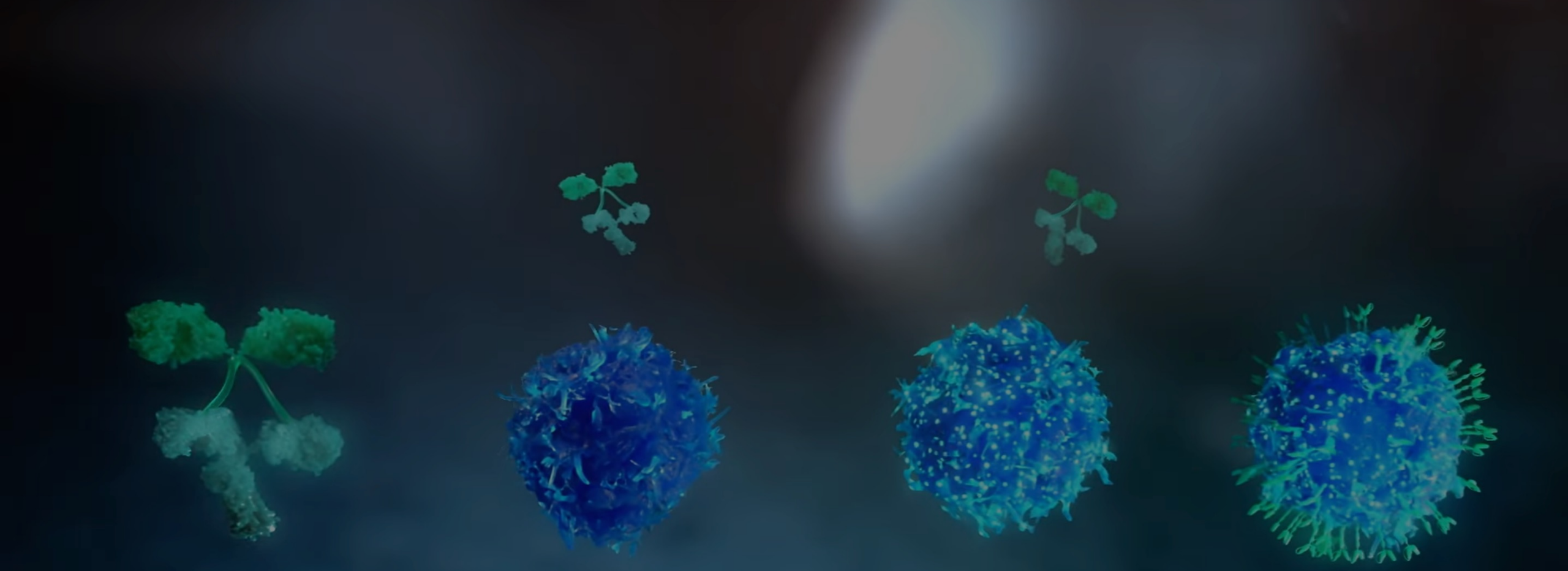


Aligning & Prioritizing Gene Editing with Functional Goals to Drive the Next Generation of iPSC-Derived NK Therapy

Wei Li, Ph.D.
Chief Scientific Officer

March 30th, 2023
Innate Killer Summit

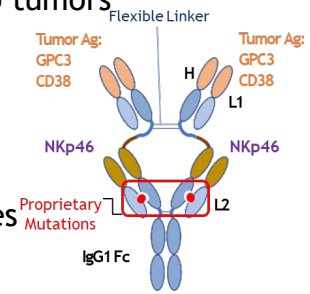
Cytovia Therapeutics

Two Complementary Platforms to Unlock the Power of NK Therapeutics

First-in-class company combining bispecific antibody & gene-edited, iPSC-derived NK cell platforms

Flex-NK™ Bispecific Antibody Platform

- Binding to NKp46 that allows recruitment of NK cells into tumors
  - NKp46 express in tumor infiltrating NK cells
- Proprietary quadrivalent antibody platform
- Ability to target a wide range of tumor antigens
- Potential to combine with multiple therapeutic modalities
- Flexible linker that facilitates multifunctional binding

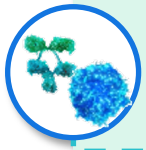


Edited iPSC-Derived NK (iNK) Cell Platform

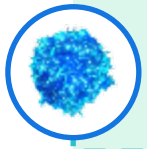
- Streamlined production of homogenous gene-edited off-the-shelf NK Cells
- Edits focusing on improved physical and functional persistence, and improved resistance to TME
- Cellectis partnership to enable custom TALEN® gene-editing
- UCSF research partnership to enable optimal integration loci for NK
- IP & licenses covering technology and targets

Multiple Therapeutic Modalities

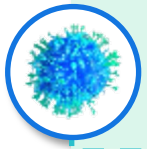
Flex-NK™ Bispecific Antibody



Unedited iNK Cell pre-complexed with Flex-NK™ Bispecific Antibody



Edited iNK Cell



CAR-iNK Cell

Multiple Therapeutic Franchises

GPC3 Franchise		EGFR	CD38 Franchise	
Hepatocellular Carcinoma (HCC)	Other Solid Tumors expressing GPC3	Glioblastoma Multiforme (GBM)	Multiple Myeloma (MM)	Cutaneous T-Cell Lymphoma (CTCL) & other CD38 expressing hematological tumors

Why NK Cells?

Pros

- Outstanding safety profile
  - Low CRS
  - No GvHD
  - Low neurotoxicity
- HLA-independent killing - Natural allogeneic therapy
- Inherent ready to go antigen-independent innate immunity against tumor cells – helpful against antigen-escape
- Cancer stem cells and solid tumors tend to have low MHC-1 – more sensitive to NK cells

Challenges:

- Short persistence
- Inactivated in TME (Tumor Microenvironment)
- Insufficient infiltration to tumors
- Concern of potential allo-rejection as an allogeneic therapy

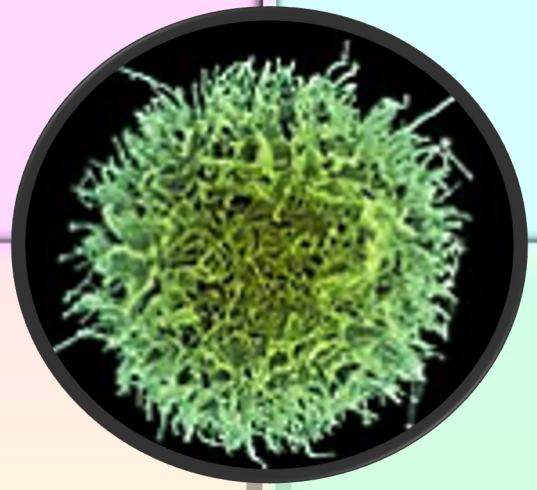
Potential Edits to Create Super NK Cells

Effector Function

Prevent exhaustion
Overcome TME
Improve metabolic status

Tumor Recognition

Addition of CAR
Overcome shedding



Persistence

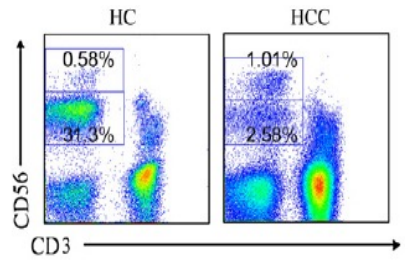
Improve proliferation
Overcome allo-rejection
Overcome fratricide

Homing

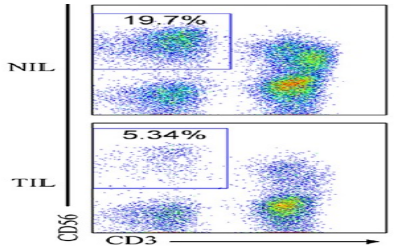
Improve trafficking
Improve tumor infiltration

NK Cell Status in HCC

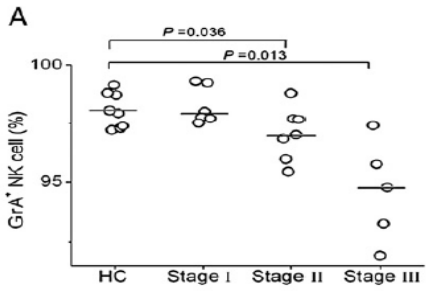
HCC Blood NK



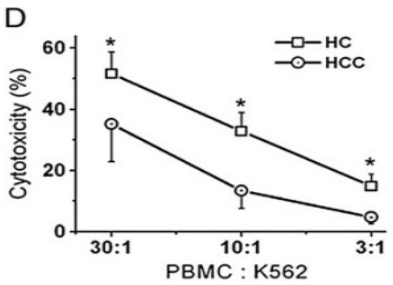
HCC Tumor Infiltrating NK cells



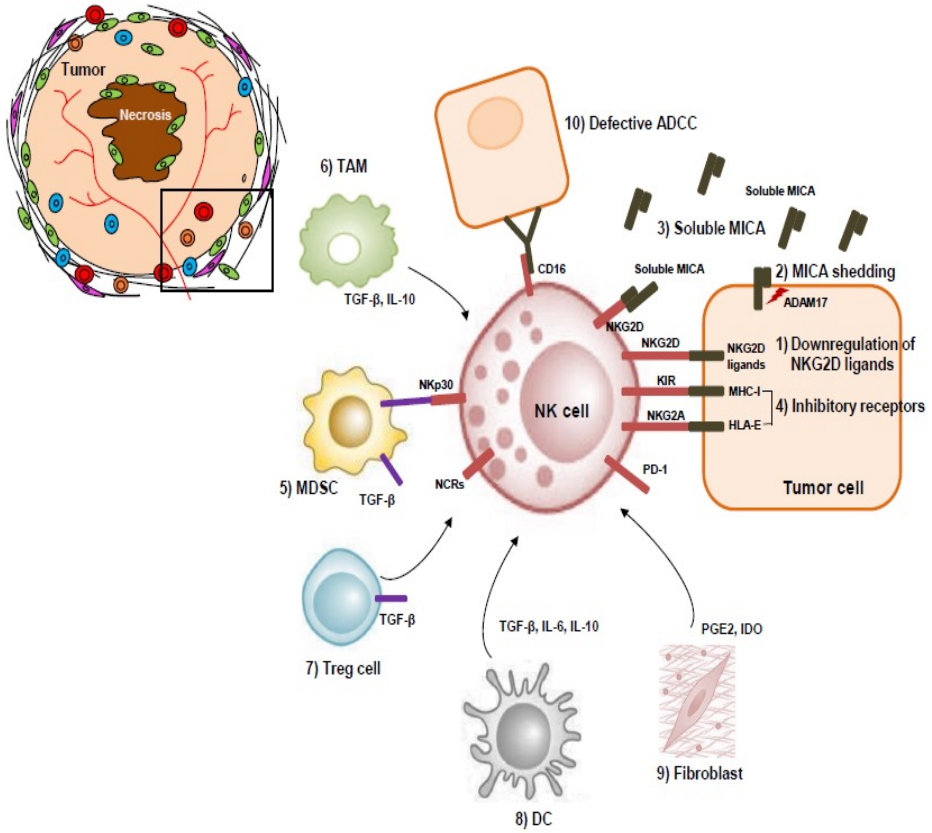
HCC Granzyme A



HCC NK Cytotoxicity

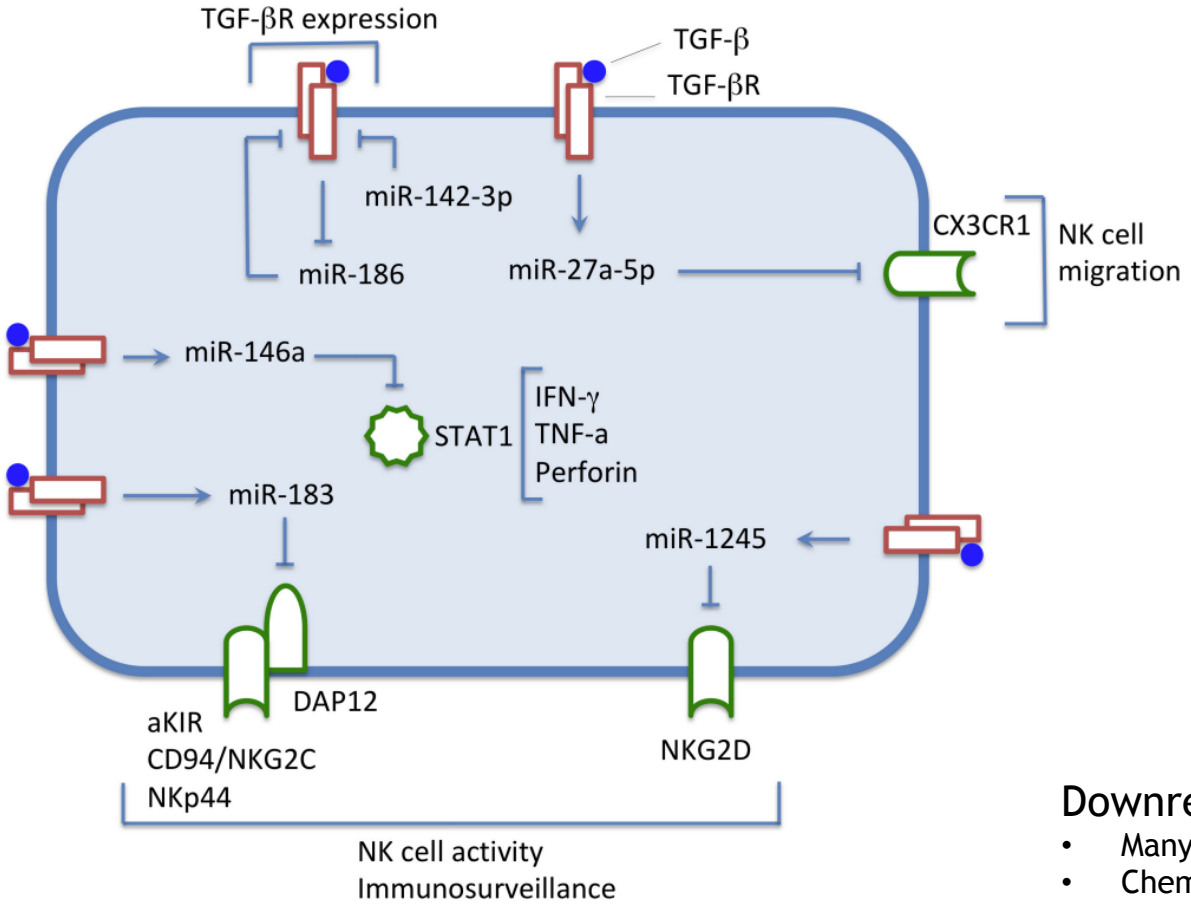


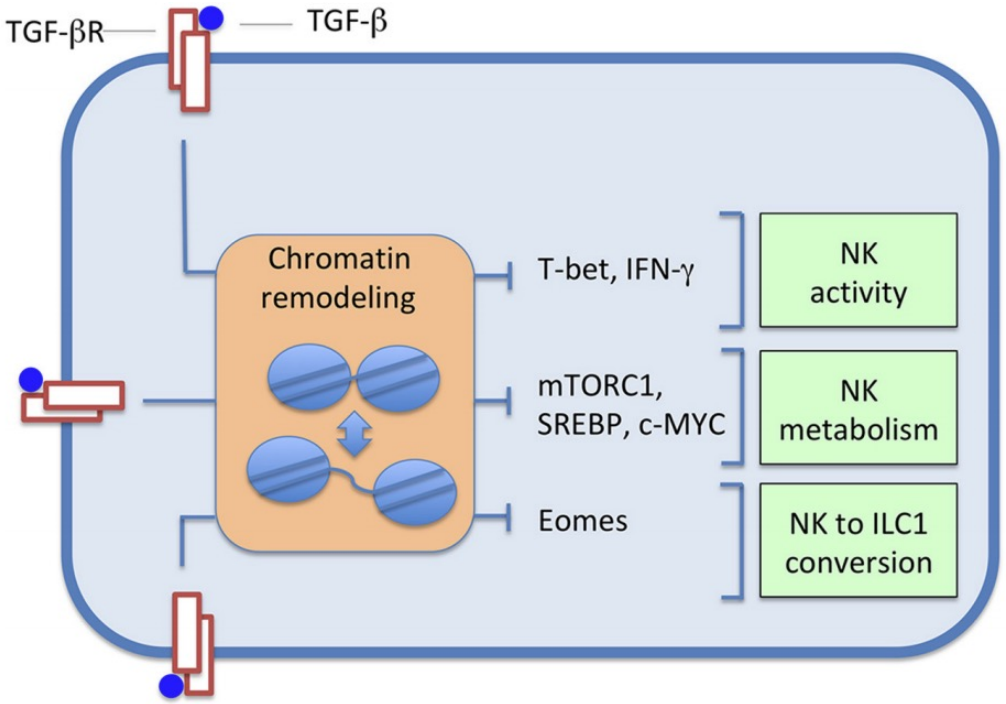
HCC NK cell dysfunction mechanisms



Cai L et al Clin Immunol 2008
Sung P et al Int J Mol Scien 2018

TGF-β Downregulates Various Key Functions of NK Cells in TME





Source: S. Regis, et. al., *Frontiers in Immunology* 2020, 11, 311

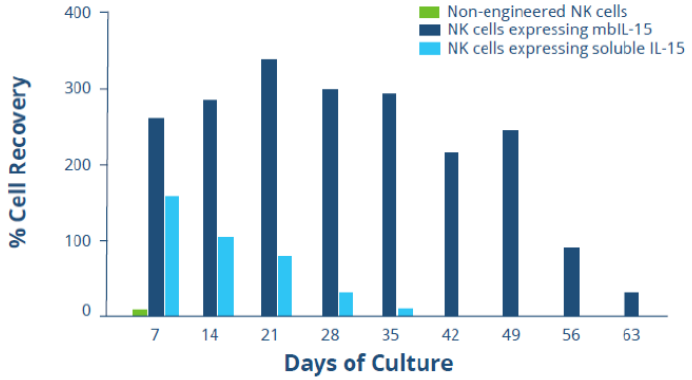
Downregulates:

- Many activating receptors – reduce cytotoxicity
- Chemokine receptors – affect migration
- IFN-r production
- Metabolism

Promotes conversion to less cytolytic ILC1

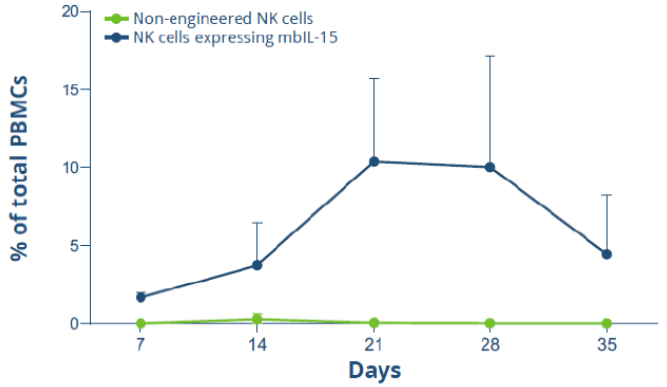
Persistence of NK Cells with IL-15 KI

In vitro persistence

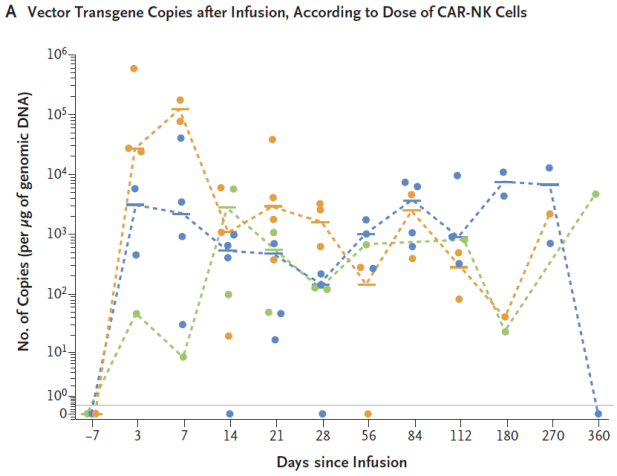


Source: Imamura, Blood 2014

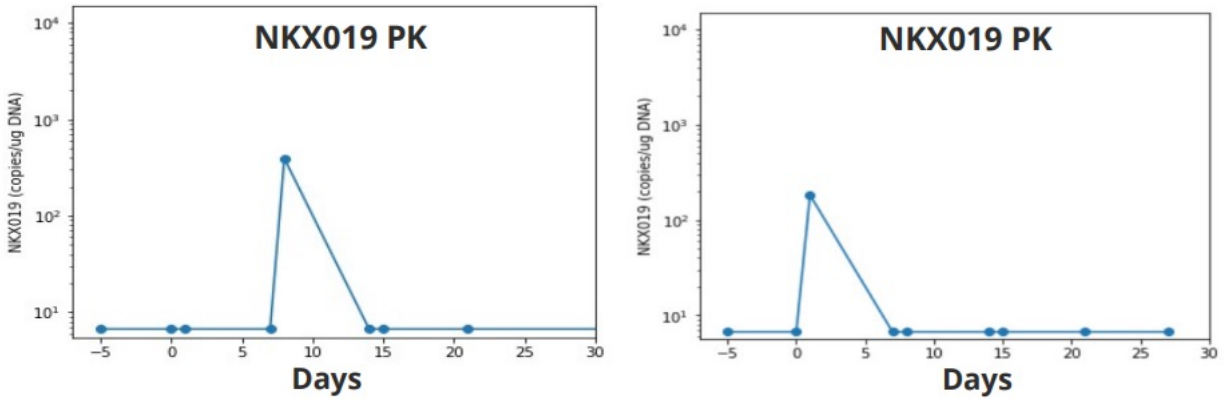
In vivo persistence and expansion in NSG mice



Source: Nkarta. N = 5 per arm.

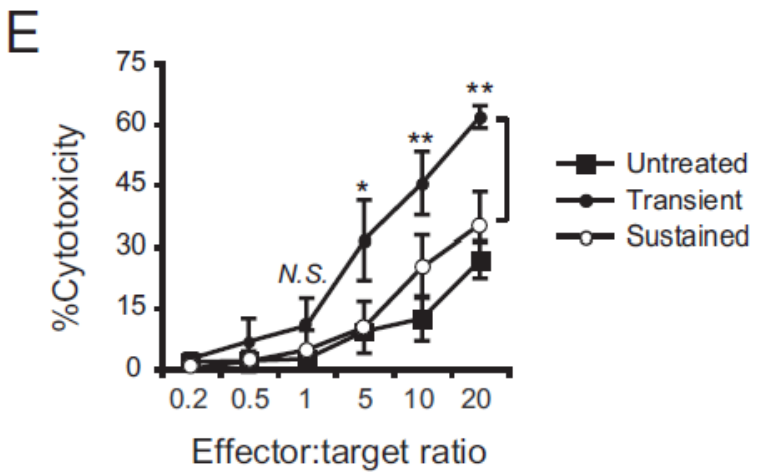


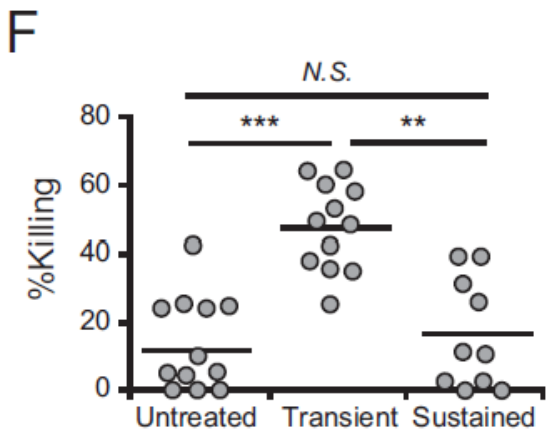
Source: Liu et al, N Engl J Med 2020; 382: 545-53

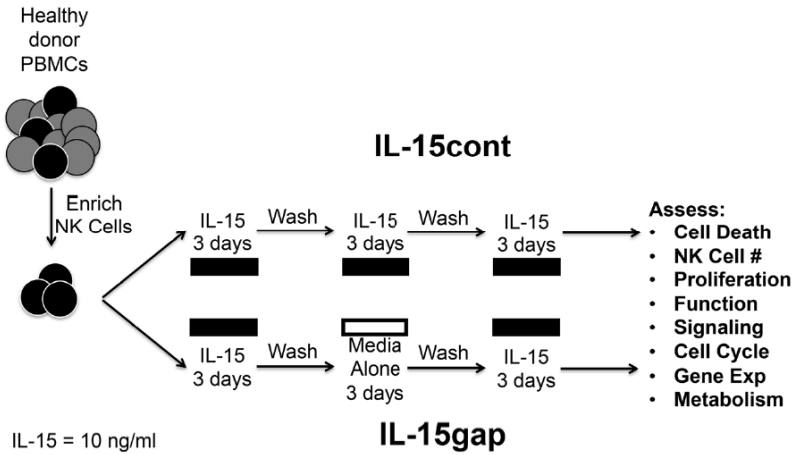


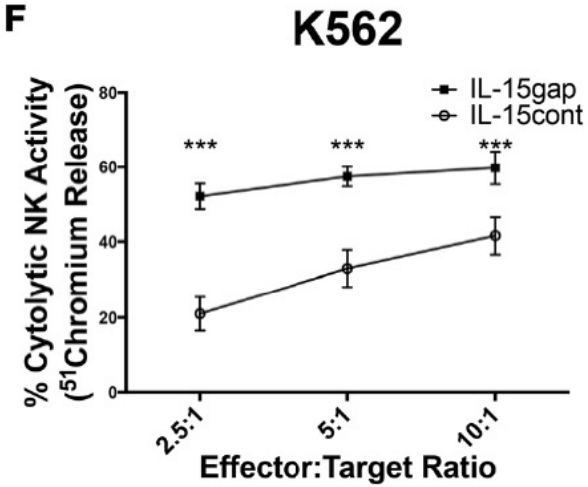
Source: NKarta

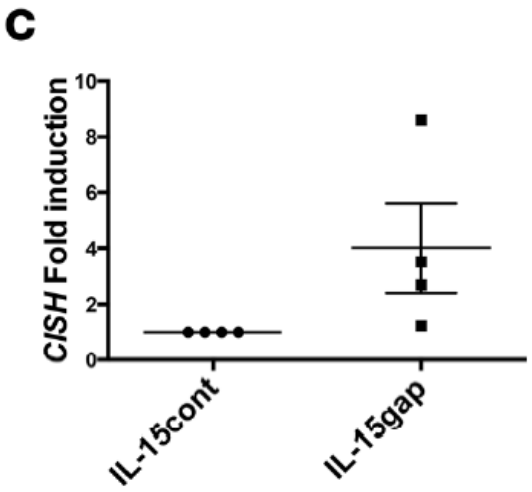
Sustained Stimulation with IL-15/IL-15Ra Complexes Impaired NK Cell Functions







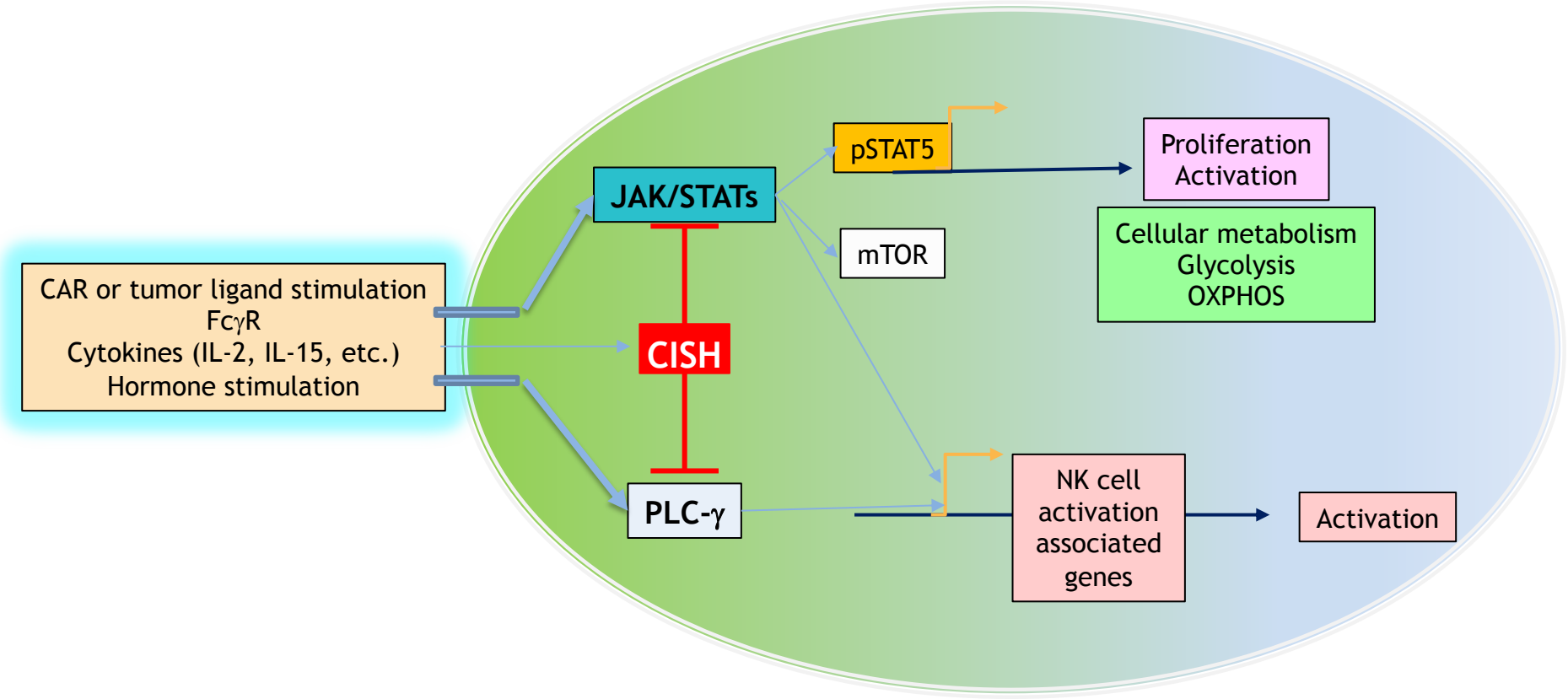




CISH is not the dominant determinant of functional differences between the IL15cont– and IL-15gap–treated NK cells

Elpek, et. al., PNAS, 2010, Vol. 107, No. 50, 21647-21652
Felices, et. al., JCI Insight, 2018; 3(3): e96219

CISH Downregulates NK Cell Functions via Various Pathways

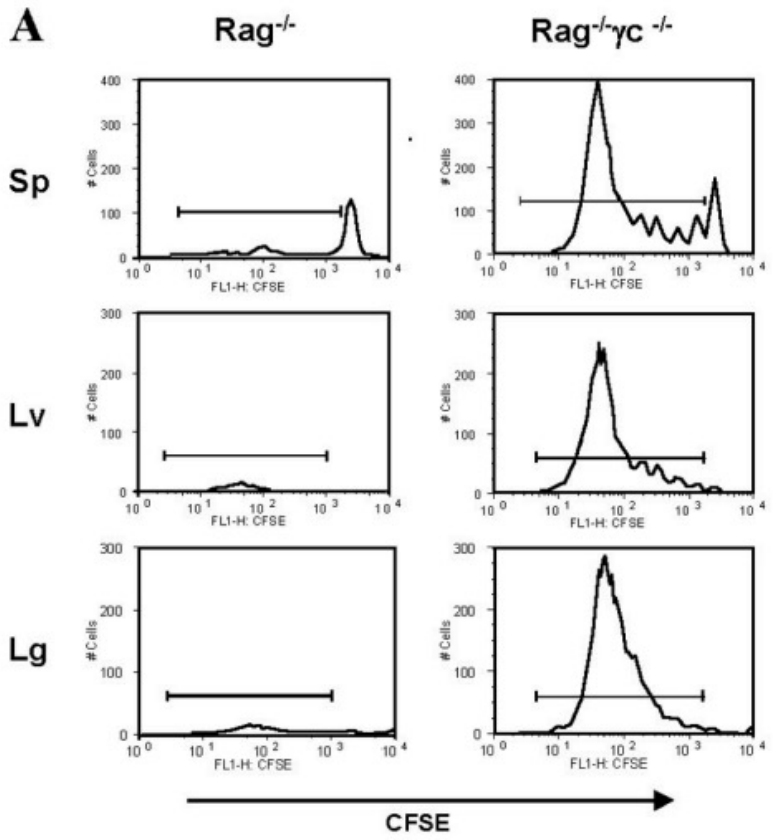


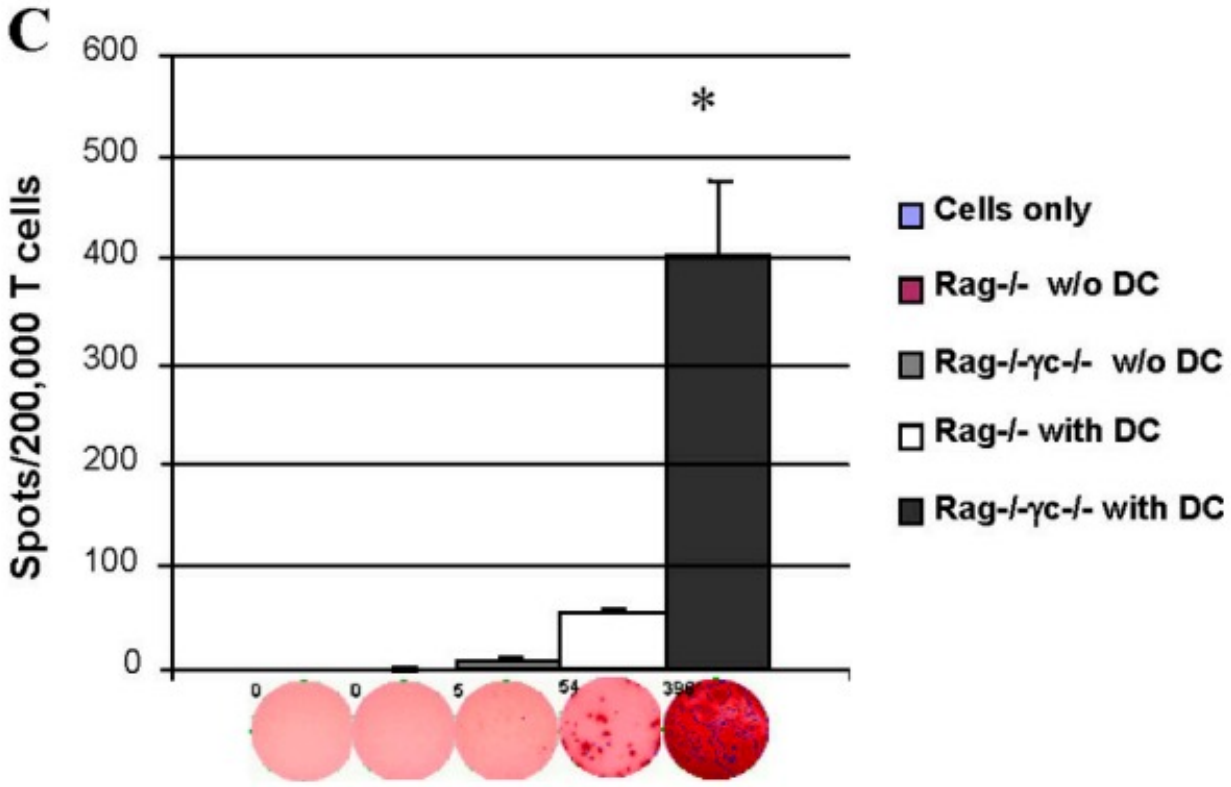
CISH KO:

- Favors IL-15 signaling
- Upregulate cell-cycling and activation pathways
- Decrease the upregulation of TIGIT

Bernard et al, J Immunother Cancer 2022, 10: e004244
Zhu et al, 2020 Cell Stem Cell 27, 224-237
Aman et al, 1999, JBC, Vol 274, No. 42, 30266-30272
Upshaw et al, J Immunol., 2005 Jul 1; 175(1): 213-8
Palmer et al, J. Exp. Med. 2015 Vol. 212 No. 12, 2095-2113

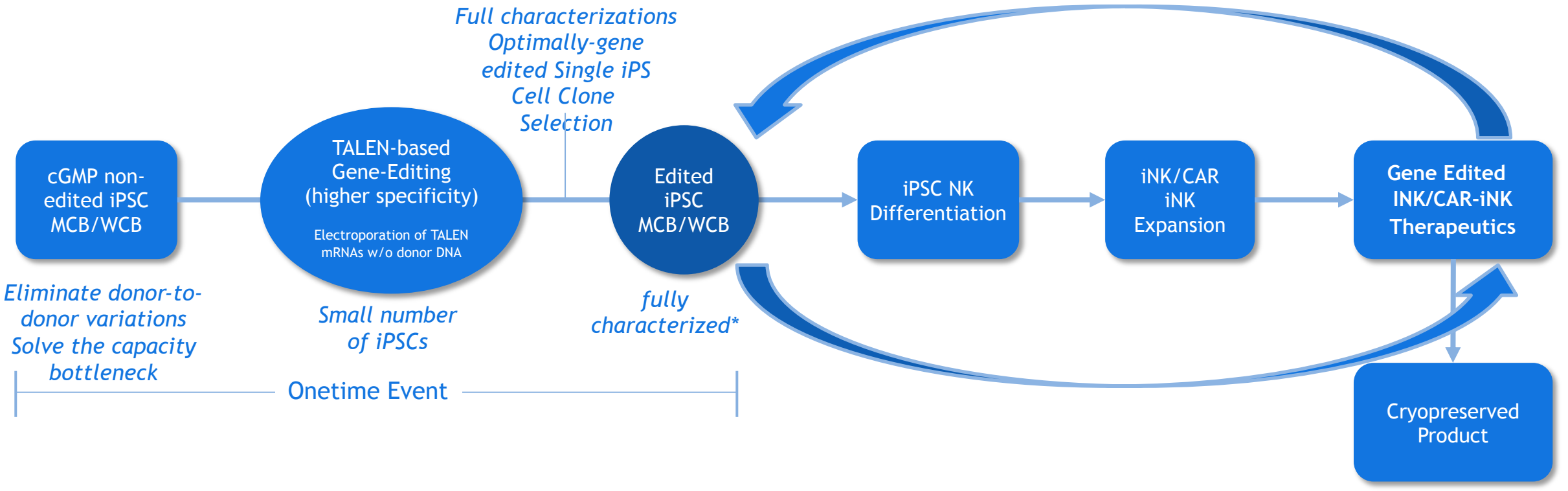
NK Cells Can Inhibit Alloreactive T-cell Activation via Killing of Allogeneic Dendritic Cells





JEM, Vol. 203, No. 8, 2006
J Immunol (2002) 169 (6)
Nature Med. 11, 1059-1065 (2005)
Blood, 2008, Vol. 112, No. 3

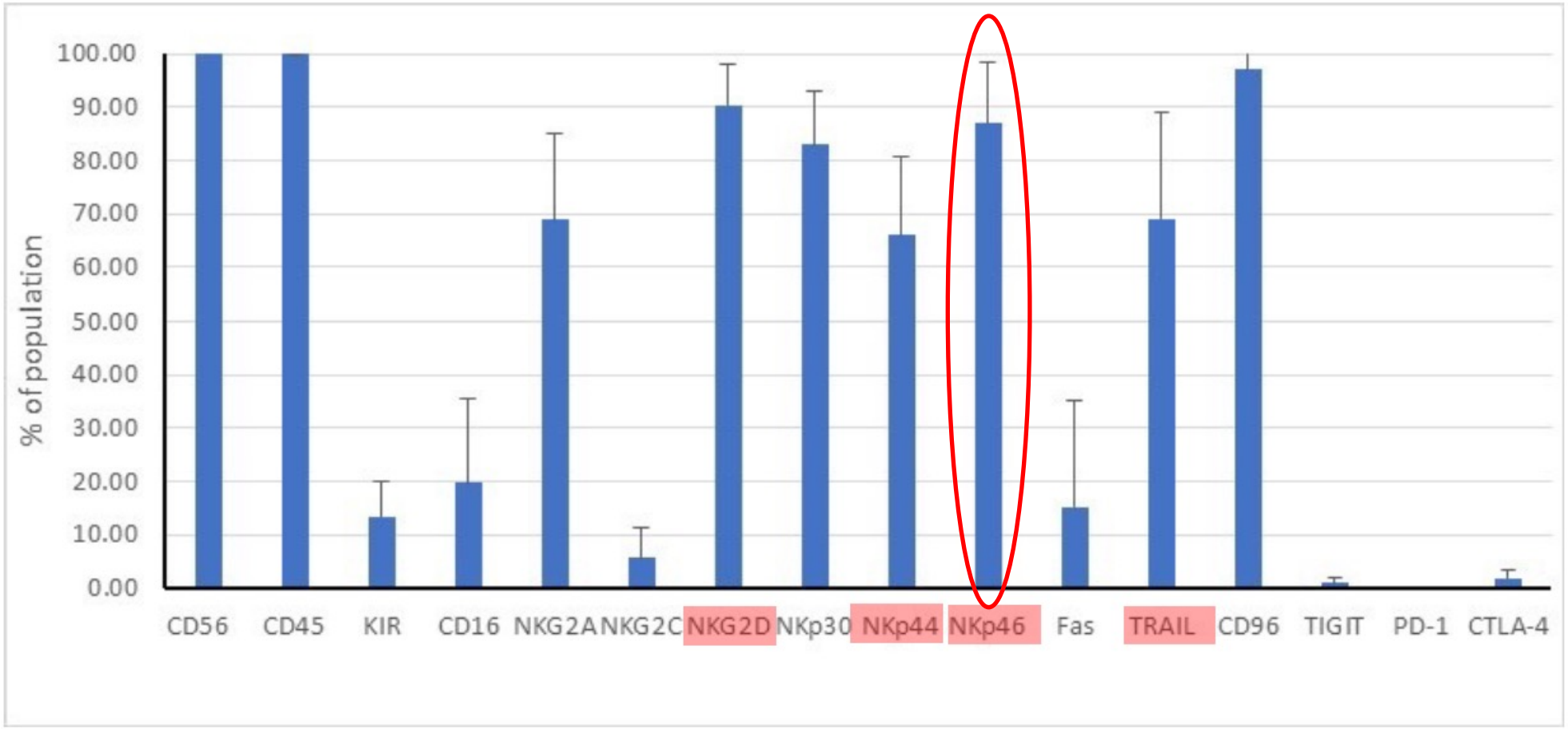
Cytovia has a Fully-Integrated In-House Process Development Capabilities for Gene-edited iNK / CAR-iNK Cell Platform



Amplifies when multiple gene-edits required.

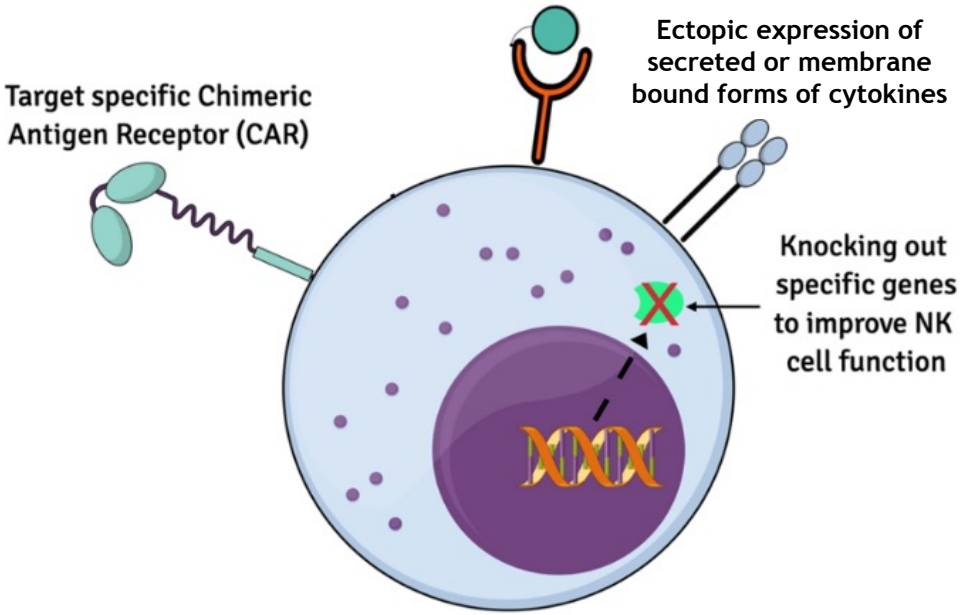
Safer for performing multiplex gene-editing

Phenotype of Non-edited iNK Cells



TALEN® Gene Editing Strategies to Improve the Performance of iNK Cells

Cytovia pursuing Multiple Specific Gene Edits to support a differentiated iNK/CAR iNK pipeline[1,3]



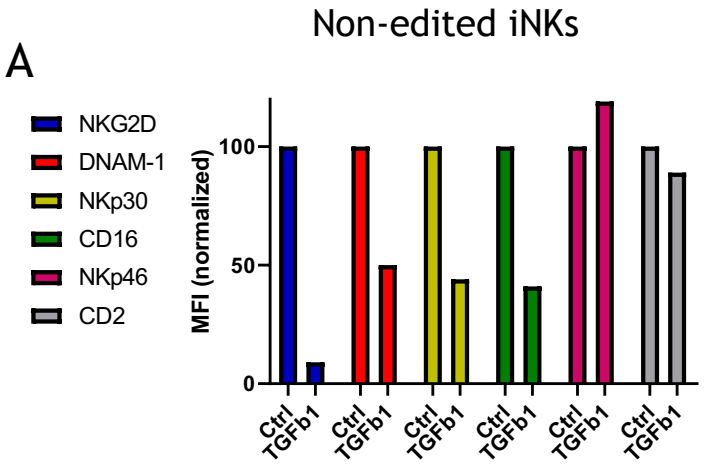
Edit Strategy: Balancing stimulation and exhaustion/senescence

- **IL-15 pathway** (and other cytokines) stimulate NK cell expansion and cytotoxic functions and have also been shown to mitigate immunosuppression
- **TGFß pathway Knock-Out** reduces immunosuppressive signaling
- **NK cell specific CAR** directs cells to the tumor and improves anti-tumor activity
- **CISH Knock-Out** improves NK cell function by reducing negative regulation of IL15 by CISH (pending licensing agreement)
- **CD38 Knock-out** in iNK cells support combination with CD38 FLEX-NK™ Bispecific Antibodies and as backbone of CD38 CAR iNK

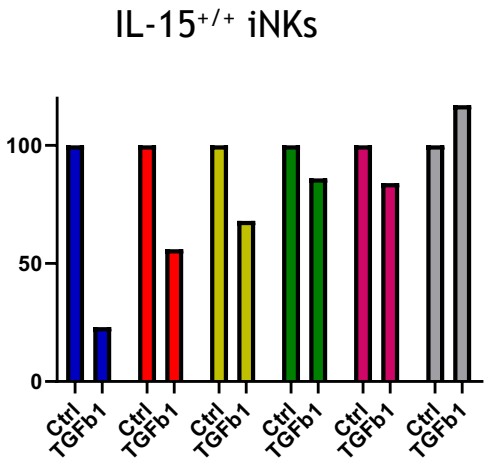
1) Source and Graphic (modified): Front. Immunol., 03 February 2022, Sec. Cancer Immunity and Immunotherapy
https://doi.org/10.3389/fimmu.2022.841107

iNK Cells with TGFβR2 KO are Resistant to TGFβ's Suppressive Regulation

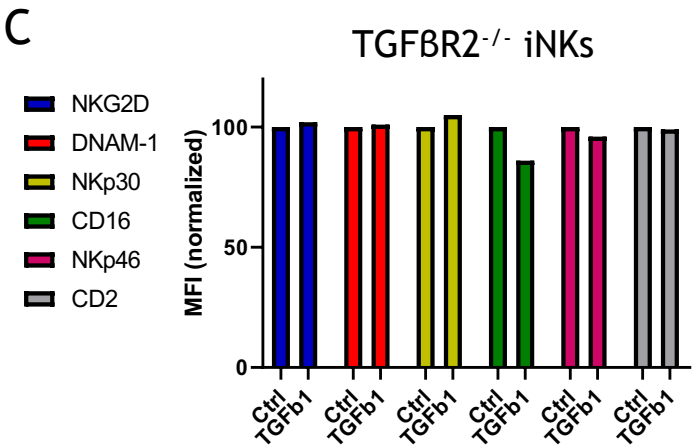
A



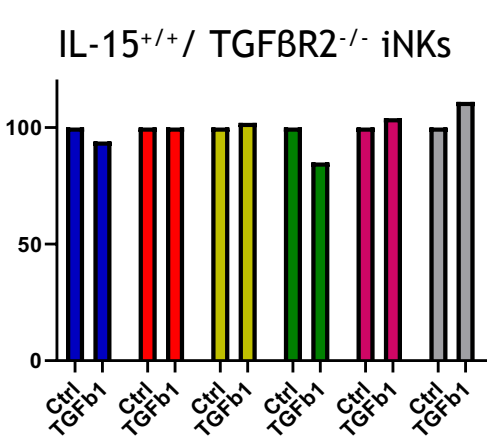
B



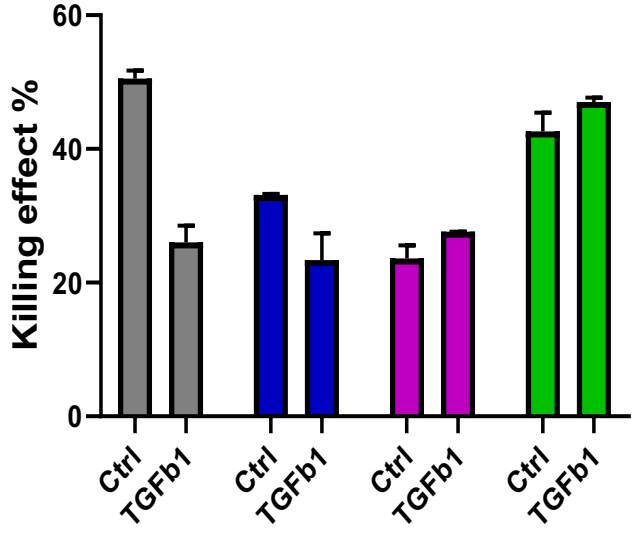
C



D

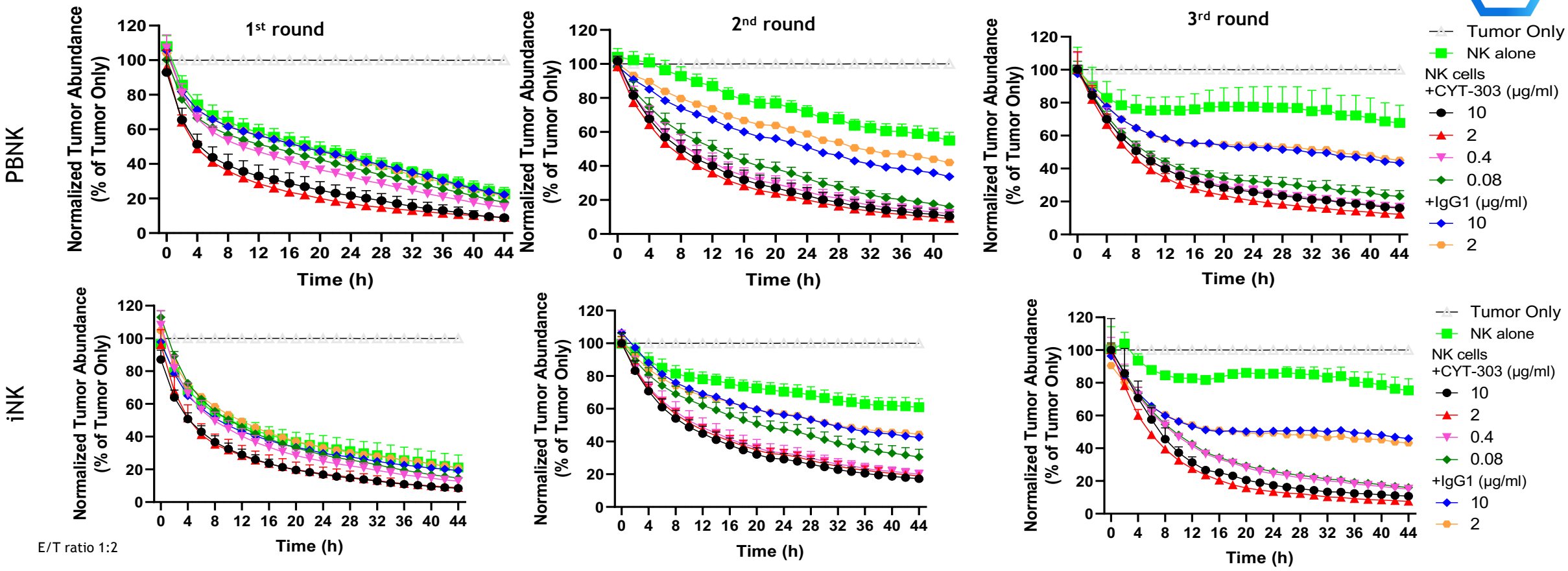


E



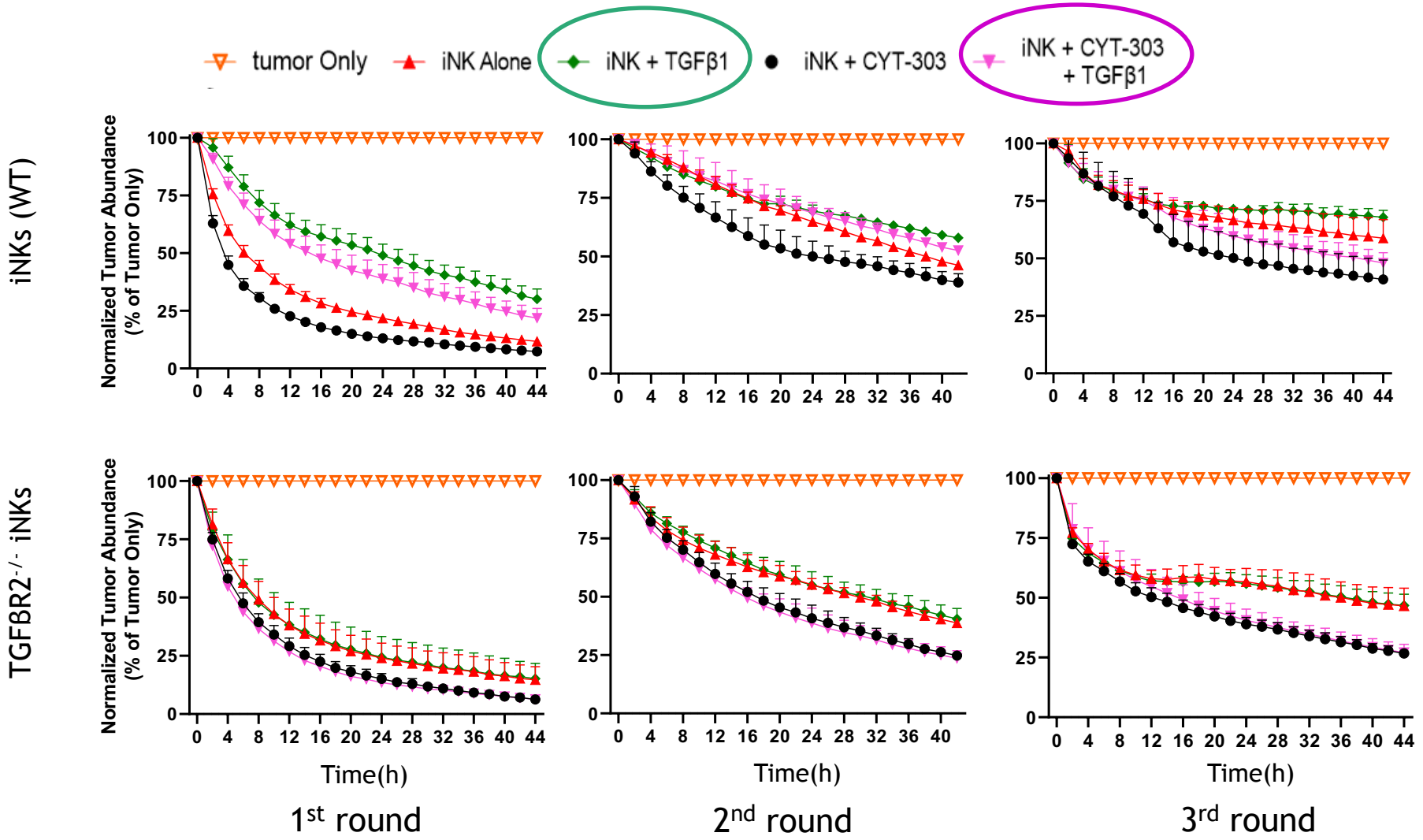
Edited iNK clones	1	2	3	4
IL-15 KI	-	+	-	+
TGFβR2 KO	-	-	+	+

Dysfunction of NK Cells During Serial Killing

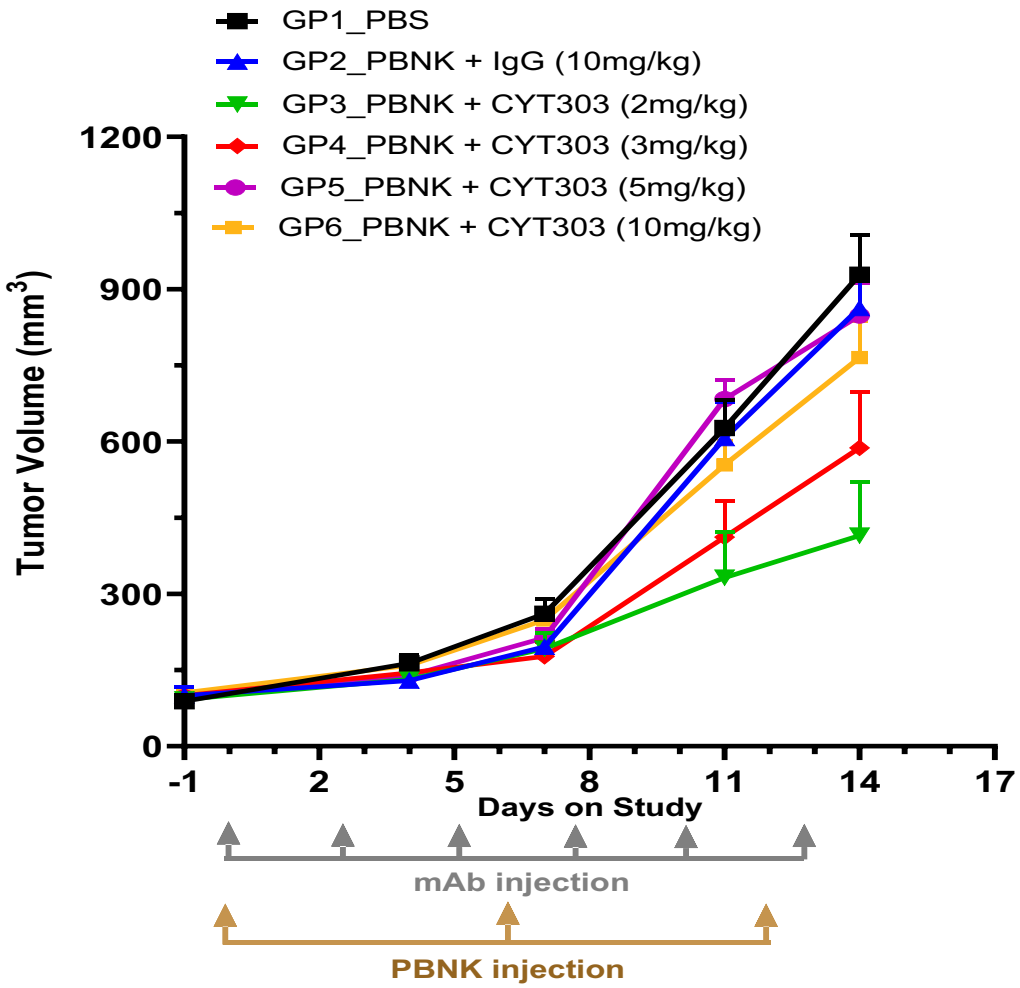


- Both PBNK or iNK cells alone showed gradual reduction / dysfunction of the cells over time in serial killing of Hep3B tumors
- Our NKp46 NK engager reversed dysfunction of PBNKs and iNKs and enhanced serial killing of Hep3B tumors in a dose dependent manner
- Same phenomenon observed for CYT-338

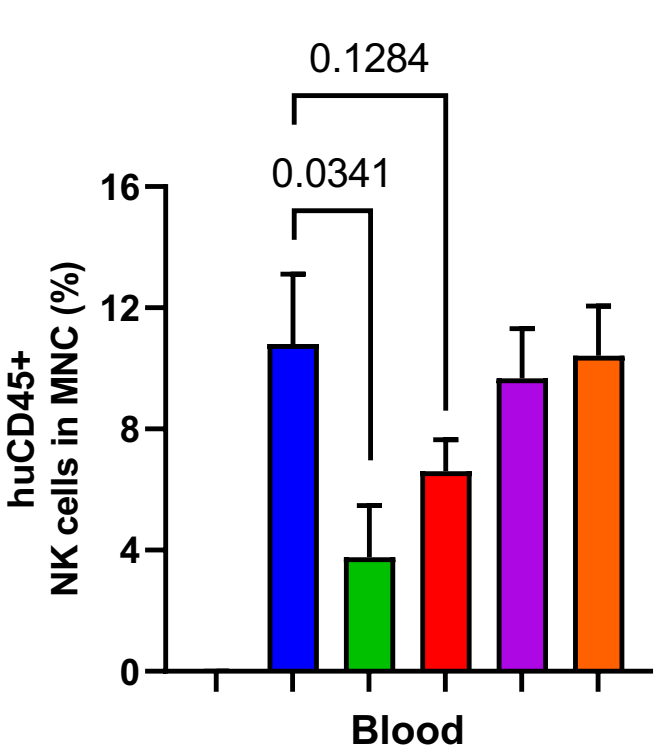
Our NKp46 NK Engagers Enhance Killing & Reverses Dysfunction of NK Cells During Serial Killing Even in the Presence of TGFβ

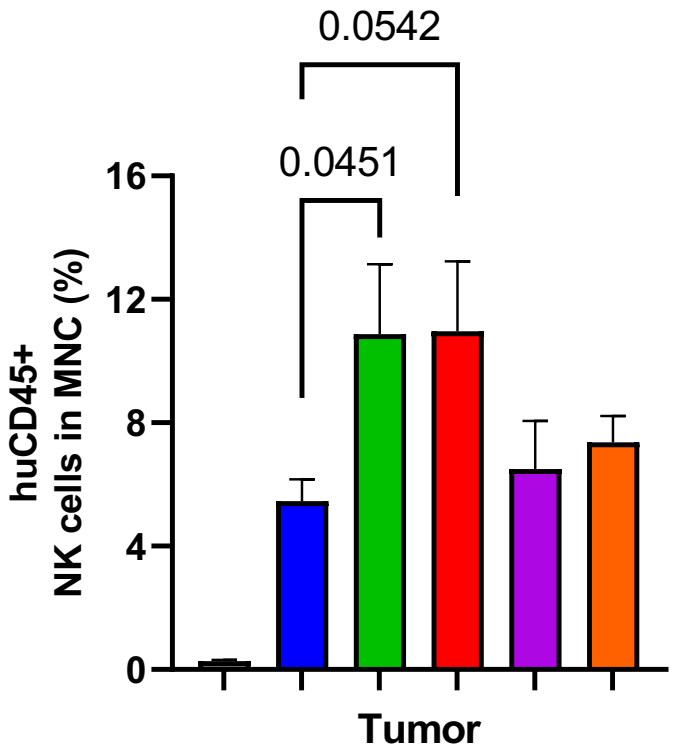


CYT-303 Can Help Recruit PBNK into Tumors



Hep3B HCC mouse model





Summary

Flex-NK™ Bispecific Antibody Platform

- NKp46 – Expresses in TME
- Tetravalent, Flexible linker, Full Fc
- Manufacturability demonstrated

Edited iPSC-Derived NK (iNK) Cell Platform

- Edits strategy: balance stimulation and exhaustion/senescence and address TME
- Cellectis partnership - TALEN® gene-editing
- UCSF partnership to enable optimal integration loci for NK

- **Improve persistence both physically and functionally**
- **Resistant to suppressive tumor microenvironment**
- **Improve tumor infiltration**

Internal R&D Team and Scientific Partnerships to Accelerate Development of Next Generation NK Therapeutics

Cytovia Therapeutics

R&D Facility & Team in Boston Area for Cell Therapy, Antibody Process Development, and Future cGMP Manufacturing

- Cellectis[1] — EDITING LIFE
- The Hebrew University of Jerusalem
- UCSF — University of California San Francisco
- NYSCF[2] — The New York Stem Cell Foundation
- CytoImmune Therapeutics[2]
- NIH National Cancer Institute
- Inserm — Institut national de la santé et de la recherche médicale