NKTR-358: a selective, first-in-class IL-2 pathway agonist, which increases number and suppressive function of regulatory T cells for the treatment of immune inflammatory disorders

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INTRODUCTION

- Impaired IL-2 production and regulatory T cell (Treg) dysfunction have been implicated as immunological mechanisms in multiple autoimmune diseases
  - Low-dose IL-2 is used to stimulate Tregs for clinical benefit
    - Poor pharmacokinetics (PK) necessitates daily delivery of low-dose IL-2
    - Treg increases are modest and short-lived
- Nektar Therapeutics has developed NKTR-358, a novel product with the goal of selectively restoring Treg homeostasis
  - Utilizes the aldesleukin (Proleukin®) amino acid sequence
  - Chemically conjugated with stable polyethylene glycol (PEG) moieties
  - Intended for low dose, subcutaneous administration
  - Minimal impact on conventional T cell (Tcon) function

METHODS

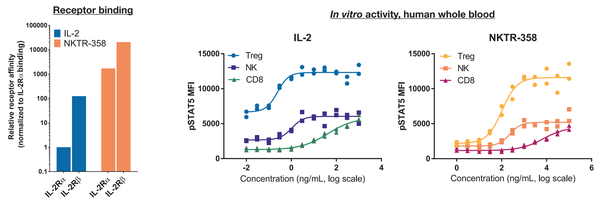
***In vitro* –** The affinity to the IL-2 receptor was assessed by surface plasmon resonance with chips bearing human IL-2Rα or IL-2Rβ. Average K_D values are expressed relative to the affinity of IL-2 to IL-2Rα. Activity in human whole blood was assessed following incubation with either IL-2 or NKTR-358, and measured by pSTAT5 induction in multiple lymphocyte populations using flow cytometry.

Pharmacodynamic and functional assessments – Following a single subcutaneous administration of NKTR-358, or IL-2 administered daily for 5 days, changes in blood lymphocyte numbers, activation and proliferation were measured by flow cytometry. The effect of NKTR-358 on the suppressive capacity of Treg was determined in an *ex vivo* functional assay in which splenic Treg isolated from treated mice were assessed for their ability to suppress the proliferation of naïve Tcon after being co-cultured at a range of Treg:Tcon ratios from 1:2 to 1:512 for three days.

Efficacy models – The ability of Treg induced by NKTR-358 administration to suppress T cell antigen-driven inflammation *in vivo* was assessed in a model of delayed-type hypersensitivity (DTH). Balb/c mice were sensitized with a subcutaneous administration of keyhole limpet hemocyanin (KLH) in an emulsion containing complete and incomplete Freund's adjuvant. Subcutaneous administration (q3d) of NKTR-358 or Cyclosporin A (10 mg/kg, qd) was initiated on day 0 and continued through day 8, with an intradermal challenge of KLH administered on day 5, and ear swelling measured for four days. The specificity of response was assessed after an additional 3-4 weeks with no treatment by either KLH rechallenge or conducting sensitization and challenge with an unrelated antigen (ovalbumin, OVA). A model of cutaneous hypersensitivity (CHS) was also utilized in cynomolgus monkey, where animals were sensitized at week -6, -4, and week -2 to tetanus toxoid, while NKTR-358 was administered every two weeks. Following a third sensitization, skin spot area, erythema and edema were measured. Efficacy in a model of systemic lupus erythematosus (SLE) was assessed using MRL/MpJ-Fas^{lpr} mice. Starting at week 8, NKTR-358 was administered q3d with urine protein level monitored weekly. At the end of the study (week 20), plasma autoantibody, blood urea nitrogen and Treg levels were assessed.

RESULTS

NKTR-358 demonstrates attenuated affinity for the IL-2Rβ relative to IL-2Rα and favors activation of Treg over Tcon

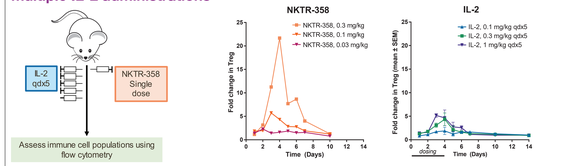


NKTR-358 displays attenuated IL-2 receptor binding with greater relative affinity for IL-2Rα than native IL-2. Cellular activity demonstrates that Treg are far more sensitive to NKTR-358 than NK and CD8 T cells.

MFI – mean fluorescence intensity

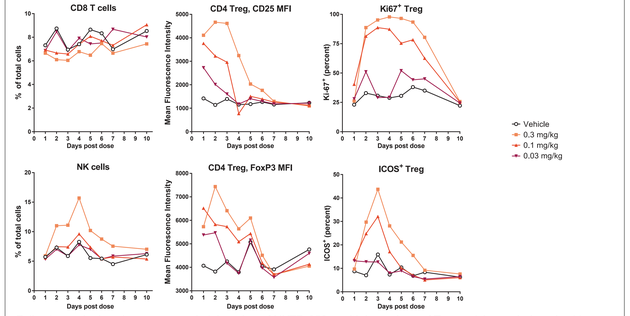
RESULTS

A single administration of NKTR-358 promotes greater Treg mobilization than multiple IL-2 administrations



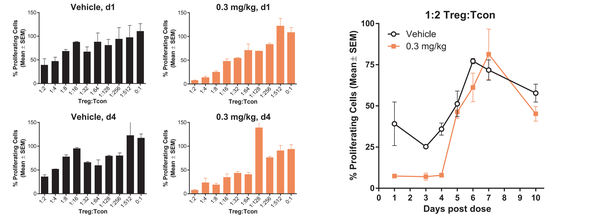
NKTR-358 stimulates a greater magnitude and duration of Treg mobilization following a single subcutaneous administration than five daily administrations of IL-2.

NKTR-358 promotes selective Treg activation



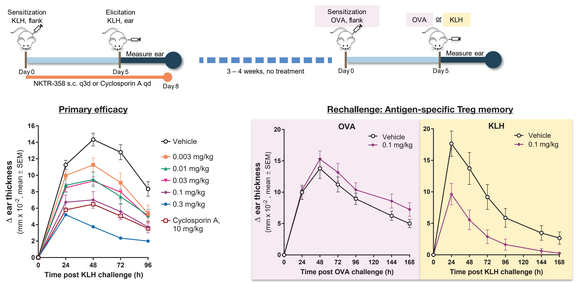
Following a single subcutaneous administration of NKTR-358, multiple markers of Treg activity are induced, with minimal activity on other lymphocyte subsets.

NKTR-358 increases Treg suppressive function



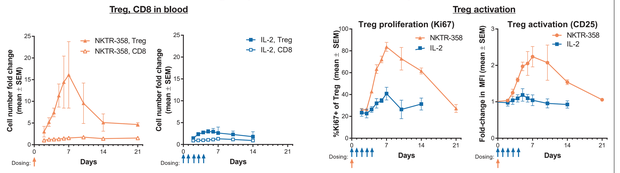
Splenic Treg from NKTR-358 treated mice, co-cultured in varying ratios of Tcon, increase their suppressive capacity, with the greatest increases in function occurring in the first four days following a single subcutaneous administration.

NKTR-358 suppresses antigen-driven inflammation in the mouse DTH model



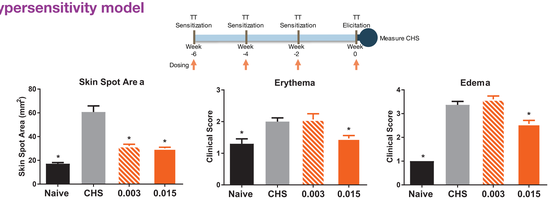
The ability of NKTR-358 to suppress antigen-induced inflammation *in vivo* was shown using a delayed-type hypersensitivity model with the KLH antigen. The durability and specificity of the response in treated mice was shown by KLH rechallenge with no additional treatment. Mice sensitized and challenged to the unrelated OVA antigen yielded normal immune responses.

Preferential and sustained Treg expansion in non-human primates



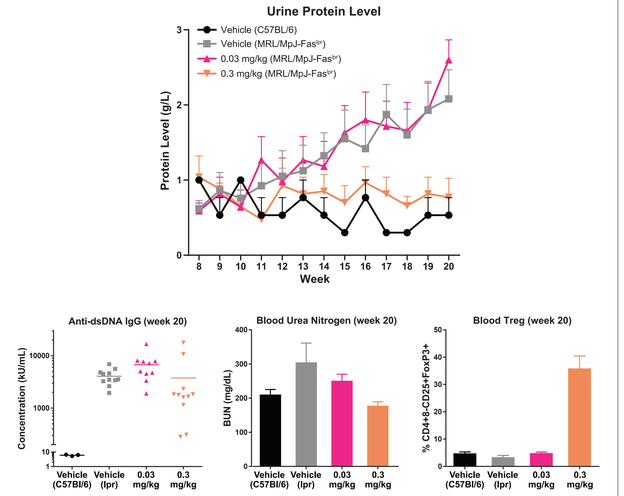
In cynomolgus monkey, a single subcutaneous NKTR-358 (0.025 mg/kg) administration led to significant Treg mobilization, which was sustained in duration for >14 days, and was concomitant with markers of proliferation and activity. This effect was far greater than an equivalent dose of IL-2 (0.005 mg/kg) delivered daily for five days. Minimal CD8 activity was noted.

Suppression of antigen-driven inflammation in a non-human primate cutaneous hypersensitivity model



In cynomolgus monkey, NKTR-358 delivered subcutaneously every two weeks (0.003 and 0.015 mg/kg) was efficacious in a model of cutaneous hypersensitivity using sensitization and challenge of the tetanus toxoid (TT). *P<0.05

NKTR-358 is efficacious in a mouse model of systemic lupus erythematosus



NKTR-358 administered subcutaneously (0.3 mg/kg q3d) from week 8 to 20 showed efficacy in the mouse MRL/MpJ-Fas^{lpr} lupus model. To monitor disease progression, urine protein level was measured weekly. At study end (week 20), the highest dose of NKTR-358 led to reduced anti-dsDNA antibodies and blood urea nitrogen levels, an effect associated with significant and sustained Treg mobilization.

CONCLUSIONS

- NKTR-358 delivers sustained, preferential activation of Tregs; in cynomolgus monkey this effect is sustained for >14 days
- NKTR-358 is currently being investigated in a Phase 1 study in healthy subjects to measure Treg mobilization, functional activity, PK, and safety, with the goal of establishing a range of dose levels to advance into a multiple-ascending dose trial in patients with SLE
- Nektar Therapeutics and Eli Lilly and Company have entered into a strategic collaboration to develop and commercialize NKTR-358