NKTR-288, a PEGylated Interferon Gamma Drug Candidate for the Treatment of Cancer

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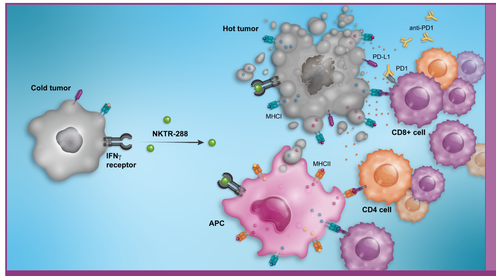
Introduction

- IFNγ is a cytokine that is released by immune cells in response to activation and enhances the processing and presentation of neoantigens on tumor and antigen presenting cells.
- IFNγ modifies the tumor microenvironment (TME), including but not limited to increasing cytokine release, polarizing macrophages towards pro-inflammatory phenotype, inhibiting Th2 cell differentiation, and inducing Treg fragility.
- Lack of response to checkpoint inhibitors (CPI) is correlated with low interferon gamma (IFNγ)-related gene expression signatures[1,2] and PD-L1 status[3].
- Exogenous IFNγ has great potential as a tumor-targeting immunotherapy, however, the use of native IFNγ has been challenging due to its pleiotropic activities and unfavorable balance between activity and toxicity.
- NKTR-288 is a site-specific polymer-conjugated IFNγ mutein that is designed to maximize sustained MHC class I induction on tumor cells *in vivo* by tuning receptor potency while enhancing systemic exposure.
- Here we describe the design and discovery of NKTR-288 and explore its pharmacological properties in comparison to native IFNγ.



TME modifications induced by IFNγ

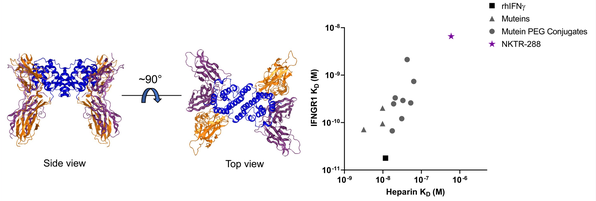
- ↑ Tumor MHCI
- ↑ Antigen presenting cell (APC) MHCII
- ↑ T cell Chemokines
- ↑ Inflammatory macrophage polarization
- ↑ Th1 differentiation
- ↑ Treg fragility

NKTR-288 directly targets both tumor cells and immune cells in the TME. Engagement of the IFN gamma receptors activates JAK-STAT1 signaling, inducing the interferon gamma response that is crucial for anti-tumor immunity.

Results

Design & Selection of NKTR-288

A panel of human IFNγ muteins, with varying distance from receptor and heparin interaction sites, was generated for site-specific polyethylene glycol (PEG) conjugation and screening.



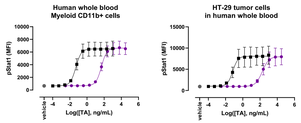
Structural model (PDB:6E3L[4]) of IFNγ dimer bound to IFNGR1 and IFNGR2 receptors. Not shown are heparin binding motifs in the dynamic C-terminal region.

Dissociation constants (K_D) for recombinant human IFNγ, muteins, and PEG conjugated muteins binding to IFNGR1 receptor or heparin were measured using surface plasmon resonance.

Results

In Vitro Potency

NKTR-288 is a full agonist that maintains decreased potency relative to IFNγ on tumor cells and myeloid cells.

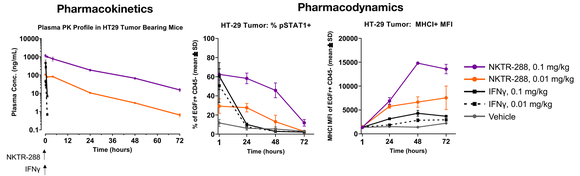


Human whole blood mixed with HT-29 tumor cells n=4 donors	EC_{50} ng/mL: geomean (95% CI)		E_{max} % normalized with IFNg value	
	CD11b+ myeloid cells	HT-29 tumor cells	CD11b+ myeloid cells	HT-29 tumor cells
IFNγ	0.051 (0.039 - 0.067)	0.071 (0.059 - 0.084)	100	100
NKTR-288	47.5 (39 - 57)	186 (138 - 251)	102	99

HT-29 (colorectal adenocarcinoma) tumor cells were mixed with human whole blood and then stimulated for 15 minutes with the indicated concentrations of either INFγ or NKTR-288. The pSTAT1 mean fluorescent intensity (MFI) in HT-29 tumor cells and CD11b+ myeloid cells was measured in the same sample by flow cytometry. EC50 values were calculated from concentration-response curves using a 4-parameter fit.

In Vivo PKPD

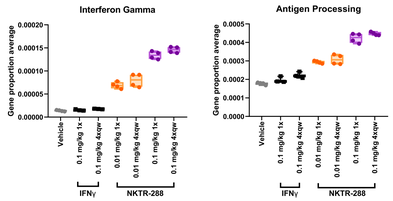
Single dose NKTR-288 shows increased concentration at time zero (C_0), extended exposure and enhanced dose dependent signaling (pSTAT1) and activity (MHCI) vs. single dose IFNγ in HT-29 xenograft model.



PK Parameters	Dose (mg/kg)	C_0 (ng/mL)	$T_{½}$	$AUC_{inf\ obs}$ (hr*ng/mL)
NKTR-288	0.1	1150	11.8 hours	17500
NKTR-288	0.01	106	11.9 hours	1470
IFNg	0.1	601	~9 minutes	211
IFNg	0.01	57.5	~9 minutes	21

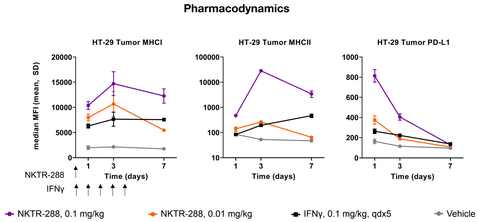
SCID mice (n=4/group) with subcutaneous HT-29 (colorectal adenocarcinoma) tumors (~100 mm³) received a single dose of 0.01 or 0.1 mg/kg NKTR-288 or IFNγ intravenously. Peripheral blood was collected at various timepoints to assess plasma drug concentration by immunoassay, and tumors were collected at day 1, 2, or 3 to quantify pSTAT1 and cell surface MHCI by flow cytometry.

RNA sequencing of human xenograft tumors shows broad and sustained interferon-related response to NKTR-288. No desensitization of response is observed with repeat dosing.



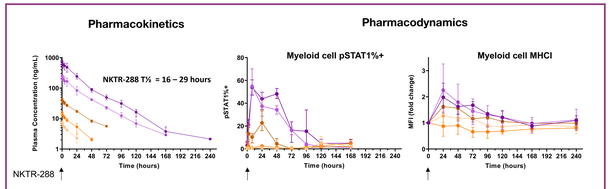
SCID mice (n=4/group) with subcutaneous A549 (lung carcinoma) xenografts were grown to 100 mm³ before randomization on Day 0. Test articles were dosed once on day 21 or weekly on days 0, 7, 14, 21. Total RNA was extracted from tumors 24 hours after the final dose and analyzed by RNAseq. Boxplots show interferon gamma[1] and antigen processing[2] gene signature scores after single or repeat dosing of IFNγ or NKTR-288.

Single dose NKTR-288 activity is superior to daily repeat treatment of IFNγ at an equivalent dose. NKTR-288 induction of MHCI and MHCII is prolonged relative to PD-L1.



SCID mice (n=4/group) with subcutaneous HT-29 tumors (~100 mm³) received a single dose of 0.01 or 0.1 mg/kg NKTR-288, or 5 daily doses of 0.1 mg/kg IFNγ. Tumors were collected at day 1, 3, or 7 to assess cell surface MHCI, MHCII, and PD-L1 mean fluorescence intensity (MFI) by flow cytometry.

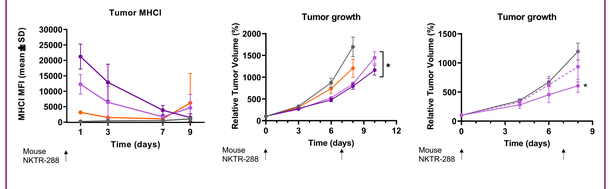
NKTR-288 has sustained pharmacology in NHP, showing exposure-driven induction of STAT1 signaling and MHCI upregulation on peripheral blood myeloid cells.



Cynomolgus monkeys (n=3/group) were dosed with NKTR-288, IV, at 0.3, 1, 3, 10, or 30 mg/kg. Blood was collected at various timepoints to assess plasma drug concentration by immunoassay, as well as peripheral blood myeloid cell (CD66-, CD11b+) STAT1 phosphorylation and MHCI expression by flow cytometry.

In Vivo PD & Efficacy

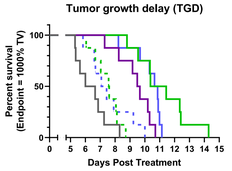
NKTR-288 mouse surrogate dosing shows sustained MHCI upregulation and has significant T cell dependent anti-tumor activity as a single agent in a syngeneic B16F10 efficacy model.



For the PD study subcutaneous B16F10 (murine melanoma) tumor bearing C57BL/6 mice received a single dose of mouse NKTR-288 (0.1, 0.3, 0.6 mg/kg, IV). Tumors were collected at day 1, 3, 7, or 9 to assess MHCI MFI by flow cytometry. For the efficacy study, mice were treated with mouse NKTR-288 (0.1, 0.3, 0.6 mg/kg, IV, weekly). In another study, T cells were depleted with anti-CD4 and -CD8 antibodies prior to dosing (confirmed by flow cytometry on blood, spleen and tumor) to show immune system requirement for efficacy. Relative tumor volumes normalized to starting tumor size were graphed. (*, p<0.05 relative to vehicle treatment, one-way ANOVA).

In Vivo Efficacy

NKTR-288 mouse surrogate dosing shows anti-tumor activity as a single agent and in combination with CPI in a syngeneic B16F10 efficacy model.



Treatment	TGI (%)	TGD (%)
Mouse NKTR-288, 0.6 mg/kg, qwx2	57*	52*
αPD-L1, 400 μg, BIWx2	ns	ns
αPD-1, 400 μg, BIWx2	ns	ns
Mouse NKTR-288 + αPD-L1	68*	71***
Mouse NKTR-288 + αPD-1	73*	72***
Vehicle	0	0

Subcutaneous B16F10 tumor bearing C57BL/6 mice (n=8/group) were treated with Mouse NKTR-288 (0.6 mg/kg, IV, qwx2) and/or anti-mouse PD-1 or PD-L1 antibodies (400mg, IP, BIWx2) starting treatment at mean tumor volume of ~100mm³. Relative tumor volumes normalized to starting tumor size were measured to quantify tumor growth inhibition (TGI) by treatments. Tumor growth delay (TGD) in drug treated animals was evaluated relative to vehicle treatment by measuring tumor volume (TV) and tumor volume decupling time (TVDT) indicating time to reach 1000%TV growth from baseline as predefined survival endpoint.
(* p<0.05, *** p<0.0001, ns = not significant relative to vehicle, one-way ANOVA).

Conclusion

- NKTR-288 is an optimized IFNγ pathway agonist
  - Precision control of receptor affinity reduces potency and target mediated drug disposition (TMDD)
  - Decreased affinity to heparin increases bioavailability compared to IFNγ
- NKTR-288 has similar but enhanced pharmacological properties compared to IFNγ
  - Durable exposure
  - Enhanced dose-dependent signaling and pharmacodynamic response in human xenograft models and in non-human primates
  - Well tolerated at pharmacologically active doses in non-human primates, well below the MTD of 1 mg/kg
  - Mouse NKTR-288 induces MHCI in a fast-growing, cold syngeneic tumor model and has T cell dependent anti-tumor efficacy alone and in combination with checkpoint inhibitors
- NKTR-288 may enhance tumor immunogenicity through increased neoantigen display, interferon gamma related gene signatures, and PD-L1; all of which are correlated with response to checkpoint blockade.
- Unlike CPIs and other T/NK cell-based cytokine approaches which induce immune cell secretion of IFNγ, NKTR-288 activity may not be dependent on pretreatment T cell infiltration level in the tumor.

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DISCLOSURES

Damon Hamel is an employee and shareholder of Nektar Therapeutics: dhamel@nektar.com