NK Cells

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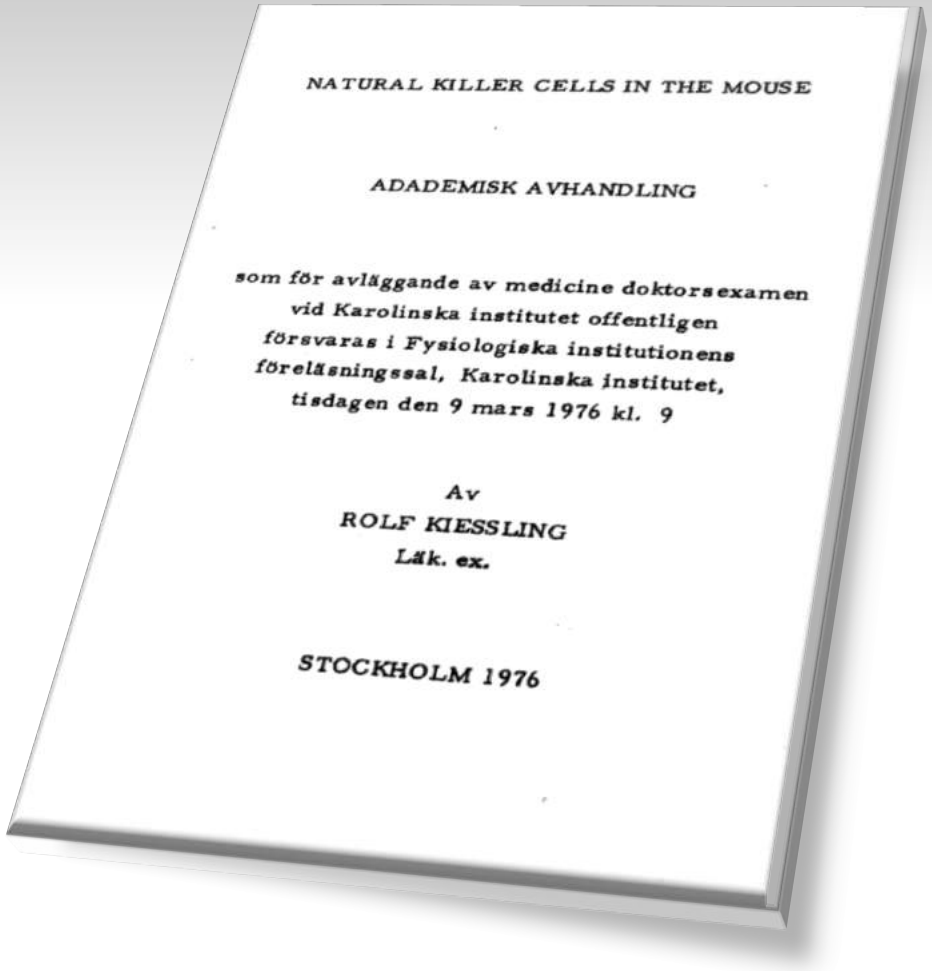
Natural Killer cells



NATURAL KILLER CELLS IN THE MOUSE

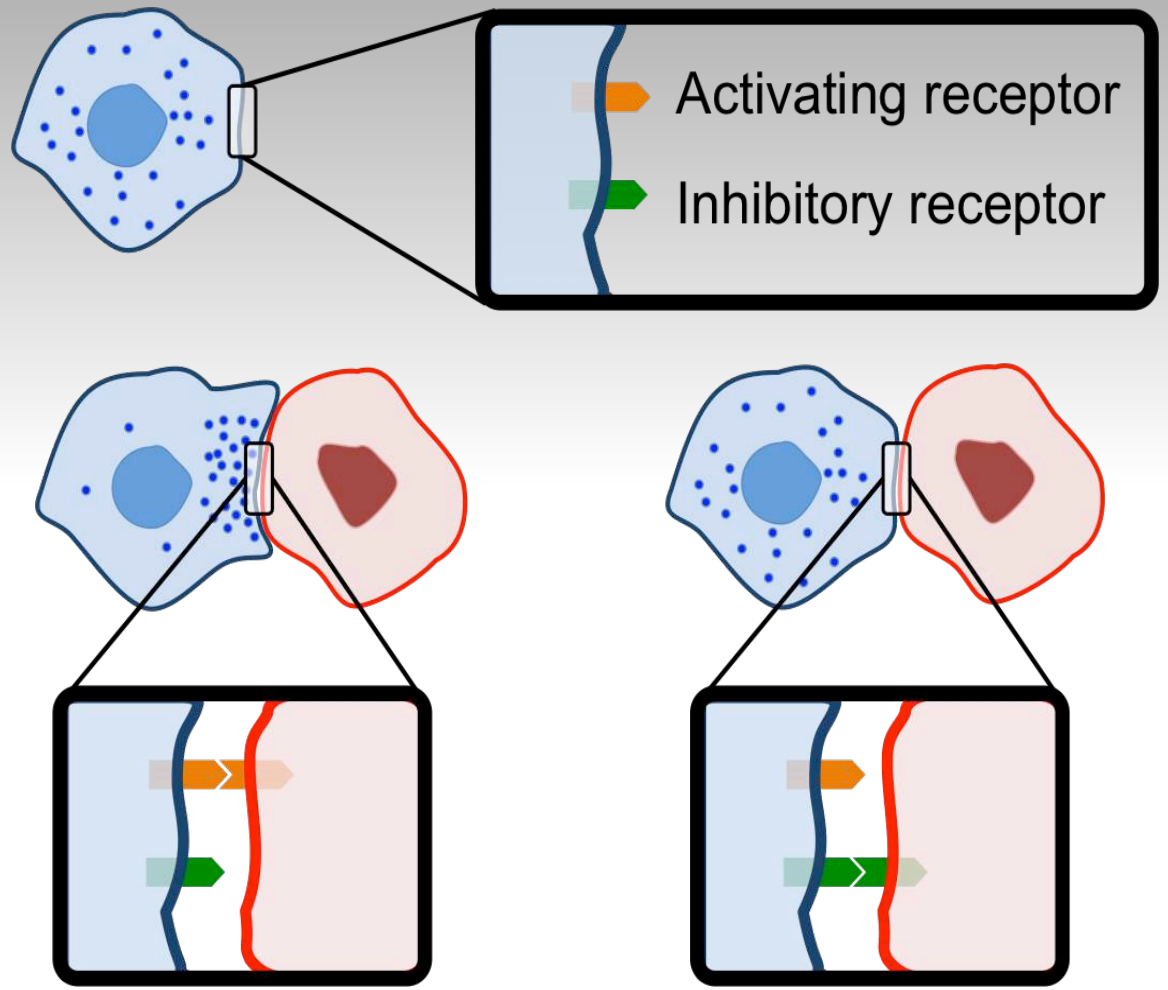
ADADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen
vid Karolinska institutet offentligen
försvaras i Fysiologiska institutionens
föreläsningssal, Karolinska institutet,
tisdagen den 9 mars 1976 kl. 9

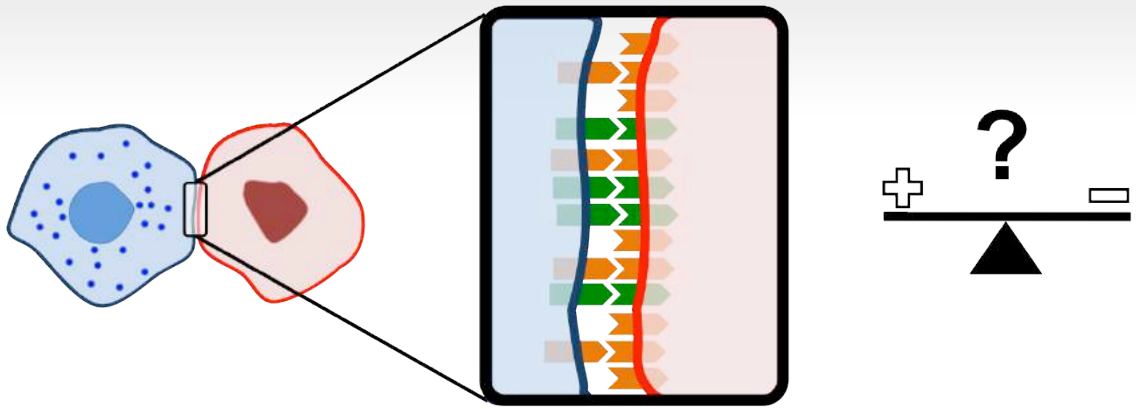
Av
ROLF KIESSLING
Läk. ex.

STOCKHOLM 1976

Natural Killer Cells



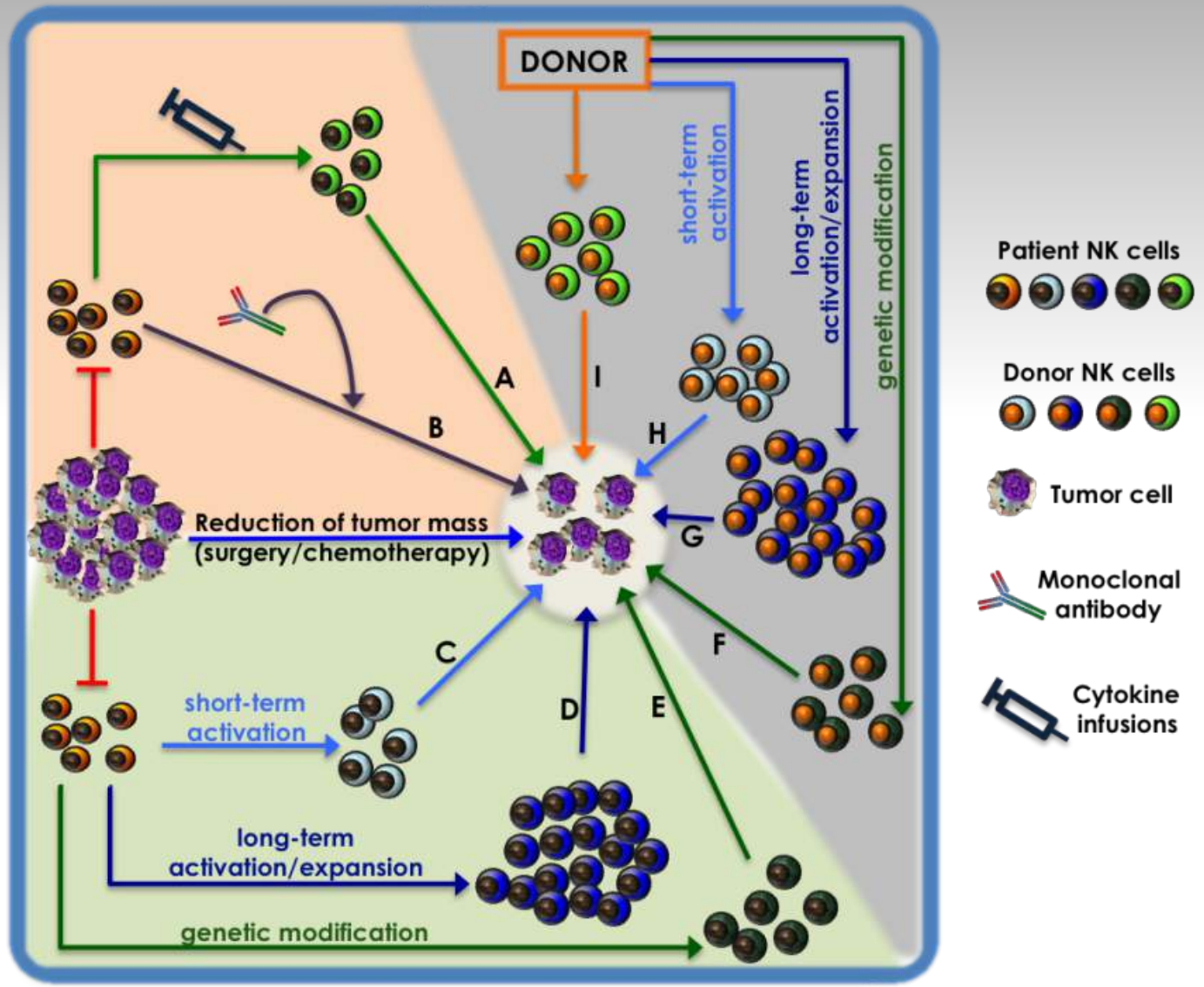
What really goes on



NK cell abnormalities

Abnormality	Disease	
Decreased cytotoxic activity of NK cells	NSC lung Ca **HCC** **CRC** H&N Ca Breast Ca Squamous cell Ca Bronchogenic Ca	Cervical Ca Ovarian Ca **AML** ALL **B-CLL** CML **MM**
Defective expression of activating receptors	**HCC** M. melanoma	**AML** **MM**
Defective NK cell proliferation	**Renal Ca** **Neuroblastoma**	Nasopharyngeal Ca CML
Increased number of $CD56^{bright}$ NK cells	H&N Ca	Breast Ca
Defective expression of signalling molecules	Cervical Ca **CRC** Ovarian Ca	Prostate Ca **AML** CML
Decreased NK cell counts	Nasopharyngeal Ca **Neuroblastoma**	CML ALL (Pediatric)
Defective cytokine production	**AML** ALL	CML

NK cell therapy overview

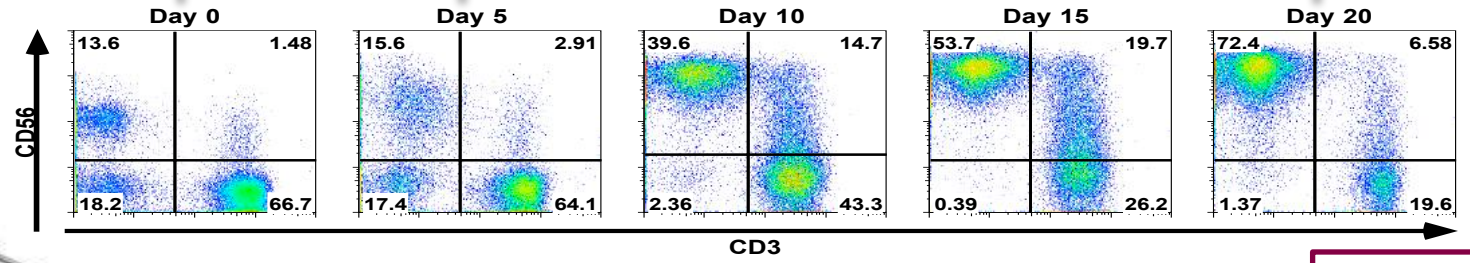


Sutlu & Alici, JIM 2009

The expansion process

Day 0
CellGro SCGM
5% Human AB serum
IL-2 (500 U/ml)
OKT3 (10 ng/ml)

Detailed phenotypic characterization of NK cells
Degranulation and cytotoxicity assays



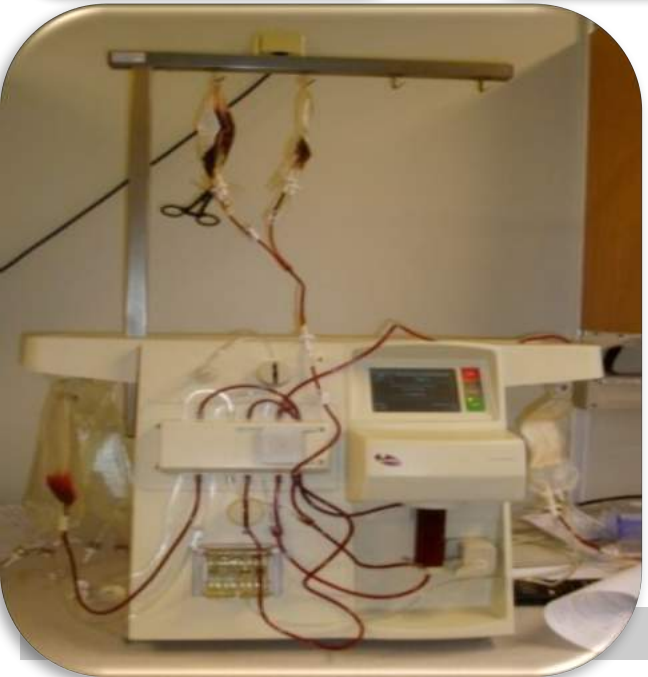
Day 20

Every 2-3 days
Additional medium with IL-2, without OKT3

cGMP certified expansion process








A phase-I clinical trial with expanded NK cells

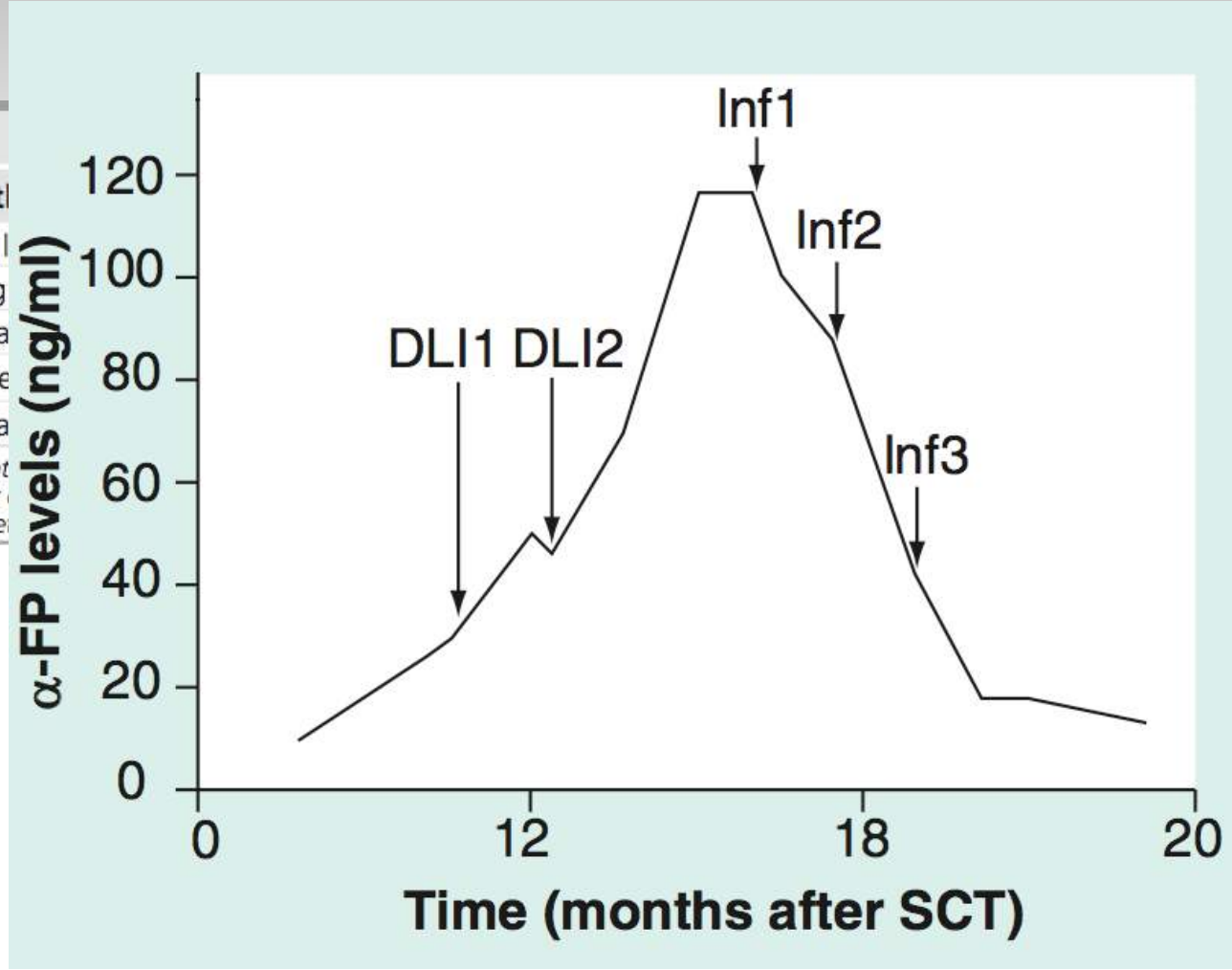
Patient	Diagnosis	Sex	Age (years)	PBL chimerism	Immuno-suppression	Location of metastases[§]	Time since last DLI (months)	GvHD	Additional tumor debulking (months)	KIR allo-reactivity
P1	CRC	M	67	DC	No	1, 2, 3, 4	7	No	RFA liver met/(3.5)	No
P2	HCC	M	48	DC	Yes*	3, 4	2	No	0	No
P3	RCC	M	50	DC	No	1, 2, 3, 4	13	Limited cGvHD[#]	RFA liver met/(16) IRD lung met/(16)	No
P4	CLL	F	59	100% recipient CD19$^+$ cells	Yes[‡]	5	11[¶]	No	0	No
P5	RCC	M	54	DC	No	1,3	26	Limited cGvHD[#]	surgery of lung met/(2)	Yes

**Tacrolimus 0.006–0.1 mg/kg BW; [‡]Prednisolon 0.8 mg/kg BW; [§]1 = lymph node (LN) mediastinum; 2 = liver; 3 = lung; 4 = pleura; 5 = LN abdomen; [¶]After booster stem cell infusion; [#]No active chronic GvHD 11 months prior to the first NK/NK-like T-cell infusion.*
All donors were siblings. KIR alloreactivity was determined by donor KIR ligand missing in the recipient.
BW: Body weight; CLL: Chronic lymphocytic leukemia; CRC: Colorectal carcinoma; DC: Total donor chimera of CD3$^+$, CD19$^+$, CD33$^+$ cells; DLI: Donor lymphocyte infusion; F: Female; GvHD: Graft-versus-host disease; HCC: Hepatocellular carcinoma; IRD: Stereotactic irradiation; KIR: Killer immunoglobulin-like receptor; M: Male; met: Metastases; PBL: Peripheral blood lymphocytes; RCC: Renal cell carcinoma; RFA: Radiofrequency ablation.

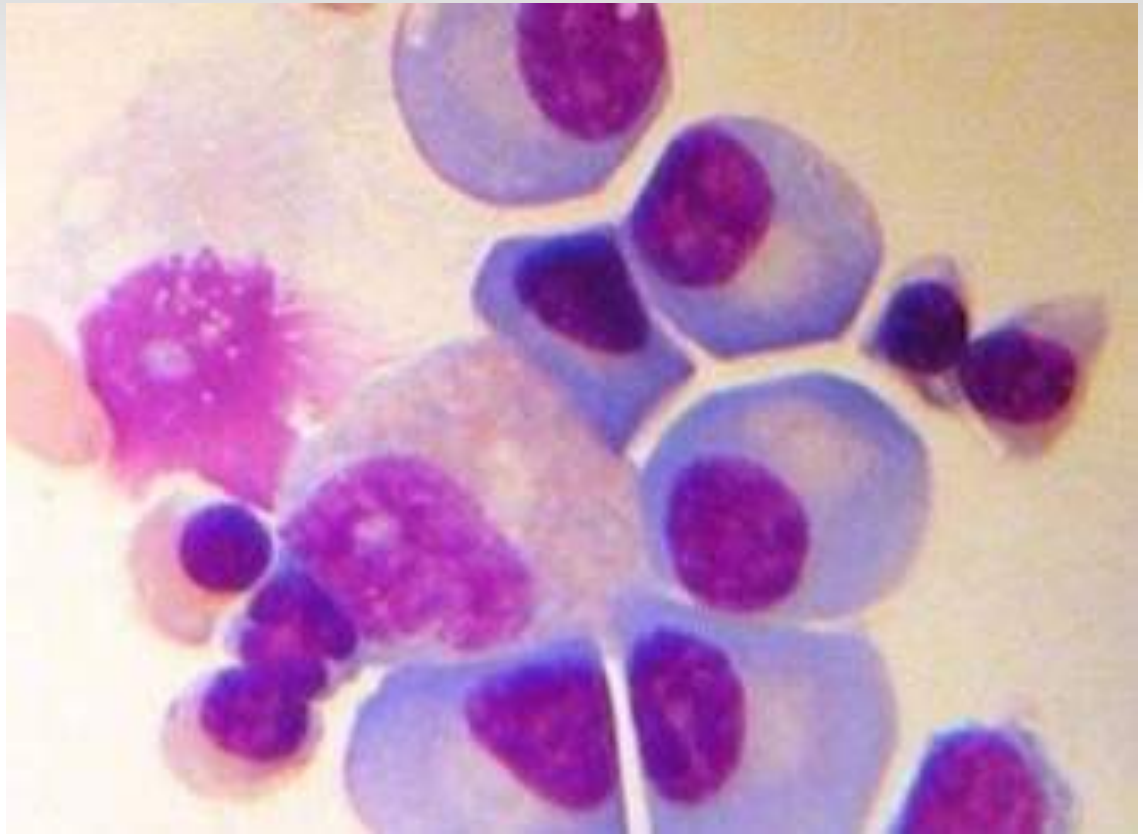
Patient follow-up

Patient	3 month	utcome 6 months e last infusion
P1	P (lung, l	
P2	SD (lung	
P3	P (pleura	
P4	P (LN me	
P5	P (pleura	

**RECIST: Response Evaluat*
†Fatal outcome as a result
LN: Lymph node; met: Me



Multiple Myeloma

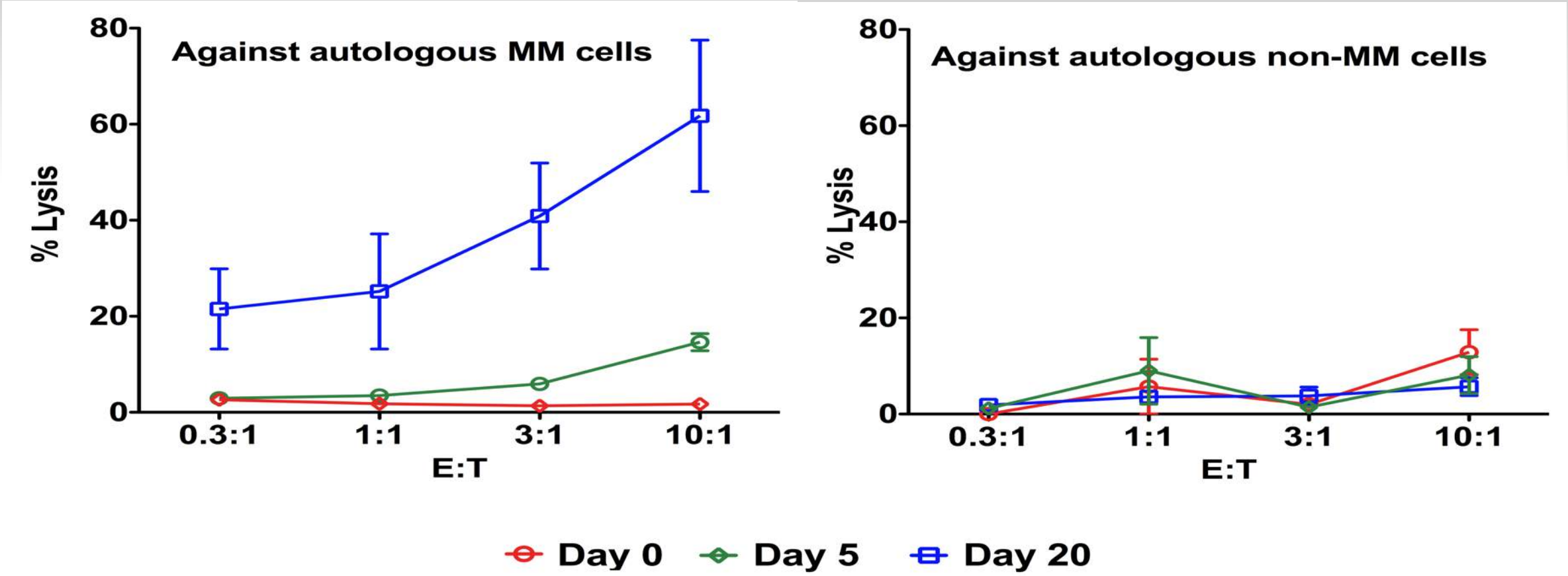


- The lifetime risk of getting MM is 1 in 159 (0.63%).
- In 2015:
  - 20,000 new cases
    - 11,000 men and 9,000 women
  - 10,650 deaths
- 5-year survival rate: 35%
- 10-year survival rate: <2%

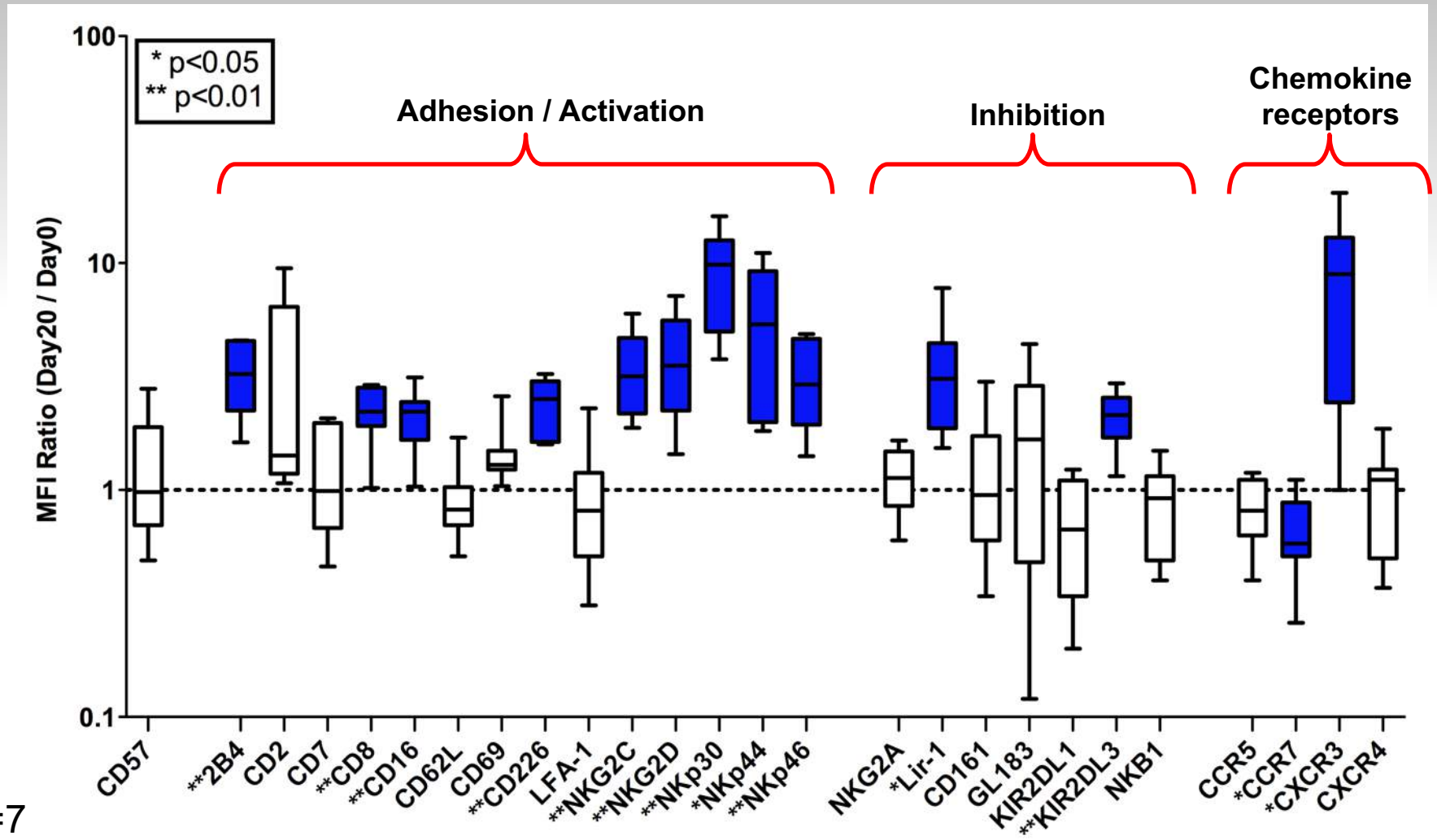
Natural Killer Cells in MM

- Various immune dysfunctions are observed in MM patients
- Tumor-induced immune dysfunctions regarding NK cells in MM:
  - Increased level of soluble IL-2 receptors
  - High levels of M-component
  - Defective expression of activating receptors
  - Impaired NK cell cytotoxicity and abnormal NK cell counts
- Adoptive transfer of IL-2 activated NK cells prolongs survival in animal models of MM

Expanded cells have cytotoxicity against autologous MM cells

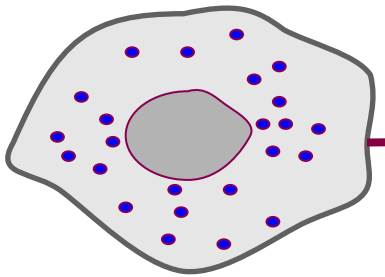


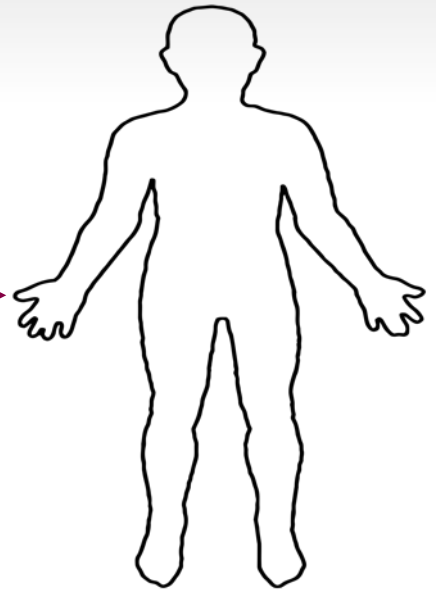
Phenotypic changes



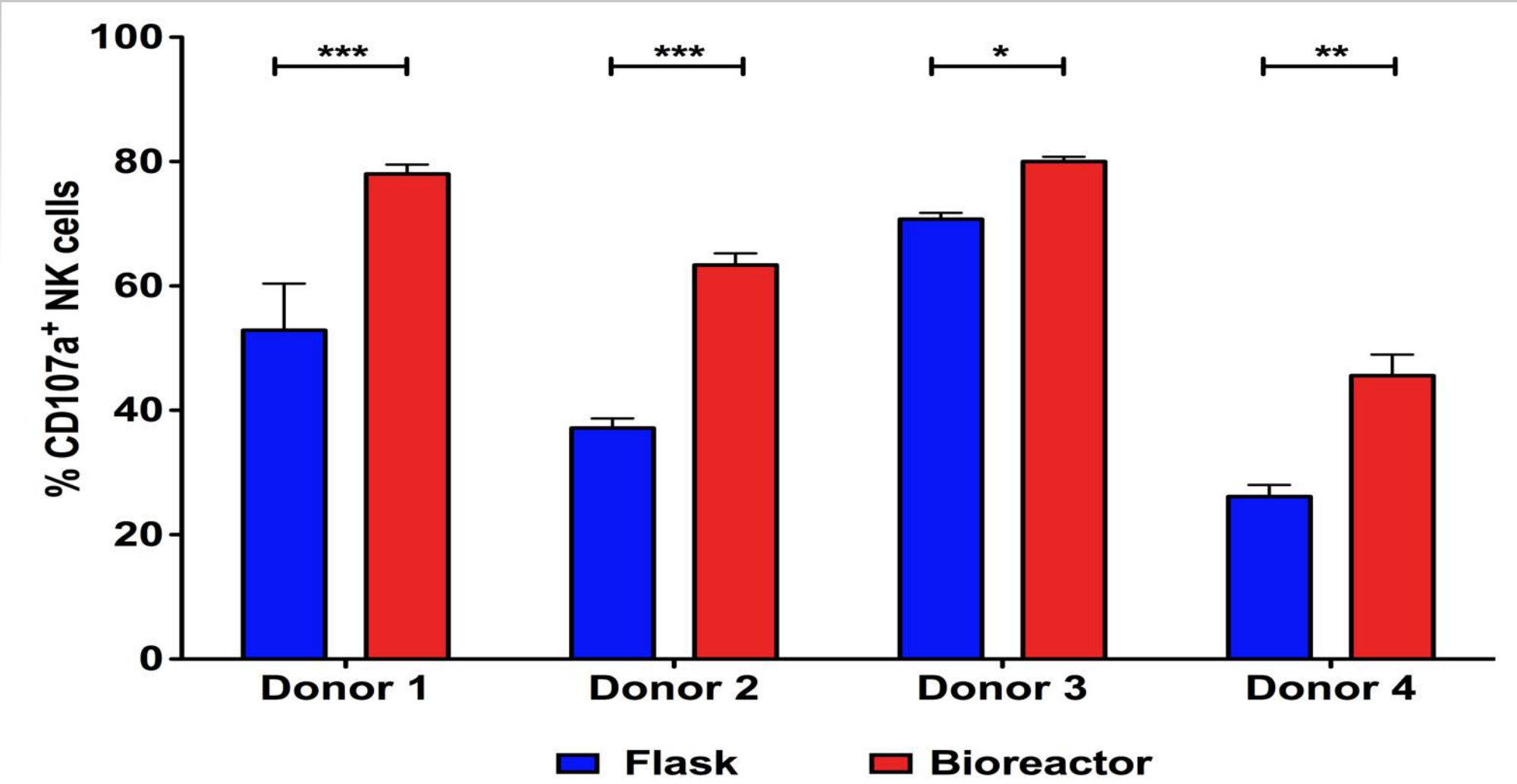
N=7

Different strategies for scaling up

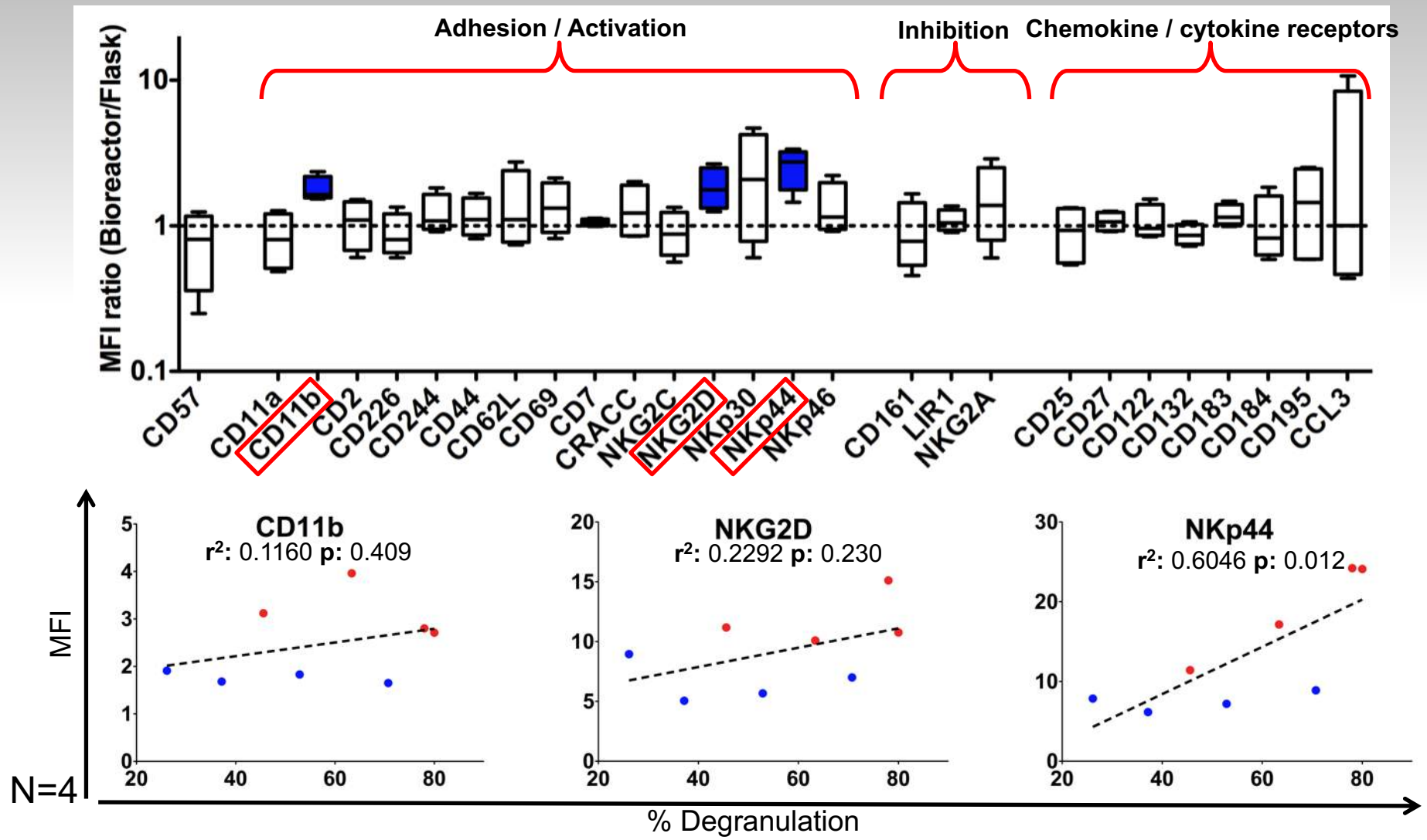




NK cells from bioreactor expansions degranulate more efficiently

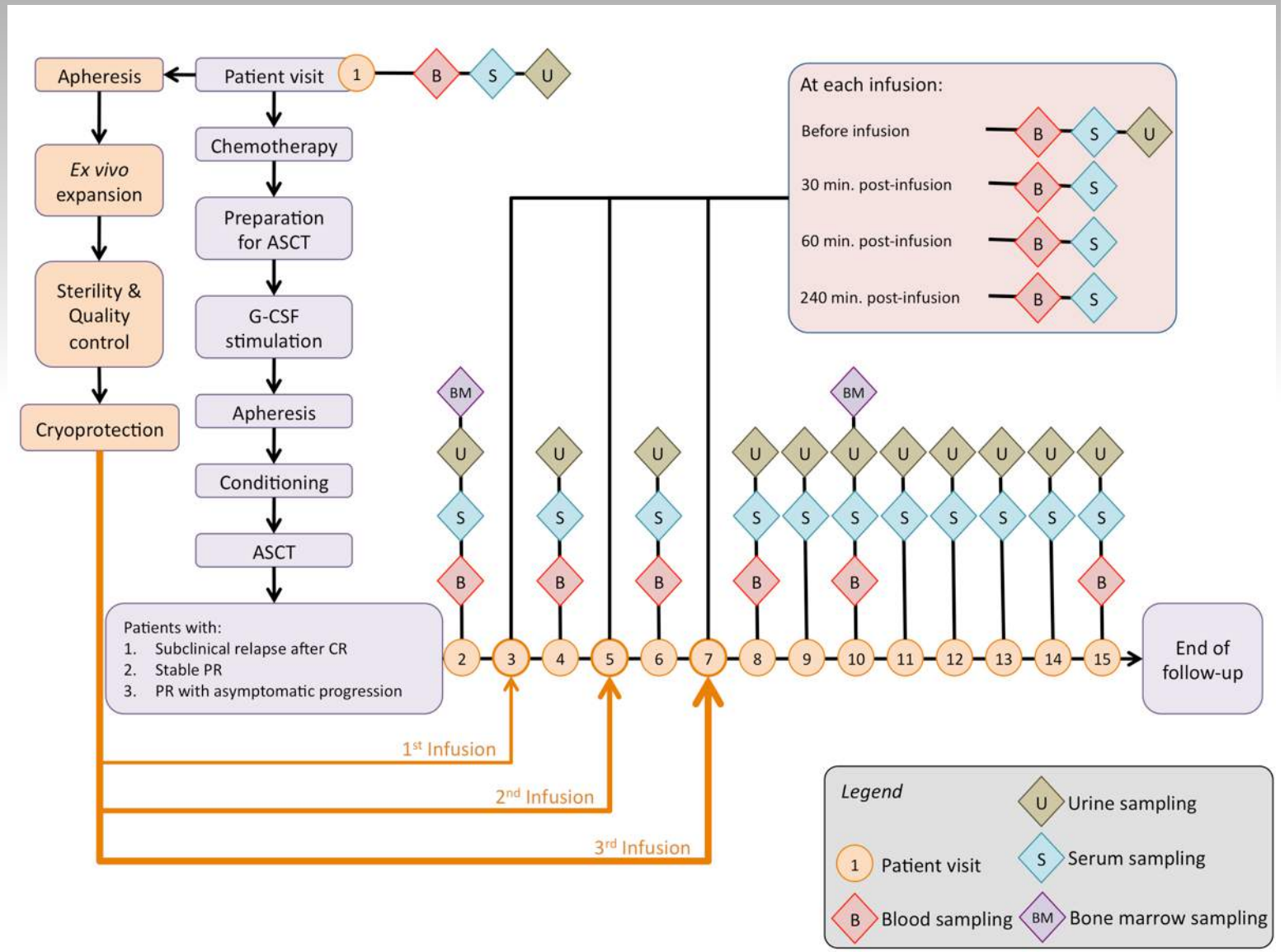


Phenotypic differences

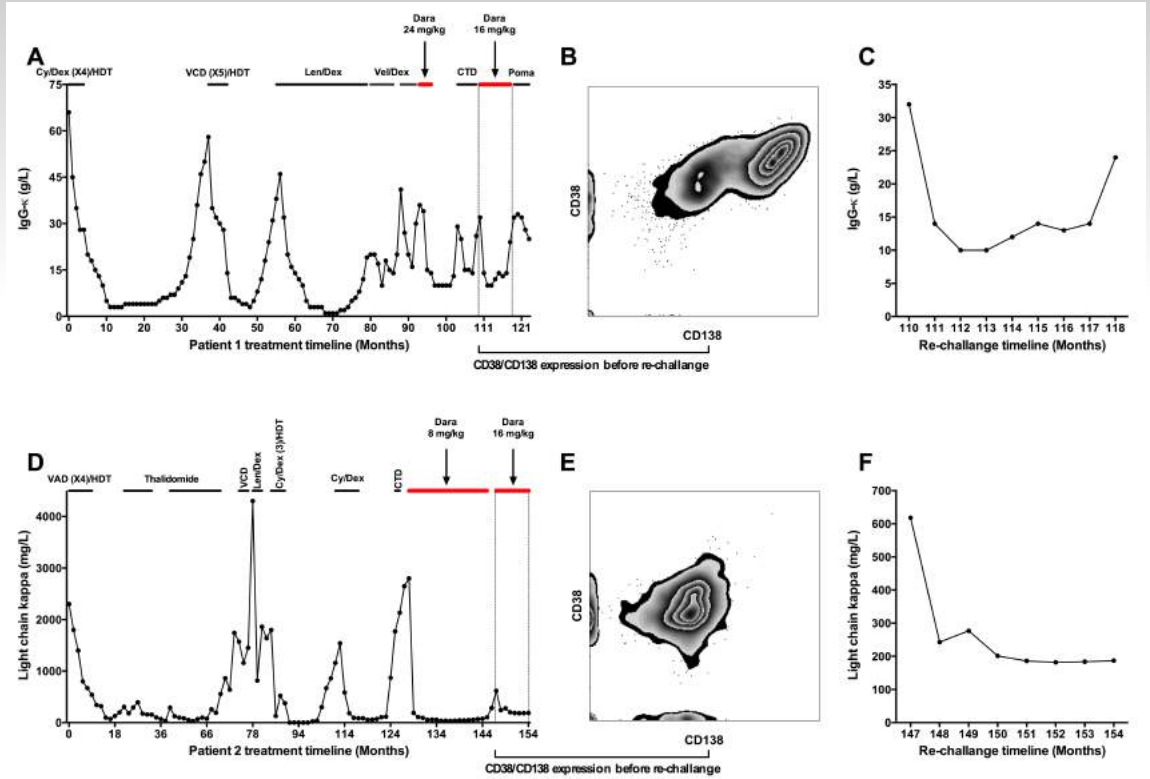


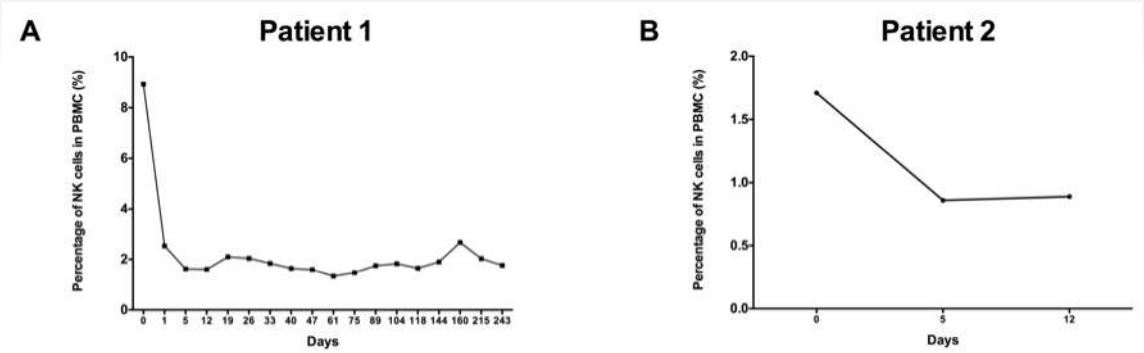
ACP-001

- First-in-man, Phase I/II
- Open, single arm study
- Primary objective:
  - Safety and tolerability
- Secondary objective:
  - Effect on serum Ig levels
- Inclusion:
  - 20 MM patients eligible for ASCT
- 3 escalating infusions/patient (Weekly)
  - 10^6, $5X10^7$ and 10^8 cells/kg
- Evaluation:
  - 4 weeks after infusion,
  - 6 months follow up.

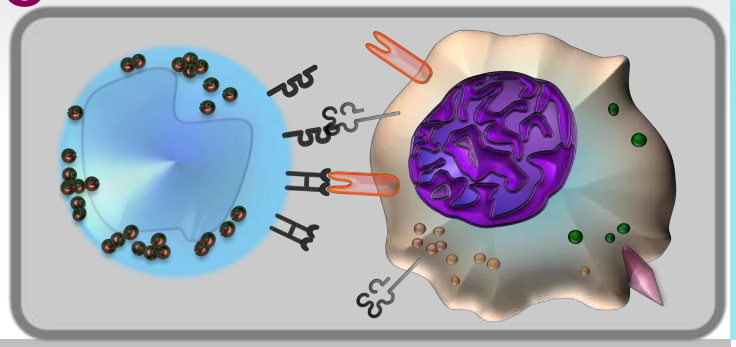


Daratumumab treatment

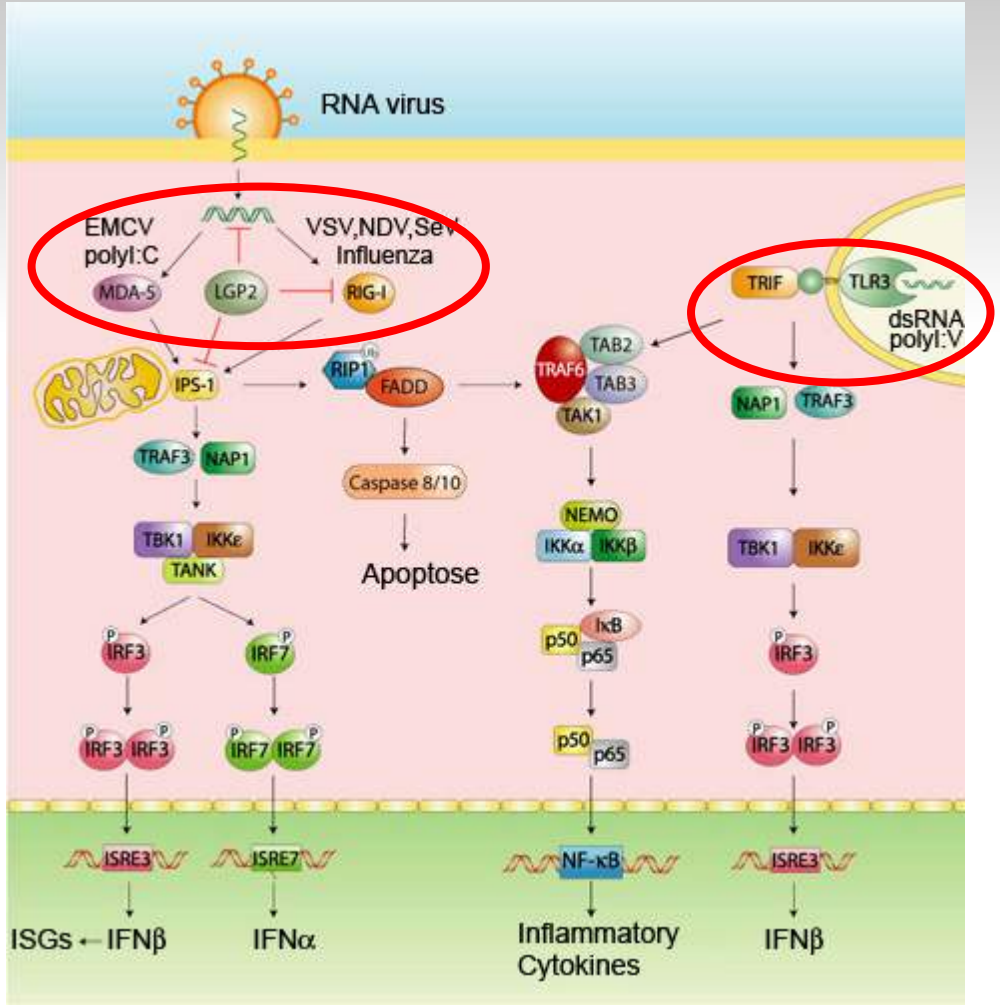




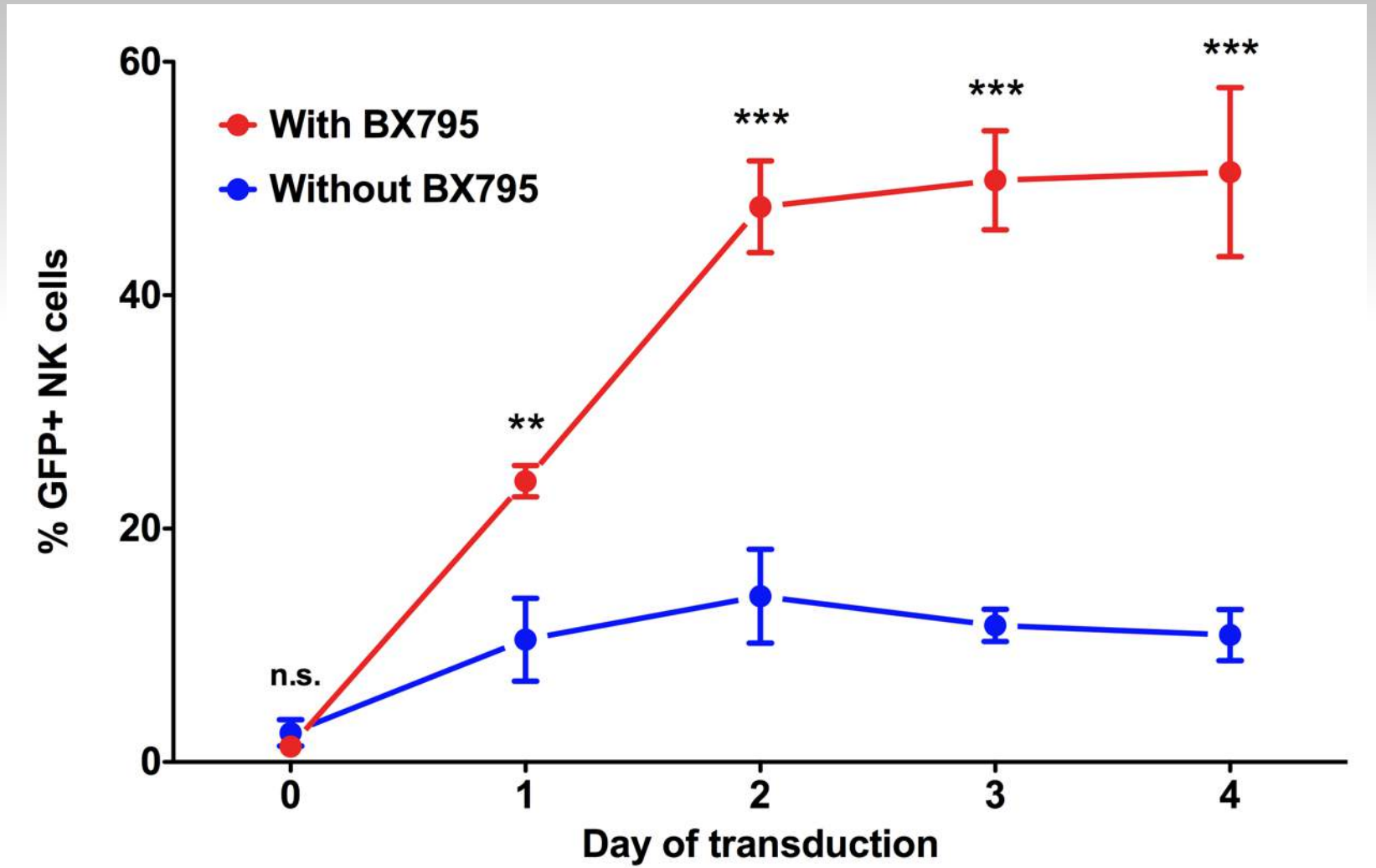
Improving lentiviral and retroviral gene delivery to NK cells



How could we help the viral gene delivery process?

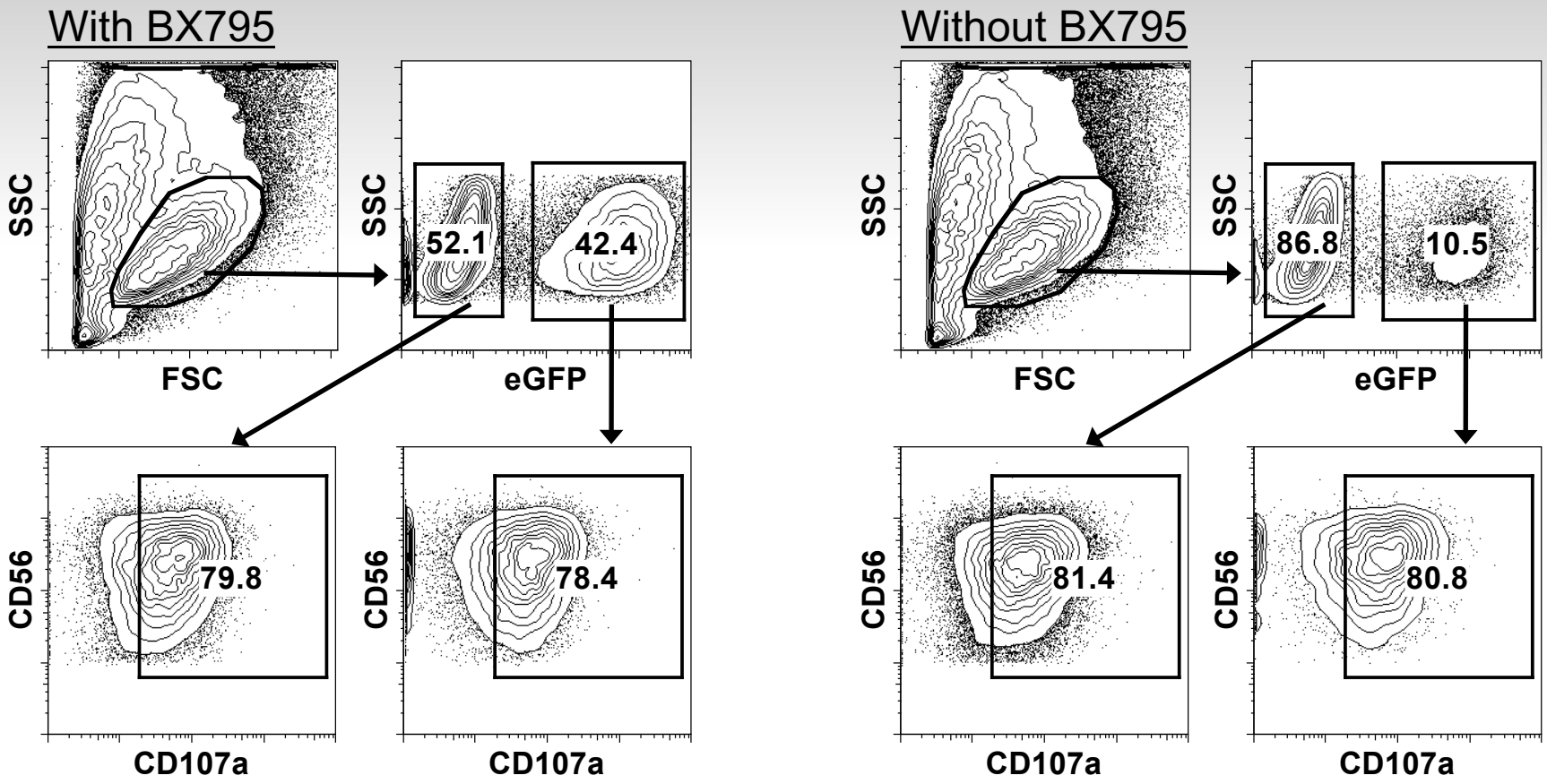


Time dynamics of lentiviral transduction on NK cells



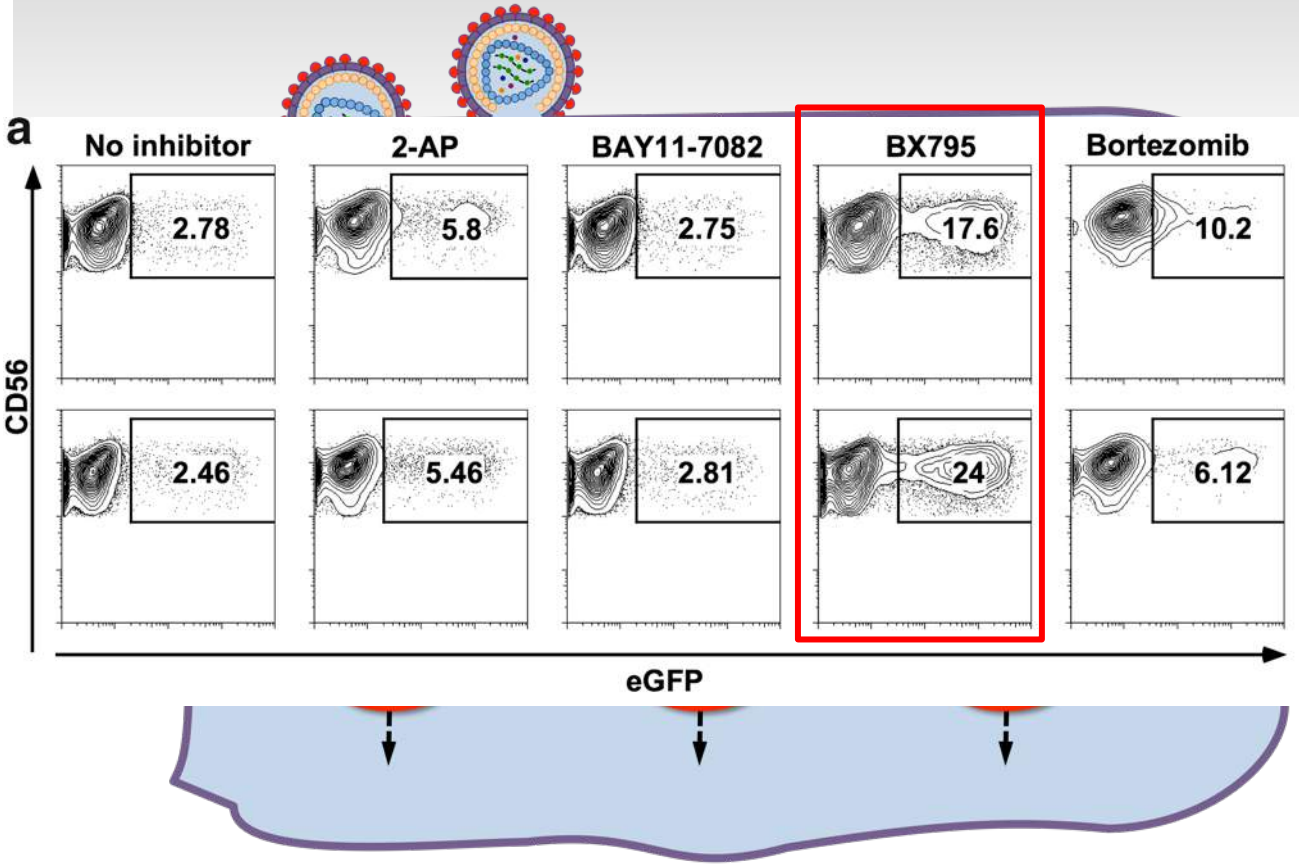
N=4

Transduction process does not effect NK cell function

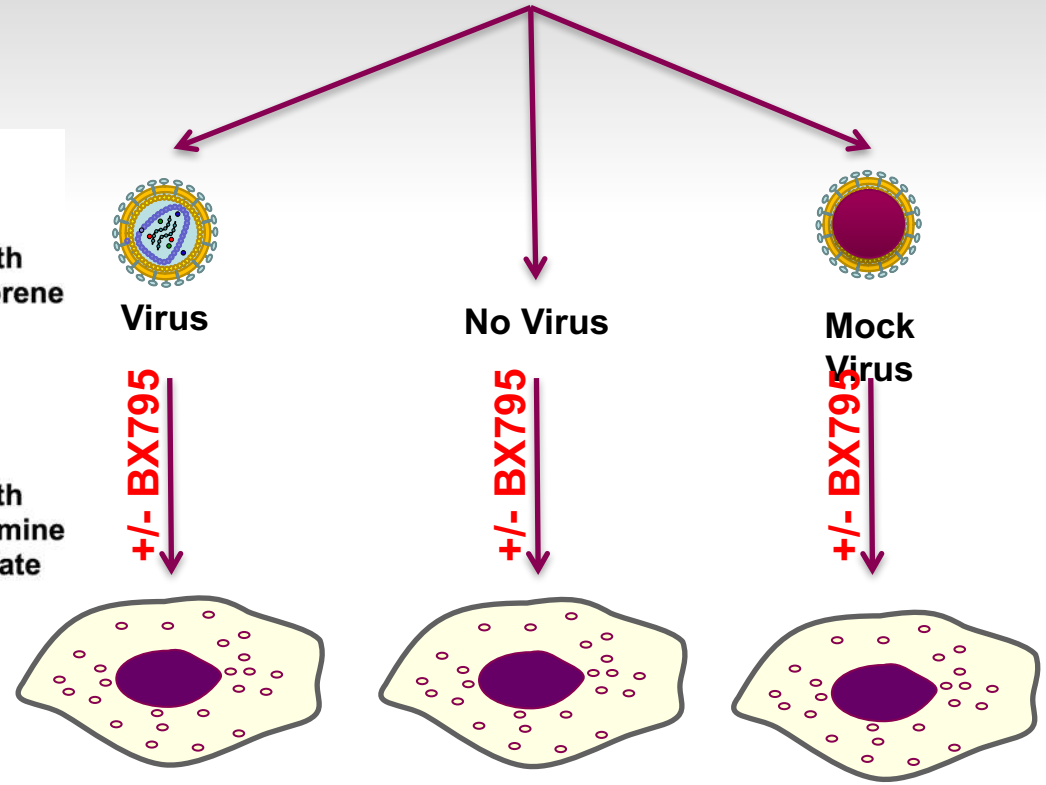


NK cell genetic modification

Intracellular anti-viral defense mechanisms can limit viral gene delivery in NK cells and this can be reduced by using small molecule inhibitors



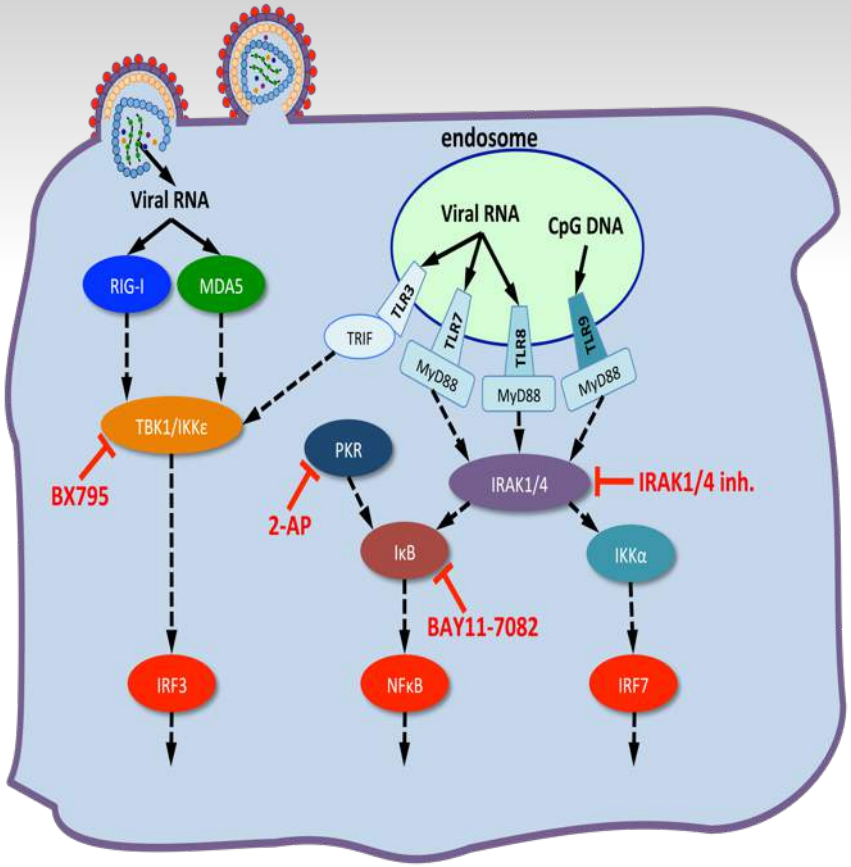
Genomics & Bioinformatics
Differential gene expression analysis

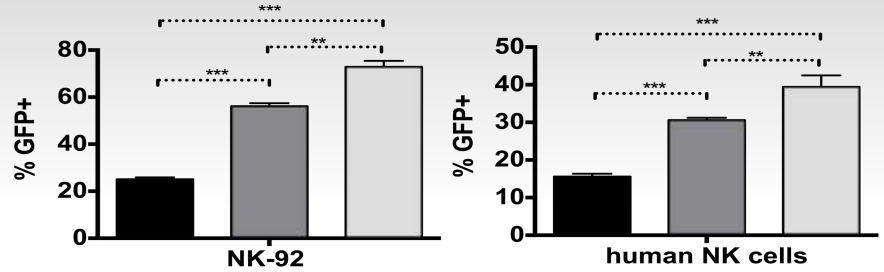


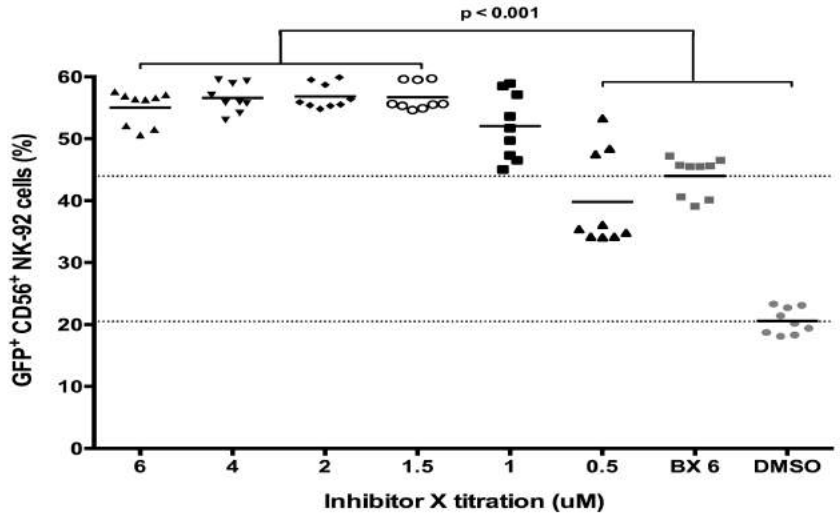
Sutlu 2012, Hum . Gene Ther.

A more targeted molecule: VyOxo

Enhancing lentiviral gene delivery to NK cells: Vy-Oxo





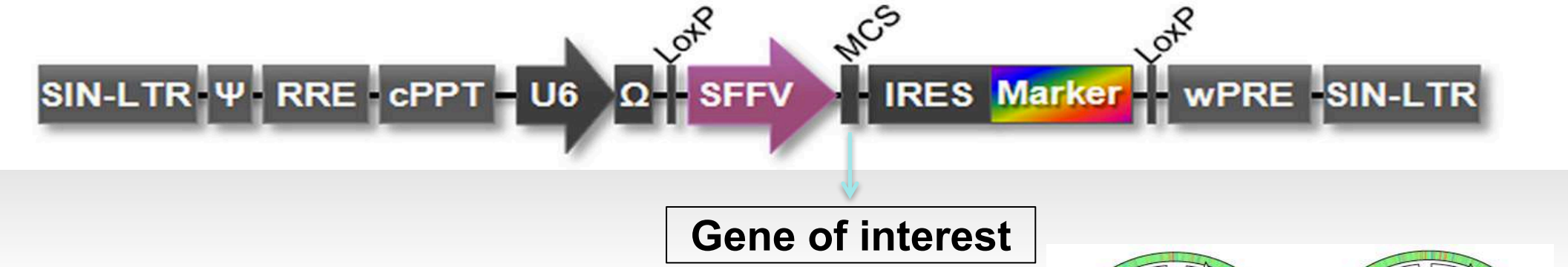


NK-Tumor interactions

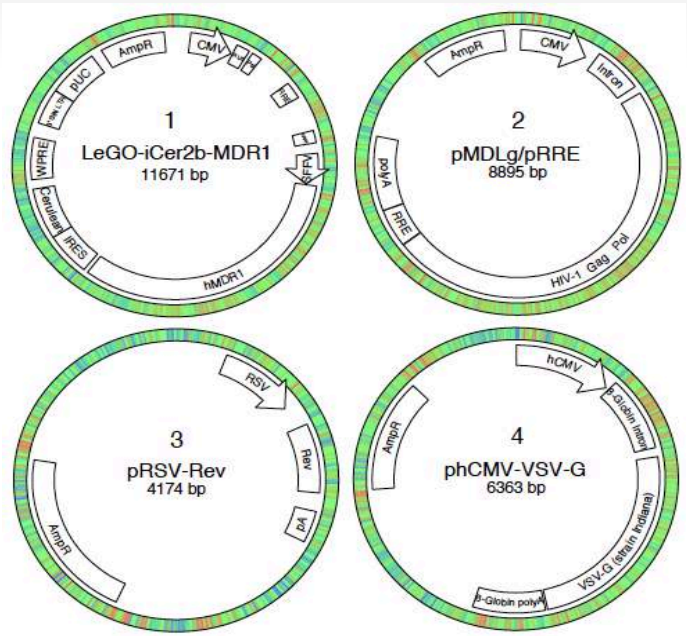
Genetic modification of NK cells from patients with hematological malignancies

- Immunotherapy with genetically modified NK cells
- Increase detection of patient-specific target ligands

LeGo-Vectors



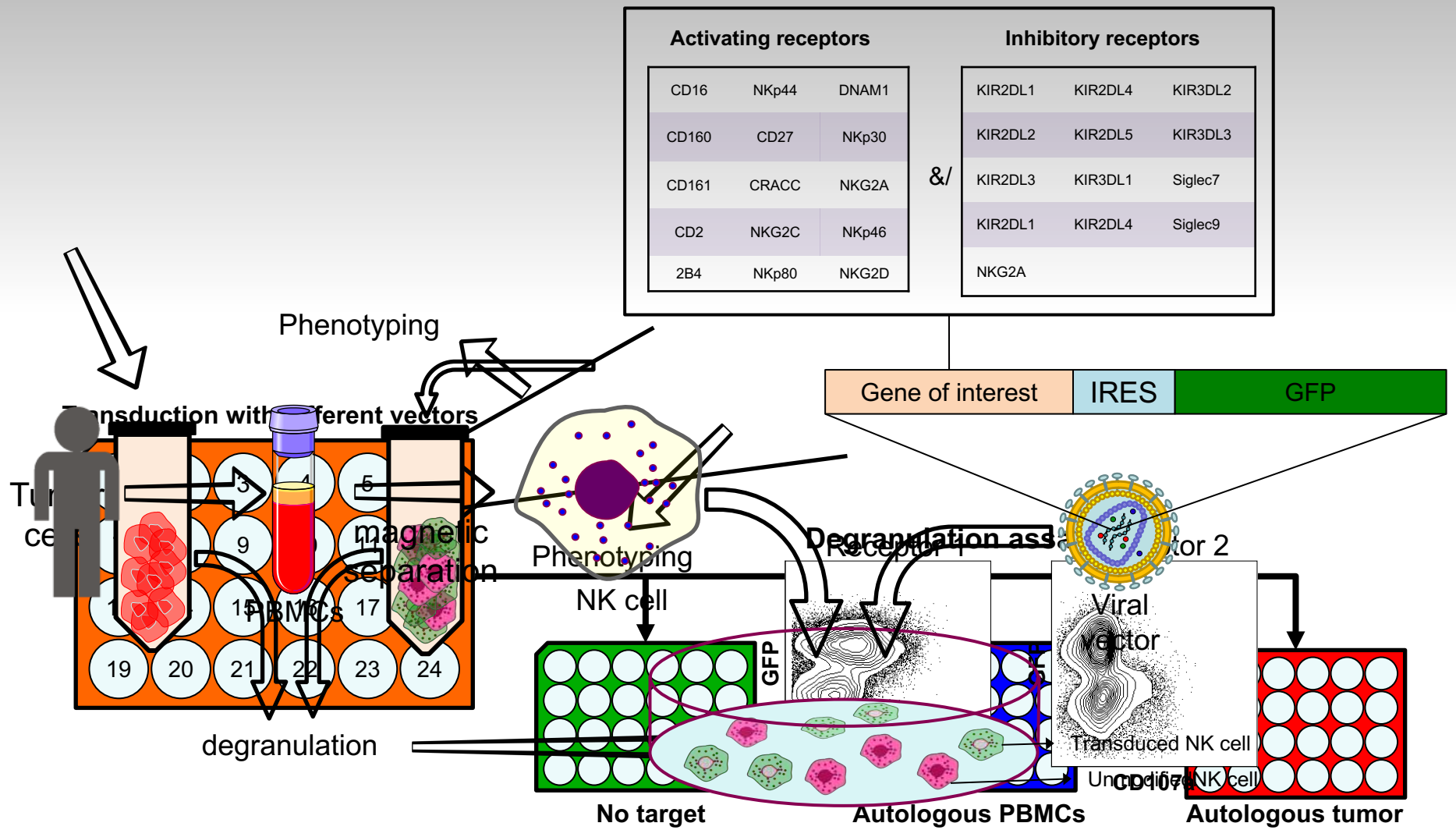
- Self- inactivating-vector of the 3rd generation
- replication incompetent
- can infect a broad group of cell types
- Backbone, packaging, envelope and regulation plasmid
- Pseudotyped with VSV-G
- HEK293FT as producer cell line



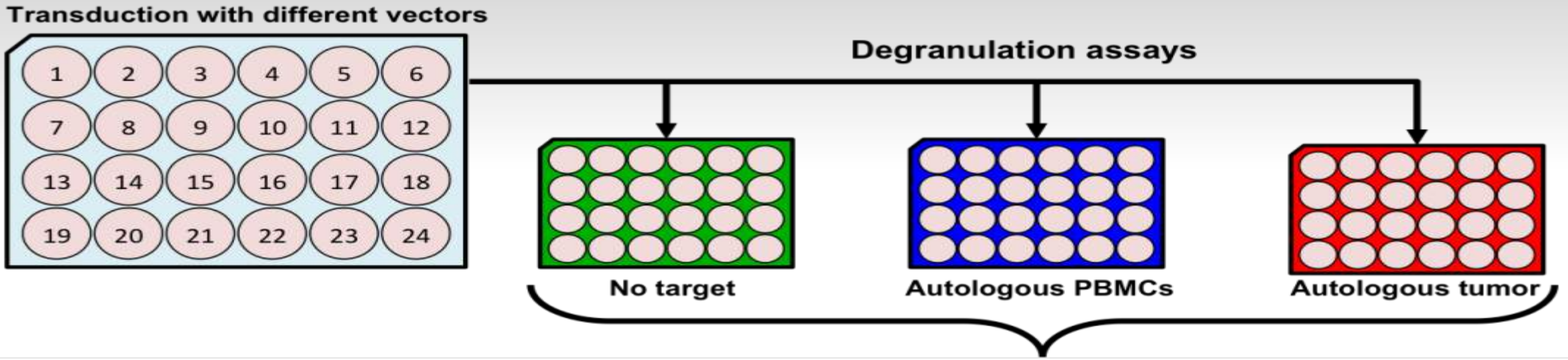
Genetically modified NK cells

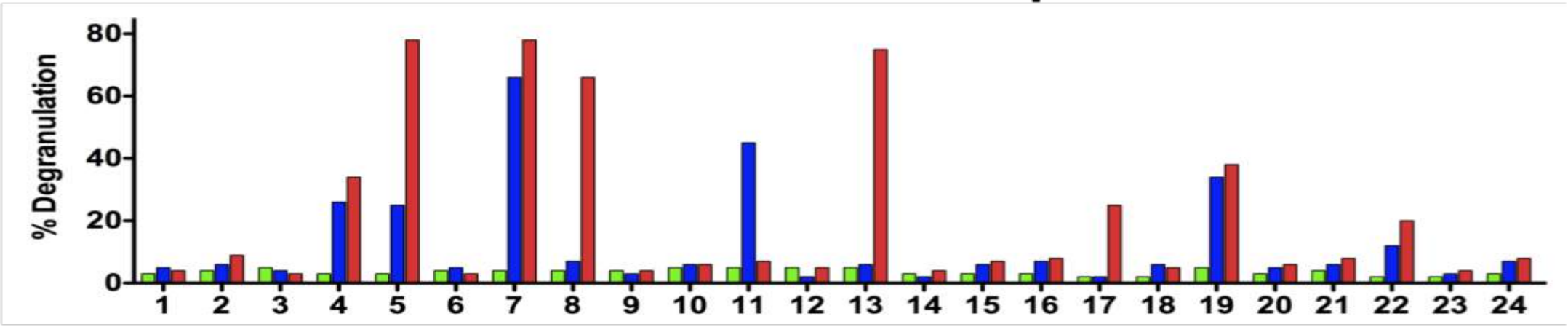
- CD94
- CD16a
- CD160
- CD161
- CD2
- DNAM1
- 2B4
- 2B4T
- CD27
- CRACC
- Siglec7
- Siglec9
- NKp46
- NKp44
- NKp30
- NKG2A
- NKG2C
- NKG2D
- NKG2E
- NTBA
- NKp80
- KIR2DL1
- KIR2DL2
- KIR2DL3
- KIR2DL4
- KIR2DL5
- KIR3DL1
- KIR3DL2
- KIR3DL3

Protocol overview



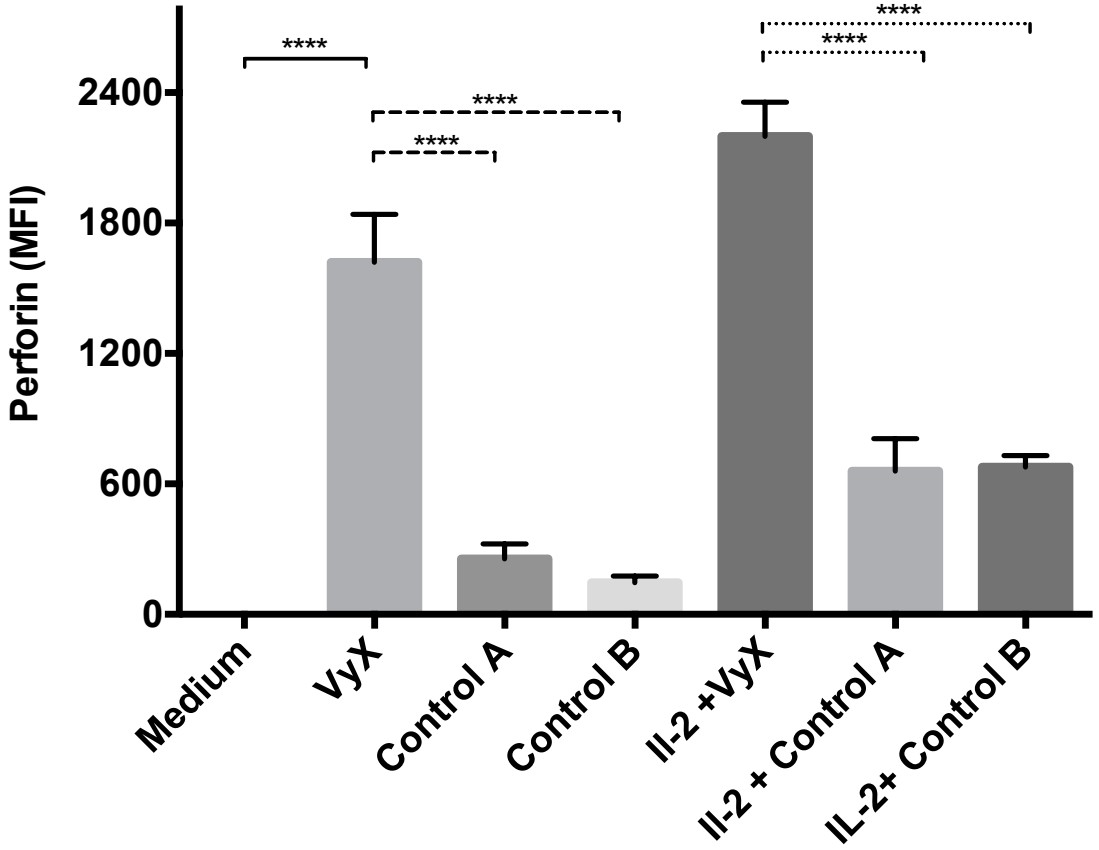
Goal: Patient-specific data

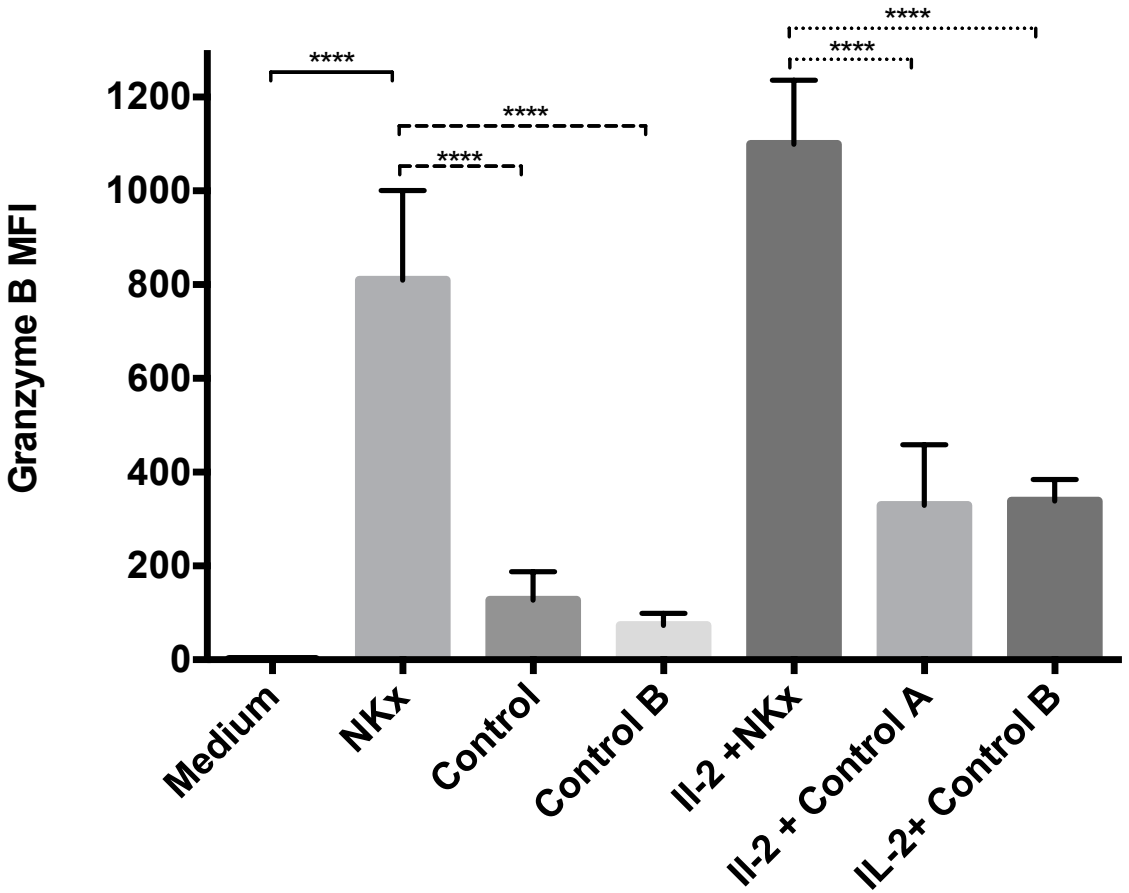




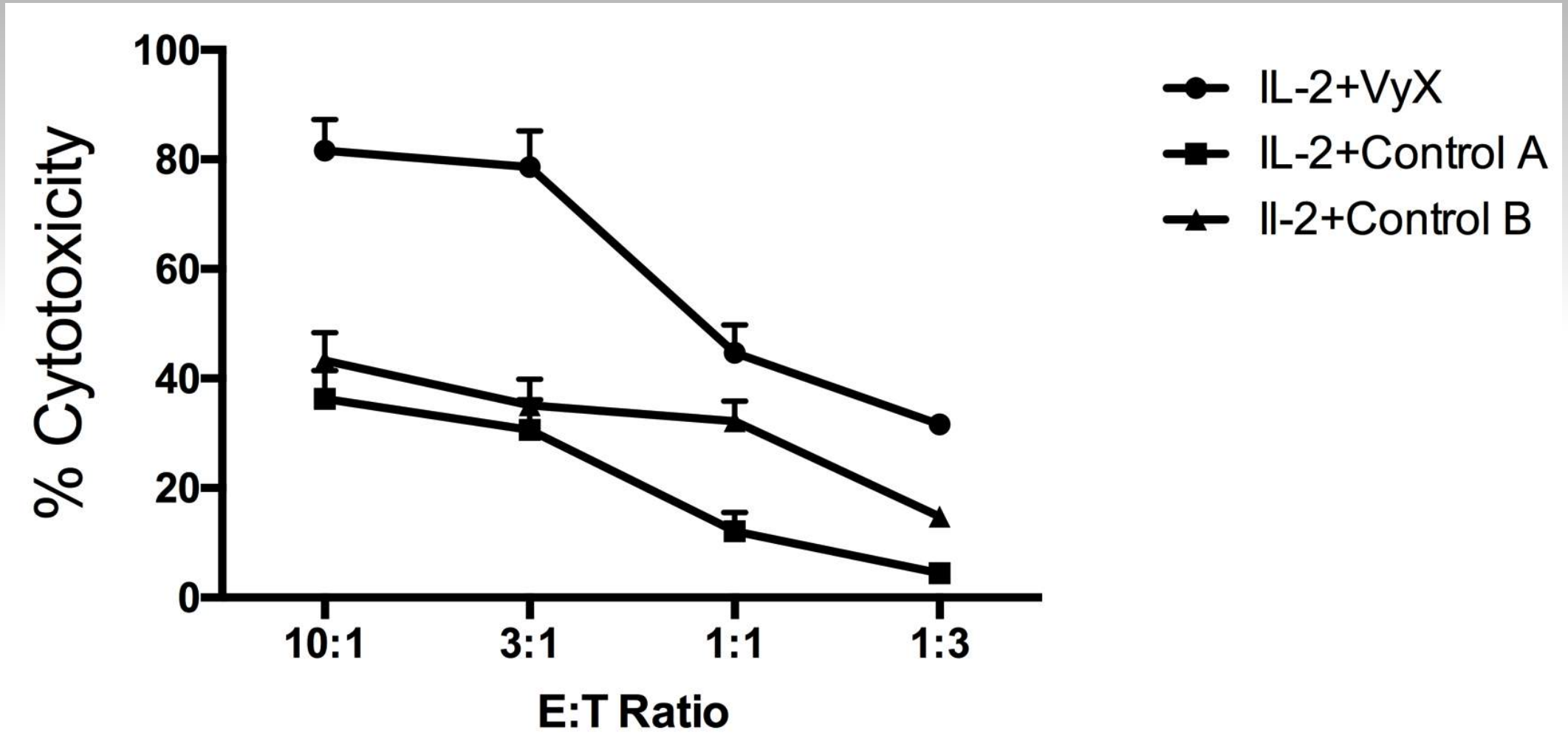
A new discovery: VyX

Vy-X effects *in vitro*

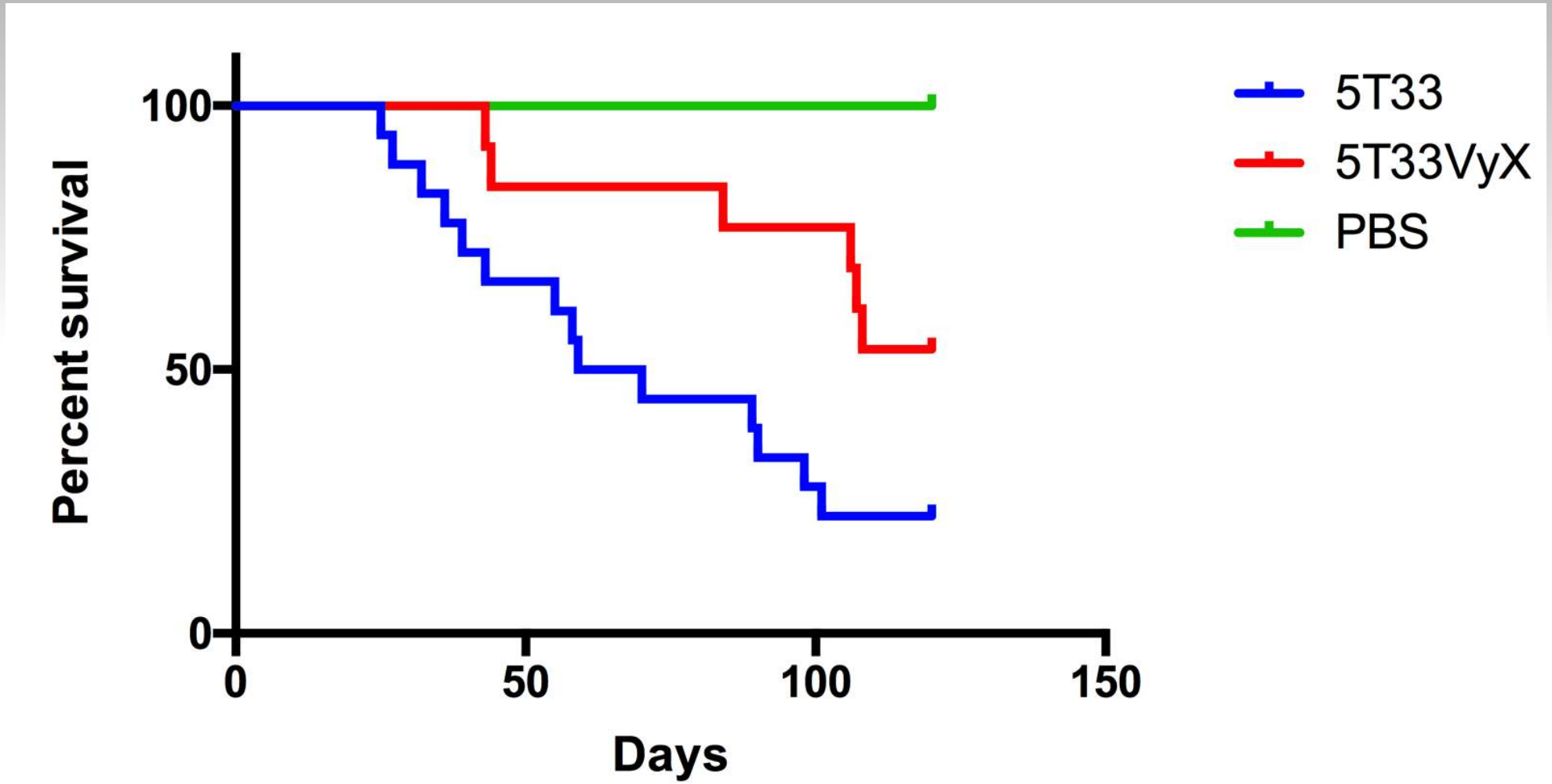




16h Cyototoxicity assay



Direct VyX in vivo delivery



MM patient

Peripheral blood

Bone marrow Aspiration

NK cell isolation

T cell isolation

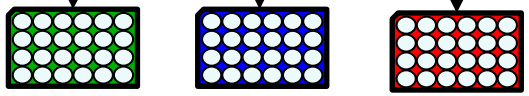
Lentivirus-based high-throughput expression of NK cell receptors

T cell expansion

CD138+ Plasma cell isolation

MSC isolation

Degranulation assays



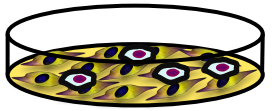
No target

PBMCs

MM cells

- Full genome sequencing
- mRNA sequencing

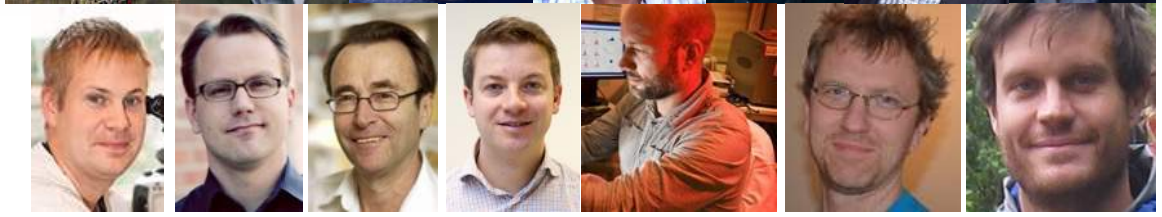
High-throughput drug screening of MM cells co-cultured with cells



Identification of genetic predispositions to treatment resistance to identify optimum personalized therapy for MM patient

Acknowledgments









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