A novel therapeutically active anti-TNFR2 agonistic antibody promotes Treg proliferation and induction of Treg functional markers

POS0465

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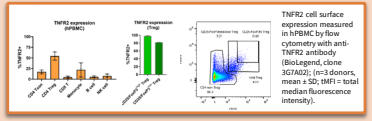
BACKGROUND

- TNFR2 signaling has been shown to be an important gatekeeper of inflammation and its absence or deficit is associated with a broad range of autoimmune diseases.
- TNFR2 is highly expressed on regulatory T cells (Tregs), essential immune cells for maintaining immune tolerance and preventing autoimmunity.
- Functional stimulation of Tregs through selective TNFR2 targeting could provide novel therapies for chronic inflammatory diseases.

TNFR2 expression in human PBMC and CD4+ Treg subsets



TNFR2 cell surface expression measured in hPBMC by flow cytometry with anti-TNFR2 antibody (BioLegend, clone 3G7A02); (n=3 donors, mean ± SD; tMFI = total median fluorescence intensity).

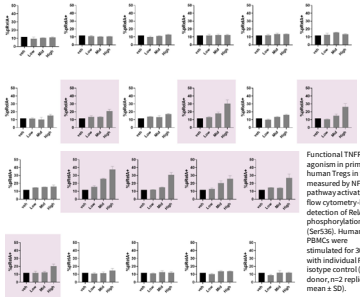
OBJECTIVES

To screen computationally-designed anti-TNFR2 antibodies for agonistic activity and assess lead candidates for:

- In vitro pharmacology using human primary Tregs
- In vivo pharmacodynamics and efficacy in humanized inflammation models

RESULTS

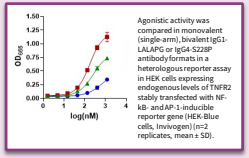
Screening: Single-arm (monovalent) TNFR2 agonists promote NFκB activation



Functional TNFR2 agonism in primary human Tregs in vitro measured by NFkB pathway activation via flow cytometry-based detection of RelA phosphorylation (Ser536). Human PBMCs were stimulated for 30 min with individual Fabs or isotype control (single donor, n=2 replicates, mean ± SD).

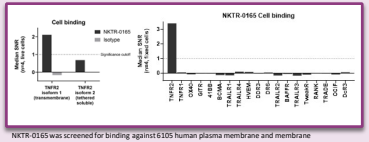
RESULTS

NKTR-0165 displays TNFR2 agonism in bivalent antibody formats



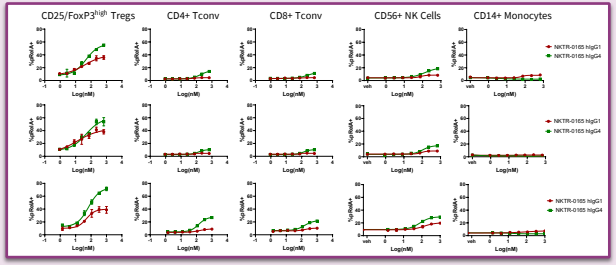
Agonistic activity was compared in monovalent (single-arm), bivalent IgG1-LALAPG or IgG4-S228P antibody formats in a heterologous reporter assay in HEK cells expressing endogenous levels of TNFR2 stably transfected with NF-kB- and AP-1-inducible reporter gene (HEK-Blue cells, Invivogen) (n=2 replicates, mean ± SD).

NKTR-0165 is highly selective for TNFR2 binding



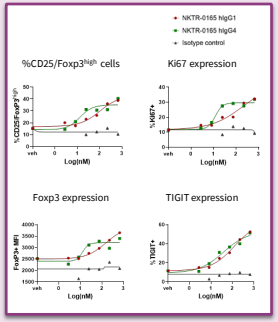
NKTR-0165 was screened for binding against 6105 human plasma membrane and membrane tethered secreted proteins (Retrogenix Cell Microarray).

NKTR-0165 induces pRelA activation selectively in primary human Tregs and not in conventional T cells, NK cells and CD14+ monocytes



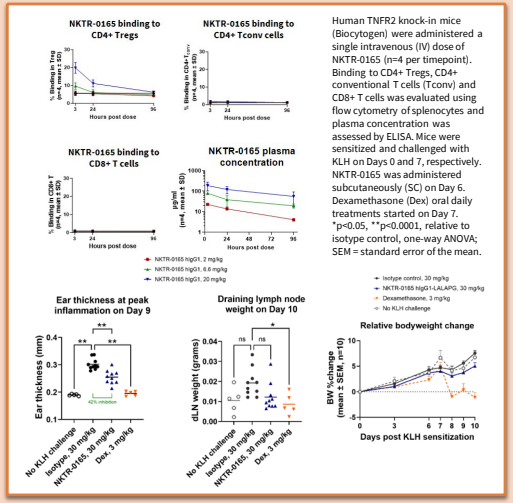
Human PBMCs were stimulated for 30 min with increasing concentrations of NKTR-0165. NFkB pathway activation was detected by flow cytometry to assess phospho-RelA (Ser536) after gating on Tregs, conventional CD4 or CD8 T cells (Tconv), NK cells, and monocytes (three donors, n=2 replicates, mean ± SD).

TNFR2 agonism by NKTR-0165 increases human primary Treg proliferation and upregulation of FoxP3 and TIGIT



Human PBMC were cultured in standard growth media with increasing concentrations of NKTR-0165 without TCR stimulation or IL-2 supplementation for 5 days. Proliferation and induction of functional activation markers (FoxP3 and TIGIT) were assessed on Tregs using flow cytometry at the end of the 5-day culture period. Representative data from experiments across n=4 PBMC donors (mean, n=2 replicates).

NKTR-0165 is selective for Tregs in hTNFR2 knock-in mice and reduces inflammation in a KLH-induced DTH model



Human TNFR2 knock-in mice (Biocytogen) were administered a single intravenous (IV) dose of NKTR-0165 (n=4 per timepoint). Binding to CD4+ Tregs, CD4+ conventional T cells (Tconv) and CD8+ T cells was evaluated using flow cytometry of splenocytes and plasma concentration was assessed by ELISA. Mice were sensitized and challenged with KLH on Days 0 and 7, respectively. NKTR-0165 was administered subcutaneously (SC) on Day 6. Dexamethasone (Dex) oral daily treatments started on Day 7. *p<0.05, **p<0.0001, relative to isotype control, one-way ANOVA; SEM = standard error of the mean.

CONCLUSIONS

- **NKTR-0165 shows selective TNFR2 binding and receptor agonism in human Tregs with minimal binding and signaling activity in other TNFR2 expressing immune cells in vitro and in vivo.**
- **NKTR-0165 agonistic activity enhances Treg lineage stabilization and upregulates expression of proteins involved in proliferation and Treg function.**
- **NKTR-0165 demonstrates therapeutic efficacy in a KLH-DTH model in human TNFR2 knock-in mice.**
- **NKTR-0165 has the potential to selectively enhance Treg function through a novel agonistic mechanism and may offer a new approach for the treatment of chronic inflammatory diseases.**