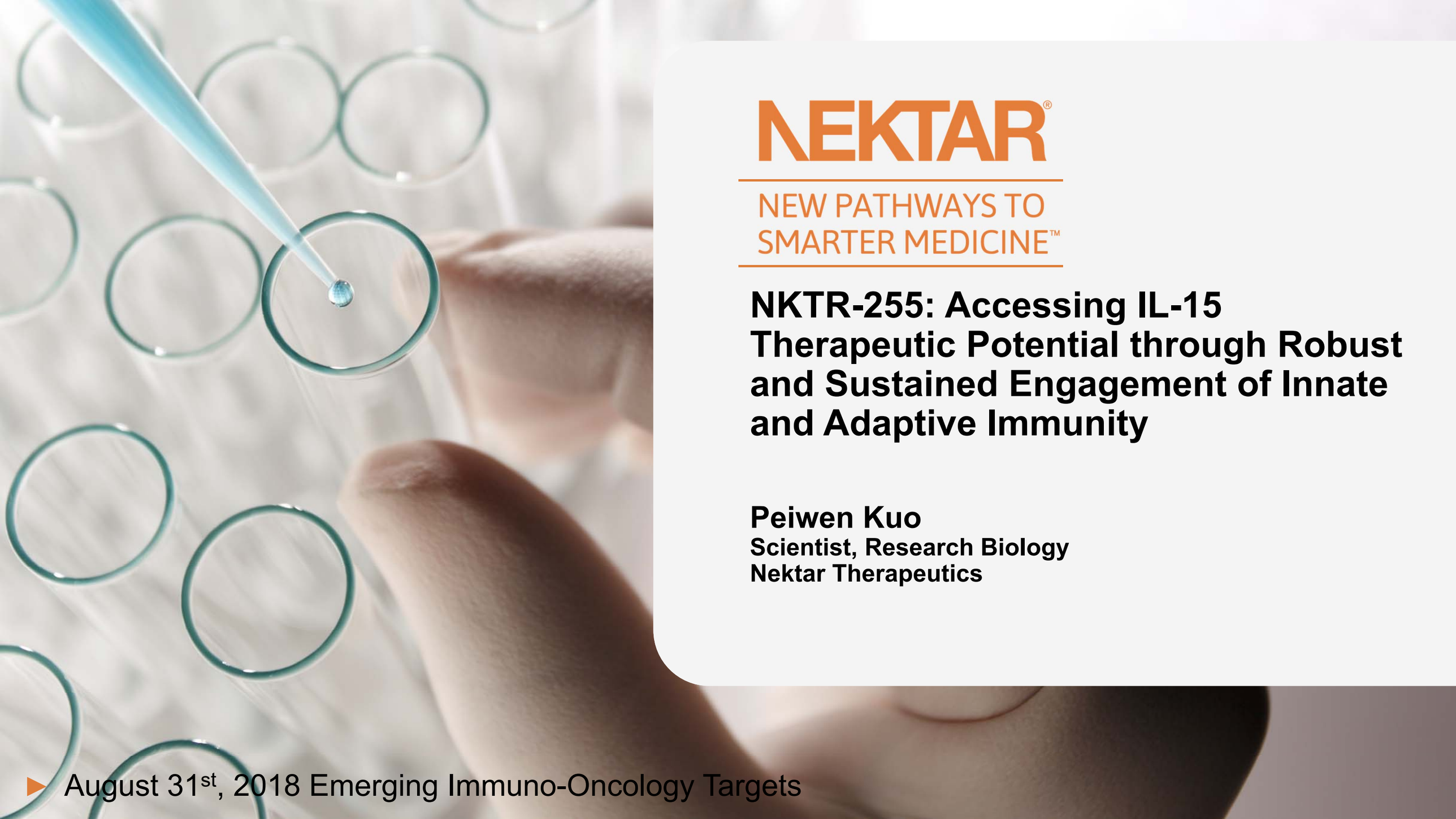


NEKTAR®

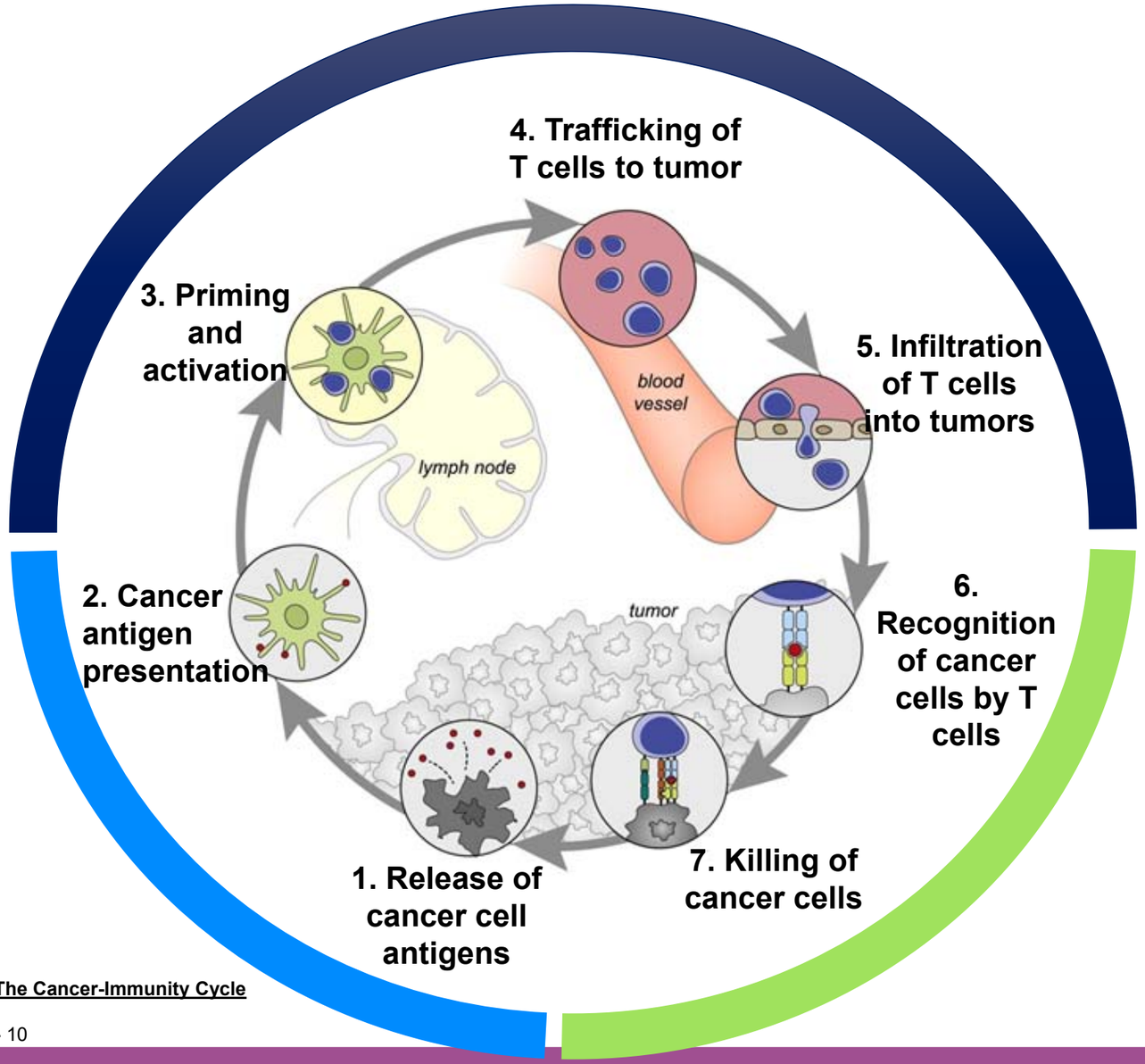
NEW PATHWAYS TO SMARTER MEDICINE™

NKTR-255: Accessing IL-15 Therapeutic Potential through Robust and Sustained Engagement of Innate and Adaptive Immunity

Peiwen Kuo
Scientist, Research Biology
Nektar Therapeutics

August 31st, 2018 Emerging Immuno-Oncology Targets

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

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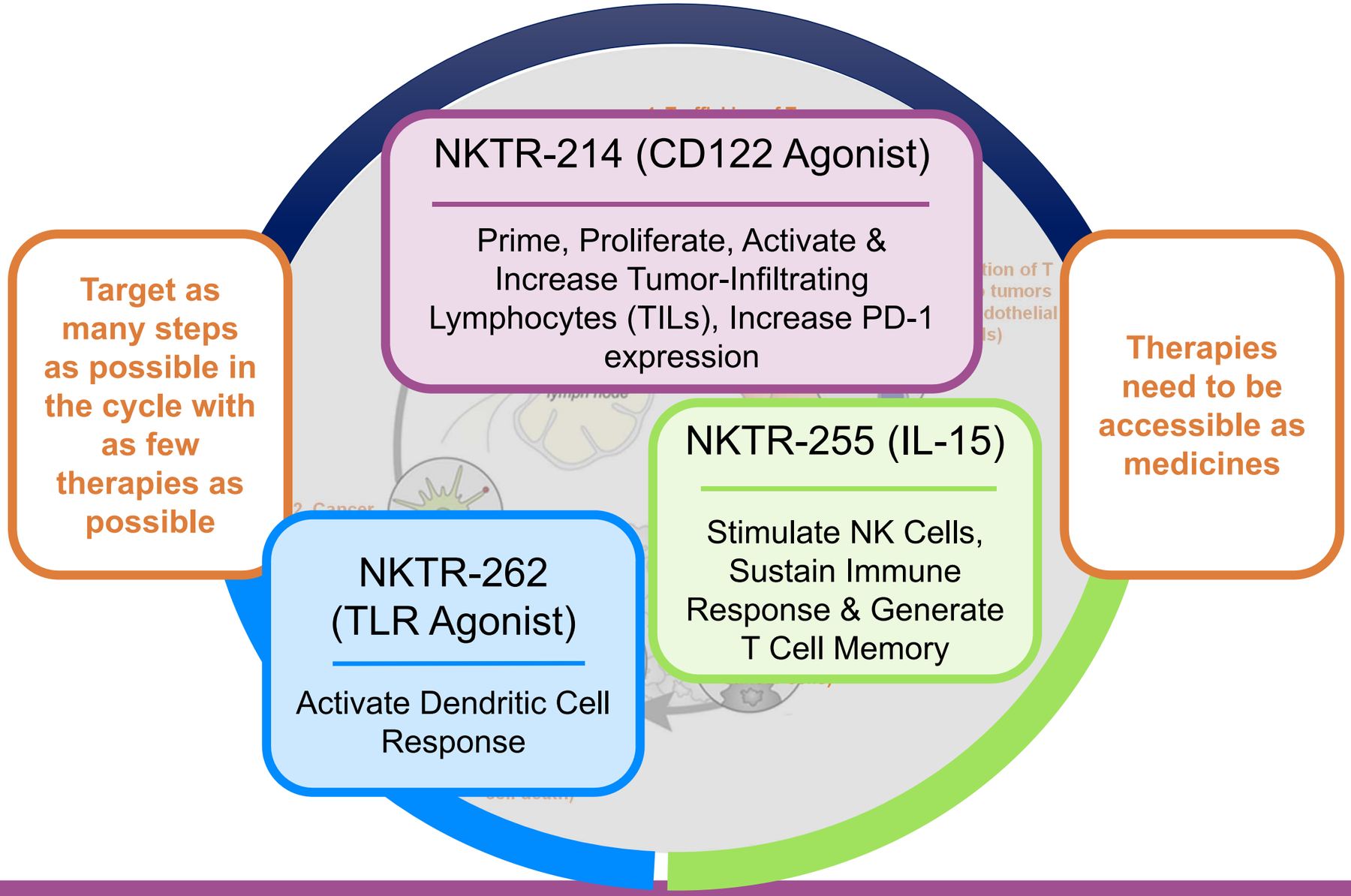
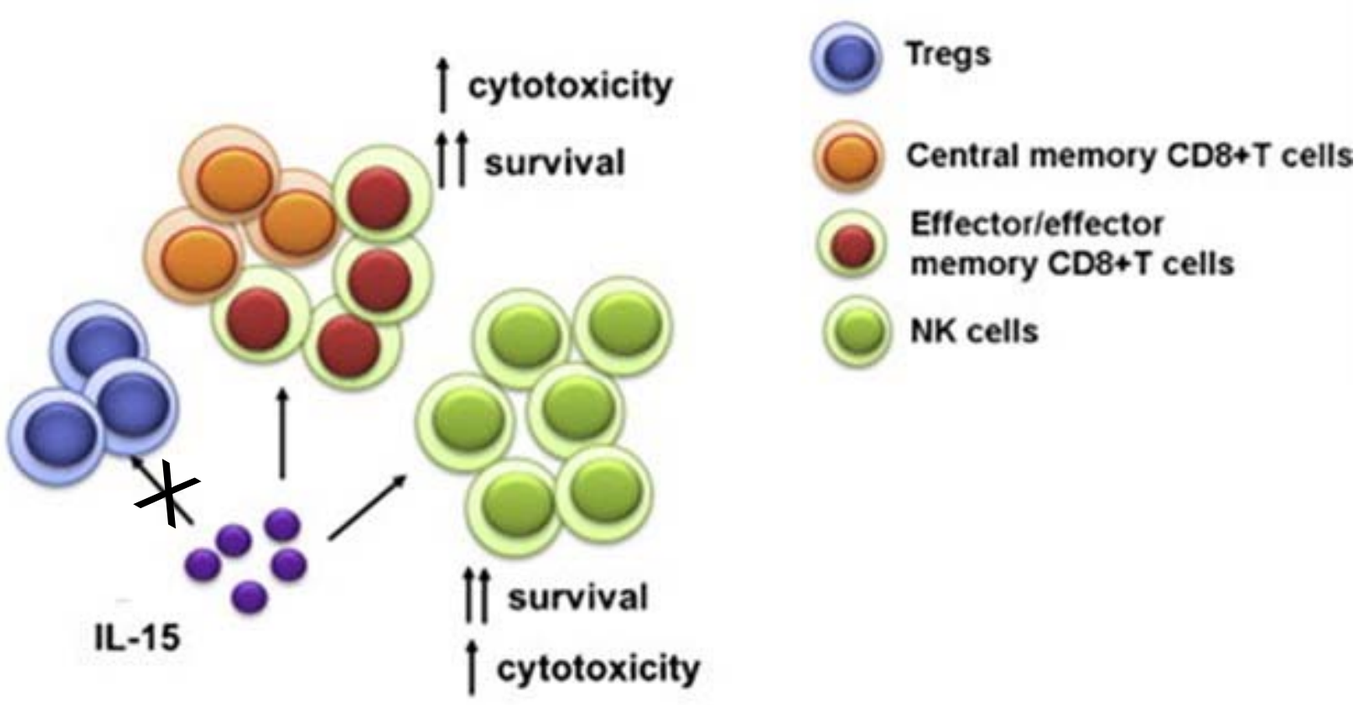
Nektar's immuno-oncology strategy to create therapies that cover the immunity cycle



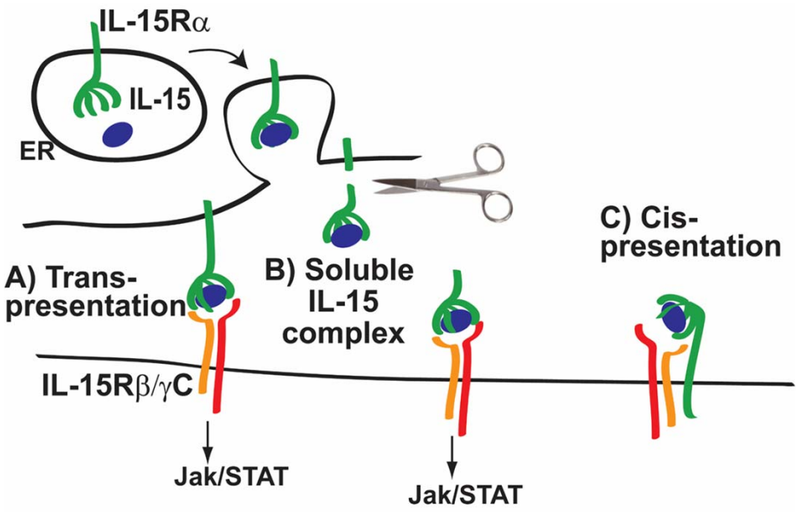
The potential of IL-15 in immuno-oncology



- 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
- Induces survival of CD8 central memory and stem cell memory cells
- Essential factor for NK development and homeostasis
- In vitro, IL-15 can reverse tumor-induced NK dysfunction
- Does not induce Tregs

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 (e.g. 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

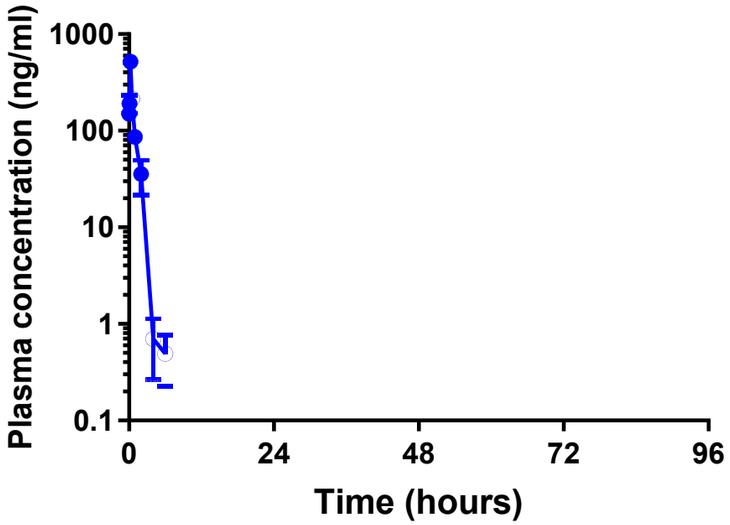


(Stonier and Schluns, 2010)

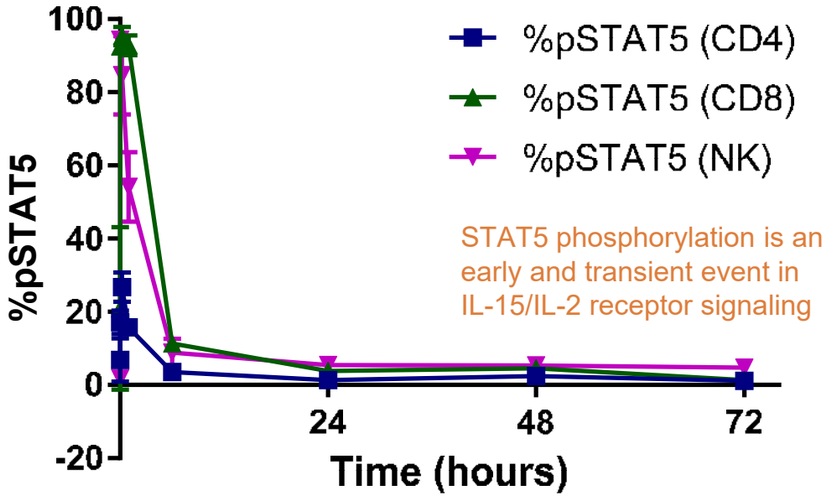
- IL-15/IL-15Rα fusion protein as a therapeutic signals in an IL-15Rα independent manner, loses biological context

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
- Potential to have Cmax-related toxicity

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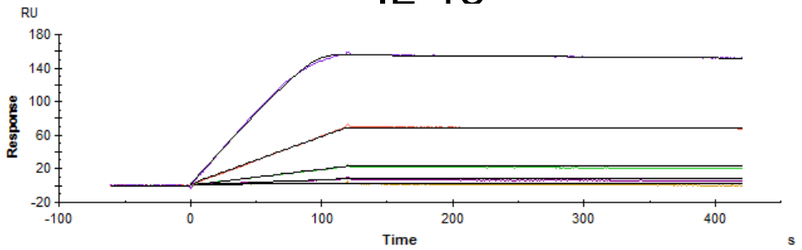
NKTR-255 – polymer conjugated IL-15

- 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 activation and proliferation
  - Supports CD8 T-cell survival and memory formation
  - Shows single-agent efficacy in syngeneic tumor models

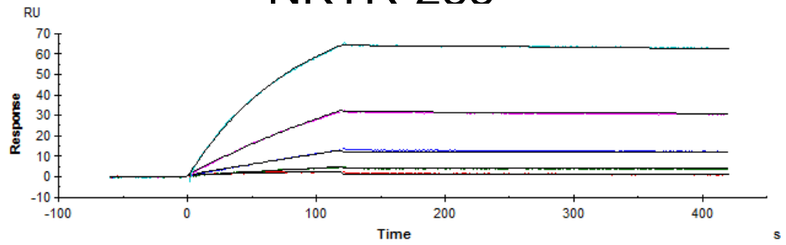
NKTR-255 is first potential medicine to access the IL-15 pathway by preserving receptor binding to IL-15Rα with antibody-like dosing

NKTR-255 retains affinity for IL-15Rα

IL-15



NKTR-255



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 selected to arrive at an optimized combination of design goals
- NKTR-255 affinity for IL-15Rα is ~10X weaker than IL-15

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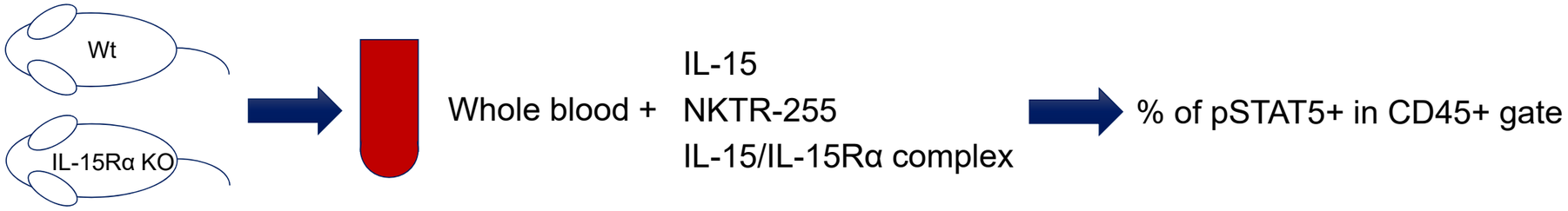
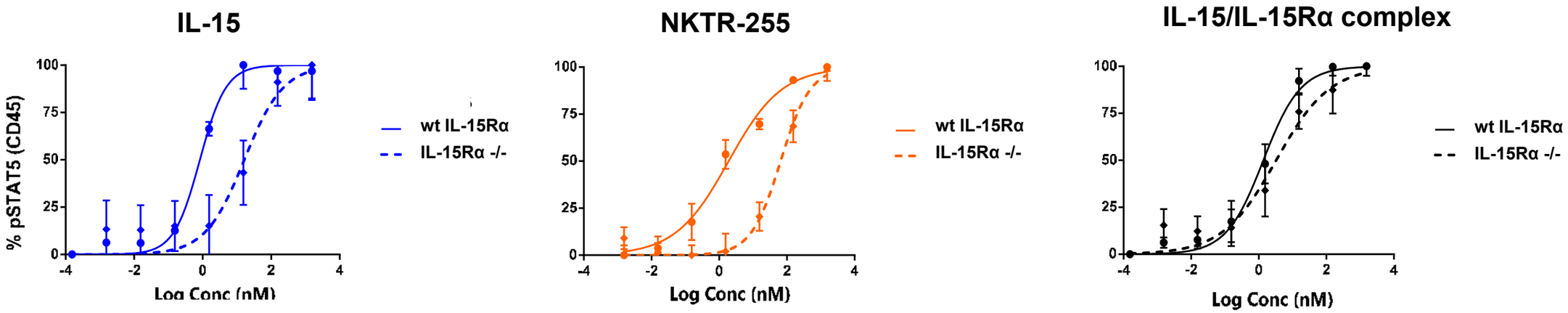
Affinity measured by BIAcore, using IL-15Rα:Fc captured by immobilized anti-Fc
NKTR-255 interaction with β and αβ were also ~8-10X weaker than IL-15

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	

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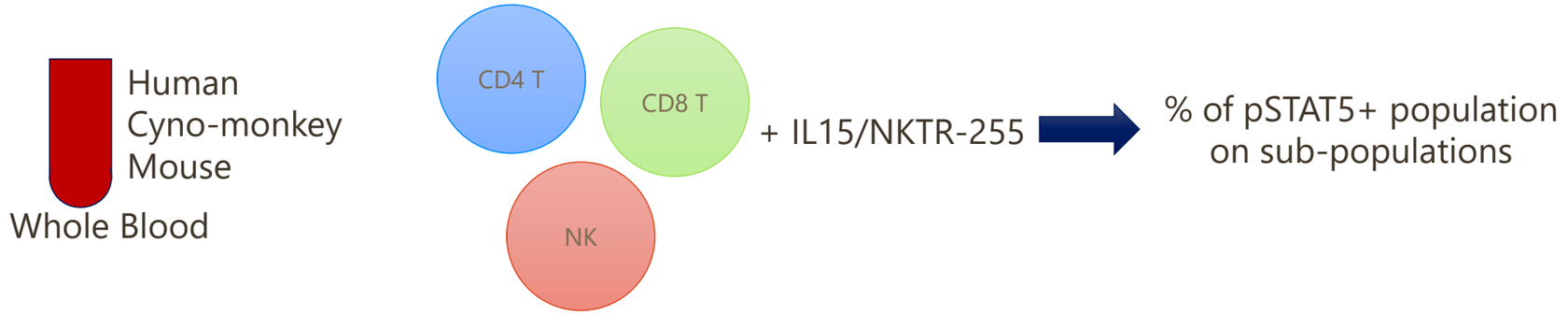
NKTR-255 signaling is dependent on IL-15Rα as is IL-15

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

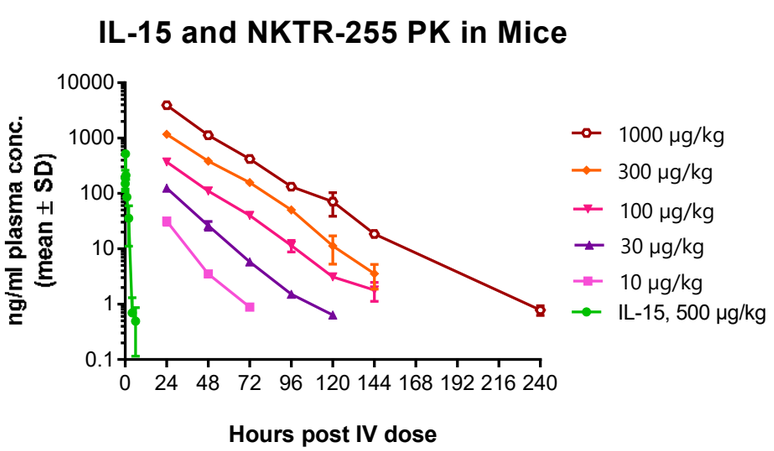
- In human, NKTR-255 exhibits equal potency across three populations
- NKTR-255 potency to NK cells is preserved across species except for mouse
- NKTR-255 potency to T cells is different depending on species
- NKTR-255 species difference is similar to that for IL-15
- Varying potency across species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

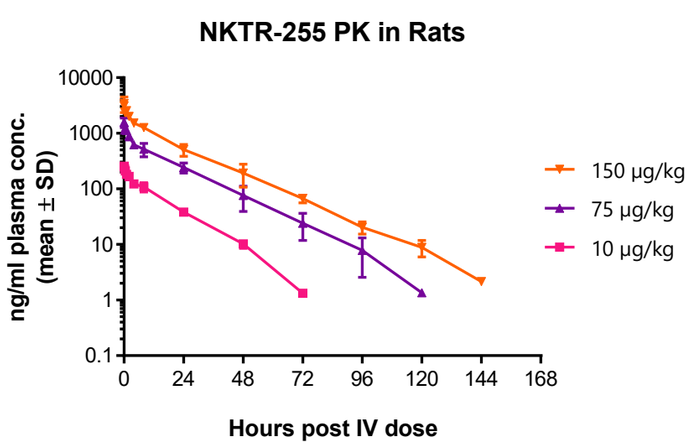
NKTR-255 binds IL-15Rα to engage the IL-15 pathway

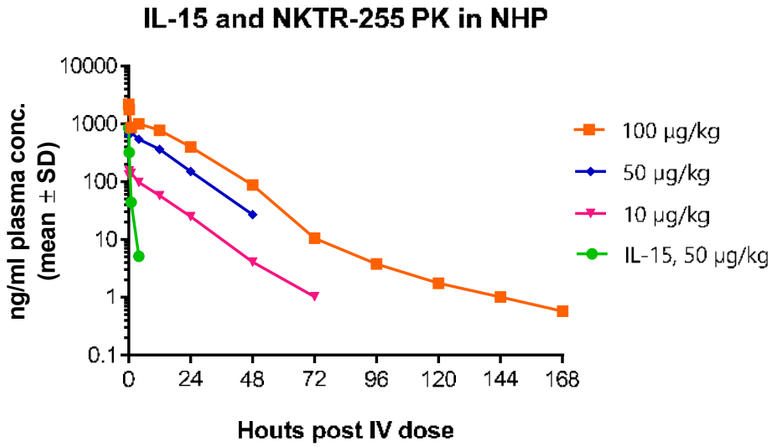
- NKTR-255 retains IL-15Rα binding
  - SPR data supports that binding to IL-15Rα is maintained, with an affinity that is ~10x weaker than IL-15
- NKTR-255 binding to IL-15Rα is essential for its activity
  - JAK/STAT signaling is abrogated in the absence of IL-15Rα
- NKTR-255 signaling is preserved across species and species difference is similar between NKTR-255 and IL-15

NKTR-255: sustained PK and robust PD in rodents and non-human primates

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)

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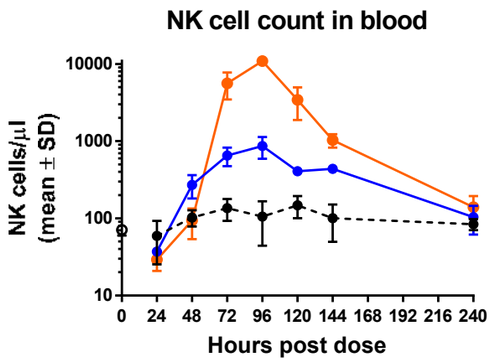
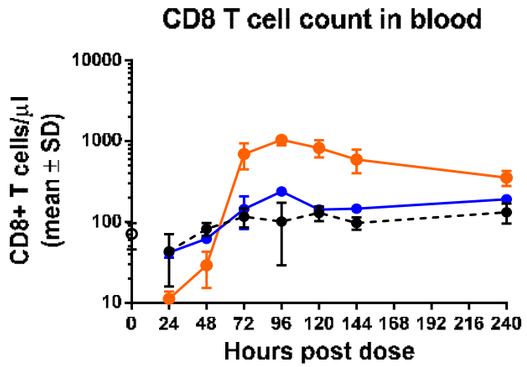
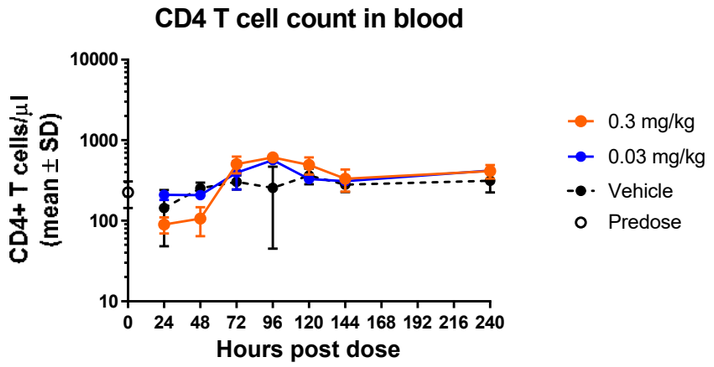
Note: Parent IL-15 was not dosed in the Rat PK study; No apparent gender differences are noted in the Cyno study

NKTR-255 drives prolonged signaling and proliferation in NK cells and CD8 T cells in mice after a single dose

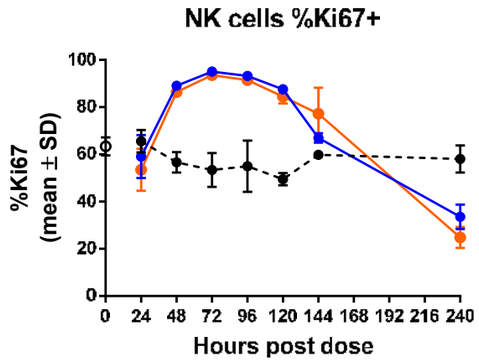
Cell expansion

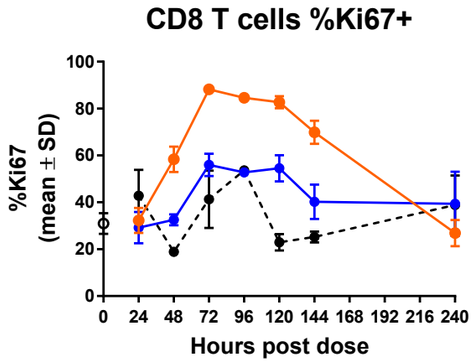


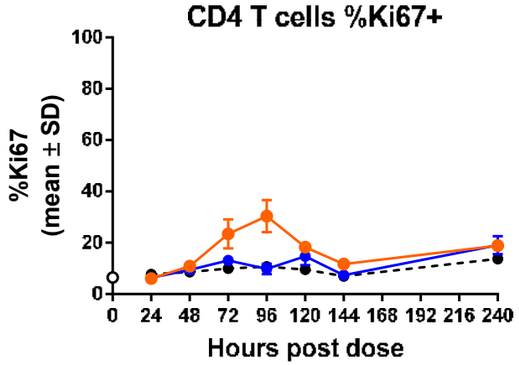




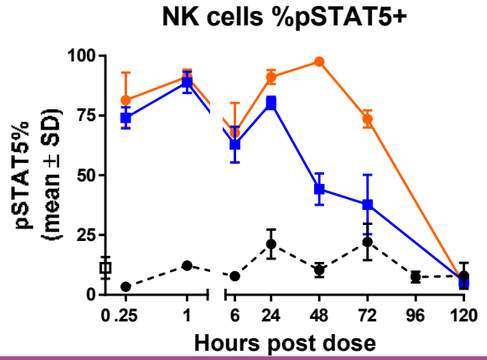
Ki67+

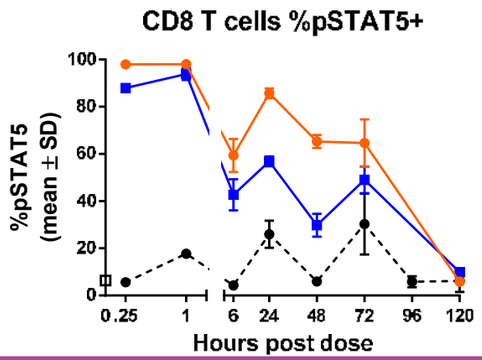


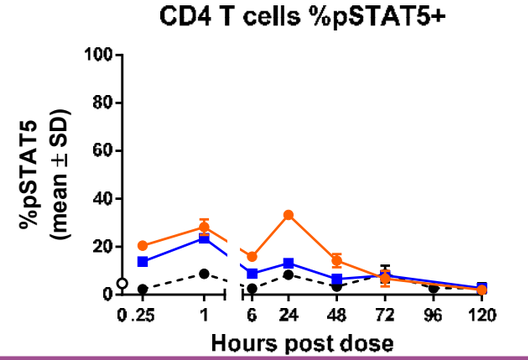




pSTAT5+







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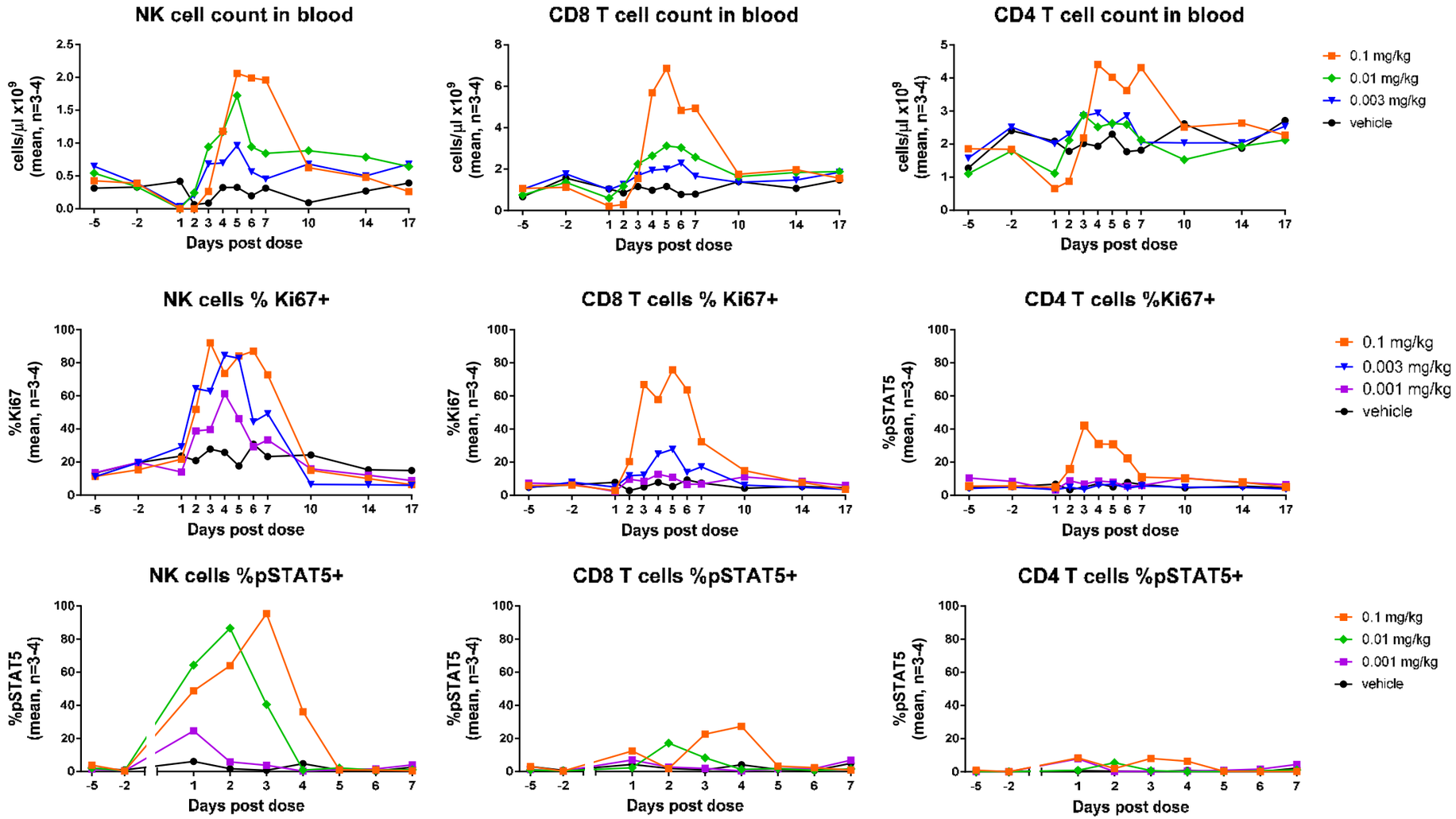
NKTR-255 expands CD8 and NK cells in vivo in NHPs

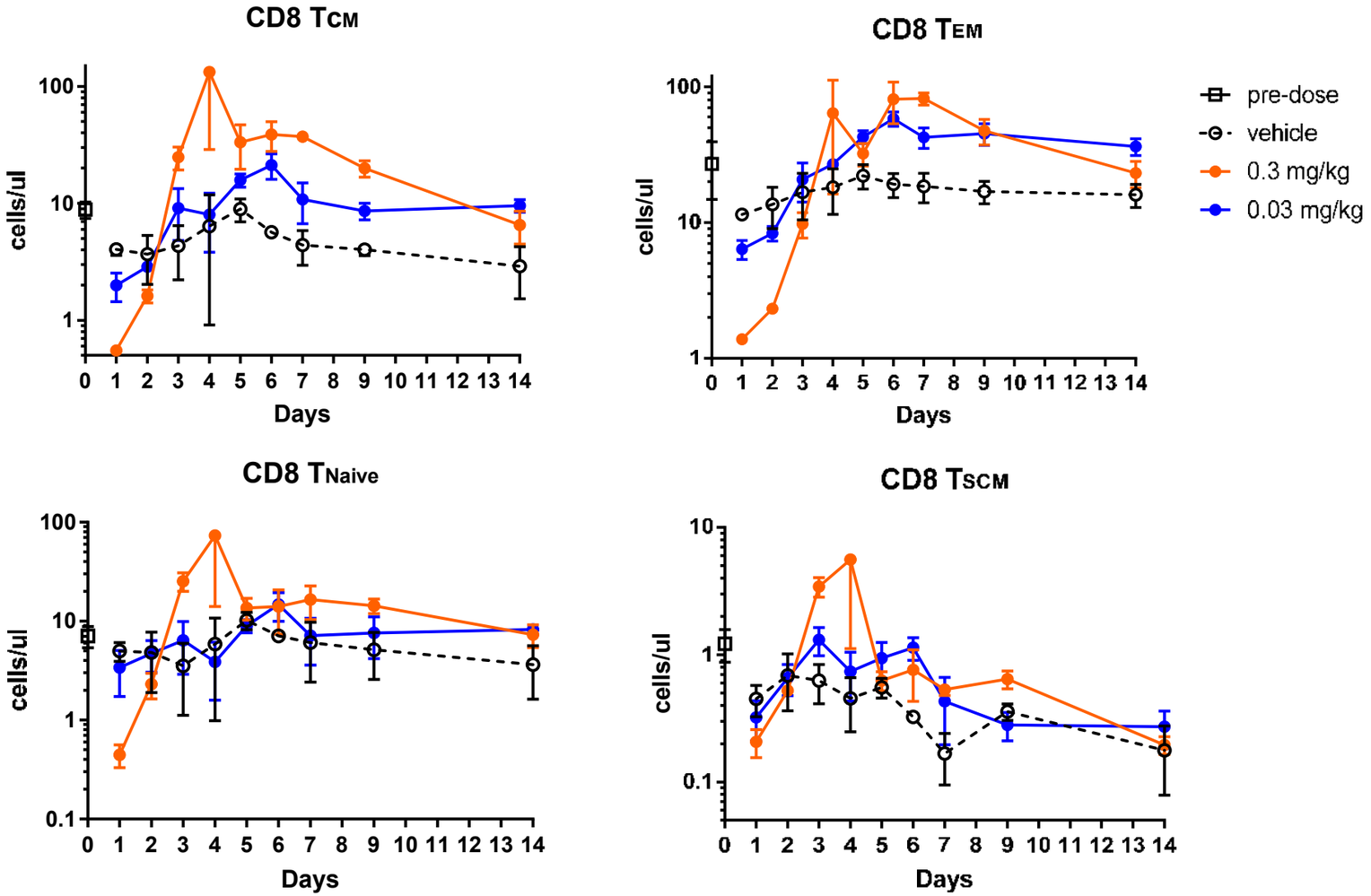
CD8 and NK cell expansion detected at 0.01 mg/kg dose level

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

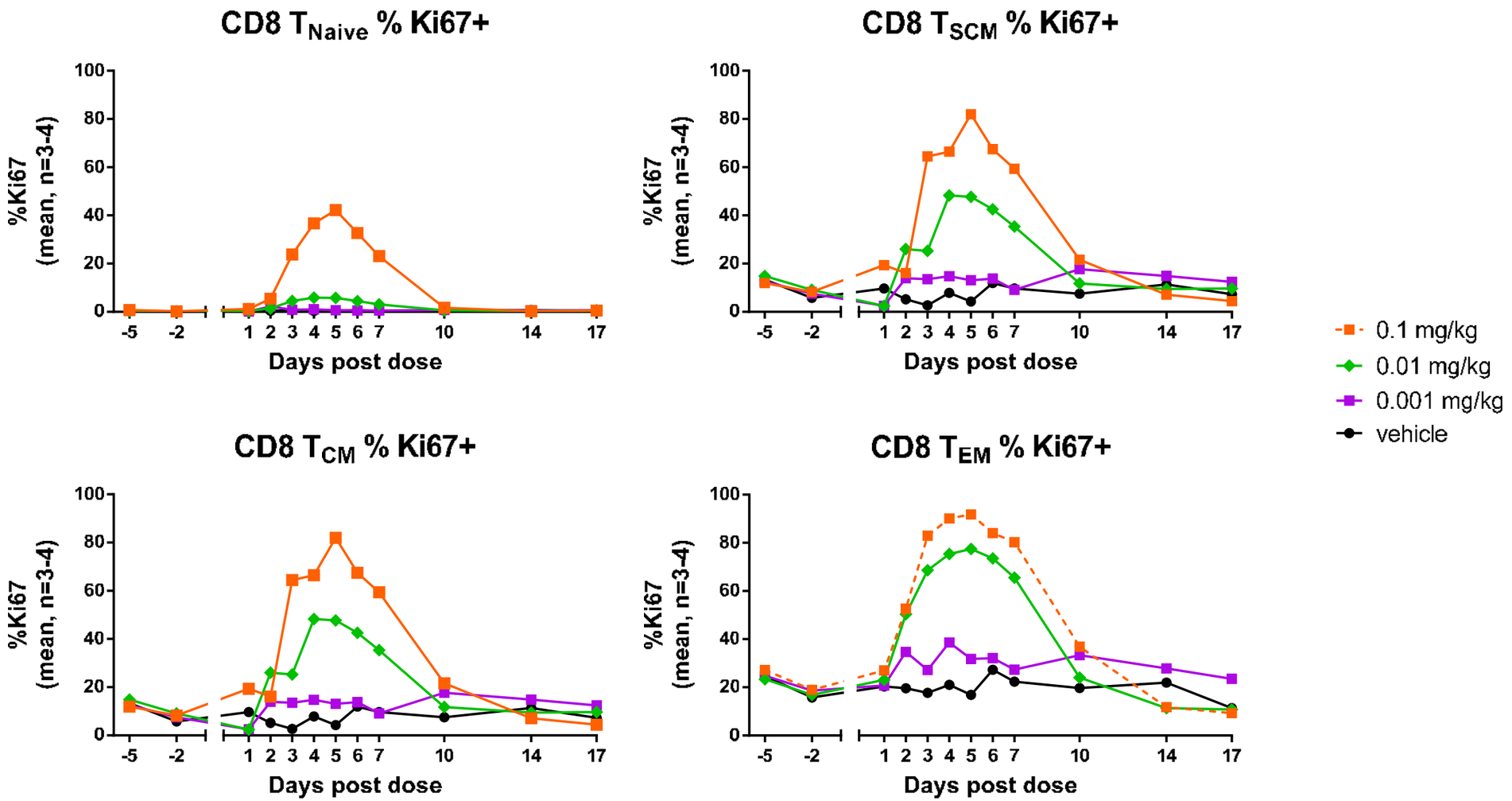


Cynomolgus monkeys NKTR-255 IV dose response PD: blood stained for leukocyte surface markers and pSTAT5 + Ki67, measured by flow cytometry

Memory CD8 T cells are highly sensitive to NKTR-255, exhibiting robust expansion after a single dose in mice



NHP CD8 memory populations are sensitive to NKTR-255



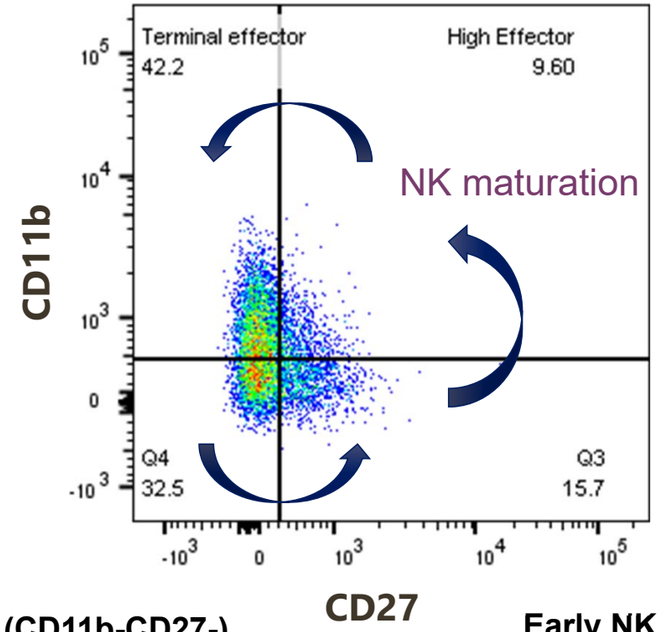
Cynomolgus monkeys NKTR-255 IV dose response PD: blood stained memory markers (CD45RA, CD197, CD95) in CD8 parent gate.

NKTR-255 expands NK subpopulations in mice

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

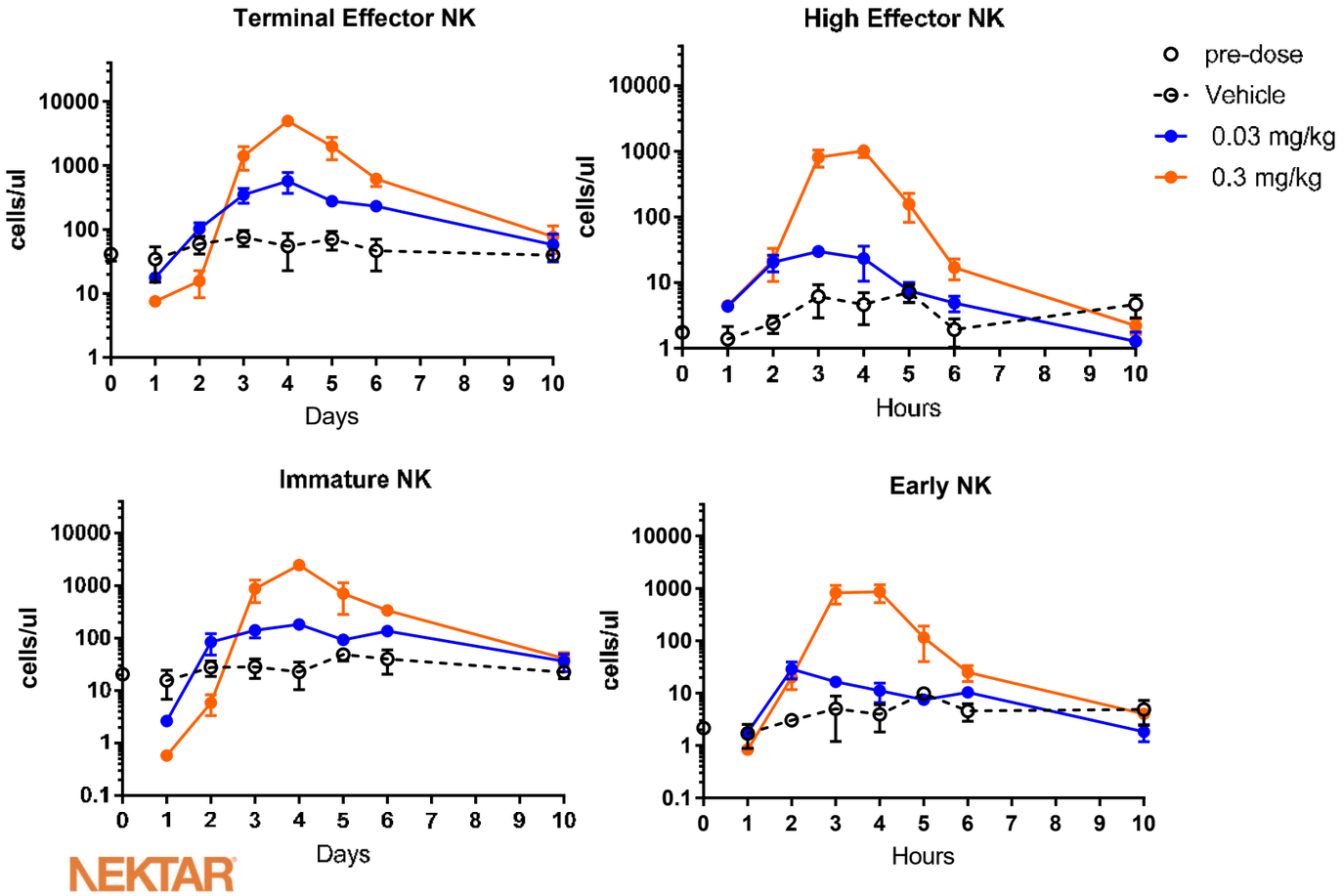


Immature (CD11b-CD27-)
Potential to differentiate

Early NK (CD11b-CD27+)
High cytokine secretion

NKTR-255 expands NK subpopulations in mice

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



Terminal Effector (CD11b+CD27+)
Most mature
Activation tightly regulation
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High Effector (CD11b+CD27+)
High cytokine secretion
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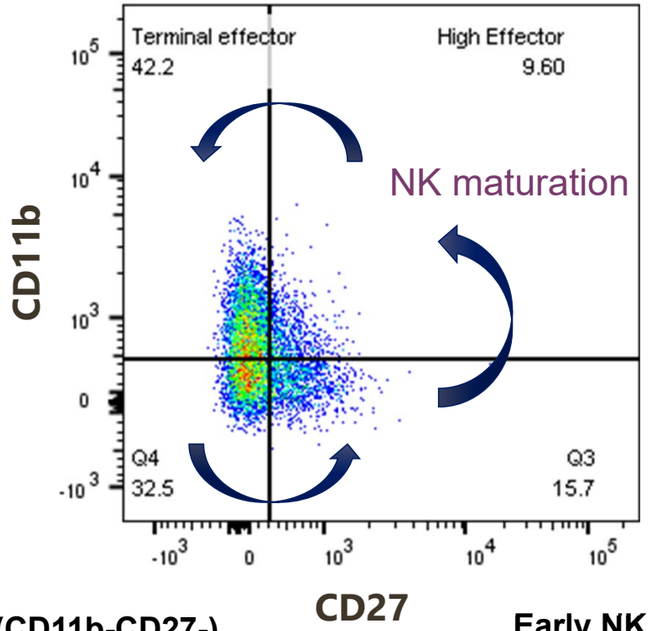

Immature (CD11b-CD27-)
Potential to differentiate

Early NK (CD11b-CD27+)
High cytokine secretion

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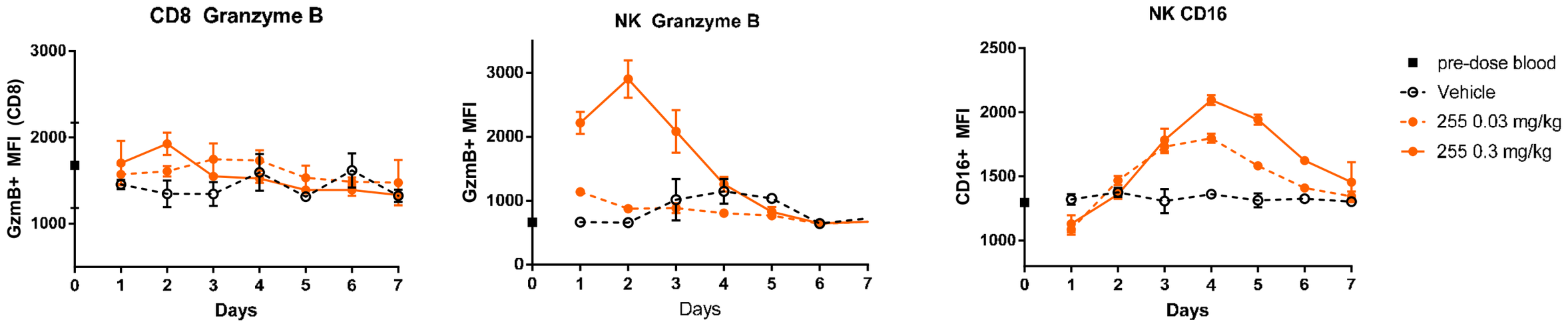
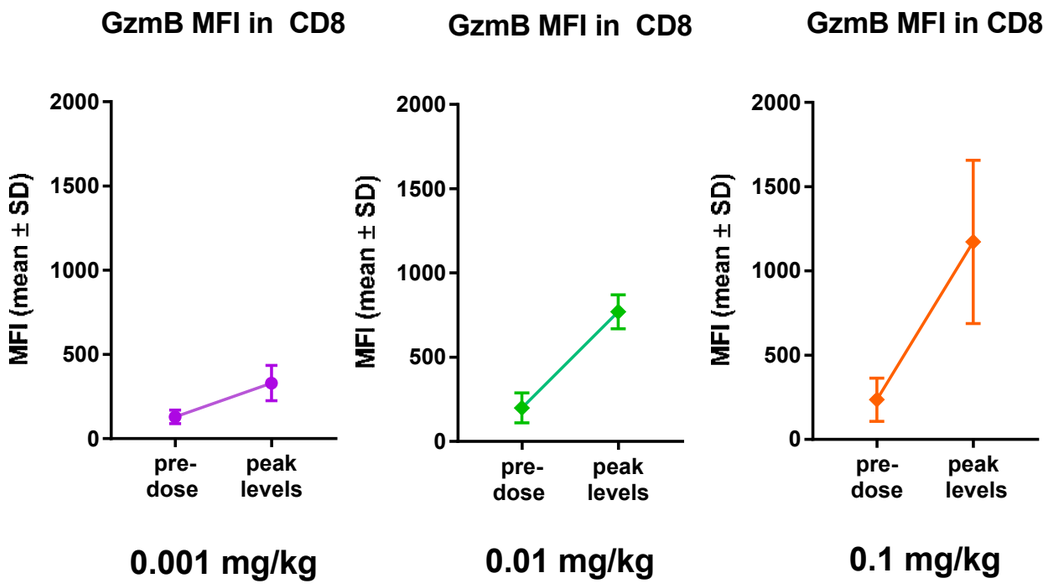
NKTR-255 increases effector protein expression in expanded NK and CD8 T cells in mice

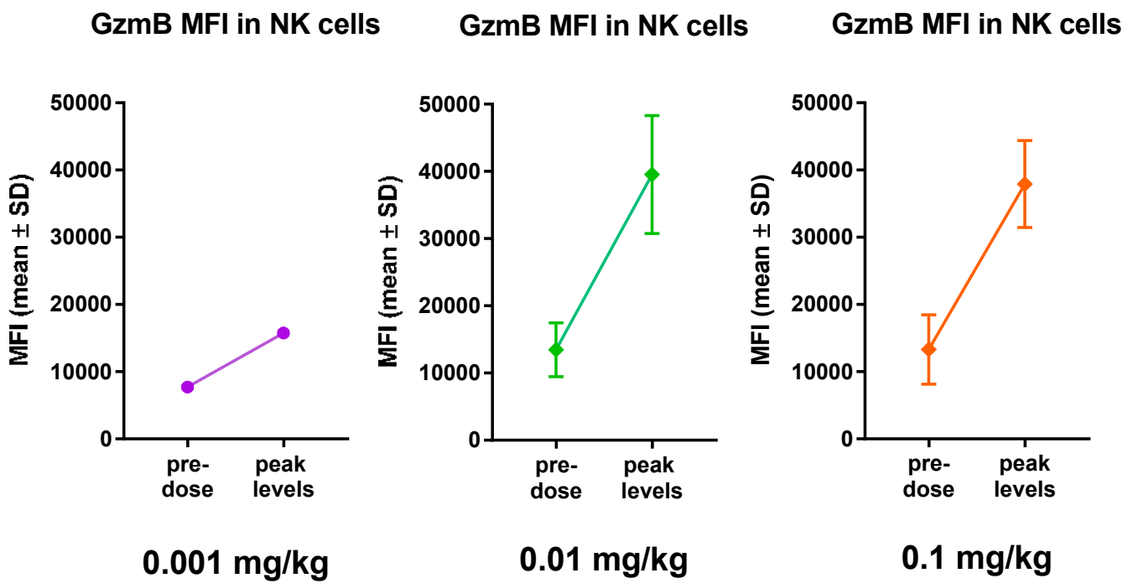


NKTR-255 increases levels of cytotoxic enzymes in NK and CD8 T cells in NHPs

Granzyme MFI in CD8 T cells



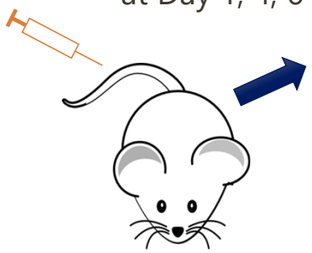
Granzyme B MFI in NK cells



NKTR-255 increases protein levels of cytolytic enzymes in both CD8 and NK cells

NKTR-255 enhances murine splenic NK cytotoxicity

NKTR-255



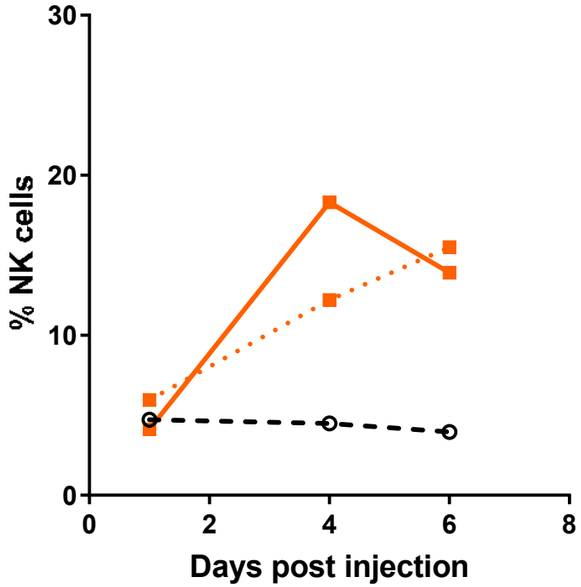
Spleens harvested at Day 1, 4, 6

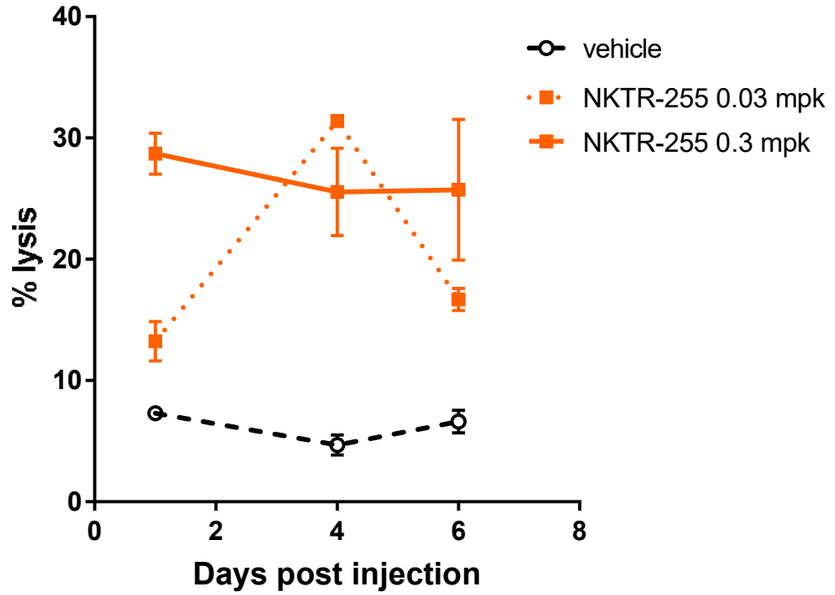
Effector cells: NK cells isolated from spleens

Target cells: YAC-1 cells labeled with PKH26

Co-culture at 12.5:1 (E:T ratio) for 4 hr

Label cells with 7-AAD; Analyze lysis of target cells via flow cytometry





A single dose of NKTR-255 boosts NK cell killing for at least 6 days (0.3 mg/kg)

NKTR-255 has extended PK and drives robust NK and CD8 T cell responses

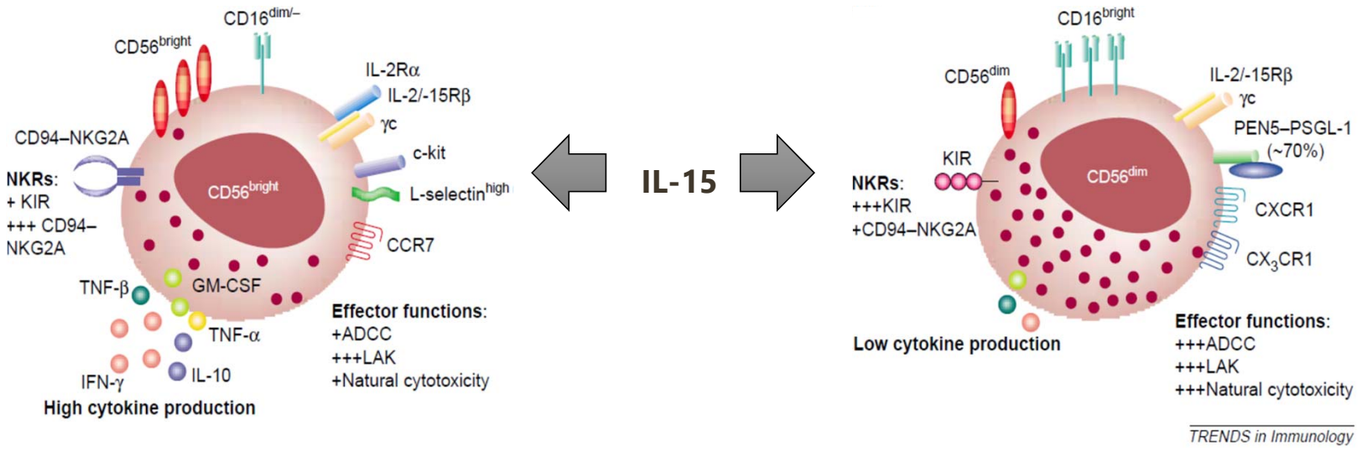
- NKTR-255 exhibits substantially superior PK over conventional IL-15
  - Prolonged plasma exposure in rodents ($T_{1/2}$ ~14-18hrs) and NHP ($T_{1/2}$ ~30hrs) vs IL-15 ($T_{1/2}$ <1hr)
- CD8 T and NK cells are responsive to NKTR-255 stimulation in vivo in mice and NHPs
  - NK cells are highly sensitive: %pSTAT5 and %Ki67 increases measurable at lowest tested dose level (0.001 mg/kg) in NHP
  - CD8 T cells are very responsive: %Ki67 and cell expansion detectable at 0.003 and 0.01 mg/kg in NHP, respectively
- Higher sensitivity to NKTR-255 in CD8 memory T cells relative to naïve CD8 T cells
  - Graded sensitivity within memory subpopulations in mouse and cyno, (e.g. cyno T_{EM}>T_{SCM}>T_{CM}>T_{Naive}) in proliferative response in NHP
- CD4 T cells are less responsive to NKTR-255 stimulation in mice and NHPs
- NKTR-255 increases levels of cytotoxic enzymes to promote cytolytic function
  - In mice, NKTR-255 engages NK cells at all levels of maturation and increases Granzyme B and CD16 expression
  - NKTR-255 enhances the cell-killing ability of murine splenic NK cells
  - In mouse and NHP, NKTR-255 increases Granzyme B expression in both CD8 T and NK cells

NKTR-255: engaging NK and CD8 T cells to boost single agent anti-tumor efficacy

NKTR-255 driving IL-15 anti-cancer immunotherapy via NK and CD8 biology

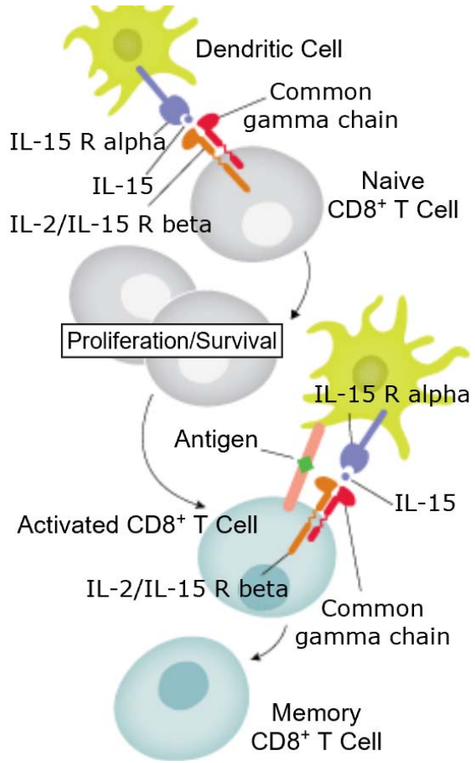
NK cell biology

- 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

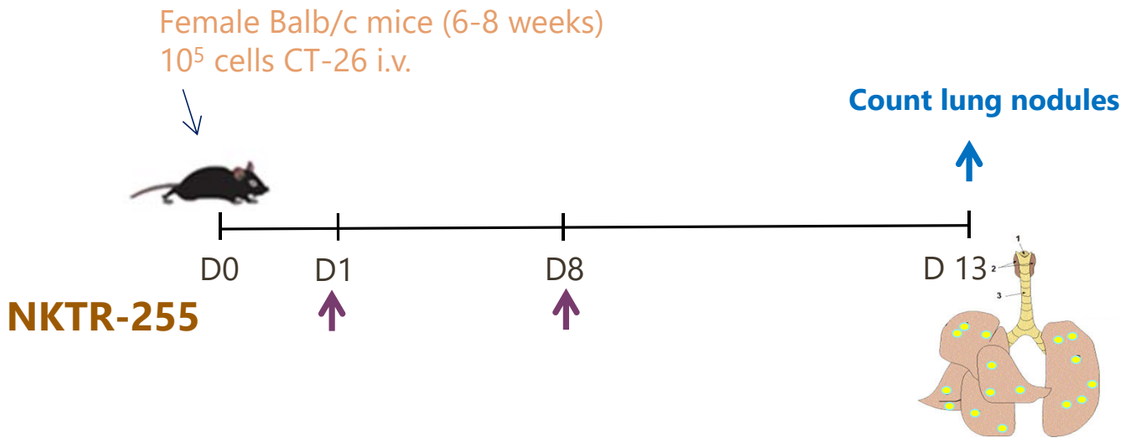


CD8 cell biology

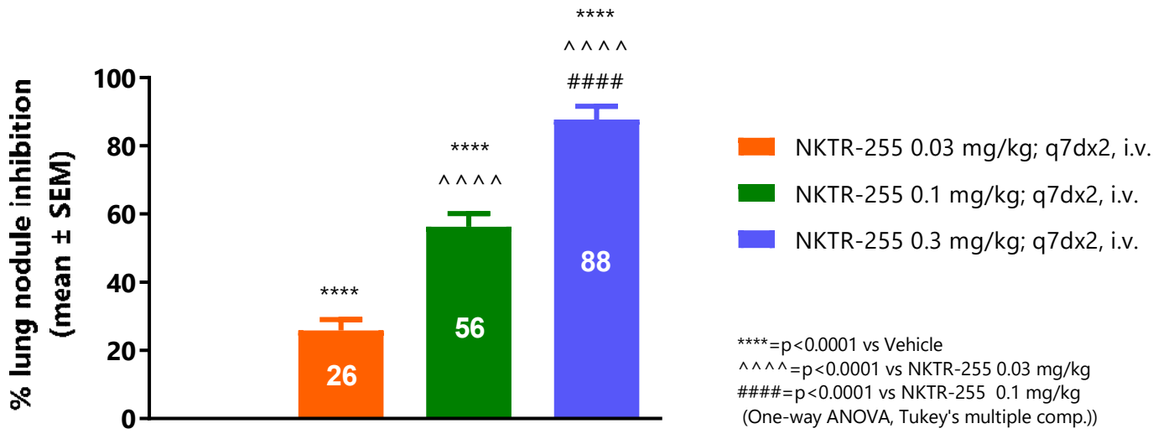
- Supporting memory CD8 T cell longevity and function
  - IL-15 maintains Ag-specific effector CD8 T cells after the contraction phase by promoting their survival and proliferation



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

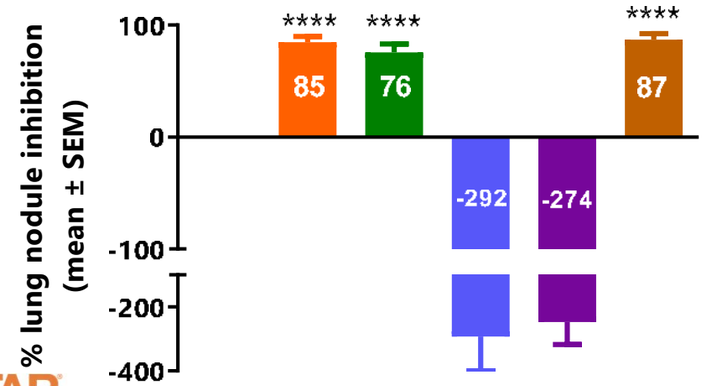


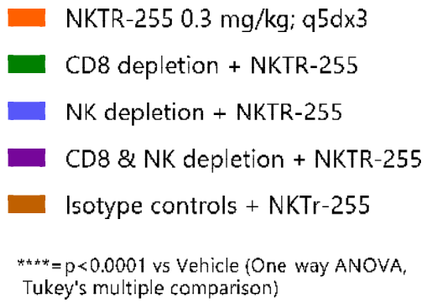
Lung nodules growth inhibition



****=p<0.0001 vs Vehicle
^^^^=p<0.0001 vs NKTR-255 0.03 mg/kg
####=p<0.0001 vs NKTR-255 0.1 mg/kg
(One-way ANOVA, Tukey's multiple comp.))

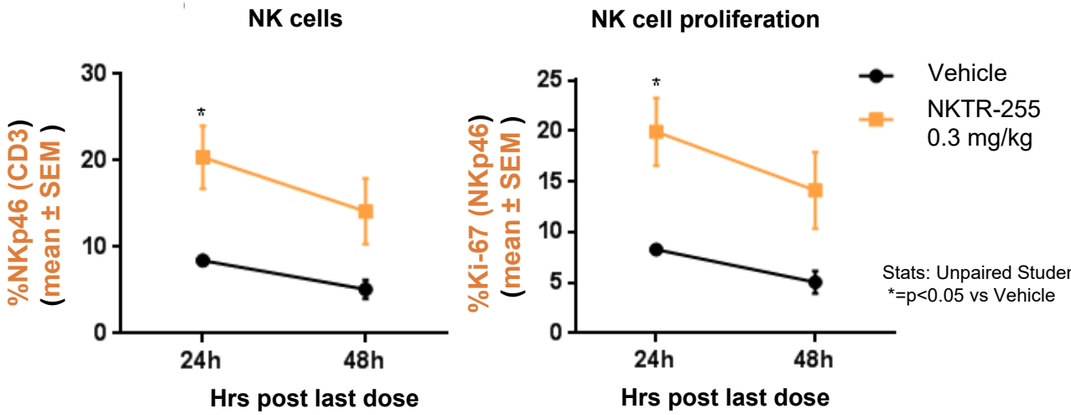
NKTR-255 efficacy in disseminated CT26 model is driven by NK cell activity





****=p<0.0001 vs Vehicle (One way ANOVA, Tukey's multiple comparison)

NK cell proliferation in the lung



Stats: Unpaired Student *t* test ;
*=p<0.05 vs Vehicle

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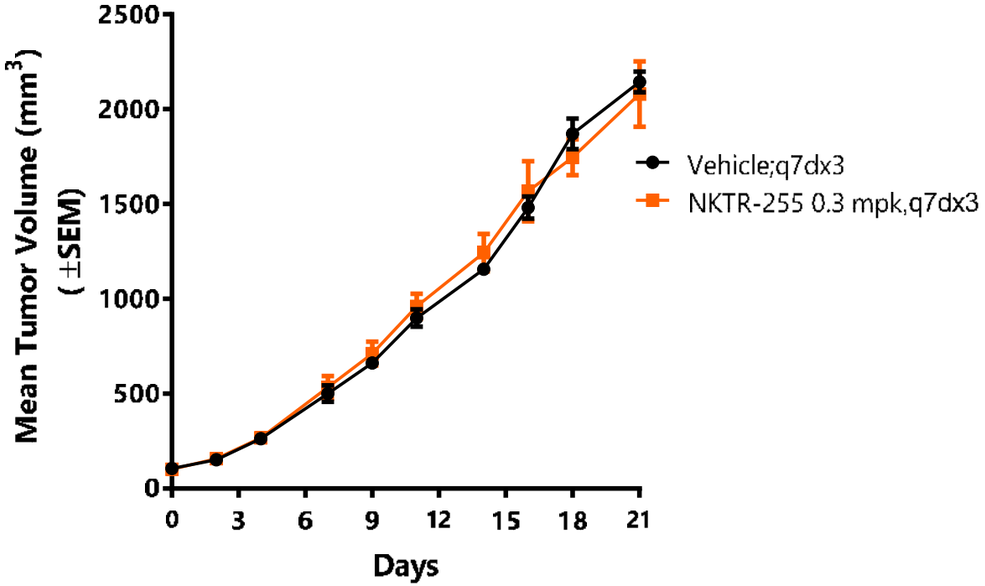
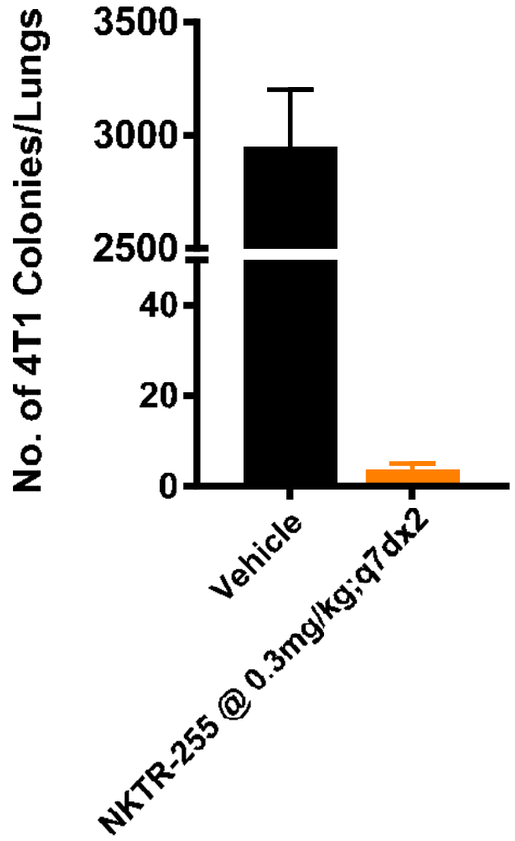
NKTR-255 inhibits spontaneous metastasis in the 4T1 model

Mean tumor volumes of 4T1 subcutaneous tumors on Day 21



Number of spontaneous metastatic colonies on Day 14 in lungs



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Conclusion

- NKTR-255 overcomes many limitations of IL-15
  - Improved PK to allow infrequent administration
  - Provides sustained IL-15 PD activity from a single dose
  - Achieves full breadth of signaling profile as expected for IL-15
- By design, NKTR-255 maintains binding affinity for IL-15Rα
- NKTR-255 promotes the proliferation of CD8 memory T cells and induces vast NK cell expansion and increased cytotoxic activity
- NKTR-255 has stand-alone efficacy in NK-driven metastasis models
- NKTR-255 provides access to the immunotherapeutic potential of the IL-15 pathway by enhancing both NK and CD8 anti-tumor responses.