Selective Induction of Activated Regulatory T-Cells in Healthy Volunteers by NKTR-358, a Novel IL-2 Conjugate Treg Stimulator, in Development for the Treatment of Autoimmune Diseases

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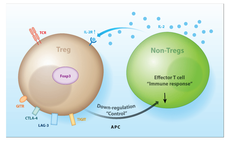
Background

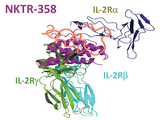
IL-2 is Critical for Treg Expansion, Function and Control of Immune Responses by Regulatory T-cells (Tregs)

Many autoimmune disorders, including systemic lupus erythematosus (SLE), are associated with:

- Reduced Treg numbers
- Impaired Treg function
- Reduced systemic IL-2



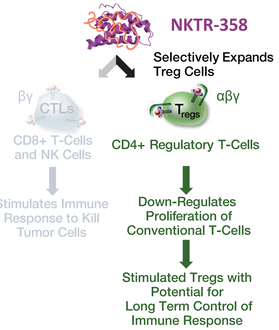
NKTR-358: PEG-conjugated rhIL-2 Selectively Induces Tregs and Their Suppressive Activity



Compared with IL-2, PEG-conjugation in NKTR-358:

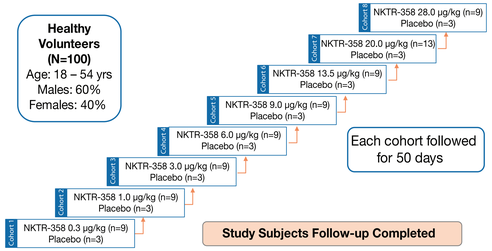
- Alters binding profile of NKTR-358 with lower binding affinity to IL-2Rβ and different binding bias for IL-2Rα & IL-2Rβ
- Imparts selectivity for effect on Tregs over conventional T-cells (Tcon)
- Increases half-life

NKTR-358 has shown activity in animal models of SLE and cutaneous hypersensitivity[1]



Methods

Study Design: Randomized Double-blind Study of Subcutaneous Single-Ascending Doses (SAD) of NKTR-358 in Healthy Volunteers



Study Objectives

- Assess the effects of subcutaneous administration of single-ascending doses of NKTR-358 in healthy volunteers on:

Primary

- Safety and tolerability in subjects as evaluated by:
  - Adverse events
  - Vital signs
  - Clinical safety lab results
  - Cytokine levels

Secondary

- Time course and extent of changes in the numbers and activity of Tregs, Tcons, T cell subsets, and NK cells
- Pharmacokinetics (PK) of NKTR-358
- Other immunological effects: cytokine levels, peripheral blood cell populations, serum proteins and gene expression

- Pharmacodynamic studies conducted to measure selective induction of Tregs and to further characterize their activity following NKTR-358 administration

Assay Methodology

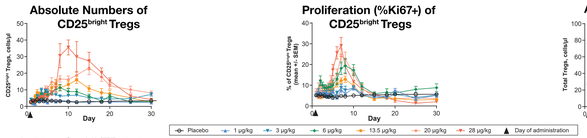
- Immunophenotyping by multicolor flow cytometry to quantify multiple immune cell subsets, using whole blood collected at multiple time points pre- and post-NKTR-358 administration
  - **Total Tregs:** CD4+FoxP3+CD25+ total Tregs, evaluated as absolute numbers and percentage of CD4+ or CD3+ T cells
  - **CD25^{bright} Tregs:** Treg subpopulation with highest CD25 expression; expected to have highest suppressive capacity
  - **Ki67:** marker of proliferation
  - **ICOS, CTLA4, Helios:** markers of Treg activation
- Evaluation of epigenetic modifications conducted with Epiontis ID qPCR-based assay, to monitor methylation status of Treg-specific demethylation region (TSDR) of FoxP3 gene, using whole blood collected at multiple time points from pre-dose through Day 50 post-dose
- Gene expression measured with NanoString platform, with whole blood collected at multiple time points from pre-dose through Day 15 post-dose

Results

NKTR-358 was Safe and Well Tolerated in Healthy Volunteers

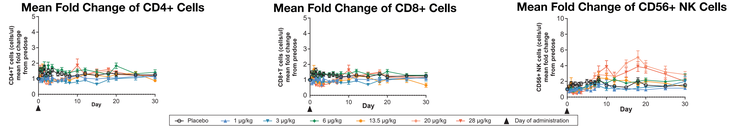
- No dose-limiting toxicities, deaths, or AEs leading to study discontinuation
- No clinically significant vital sign, ECG, or physical examination abnormalities
- Adverse events primarily limited to mild or moderate (Grade 1 or 2) injection site reactions
- 4 subjects experienced Grade 1 mild events of headache
- 1 subject at the highest dose tested (28.0 µg/kg) experienced mild (Grade 1) signs and symptoms of vomiting, diarrhea, anorexia, tachycardia, and myalgia attributed to elevated cytokine levels
- No anti-drug antibodies detected
- NKTR-358 C_{max} and AUC values demonstrated dose proportional increase, with maximal concentrations reached in 5-7 days, and with an estimated half-life of 8-11 days

NKTR-358 Leads to Sustained, Dose-dependent Increases in Numbers and Proliferation (%Ki67+) of Total and CD25^{bright} Tregs

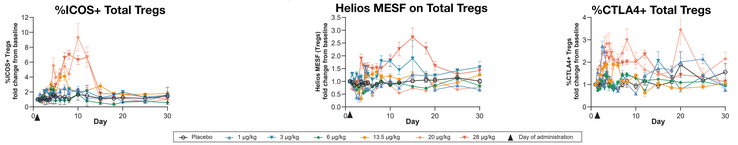


- At 28 µg/kg NKTR-358:
  - 3.5-fold mean peak increase (above predose levels) in numbers of total Tregs and 17-fold mean peak increase in numbers of CD25^{bright} Tregs, suggesting a large increase in most suppressive Treg population
  - Treg levels peak at Days 10-12 and do not return to baseline until Days 20-25 following administration
  - 6-fold mean increase in Ki67+ CD25^{bright} Tregs above predose value

No Changes in Numbers of Tcon Cells and Low-level Increases in Numbers of CD56+ NK Cells in Response to NKTR-358



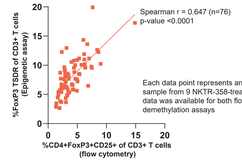
NKTR-358 Increases Expression of Treg Activation Markers



- Sustained increase in percentage of Treg activation markers CTLA4+ and ICOS+ at 20 and 28 µg/kg NKTR-358
- Increase in Helios expression (MESF) on Total Tregs at highest dose of NKTR-358
- Preliminary results indicate suppressive activity observed in ex vivo Treg suppression assay performed with whole blood collected from a limited number of subjects at 8-11 days post-NKTR-358 administration (data not shown)

Identification of NKTR-358-induced Tregs Supported by Correlation with Demethylation Status of FoxP3 Gene

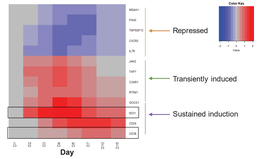
Correlation of % Total Tregs and % Demethylated FoxP3 Gene at 28 µg/kg



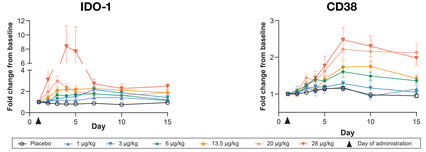
- Published studies have demonstrated that an epigenetically active (demethylated) FoxP3 gene is observed solely in Tregs and not in activated, conventional (non-Treg) CD4+ T cells[2]
  - FoxP3 expressed transiently in activated conventional T cells
  - Constitutive FoxP3 expression in Tregs regulated at epigenetic level by demethylation of TSDR in FoxP3 gene
- After treatment with 28 µg/kg NKTR-358, significant correlation observed between NKTR-358-induced Tregs identified by flow cytometry (CD4+CD25+FoxP3+ cells) and Tregs identified by epigenetic analysis (% demethylation of FoxP3 TSDR)

Genes Associated with Treg Regulation Show Correlation with Treg Induction

Pattern of Differential Expression of 13 Correlated Genes at 28 µg/kg



Dose-dependent Changes in 2 Genes Correlated with Treg Induction



- Increase in number and magnitude of differentially expressed genes in response to NKTR-358 administration
- 84 genes identified that show consistent trend of dose-dependent changes in expression in response to NKTR-358 administration
- NKTR-358-dependent differential expression of 13 genes significantly correlated with induction of Tregs as measured by flow cytometry

Conclusions

- Safe and well tolerated in this first in human study
- Marked dose-dependent expansion of numbers and proliferating CD25^{bright} Treg cells, as demonstrated by flow cytometric and epigenetic analyses
- The induction of Tregs is selective, with no measurable changes in numbers and percentages of CD4+ and CD8+ Tcons at all doses tested and low-level increases of NK cell numbers at highest doses tested
- Tregs induced by NKTR-358 are activated, as measured by flow cytometry and RNA expression analyses
- Data provide strong support for studying NKTR-358 in autoimmune and inflammatory diseases
- NKTR-358 is also being evaluated in a multiple ascending dose MAD study in patients with systemic lupus erythematosus (NCT03556007) and two additional Phase 1b studies in adults with psoriasis (NCT04119557) and atopic dermatitis (NCT04081350)

References

1. Langowski J, et al. *Arthritis Rheumatol.* 2017;69 (suppl 10).
2. Baron U, *Eur J Immunol.* 2007;37:2378.

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