gamida cell

Shifting From T to NK Immunotherapy

Aviad Pato, PhD

PEGS 2021
Immunotherapy Safety & Efficacy

Agenda

- **Natural Killer Cells (NKs) And Immunotherapy**
- **NK Genetic Modification**
- **Gamida-Cell NAM-NKs**
- **Gamida-Cell mRNA NK Immunotherapy**

Agenda

- **Natural Killer Cells (NKs) And Immunotherapy**
- **NK Genetic Modification**
- **Gamida-Cell NAM-NKs**
- **Gamida-Cell mRNA NK Immunotherapy**

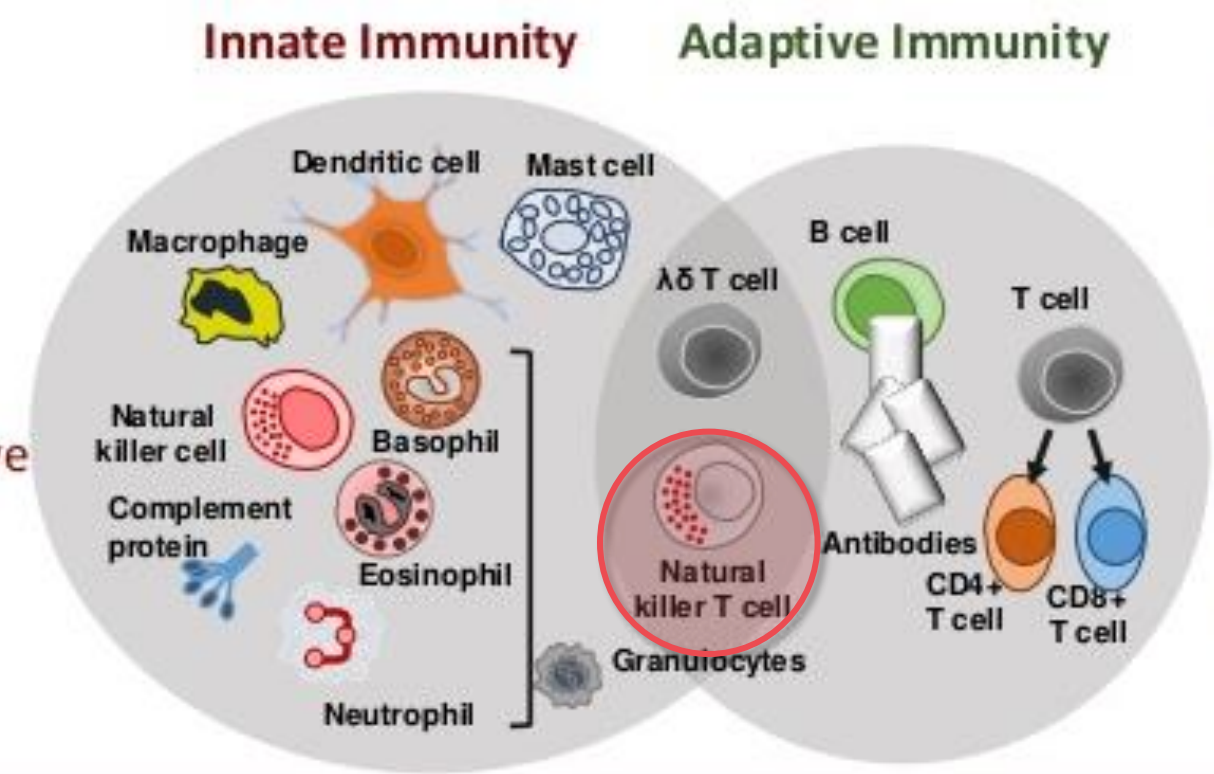
Immune System and Natural Killer Cells

Nonspecific

First line of defense

WBCs (natural killer cells, neutrophils)

Activation of adaptive response



Specific

Adapts specifically to diverse stimuli

B-cell antibody production

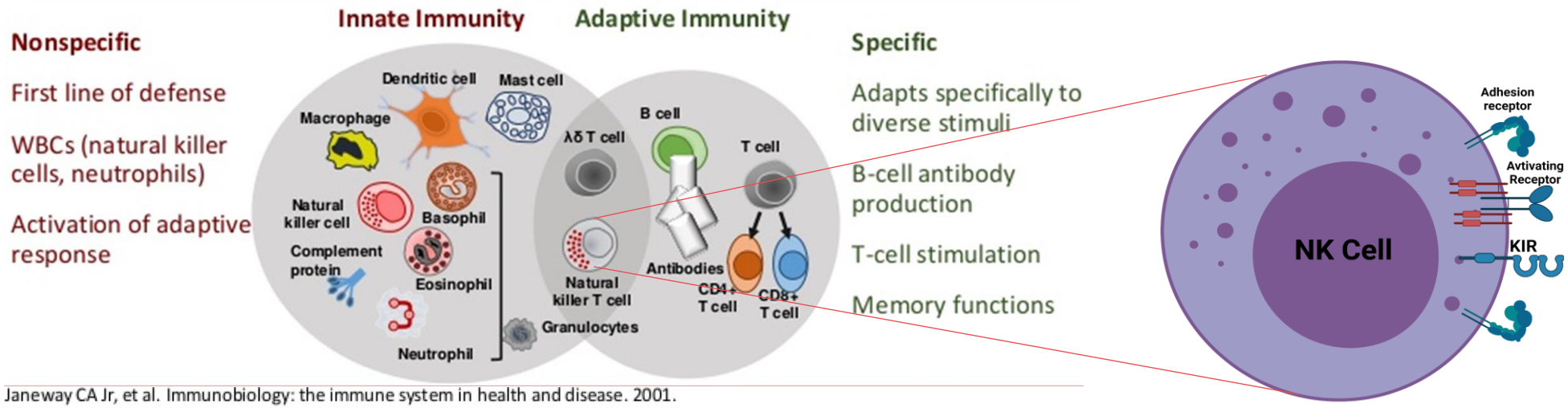
T-cell stimulation

Memory functions

Janeway CA Jr, et al. Immunobiology: the immune system in health and disease. 2001.

Natural Killer Cells - Function and Response

- Lymphocytes in the same family as T and B cells, originating from a common progenitor.
- Rapidly respond to a wide variety of pathological challenges.
- Kill virally infected cells, detect and control early signs of cancer.
- Secrete proinflammatory cytokines to enhance the immune response.
- **Kill tumor cells without priming, prior activation or antigen presentation.**



Janeway CA Jr, et al. Immunobiology: the immune system in health and disease. 2001.

Natural Killer cells - The Next Great Cancer Immunotherapy

Natural killer cells (yellow) attack a cancer cell. EYE OF SCIENCE/SCIENCE SOURCE

Engineered natural killer cells may be the next great cancer immunotherapy

"CAR natural killer (CAR-NK) cells could be safer, faster to produce, and cheaper, and they may work in situations where T cells falter" ***Science***. *Sep 13,2018*

Chimeric Antigen Receptor- T Vs NK

	T Cells	NK Cells
“Off the shelf”	No - Autologous T	Yes - Allogeneic donor NK

Agenda

- Natural Killer Cells (NKs) And Immunotherapy
- **NK Genetic Modification**
- Gamida-Cell NAM-NKs
- Gamida-Cell mRNA NK Immunotherapy

NKs Genetic Modifications

- Different systems - viral and nonviral technologies
- Many viral vector systems have been developed however the transduction efficiency in NKs is less superior than those developed for T cells
- GMP-grade viral vector production for clinical therapy is cost intensive and requires high safety standards
- During the last decade mRNA electroporation has increasingly been used for NK cell genetic modification
- Significantly high expression of CD19-CARs in NK (cell lines and primary) were achieved by using mRNA with minimal effects on cell viability

CAR-NK in Clinical Trials

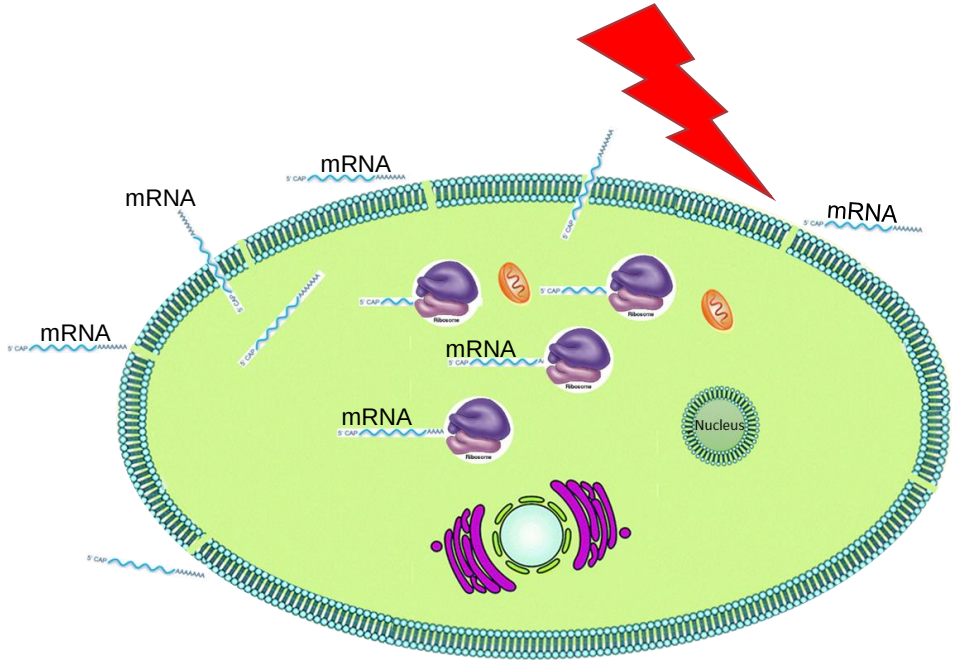
No.	NCT	Start Year	Stage	Tumors	Target	NK source	sponsor locations	CAR structure	Gene transfer
Trials completed									
1	NCT00995137	2009	I	B-ALL	CD19	PB-NK	St. Jude Children's Research Hospital, US	ScFv-CD8αTM-CD137-CD3ζ	mRNA electroporation
2	NCT02944162	2016	I/II	AML	CD33	NK92	PersonGen BioTherapeutics (Suzhou) Co., Ltd., China	ScFv-CD28-CD137-CD3ζ	LV*
Trials actively recruiting									
1	NCT01974479	2013	II	B-ALL	CD19	PB-NK	National University Health System, Singapore	ScFv-CD8αTM-CD137-CD3ζ	mRNA electroporation
2	NCT02742727	2016	I/II	Lymphoma, leukaemia	CD7	NK92	PersonGen BioTherapeutics (Suzhou) Co., Ltd., China	ScFv-CD28-CD137-CD3ζ	electroporation
3	NCT02839954	2016	I/II	Solid tumour	MUC1	NK92	PersonGen BioTherapeutics (Suzhou) Co., Ltd., China	ScFv-CD28-CD137-CD3ζ	LV
4	NCT02892695	2016	I/II	Lymphoma, leukaemia	CD19	NK92	PersonGen BioTherapeutics (Suzhou) Co., Ltd., China	ScFv-CD28-CD137-CD3ζ	LV
5	NCT03056339	2017	I/II	B-lymphoma	CD19	UCB-NK	MD Anderson, US	iCasp9-ScFv-CD28-CD3ζ-IL-15	RV**
6	NCT03383978	2017	I	GBM	HER2	NK92	Johann Wolfgang Goethe University Hospital, Germany	ScFv-CD28-CD3ζ	LV
7	NCT03415100	2018	I	Metastatic solid tumour	NKG2DL	PB-NK	The Third Affiliated Hospital of Guangzhou Medical University, China	ScFv-CD8αTM-CD3ζ; ScFv-CD8αTM-DAP12	mRNA electroporation
8	NCT03656705	2018	I	NSCLC	NR	NK92	Xinxiang medical university, China	NR	RV/LV
9	NCT03940833	2019	I/II	R/R multiple myeloma	BCMA	NK92	Asclepius Technology Company Group (Suzhou) Co., Ltd., China	NR	LV
10	NCT03941457	2019	I/II	Pancreatic Cancer	ROBO1	NK92	Asclepius Technology Company Group (Suzhou) Co., Ltd., China	NR	LV
11	NCT03940820	2019	I/II	Solid tumour	ROBO1	NK92	Asclepius Technology Company Group (Suzhou) Co., Ltd., China	NR	LV
12	NCT04245722	2020	I	B-cell lymphoma, CLL	CD19	iPSC (FT596)	Fate Therapeutics, San Diego, USA	scFv-NKG2D-2B4-CD3ζ-IL-15/R-hnCD16	LV

G. Xie et al. / EBioMedicine 59 (2020) 102975

All clinical trials using peripheral blood (PB) NKs to express CARs were generated using mRNA electroporation

mRNA Electroporation

- No transgene integration into the genome
- Homogenous protein expression
- High transfection efficiency
- Rapid expression of protein
- Transiency of the expression (+/-)
- Absence of an influence on cell viability
- Ability to introduce several proteins at the same time
- Scalability of the method
- GMP compatibility



This method creates a highly reproducible and easy-to-validate product that can be used for cellular immunotherapies

Agenda

- Natural Killer Cells (NKs) And Immunotherapy
- NK Genetic Modification
- **Gamida-Cell NAM-NKs**
- Gamida-Cell mRNA NK Immunotherapy

Gamida-Cell 'Off-The-Shelf' Peripheral-Blood NKs

- Peripheral blood NKs are highly functional and cytotoxic; however, they are in their last lineage stage and display elevated markers of exhaustion
- Nicotinamide (NAM), a vitamin B3 derivative, is the key component in culturing Gamida-Cell NKs
- NAM rejuvenates NKs during expansion and cryopreservation
- Clinical responses have been observed in a phase 1 trial of GDA-201 (Gamida-Cell NAM-NKs) in patients with refractory non-Hodgkin lymphoma (Bachanova, et. al., Blood 134:777, 2019)

Gamida-Cell 'Off-The-Shelf' NAM-NK Manufacture

Allogenic NK cells collected by apheresis

HEALTHY DONOR

Seed $CD3^-$ cells from apheresis material

DAY 0

Proprietary expansion with NAM +IL-15 + autologous irradiated $CD3^+$ feeder cells

Highly functional NK cells: ~50-100 billion NK cells with purity >99%

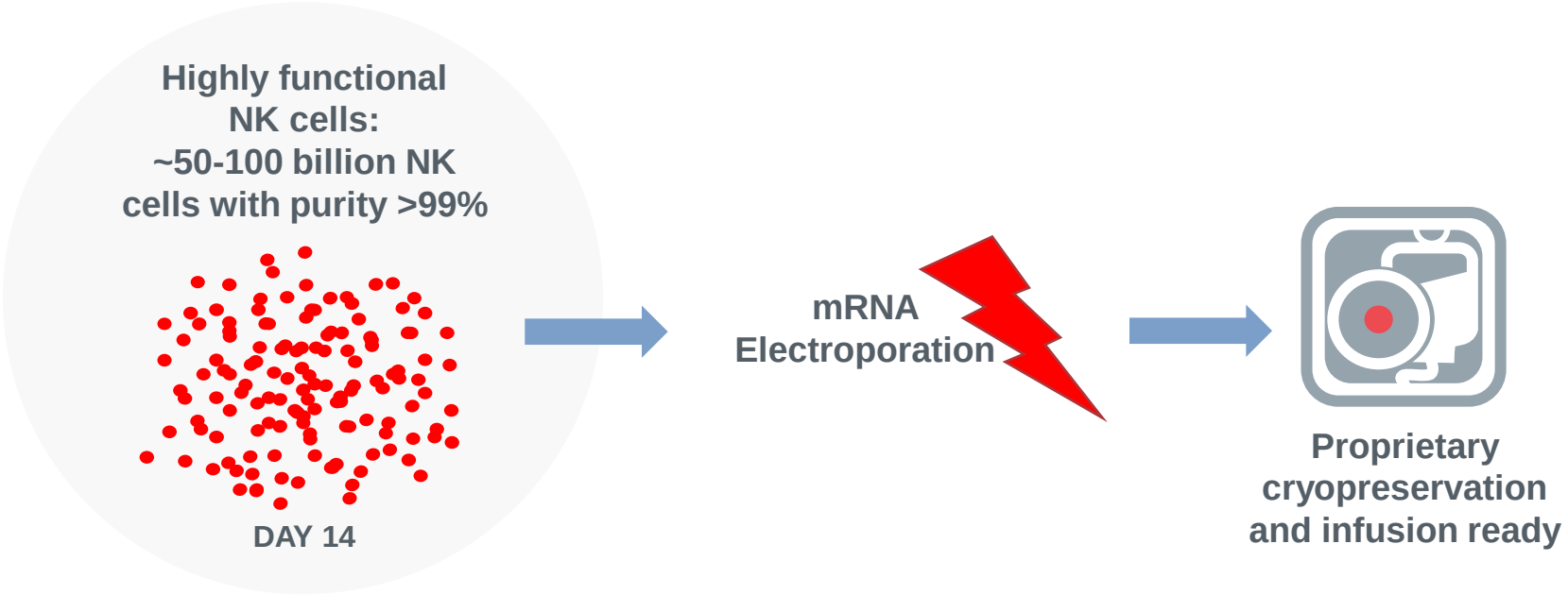
DAY 14

GDA-201

Proprietary cryopreservation and infusion ready

NAM rejuvenates NKs during expansion and cryopreservation

‘Off-The-Shelf’ mRNA-Manipulated NAM-NKs



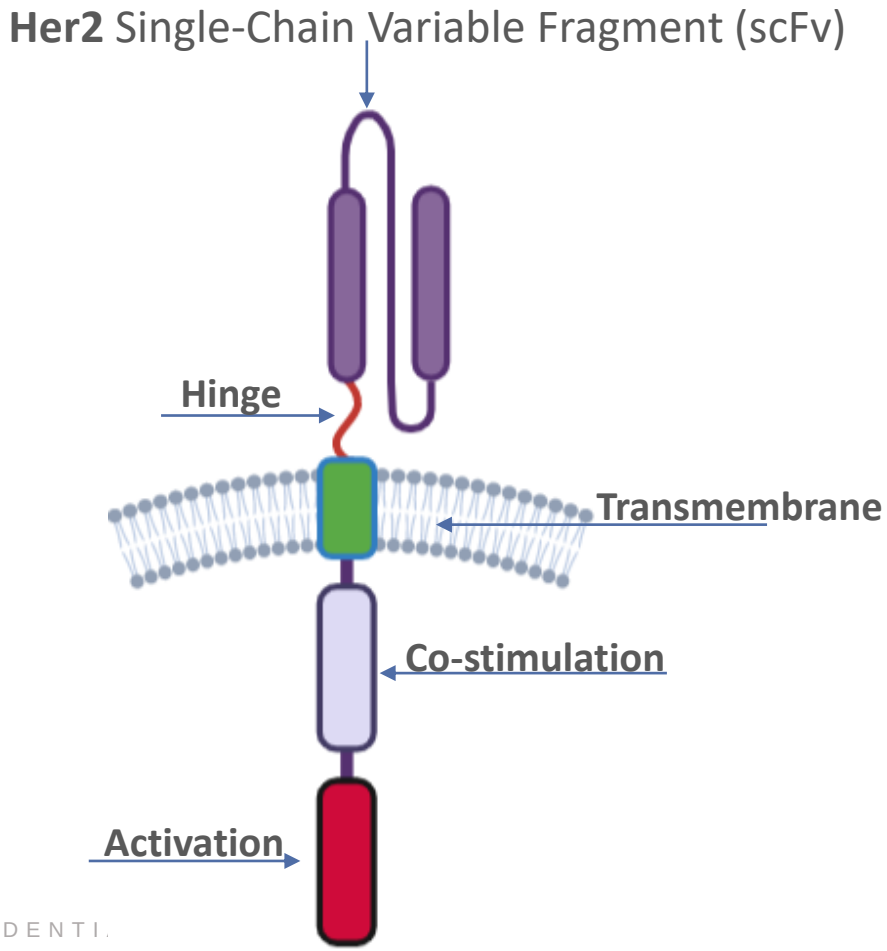
mRNA NAM-NK electroporated at harvest

Agenda

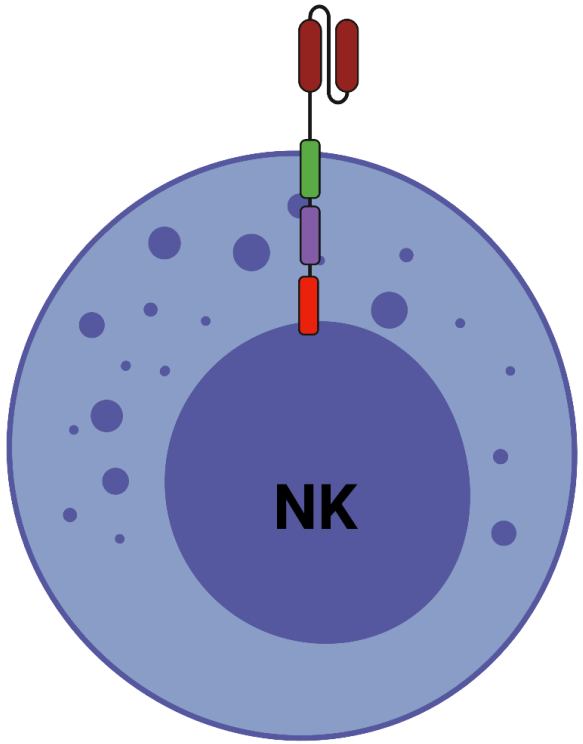
- Natural Killer Cells (NKs) And Immunotherapy
- NK Genetic Modification
- Gamida-Cell NAM-NKs
- **Gamida-Cell mRNA NK Immunotherapy**

Gamida-Cell Chimeric Antigen Receptor mRNA Constructs

Gamida-cell developed multiple CAR-NKs to target and activate NKs against Her2^{+} tumors

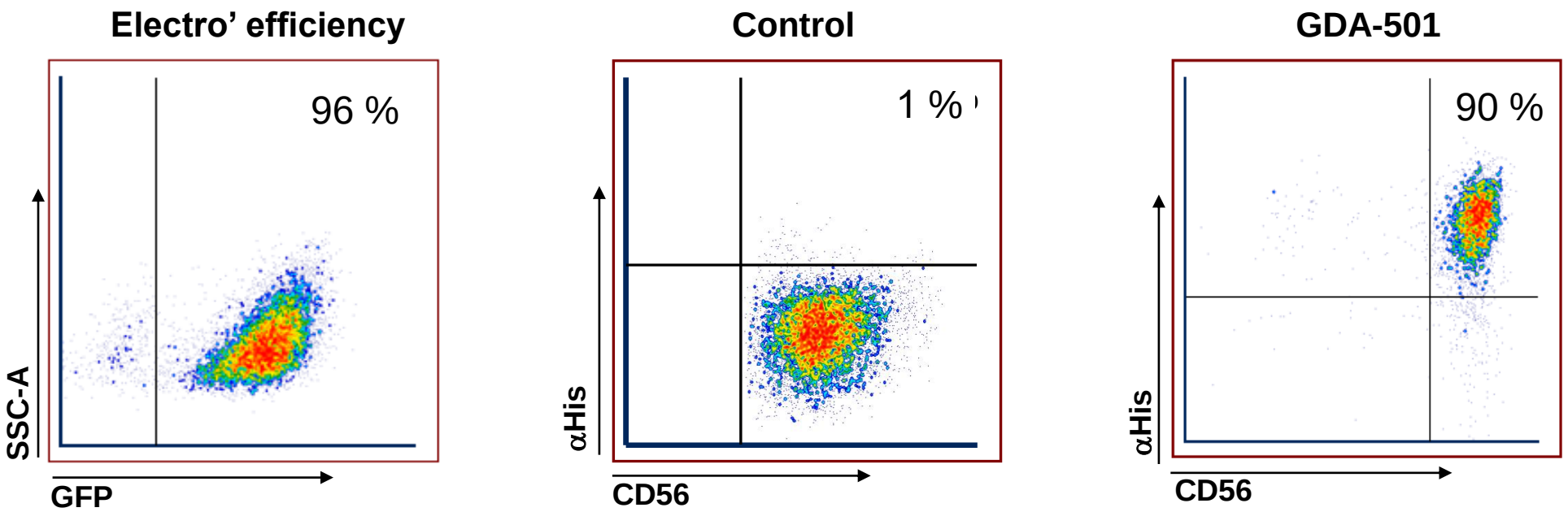


GDA-501: αHer2 CAR NK

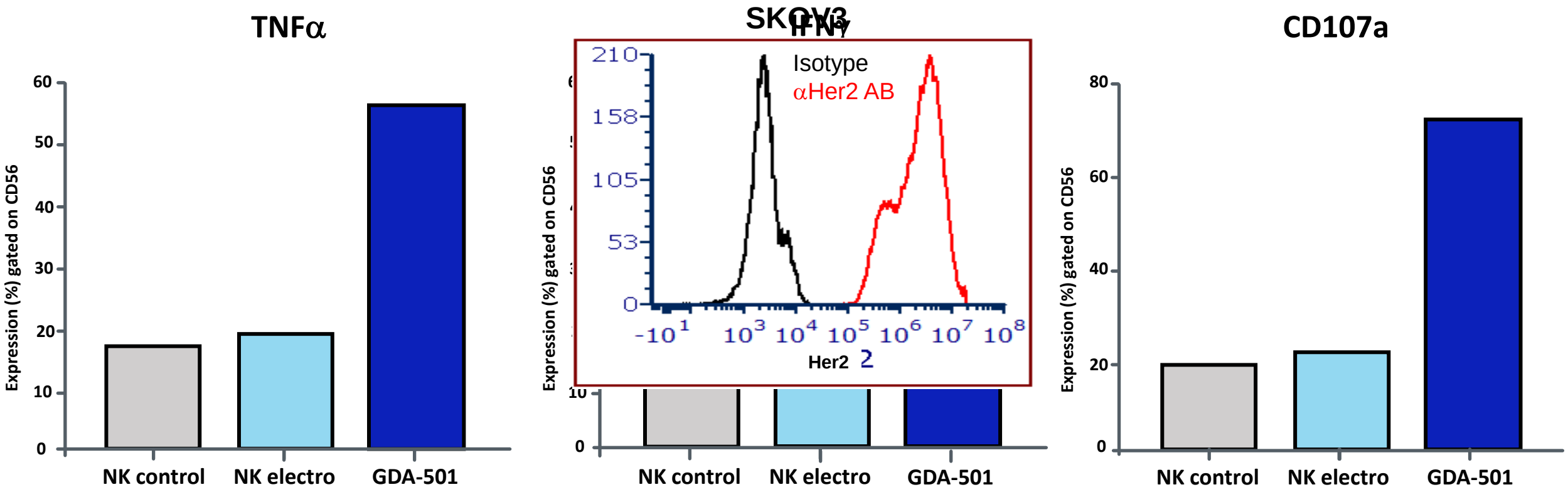


GDA-501: NAM-NK αHer2 mRNA CAR Construct

The CAR construct is expressed by NK cells and recognizes the Her2 protein

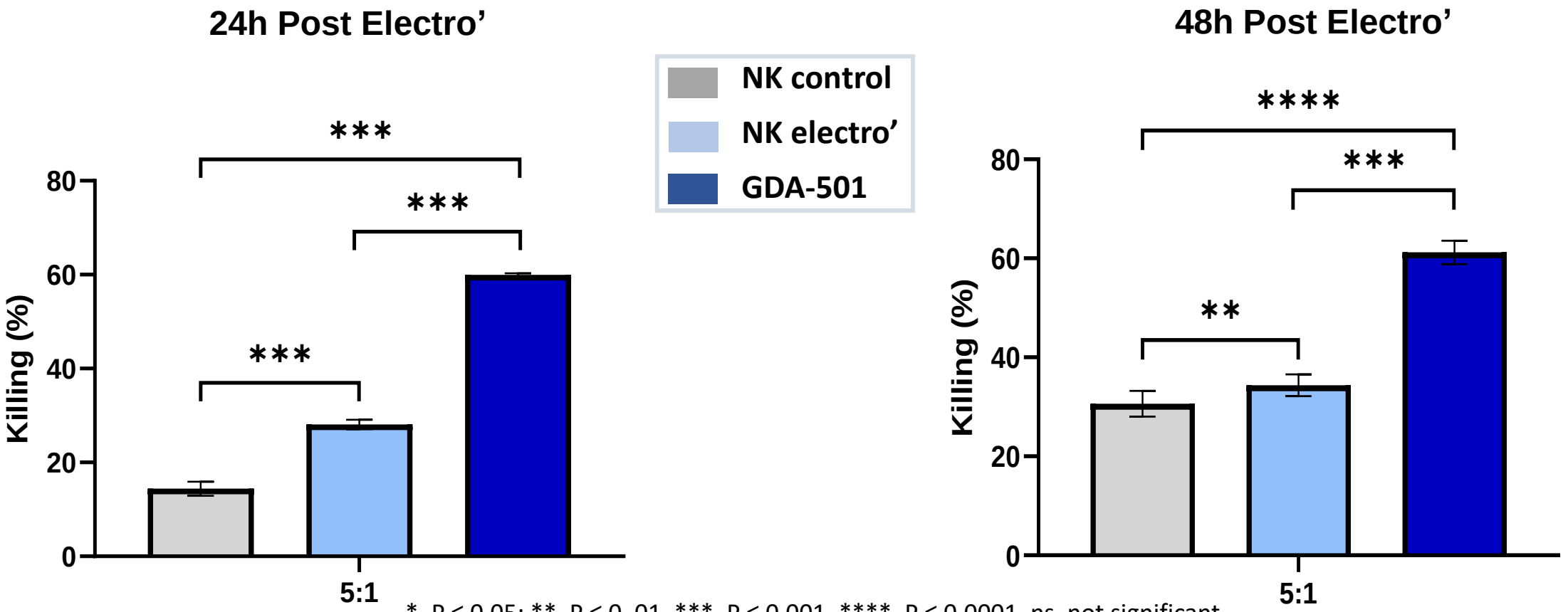


GDA-501 Enhances Activity Against a Her2 Tumor Expressing Cell Line



αHer2 CAR-NKs upregulate the degranulation marker CD107a and expression of inflammatory cytokines when cultured with SKOV3

GDA-501 Increases Killing Against a Her2 Tumor Expressing Cell Line



*, P ≤ 0.05; **, P ≤ 0. 01. ***, P ≤ 0.001. ****, P ≤ 0.0001. ns, not significant

Killing assay - 24h and 48h after the electroporation followed by a 6hr co-culture of NK cells with SKOV3 cell line

Combined Genetic Engineering
CISH Knockout & mRNA Membrane-bound IL-15 Expression

CISH

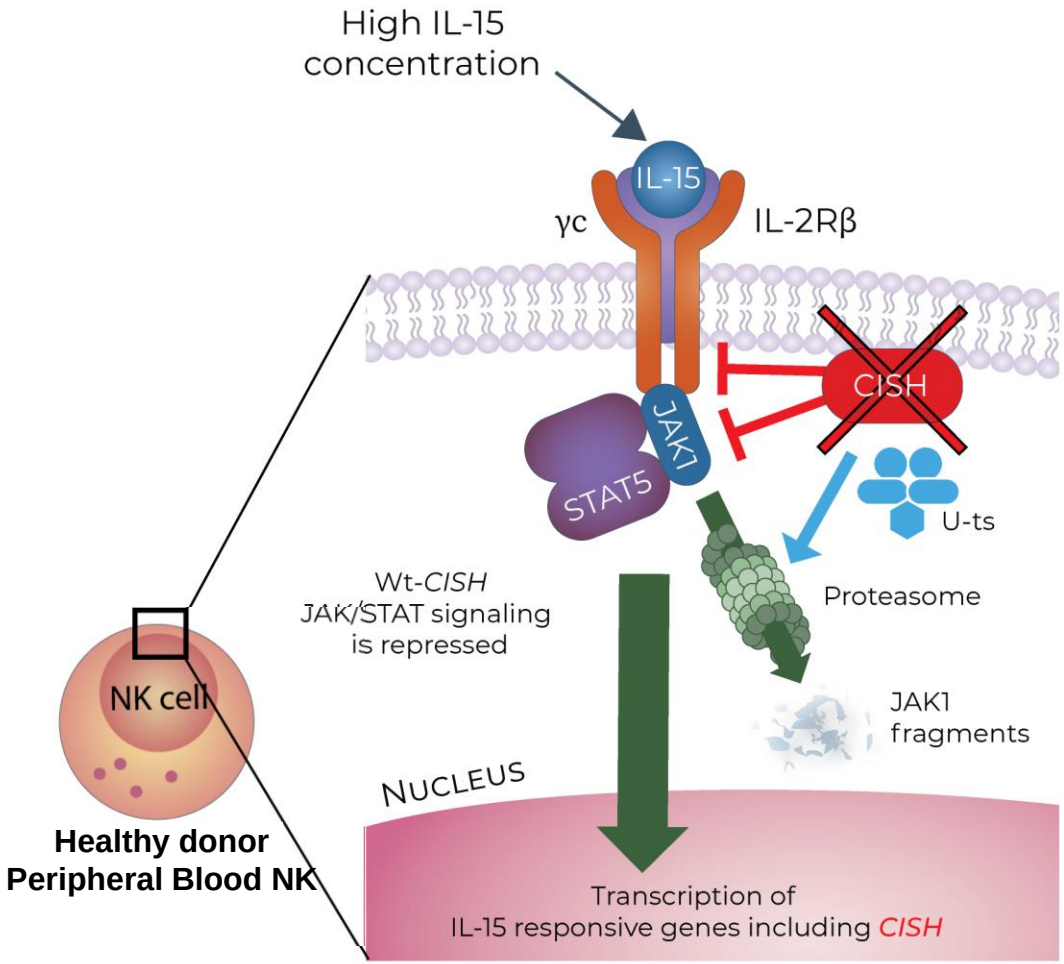
- *CISH* is a key suppressor of IL-15 signaling which acts as negative feedback loop
- *CISH* deletion increases sensitivity to IL-15 by lowering the NK activation threshold

IL-15

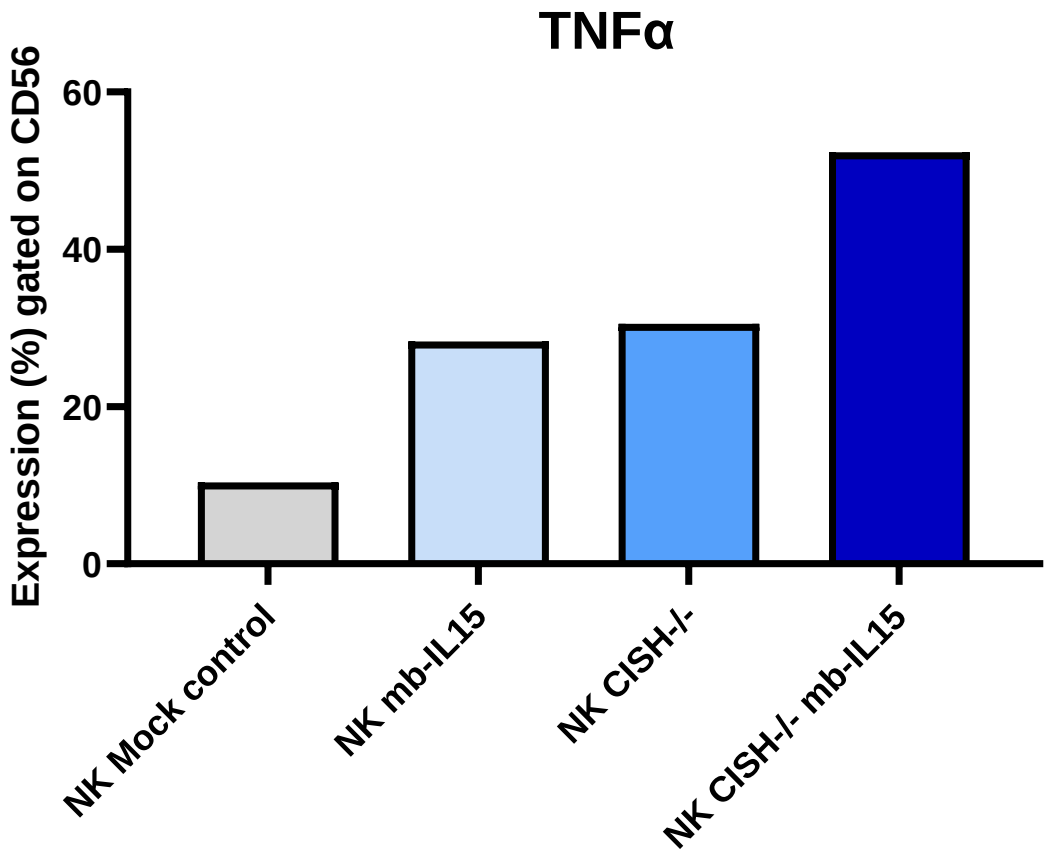
- The lack of persistence of infused NK cells is a principal limitation of adoptive immunotherapy
- The most important cytokine for NK activation, persistence, and proliferation
- NKs equipped with membrane-bound IL-15 (mbIL-15) will be fully autonomous and will obviate the need for patient IL-2 administration regimen

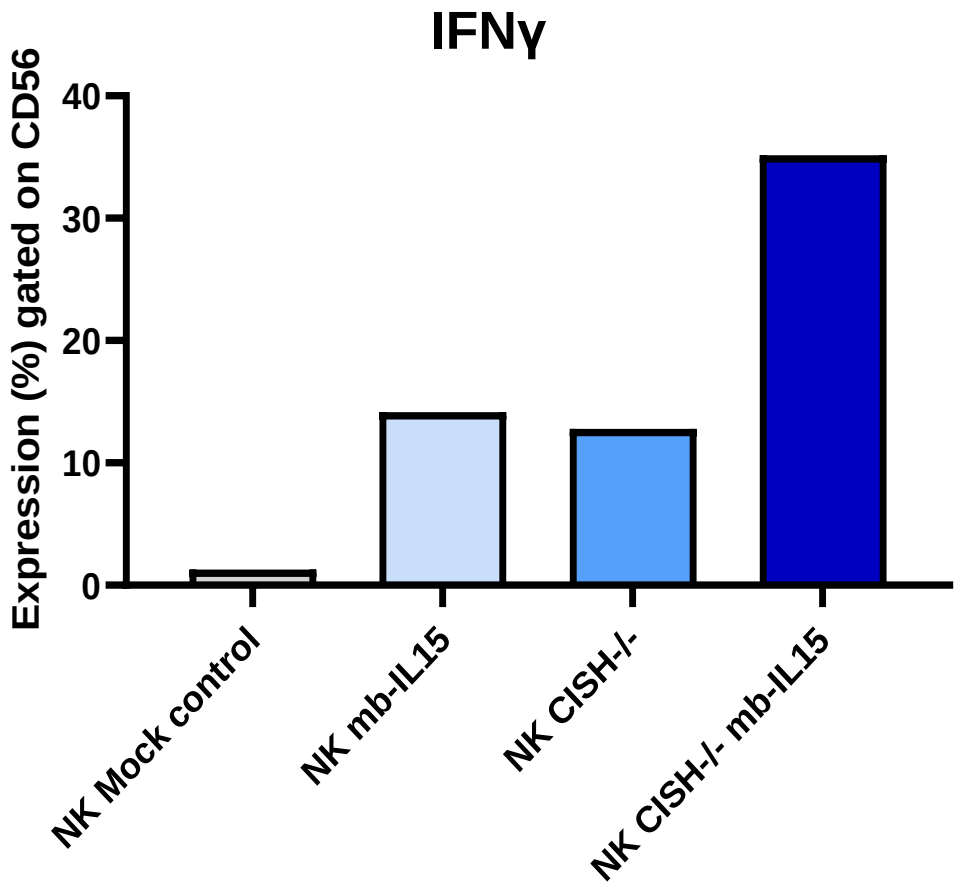
Combining *CISH* KO with mbIL-15 mRNA can improve NK activation, persistence and killing capacity

GDA-301: CISH KO & MbIL-15
Combined Genetic Approach



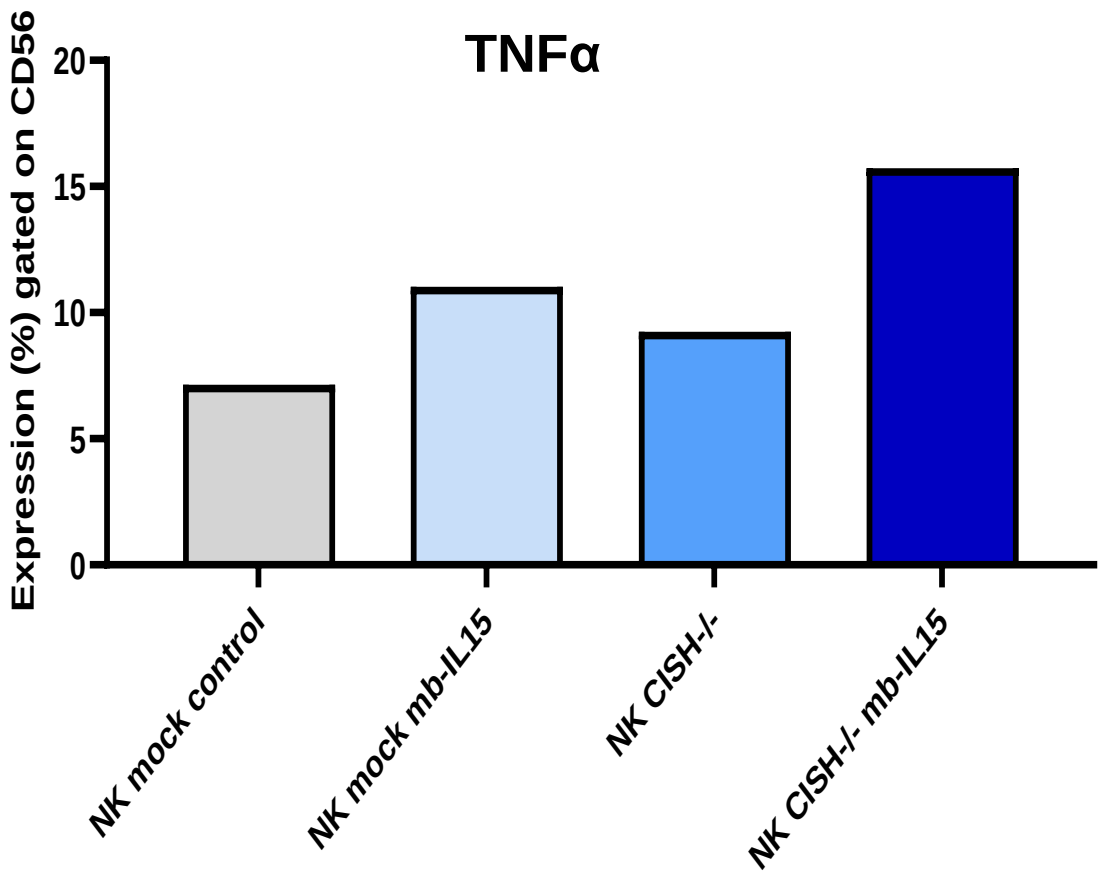
GDA-301 Enhances Activity Against a Chronic Myelogenous Leukemia Cell line (K562)

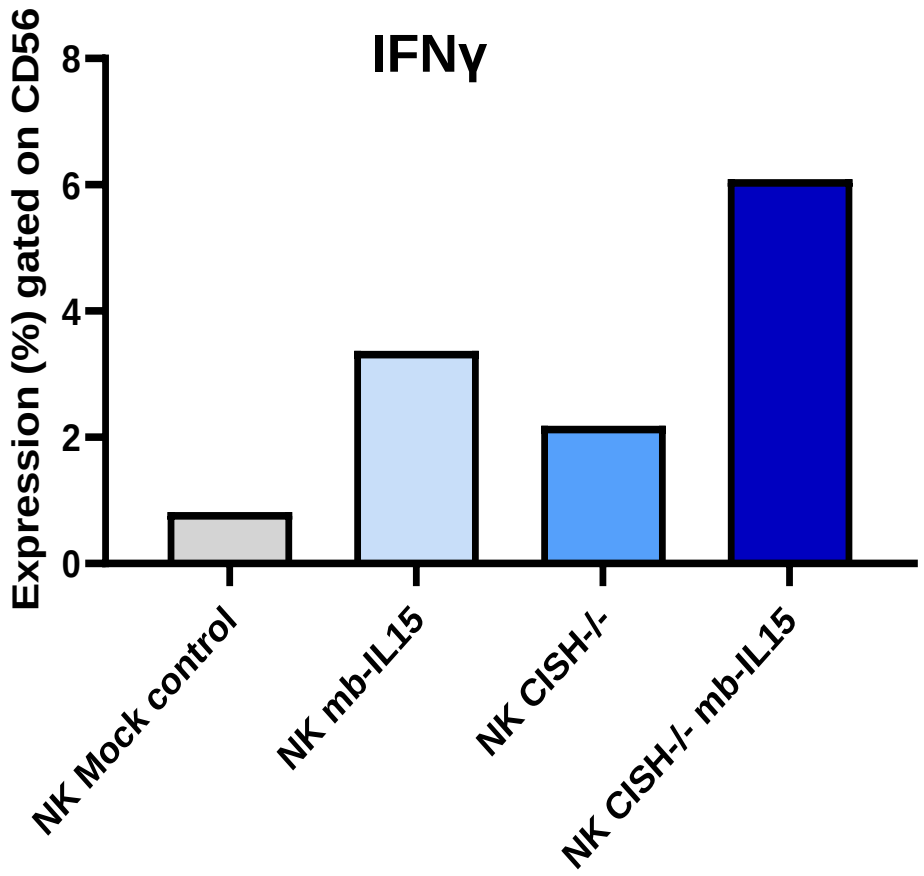




GDA-301 Increases the expression of pro-inflammatory cytokines when cultured with K562

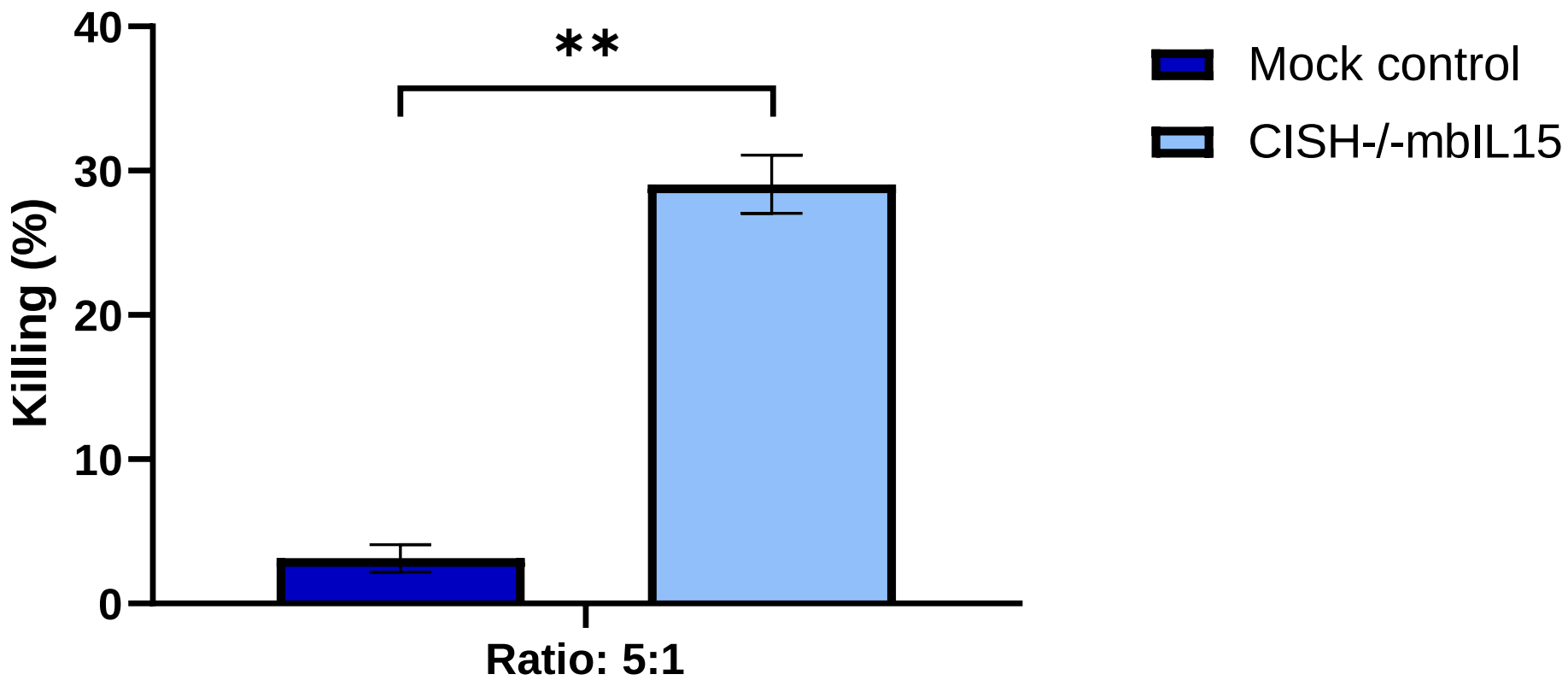
GDA-301 Enhances Cytotoxicity Function Against a Multiple Myeloma Cell line (RPMI)





GDA-301 Increases the expression of pro-inflammatory cytokines when cultured with MM RPMI

GDA-301 Increases Killing Against Multiple Myeloma Cell Line (RPMI)



GDA-301 Increases the killing capacity, 24 h post electro' when cultured for 6h with MM RPMI

Summary

- The evolving field of NK cellular immunotherapy is modifying what has been learned from T cell gene editing and applying said knowledge to NK cell manipulation
- Gamida-Cell utilizes mRNA delivery by electroporation, enabling highly efficient expression of cytokines, and CAR constructs into NAM-expanded, allogeneic NK cells
- This technique can be easily applied to generate improved “off-the-shelf” NK cancer therapeutics
- **Gamida-cell NK mRNA gene engineering allows manipulations that hold the potential for efficient and safe clinical immunotherapy applications**

Acknowledgment

gamida Cell

Julian Adams – CEO

Ronit Simantov- CMO

Yona Geffen- V.P R&D

Research Team

Astar Hailu

Avishay Edri

Dima Yackoubov

Julia Rifman

Nurit Brickman

Orit Berhani Zipori

Sherri Cohen

Bar-Ilan University

Ayal Hendel

Nimrod Ben Haim

Tradition of Excellence

Bar-Ilan University

Thank you