

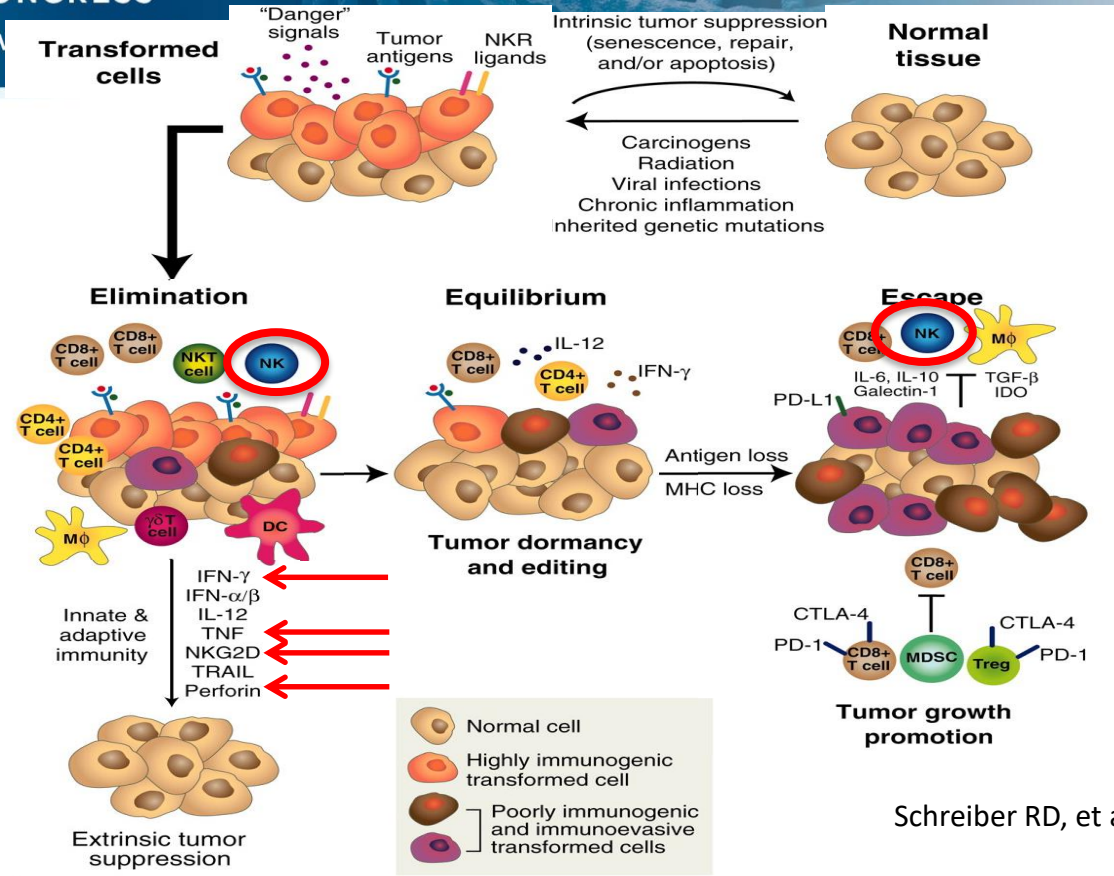
# **Novel approaches for NK cell based cancer immunotherapy**

Francisco Borrego

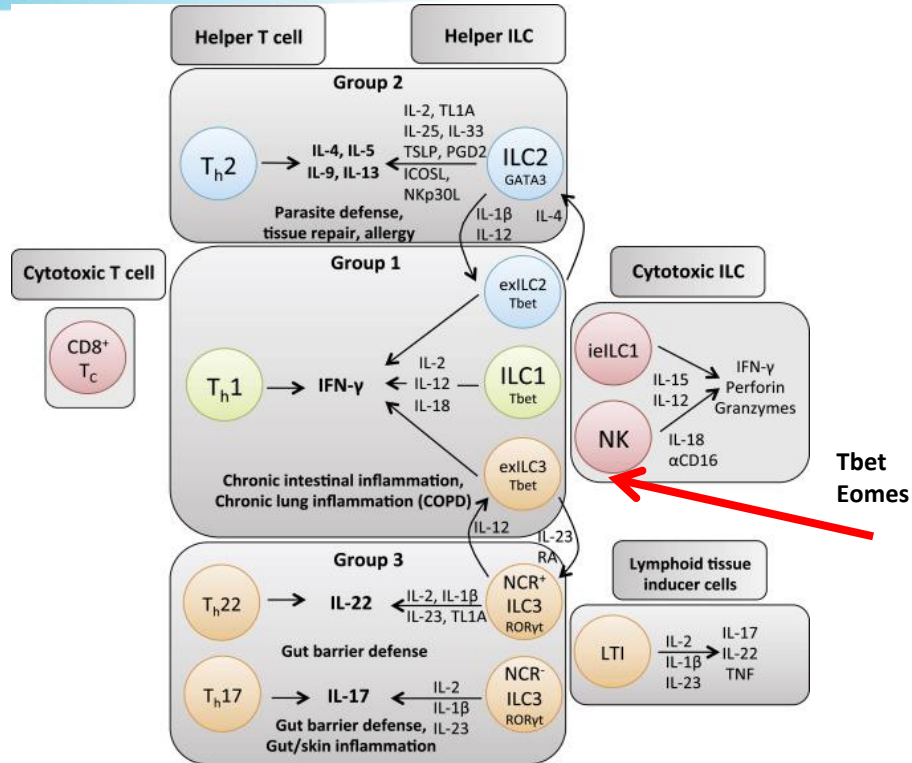
Ikerbasque Research Professor

Biocruces Bizkaia Health Research Institute

Basque Center for Blood Transfusion and Tissues

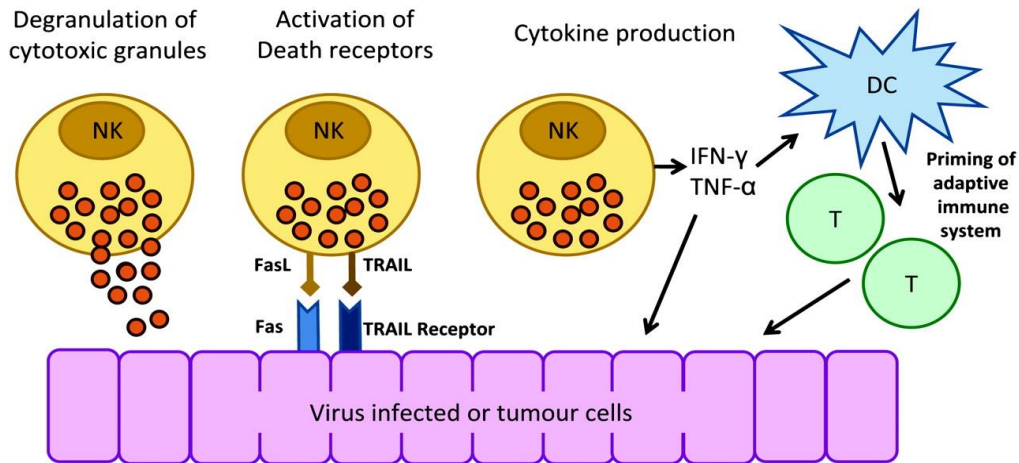


Schreiber RD, et al.

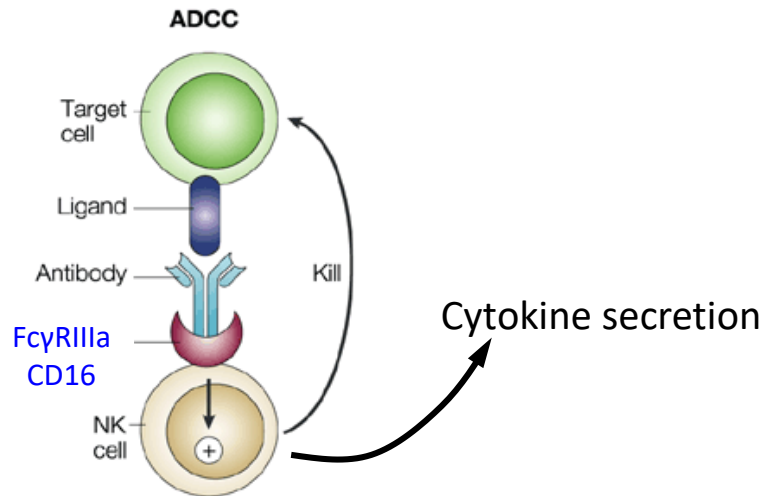


- Innate Immune System\*
- Classification: **Innate Lymphoid cells (ILCs)**
  - Counterpart of T lymphocytes
  - ILC1 Group
  - Classification based on cytokine production and transcription.
- Lack of specific antigen receptors

# MECHANISMS OF KILLING



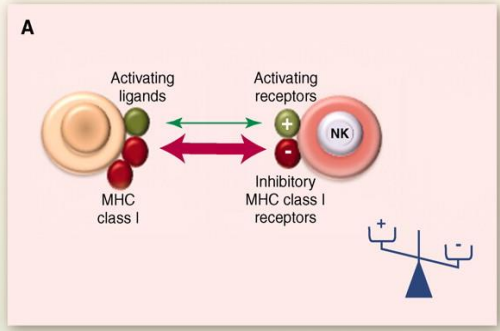
Adapted from Cheent K, et al.



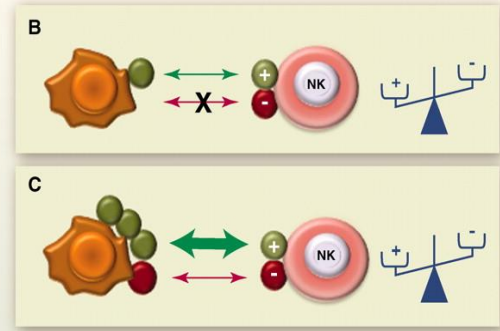
Adapted from Yokoyama WM, et al.

# Dynamic Regulation

NK cell effector function  
 ↙ NK cell tolerance  
 ↘ NK cell activation



Healthy cells



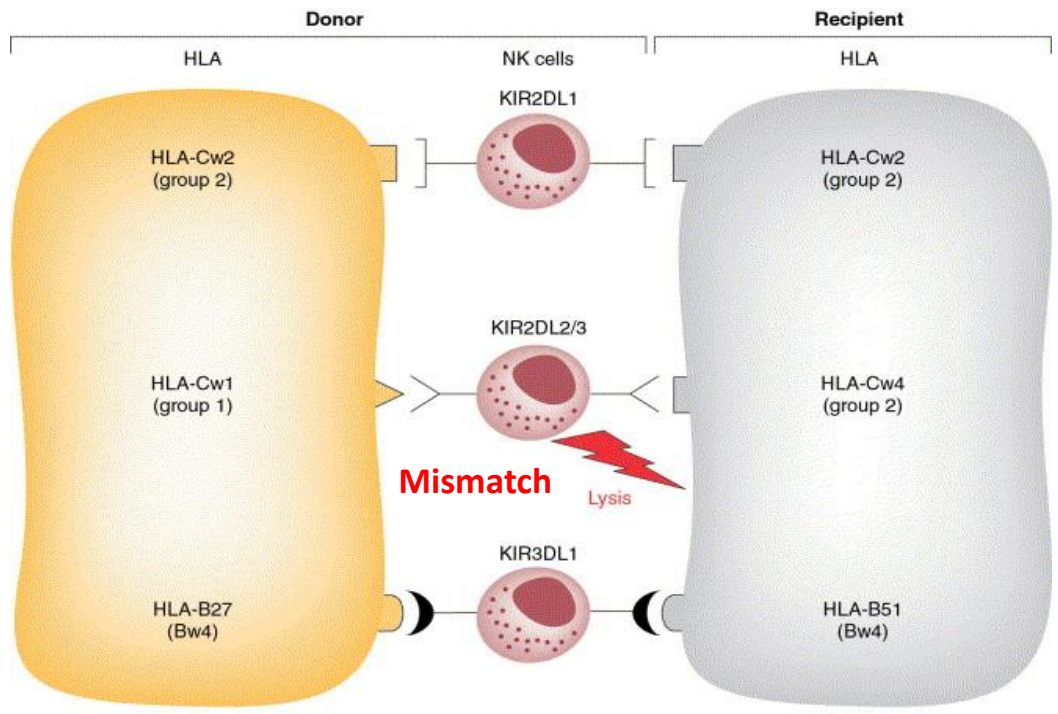
Cells "in distress"

Activating receptors		Inhibitory receptors	Chemotactic receptors
<b>Receptors</b>	<b>Adaptors</b>	h KIR-L h LILRB1 CD94/NKG2A m Inh. Ly49 m NKR-P1B m NKR-P1D KLRG-1 TIGIT CEACAM-1 LAIR-1	CCR2 CCR5 CCR7 CXCR1 CXCR3 CXCR4 CXCR6 CX3CR1 h Chem23R S1P5
NKp46 CD16 h NKp30 h NKp44 h NKp80 m NKR-P1C NKG2D m NKG2D-S h KIR-S m Act. Ly49 CD94/NKG2C CRACC Ly9 CD84 NTBA 2B4	FcγR CD3ζ, FcγR CD3ζ, FcγR DAP12 - FcγR DAP10 DAP12 DAP12 DAP12, DAP10 DAP12 SAP, EAT2 SAP SAP, EAT2 SAP SAP, EAT2, ERT		
			<b>Adhesion receptors</b>
			CD2 DNAM-1 β1 integrins β2 integrins
			<b>Cytokine receptors</b>
			IL-1R IL-2R IL-12R IL-15R IL-18R IL-21R IFNAR

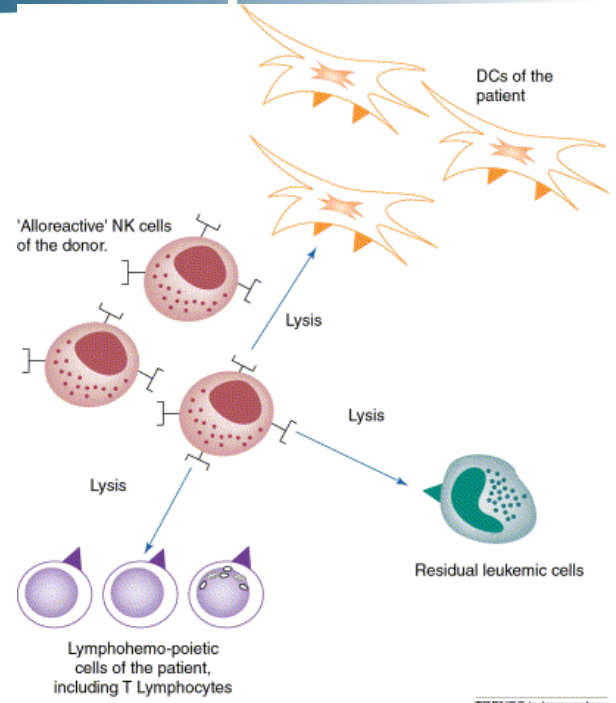
Vivier E, et al.



# Beneficial Effect of NK Cells on Haploidentical HSC Transplantation



TRENDS in Immunology

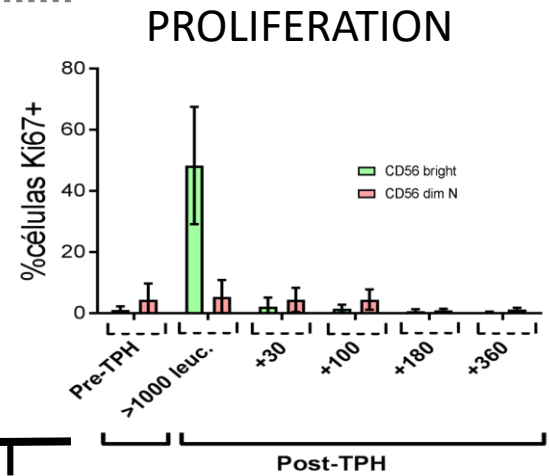
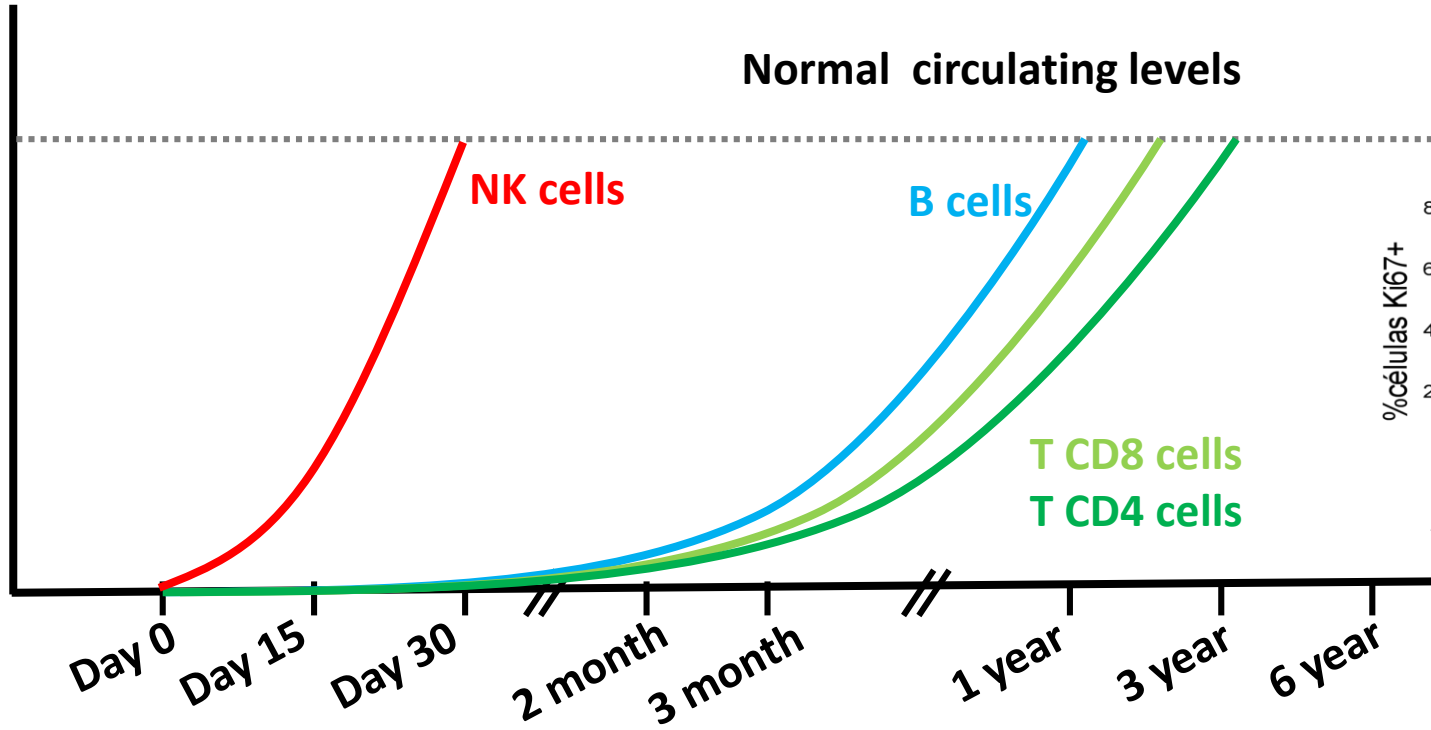


TRENDS in Immunology

Graft vs Leukemia  
Graf vs Host

**YES**  
**NO**

# Immune Reconstitution after HSC Transplantation



Orrantia A, et al.

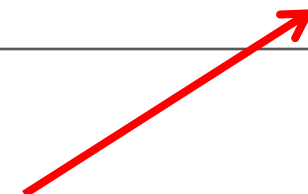
Adapted from Porrata LF, et al.

# Effectiveness of NK Cell Alloreactivity in mismatched HSC Transplantation

KIR ligand incompatibility in GVH direction	No	Yes
Number of transplants	58	34
Donors displaying antirecipient NK clones	1/58	34/34*
Disease		
ALL	21	14
AML	37	20
Transplantation outcomes		
Rejection	15.5%	0%*
Acute GVHD, $\geq$ grade II	13.7%	0%*
Probability of relapse at 5 years		
ALL	90%	85%
AML	75%	0%**

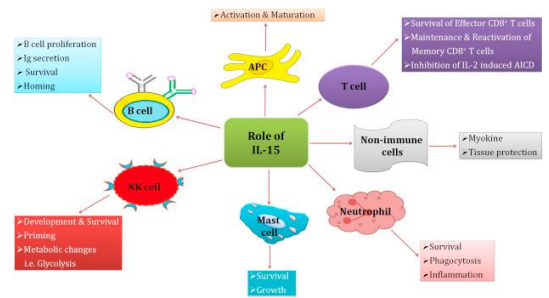
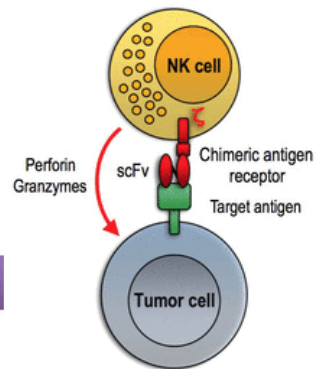
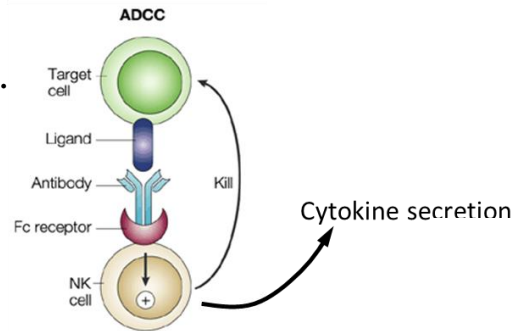
$P \leq 0.01$ ; \*\* $P < 0.0008$  (22).

Ruggeri L, et al.



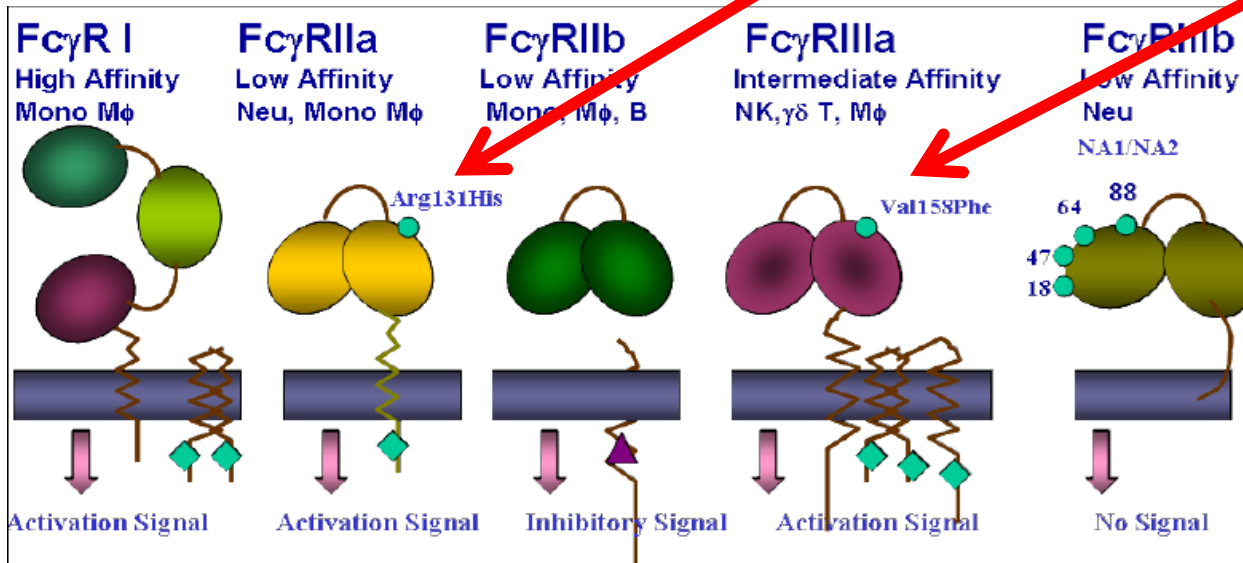
# NK Cells in Tumor Immunotherapy

- Via antibody-cell dependent mediated cytotoxicity (ADCC).
  - Systemic administration.
- Autologous NK cells.
  - Expansion *in vivo* with cytokines (i.e. IL-15).
  - Adoptive transfer after activation/expansion *ex vivo*.
  - iPSC derived NK cells.
- Allogenic NK cells.
  - Adoptive transfer after activation/expansion *ex vivo* or unstimulated donor NK cells.
  - Expansion *in vivo* after transfer.
- “Off-the-shelf”.
  - NK cell lines: NK-92.
  - iPSC derived NK cells.
- Genetic modifications of NK cells.
  - Cytokine transgenes, CARs.

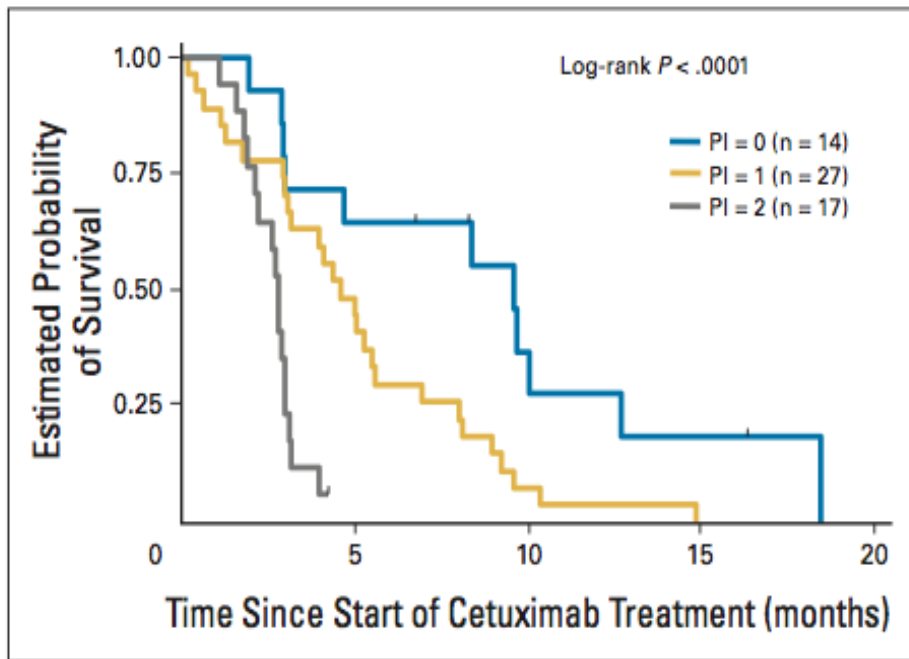


# Fcγ Receptors (FcγR)

- Phagocytosis
- ROS/NO
- Cytokines/  
Chemokines
- Increase APC Fx
- Phagocytosis
- ROS/NO
- Cytokines/  
Chemokines
- Inhibitory  
signaling
- IC mediated  
phagocytosis
- Antibody-  
dependent cellular  
cytotoxicity (ADCC)
- IC mediated  
phagocytosis



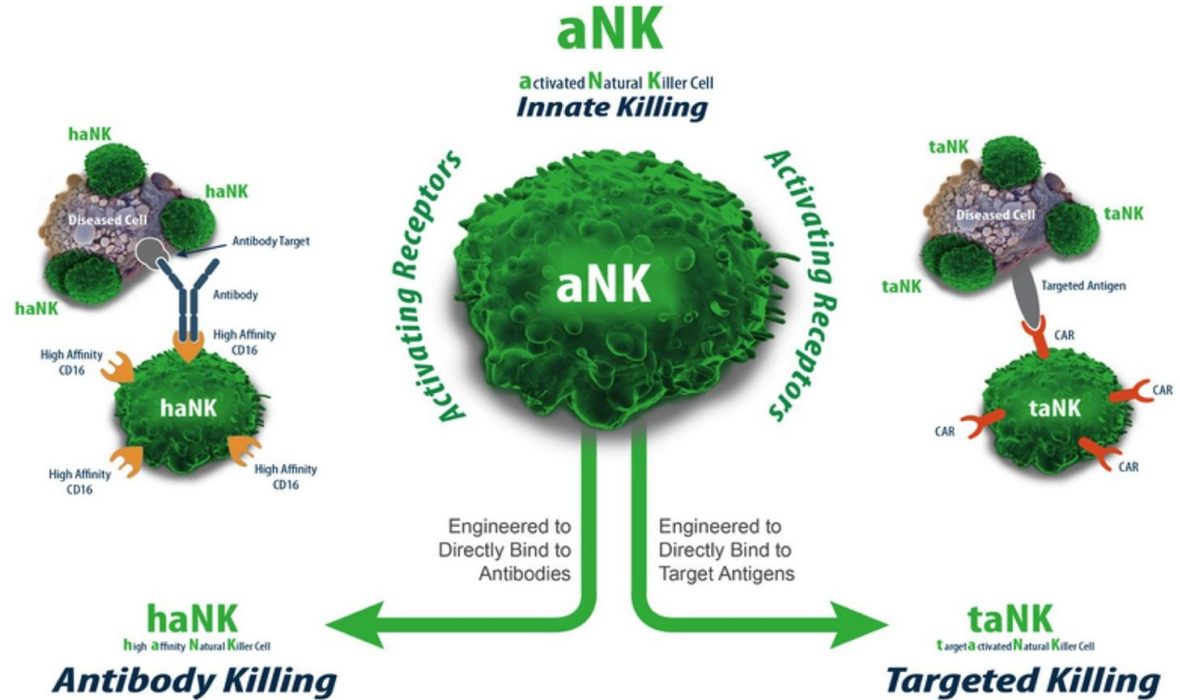
# FcγR polymorphisms are Prognostic Factors



- **PI=0**: Two favorable prognostic factors (wt-KRAS and FcγRIIa-131H/H and/or FcγRIIIa-158V/V genotypes).
- **PI=1**: One favorable prognostic factor (ie, wt-KRAS and FcγRIIa-131R and FcγRIIIa-158F genotypes; mutated KRAS and FcγRIIa-131H/H and/or FcγRIIIa-158V/V genotypes).
- **PI=2**: No favorable prognostic factors (ie, mutated KRAS and FcγRIIa-131R and FcγRIIIa-158F genotypes).

# NK cells and NK cell lines in adoptive cell therapy

- Reduced risk of cytokine storm
- NK cell lines (NK-92) and iPSC derived NK cells:
  - Large supply
  - Off-the-shelf
  - Relatively easy to engineer
  - Reduced prize
  - Less toxicity



Klingemann et al.

# CAR Expressing NK Cells

	CAR-NK cells	CAR-T cells
Sources	Cord blood, peripheral blood, iPSC, cell lines	Cord blood, peripheral blood, iPSC
Expansion	Flasks or bag-based expansion systems with cytokines (IL-2, IL-12, IL-15, IL-18, IL-21); or feeder cell lines (engineered K562 cells)	Flasks or bag-based expansion systems with cytokines (IL-2 or IL-7)
Use	Autologous; allogeneic possible	Autologous; allogeneic with MHC match
Engineering methods	Viral transduction, electroporation/nucleofection, nanoparticles, trogocytosis	Viral transduction, electroporation/nucleofection, nanoparticles, trogocytosis
Transfection efficiencies	Low even with viral vectors	Higher than for NK cells
Adoptive transfer considerations	Limited persistence	GvHD Cytokine storm Suicide genes needed

Clinical trial identifier	NK cell source	Target antigen	Disease	Study location
NCT02944162	NK-92 cell line	CD33	AML	China
NCT02892695	NK-92 cell line	CD19	CD19 positive B cell malignancies	China
NCT02742727	NK-92 cell line	CD7	CD7 positive leukemia or lymphoma	China
NCT02839954	NK-92 cell line	MUC1	MUC1 positive solid tumors (colorectal, gastric, pancreatic, NSCLC, breast, glioma)	China
NCT03056339	Cord blood	CD19	CD19 positive leukemia or lymphoma	MDACC, USA



good safety profile

Tang X, et al.

AML, acute myeloid leukemia; MDACC, MD Anderson Cancer Center; NK, natural killer; NSCLC, non-small cell lung cancer.

### T cell- and B cell-independent adaptive immunity mediated by natural killer cells

Jacqueline G O'Leary<sup>1-3</sup>, Mahmoud Goodarzi<sup>1,3</sup>, Danielle L Drayton<sup>1,3</sup> & Ulrich H von Andrian<sup>1</sup>

It is commonly believed that only T lymphocytes and B lymphocytes expressing recombination-dependent antigen-specific receptors mediate contact hypersensitivity responses to haptens. Here we found that mice devoid of T cells and B cells demonstrated substantial contact hypersensitivity responses to 2,4-dinitrofluorobenzene and oxazolone. Those responses were adaptive in nature, as they persisted for at least 4 weeks and were elicited only by haptens to which mice were previously sensitized. No contact hypersensitivity was induced in mice lacking all lymphocytes, including natural killer cells. Contact hypersensitivity responses were acquired by such mice after adoptive transfer of natural killer cells from sensitized donors. Transferable hapten-specific memory resided in a Ly49C-I<sup>+</sup> natural killer subpopulation localized specifically in donor livers. These observations indicate that natural killer cells can mediate long-lived, antigen-specific adaptive recall responses independent of B cells and T cells.

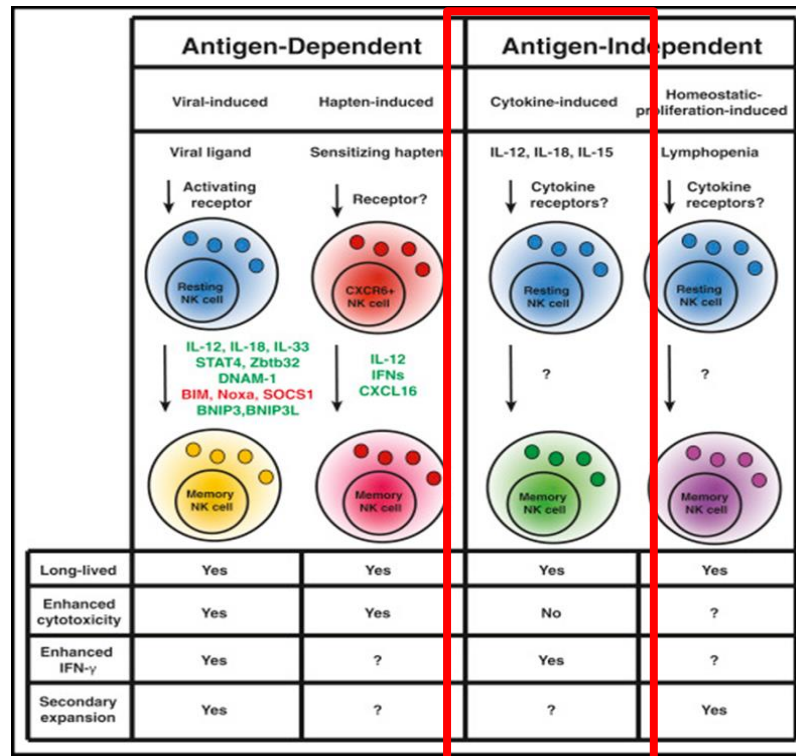
### Adaptive immune features of natural killer cells

Joseph C. Sun<sup>1</sup>, Joshua N. Beilke<sup>1</sup> & Lewis L. Lanier<sup>1</sup>

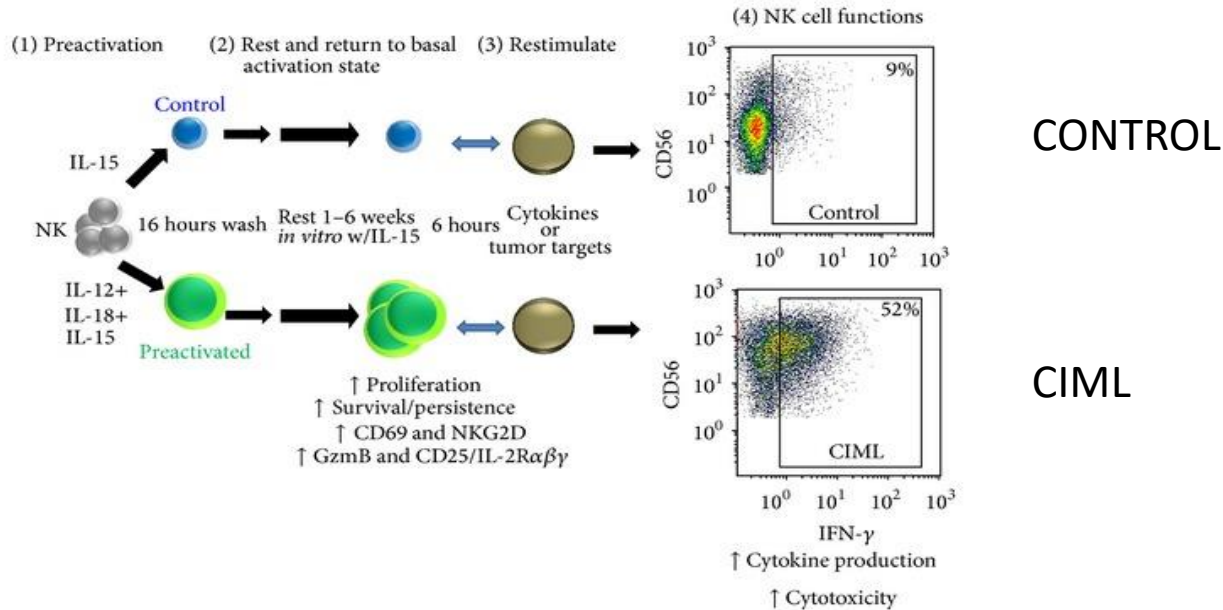
In an adaptive immune response, naive T cells proliferate during infection and generate long-lived memory cells that undergo secondary expansion after a repeat encounter with the same pathogen. Although natural killer (NK) cells have traditionally been classified as cells of the innate immune system, they share many similarities with cytotoxic T lymphocytes. We use a mouse model of cytomegalovirus infection to show that, like T cells, NK cells bearing the virus-specific Ly49H receptor proliferate 100-fold in the spleen and 1,000-fold in the liver after infection. After a contraction phase, Ly49H-positive NK cells reside in lymphoid and non-lymphoid organs for several months. These self-renewing 'memory' NK cells rapidly degranulate and produce cytokines on reactivation. Adoptive transfer of these NK cells into naive animals followed by viral challenge results in a robust secondary expansion and protective immunity. These findings reveal properties of NK cells that were previously attributed only to cells of the adaptive immune system.

# Adaptive (memory) NK Cells

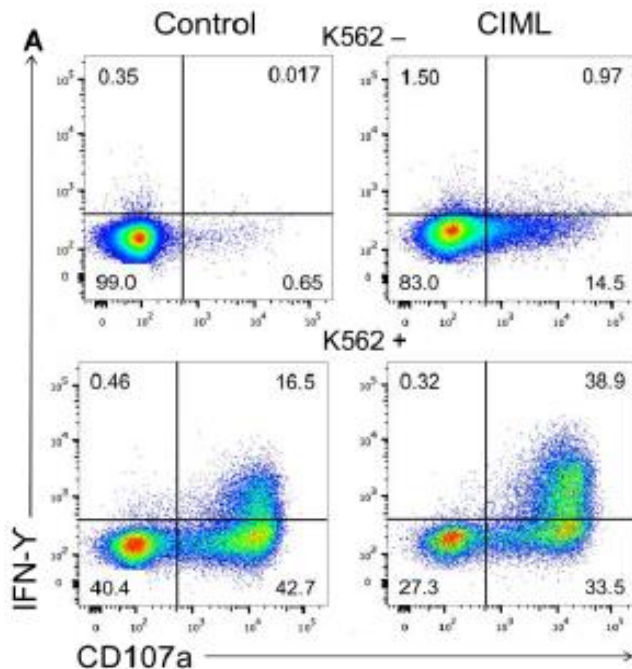
- Cytokine-Induced Memory-Like NK cells or **CIML NK cells**.
- CIML NK cells are characterized by increased effector functions after a resting period.



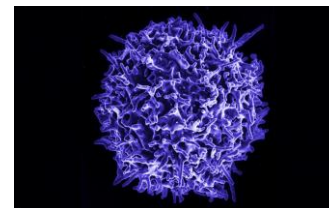
# CIML NK Cells



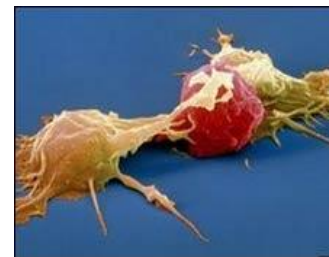
# CIML NK Cells



WITHOUT  
TUMOR CELLS

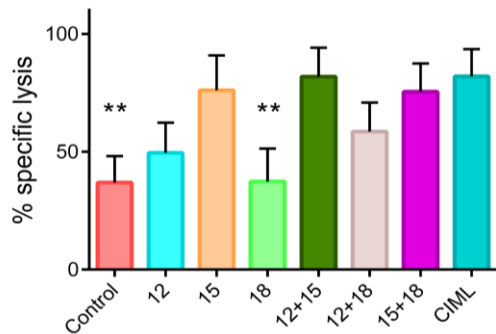


WITH TUMOR  
CELLS

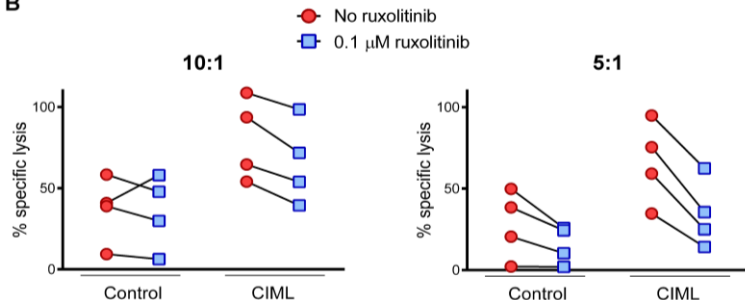


Odriozola I, et al.

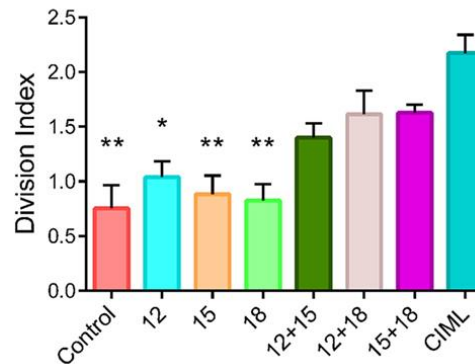
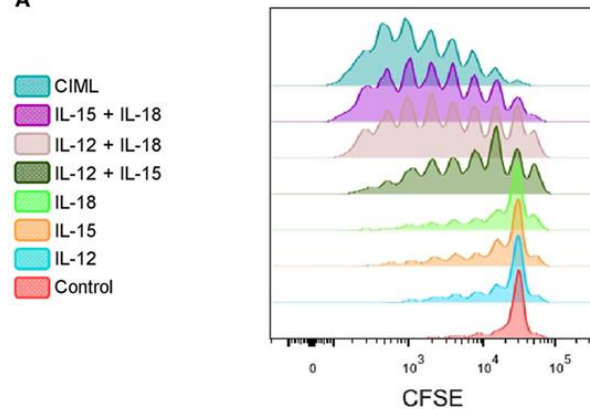
A



B



A



Terrén I, et al.



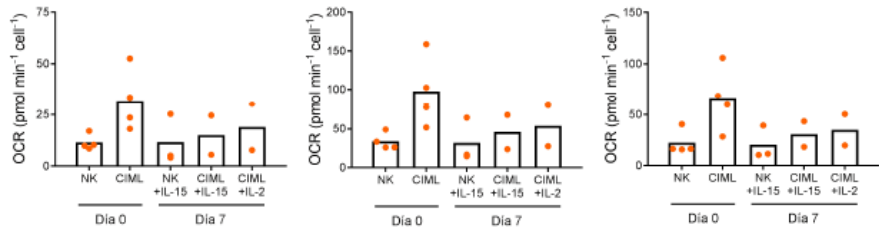


# CIML NK Cells

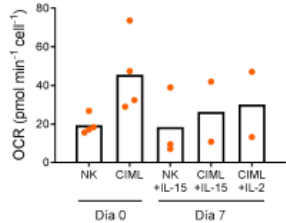
## Basal Respiration

## Maximal Respiration

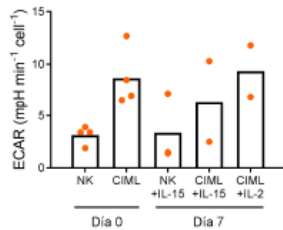
## Spare Capacity



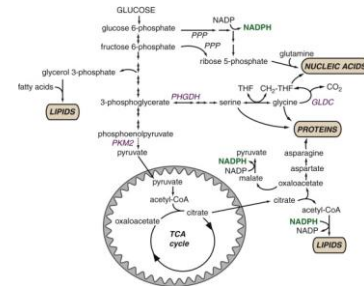
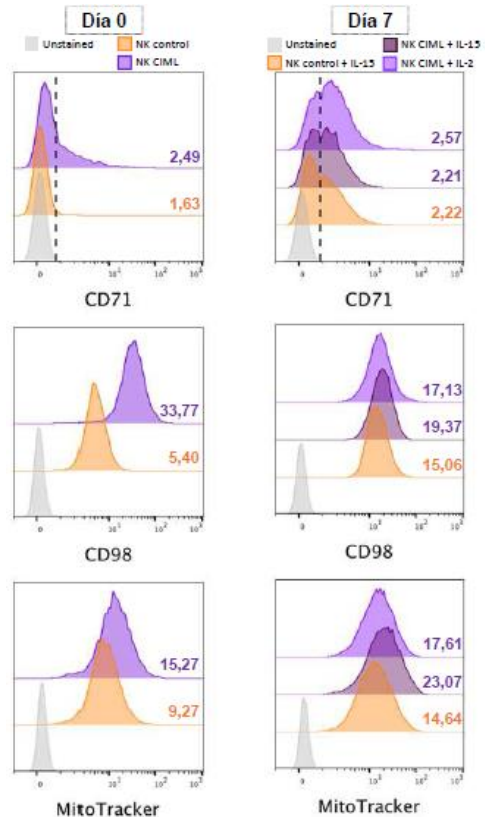
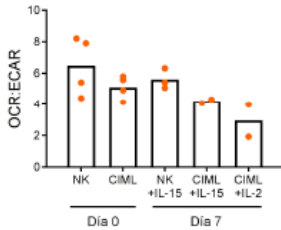
## OCR



## ECAR



## ratio OCR:ECAR



Terrén I, et al.

# Clinical Trials with CIML NK Cells

Day -1: HLA-  
Haploidentical donor  
leukapheresis



NK cell purification



rhIL-12 +  
rhIL-15 +  
rhIL-18



Cell dose levels:  
1:  $0.5 \times 10^6$ /kg  
2:  $1.0 \times 10^6$ /kg  
3: Maximum generated  
(capped at  $10 \times 10^6$  / kg)

Patient



Infuse CIML NK cells



Fludarabine  $25\text{mg}/\text{m}^2 \times 5$   
Cyclophosphamide  $60\text{ mg}/\text{kg} \times 2$

Day 0

In vivo  
Expansion  
Low Dose IL-2  
qOD x 2 weeks

Monitor for:

- Adverse events
- Response
- CIML NK cell
  - Persistence
  - Proliferation
  - Function

RESEARCH ARTICLE

CANCER

## Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia

Rizwan Romee,<sup>1\*</sup> Maximilian Rosario,<sup>1,2\*</sup> Melissa M. Berrien-Elliott,<sup>1\*</sup> Julia A. Wagner,<sup>1</sup> Brea A. Jewell,<sup>1</sup> Timothy Schappe,<sup>1</sup> Jeffrey W. Leong,<sup>1</sup> Sara Abdel-Latif,<sup>1</sup> Stephanie E. Schneider,<sup>1</sup> Sarah Willey,<sup>1</sup> Carly C. Neal,<sup>1</sup> Liyang Yu,<sup>3</sup> Stephen T. Oh,<sup>3</sup> Yi-Shan Lee,<sup>2</sup> Arend Mulder,<sup>4</sup> Frans Claas,<sup>4</sup> Megan A. Cooper,<sup>5</sup> Todd A. Fehniger<sup>1†</sup>

# Clinical Trials with CIML NK Cells

UPN	Dose level	Gender	Age (years)	WHO diagnosis	Number of previous therapies	Pretreatment BM blast (%)	KIR-ligand mismatch	IWG response	DLT	GVHD
001	1	M	73	M2	2	16	Yes	TF-PD	No	No
006	1	M	70	M0	3	28	Yes	TF-PD	No	No
007	1	M	77	M0	1	47	Yes	CR	No	No
008	2	M	76	t-AML	3	17	Yes	TF-PD	No	No
009	2	F	73	M1	3	80	No	MLFS	No	No
012	2	F	71	M5	3	15	Yes	CR	No	No
017	3	M	64	t-AML	3	69	Yes	TF-PD	No	No
019	3	F	71	M5	4	15	Yes	CR	No	No
020	3	M	60	MDS-AML	1	13	Yes	CRI	No	No

DLT: dose-limiting toxicity.

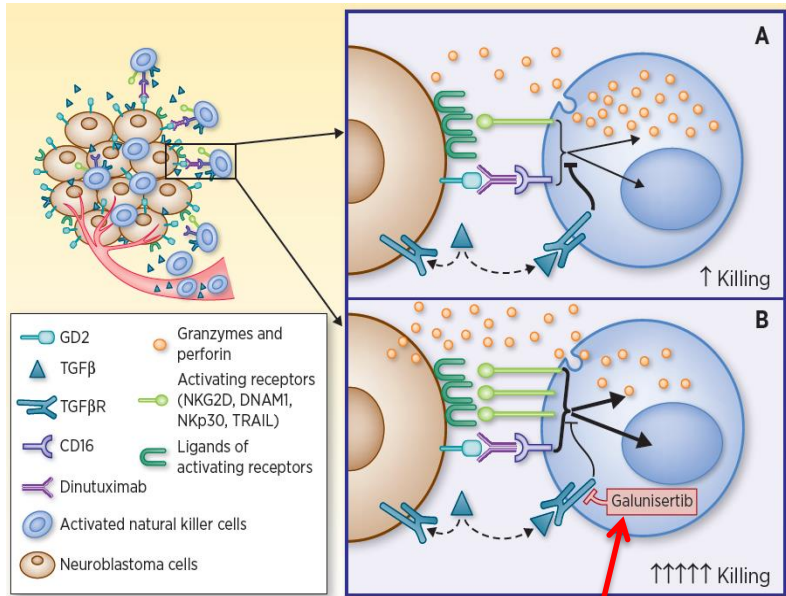
TF-PD: treatment failure due to progressive disease.

CR: complete remission.

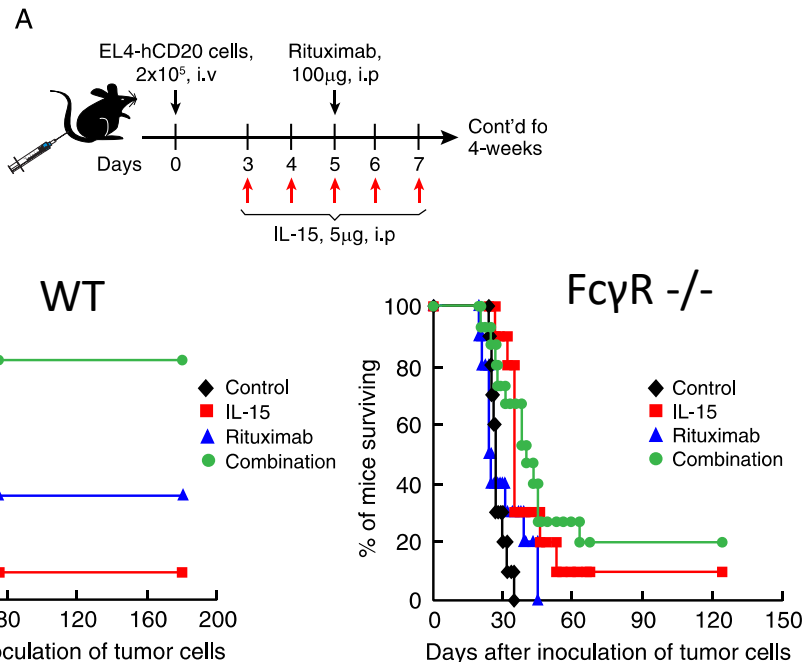
MLFS: morphologic leukemia-free state.

Cri: CR with incomplete blood count recovery.

# Combination Therapy with NK Cells

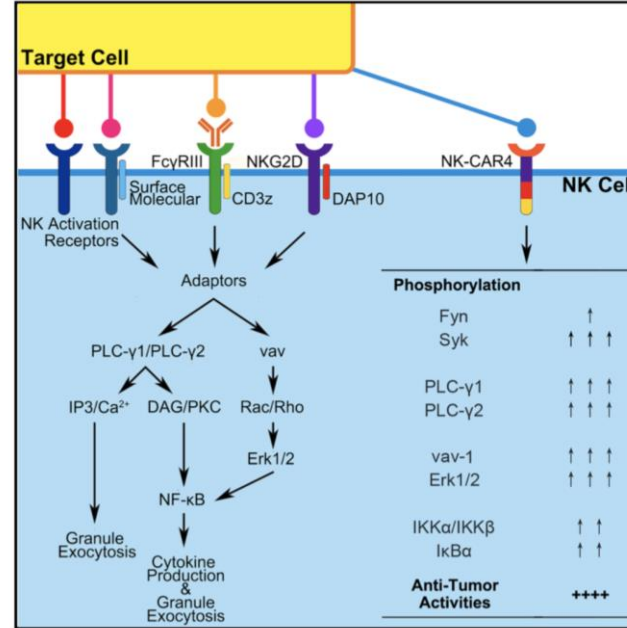
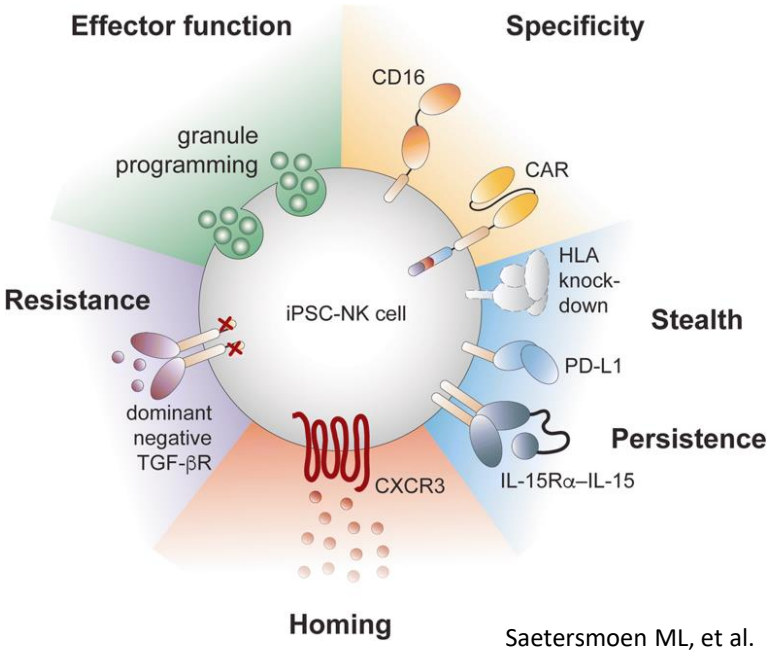


Zenarruzabeitia O, et al.



Zhang M, et al.

# Engineering of a Synthetic Killer



- Activity similar to that of CAR-expressing T cells.
- Less toxicity.



## Immunopathology Group

## Collaborations

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Ane Orrantia  
Laura Santos-Díaz  
Iñigo Terrén  
Joana Vitallé  
Olatz Zenarruzabeitia

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Bioef  
Biodonostia  
CIC BiomaGune  
CVTTH  
IBiS  
IdiPaz  
CSIC  
FDA  
NIH



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# ikerbasque

