



NEW PATHWAYS TO
SMARTER MEDICINE™

**Selective Expansion of
Regulatory T-Cells by a
Novel IL-2 Conjugate,
NKTR-358, Being Developed
for the Treatment of
Autoimmune Diseases**

Christie Fanton, PhD

Presenter Disclosure Information

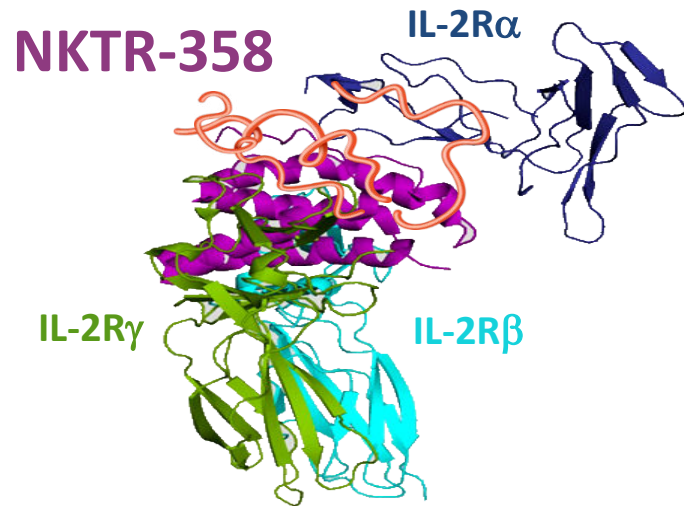


Christie Fanton, PhD
Nektar Therapeutics

The following relationships exist related to this presenter:

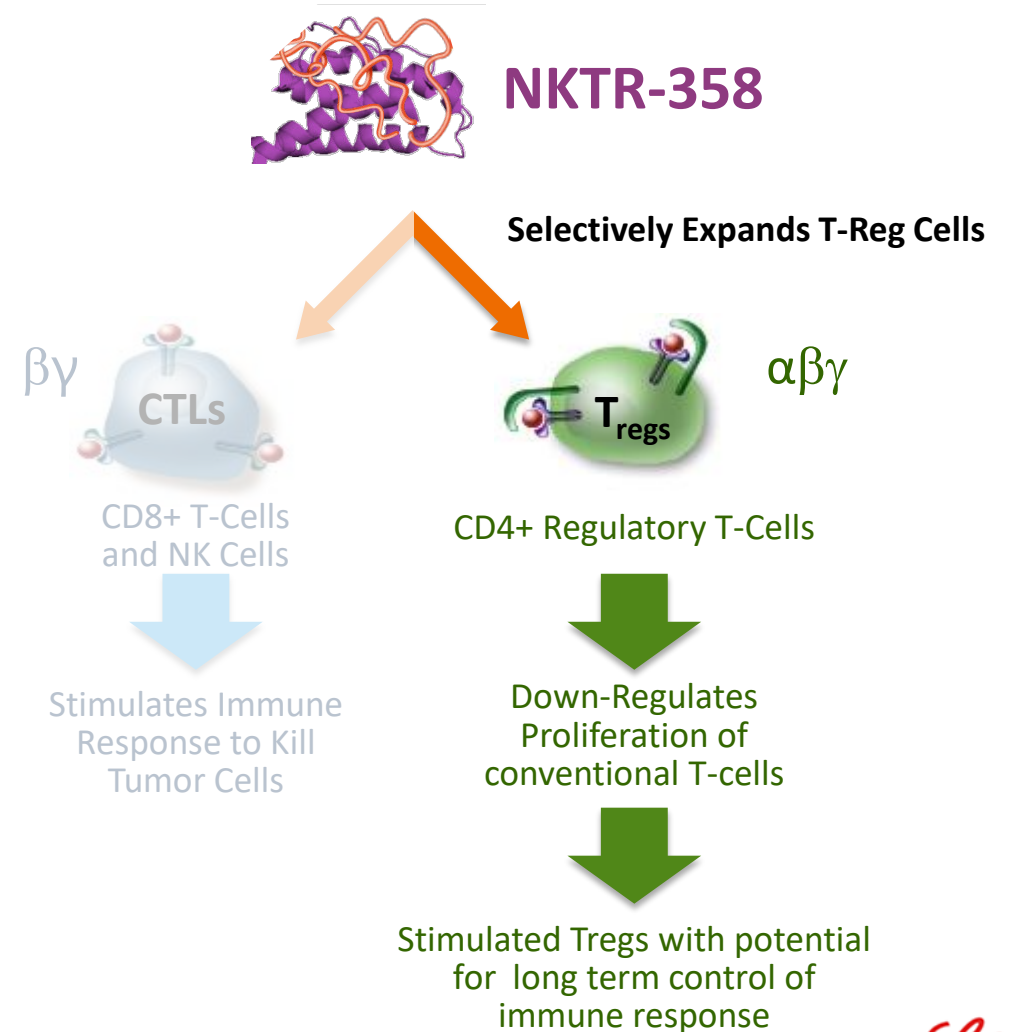
- C. Fanton is an employee of Nektar Therapeutics and owns shares of the company

NKTR-358: PEG-conjugated rhIL-2 Selectively Induces Regulatory T Cells (Tregs) and Their Suppressive Activity



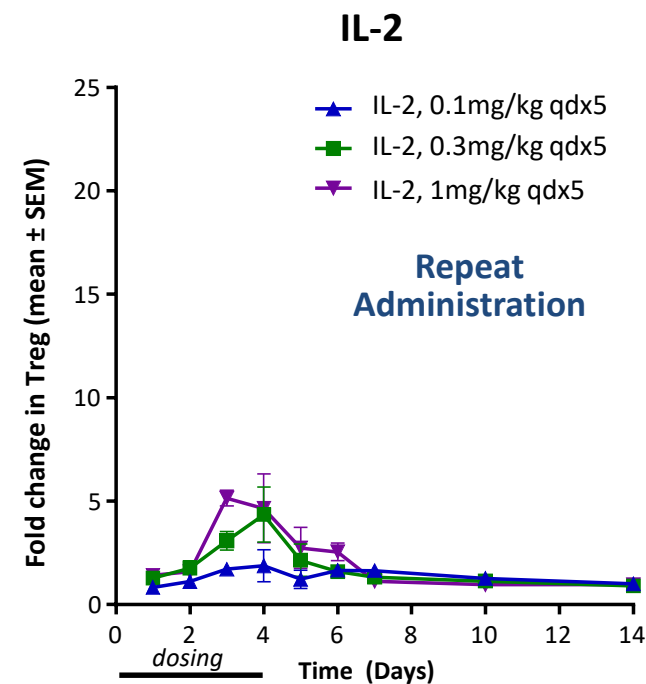
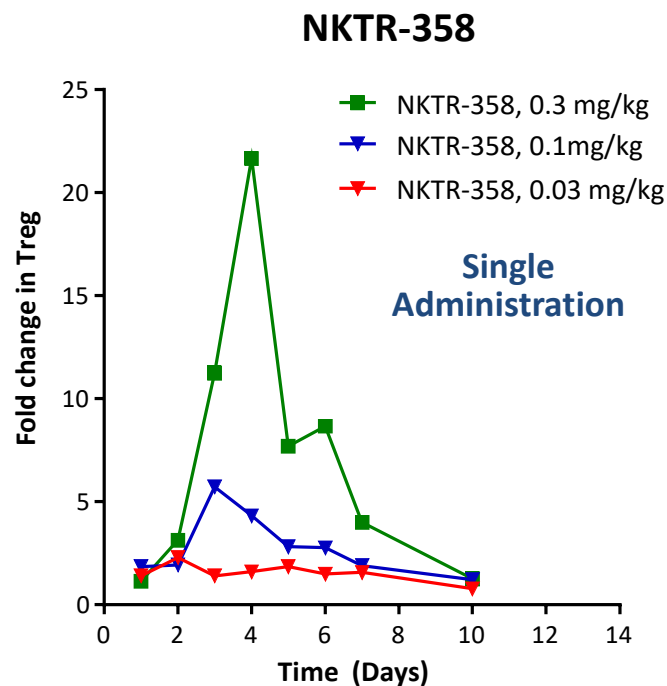
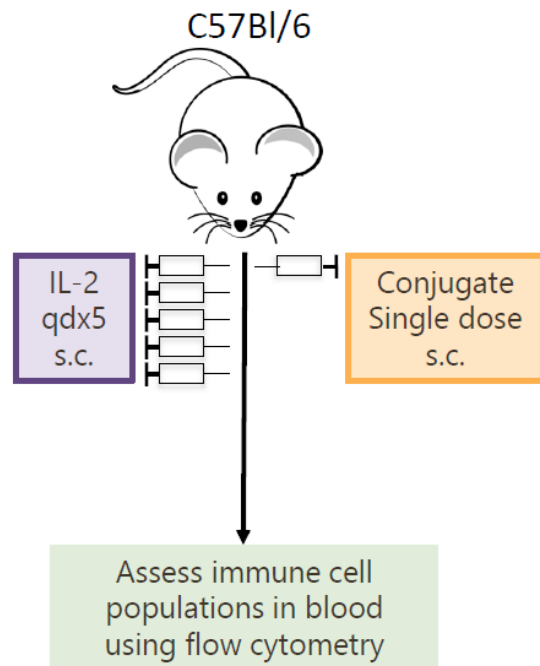
PEG-conjugation:

- Stable covalent conjugation to rhIL-2 (aldesleukin sequence)
- Increases half life (vs IL-2)
- Alters binding profile of NKTR-358 (relative to IL-2) with lower binding affinity to IL-2R β and different binding bias for IL-2R α & IL-2R β
- Imparts selectivity for effect on Tregs over Tcons (vs IL-2)

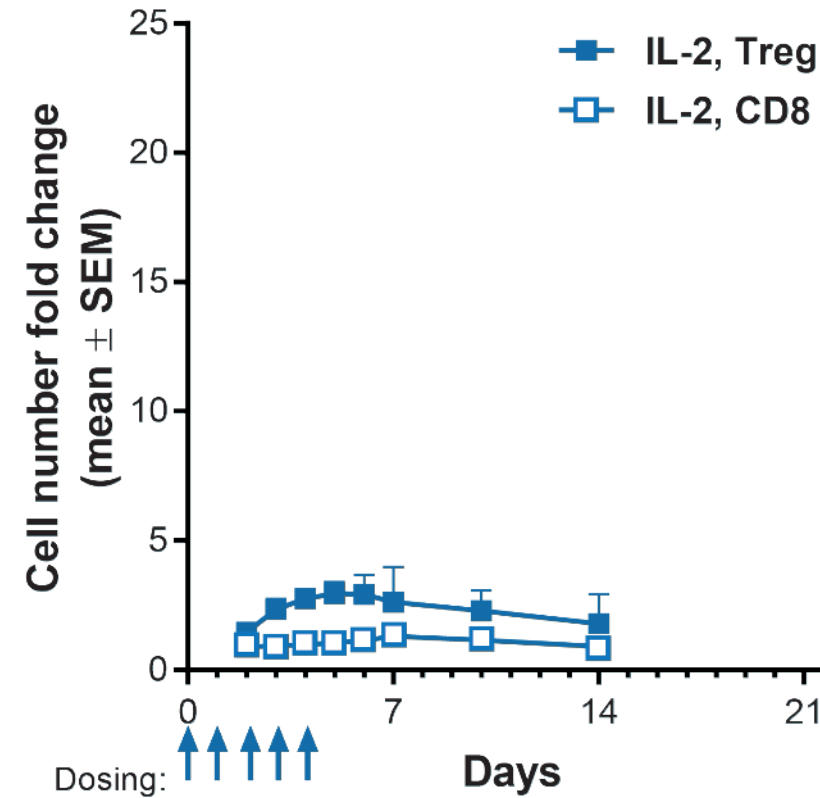
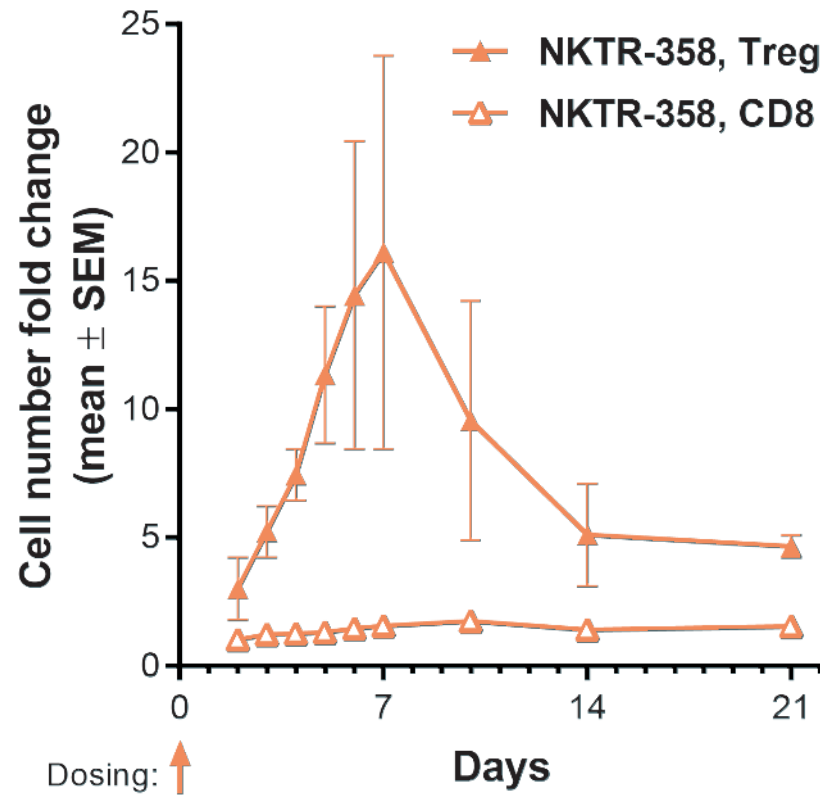


Comparison of NKTR-358 and IL-2 by *In Vivo* Screening

Single NKTR-358 administration in mouse leads to superior Treg induction compared to multiple IL-2 administrations



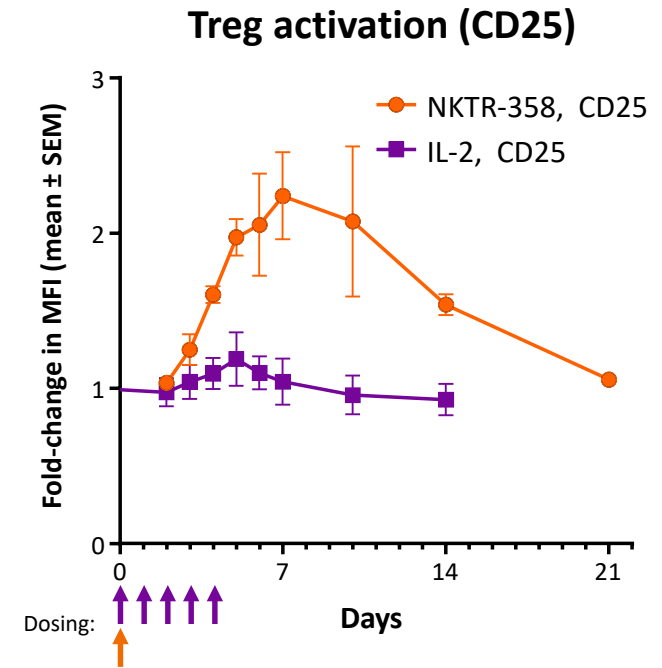
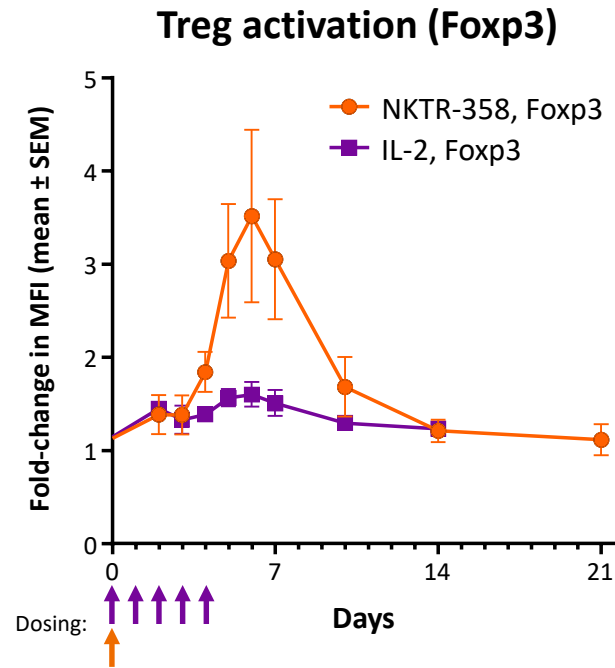
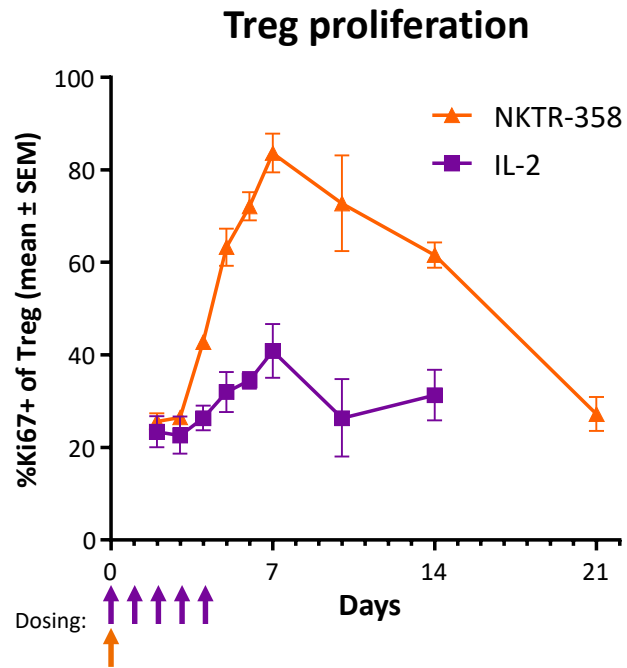
NKTR-358 Preferentially Expands Tregs in non-Human Primates



Cynomolgus monkeys administered:

- NKTR-358 (25 μ g/kg) single dose, or,
- Recombinant human IL-2 (5 μ g/kg) daily on 5 consecutive days

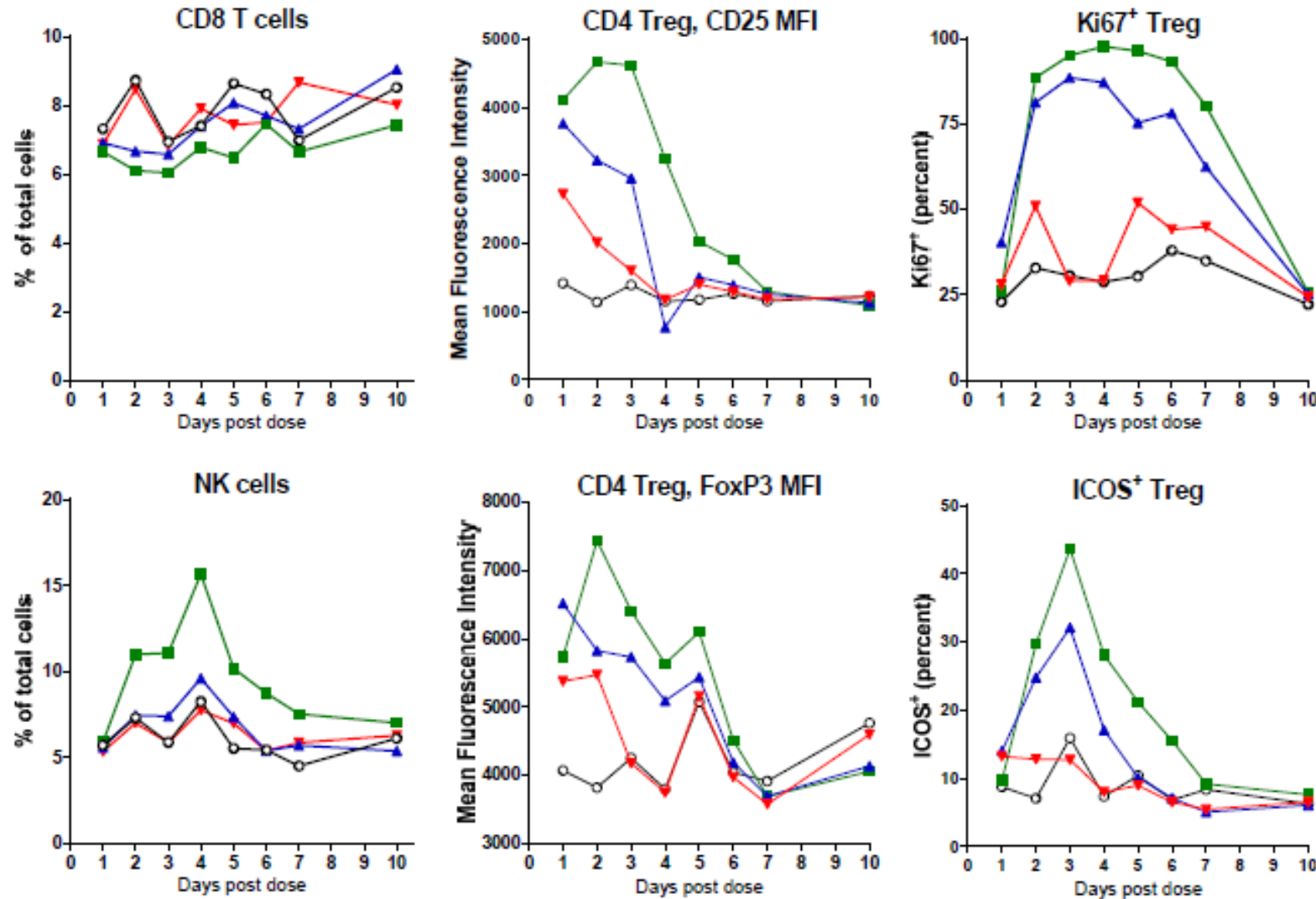
NKTR-358 Promotes Greater Treg Proliferation and Activation than IL-2 in non-Human Primates



Cynomolgus monkey: 1M + 1F

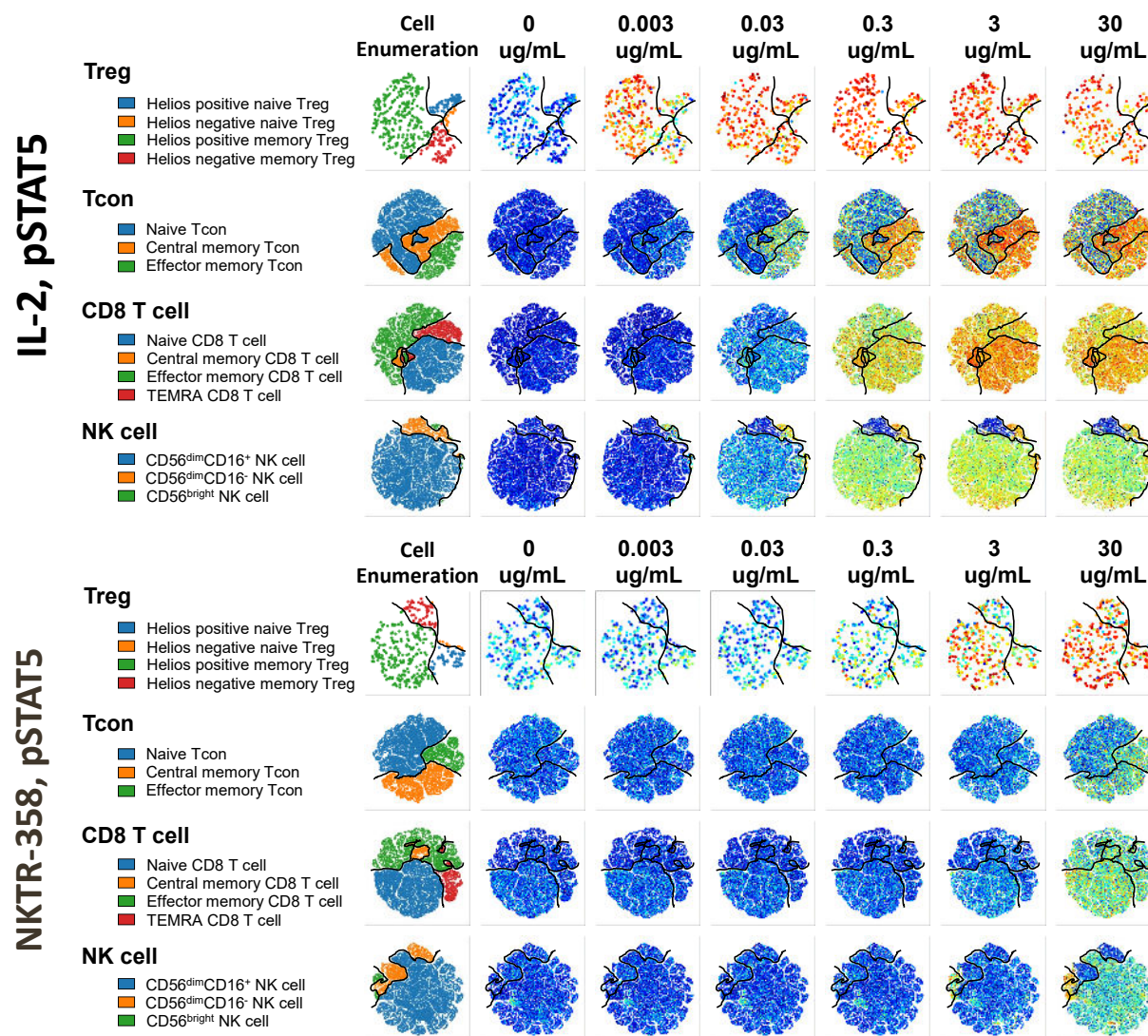
- 25µg/kg : NKTR-358 single dose vs. qdx5 for IL-2

NKTR-358 Promotes Selective Treg Proliferation and Activation In Vivo

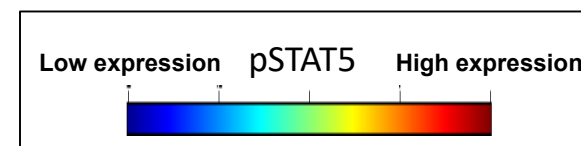


- Single dose NKTR-358 SC
- Induction of proliferation and activation markers
 - Helios, GITR, CTLA-4, CD39, CD73, OX40, and PD-1 (not shown)
 - Similar effect in blood and spleen (not shown)

NKTR-358 Favors Activation of Tregs Over Other Subsets

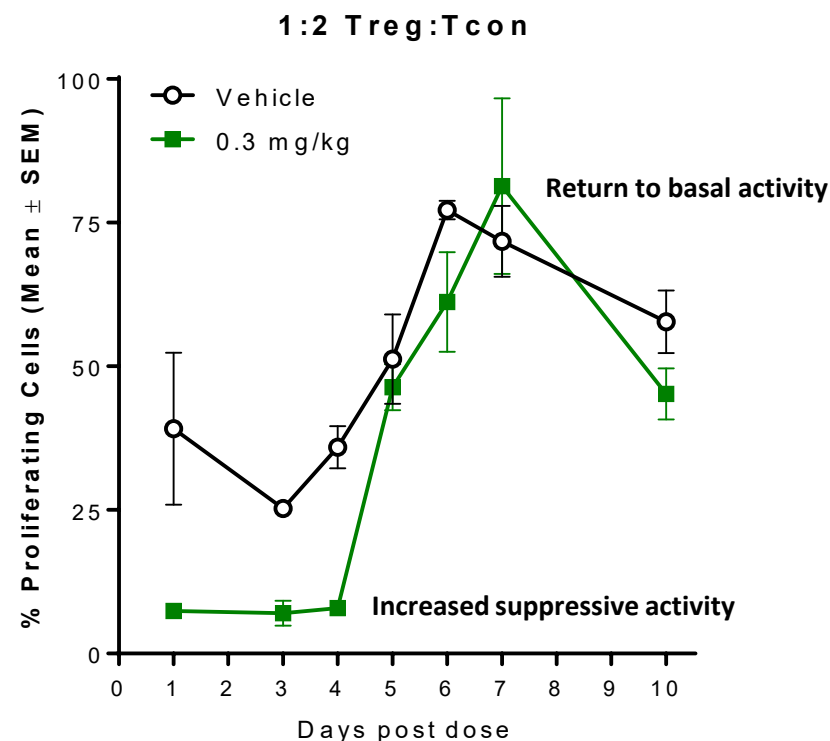
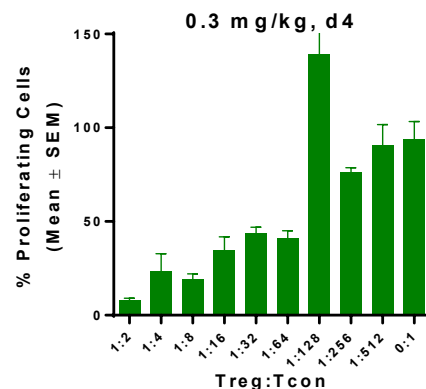
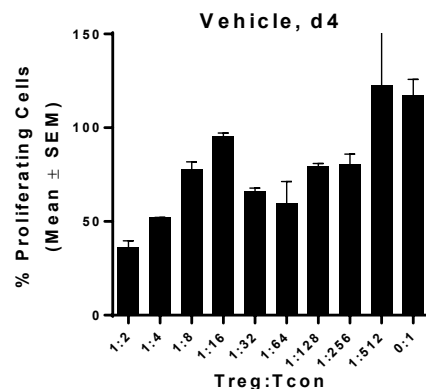
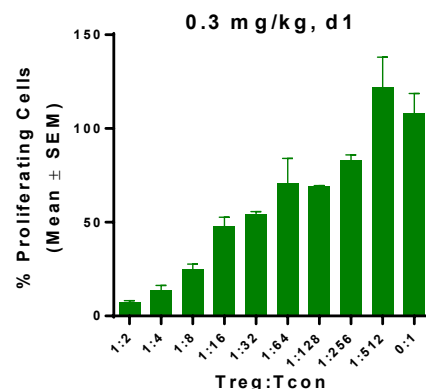
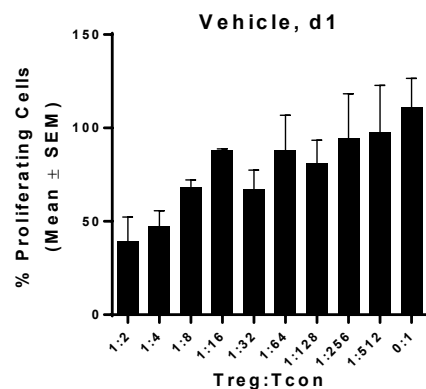


- Healthy human PBMCs
 - IL-2 or NKTR-358 for 15 min
 - Analysis by CyTOF
- IL-2 and NKTR-358 had primary effect on pSTAT5
 - No effect on pAKT, pERK, pS6, and pSTAT3 (save IL-2 on CD56+++ NK)



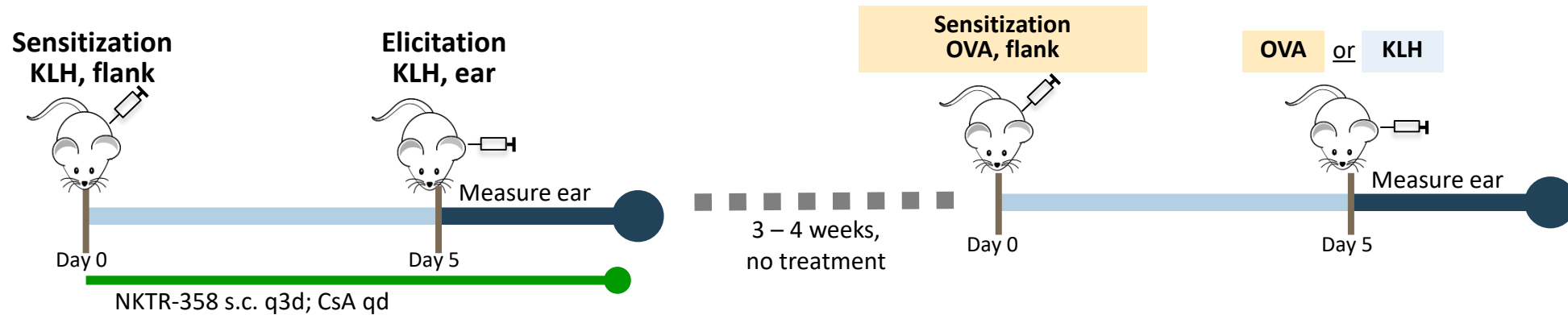
NKTR-358 Increases Treg Suppressive Activity

In vivo / *Ex vivo* T suppressor assay in mice

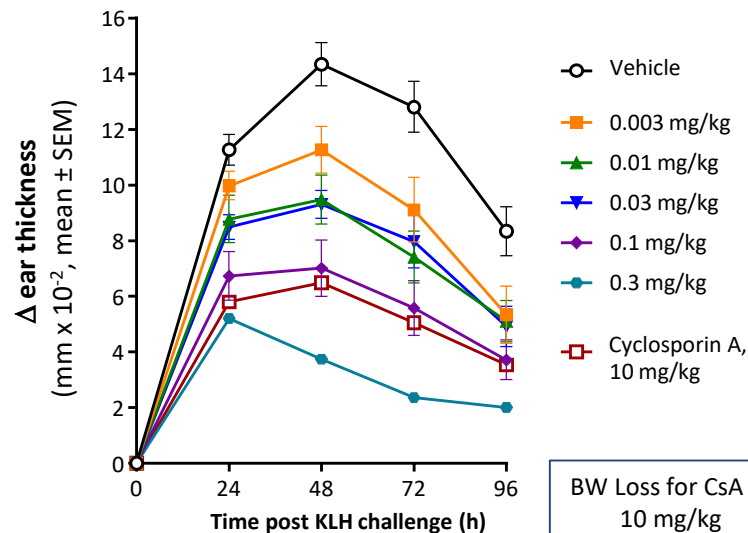


NKTR-358 Suppresses Antigen-Driven Inflammation

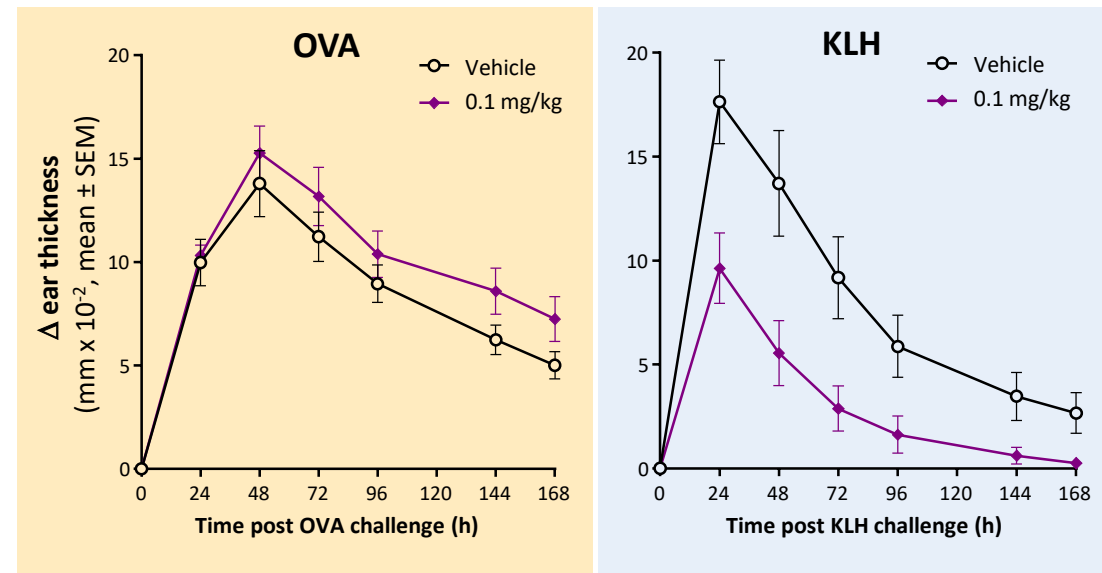
Effect is durable and antigen-specific



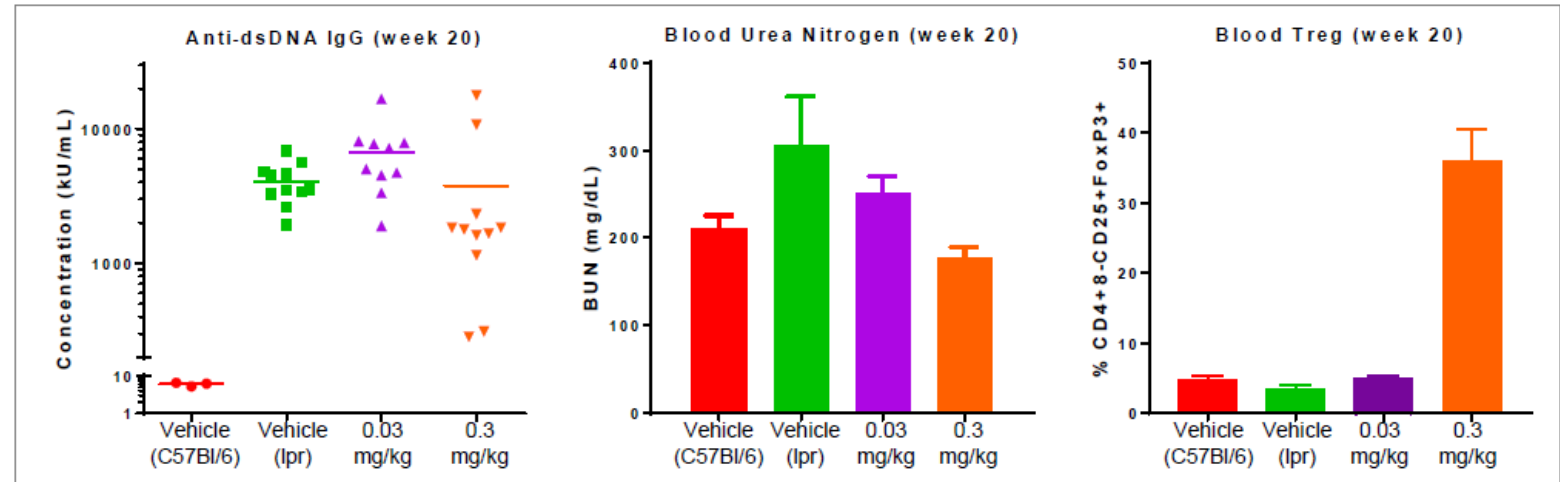
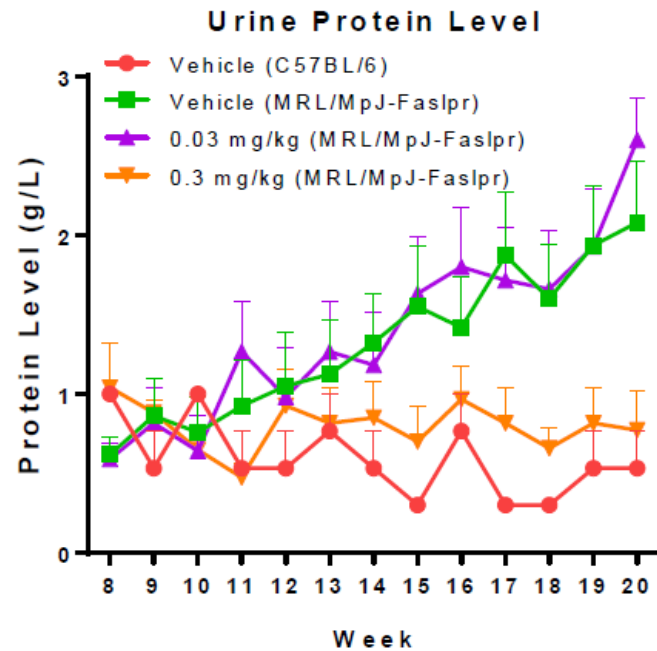
Primary efficacy



Rechallenge : Antigen-specific Treg memory



NKTR-358 Is Efficacious in a Mouse Model of SLE



- NKTR-358 demonstrated dose-dependent efficacy on multiple parameters in mouse SLE
- 0.3 mg/kg (q3d, week 8-20) reduces urine protein and blood urea nitrogen to naïve mouse parameters
- Efficacy is consistent with Treg elevation

NKTR-358: Single Ascending Dose Study Objectives

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

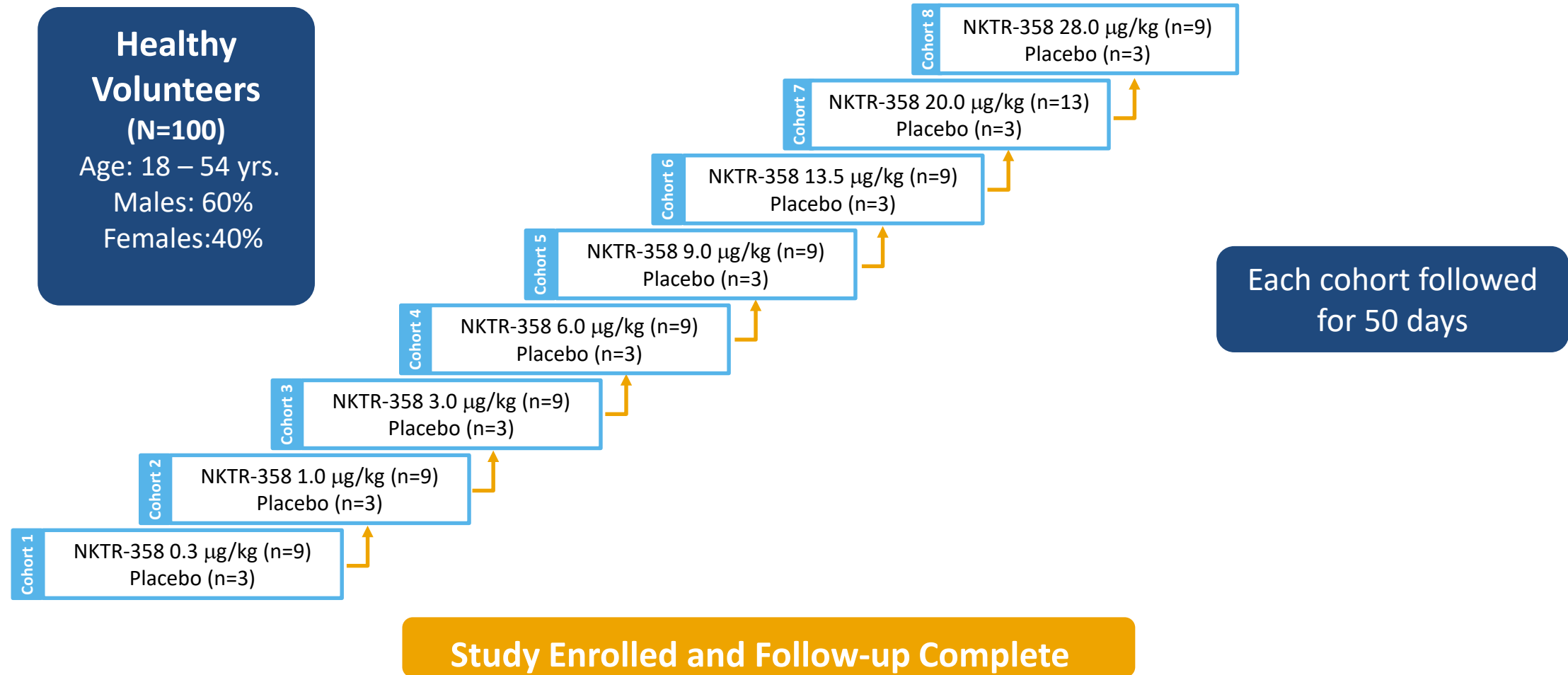
Primary

- 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, and NK cells and subsets
- Pharmacokinetics (PK) of NKTR-358
- Other immunological effects: cytokine levels, peripheral blood cell populations, serum proteins and gene expression

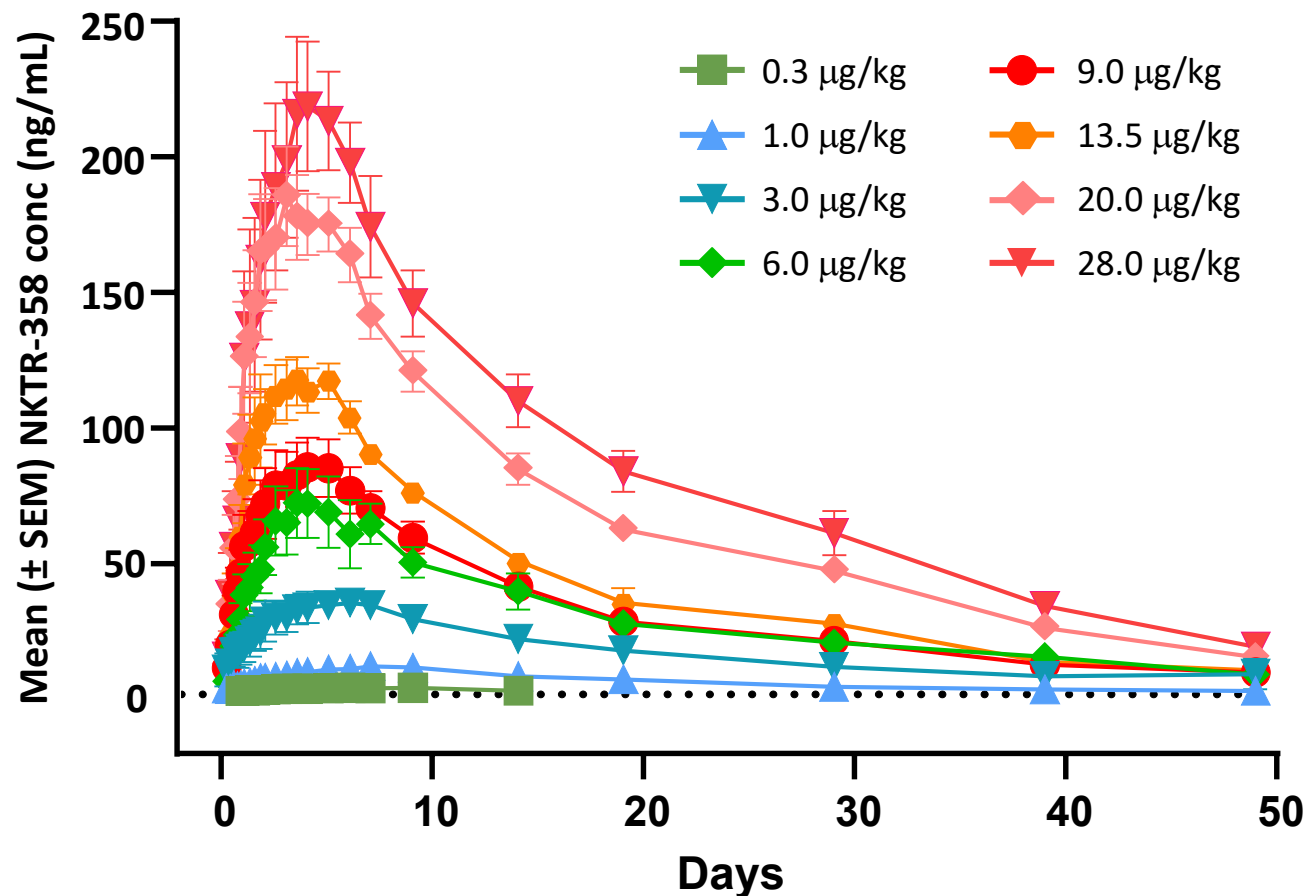
Study Design: Randomized Double-blind Study of Subcutaneous Single Ascending Doses of NKTR-358 in Healthy Volunteers



NKTR-358 SAD Study Results: NKTR-358 Was Safe and Well-Tolerated in Healthy Volunteers

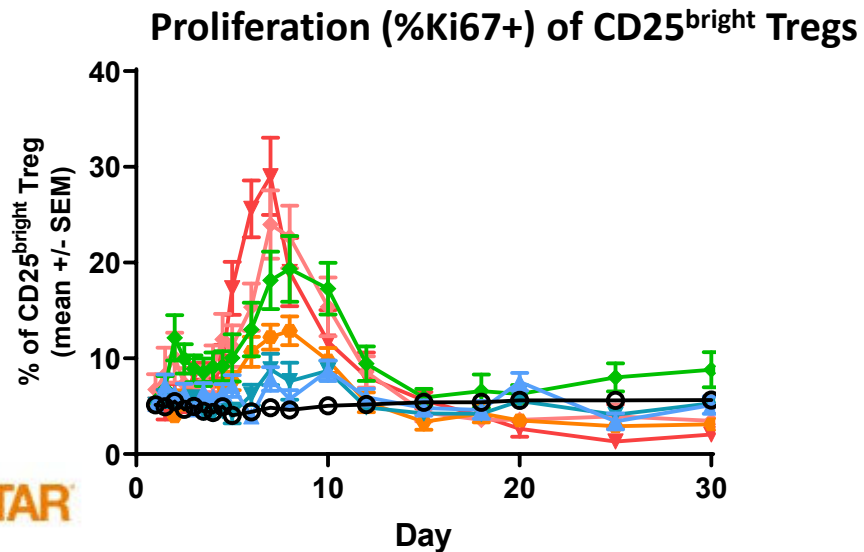
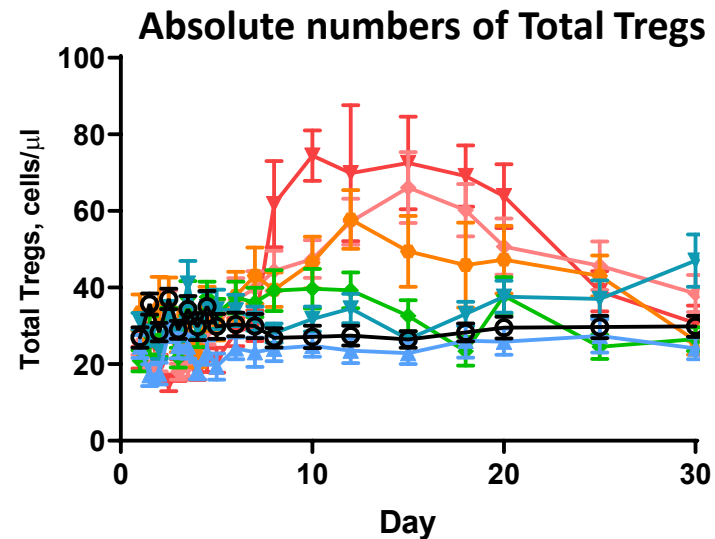
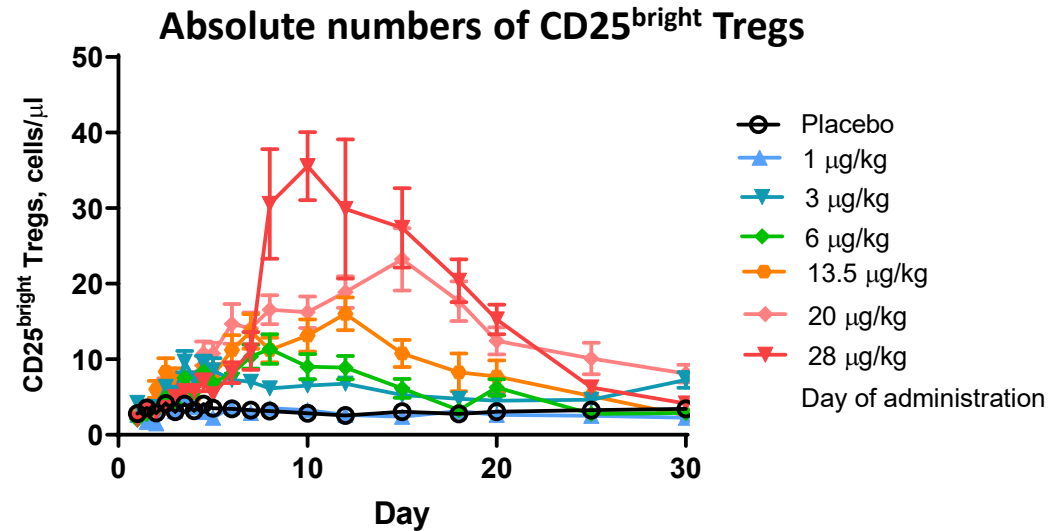
- No dose-limiting toxicities, deaths, or AEs leading to study discontinuation
- No clinically significant vital sign, ECG, or physical examination abnormalities
- Adverse events primarily limited to mild or moderate (Grade 1 or 2) injection site reactions
- 4 subjects experienced Grade 1 mild events 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 Concentration Curves Indicate Dose Proportional Pharmacokinetics



- NKTR-358 C_{max} and AUC values exhibited a dose proportional increase
- NKTR-358 concentrations reached maximum levels in 5-7 days
- NKTR-358 has an estimated elimination half-life of 8-11 days
 - half-life of IL-2 in human serum is ~5-7 minutes

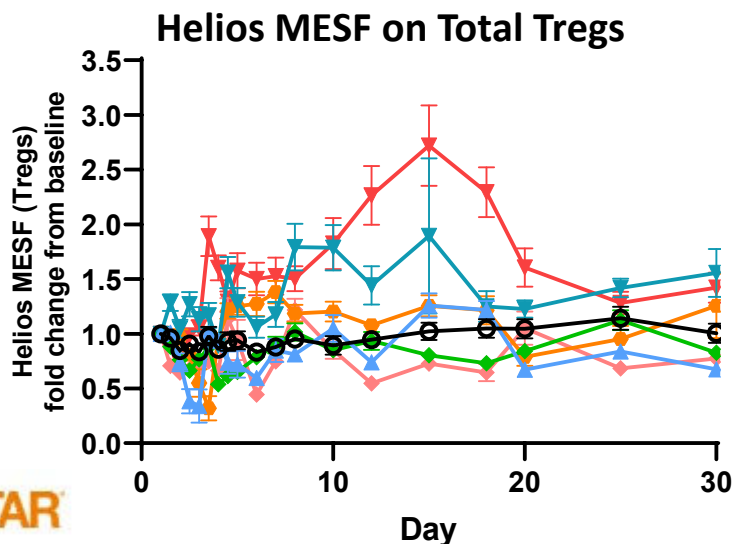
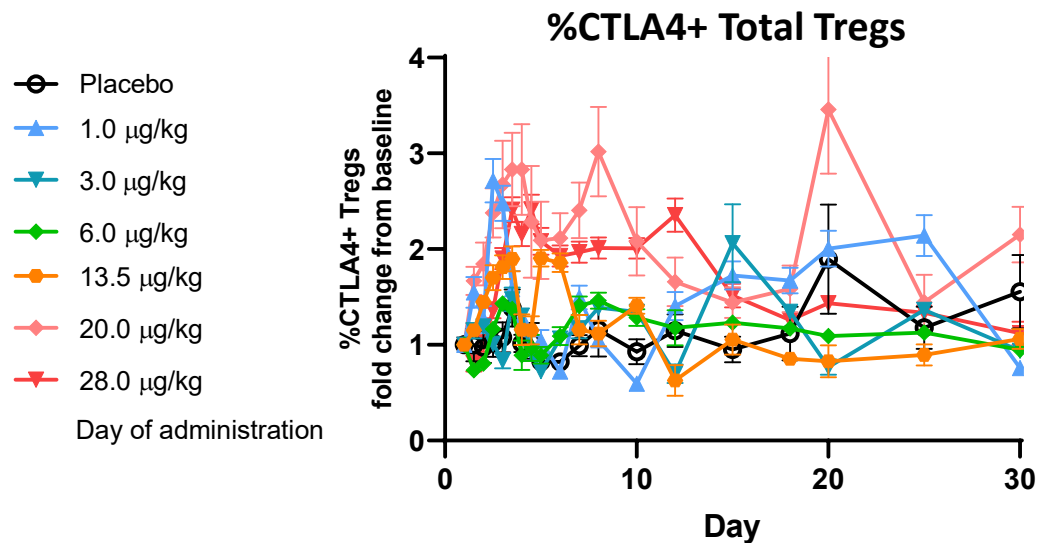
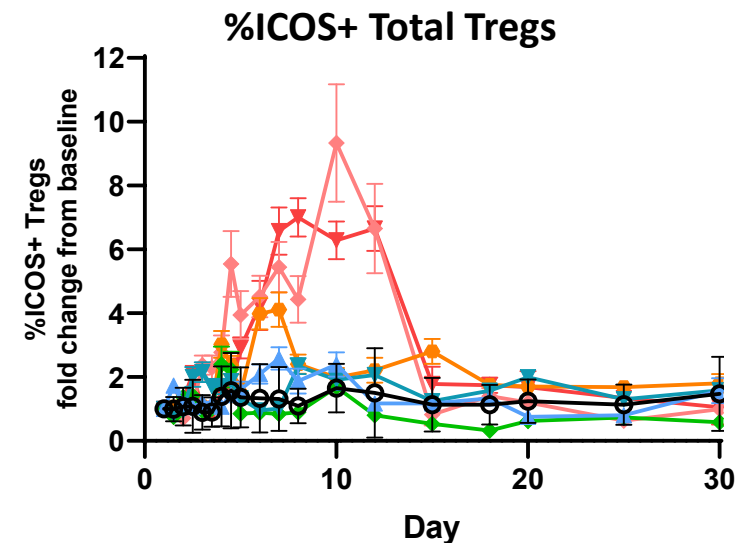
NKTR-358 Leads to Sustained, Dose-dependent Increases in Numbers and Proliferation (%Ki67+) of Total and CD25^{bright} Tregs



At 28 μ g/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

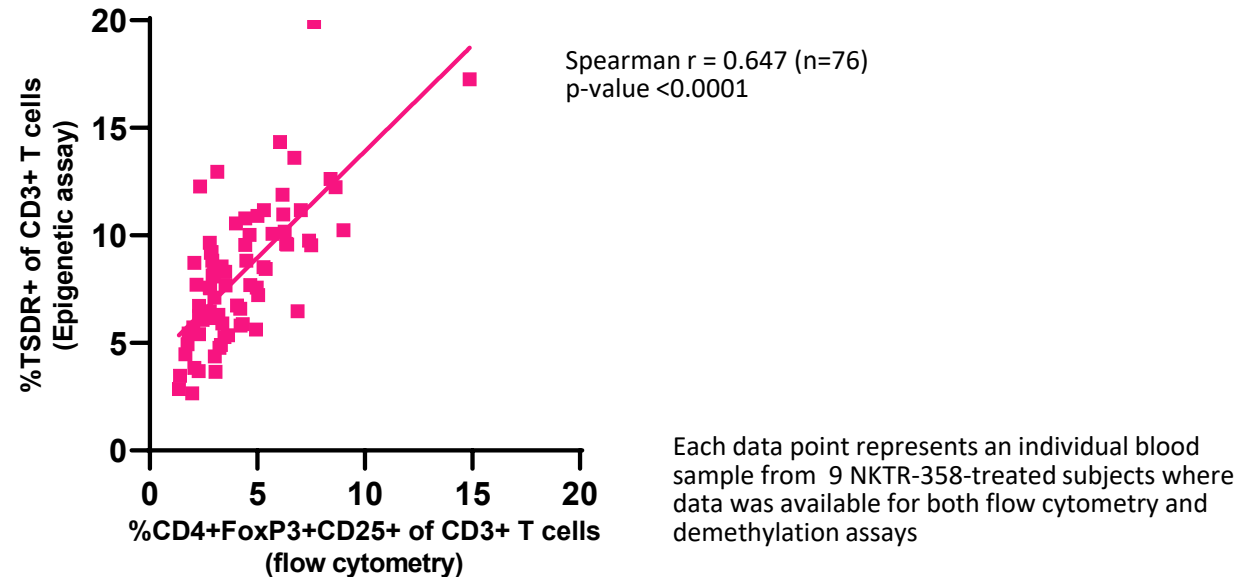
NKTR-358 Increases Expression of Treg Activation Markers



- 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

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

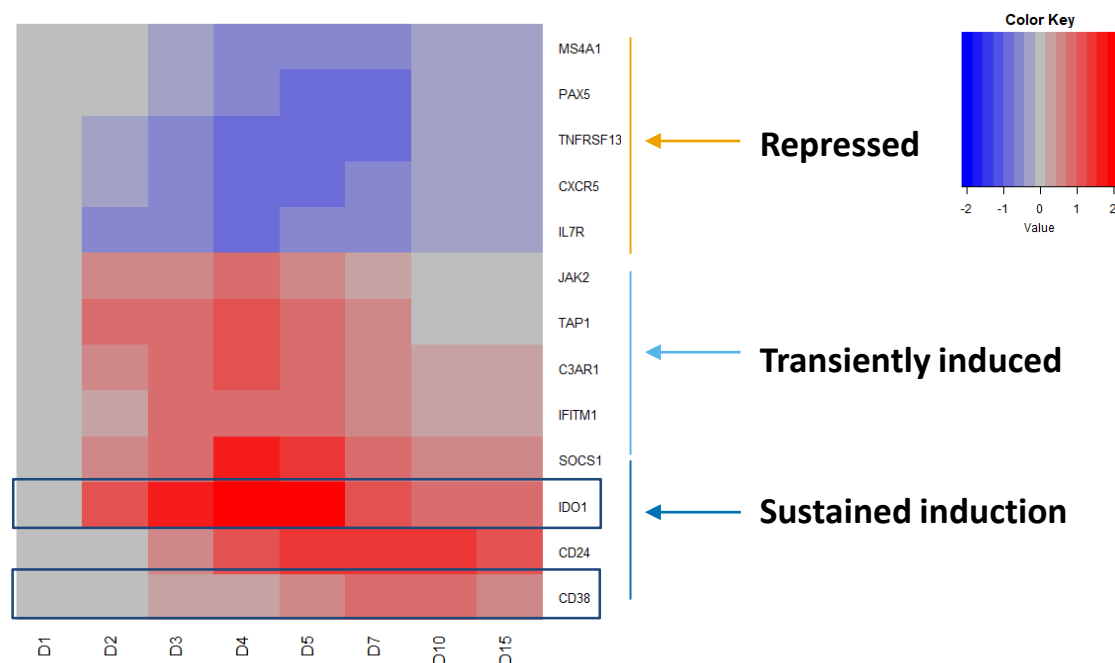
Correlation of % Total Tregs Across Assays at 28 µg/kg



- Constitutive FoxP3 expression in Tregs is regulated at the epigenetic level by demethylation of TSDR in the FoxP3 gene, and an epigenetically active (demethylated) FoxP3 gene is observed solely in Tregs and not in activated, conventional (non-Treg) CD4+ T cells
- After 28 µg/kg NKTR-358 administration, a significant correlation is observed between NKTR-358-induced Tregs identified by flow cytometry and Tregs identified by epigenetic analysis (% demethylation of FoxP3 TSDR)

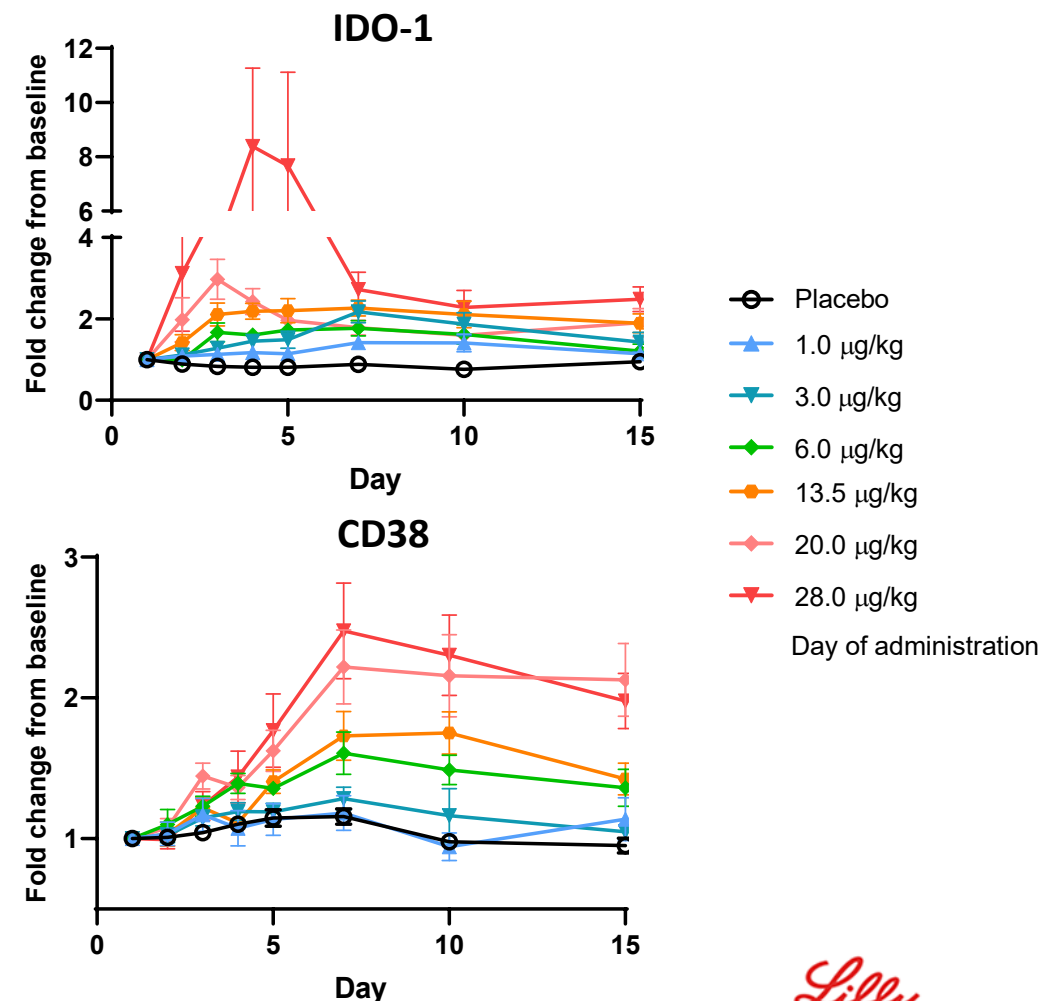
Genes Associated with Treg Regulation Show Correlation with Treg Induction

Pattern of Differential Expression of 13 Correlated Genes at 28 $\mu\text{g/kg}$

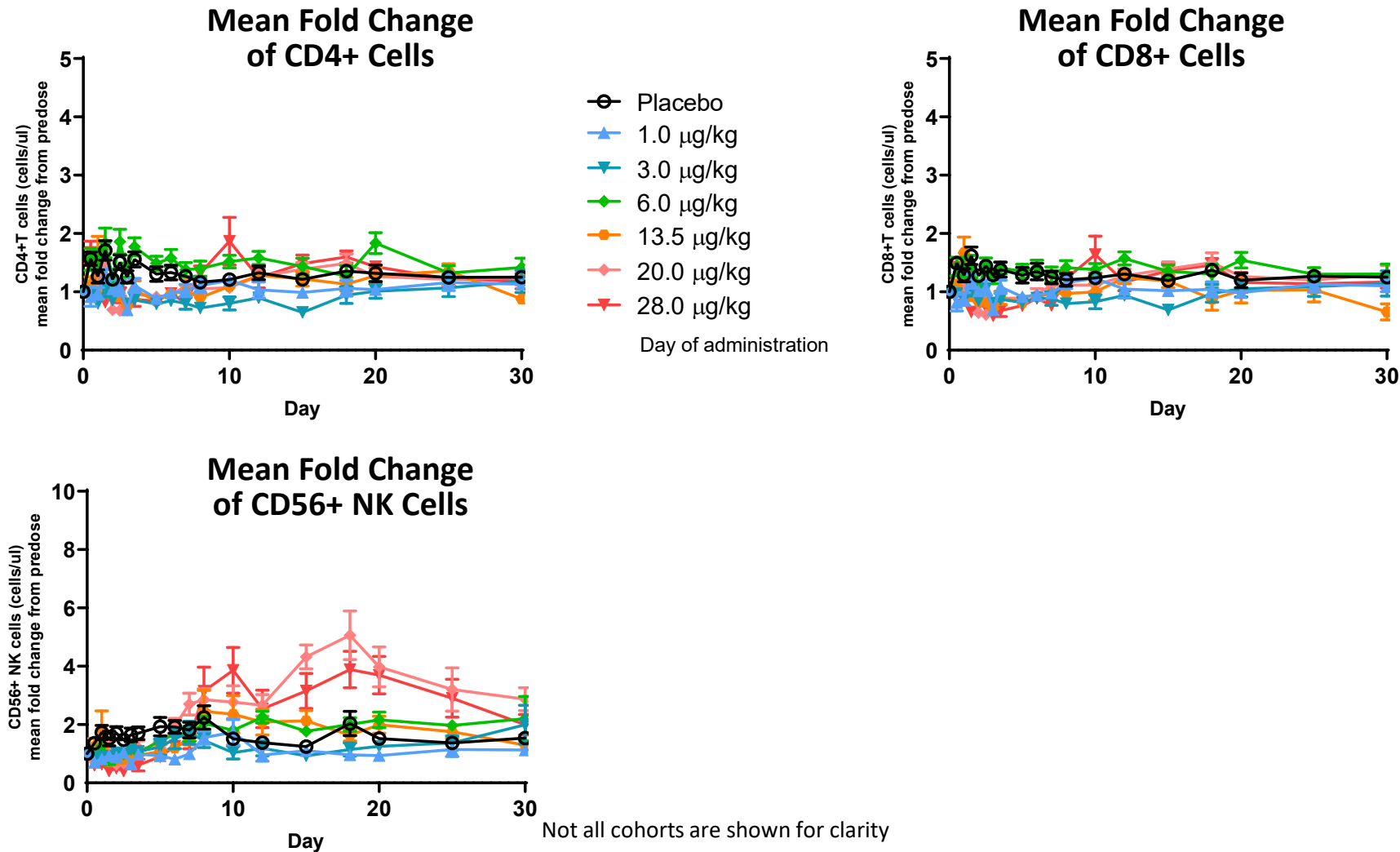


- Increase in number and magnitude of differentially expressed genes in response to NKTR-358 administration observed
- NKTR-358-dependent differential expression of 13 genes significantly correlated with induction of Tregs as measured by flow cytometry ($p < 0.05$)

Dose Dependent Changes in 2 Genes Correlated with Treg Induction

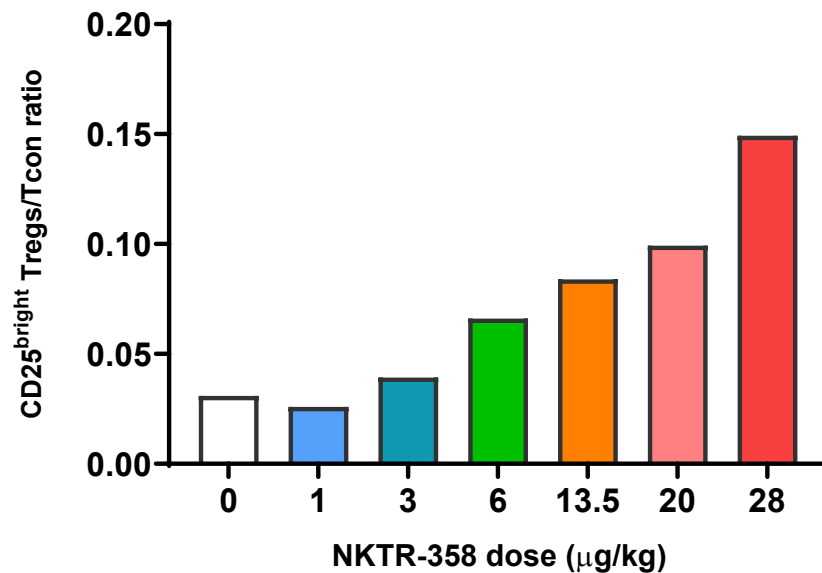


NKTR-358: No Changes in Numbers of Tcon Cells and Low-level Increases in Numbers of CD56+ NK Cells

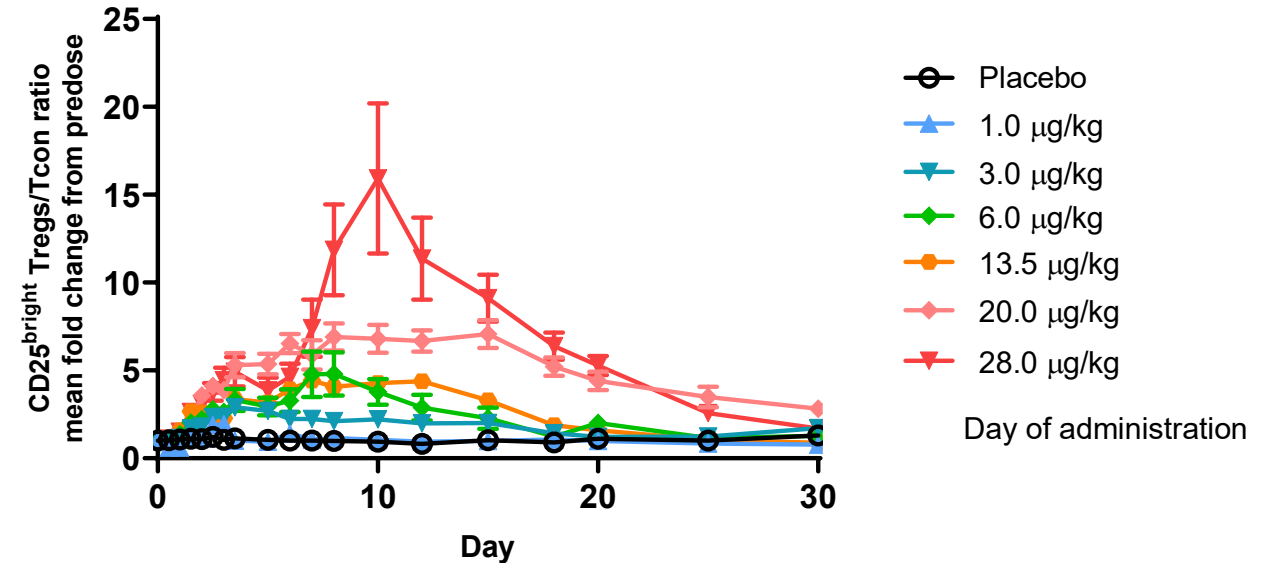


NKTR-358 Selectively Induces Tregs in a Dose-Dependent Manner

Median Peak Effect of CD25^{bright} Treg/Tcon Ratio



Mean Fold Change in CD25^{bright} Tregs/Tcon Cell Ratio



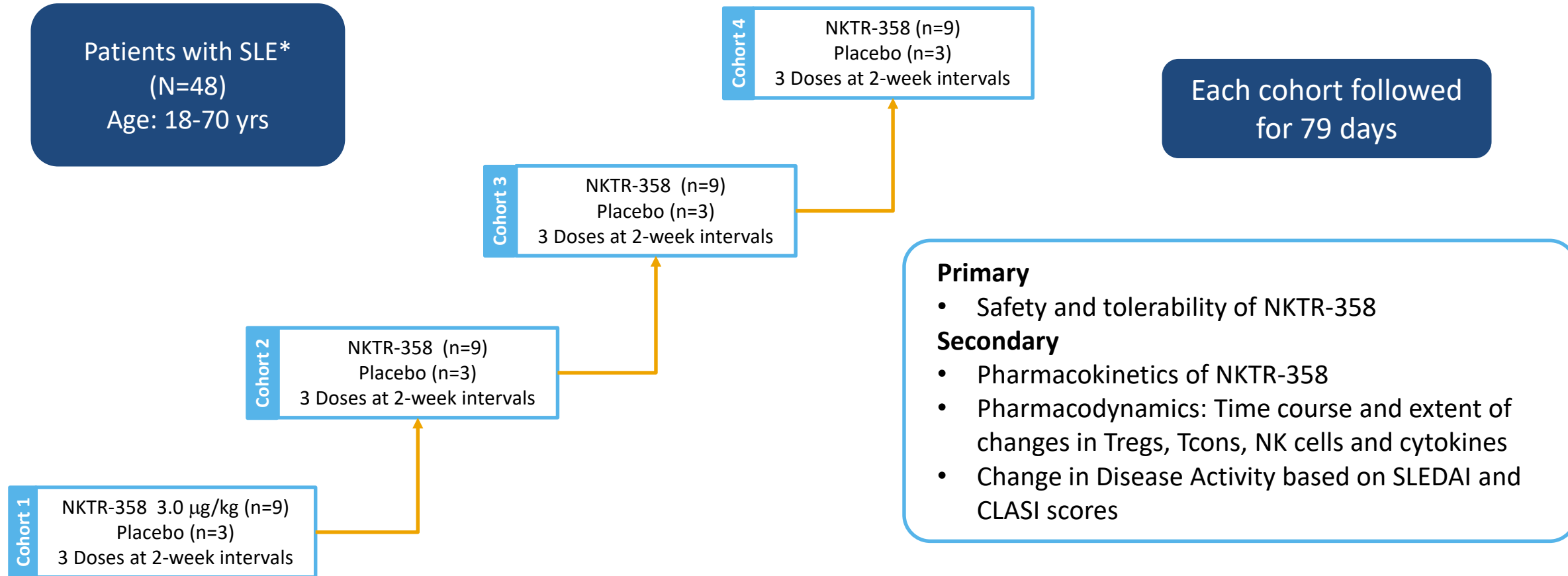
NKTR-358 administration leads to 15-fold increase in mean peak Treg: Tcon ratio over baseline at 28 µg/kg.

In this analysis Tcon cells are defined as CD8⁺ Tcells; Not all cohorts are shown for clarity

NKTR-358 SAD Study: Conclusions

- Safe and well tolerated in this first in human study
- Dose proportional pharmacokinetics and prolonged exposure with a half-life of 8-11 days
- Marked and selective dose-dependent expansion of numbers of 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 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 currently being studied in a multiple ascending dose clinical trial in patients with SLE and additional studies in other inflammatory diseases are ongoing or planned

Ongoing Phase 1b Study of Subcutaneous Multiple Ascending Doses of NKTR-358 in Patients With SLE



*Diagnosis of adult SLE according to 1997 ACR criteria for at least 6 months