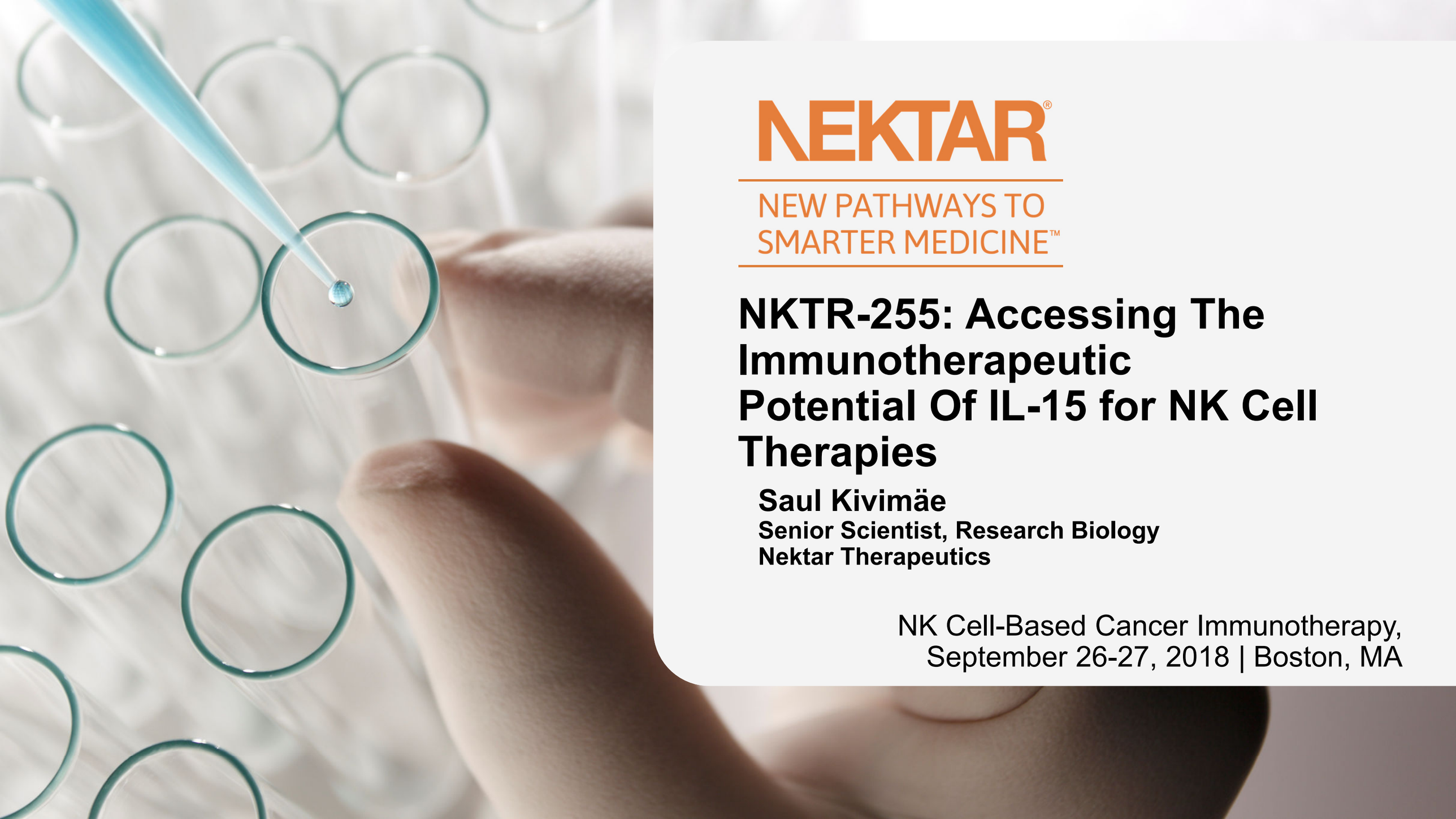

NEKTAR®

NEW PATHWAYS TO SMARTER MEDICINE™

NKTR-255: Accessing The Immunotherapeutic Potential Of IL-15 for NK Cell Therapies

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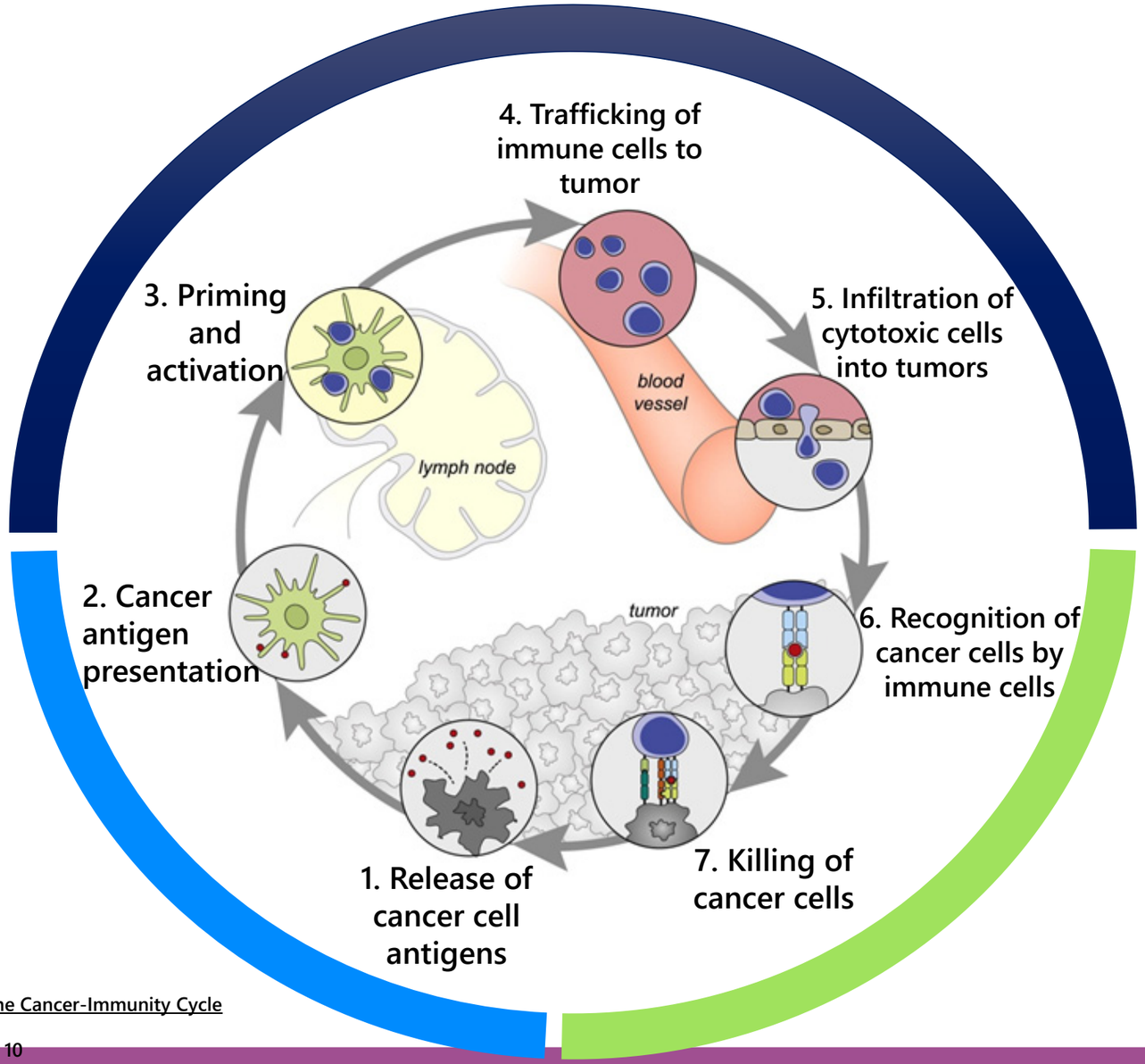
Senior Scientist, Research Biology
Nektar Therapeutics

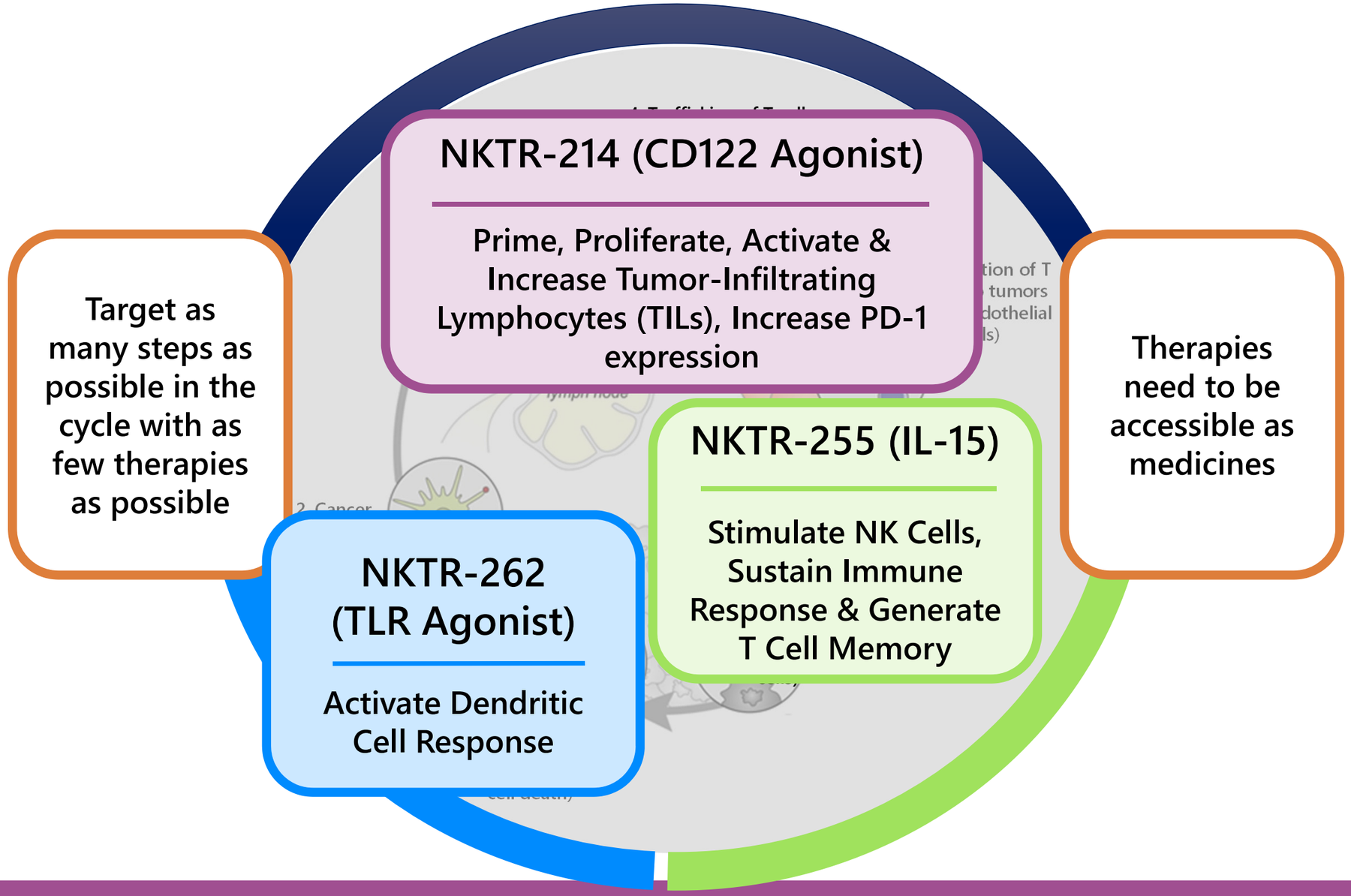
NK Cell-Based Cancer Immunotherapy,
September 26-27, 2018 | Boston, MA

The immunity cycle and multiple points of intervention for I-O therapies

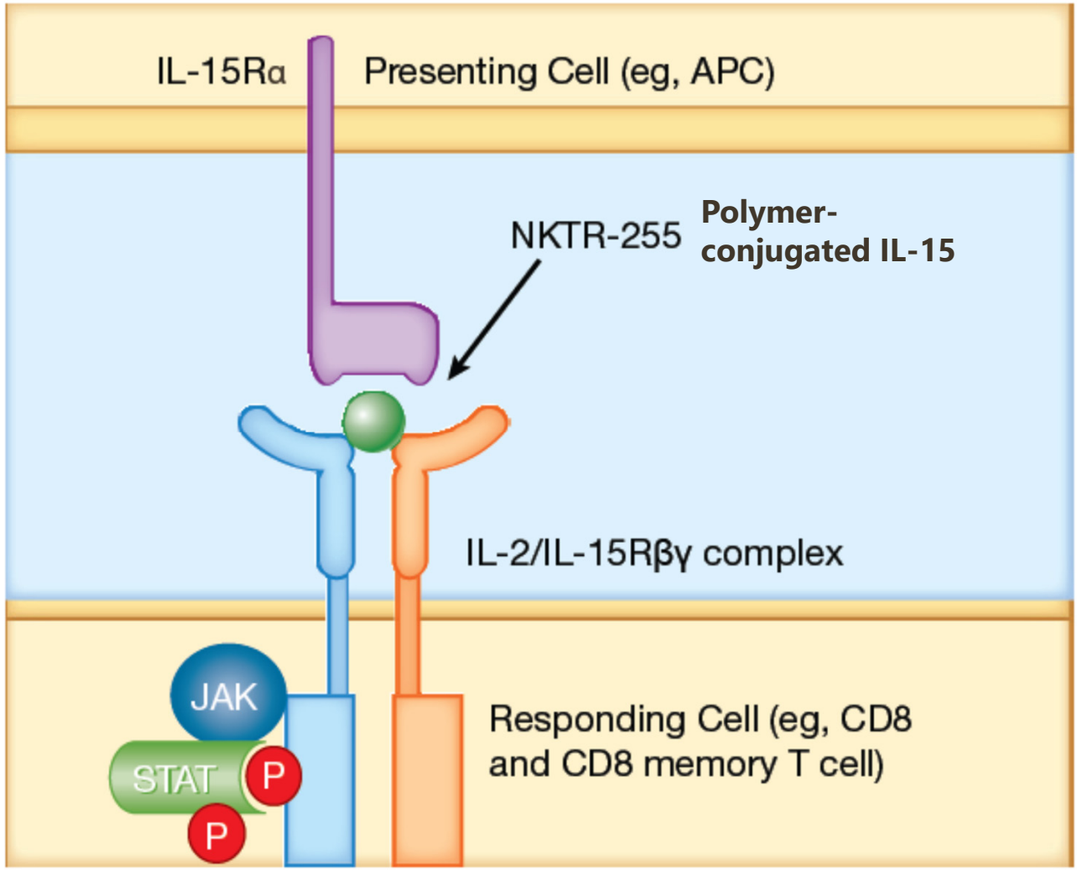


Source:
Oncology Meets Immunology: The Cancer-Immunity Cycle
Chen and Mellman
Immunity, Volume 39, Issue 1, 1 - 10

Nektar's immuno-oncology strategy to create therapies that cover the immunity cycle



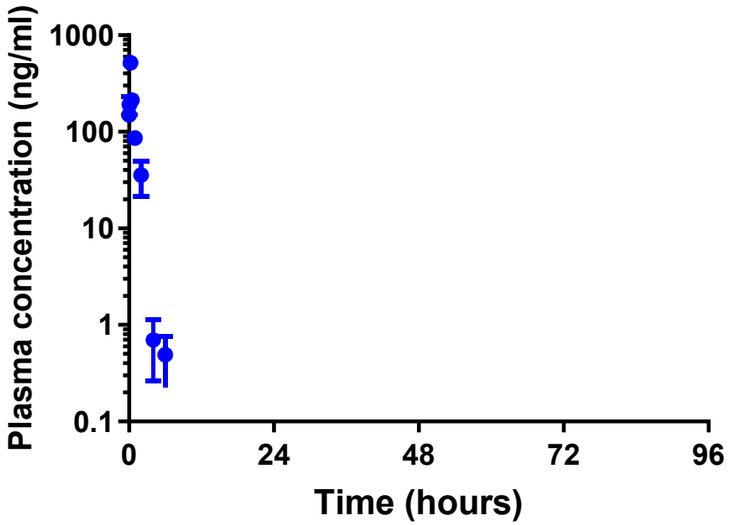
The potential of IL-15 in immuno-oncology



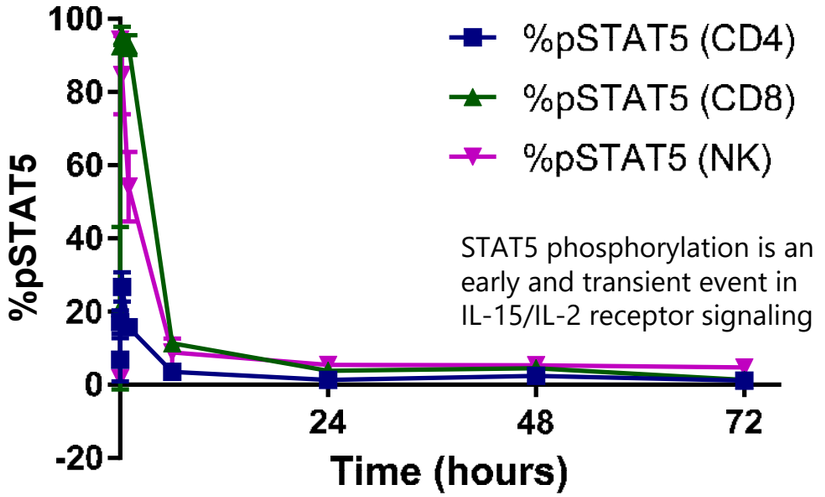
- IL-15 is a pleiotropic cytokine with roles in innate and adaptive immunity
- Identified by NCI as one of the most promising immuno-oncology agents
- Key role in formation and maintenance of immunological memory
- Essential factor for NK development and homeostasis
- In vitro, IL-15 can reverse tumor-induced NK cell dysfunction

The challenge to therapeutic use of IL-15

- IL-15 displays rapid clearance from plasma
- In vivo signaling activity is similarly short-lived



Mouse PK: IL-15 0.5mpk i.p., serum assayed by ELISA



Mouse PD: IL-15 0.3mpk i.p., whole blood stained for leukocyte surface markers and pSTAT5, measured by flow cytometry

- Requires daily dosing or multi-day continuous infusion for optimal activity with high Cmax-related toxicity

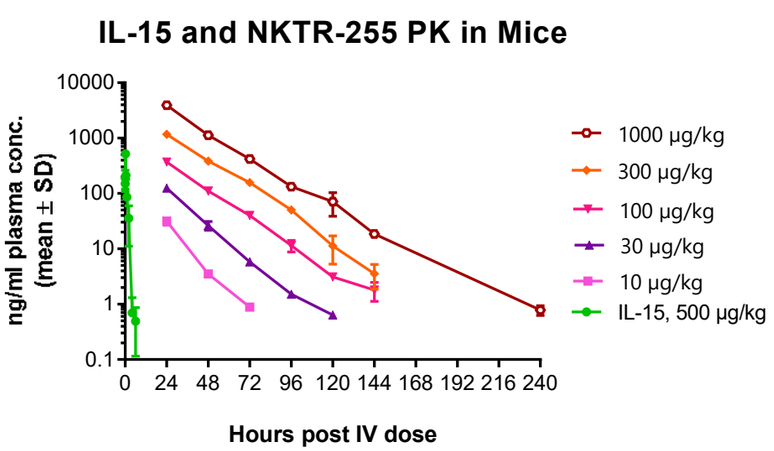
NKTR-255 – polymer conjugated IL-15

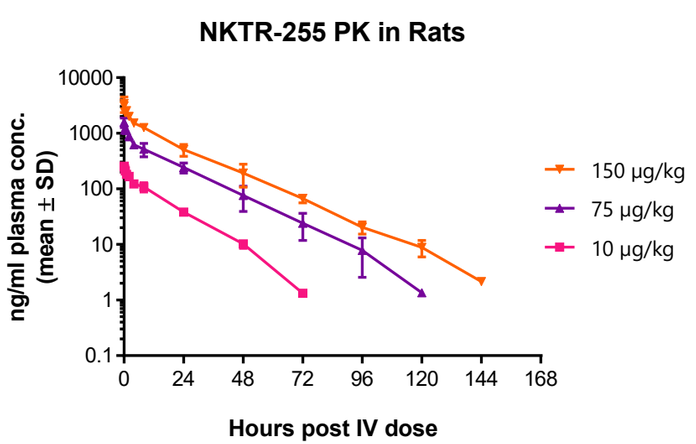
- Design Goals:
  - Improve PK and PD to sustain IL-15 activity and achieve large pharmacodynamic effect without need for daily dosing
  - Retain binding to IL-15Rα to maintain full spectrum of IL-15 biology
  - No mutagenesis or complex to soluble IL-15Rα

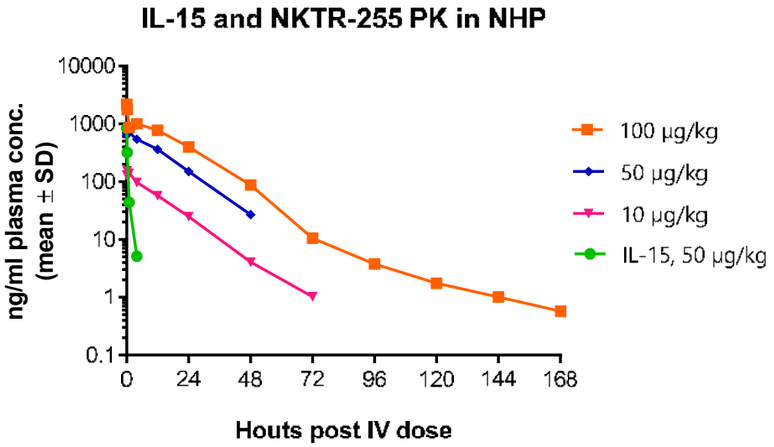
- As a result, NKTR-255:
  - Stimulates NK cell activation and proliferation
  - Supports CD8 T-cell survival and memory formation
  - Shows efficacy in various syngeneic tumor models

NKTR-255 is first potential medicine to access the IL-15 pathway by preserving receptor binding to IL-15Rα with antibody-like dosing

NKTR-255 achieves sustained plasma exposure in mice, rats and NHP after single dose







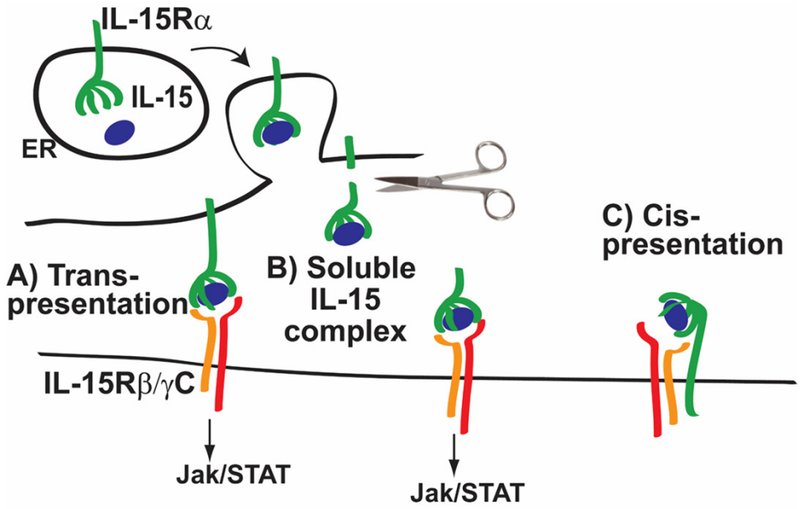
- PEGylation Significantly Improved NKTR-255 PK Profiles:
  - PEGylation significantly enhanced plasma exposure and reduced total clearance
  - Extended plasma exposure across the species on single dose (Mice, Rat and NHP)

- NKTR-255 Half-life ($t_{1/2}$):
  - Mouse: ~14 hrs
  - Rat: ~18 hrs
  - Monkey: ~30 hrs (100µg/kg)

Note: Parent IL-15 was not dosed in the Rat PK study; No apparent gender differences are noted in the Cyno study

Binding to IL-15Rα is required to access the biological functions of IL-15

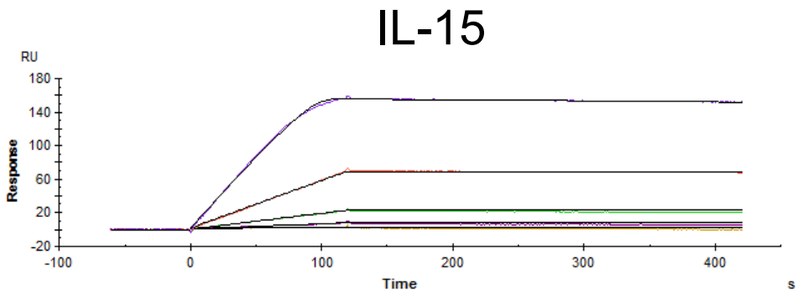
- Three potential modes of interaction
  - Trans-presentation: IL-15 binds to IL-15Rα on one cell (eg. DC) then signals through Rβγ on a second cell (eg. T-cell)
  - Cis-presentation: soluble IL-15 binds to IL-15Rα and Rβγ on the same cell
  - Binding of soluble complex: soluble IL-15:IL-15Rα heterodimer binds to Rβγ

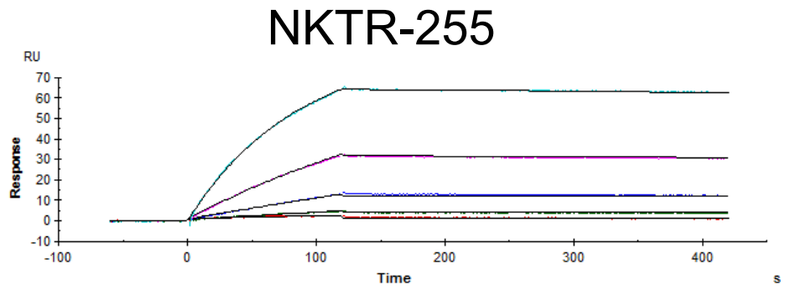


(Stonier and Schluns, 2010)

- Design NKTR-255 to maintain IL-15 biological context
  - IL-15/IL-15Rα therapeutic fusion protein signals in an IL-15Rα independent manner, loses biological context

NKTR-255 retains affinity for IL-15Rα



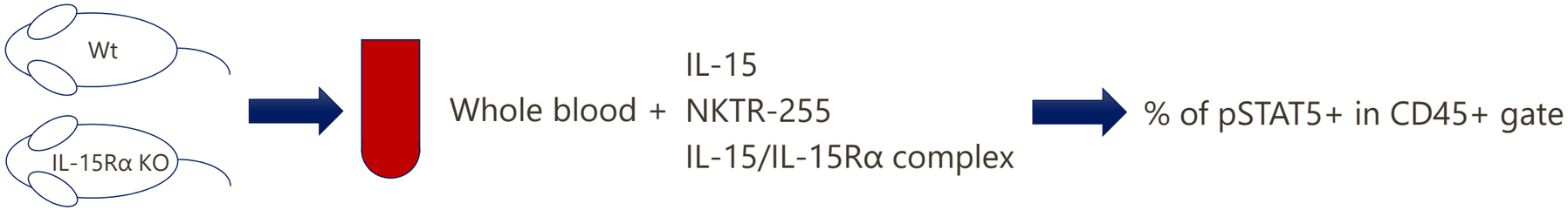


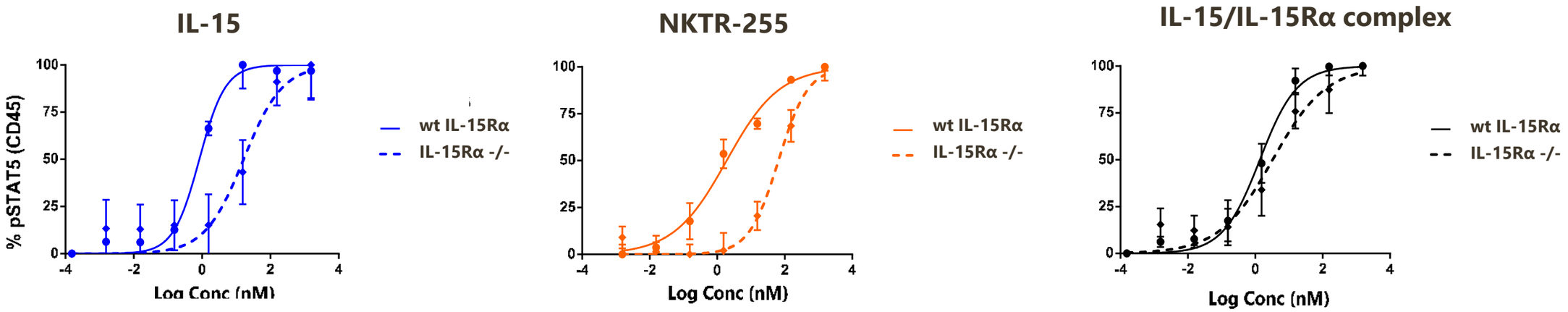
Conjugate	k_{on} (M^{-1} s^{-1})	k_{off} (s^{-1})	K_D (pM)
IL-15	7.88×10^{6}	1.33×10^{-4}	16.2
NKTR-255	1.08×10^{6}	1.69×10^{-4}	182

- Many conjugates were screened for ability to bind IL-15Rα
- Conjugation chemistry parameters were carefully optimized
- NKTR-255 affinity for IL-15Rα is ~10X weaker than IL-15

Affinity measured by BIAcore, using IL-15Rα:Fc captured by immobilized anti-Fc
NKTR-255 interaction with β and αβ were also ~8-10X weaker than IL-15

NKTR-255 signaling is mediated via IL-15Rα

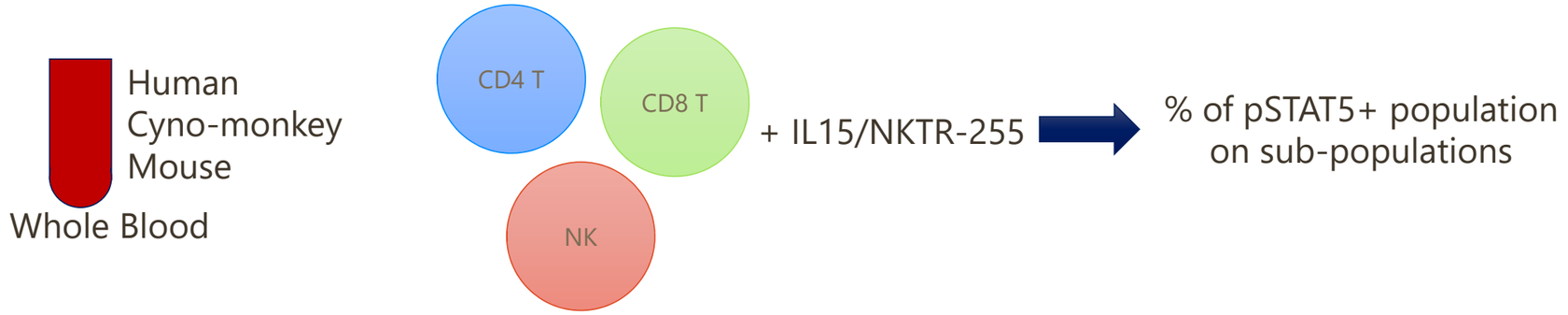




Treatment	IL-15		NKTR-255		IL-15/IL-15Rα complex	
	WT	KO	WT	KO	WT	KO
pSTAT5 EC50 (ng/ml)	15.58	347.3	26.23	1220	20.35	54.23
EC50 ratio	22.29		46.51		2.66	

NKTR-255 signaling is dependent on IL-15Rα as with IL-15

NKTR-255 drives IL-15-like signaling across species



IL-15 EC50 (ng/ml)	NK	CD8 T	CD4 T
Human	0.48	1.5	1.6
Cyno-monkey	0.24	2.6	4.0
Mouse	1.8	0.27	1.2

NKTR-255 EC50 (ng/ml)	NK	CD8 T	CD4 T
Human	5.1	4.9	5.3
Cyno-monkey	6.9	39	53
Mouse	42	3.4	19

- In human, NKTR-255 exhibits equal potency across three populations
- NKTR-255 potency to NK cells is preserved across species except for mouse
- NKTR-255 potency to T cells is different depending on species
- NKTR-255 species difference is similar to that for IL-15
- Varying potency across species attributed to differences in IL-15 Rα expression level

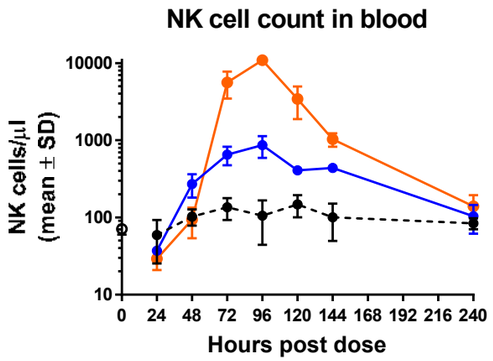
Conclusions: NKTR-255 PK and target engagement

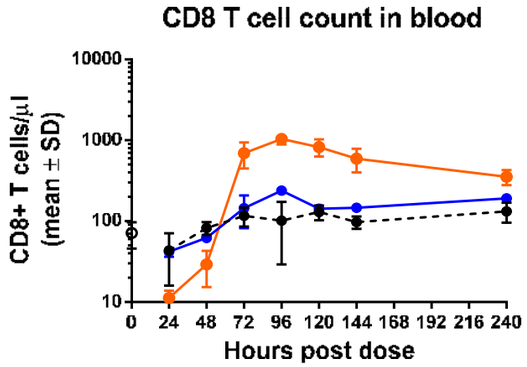
- NKTR-255 dramatically improves IL-15 receptor agonist exposure enabling low frequency administration
- NKTR-255 enables sustained IL-15 receptor pathway engagement in NK and T cells
- NKTR-255 retains IL-15Rα binding specificity maintaining IL-15 biological context

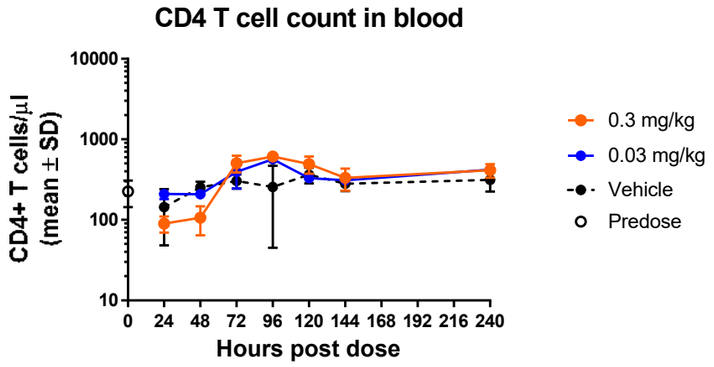
Functional characterization of NKTR-255 across immune cell subsets in rodents and non-human primates

NKTR-255 drives IL-15 receptor signaling and proliferation in CD8 T and NK cells in mice

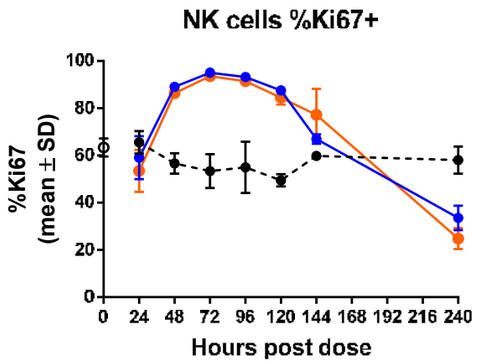
Cell expansion

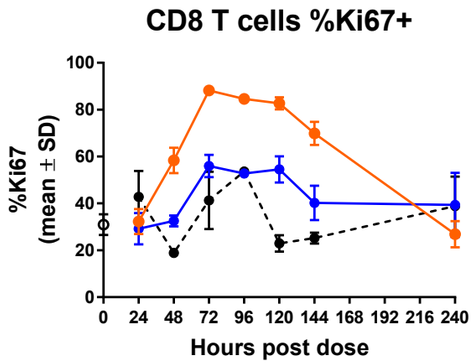


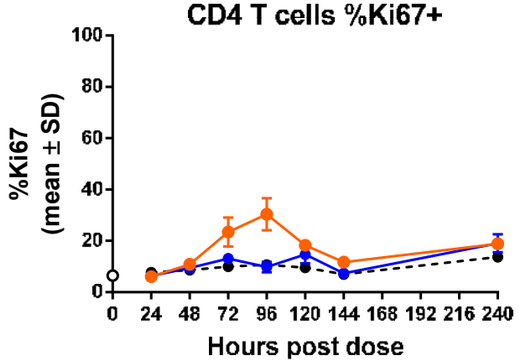




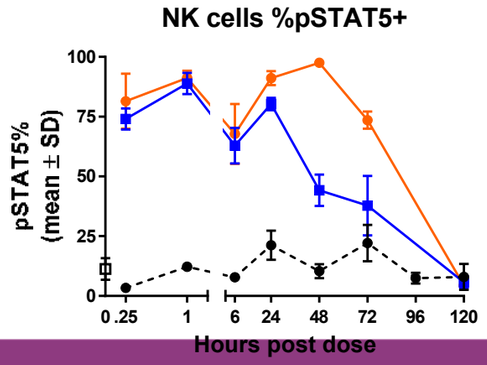
Ki67+

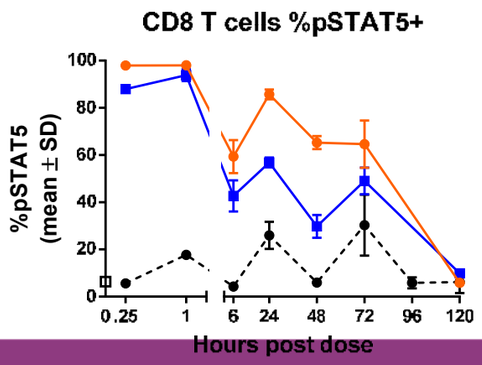


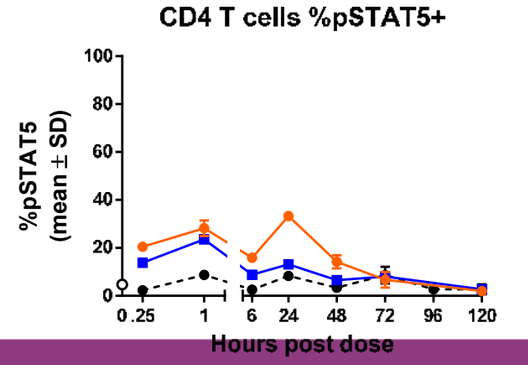




pSTAT5+







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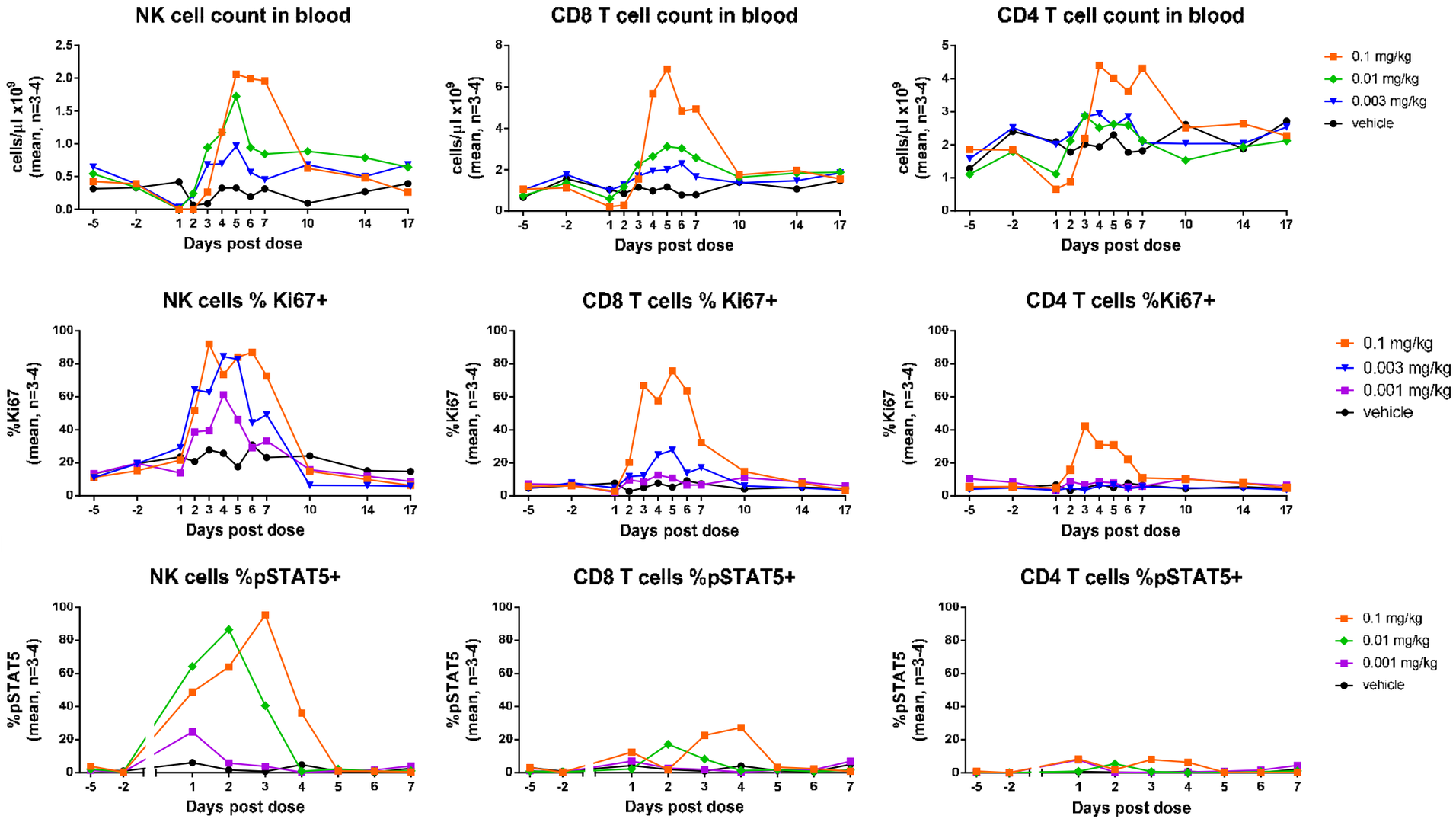
NK cells are the most sensitive in NKTR-255 dose response compared to CD4 and CD8 T cells in vivo in NHPs

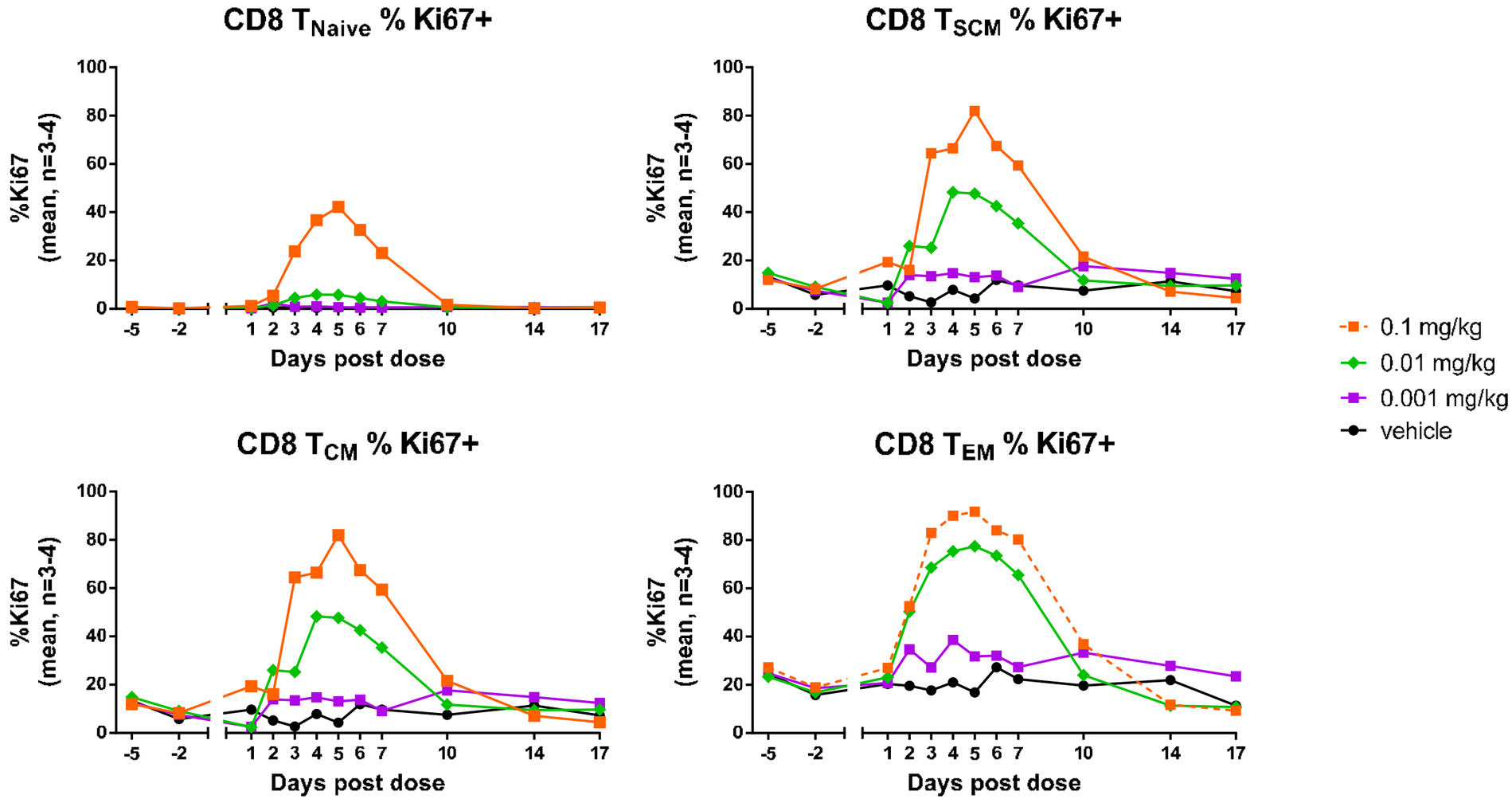
NK cell expansion is detected at 0.01 mg/kg dose level

NK cells Ki-67 and pSTAT5 induced at lowest dose level (0.001mg/kg).



Cynomolgus monkeys NKTR-255 IV dose response PD: blood stained for leukocyte surface markers and pSTAT5 + Ki67, measured by flow cytometry

Increased NKTR-255 sensitivity in CD8 T cell memory populations compared to naïve CD8 T cells in vivo in NHPs



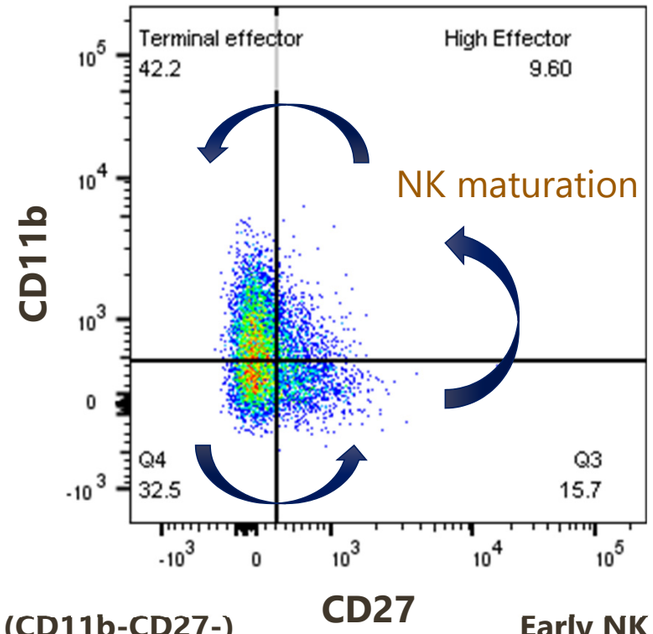
Cynomolgus monkeys NKTR-255 IV dose response PD: blood stained memory markers (CD45RA, CD197, CD95) in CD8 T parent gate.

NKTR-255 expands NK cell subpopulations

NK cells at all stages of maturation are highly responsive to NKTR-255

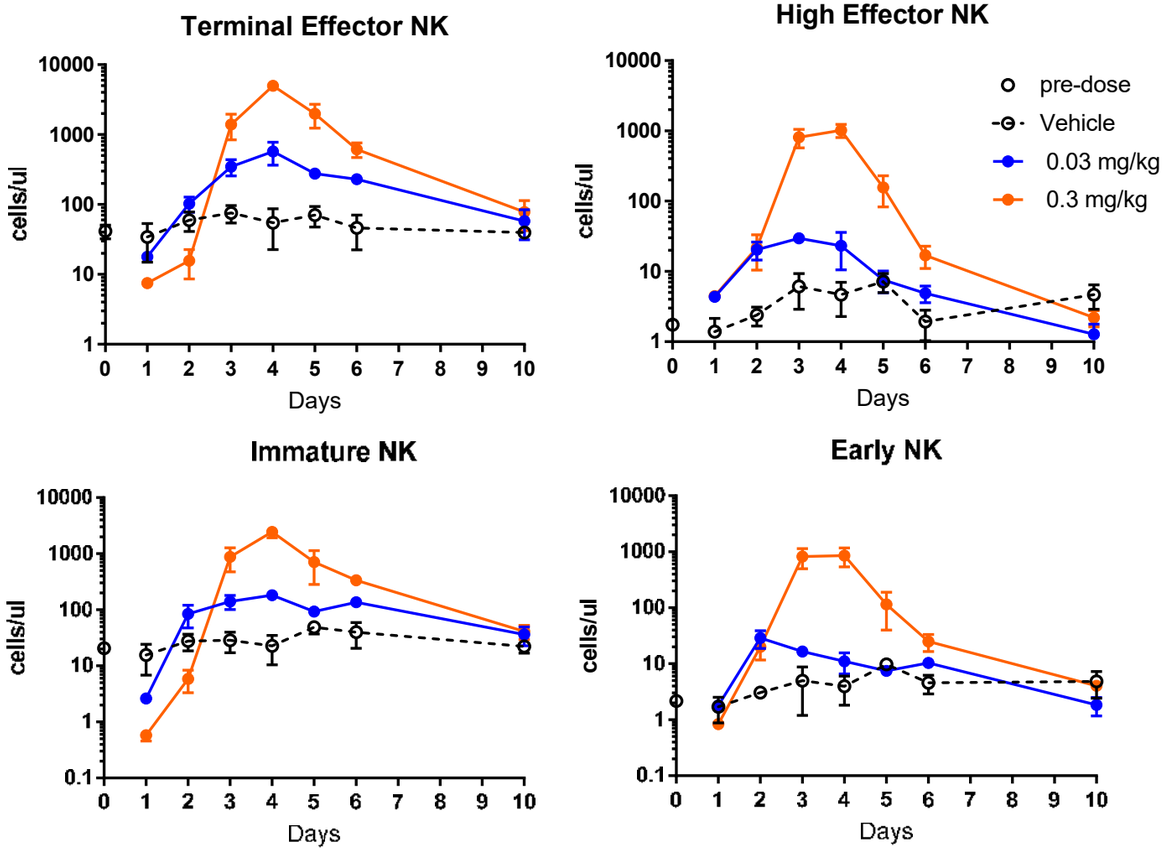
Terminal Effector (CD11b+CD27+)
Most mature
Activation tightly regulation
Higher activation threshold

High Effector (CD11b+CD27+)
High cytokine secretion
Great effector function
Lower activation threshold

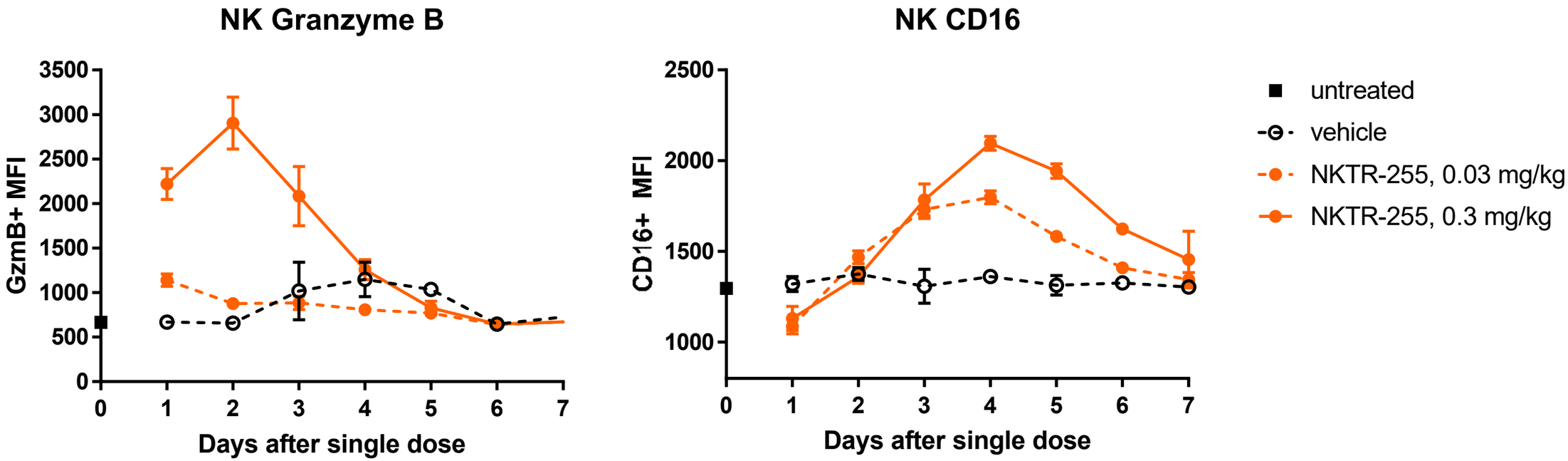


Immature (CD11b-CD27-)
Potential to differentiate

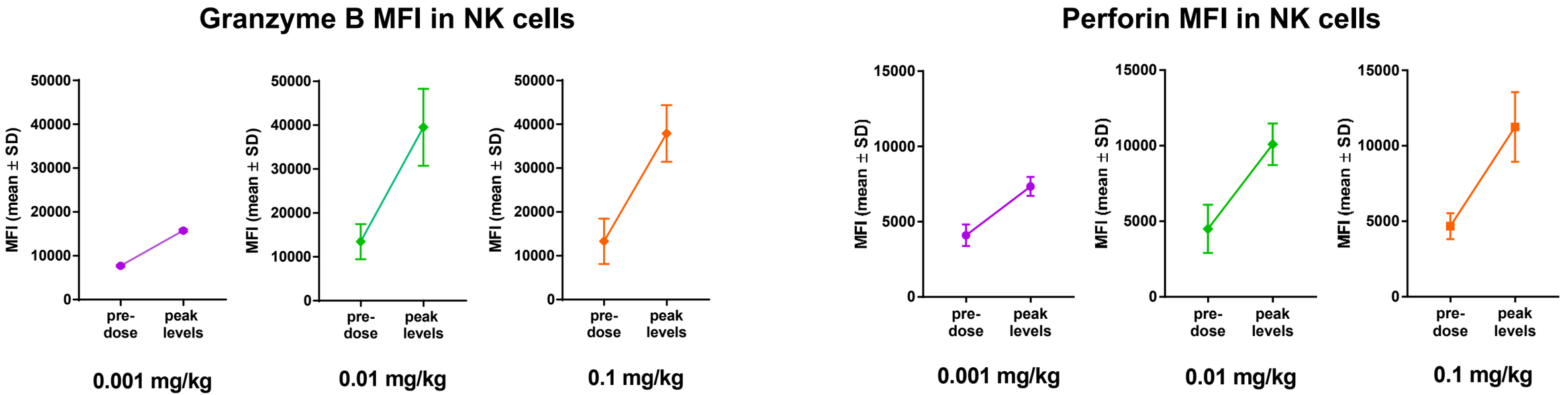
Early NK (CD11b-CD27+)
High cytokine secretion



NKTR-255 increases expression of effector functions in NK cells in mice

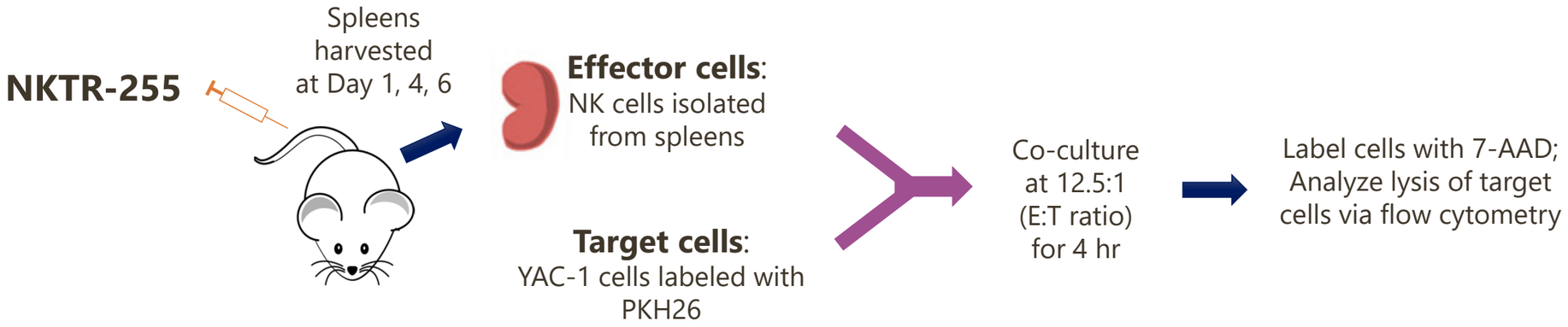


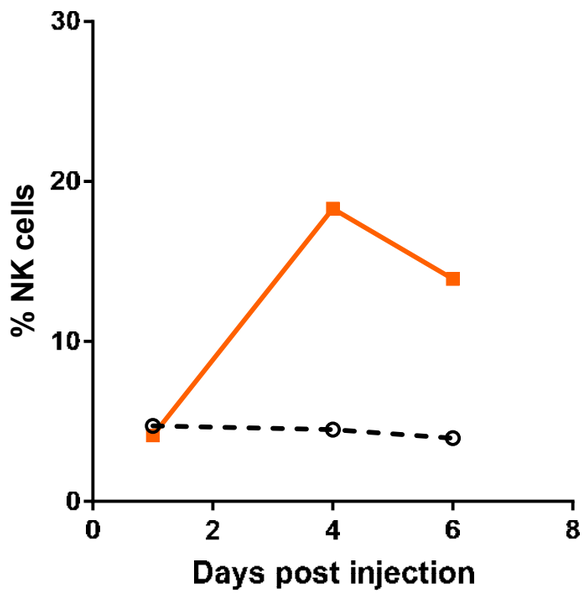
NKTR-255 increases levels of cytotoxic enzymes in NK cells in NHPs

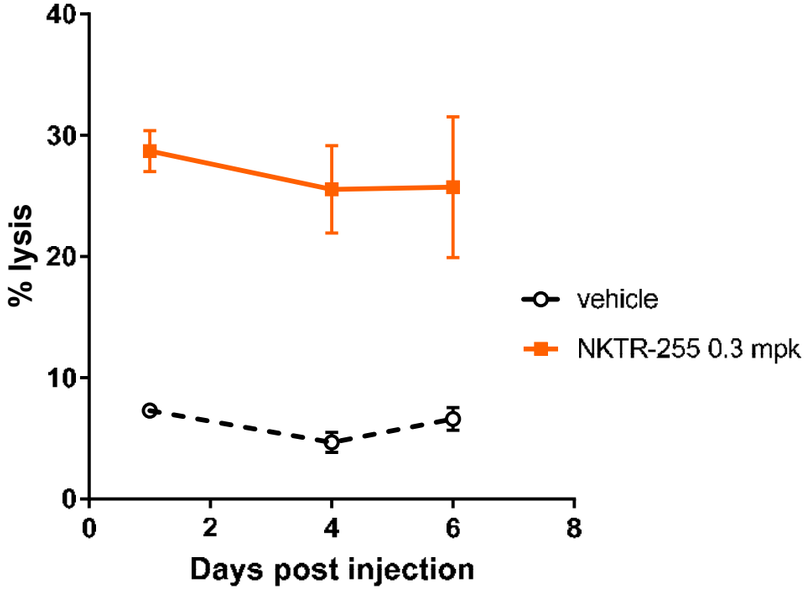


NKTR-255 increases protein levels of constitutively expressed cytolytic enzymes Granzyme B and Perforin in NK cells

NKTR-255 enhances murine splenic NK cytotoxicity







A single in vivo dose (0.3 mg/kg) of NKTR-255 sustains NK cell killing activity for at least 6 days ex vivo

In vivo comparison summary of NK vs T cell responses to NKTR-255

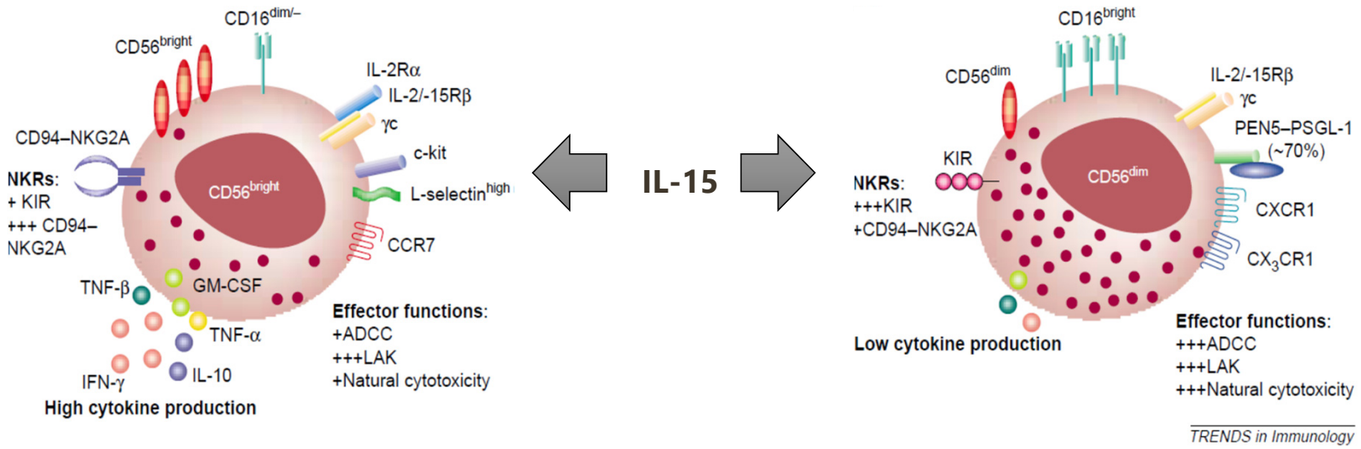
- NK cells most sensitive to NKTR-255 stimulation in vivo in mice and NHPs
  - %pSTAT5 and %Ki67 increases measurable at lowest tested dose level (0.001 mg/kg) in NHP
  - Lowest dose level requirement for effective in vivo expansion in NK cells
- NKTR-255 increases intracellular levels of cytotoxic enzymes and prolongs cytotoxic activity of NK cells
- Higher sensitivity to NKTR-255 in CD8 memory T cells relative to naïve CD8 T cells
  - Graded sensitivity within memory subpopulations (T_{EM}>T_{SCM}>T_{CM}>T_{Naive}) in proliferative response
- CD4 T cells are the less responsive T cell population to NKTR-255 stimulation in mice and NHPs

NKTR-255 in vivo anti-tumor efficacy

NKTR-255 has potential to enhance anti-cancer immunotherapy via both NK and CD8 cells

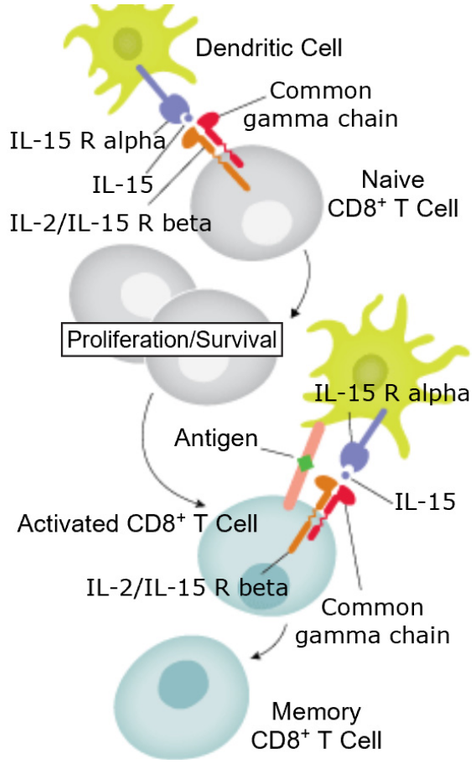
NK cell biology

- Activation balance among human NK subtypes
  - IL-15 is similarly potent to regulatory CD56^{bright} and cytotoxic CD56$^{dim/null}$ NK sub-populations
  - IL-15 pre-activated NK cells show sustained function

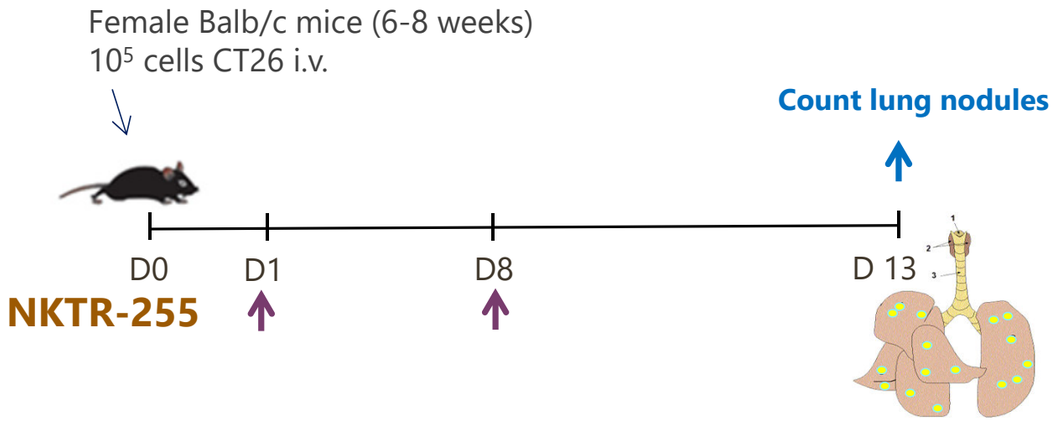


CD8 cell biology

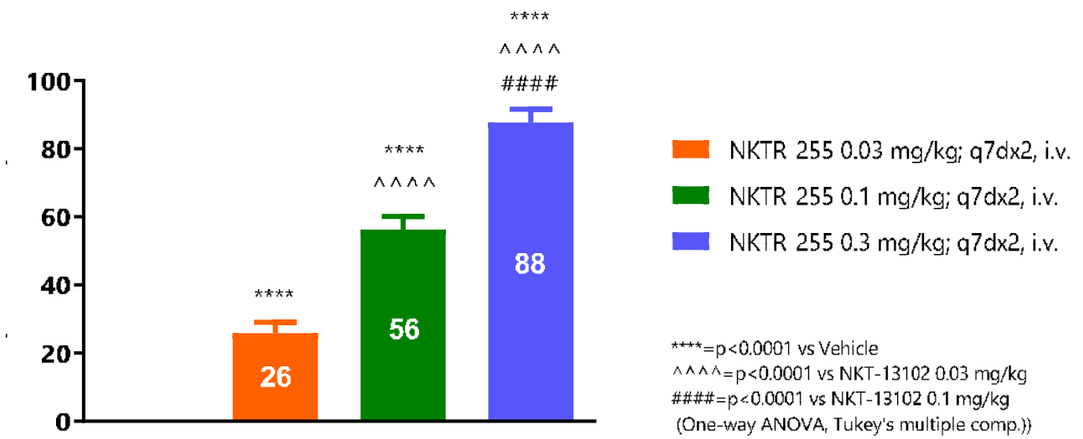
- Supporting memory CD8 T cell longevity and function
  - IL-15 maintains Ag-specific effector CD8 T cells after the contraction phase by promoting their survival and proliferation



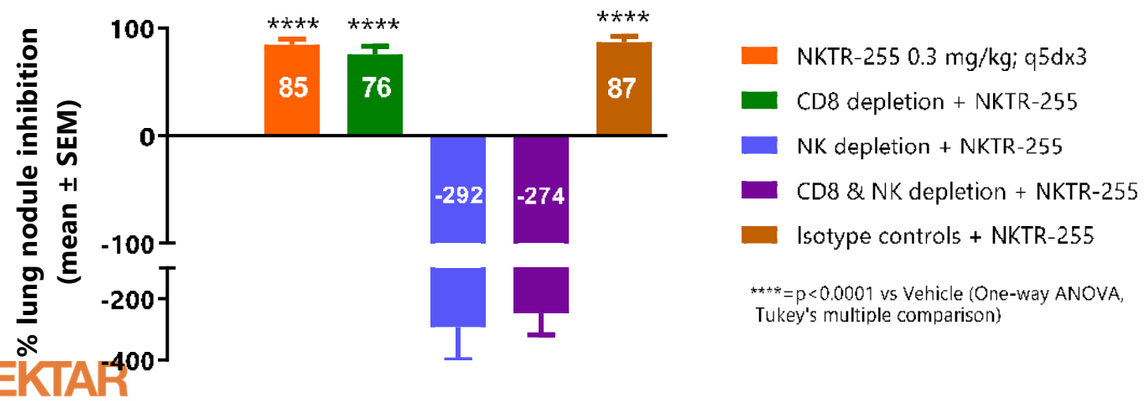
NKTR-255 enhances NK cell-dependent anti-tumor efficacy in disseminated CT26 lung metastasis model



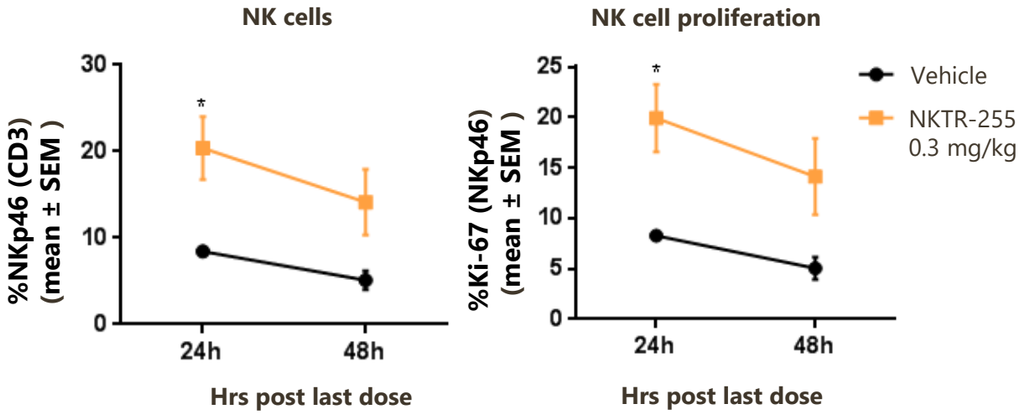
Lung nodules growth inhibition



NKTR-255 efficacy in disseminated CT26 model is NK cell dependent

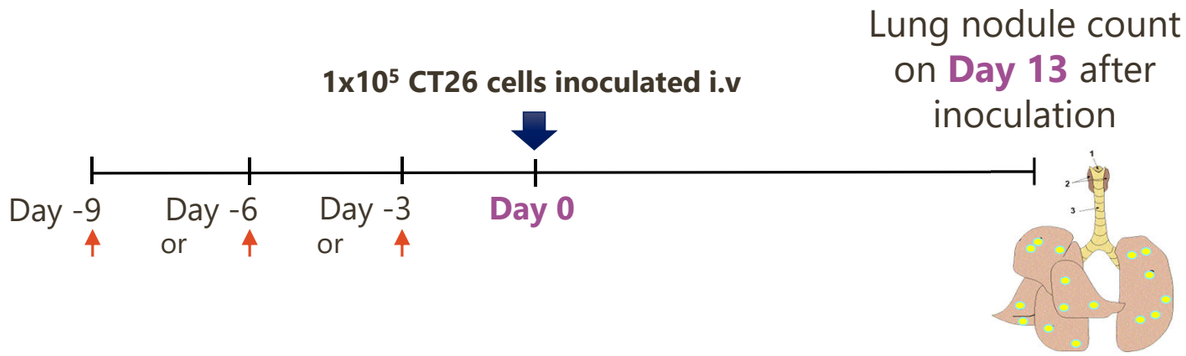


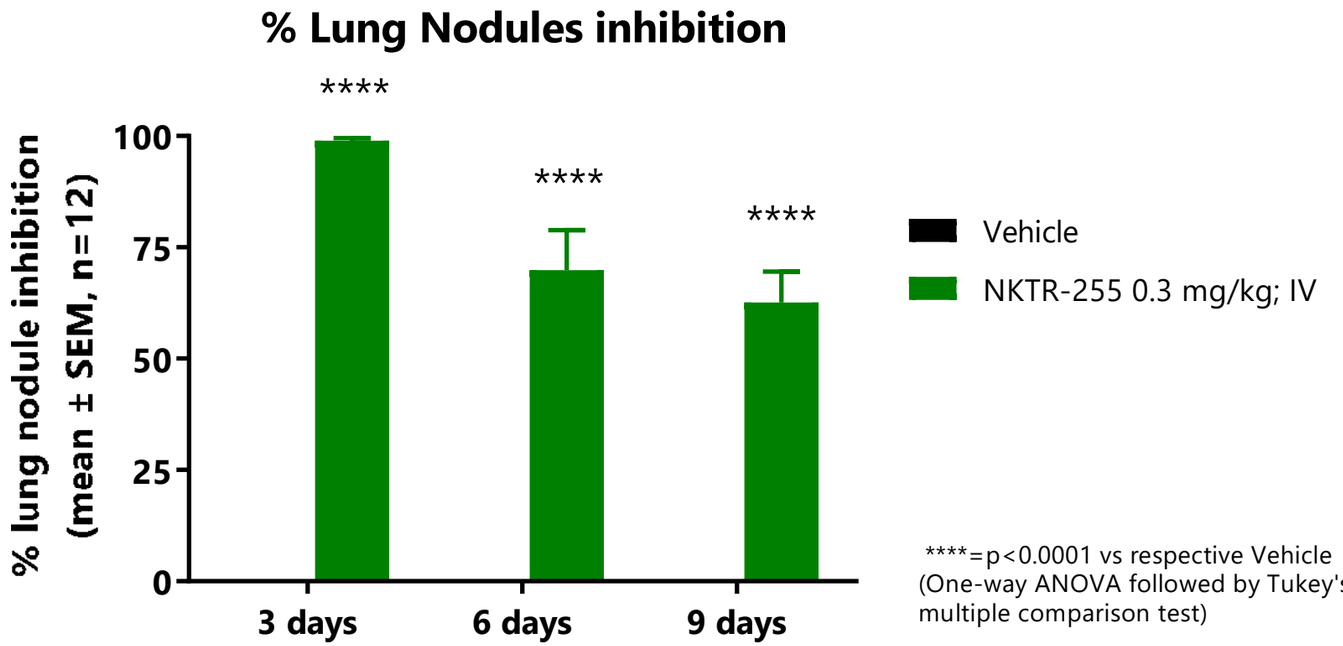
NK cell proliferation in the lung



A single dose of NKTR-255 provides sustained protection against lung colonization by circulating tumor cells

Prophylactic NKTR-255: single dose treatment

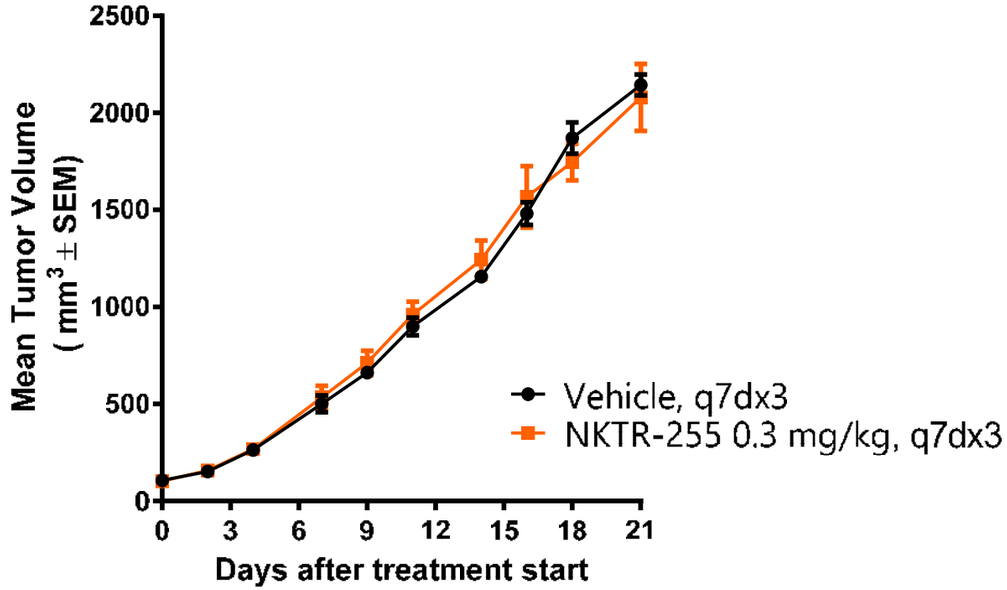




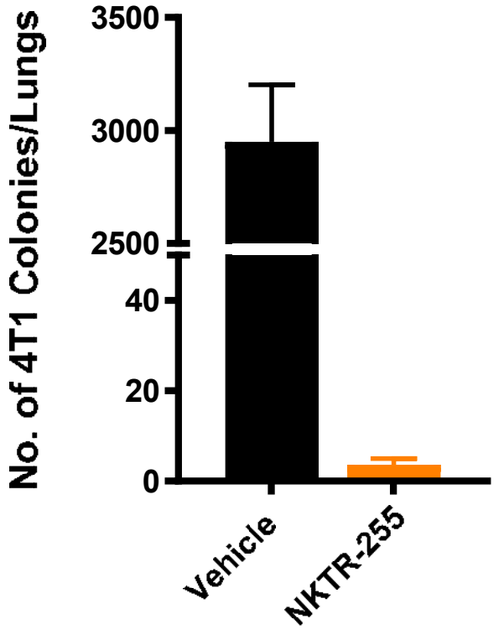
****=p<0.0001 vs respective Vehicle (One-way ANOVA followed by Tukey's multiple comparison test)

NKTR-255 inhibits establishment of spontaneous lung metastasis in 4T1 tumor model

Mean tumor volumes of subcutaneous primary tumors



Number of spontaneous metastatic colonies on Day 14 in lungs



Summary

- NKTR-255 may overcome many limitations of IL-15 as a therapeutic agent
  - Improved PK to allow infrequent administration
  - Provides sustained IL-15 PD activity from a single dose
  - Achieves full breadth of signaling profile characteristic to IL-15
- By design, NKTR-255 maintains binding affinity for IL-15Rα
- NKTR-255 promotes the proliferation of memory T cells and induces significant NK cell expansion and increased cytotoxic activity
- NKTR-255 enhances NK dependent anti-tumor immune responses in vivo
- NKTR-255 enables access to the immunotherapeutic potential of the IL-15 pathway by enhancing expansion and activation of both NK and CD8 T cells