

Inmunoterapia basada en células NK

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- 1. NK cell: the interface between innate and adaptive**
- 2. Missing self hypothesis**
- 3. Induced self hypothesis**
- 4. Memory induced NK cells**
- 5. The T-NK approach**

NK cell: the interface between innate and adaptive

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Rolf Kiessling (Karolinska Institute)



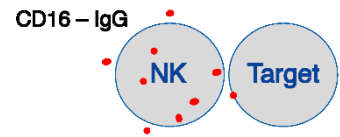
Ronald B Herberman 1940-2013 (NCI)



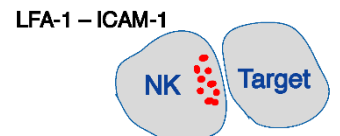
"Natural" killer cells in the mouse. II. Cytotoxic cells with specificity for mouse Moloney leukemia cells. Characteristics of the killer cell.

[Kiessling R](#), [Klein E](#), [Pross H](#), [Wigzell H](#).
Eur J Immunol. 1975 Feb;5(2):117-21.

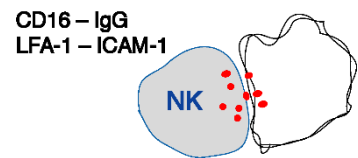
Natural cytotoxic reactivity of mouse lymphoid cells against syngeneic and allogeneic tumors. I. Distribution of reactivity and specificity. [Herberman RB](#), [Nunn ME](#), [Lavrin DH](#).
Int J Cancer. 1975 Aug 15;16(2):216-29.



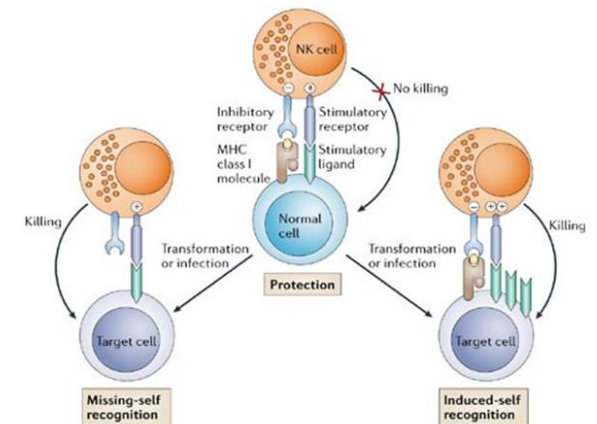
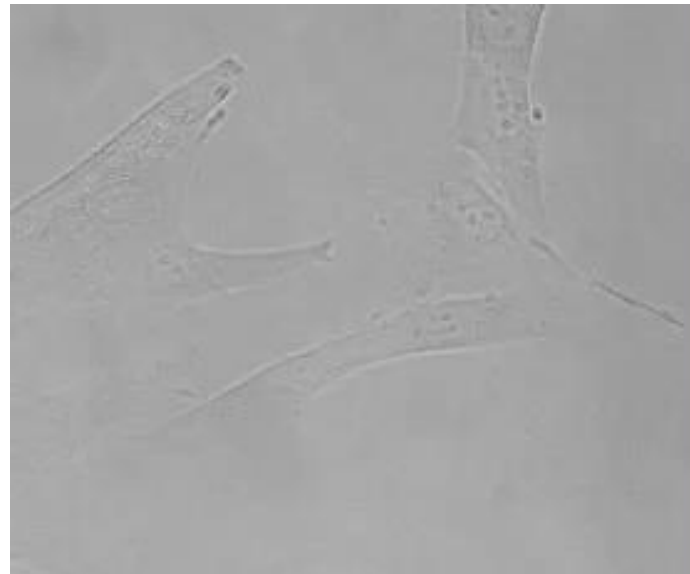
Degranulation



Adhesion, granule polarization

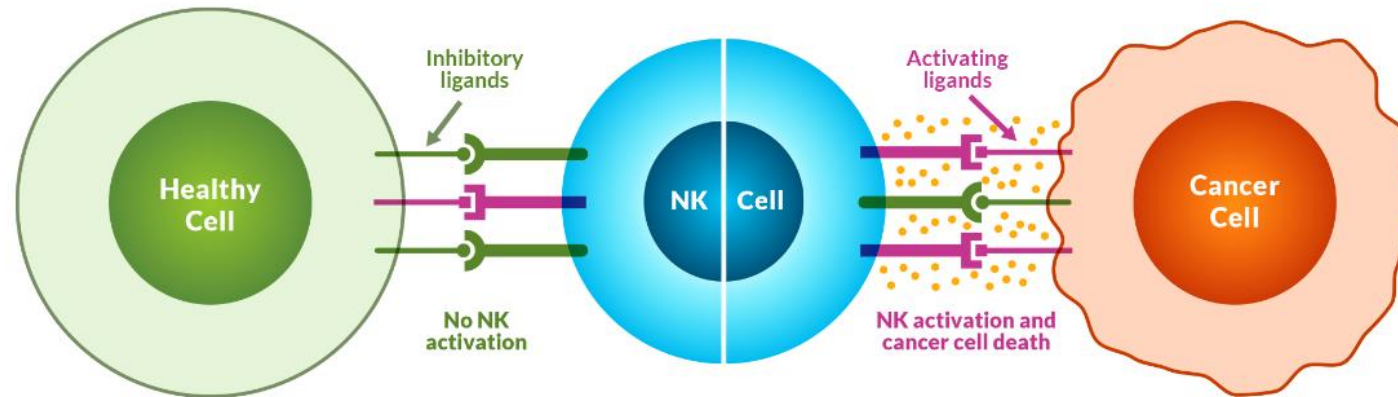


Adhesion, granule polarization, degranulation, and efficient target lysis



Bryceson *et al.* (2005) *J Exp Med*
Bryceson *et al.* (2009) *Blood*

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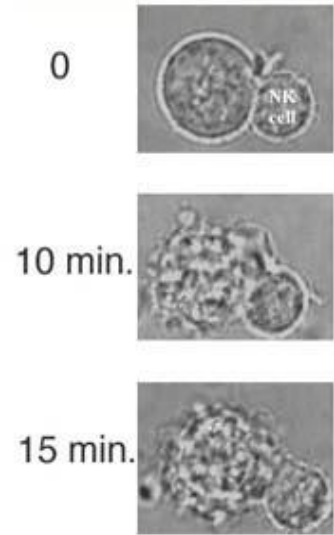
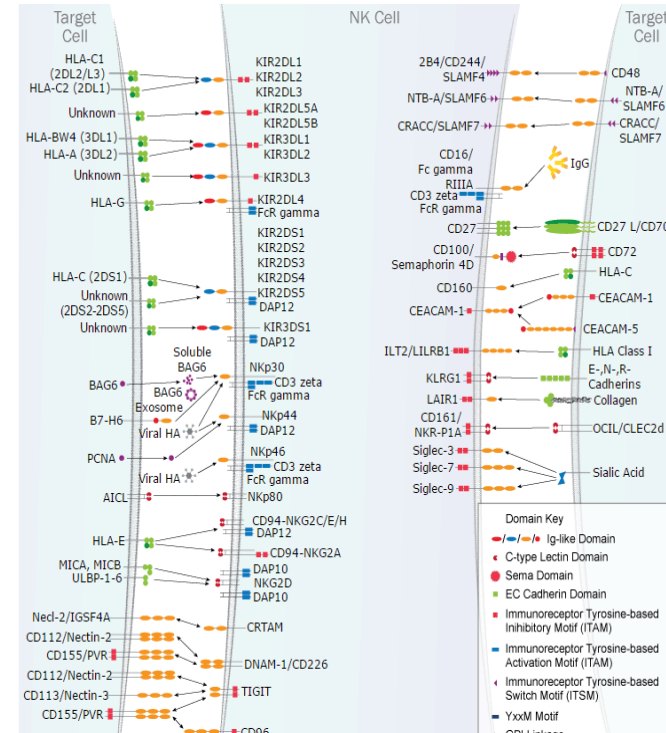
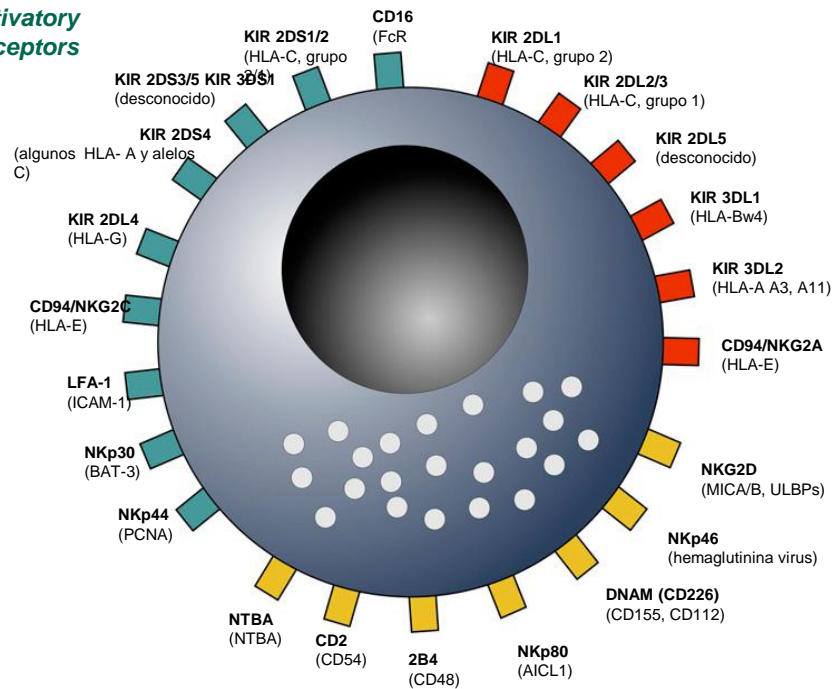
NK cells are the body's first line of defense against infections and diseases with an innate ability to rapidly seek and destroy abnormal cells.

NK cell therapy has the potential to

- ✓ Target multiple pathogenic antigens with measurably more efficient cytotoxicity
- ✓ Be better controlled to reduce risk of cytokine storms
- ✓ Be produced from a variety of sources without relying on patient-specific immune cells

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Activatory Receptors



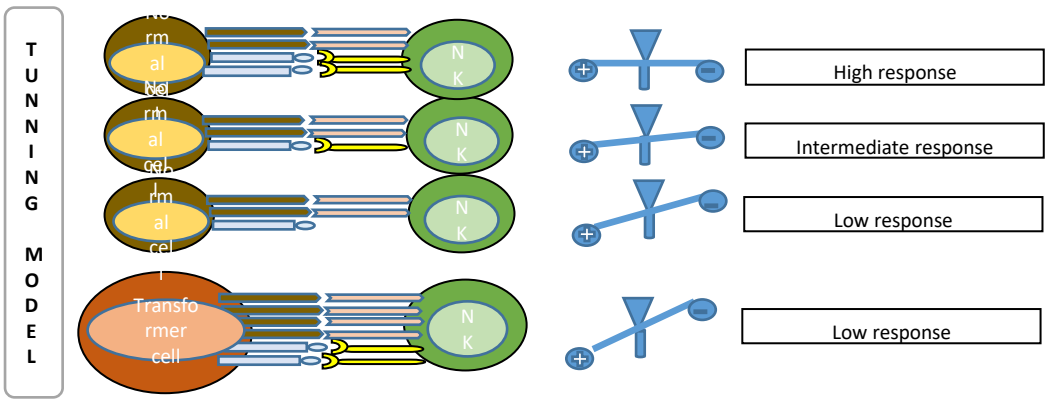
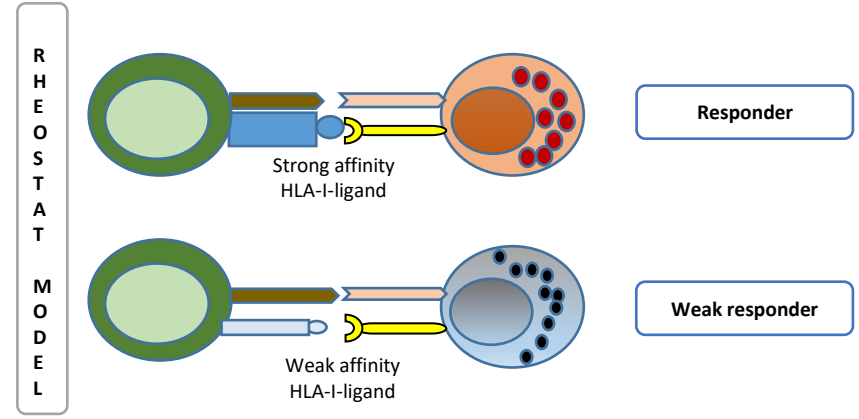
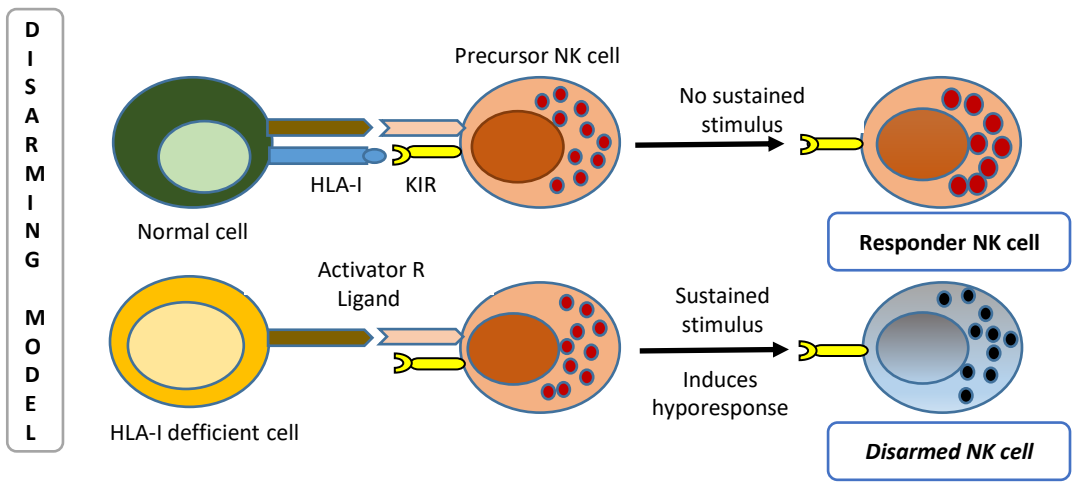
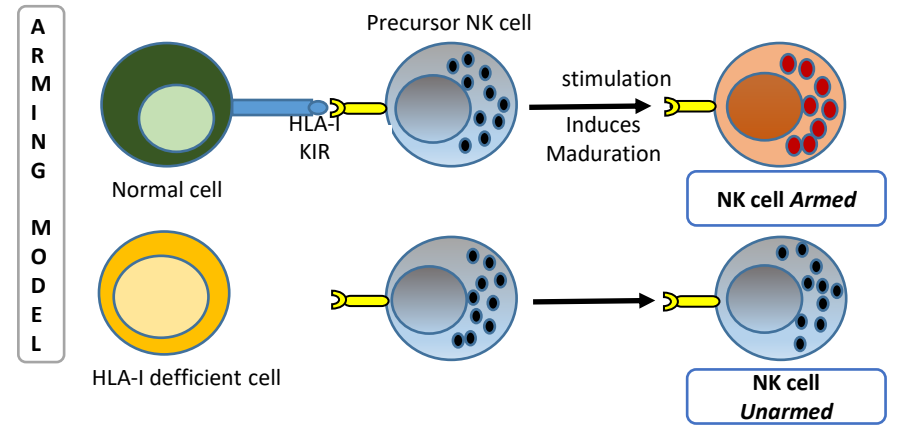
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Nature Reviews | Immunology

- ✓ These functions are performed in the context of a learning process ("licensing") regulated mainly by inhibitory KIR receptors and their ligands (HLA class I molecules, in humans).
- ✓ In a basal situation the cells of the different tissues express their own ligands (self), HLA class I, so they are protected.

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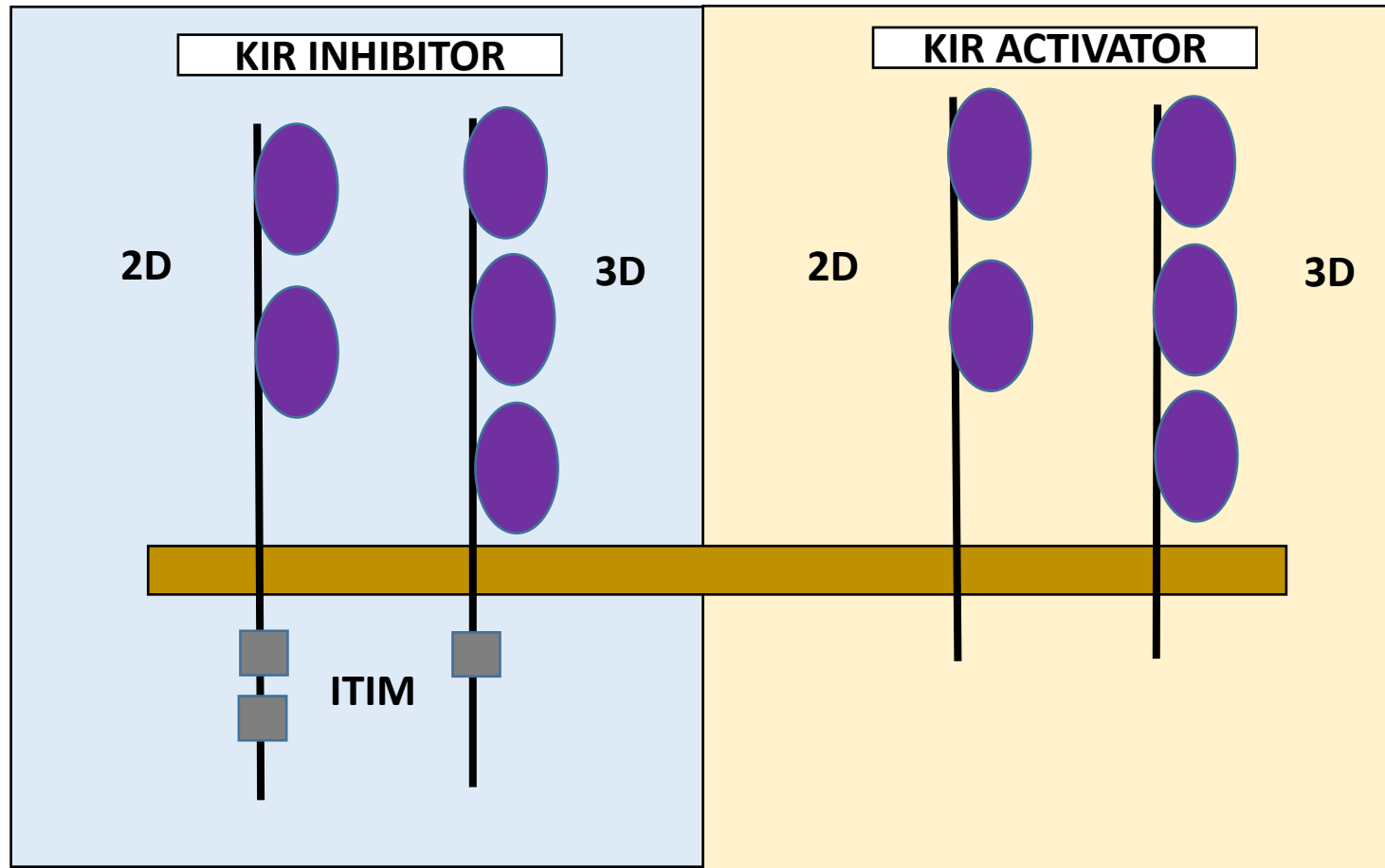
THEORIES

- Arming Model
- Disarming Model
- Rheostat Model
- Tuning Model

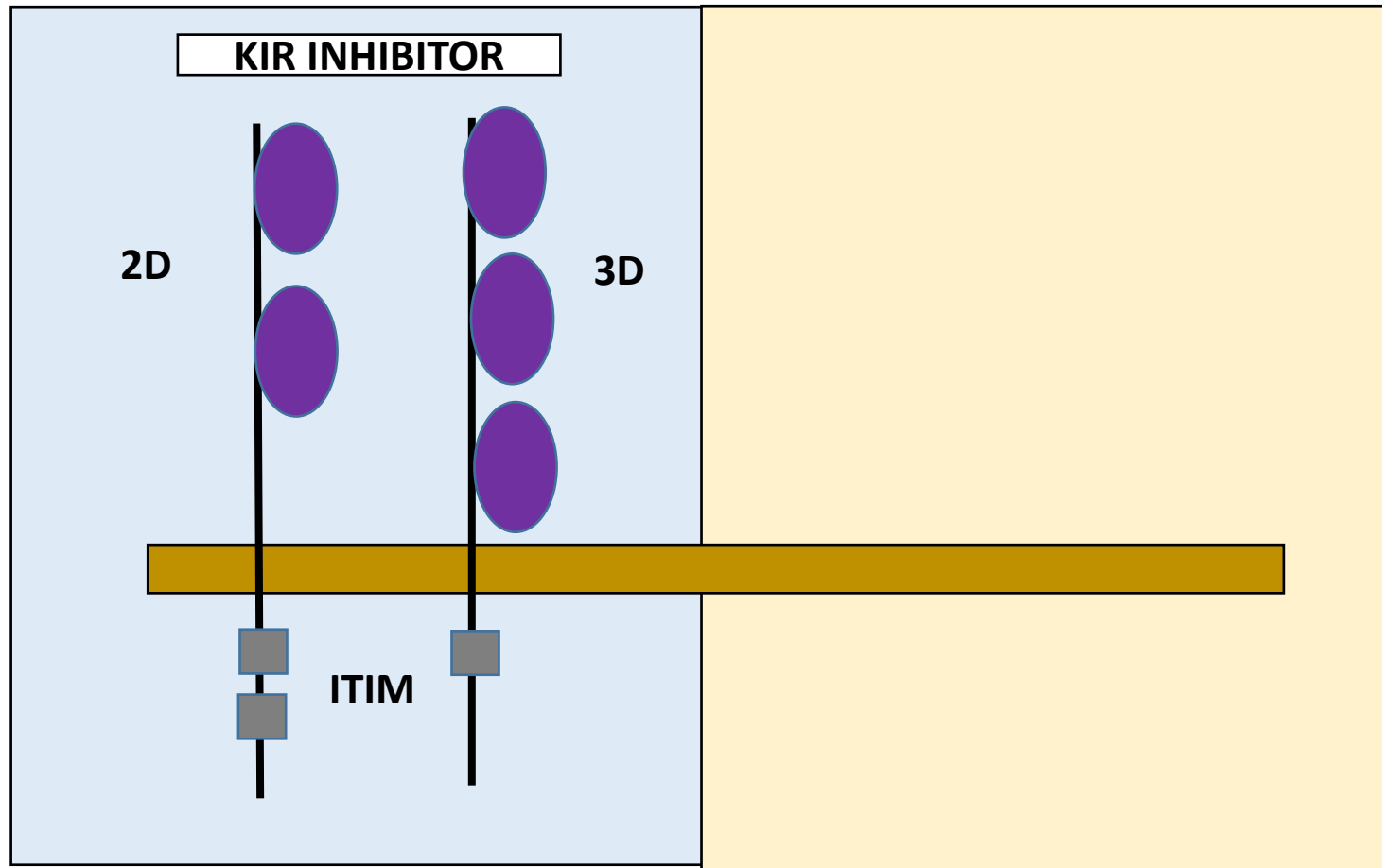


2. Missing self hypothesis

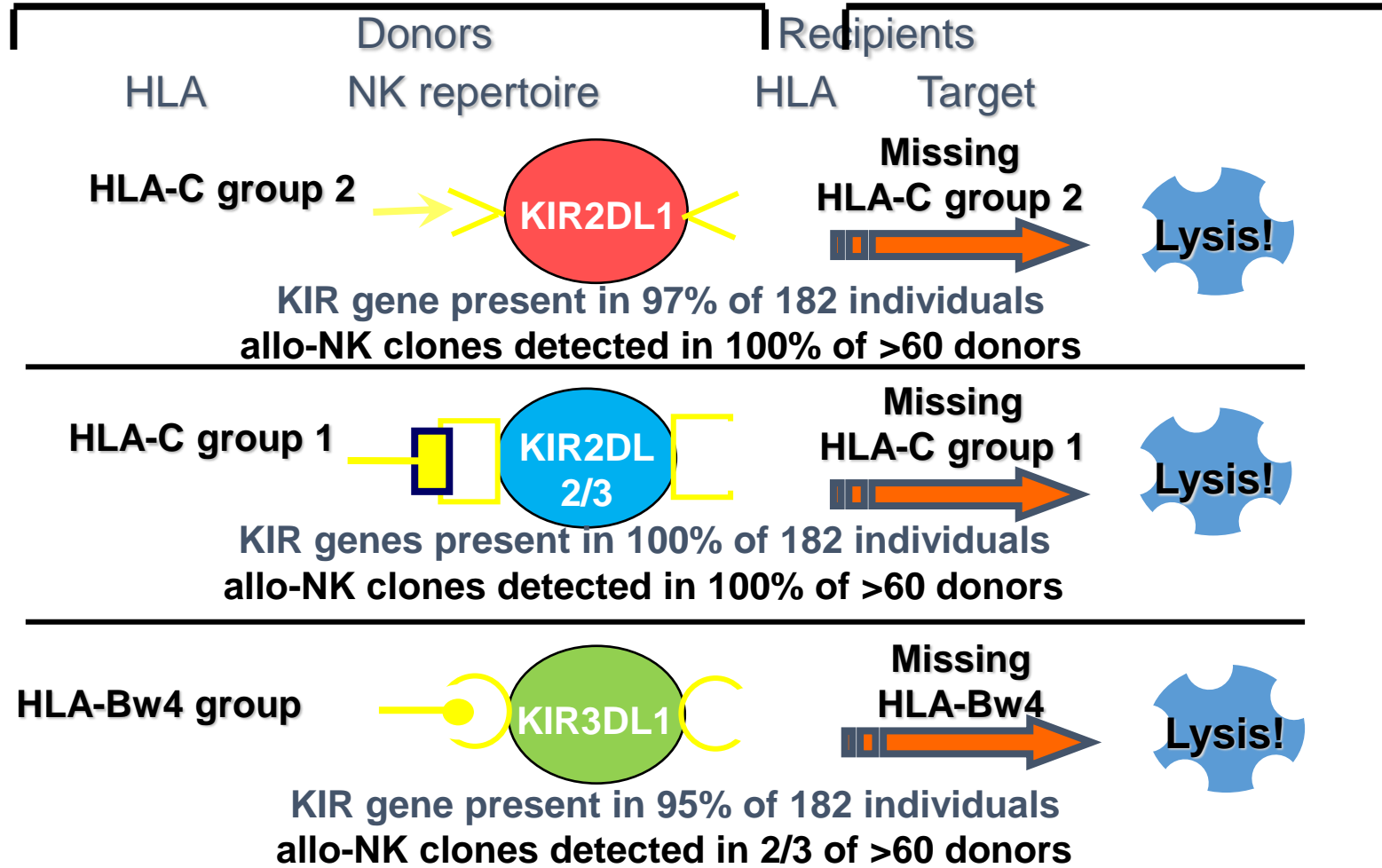
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Inmunoterapia basada en células NK



Inmunoterapia basada en células NK



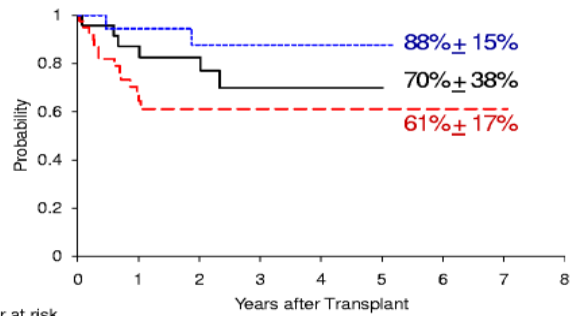
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Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants

Loredana Ruggeri,¹ Marusca Capanni,¹ Elena Urbani,¹ Katia Perruccio,¹ Warren D. Shlomchik,² Antonella Tosti,¹ Sabrina Posati,¹ Daniela Rogaia,¹ Francesco Frassoni,³ Franco Aversa,¹ Massimo F. Martelli,¹ Andrea Velardi^{1*}

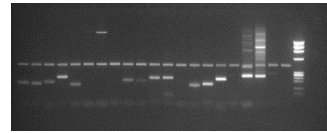
— Sibling - - - Unrelated ···· Haploidentical

Survival of Recent Cohorts

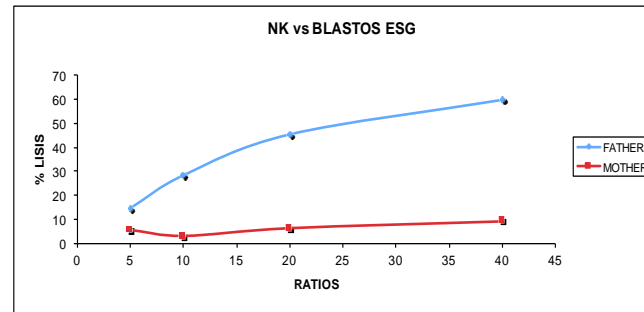
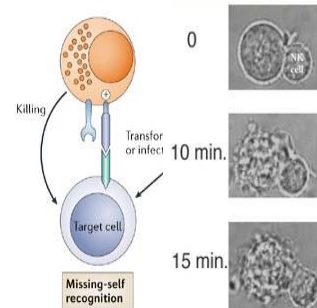
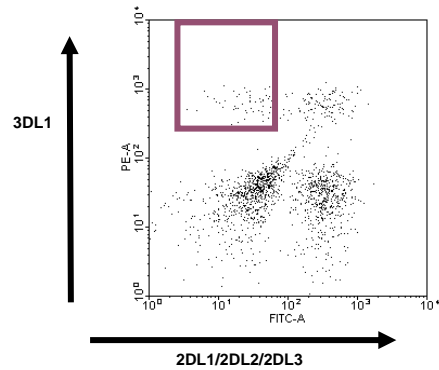


Leung W. Blood. 2011

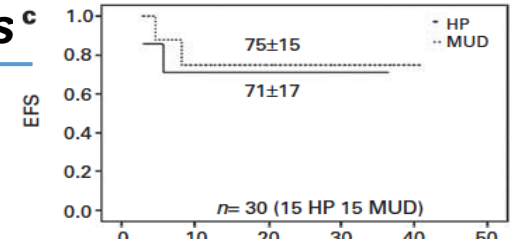
NK cell alloreactivity based in *missing self hypothesis*^c



	HLA-B	HLA-C	Missing ligands	KIR alloreactivity	ALL killing
Patient	08:01:01/18:01:01	05:01:01/07:01:01			
Father	18/49	05/07			
Mother	08/18	12/07			
Patient	Bw6/Bw6	Asn80/Lys80	Bw4	KIR3DL1 unlicensed	No
Father	Bw4/Bw6	Asn80/Lys80	No	KIR3DL1 licensed	Yes
Mother	Bw6/Bw6	Asn80/Asn80	Bw4/Lys80	KIR3DL1/2d1 unlicensed	Anergy
Patient	Bw6/Bw6	C1/C2	Bw4	KIR3DL1 unlicensed	No
Father	Bw4/Bw6	C1/C2	No	KIR3DL1 licensed	Yes
Mother	Bw6/Bw6	C1/C1	Bw4/C2	KIR3DL1/2d1 unlicensed	Anergy

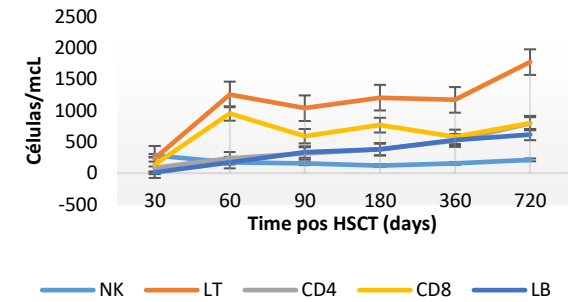


Pérez Martínez A et al. PBC 2009.

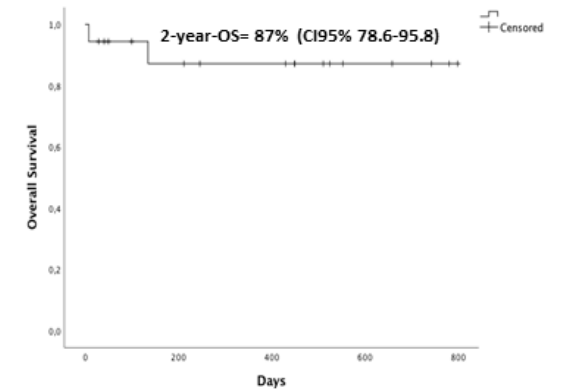


Pérez-Martínez A. BMT 2012

Immune reconstitution from KIR A donor



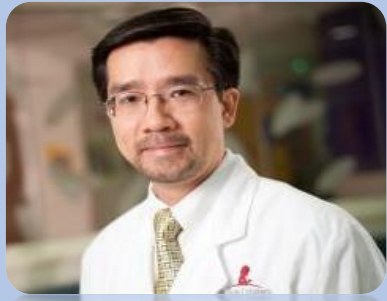
Escudero A et al. BBMT 2018.



Gassior M. Submitted 2020

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Wing Leung



- **Determinants of antileukemia effects of allogeneic NK cells.** Leung W, et al. J Immunol 2004; 172:644 – 650.
- **Comparison of killer Ig-like receptor genotyping and phenotyping for selection of allogeneic blood stem cell donors.** Leung W et al J Immunol 2005; 174:6540 – 6545

Jeffrey S Miller



- **Missing KIR ligands are associated with less reapse an increased graft-versus-host disease (GVHD) following unrelated donor allogeneic HCT.** Miller JS, Cooley S, Parham P, etal. Blood 2007; 109:5058 – 5061.

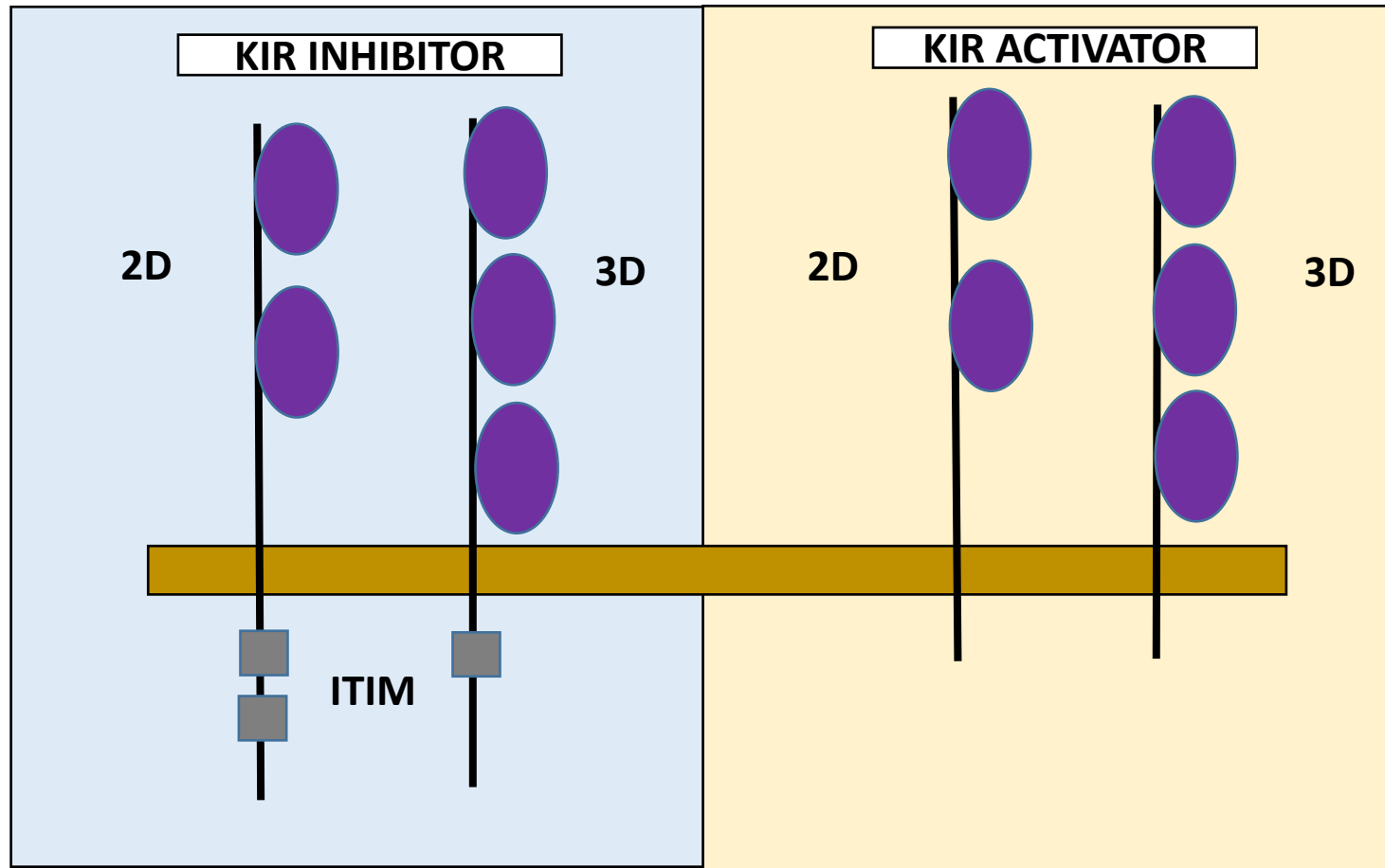
Rupert Handgretinger



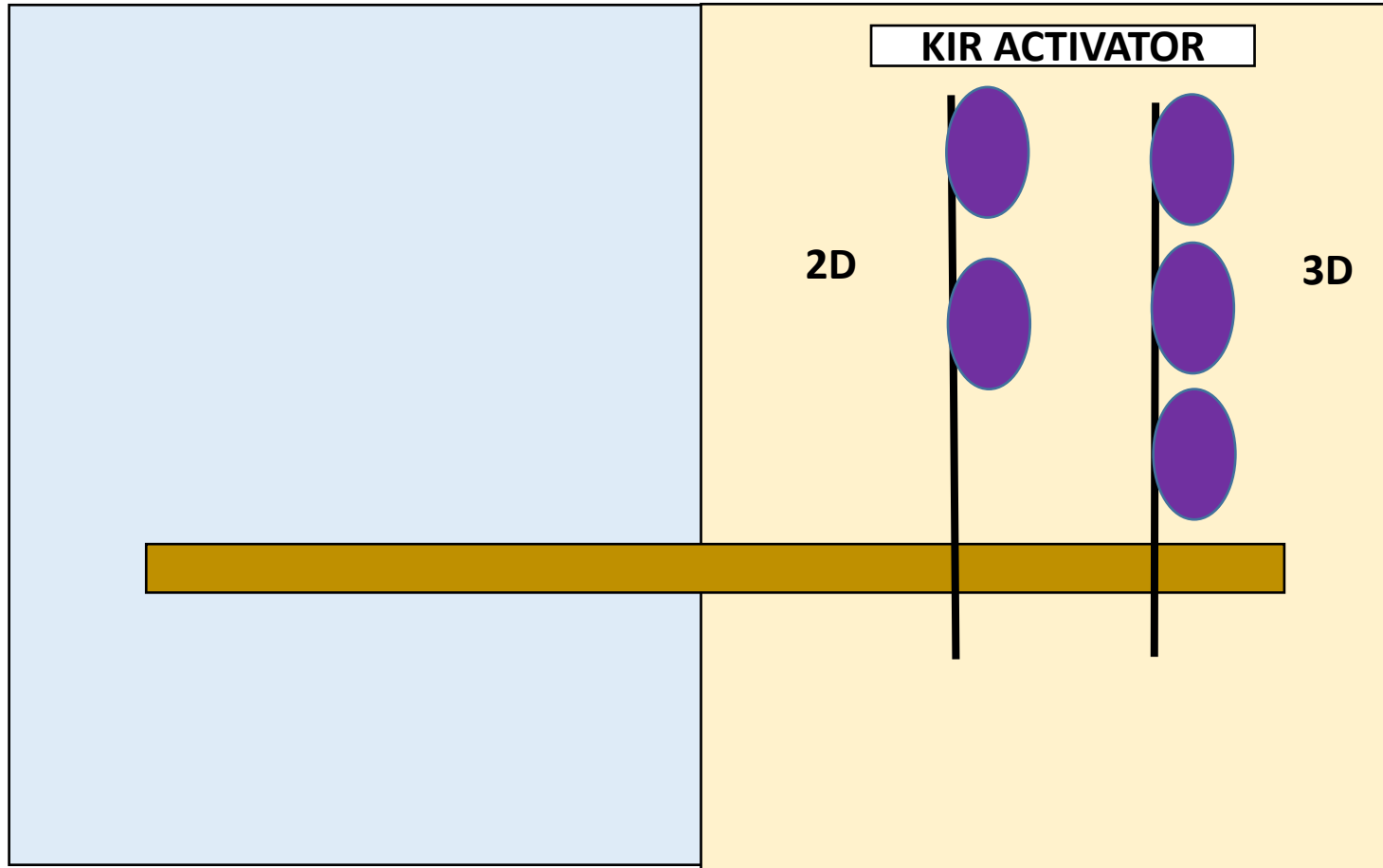
- **KIR B haplotype donors confer a reduced risk for relapse after haploidentical transplantation in children with ALL.** Oevermann L, et al. Blood. 2014 Oct23;124(17):2744-7.
- **Tumor-priming converts NK cells to memory-like NK cells** Marina Palet al. Oncoimmunol. 2016.

KIR Donr Haplotyè	Score B content	CEN	TEL
A/A	0	A/A	A/A
B/x	1	A/A	A/B
		A/B	A/A
	2	A/A	B/B
		A/B	A/B
		B/B	A/A
	3	A/B	B/B
		B/B	A/B
	4	B/B	B/B

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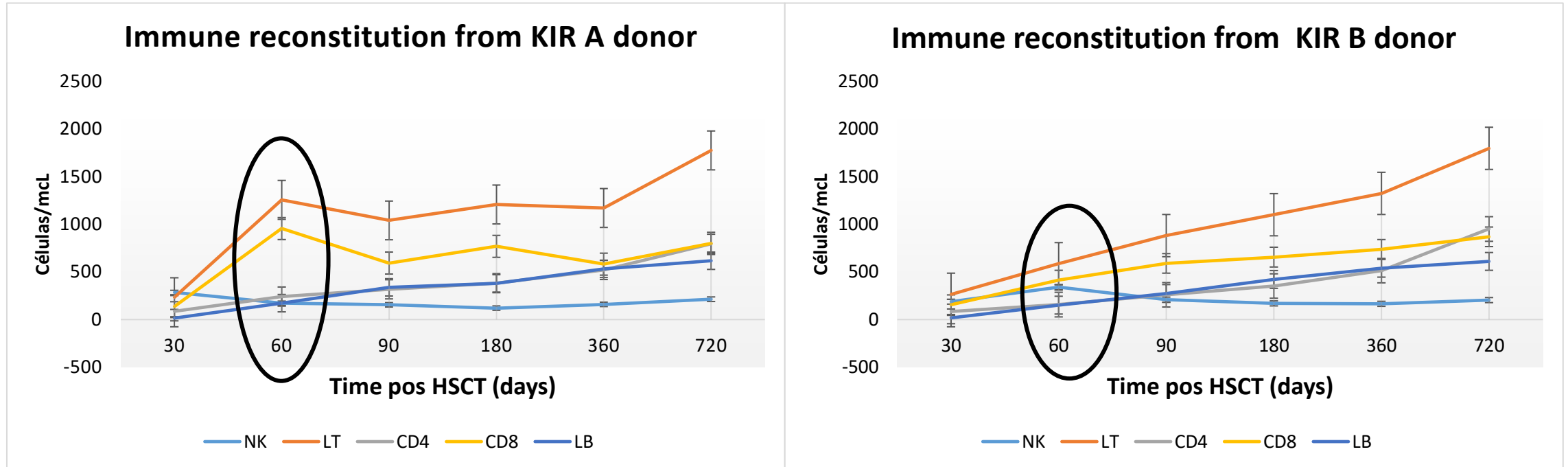
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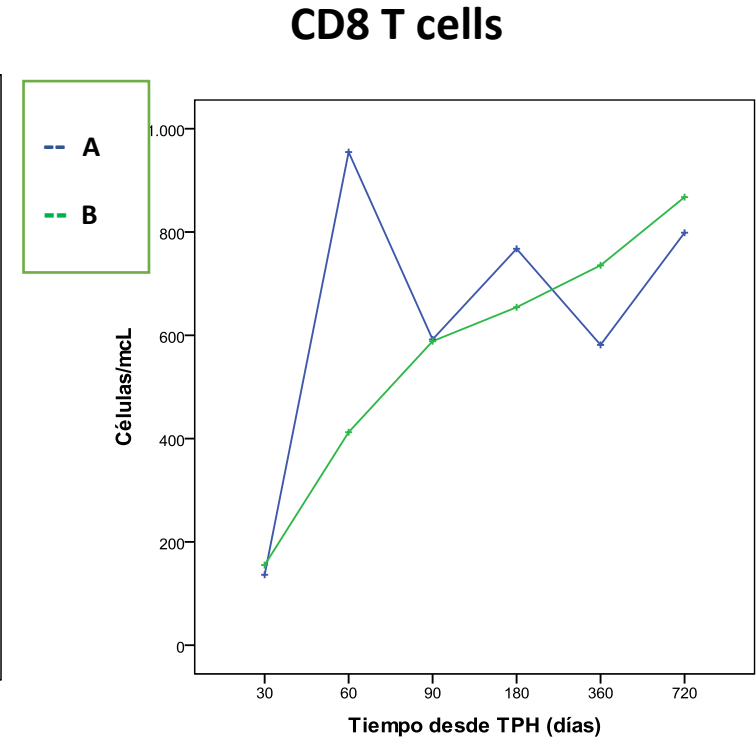
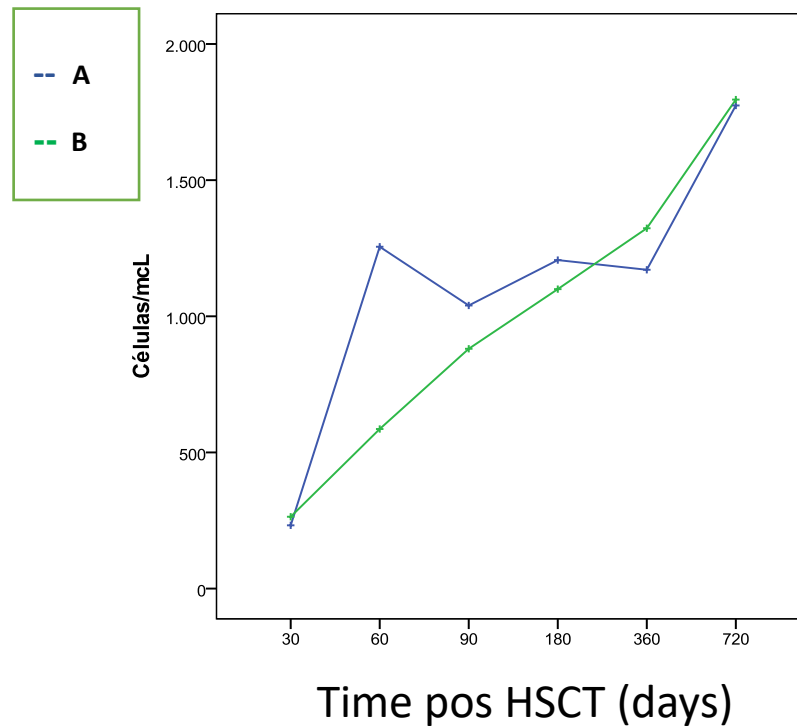
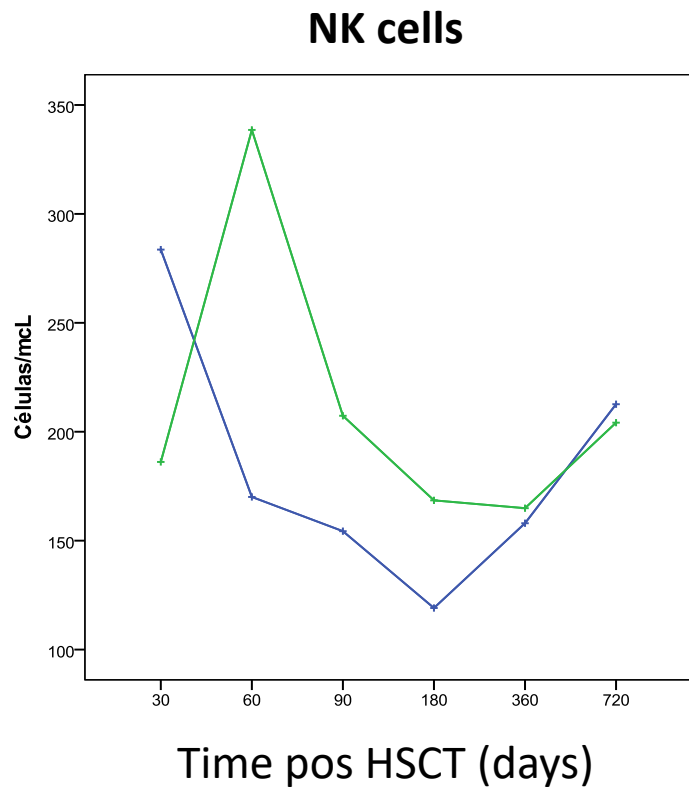
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		CENTROMERIC									TELOMERIC										
Haplotype	CEN	3DL3	2DS2	2DL2	2DL3	2DL5B	2DS3/5	2DP1	2DL1	3DP1		2DL4	3DL1	3DS1	2DL5A	2DS3/5	2DS1	2DS4	3DL2	TEL	
A	CEN-A1																			TEL A-1	
B	CEN A-1																			TEL B-1	
	CEN B-1																				TEL A1
	CEN B-2																				TEL A-1
	CEN B-1																			TEL B-1	
	CEN B-2																				TEL B-1

Immune reconstitution after MRD depends on KIR donor repertoire



Innate immune (NK cells) and adaptive immune (T cells) compete at reconstitution



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Female, 6 year old.

T cell acute lymphoblastic leukemia:

- Debut in August 2019.
- Intermediate risk group.
- Poor response to prednisone (high risk)
- MRD day +33 (end of Induction IA) : 22.47%
- MRD + after end of treatment.
- Haplo-HSCT in April 2020.

HLA genotype of patient

A*03:01P C*07:02:01 B*07:02:01 (Bw6) DRB1*13:02:01 DQB1*06:04:01
 A*01:01P C*07:01:01 B*08:01:01 (Bw6) DRB1*03:01:01 DQB1*02:01:01

Anticuerpos anti-HLA: **1131080** (18.03.19)

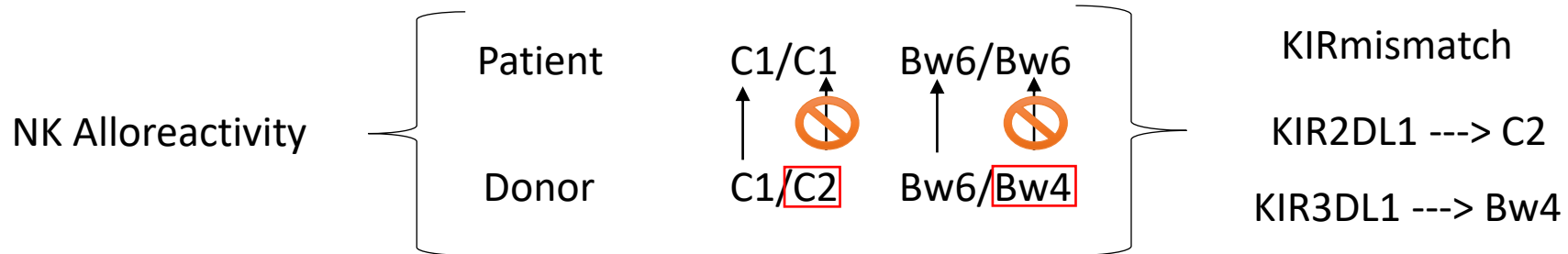
Negativos IgG anti-HLA clase I,II mediante citometría de flujo y CDC clase I

HLA genotype of donor

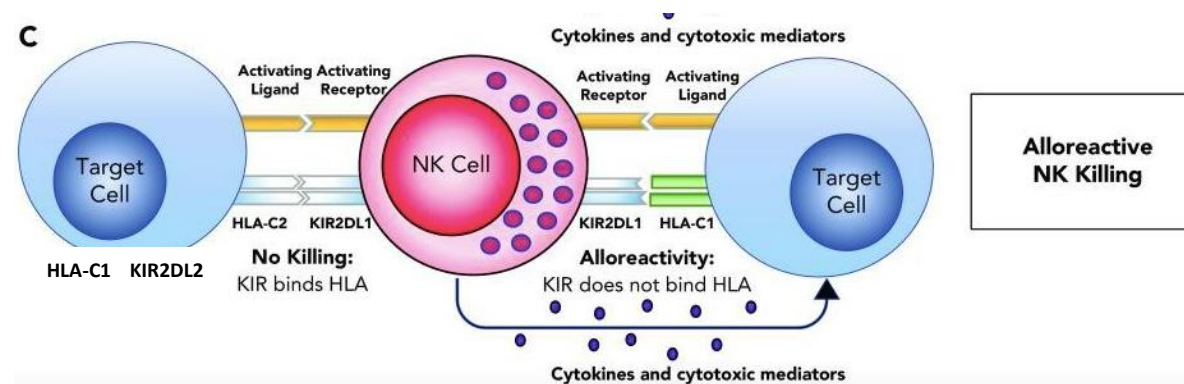
A*03 C*07 B*07 (Bw6) DRB1*13 DQB1*06
 A*24 C*15 B*51 (Bw4) DRB1*11 DQB1*03(DQ7)

Anticuerpos anti-HLA: **0131081** (18.03.19)

Negativos IgG anti-HLA clase I,II mediante citometría de flujo y CDC clase I

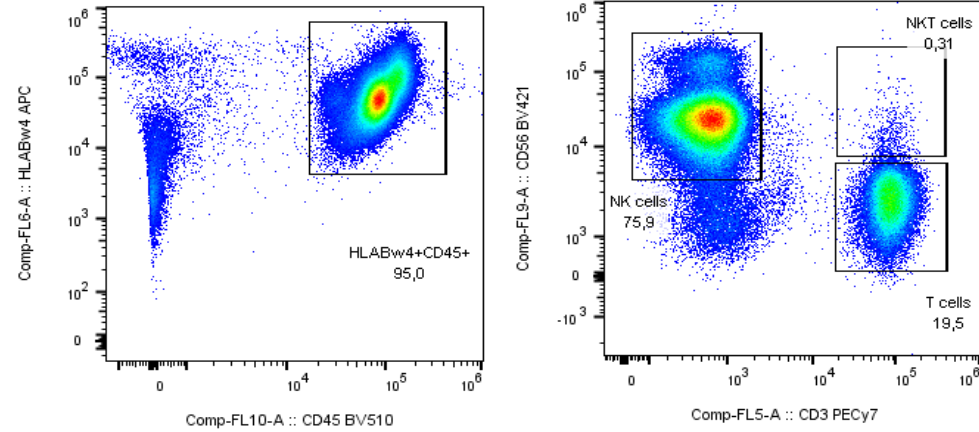
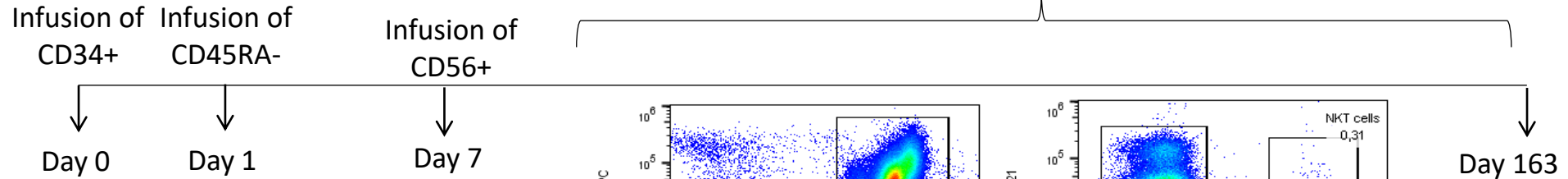


Ligand---> Receptor
KIR2DL1 ---> C2
KIR2DL2 ---> C1
KIR3DL1 ---> Bw4



Inmunoterapia basada en células NK

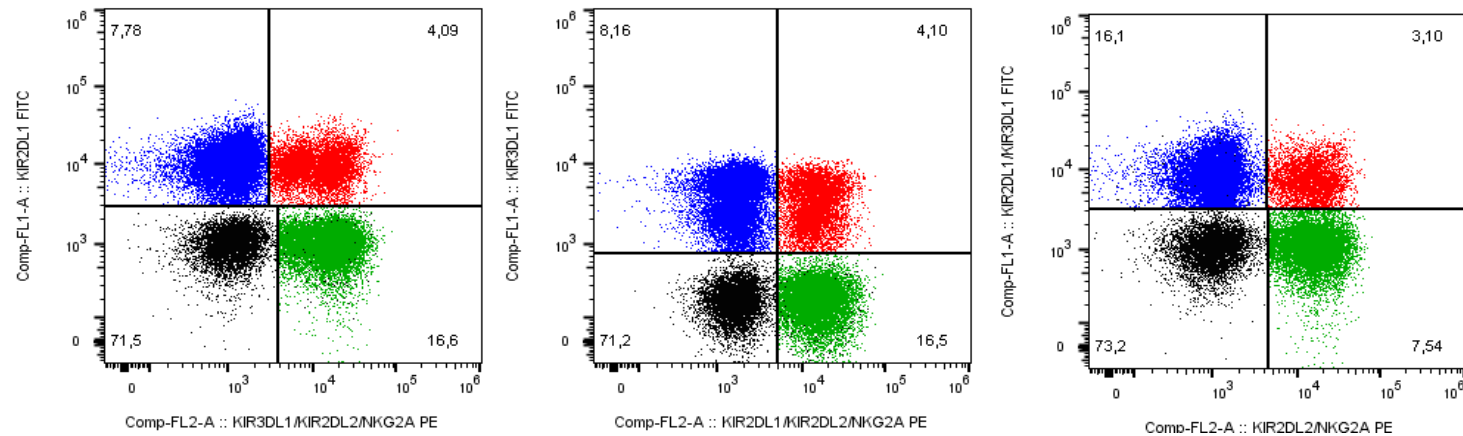
Follow up by flow citometry



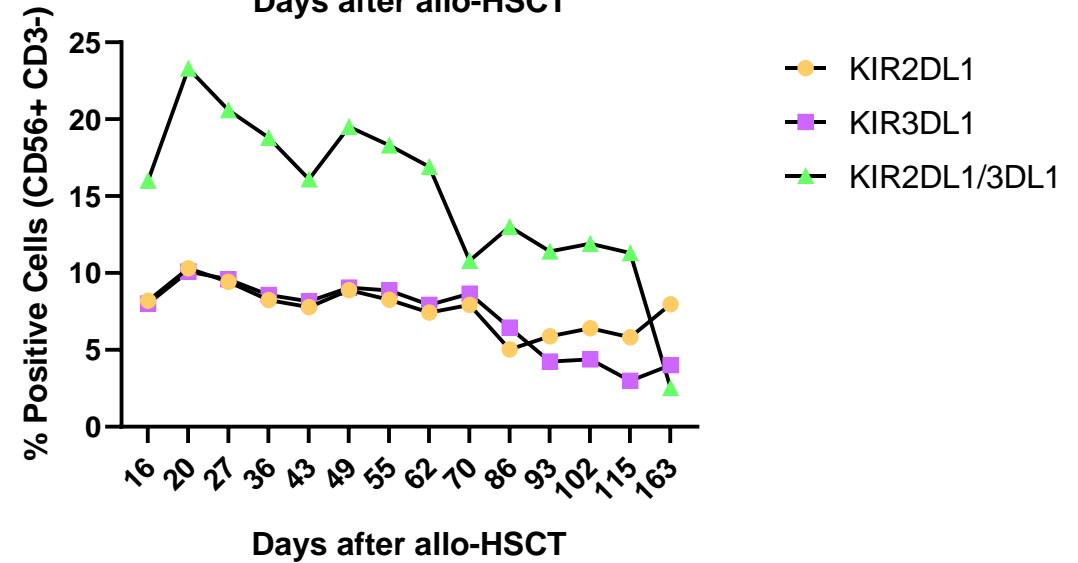
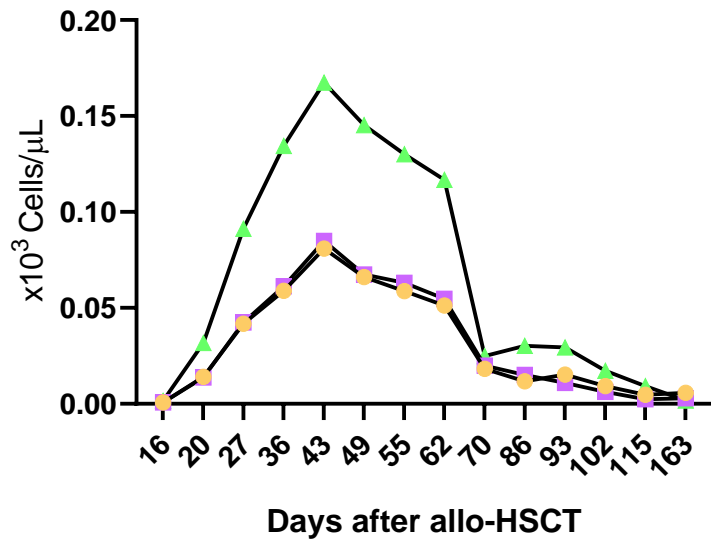
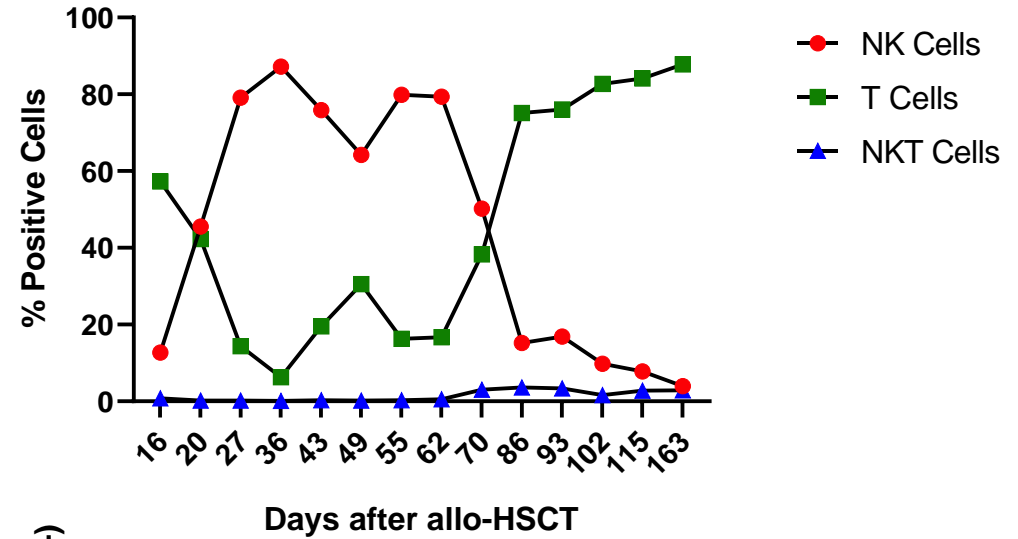
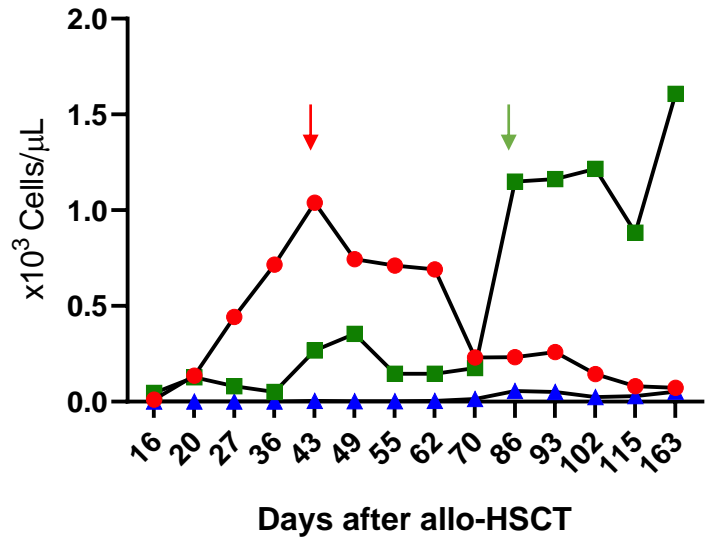
KIRmismatch

KIR2DL1 ---> C2

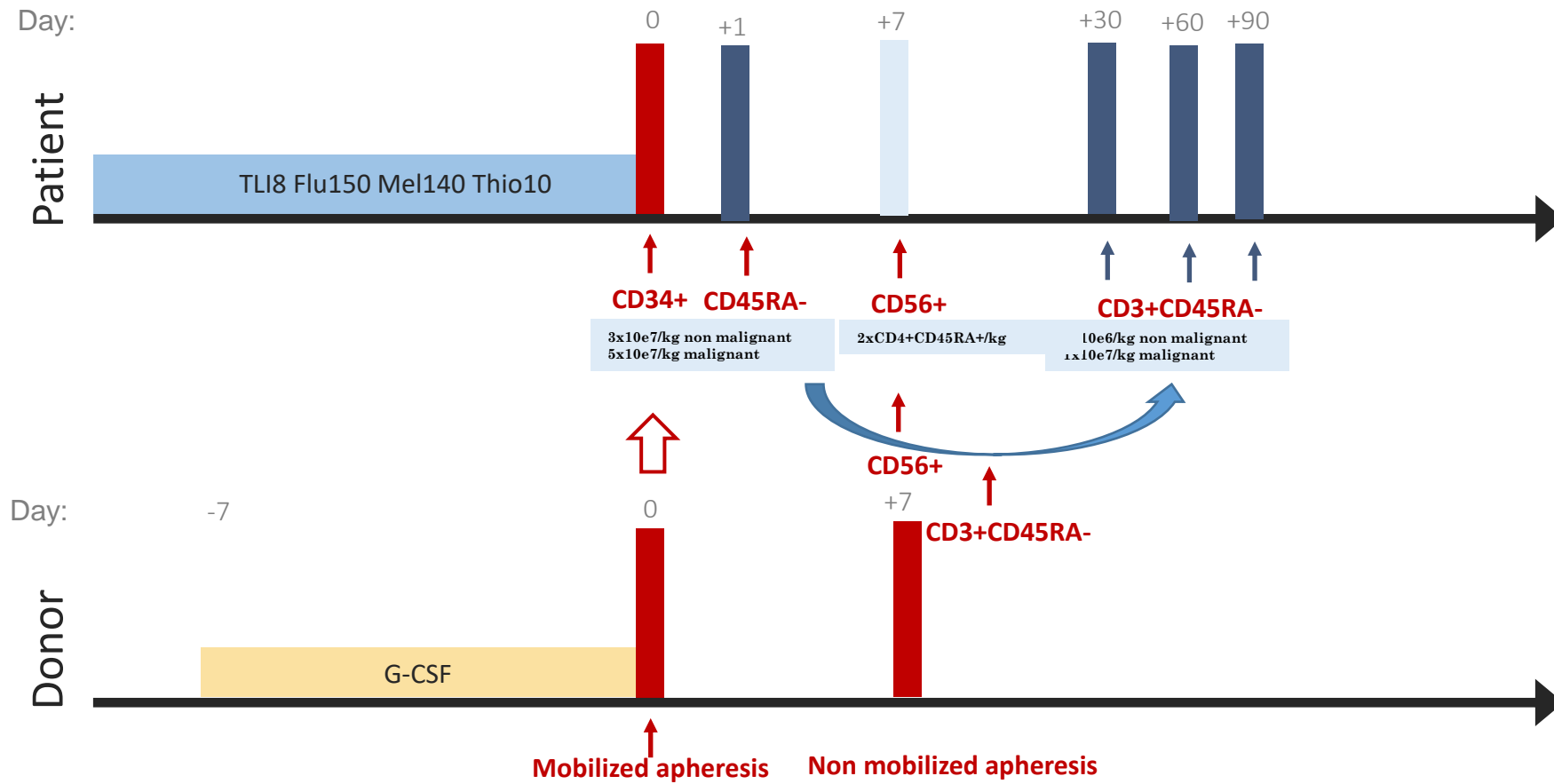
KIR3DL1 ---> Bw4



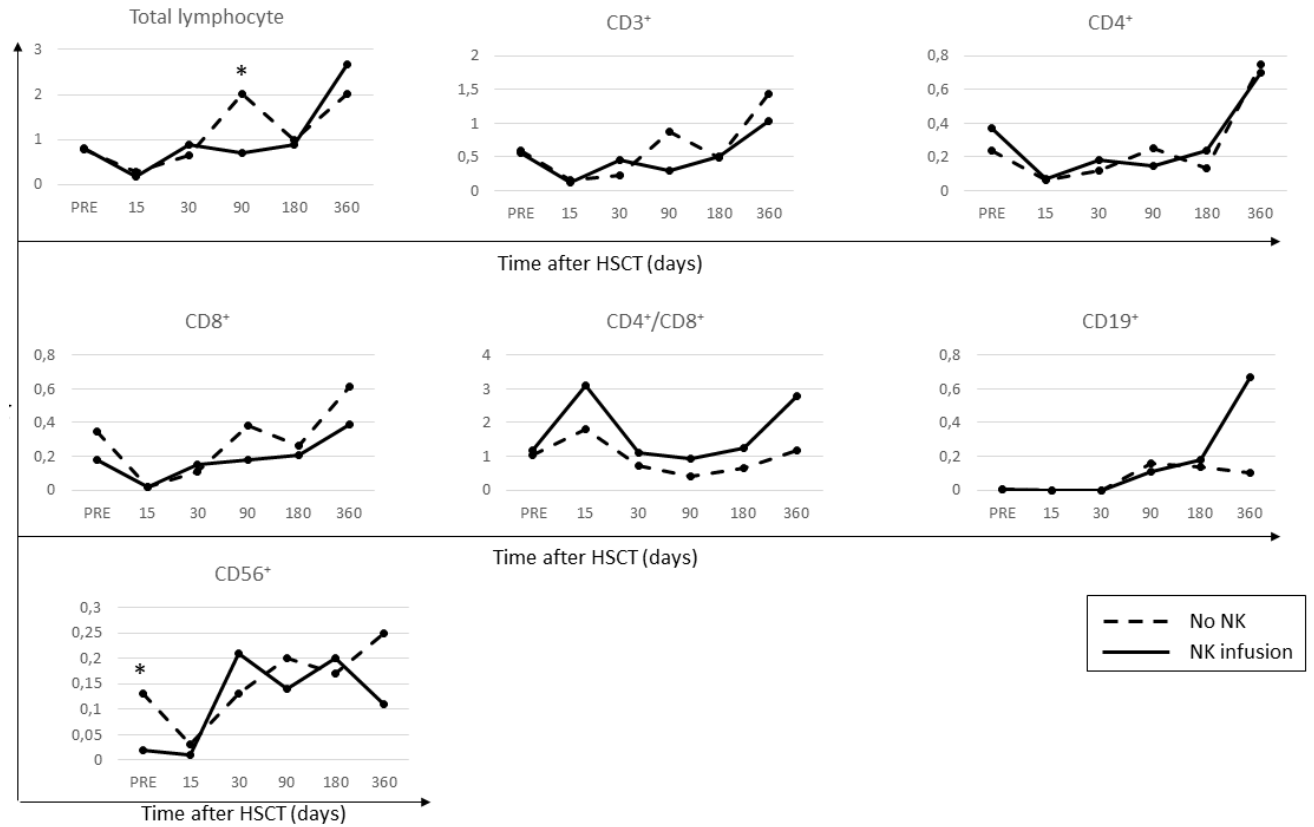
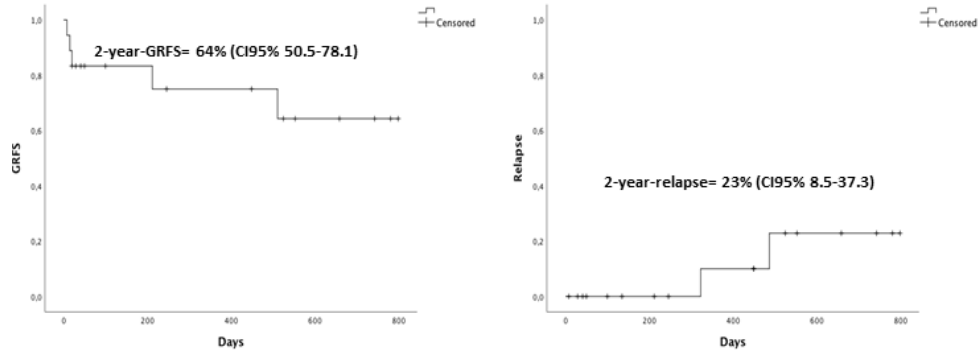
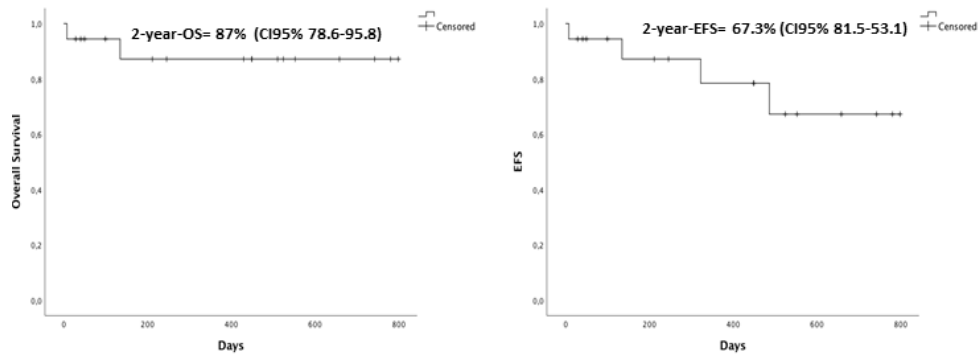
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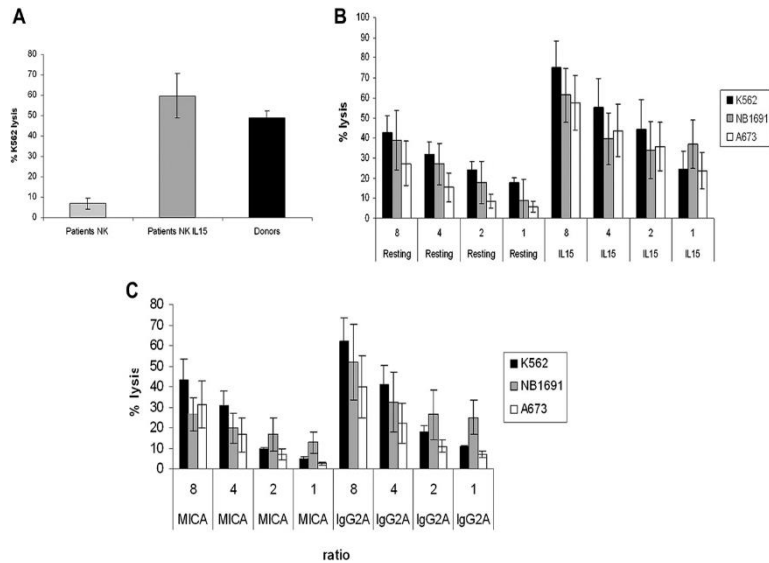
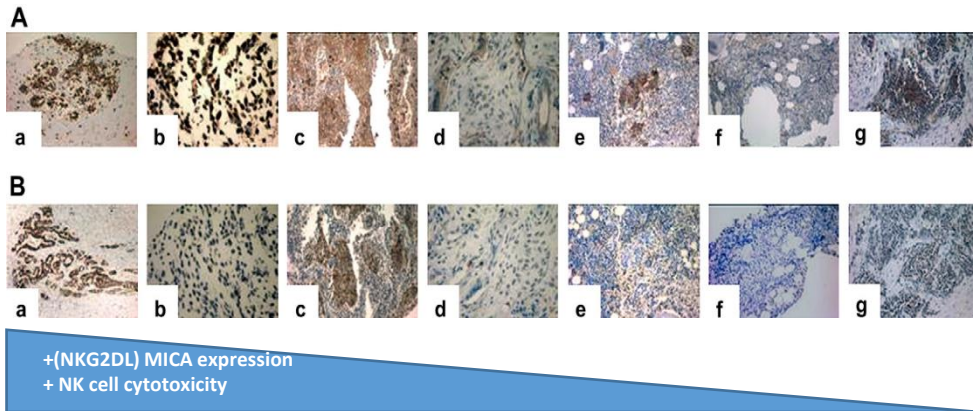
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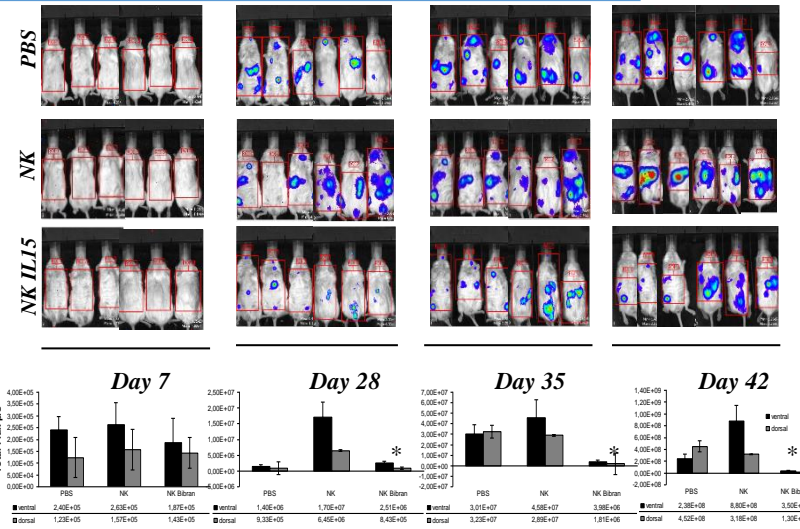
Gassior M et al. Submitted

3. Induced self hypothesis

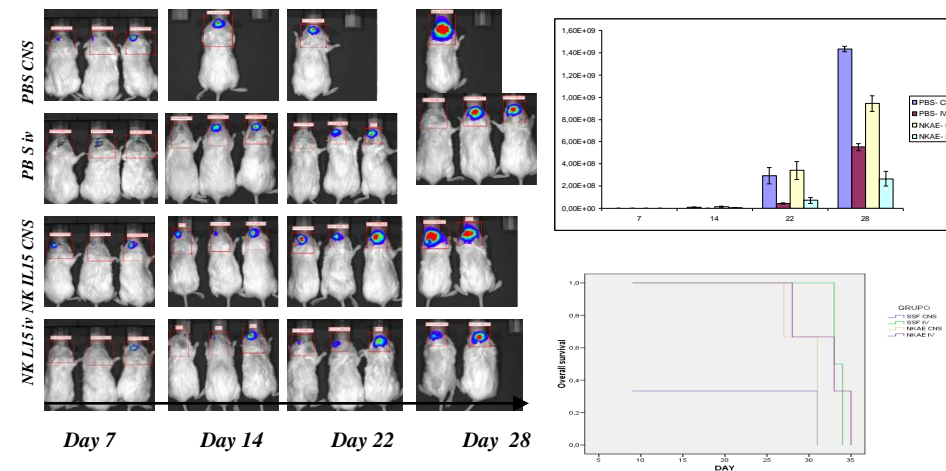
NK cell alloreactivity based in *induced self hypothesis*



Pérez-Martínez A. Exp Hematol 2012.

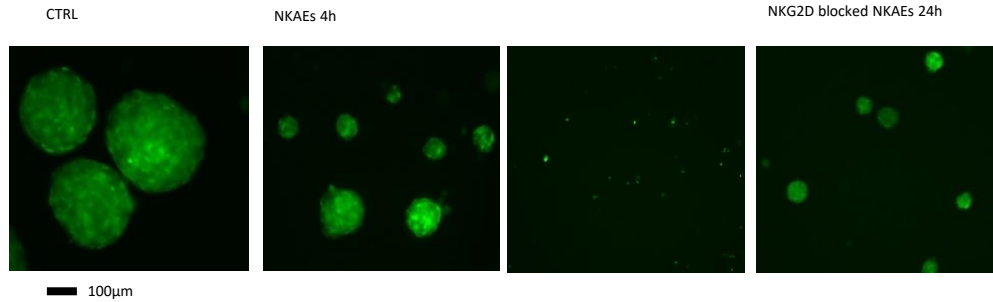


Pérez-Martínez A. Cytotherapy 2015

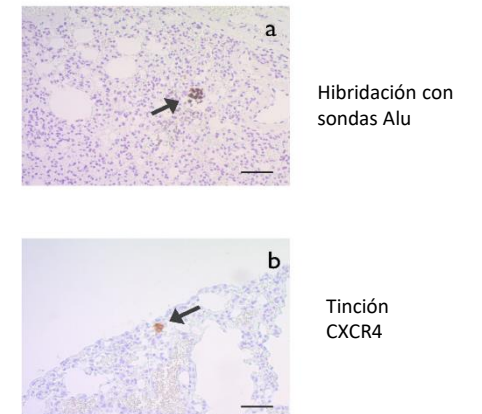
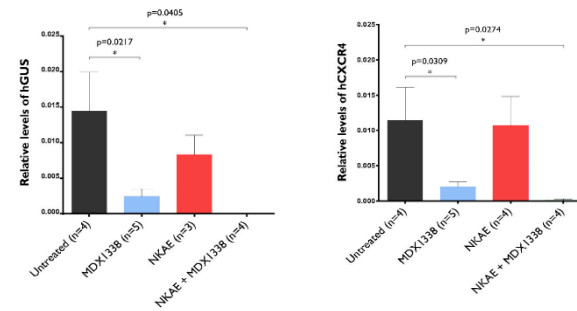
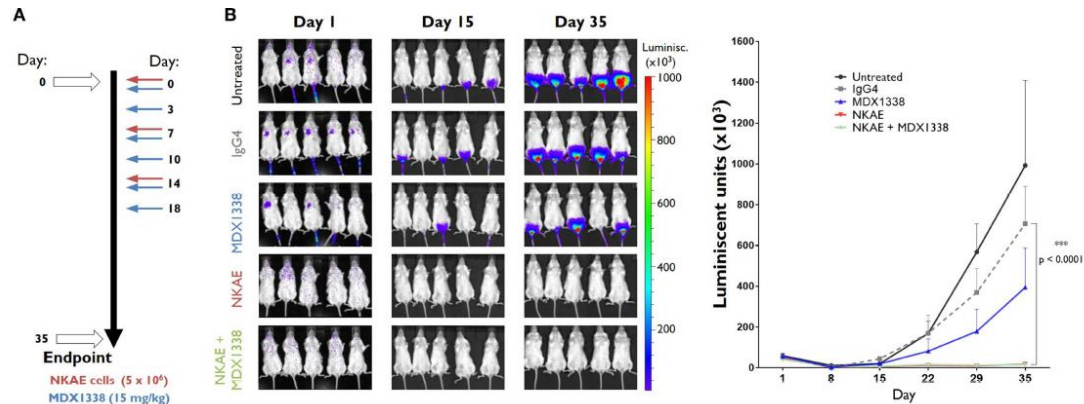
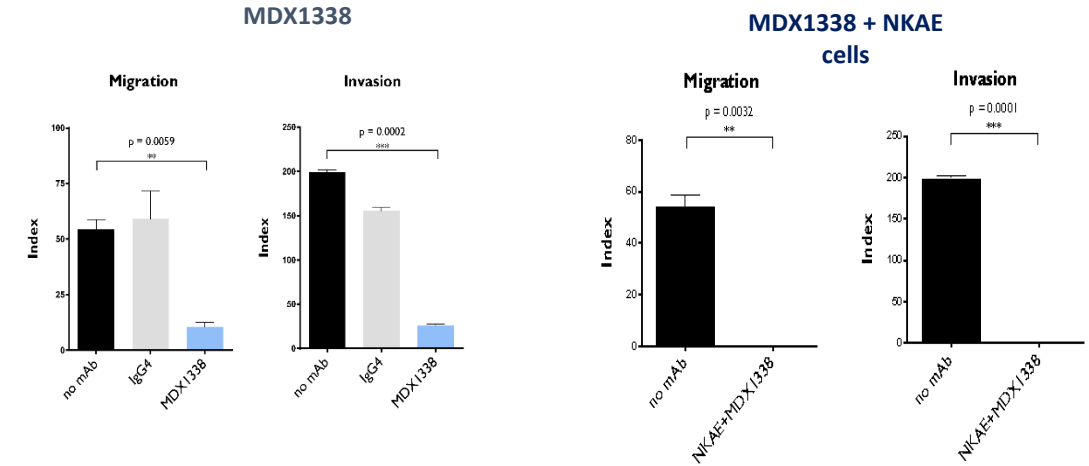


Fernández L et al. Front Oncol 2013.

NK cell target OIC and prevent migration, invasion and micrometastasis

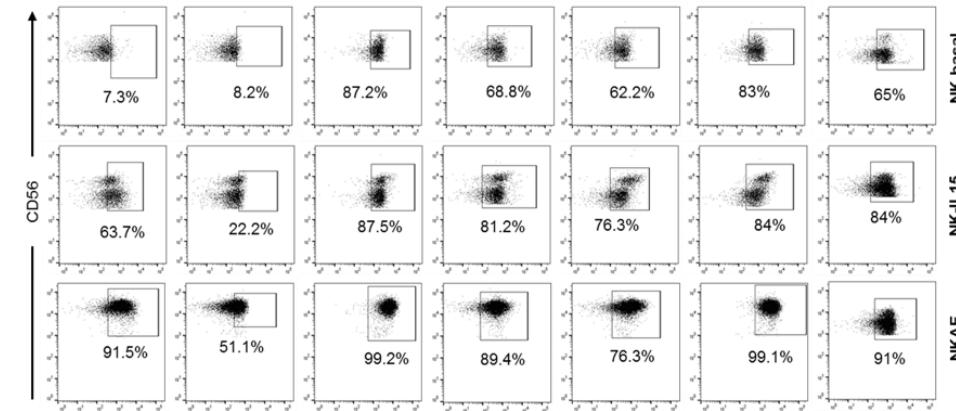
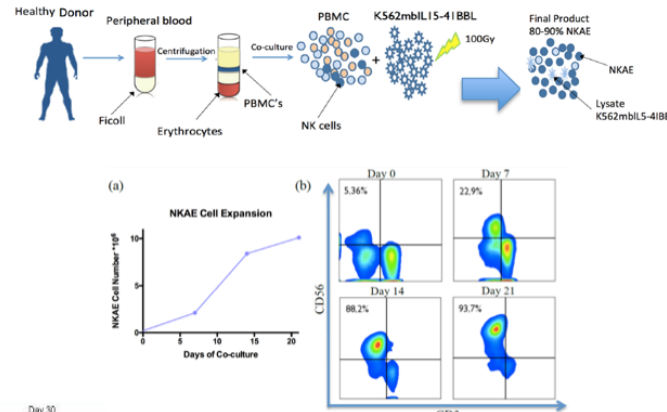
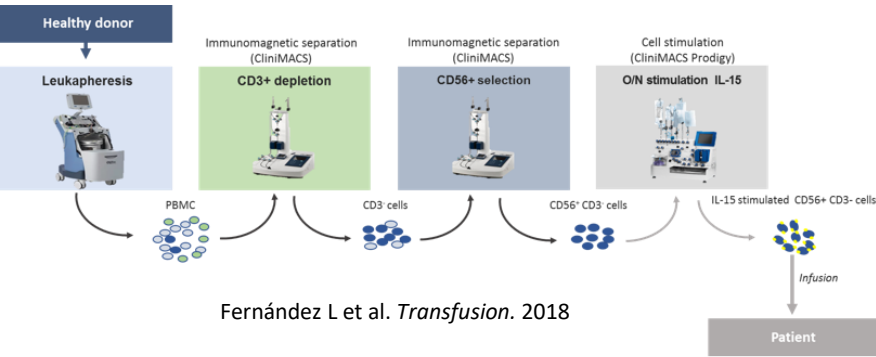
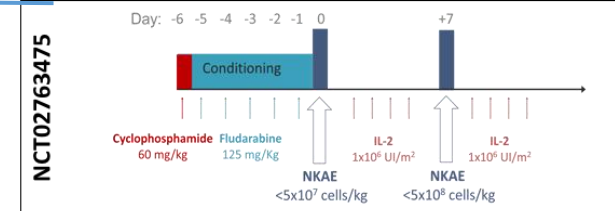
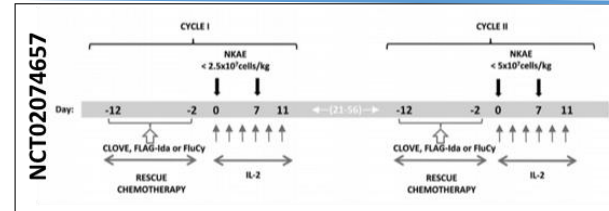
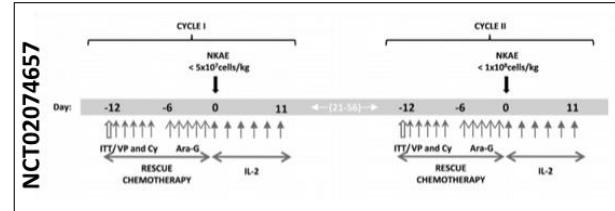
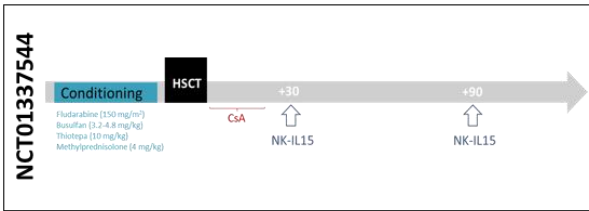


Fernández L et al. Cancer Letter 2016.



Vela M et al. Frontiers Immunology 2019.

First in human NK cell adoptive therapy for pediatric malignancies



Cytotherapy, 2015; 17: 1594–1603

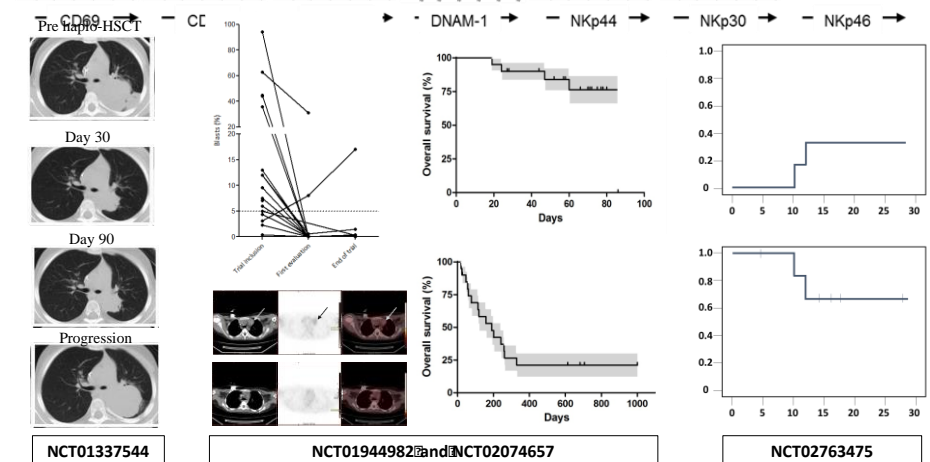
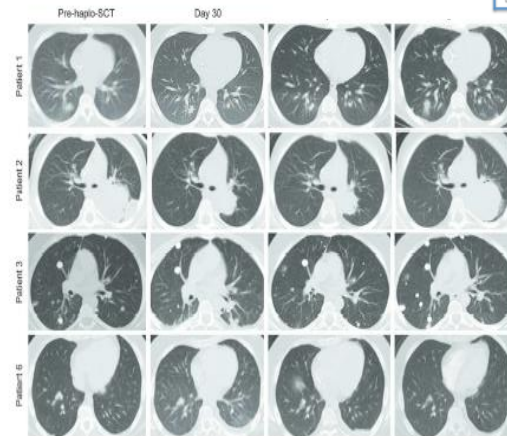
International Society for Cellular Therapy
ISCT



A phase I/II trial of interleukin-15-stimulated natural killer cell infusion after haplo-identical stem cell transplantation for pediatric refractory solid tumors

ANTONIO PÉREZ-MARTÍNEZ¹, LUCÍA FERNÁNDEZ², JAIME VALENTÍN³, ISABEL MARTÍNEZ-ROMERA¹, MARÍA DOLORES CORRAL¹, MANUEL RAMÍREZ^{4,5}, LOREA ABAD⁵, SANDRA SANTAMARÍA³, MARTA GONZÁLEZ-VICENT⁶, SARA SARVENT⁷, JULIÁN SEVILLA⁸, JOSÉ LUIS VICARIO³, INMACULADA DE PRADA⁸ & MIGUEL ÁNGEL DÍAZ²

Pérez Martínez A et al. *Cytotherapy* 2015.

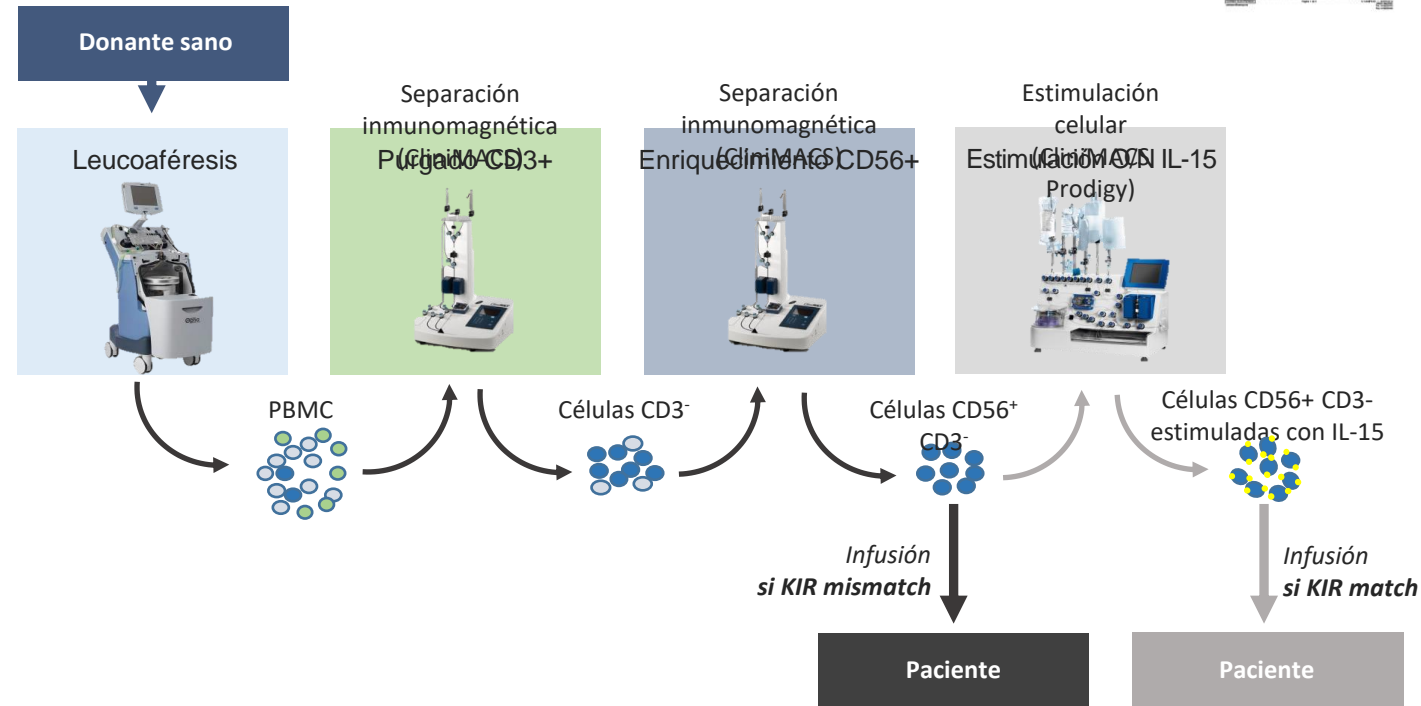
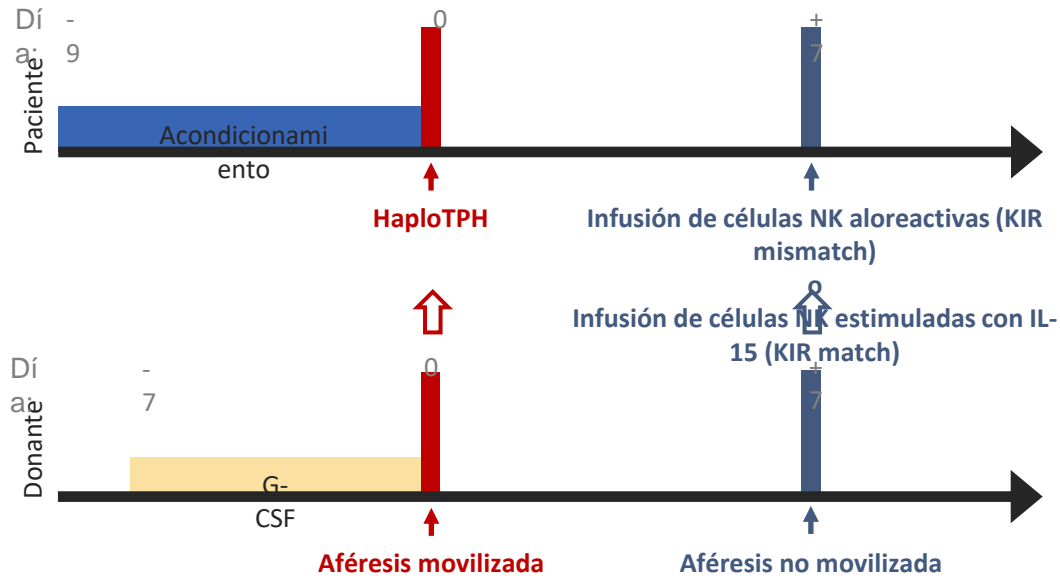


Vela et al. *Cancer Letter* 2017

González L et al. Submitted 2020

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PHINK



Resumen de la Unidad

Objetivo: Desarrollar un protocolo de inmunoterapia con células NK aloreactivas (KIR mismatch) y células NK estimuladas con IL-15 (KIR match) en pacientes con leucemia aguda y mieloma múltiple.

Justificación: Las células NK son capaces de reconocer y destruir células tumorales que expresan moléculas de estrés. La combinación de células NK aloreactivas y células NK estimuladas con IL-15 puede mejorar la eficacia de la inmunoterapia.

Metodología: Se utilizará un protocolo de leucoaféresis para obtener células NK de donantes sanos. Las células NK serán procesadas en el laboratorio de inmunología y se infundirán al paciente.

Resultados: Se espera que la inmunoterapia con células NK aloreactivas y células NK estimuladas con IL-15 mejore la supervivencia y la calidad de vida de los pacientes.

Conclusiones: La inmunoterapia con células NK aloreactivas y células NK estimuladas con IL-15 es una estrategia prometedora para el tratamiento de la leucemia aguda y el mieloma múltiple.

Palabras clave: células NK, KIR mismatch, IL-15, leucemia aguda, mieloma múltiple.

- Feasible, Safety, Efficacy, Cheap, Allow combining strategies and timing
- However, limited clinical benefits because
 1. Obtaining large numbers of fully activated NK cells can be challenging
 2. NK cell exhaustion
 3. Limited in vivo expansion
 4. Lack of antitumor memory
 5. Cryopreservation often impairs functionality
 6. Poor ability to reach solid tumours
 7. Suppression by tumour microenvironment (TME)
- Opportunities to improvement: from Natural Killer cells to Artificial Executors
 1. Induced memory NK cells
 2. Resident memory
 3. Bikes, Trikes...
 4. CAR-T, CAR-NK

- Feasible, Safety, Efficacy, Cheap, Allow combining strategies and timing
- However, limited clinical benefits because

4. Lack of antitumor memory

- Opportunities to improvement: **from Natural Killer cells to Memory NK cells**

1. **Induced memory NK cells**

4. Memory induced NK cells

Why using ML-NK-based therapy instead of CAR-T?

1

Innate hability to recognize some tumors

2

Enhanced activity after pre-activation (ML-NKs)

3

NK transfer does not cause Graft versus host disease

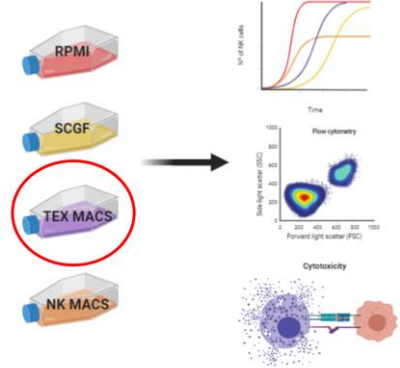
Sci Transl Med. 2016 September 21; 8(357): 357ra123. doi:10.1126/scitranslmed.aaf2341.

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

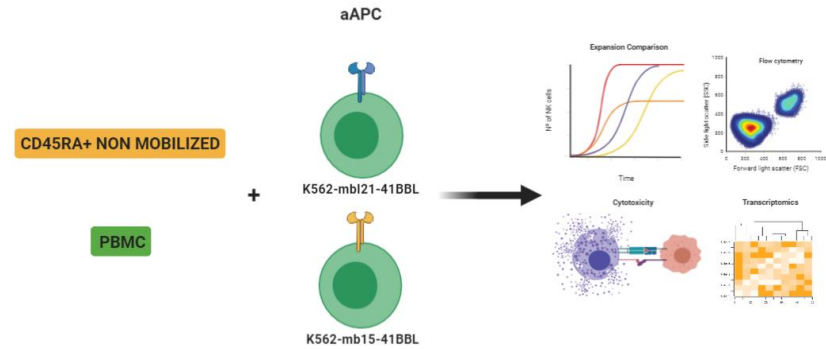
Rizwan Romee^{1,*}, Maximillian Rosario^{1,2,*}, Melissa M. Berrien-Elliott^{1,*}, Julia A. Wagner¹, Brea A. Jewell¹, Timothy Schappe¹, Jeffrey W. Leong¹, Sara Abdel-Latif¹, Stephanie E. Schneider¹, Sarah Willey¹, Carly C. Neal¹, Liyang Yu³, Stephen T. Oh³, Yi-Shan Lee², Arend Mulder⁴, Frans Claas⁴, Megan A. Cooper⁵, and Todd A. Fehniger^{1,†}

Inmunoterapia basada en células NK

Optimization of cell growth culture media



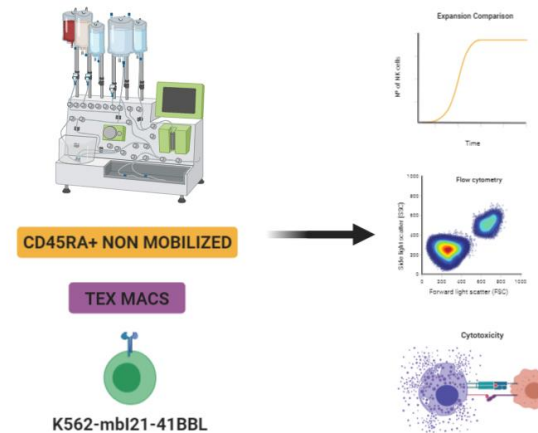
Expansion of NKAЕ cells by using different aAPC



Use of CD45RA+ cells as source of NK cells to obtain NKAЕ



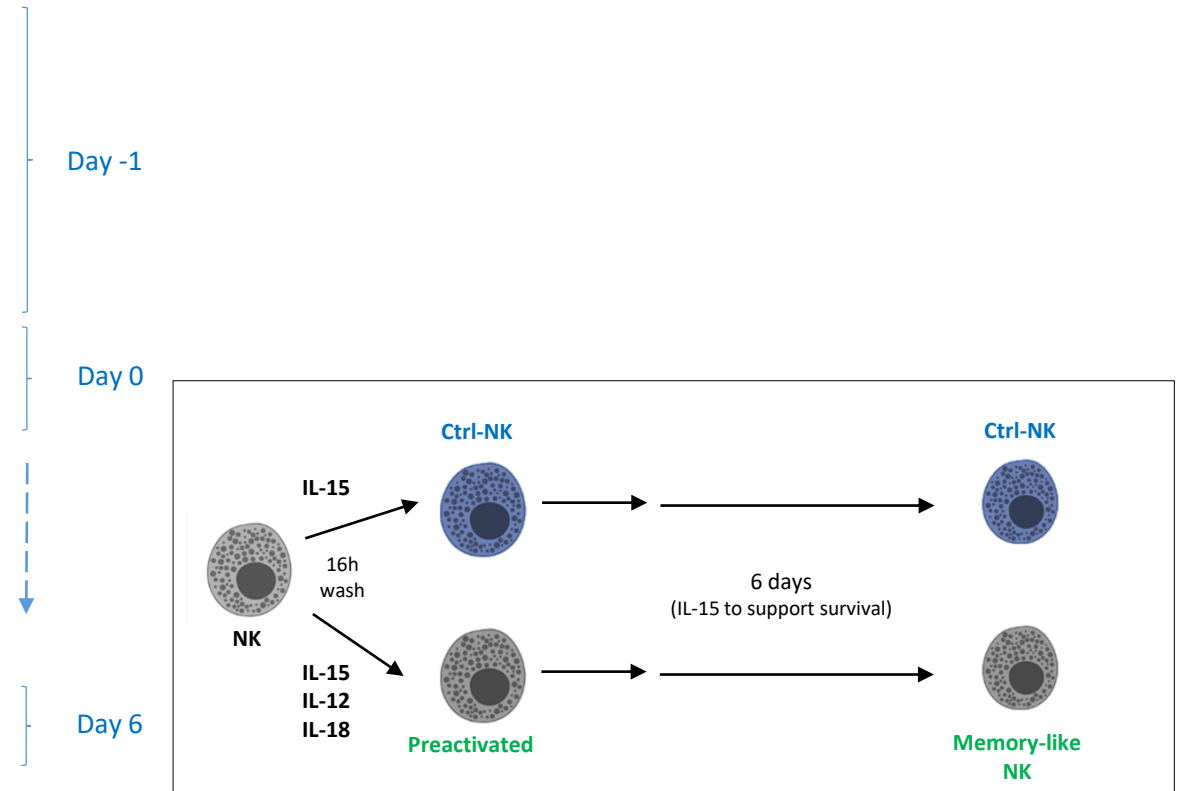
Production of clinical grade NKAЕ cells in CliniMACS Prodigy



Inmunoterapia basada en células NK

Generating ML-NK cells

- To obtain blood sample or buffy coat
- NK cells purification with RosetteSep kit (manufacturer instructions)
- NK cells culture with ILs:
 - Ctrl NKs – IL-15 1ng/mL
 - preML-NKs – IL-15 50 ng/mL, IL-12 10 ng/mL, IL-18 50 ng/mL16h in culture
- Wash ILs: washed **three times** in PBS 1X
- Culture NK cells (IL-15 1ng/mL for both) **at $3-5 \times 10^6$ c/mL**
- Refresh medium each 2 days with IL-15 1ng/mL to support survival (both)
- Day 6: cells differentiated to ML-NKs

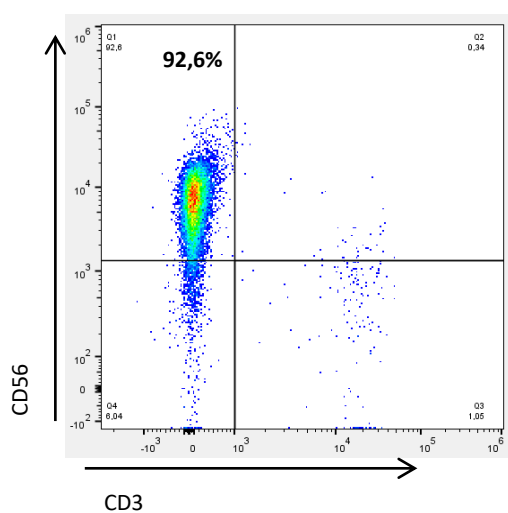
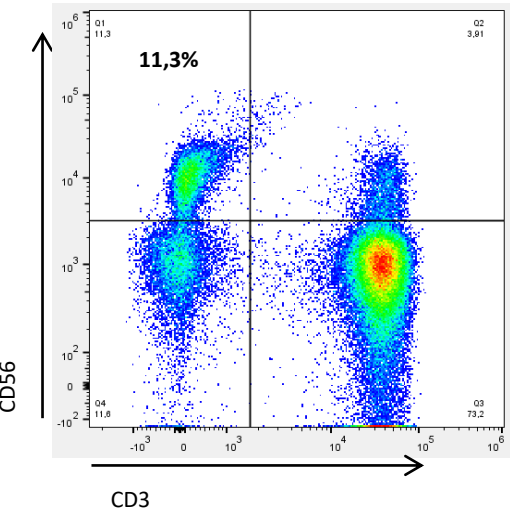
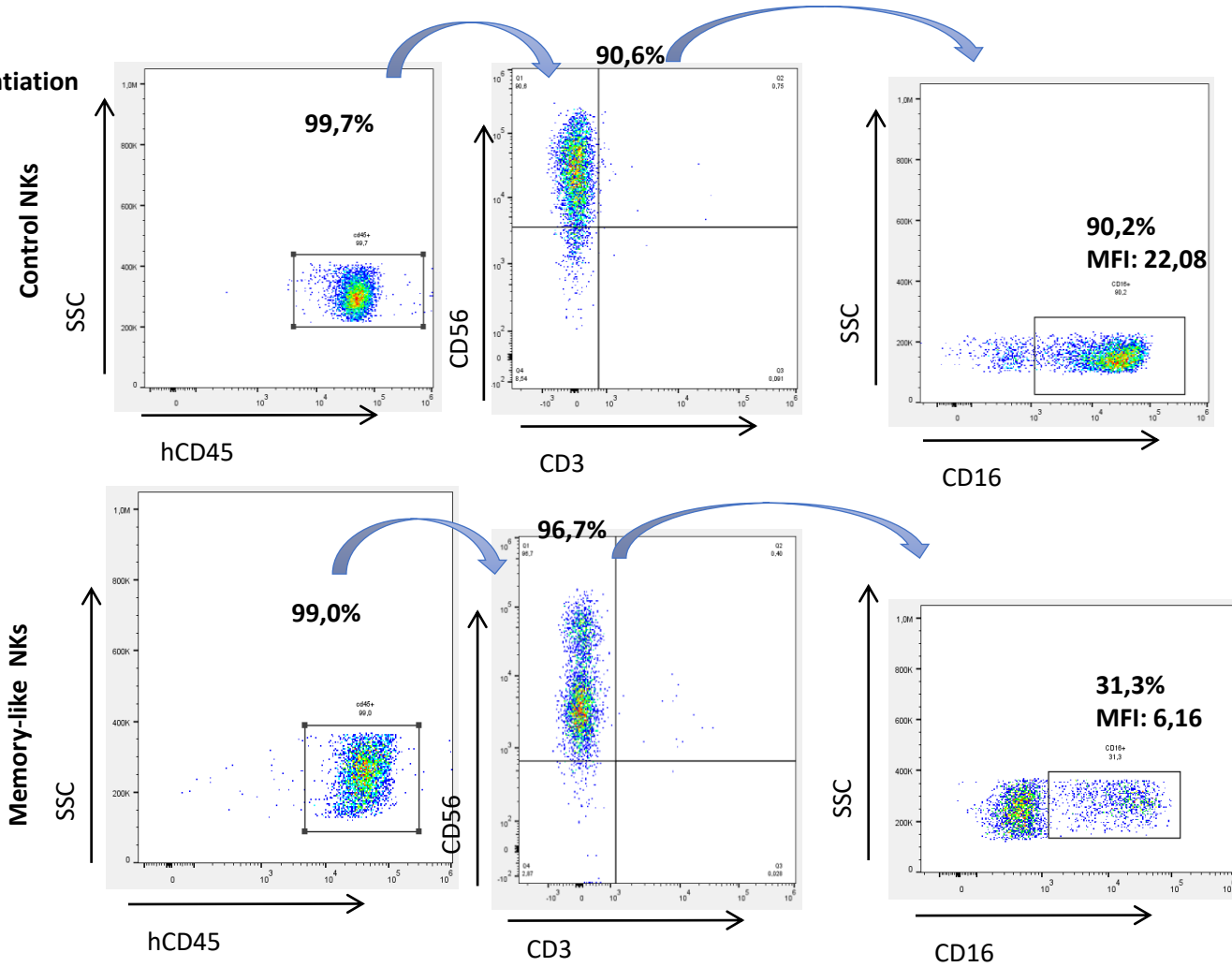


Inmunoterapia basada en células NK

NK cells at day 6 of differentiation

NK cells in buffy coat

NK cells after RosetteSep purification



5. The T-NK approach

The NEW ENGLAND JOURNAL *of* MEDICINE

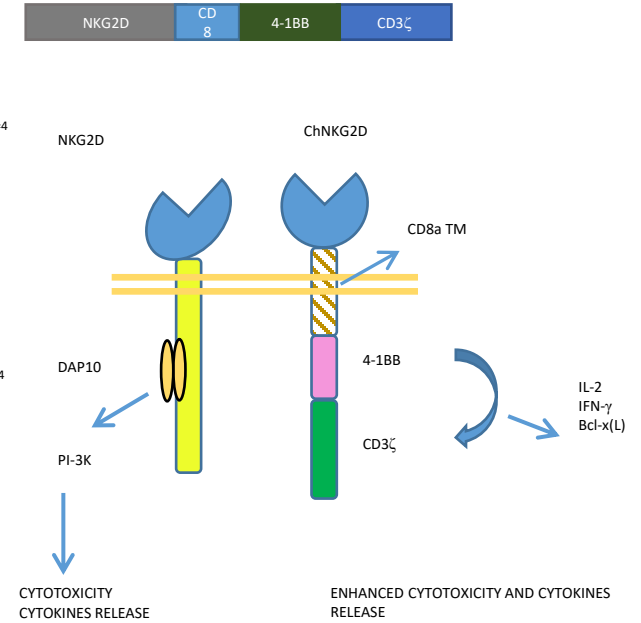
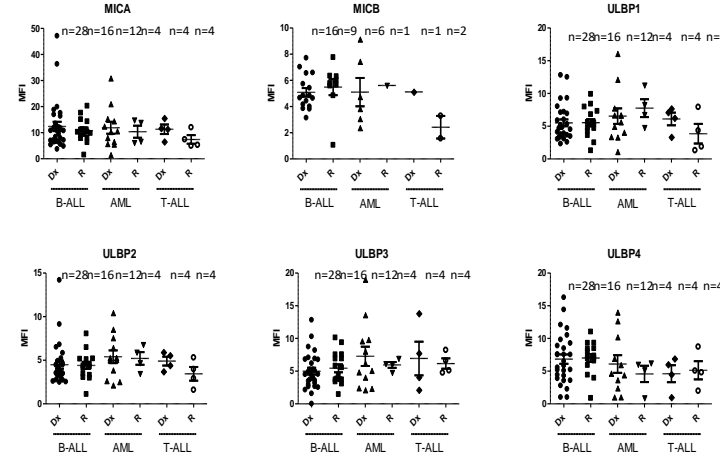
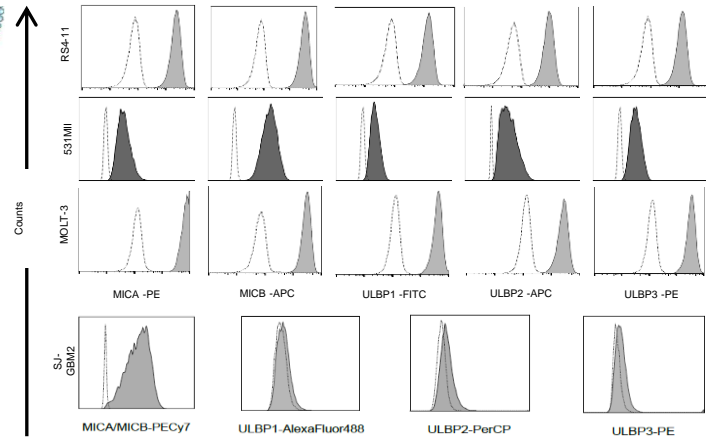
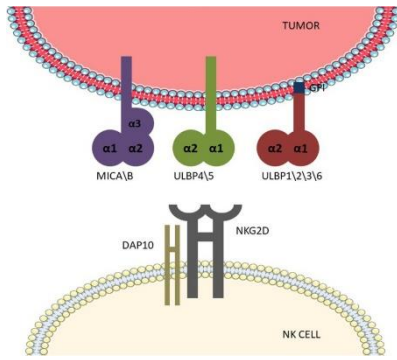
ORIGINAL ARTICLE

Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors

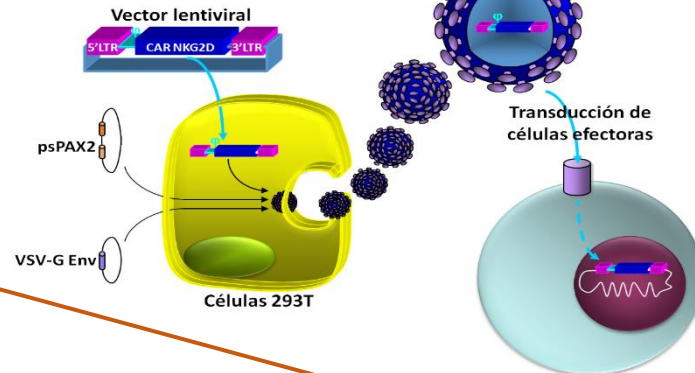
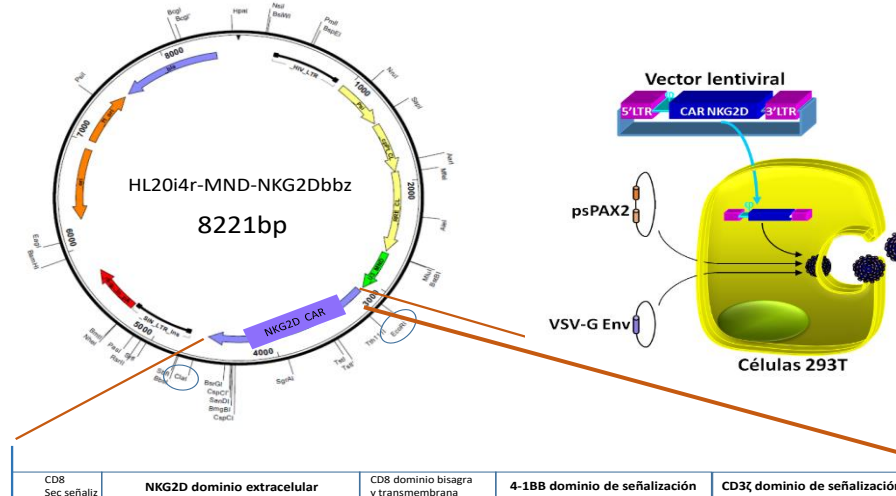
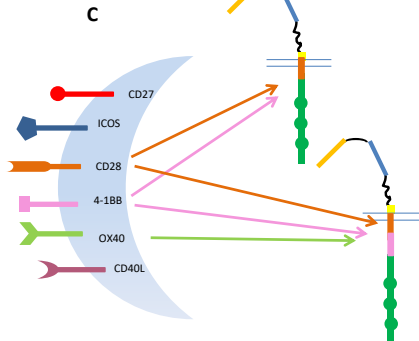
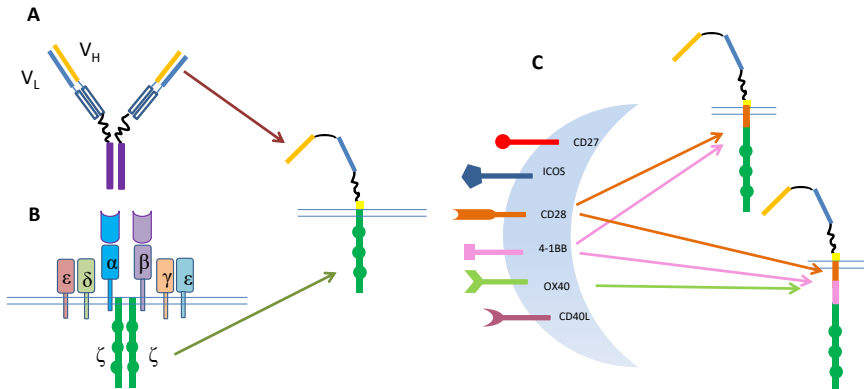
Enli Liu, M.D., David Marin, M.D., Pinaki Banerjee, Ph.D.,
Homer A. Macapinlac, M.D., Philip Thompson, M.B., B.S., Rafet Basar, M.D.,
Lucila Nassif Kerbaui, M.D., Bethany Overman, B.S.N., Peter Thall, Ph.D.,
Mecit Kaplan, M.S., Vandana Nandivada, M.S., Indresh Kaur, Ph.D.,
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William Wierda, M.D., Ph.D., Michael Keating, M.D., Richard Champlin, M.D.,
Elizabeth J. Shpall, M.D., and Katayoun Rezvani, M.D., Ph.D.

Inmunoterapia basada en células NK

The T-NK approach a multiple targets model (NKG2DL)

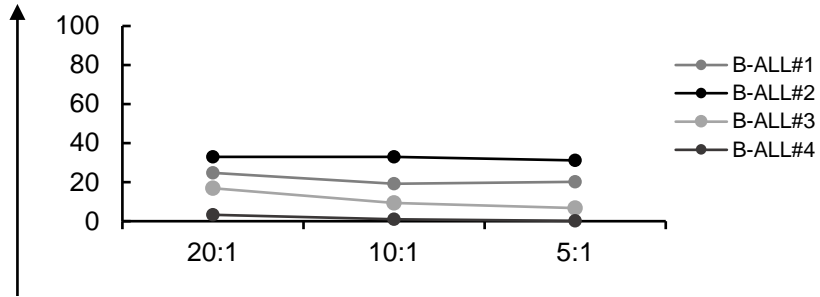


Duan S. et al. *Mol. Cancer* 2019

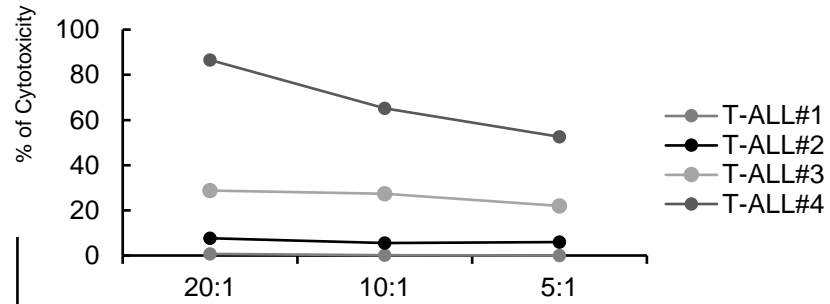


Inmunoterapia basada en células NK

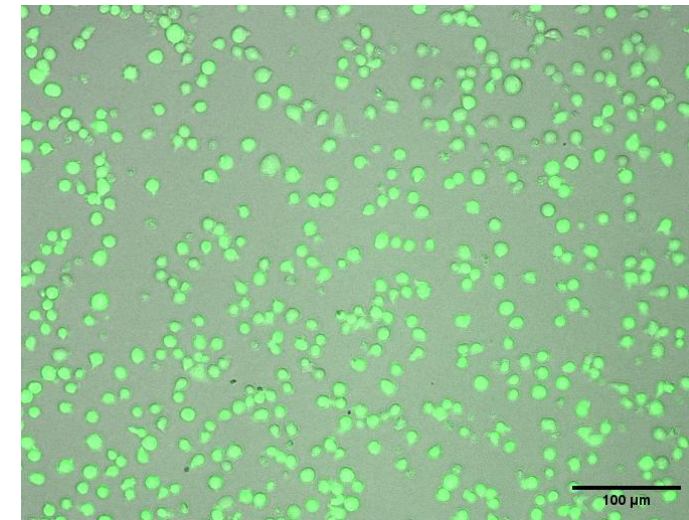
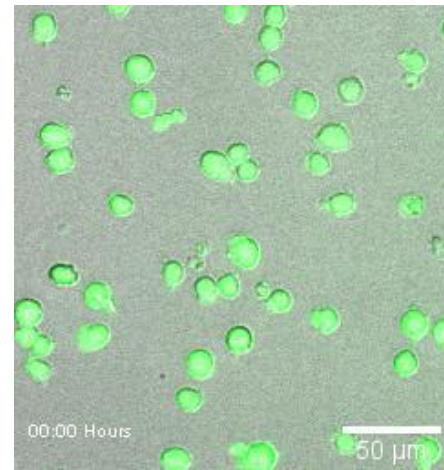
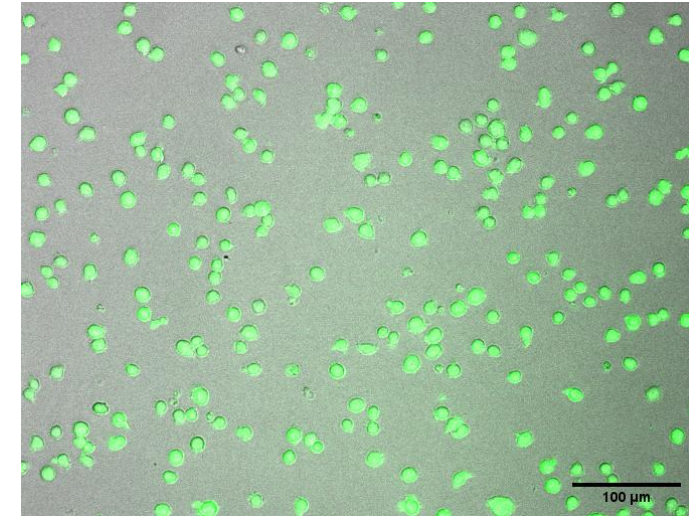
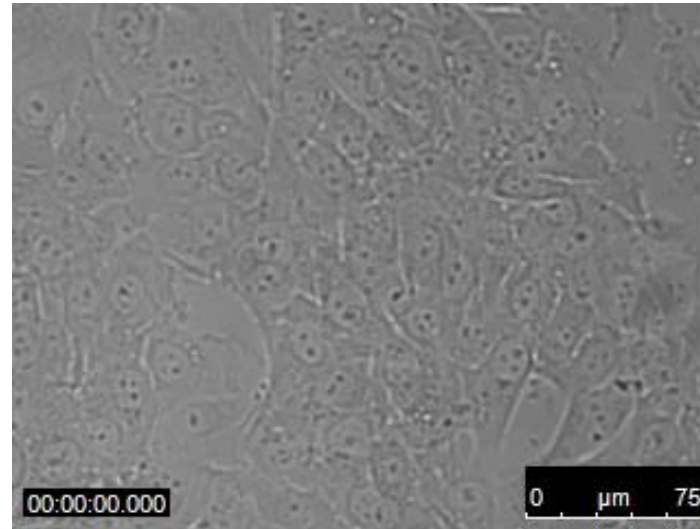
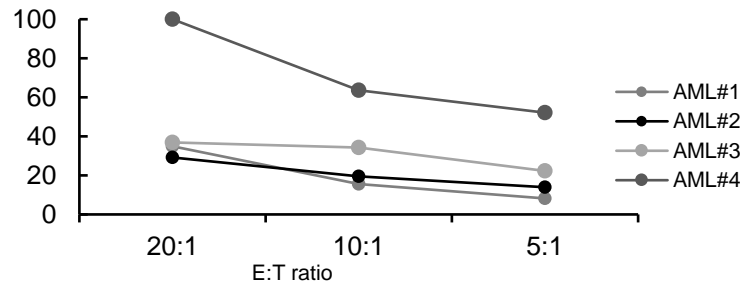
NKG2D CAR vs primary B-ALL blasts



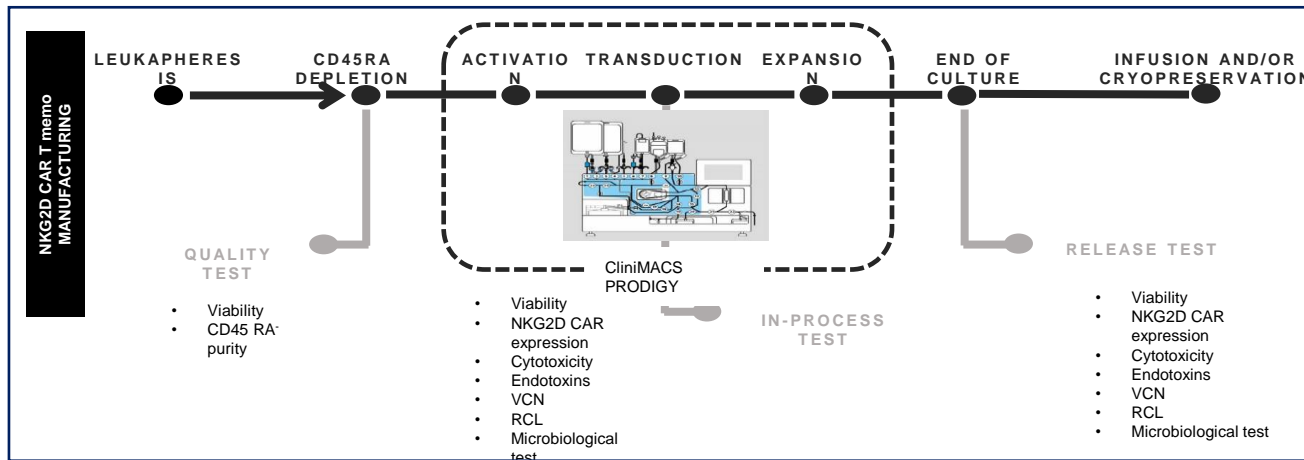
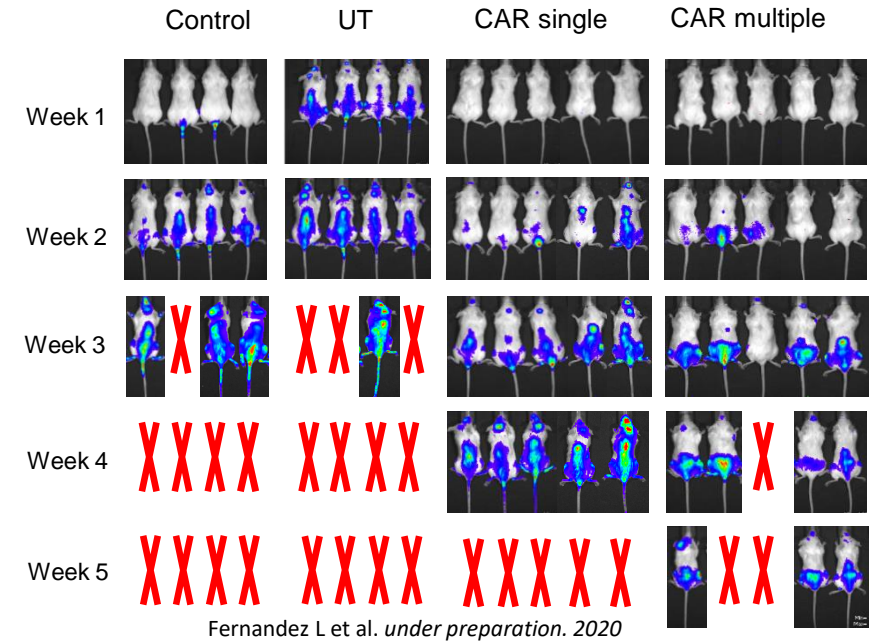
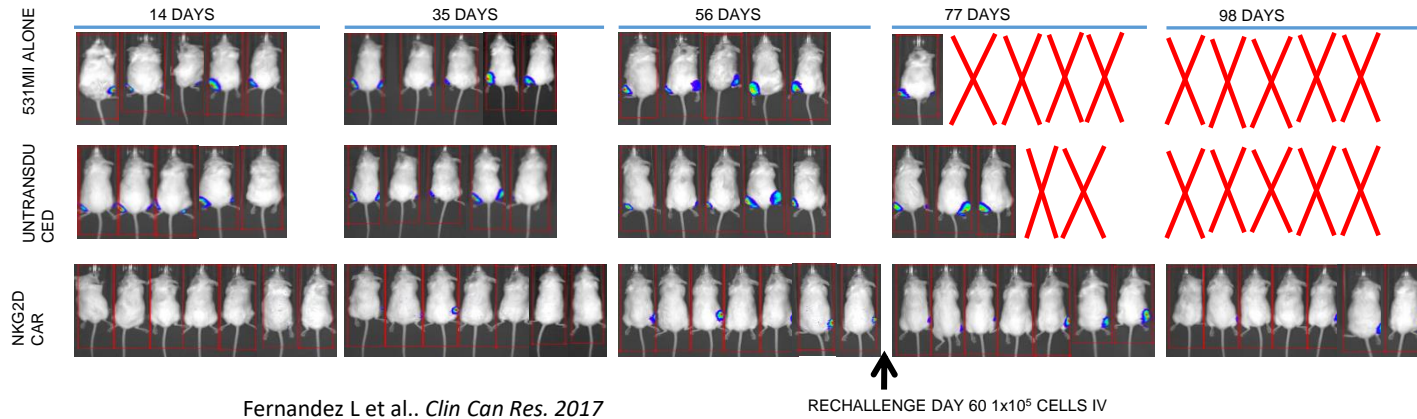
NKG2D CAR vs primary T-ALL blasts



NKG2D CAR vs primary AML blasts



Inmunoterapia basada en células NK



frontiers
in Immunology

ORIGINAL RESEARCH
published: 10 October 2018
doi: 10.3389/fimmu.2018.02661

GMP-Compliant Manufacturing of NKG2D CAR Memory T Cells Using CliniMACS Prodigy

Lucía Fernández^{1*}, Adrián Fernández¹, Isabel Mirones², Adela Escudero³, Laila Cardoso⁴, María Vela⁵, Diego Lanzarotti⁶, Raquel de Paz⁶, Alejandra Lomas^{1*}, Miguel Gallardo^{1*}, Antonio Marcos⁷, Ana Balón Romero⁸, Joaquín Martínez-López^{1*} and Antonio Pérez-Martínez^{1,9*}



” Phase I Trial of Memory T Cells Expressing and anti-NKG2D CAR T in Children, Adolescents and Young Adults with Advanced Sarcoma (CAR4SAR)”



GRUPOS COORDINADOS AECC
2019

SCIENTIFIC PROPOSAL

Coordinator Group:

Dr. Antonio Pérez-Martínez Translational Research in Pediatric Oncology, Hematopoietic Transplantation and Cell Therapy Unit, Hospital Universitario La Paz, Madrid (Spain) **on behalf of the Grupo de Inmunoterapia de la Sociedad Española de Hemato-Oncología Pediátrica.**

Dra. Lucía Fernández, Centro Nacional de Investigaciones Oncológicas (CNIO)

Dr. Javier Garcia Castro, Instituto de Salud Carlos III (ISCIII)

Dr. Carlos López Larrea, Hospital Universitario Central de Oviedo (HUCA)

Madrid, 17 de julio de 2019

”Phase II clinical trial on the use of intratumoral/intraventricular CART-NKG2D or NKIL15 cells in children, adolescent and young adults (AYA) with recurrent/refractory high grade Central Nervous System tumours (CINK-CAR)”



Coordinator Group:



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Dra. Lucía Fernández, CNIO

Dr. Bernat Soria, Universidad de Alicante

Dr. Carcas, UCICEC, Hospital Universitario La Paz

Comments and take message home

- 1. NK cells are the predominant innate lymphocyte subsets that mediate anti-tumor and anti-viral responses, and possess promising clinical utilization**
- 2. NK cells utilize inhibitory receptors, killer immunoglobulin-like receptor to develop, mature, and recognize “self” from “non-self.”**
- 3. Transformed cells increased numbers of stress-induced molecules on their surface which can be recognized by specific activating NK cell receptors, such as NKG2D, “induced self” recognition**
- 4. Novel adoptive “adaptive” and “memory” characteristics of NK cells as “antigen-experienced” NK cells should overcome main adoptive NK cell limitation**
- 5. To improve the clinical efficacy of NK cell immunotherapy has led the development of genetically engineered NK cells that express a chimeric antigen receptor (CARNK and T-NK CAR)**

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ThaNKs

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 Luisa Sisinni
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 Ana Sastre Urgellés
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 Pedro Rubio Aparicio

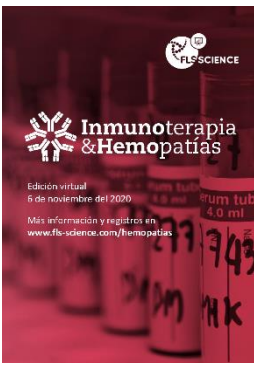
ADVANCED THERAPY UNIT PRODUCTION

Isabel Mirones PhD
 Marta Cobo
 Gema Casado

HEMATOLOGY AND CELL THERAPY

Raquel de Paz Arias PhD
 Mercedes Gassior
 Antonio Marcos
 Ana Romero





Inmunoterapia basada en células NK

Antonio Pérez-Martínez^{1,2,3,4}

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