counts. Importantly, the study was not designed to study effects on disease activity, and all analyses were limited by the small numbers of patients per cohort, minimal baseline disease activity in a proportion of patients, and the short duration of treatment (1 month). No NKTR-358 treatment-related changes in SLEDAI or joint scores were apparent. In an exploratory analysis of patients with a CLASI-A baseline score ≥4 (n = 22), a dose-dependent reduction in CLASI-A score was

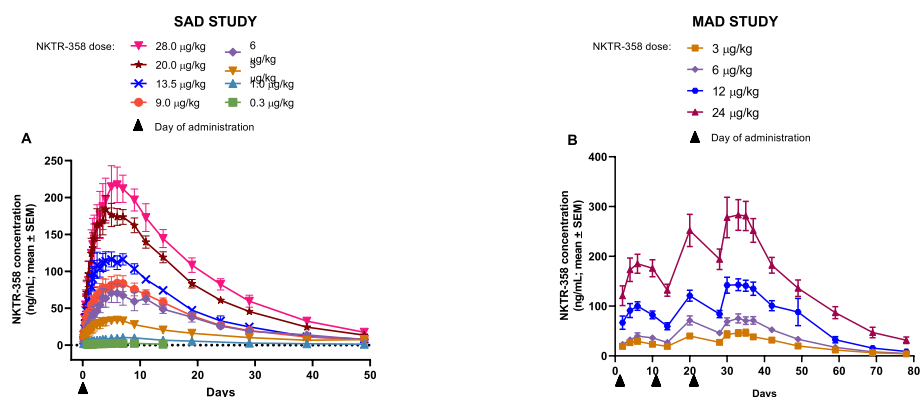


Fig. 1. Mean concentration–time profiles of NKTR-358 across dose cohorts after A) a single dose in the SAD study in healthy volunteers and B) biweekly multiple doses in the MAD study in patients with SLE.

Abbreviations: MAD: multiple ascending dose; SAD: single ascending dose; SEM: standard error of the mean.

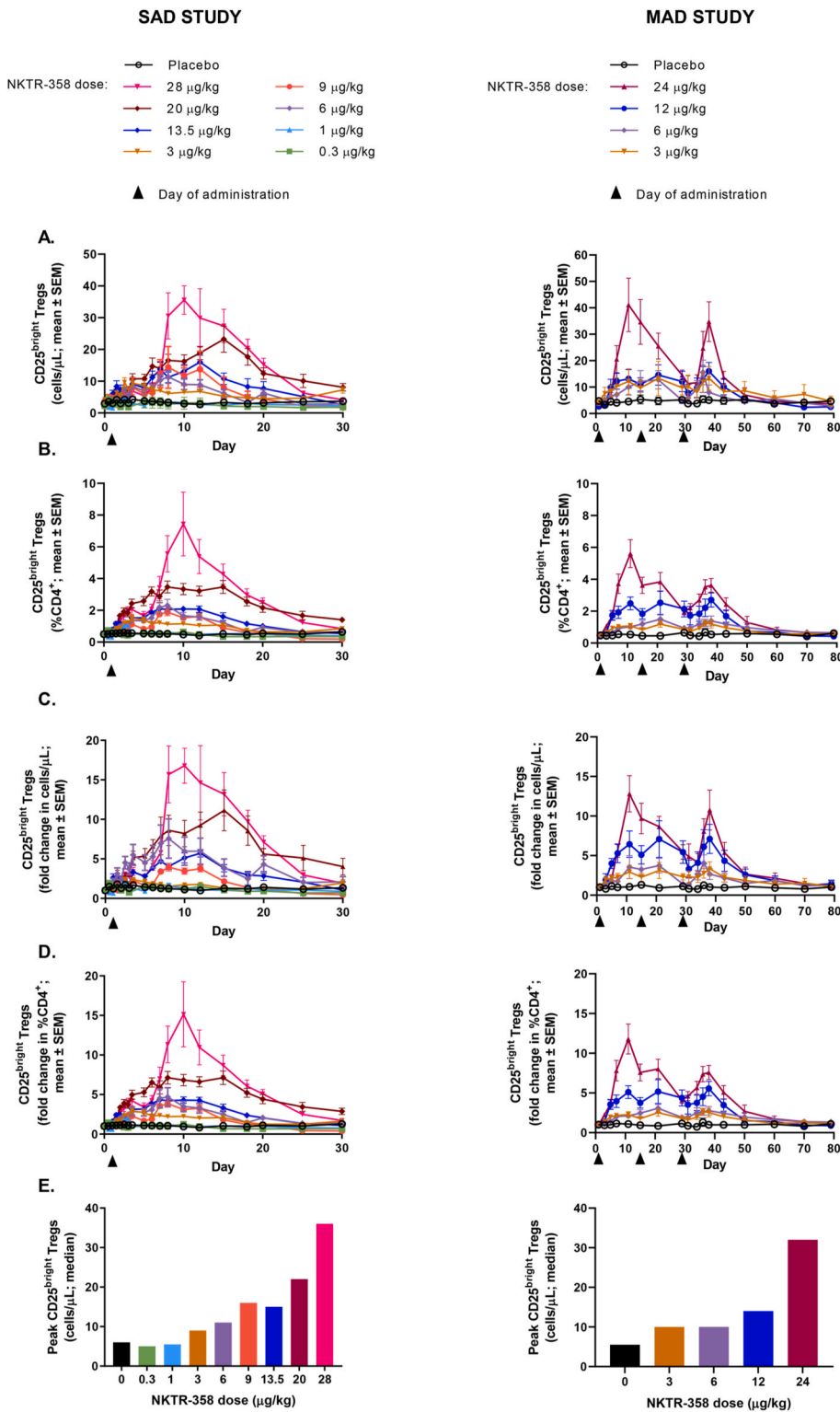


Fig. 2. Changes from baseline in the numbers and percentages of Tregs in peripheral blood after a single dose of NKTR-358 in the SAD study and after multiple doses of NKTR-358 in the MAD study. A) absolute number of CD25^{bright} Tregs; B) percentage of CD25^{bright} Tregs in total CD4⁺ T cells; C) fold-change in the absolute number of CD25^{bright} Tregs; D) fold-change in percentage of CD25^{bright} Tregs in total CD4⁺ T cells; E) peak level of post-baseline CD25^{bright} Tregs after 1 dose of NKTR-358.

Abbreviations: MAD: multiple ascending dose; SAD, single ascending dose; SEM, standard error of the mean.

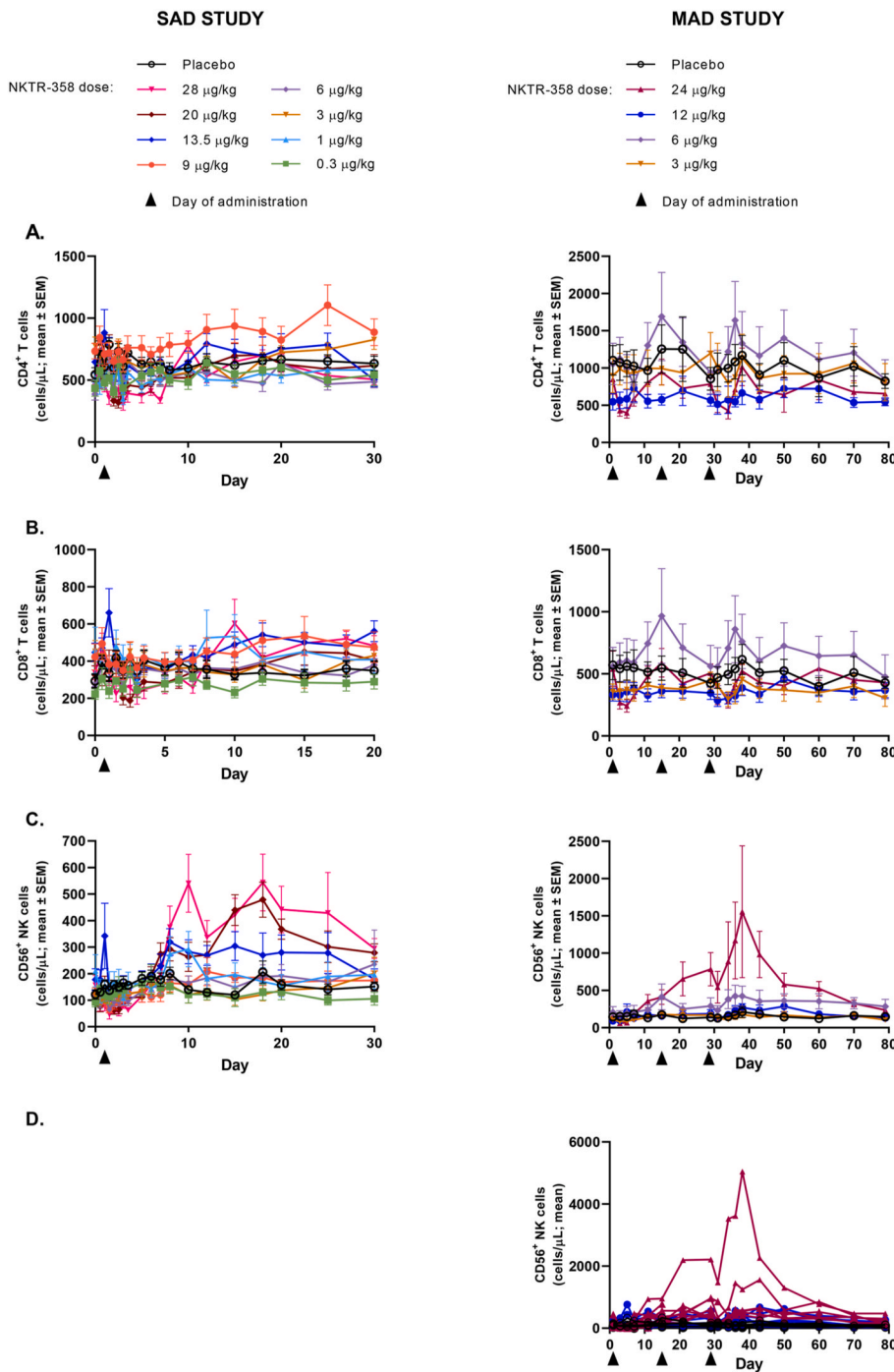


Fig. 3. Mean (SE) changes in absolute number of Tcon and NK cells in peripheral blood after a single dose of NKTR-358 in the SAD study and with multiple doses of NKTR-358 in the MAD study. A) CD4⁺ T cells; B) CD8⁺ T cells; C) total CD56⁺ NK cells; D) total CD56⁺ NK cells from individual SLE patients given the highest dose (24 μg/kg).

Abbreviations: MAD: multiple ascending dose; NK: natural killer; SAD: single ascending dose; SEM: standard error of the mean; Tcons: conventional T cells.

seen with NKTR-358 treatment (Supplementary Fig. 8). One patient (24 μg/kg) experienced a reduction in CLASI-A score from 22 at baseline to 5 by Day 43 (2 weeks after last dose), and 7 of 18 patients had a ≥4-point reduction in CLASI-A score from baseline to Day 43.

4. Discussion

The biological functions of IL-2 are pleiotropic, supporting expansion and activation of both Teffs and Tregs, and IL-2 thus has the capability of promoting or down-regulating immune responses in health and disease [5,18]. Considerable evidence has implicated deficiencies in IL-2 and in Treg numbers and function in the pathogenesis of different autoimmune diseases, including SLE [4,10–13]. IL-2’s regulation of the Teff/Treg

balance, as well as its ability to prevent the effector antibody-mediated autoimmune response and to control inflammation by blocking differentiation of pro-inflammatory T helper 17 cells, has supported the use of low-dose IL-2 in the treatment of autoimmune diseases [5]. Consistent with the well-understood immunomodulatory roles of IL-2, preliminary evidence of clinical efficacy with low-dose IL-2 in SLE and other inflammatory disease indications has been reported [11,19–21]. However, rhIL-2 has a short half-life, necessitating frequent dosing, and there is a narrow therapeutic window of selective Treg induction by IL-2 before Teffs are also induced [22,23]. NKTR-358 was identified through an *in vivo* screening campaign to overcome the shortcomings of rhIL-2, first by altering binding to the IL-2 receptor to favor Treg versus Tcon stimulation, and second by extending the

half-life [15]. Our studies not only established that NKTR-358 was well tolerated, with a favorable safety profile, but also demonstrated the desired PK profile. Importantly, selective Treg stimulation, without the induction of Tcons, was achieved in healthy volunteers, and these findings were confirmed in patients with SLE, including following multiple doses of NKTR-358. The selective, sustained Treg induction, combined with optimal PK and infrequent administration, suggest that NKTR-358 has the potential to be a safe and effective treatment option for SLE.

NKTR-358 administered subcutaneously was well tolerated at the doses tested, with a similar safety profile for both single and repeat administrations. No treatment-related grade ≥ 3 or serious TEAEs were observed, and TEAEs were primarily limited to mild or moderate injection site reactions. A safety profile dominated by local reaction at the injection site is consistent with other clinical trials with low-dose rhIL-2 [11–13], IL-2 muteins [24], and other cytokines. While increased eosinophil counts were seen in some patients at the highest dose levels, it is important to emphasize that no participants had associated clinical manifestations suggestive of a hypereosinophilic disorder, and eosinophil counts were cyclical, related to dosing, and resolved after treatment discontinuation. It is well documented that IL-2 can induce eosinophilia via IL-5 produced by proliferating type 2 innate lymphoid cells [5,25], and induction of IL-5 was observed in some participants in our studies (data not shown). Overall, there was no evidence of a clinical syndrome characteristic of severe cytokine-release syndrome in either study, nor was there an observed increased risk of AEs or laboratory abnormalities that are typically associated with high-dose rhIL-2 (aldesleukin), such as capillary leak syndrome, disseminated infections, autoimmune diseases, cardiopulmonary events, and hematologic toxicities [5,26]. Importantly, in contrast to other molecules designed to improve the half-life and Treg selectivity of IL-2 through mutation or fusion approaches, NKTR-358 utilizes the rhIL-2 aldesleukin sequence, which has minimal potential for the development of anti-drug antibodies. Indeed, no NKTR-358 anti-drug antibodies were detected in either the SAD or MAD studies.

The primary effect of NKTR-358 in our studies was a marked and selective, dose-dependent expansion of Tregs. Treg stimulation was sustained, with elevated Treg levels detectable for over 3 weeks after dosing, and restimulation was maintained through multiple administrations of NKTR-358. We focused on the CD25^{bright} Tregs, the subpopulation which has been shown to have the highest suppressive capacity and to be inversely correlated with SLE disease activity [4,5]. Effects on total Tregs were also observed, but the magnitude of expansion was less than that observed for CD25^{bright} Tregs. The functional activity of the NKTR-358-expanded Tregs in these phase 1 studies was further supported by an increase in expression of the proliferation marker Ki67, as well as an increase in expression of Helios and CTLA4, markers associated with increased Treg suppressive activity [27,28].

Importantly, we showed that the NKTR-358-dependent induction of Tregs was not associated with any meaningful changes in CD4⁺ and CD8⁺ Tcons across dose levels, and only low-level increases in total NK cells were observed in some patients at the highest doses tested. When NK cell subsets were examined in response to NKTR-358, the CD56^{bright} population showed a larger increase than the CD56^{dim} population. This result was not unexpected, as the CD56^{bright} NK cells are considered more immature than the CD56^{dim} NK cells and have been shown to constitutively express the high-affinity IL-2 receptor. This NK subpopulation is also more highly associated with production of immunoregulatory cytokines rather than high cytotoxic activity [29,30].

Despite a greater understanding of the mechanisms underlying SLE, corticosteroids and immunosuppressive agents are still considered mainstays of therapy for this disease [31,32]. Unfortunately, corticosteroids are associated with a myriad of complications that contribute to the long-term morbidity and poor outcome in some SLE patients [31,

32], and immunosuppressive drugs further contribute to the long-term risk of infection [32–34]. Newer therapeutics recently approved and in development for the treatment of SLE [33,34] primarily target immune pathways, such as cytokines and key cellular populations, with inhibitory agents that may add to the immunosuppressive risk [32]. In contrast, low-dose IL-2, NKTR-358, and other IL-2-like molecules have the ability to restore immune homeostasis in SLE patients by increasing deficient IL-2 levels and by selectively stimulating Tregs. The resulting correction of the Treg/effector T cell imbalance may provide the opportunity to control disease activity without increasing immunosuppression, and also allow for a decrease in the use of steroids and immunosuppressive agents [5,21,35].

Considerable evidence indicates that both T cells and B cells are dysregulated in SLE [36–38]. T cell-dependent stimulation of B cells contributes to excess activation and production of autoantibodies which are a key hallmark of SLE [39,40]. Deposition of immune-complexes and excessive effector T cell activity in tissues can further lead to increased chronic inflammation and long-term organ damage [39,41]. In addition to selective stimulation of Tregs and suppression of effector T cell inflammatory responses, IL-2 agents have the ability to alter the balance and function of T follicular helper cells and T follicular regulatory cells, and more directly reduce autoantibody formation and immune complex deposition [4,5,10–13,19–21]. There also is evidence that Tregs have a potential role in regulating tissue damage and regeneration, through involvement of the amphiregulin-EGFR signaling axis and other pathways [41,42].

In conclusion, NKTR-358 was well tolerated in healthy volunteers and patients with minimal-to-moderate SLE. NKTR-358 elicited substantial dose-dependent increases in circulating CD25^{bright} Tregs, with no significant changes in Tcons and low-level increases in NK cells. These immune effects extend previous preclinical studies showing the prolonged and Treg-selective action of NKTR-358. Due to the inherent limitations in phase 1 studies, including a relatively short treatment duration and a small number of patients, there was a limited ability to assess clinical disease activity in the NKTR-358 MAD study. Still, the results from this study showing dose-dependent reductions in CLASI-A scores in the subset of patients with higher baseline activity are encouraging. Together, the findings from these studies provide strong support for continued testing of NKTR-358 as a new therapeutic in SLE and other inflammatory diseases. A randomized, double-blind, placebo-controlled phase 2 study (ISLAND-SLE; NCT04433585) has been initiated to further evaluate NKTR-358 in patients with SLE.

Author statement

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Data availability statement

Data from this study may be shared with qualified researchers, upon request.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: CF, RF, VC, RL, ID, ND, CH, JG, NH, JZ and BK report financial support was provided by Nektar Therapeutics. CF reports a relationship with Nektar Therapeutics that includes: employment and equity or stocks. RF reports a relationship with Nektar Therapeutics that includes: consulting or advisory. RF reports a relationship with Genentech/Roche that includes personal fees and grants. RF reports a relationship with AstraZeneca that includes: consulting or advisory. RF reports a relationship with Mallinckrodt Pharmaceuticals that includes: consulting or advisory. VC reports a relationship with AbbVie Inc that includes: funding grants. VC reports a relationship with Amgen Inc that includes: funding grants. VC reports a relationship with Boehringer Ingelheim Corp USA that includes: funding grants. VC reports a relationship with Boston Pharmaceuticals that includes: funding grants. VC reports a relationship with Eli Lilly and Company that includes: funding grants. VC reports a relationship with EMD Serono Inc that includes: funding grants. VC reports a relationship with Genentech that includes: funding grants. VC reports a relationship with GlaxoSmithKline USA that includes: funding grants. VC reports a relationship with Merck & Co Inc that includes: funding grants. VC reports a relationship with Nektar Therapeutics that includes: funding grants. VC reports a relationship with Novartis that includes: funding grants. VC reports a relationship with Pfizer that includes: funding grants. VC reports a relationship with Roche that includes: funding grants. VC reports that Pinnacle Research Group/Anniston Medical Clinic also received funding from Astellas Pharma Global Development Inc. RL reports a relationship with Gilead Sciences Inc that includes: consulting or advisory. RL reports a relationship with Exagen Inc that includes: consulting or advisory. RL reports a relationship with Myriad Genetics Inc that includes: consulting or advisory. RL reports a relationship with Sanofi that includes: paid expert testimony and speaking and lecture fees. RL reports a relationship with Regeneron Pharmaceuticals Inc that includes: paid expert testimony and speaking and lecture fees. RL reports a relationship with Bristol Myers Squibb Co that includes: paid expert testimony and speaking and lecture fees. RL reports a relationship with AbbVie that includes: paid expert testimony and speaking and lecture fees. ND reports a relationship with Nektar Therapeutics that includes: employment and equity or stocks. CH reports a relationship with Nektar Therapeutics that includes: employment and equity or stocks. JG reports a relationship with Nektar Therapeutics that includes: employment and equity or stocks. NH reports a relationship with GlaxoSmithKline that includes: employment. DD reports a relationship with PRA Health Sciences that includes: employment. DD reports a relationship with ICON plc that includes: employment. JZ reports a relationship with Nektar Therapeutics that includes: employment and equity or stocks. BK reports a relationship with Nektar Therapeutics that includes: employment and equity or stocks. ID has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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