Selective Expansion of Regulatory T Cells in Patients with Systemic Lupus Erythematosus by a Novel IL-2 Conjugate, NKTR-358

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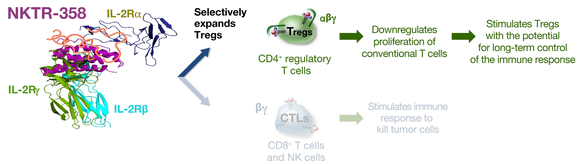
BACKGROUND

- Compared with recombinant human interleukin-2 (IL-2), PEG-conjugated NKTR-358 has:[1]
  - An altered binding profile, with a lower binding affinity for IL-2Rβ and a different binding bias for IL-2Rα and IL-2Rβ
  - Selectivity for stimulating regulatory T cells (Tregs) over conventional T cells (Tcons)
  - An increased half-life
- NKTR-358 has shown:
  - Activity in animal models of systemic lupus erythematosus (SLE) and cutaneous hypersensitivity[2]
  - Selective stimulation of Tregs in a single-ascending dose (SAD) study in healthy volunteers[3]

NKTR-358: A PEG-conjugated rhIL-2 that selectively induces Tregs and their suppressive activity

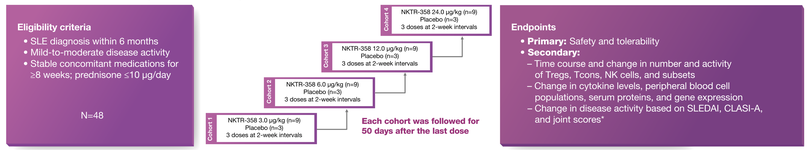


CTL, cytotoxic T lymphocyte; IL-2R, interleukin-2 receptor; NK, natural killer; rh, recombinant human; Treg, regulatory T cell.

METHODS

Study design

A randomized, double-blind, multiple-ascending dose (MAD) Phase 1b study of subcutaneous NKTR-358 in patients with mild-to-moderate SLE (NCT03556007)



CLASI-A, cutaneous lupus erythematosus disease area and severity index–activity; NK, natural killer; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index; Tcon, conventional T cell; Treg, regulatory T cell.
*This Phase 1b study design, including small numbers of patients, low entry disease activity, and short treatment duration is unlikely to support adequate assessment of disease activity effect.

Assessments

- Immunophenotyping by multicolor flow cytometry was performed to quantify multiple immune cell subsets, using whole blood collected at multiple time points pre- and post-NKTR-358 administration
  - **CD25^{bright} Tregs:** A CD4$^+$ FoxP3$^+$ CD25$^+$ Treg subpopulation with the highest CD25 expression; expected to have the highest suppressive capacity
  - **CD4$^+$ T cells:** CD3$^+$ CD4$^+$ Tcons
  - **CD8$^+$ T cells:** CD3$^+$ CD8$^+$ Tcons
  - **Natural killer (NK) cells:** CD3$^-$ CD56^{+++} CD16$^-$ (bright), CD3$^-$ CD56$^+$ CD16$^+$ (dim), and CD3$^-$ CD56$^+$ (total)
- Epigenetic modifications were evaluated with the Epiontis ID qPCR-based assay to monitor methylation status of the Treg-specific demethylation region (TSDR) of the *FoxP3* gene, using whole blood collected at multiple time points pre- and post-NKTR-358 administration
- Gene expression was measured with the NanoString platform, with whole blood collected at multiple time points pre- and post-NKTR-358 administration
- Post-hoc analyses were performed for change in cutaneous lupus erythematosus disease area and severity index–activity (CLASI-A) score for individual patients following NKTR-358 treatment

RESULTS

Baseline demographics and disease characteristics

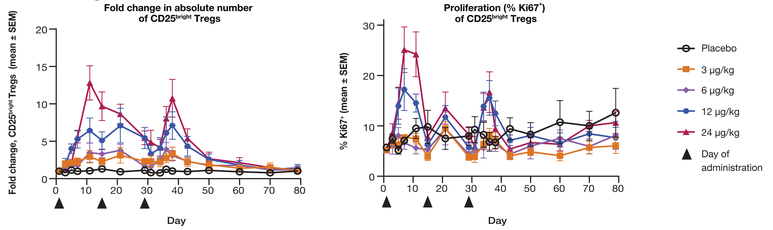
	NKTR-358 (n=36)	Placebo (n=12)
Age, years, mean (SD)	47.2 (12.5)	47.8 (8.3)
Female, n (%)	34 (94.4)	12 (100)
Body mass index, mean (SD)	26.9 (3.0)	26.7 (4.6)
Disease duration, months, mean (SD)	9.5 (8.9)	14.3 (9.7)
SLEDAI score, mean (SD, min–max)	6.0 (2.8, 0–10)	5.2 (2.7, 2–10)
CLASI-A score, mean (SD, min-max)	4.1 (4.7, 0–22)	2.7 (3.2, 0–9)
Baseline medication, n (%)		
Prednisone	12 (33.3)	4 (33.3)
Hydroxychloroquine	24 (66.7)	6 (50.0)
Methotrexate	4 (11.1)	0
Mycophenolate mofetil	1 (2.8)	2 (16.7)
Azathioprine	5 (13.9)	0

CLASI-A, cutaneous lupus erythematosus disease area and severity index–activity; SD, standard deviation; SLEDAI, systemic lupus erythematosus disease activity index.

Safety and tolerability

- No dose-limiting toxicities, deaths, or clinically significant vital sign, electrocardiogram, or physical examination abnormalities were observed
- Adverse events were primarily mild or moderate (Grade 1 or 2) injection-site reactions
- One patient in the lowest dose cohort (3 µg/kg) experienced a serious adverse event of migraine 3 weeks after the last dose of NKTR-358; it was not considered related to the study drug
- Three patients discontinued treatment (one patient [24 µg/kg] due to elevated eosinophil levels, with no clinical sequelae; two patients for reasons unrelated to adverse events)
- One patient in the highest dose cohort (24 µg/kg) demonstrated transient and mild (Grade 1) flu-like symptoms after the second and third doses that were considered related to the study drug; no clinically relevant changes in hematology, chemistry, or cytokine levels were associated with either episode, and both episodes resolved within 24 hours without treatment
- No antidrug antibodies were detected throughout the entire 50 days of follow-up

NKTR-358 led to sustained, dose-dependent increases in the number and proliferation of CD25^{bright} Tregs

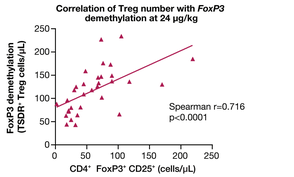


SEM, standard error of the mean; Treg, regulatory T cell.

- NKTR-358 (24 µg/kg) resulted in:
  - Maximum 12-fold mean peak increase over baseline in number of CD25^{bright} Tregs
  - Treg levels peaked at Day 10 after the first NKTR-358 dose and remained above baseline for 20–30 days following the last dose
  - Maximum 5-fold mean peak increase over baseline in Ki67$^+$ CD25^{bright} Tregs
- Treg increases observed with NKTR-358 in SLE patients were comparable with those seen at 28 µg/kg in healthy volunteers
- No overall changes in Tcon cell numbers were observed following NKTR-358 administration (data not shown)

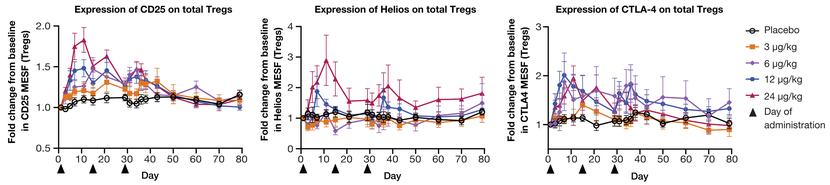
Identification of NKTR-358-induced Tregs supported by correlation with demethylation status of *FoxP3* gene

- An epigenetically active (demethylated) *FoxP3* gene is observed solely in Tregs and not in activated, (non-Treg) CD4$^+$ Tcons[4]
- After treatment with ≥6 µg/kg NKTR-358, a significant correlation was observed between NKTR-358-induced Tregs identified by flow cytometry (CD4$^+$ CD25$^+$ FoxP3$^+$ cells) and Tregs identified by epigenetic analysis (percentage of demethylation of *FoxP3* TSDR)



Each data point represents an individual blood sample from nine NKTR-358-treated patients where data were available for both flow cytometry and demethylation assays.
TSDR, Treg-specific demethylated region; Treg, regulatory T cell.

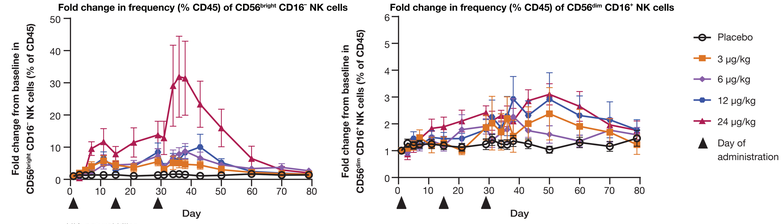
NKTR-358 (≥12 µg/kg) elicited a dose-dependent induction of Treg activation markers



CTLA4, cytotoxic T-lymphocyte-associated protein 4; MESF, molecules of equivalent soluble fluorochrome; Treg, T regulatory cell.

- A sustained induction of markers associated with increased suppressive Treg function was observed at 12 and 24 µg/kg NKTR-358
- The magnitude of increase was similar to the change observed in the SAD study in healthy volunteers

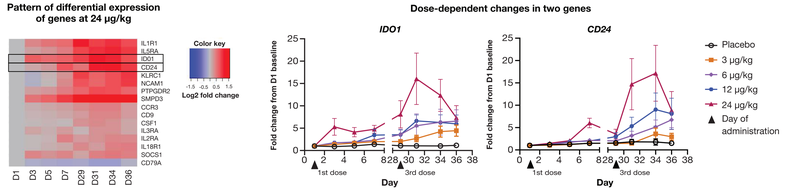
NKTR-358 led to low-level increases in CD56$^+$ NK cells in most patients at 24 µg/kg



NK, natural killer.

- NKTR-358 treatment led to low-level increases in total CD56$^+$ NK cells in most patients at 24 µg/kg, with the increase driven by two outlier individuals
- The CD56^{bright} population was more sensitive to NKTR-358 than the CD56^{dim} population, showing greater induction following NKTR-358 administration
- The ratio of CD56^{bright} to CD56^{dim} NK cells increased 16-fold over predose at 24 µg/kg

NKTR-358 led to the dose-dependent induction of genes associated with regulating immune processes and Treg function



NanoString mRNA expression analysis (Pan-Cancer Immune Profiling 770 gene panel).

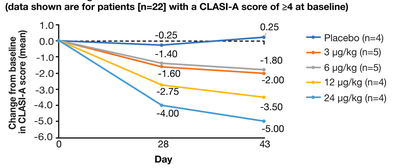
RNA samples were collected after the 1st and 3rd administration. D1, day 1.

- NKTR-358 elicited an increase in the number and magnitude of differentially expressed genes associated with Treg regulation
- Some of the differentially expressed genes overlap with those reported previously in healthy volunteers
- Repeat administration with NKTR-358 showed further induction in most dose-dependent genes

NKTR-358 demonstrated a dose-dependent reduction in CLASI-A score*

- 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)
- Seven of 18 patients had a ≥4-point reduction in CLASI-A score from baseline by Day 43
- No observed changes in SLEDAI or joint scores were noted due to the short treatment duration in this study

*In this small subset of patients, primarily with mild disease and short treatment duration.



CLASI-A, cutaneous lupus erythematosus disease area and severity index–activity.

CONCLUSIONS

- NKTR-358 was safe and well tolerated, with a similar safety profile for single and repeat doses
- A selective, dose-dependent expansion of CD25^{bright} Tregs was observed, which was maintained through multiple NKTR-358 administrations
  - Treg induction was further supported by a correlation between the number of Tregs and the extent of demethylated *FoxP3*
  - Increases in Treg activation markers (CD25, Helios, and CTLA-4) and genes associated with Treg regulation were observed with NKTR-358
- Low-level increases in NK cell numbers occurred in most patients at the highest NKTR-358 dose; the CD56^{bright} population was more sensitive than the CD56^{dim} population
- A dose-dependent reduction in CLASI-A score was seen with NKTR-358 treatment, which warrants further exploration
- These data provide strong support for continued testing of NKTR-358 in patients with SLE and other inflammatory diseases
  - A Phase 2 trial of NKTR-358 in patients with SLE is currently recruiting (NCT04433585)

REFERENCES

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DISCLOSURES

CF, ND, CH, LL, SS, BK, JZ: shareholders of Nektar Therapeutics; employees of Nektar Therapeutics. **VC:** grant/research support from Nektar Therapeutics for conducted studies; speaker bureaus: >5 years ago. **RL:** grant/research support for industry-sponsored trials; consultant for Gilead, Exagen, Myriad Rheumatology; speaker bureaus: Sanofi/Genzyme, Regeneron, Bristol-Myers Squibb, AbbVie. **RF:** grant/research support from Nektar Therapeutics to Northwell Rheumatology to conduct this study; consultant for Nektar Therapeutics. **ID** has no disclosures.

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