Methods

Study Design: Randomized Double-blind Study of Subcutaneous Single-Dosing Assays of NKTR-358 in Healthy Volunteers

- **Cohort 1**: NKTR-358 0.3 µg/kg (n=9)
- **Cohort 2**: NKTR-358 1.0 µg/kg (n=9)
- **Placebo (n=3)**
- **Cohort 3**: NKTR-358 9.0 µg/kg (n=9)
- **Placebo (n=3)**
- **Cohort 4**: NKTR-358 20.0 µg/kg (n=13)
- **Placebo (n=3)**
- **Cohort 5**: NKTR-358 28.0 µg/kg (n=9)
- **Placebo (n=3)**

**Study Objectives**

- **Primary Objectives**
  - Assay the effects of subcutaneous administration of single-dosing doses of NKTR-358 on Tregs and T-cells
  - Study the safety and tolerability in subjects evaluated for eligibility and study outcome

- **Secondary Objectives**
  - Increase in Treg cell numbers and the levels of activated Treg cells
  - Increase in Helios expression (MESF) on Total Tregs at highest dose of NKTR-358

**Assay Methodology**

- Immunophenotyping by multiplex flow cytometry to quantify multiple immune cell subsets, using whole blood collected at multiple time points
- Clinical safety test results
- Time course and extent of change in the numbers and activity of Tregs

**Antibody Selection**

- 4 mAbs targeting the FoxP3 gene, using whole blood collected at multiple time points
- A subset of 16 mAbs targeting the FoxP3 gene, using whole blood collected at multiple time points

**Preliminary Results**

- NKTR-358 Increases Expression of Treg Activation Markers
- No Changes in Numbers of T Cells and Low-level Increases in Numbers of CD69+ NK Cells in Response to NKTR-358
- NKTR-358 Leads to Sustained, Dose-dependent Increases in Numbers and Proportion of [K(4)ET] of Total and CD3+ T-cells
- NKTR-358 is Safe and Well Tolerated in Healthy Volunteers
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- NKTR-358 Increases Expression of Treg Activation Markers
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**Adverse Events**

- Adverse events primarily limited to mild or moderate (Grade 1 or 2) injection site reactions
- Two 4 subjects experienced Grade 1 mild events of headache
- One subject at the highest dose tested (28.0 µg/kg) experienced mild (Grade 1) signs and symptoms of vomiting, diarrhea, anorexia, transient myalgia and myalgia, and suggestive of elevated liver transaminases.
- No severe adverse events detected
- No drug-related antibodies detected

**Pharmacodynamic Studies**

- Increases in fold-change from baseline of 5.0-fold or greater in Ki67+ CD25bright Tregs above predose value at 28 µg/kg NKTR-358
- 6-fold mean increase in Ki67+ CD25bright Tregs above predose value from a limited number of subjects at 8-11 days post-NKTR-358 administration (data not shown)

**Results**

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

- *p*-value <0.0001
- **Correlation of % Total Tregs and %FoxP3 TSDR of CD3+ T cells**
- **%Demethylated FoxP3 Gene at 28 µg/kg NKTR-358**
- **Fold change from baseline**
- **Placebo**
- **6 µg/kg**
- **20 µg/kg**
- **30 µg/kg**
- **40 µg/kg**

**Conclusion**

- **Safety and well tolerated in the first in human study**
- Marked dose-dependent expansion of numbers and proportion of CD25bright Tregs, as demonstrated by flow cytometric and epigenetic analysis
- The induction of Tregs is selective, with no measurable changes in numbers and percentages of CD4+ and CD8+ T-cells at all doses tested and low-level increases of NK cell numbers at highest dose tested
- Tregs in human volunteers were expanded and measured by flow cytometry and RNA-sequence analysis
- 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 as an add-on therapy in subjects with systemic sclerosis (NCT03336335)