Inmunoterapia con células NK y CAR-T en el cáncer infantil

Antonio Pérez-Martínez[1,2,3,4]

[1]Translational Research in Pediatric Oncology, Hematopoietic Transplantation and Cell Therapy Unit, Hospital Universitario La Paz, Madrid (Spain), [2]Instituto de Genética Médica y Molecular (INGEMM), Hospital Universitario La Paz, Madrid (Spain), [3]Profesor Titular de Pediatría de la UAM, [4]Jefe de Servicio de Hemato-Oncología Pediátrica, Hospital Universitario La Paz, Madrid (Spain)

1. Antecedentes de la inmunoterapia

2. Inmunoterapia con células NK

3. Inmunoterapia con células CAR-T

4. Transformación de nuestro hospital

1. Antecedentes de la inmunoterapia

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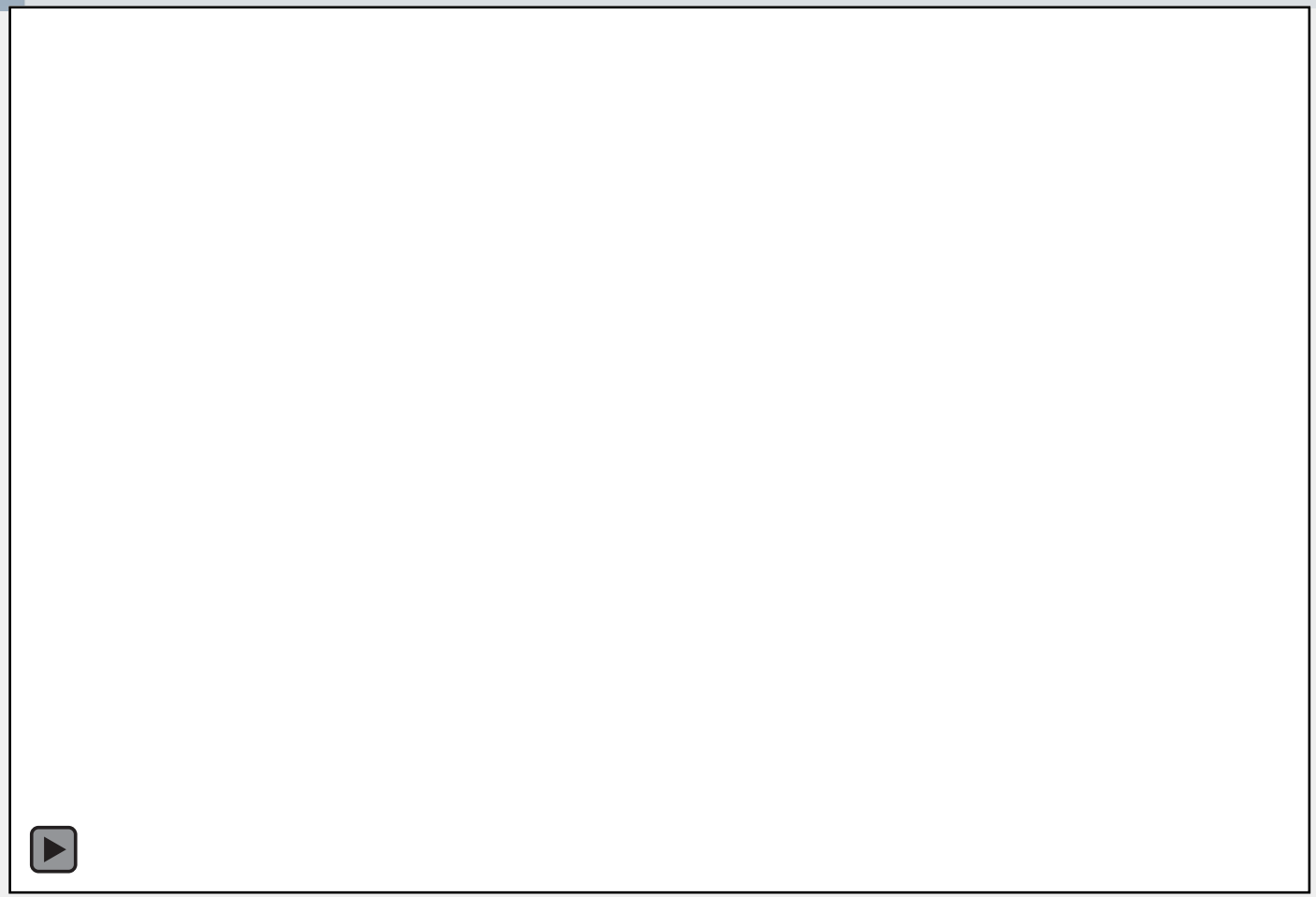
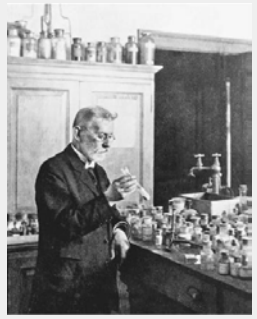
Cytotoxic lymphocyte



James A Sullivan. Quill Graphics. Charlottesville, VA, USA

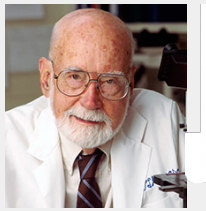
"From immune hypothesis to drug development"



Paul Ehrlich: Hypothesis that host defense forces may prevent neoplastic cells from developing tumors.

Gross and Foley: first clear demonstration of specific capability of tumours to stimulate immune response.

1909 | 1953 | 1957 | 2002 | 2012 | 2018



Innovative Therapies for Children with Cancer
ITCC
European Consortium

Next generation sequencing

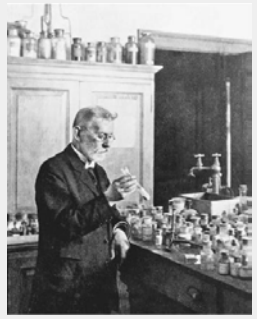
Immunotherapy

aminopterine

Total chemotherapy | **HSCT** | **Clinical trials** | **Personalized medicine** | **Cell therapy and advances therapies**

1955-1990 | **1990** | **2000** | **2005** | **2010**

"From immune surveillance to cancer immunoediting"



Paul Ehrlich: Hypothesis that host defense forces may prevent neoplastic cells from developing tumors.

Gross and Foley: first clear demonstration of specific capability of tumours to stimulate immune response.

Lewis Thomas and MacFarlane Burnet: *The theory of immune surveillance.*

Ralph Schrelber: *The theory of immunoediting.*

Tasuku Honio and James P Allison: *The checkpoint inhibitors*

1909 1953 1957 2002 2012 2018

MILESTONES IN IMMUNOTHERAPY OF CANCER

1957 Bone Marrow Trasplant

1984 IL-2 Immunotherapy

1988 Adoptive T cell

1997 Antibody therapy
2002 NK cell alloreactivity

2012 Dendritic cell vaccines
2013 Checkpoint inhibitor
2017 CART cell therapies

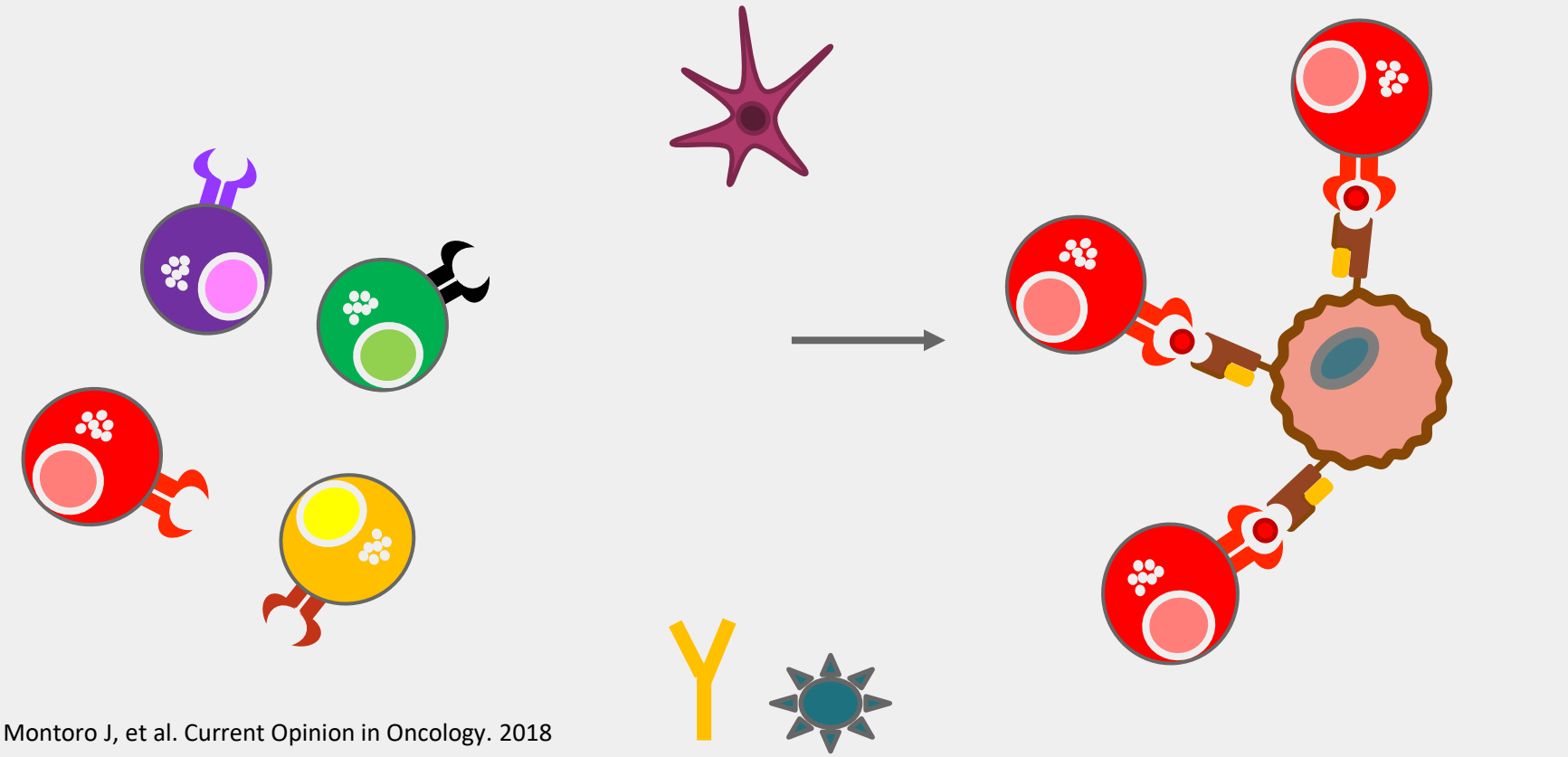
Donald Thomas

Premio Nobel

1990

Tasuku Honio James P Allison

Cytotoxic T lymphocytes



Montoro J, et al. Current Opinion in Oncology. 2018

Direct selection

APC

Non-tumor specific T-cells

Tumor specific T-cell

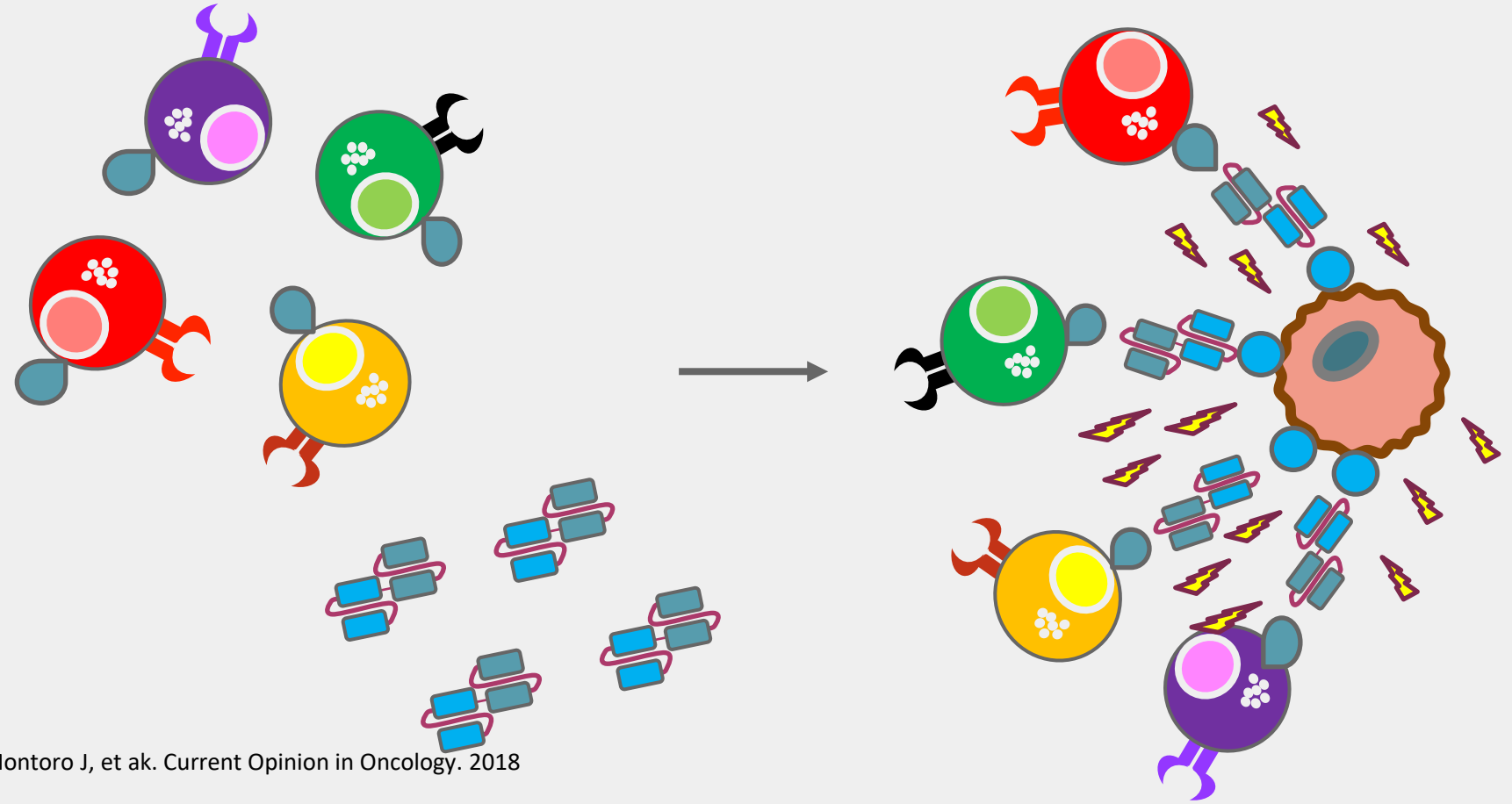
HLA

Tumor antigen

Tumor specific TCR

Tumor cell

Bi-specific antibodies



Montoro J, et ak. Current Opinion in Oncology. 2018

Perforin and granzymes

CD3xCD19 BsAb

CD19

CD3

Non-tumor specific T-cells

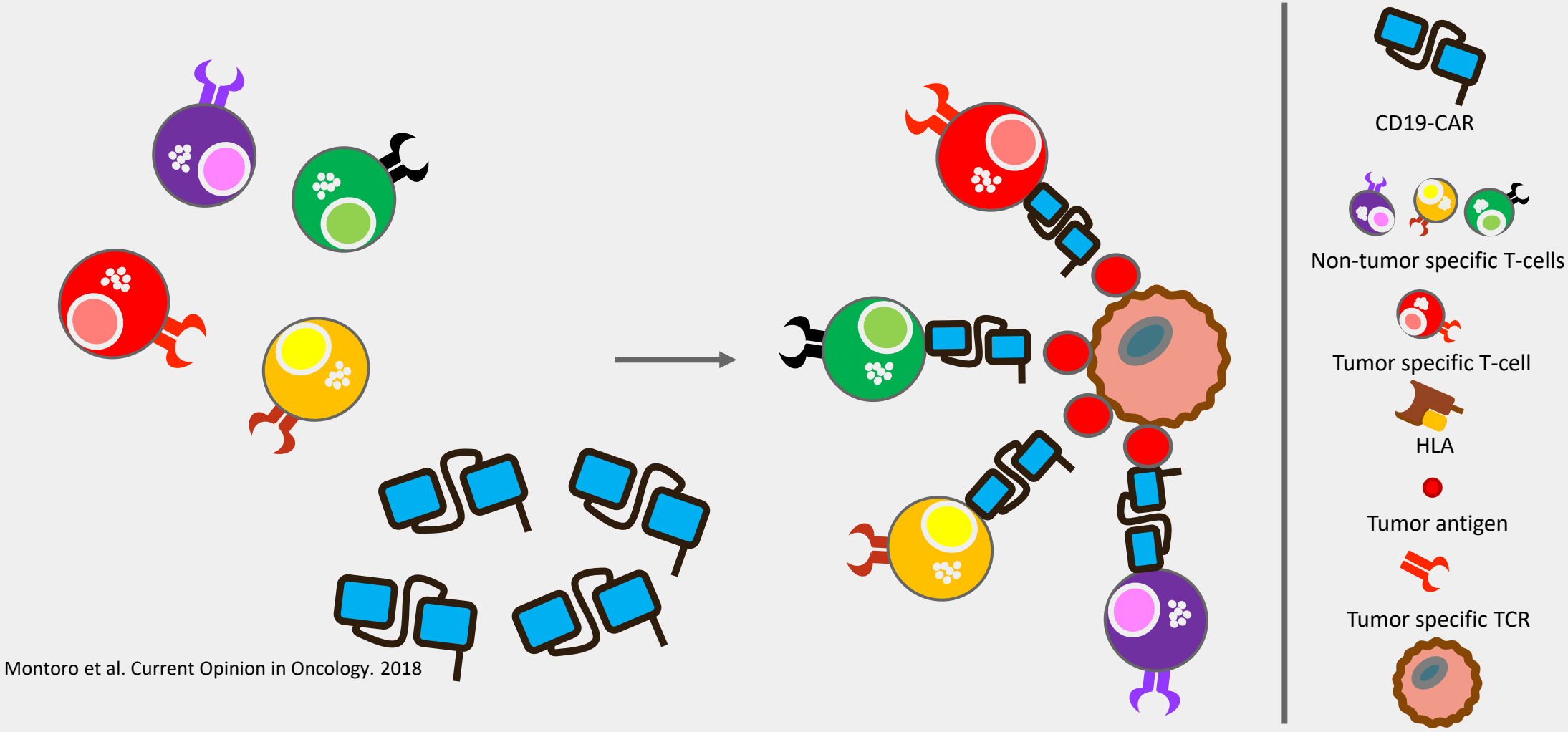
Tumor specific T-cell

HLA

Tumor antigen

Tumor cell

CHIMERIC ANTIGEN RECEPTOR (CAR)-T-CELL



Montoro et al. Current Opinion in Oncology. 2018

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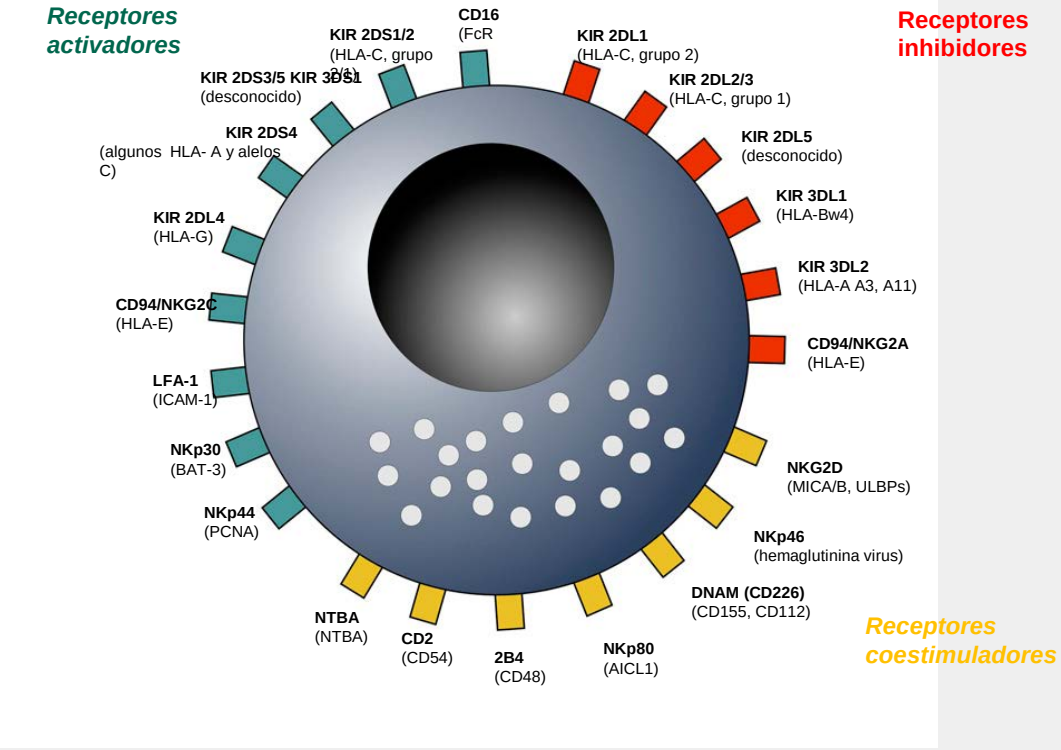
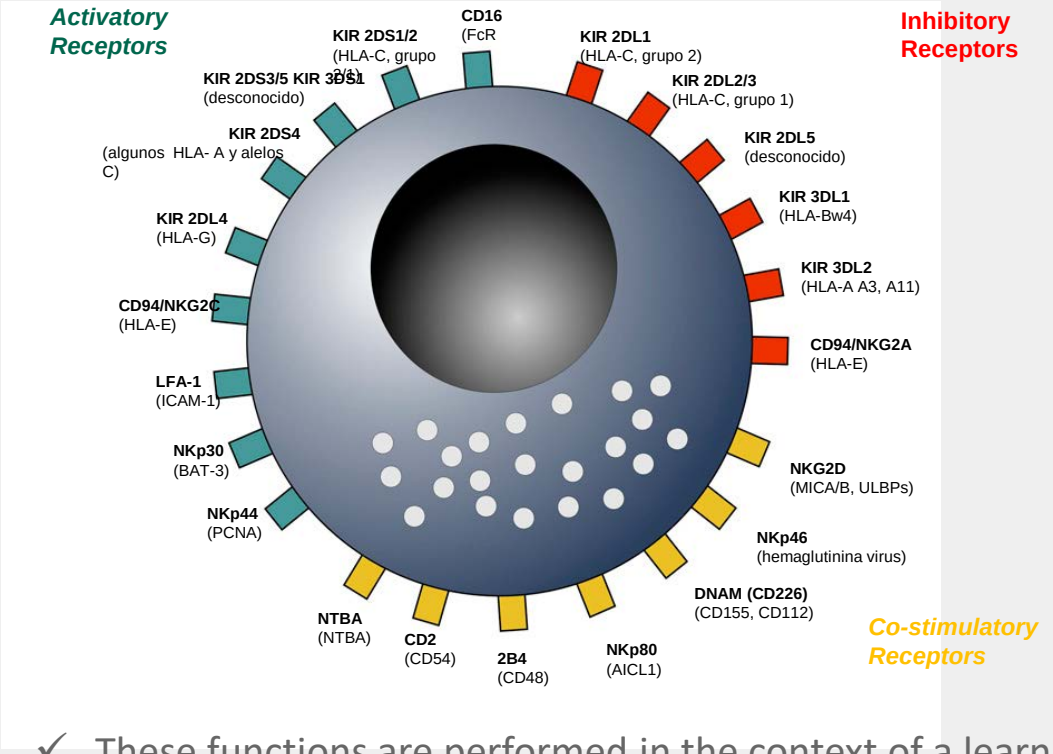
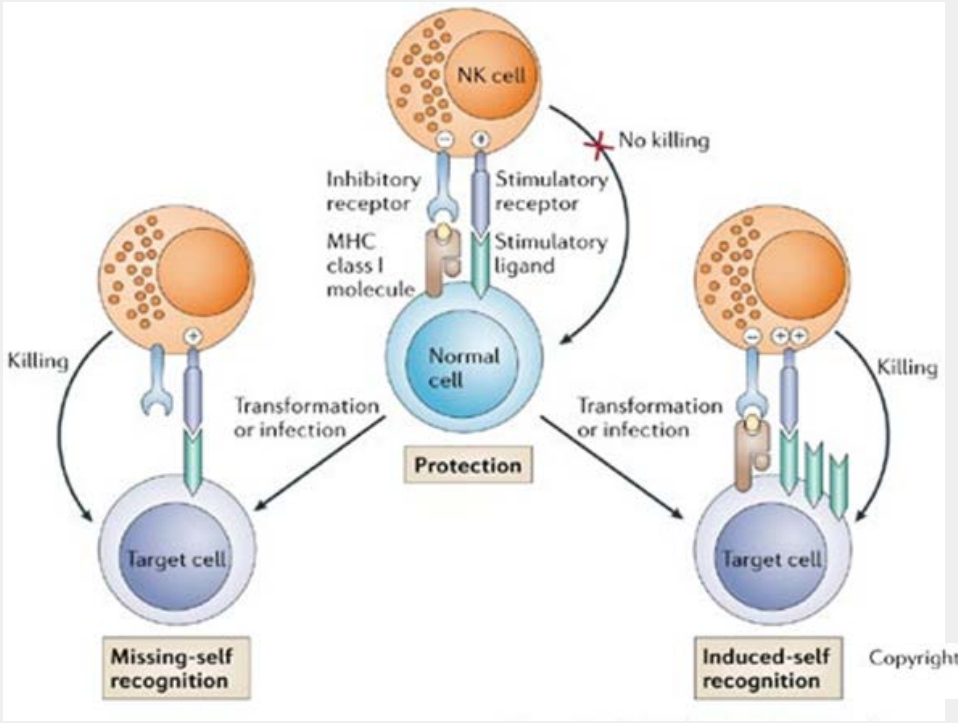
Natural Killer cells for cancer cell therapy



- ✓ Its activation is regulated by a dynamic equilibrium between the activating and inhibiting receptors and their ligands (HLA class I molecules and others).

Natural Killer cells for cancer cell therapy



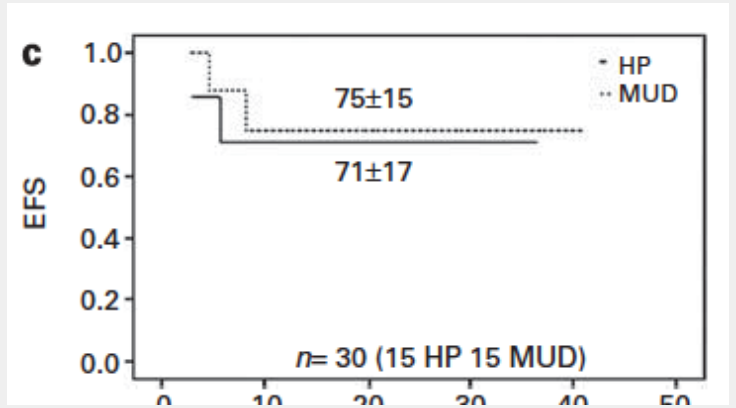


- ✓ These functions are performed in the context of a learning process ("licensing") regulated mainly by inhibitory KIR receptors and their ligands (HLA class I molecules, in humans).
- ✓ In a basal situation the cells of the different tissues express their own ligands (self), HLA class I, so they are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

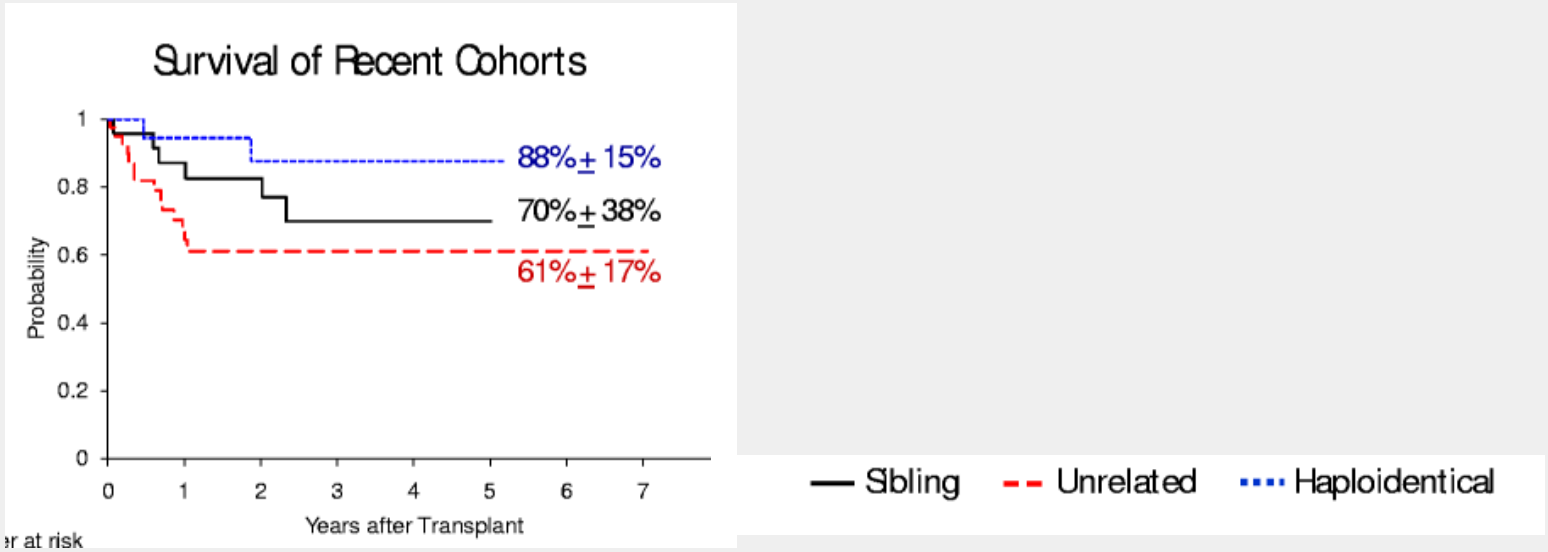
Natural Killer cells for cancer cell therapy

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]*

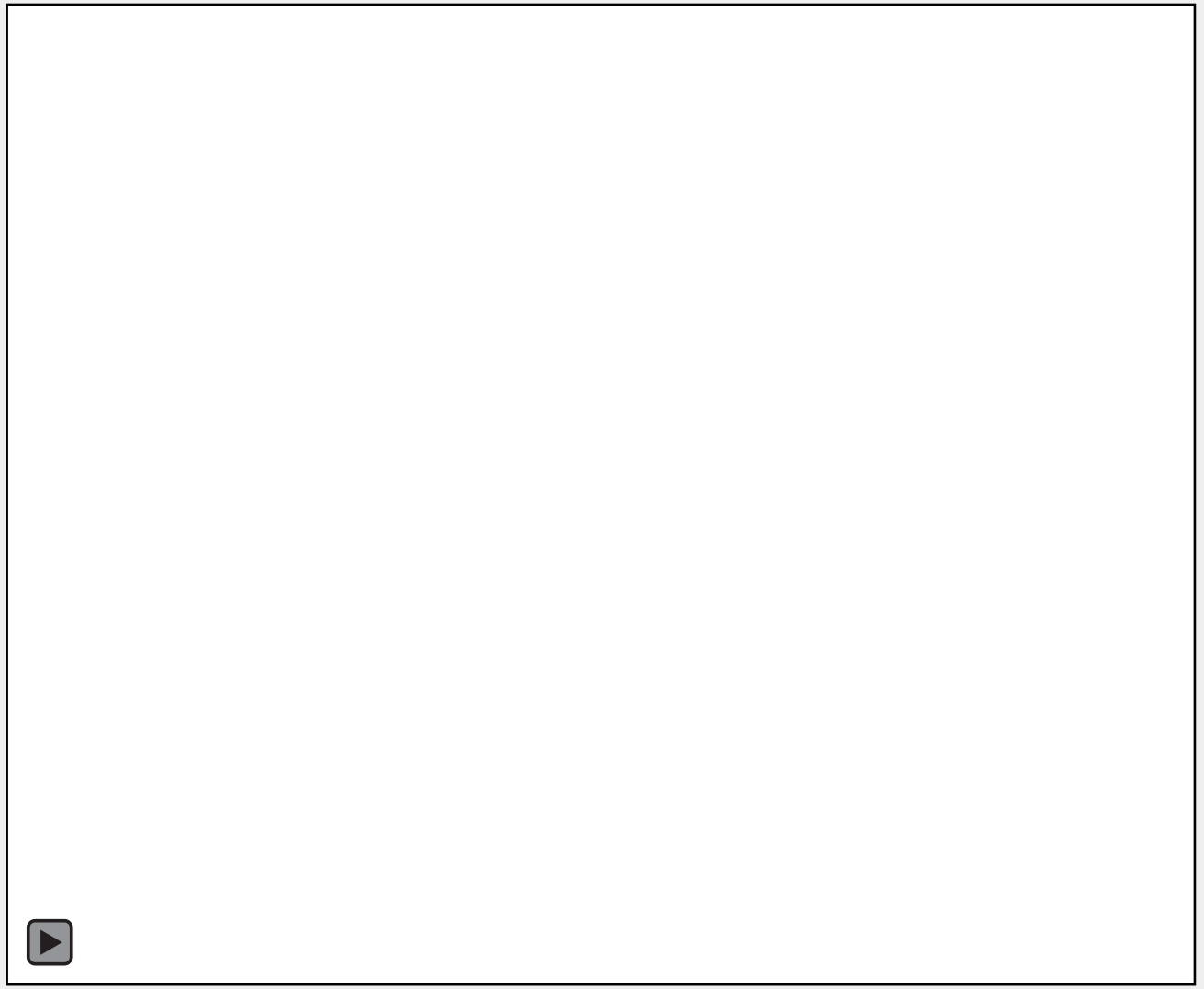


Pérez-Martínez A. BMT 2012:1-9.



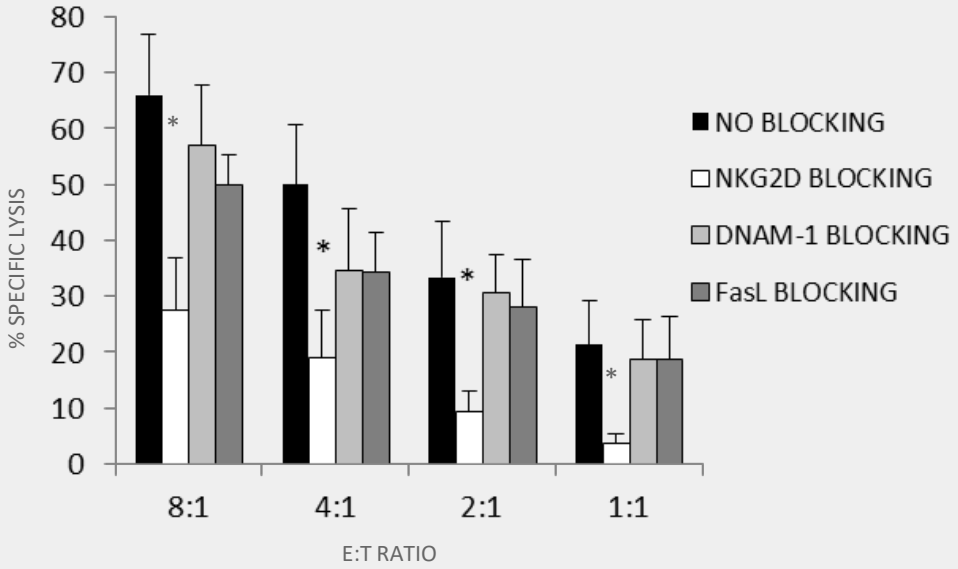
Leung W. Blood. 2011 Jul 14;118(2):223-30

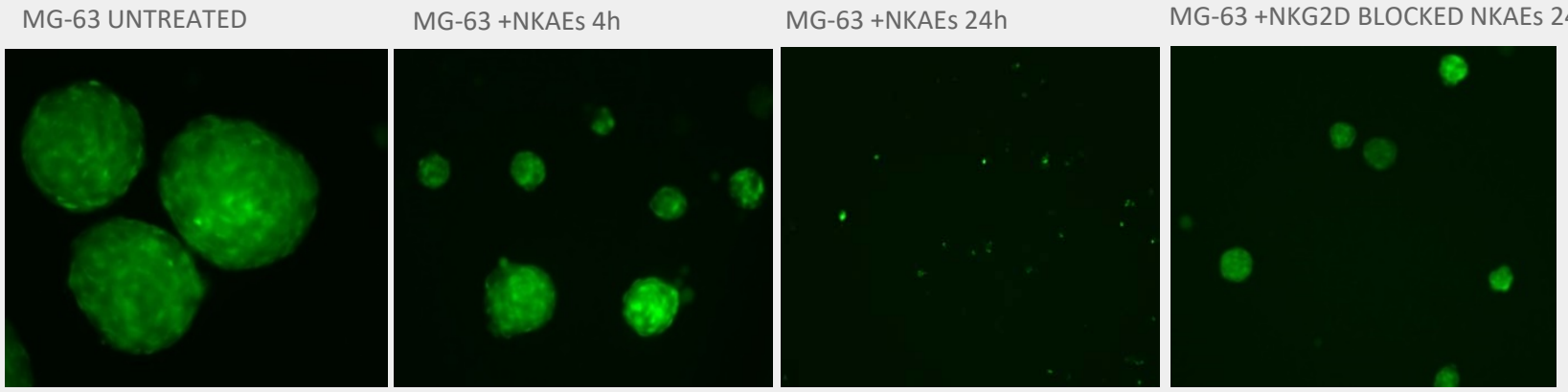
Natural Killer cells for cancer cell therapy

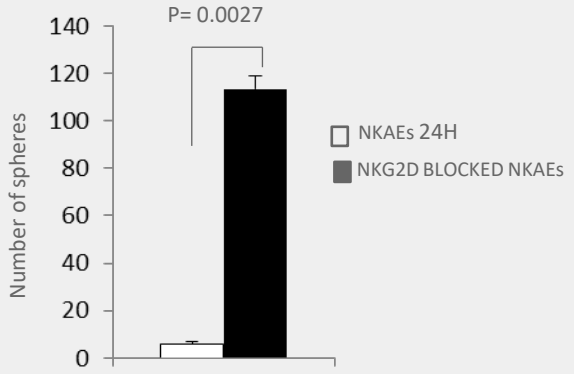


Natural Killer cells for cancer cell therapy

NKAE cells target OS TICs using NKG2D-NKG2DL interactions



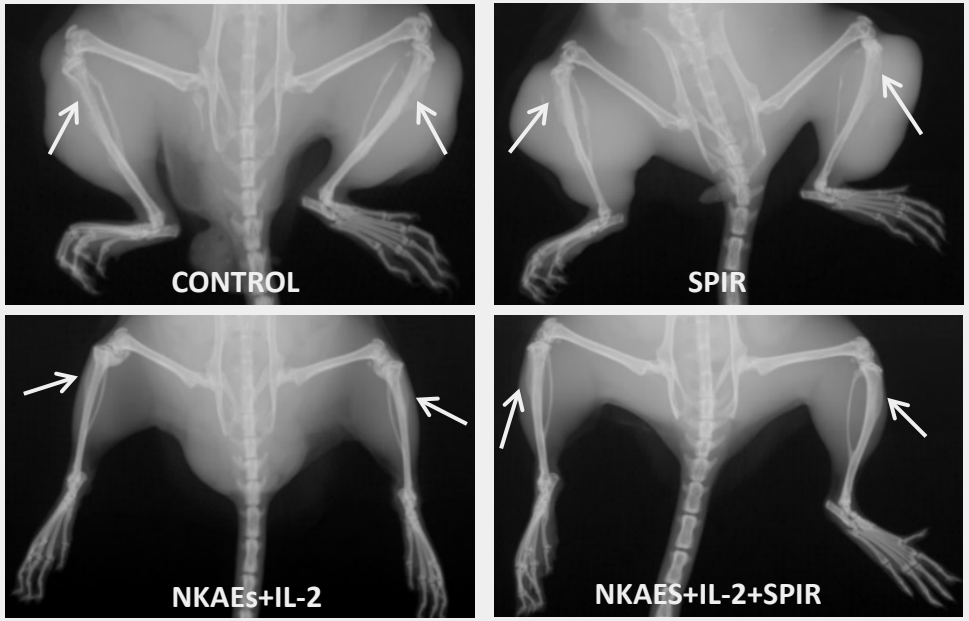


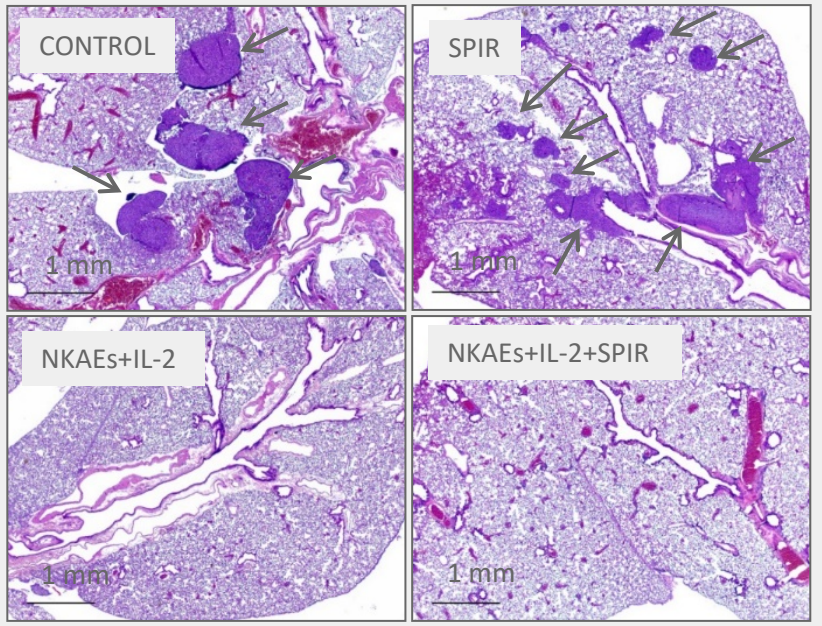


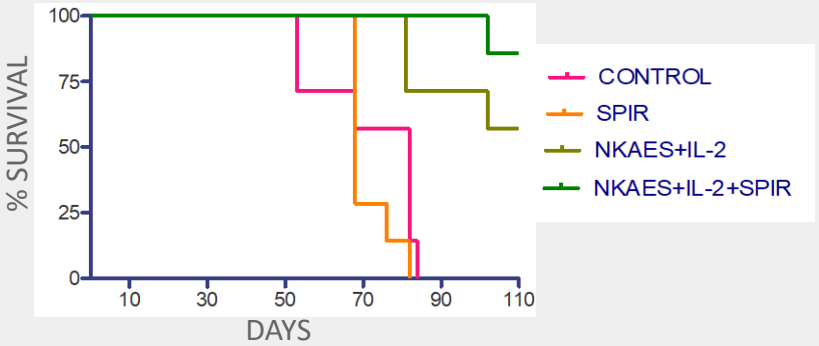
Fernández L et al. Cancer Letter 2016.

Natural Killer cells for cancer cell therapy

In vivo, NKAE cells reduce tumor burden, avoid metastases and prolong survival







Fernández L et al. Cancer Letter 2016.

Natural Killer cells for cancer cell therapy

Activation and expansion protocols:

- Overnight stimulation with IL-15

- 15-21 days co-culture with K562-mb15-4.1bbl

Natural Killer cells for cancer cell therapy

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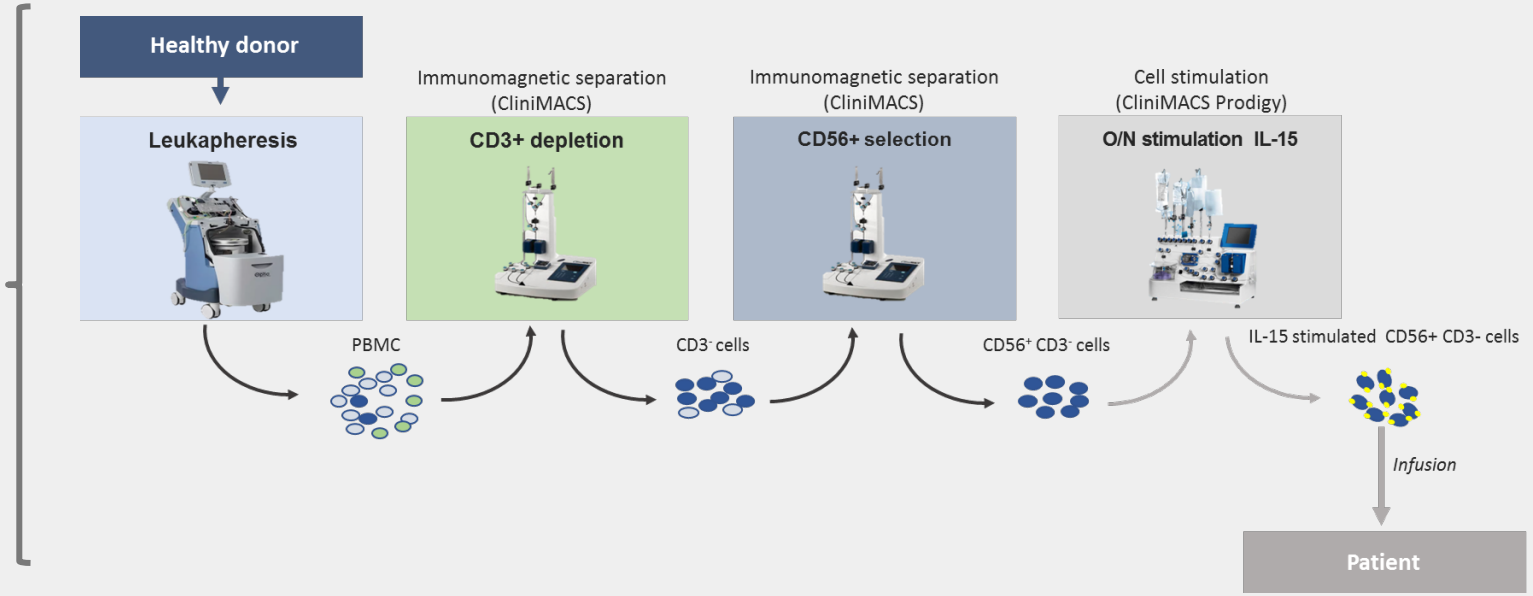

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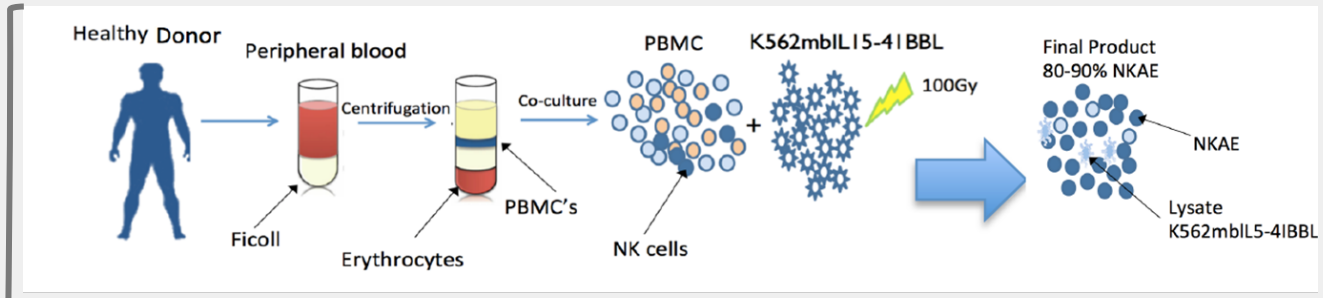

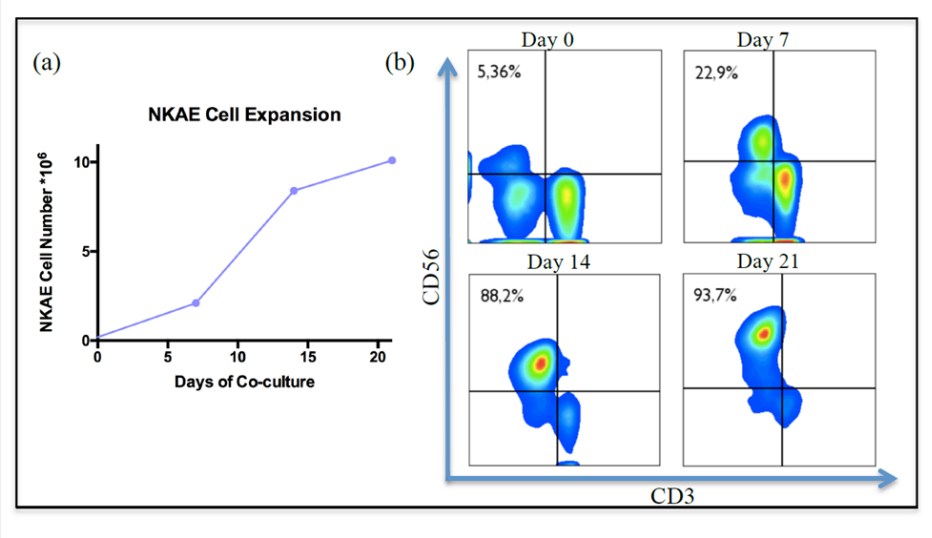

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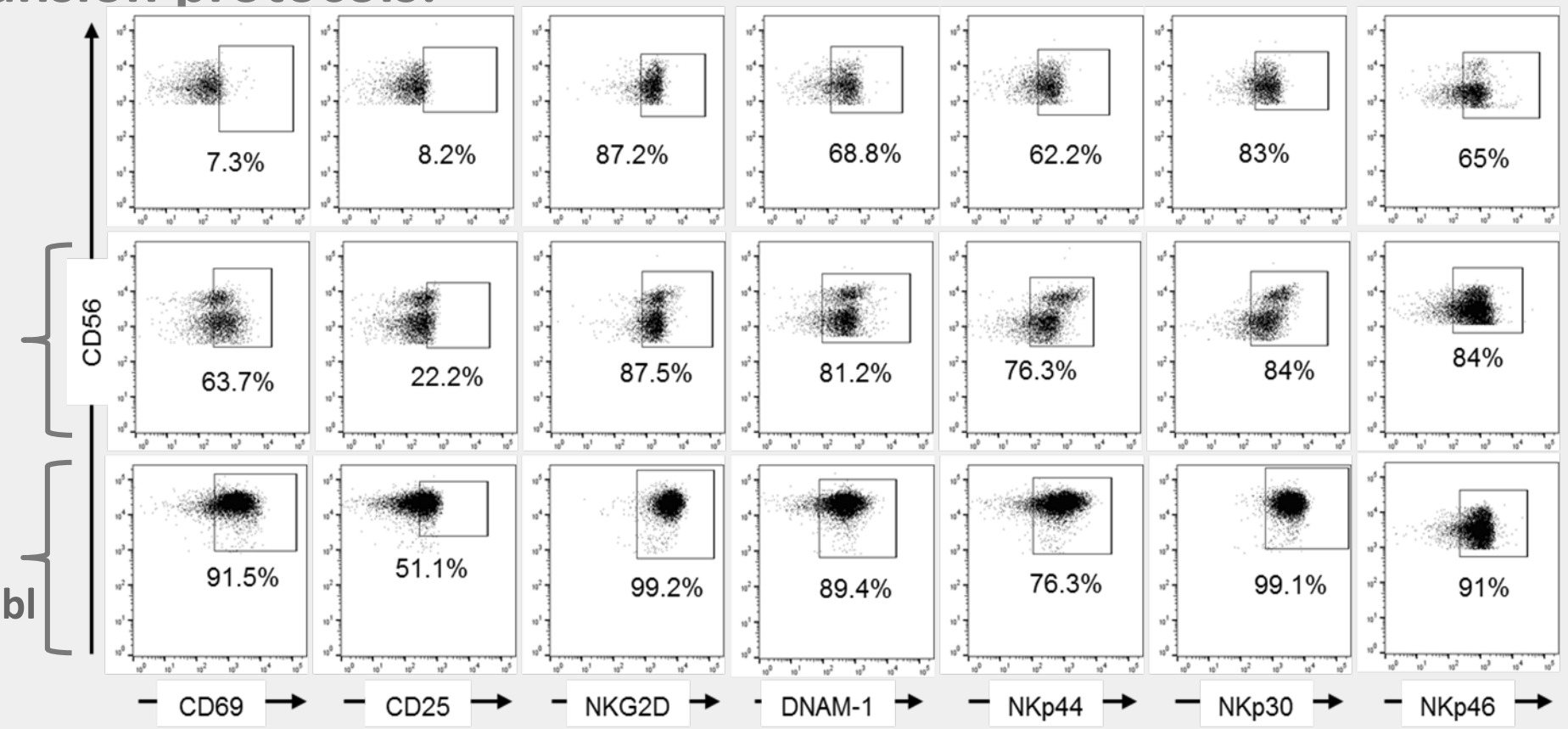
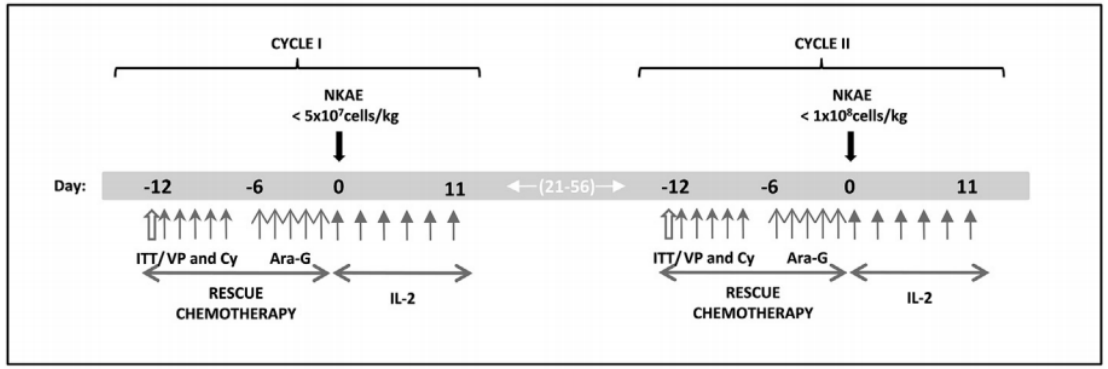


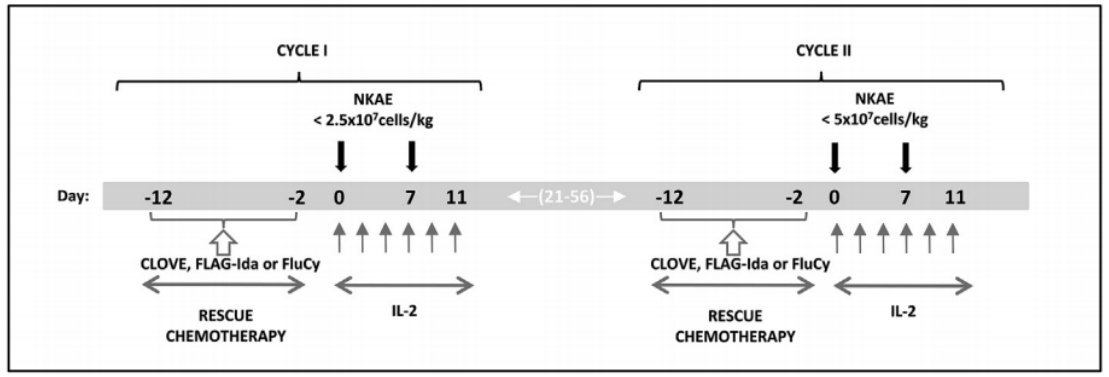
Fernández F, et al. Transfusion 2018. How do we manufacture clinical-grade interleukin-15-stimulated natural killer cell products for cancer treatment?

Natural Killer cells for cancer cell therapy

❖ HNJ-NKAES-2012



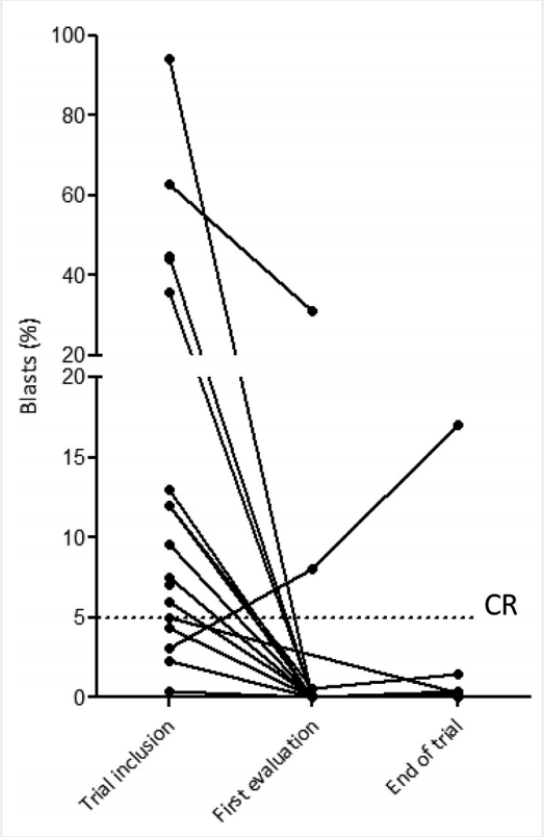
❖ LYDIA



- In both cases, two cycles of rescue chemotherapy followed by NKAE and IL-2 infusions were administered

Vela M, et al. Cancer Lett. 2018. Haploidentical IL-15/41BBL activated and expanded natural killer cell infusion therapy after salvage chemotherapy in children with relapsed and refractory leukemia.

Natural Killer cells for cancer cell therapy



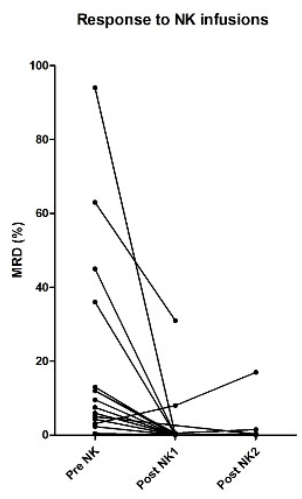
- **Bone marrow response to treatment.**

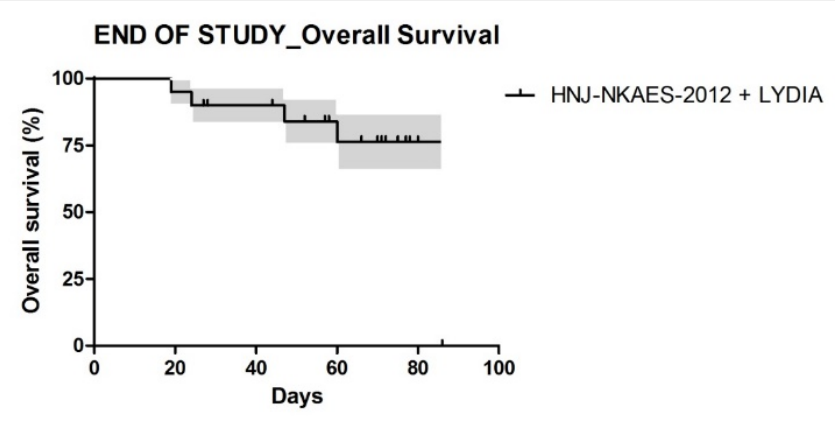
  Percentage of blasts of each patient at trial inclusion, after first treatment cycle and at he end of the study are indicated.

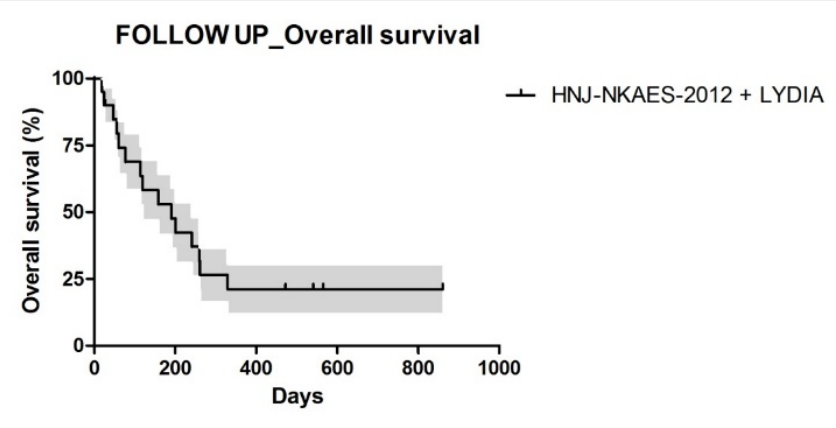
Vela M, et al. Cancer Lett. 2018. Haploidentical IL-15/41BBL activated and expanded natural killer cell infusion therapy after salvage chemotherapy in children with relapsed and refractory leukemia.

Natural Killer cells for cancer cell therapy

	NCT01944982	NCT02074657	
	HNJ-NKAEs (EudraCT: 2012-005146-38)	**LANK-2/LYDIA (EudraCT: 2012-000054-63)**	**Total**
Characteristic	7	13	20
Response (%)			
Cytological remission	3 (42)	4 (30)	7 (35)
MRD negative	2 (28)	5 (38)	7 (35)
Progression	1(16)	1 (16)	2 (10)
Died because toxicity	1(16)	3 (23)	4 (20)
Get a HSCT (%)	3 (42)	7 (53)	10 (50)
Status			
Alive without disease	1 (14)	5 (38)	6 (30)
Follow-up (days)	150 (27-230)	177 (19-350)	164 (19-350)







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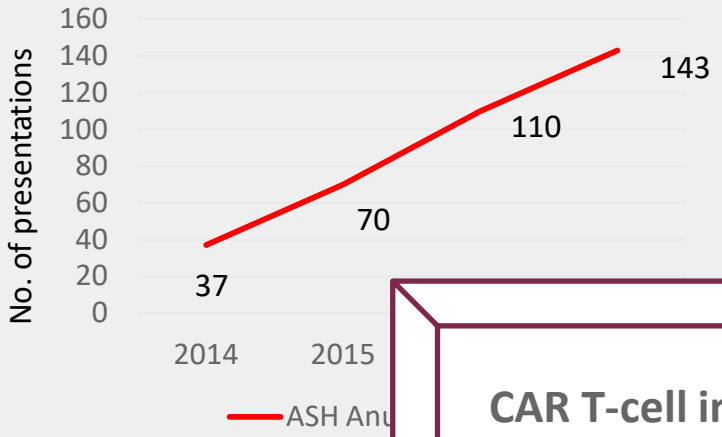
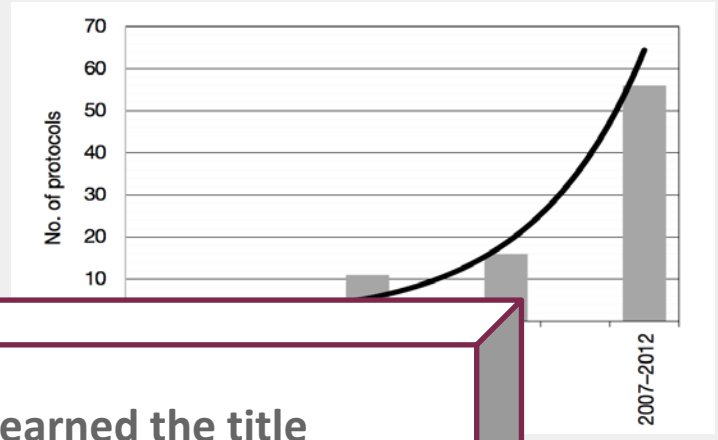
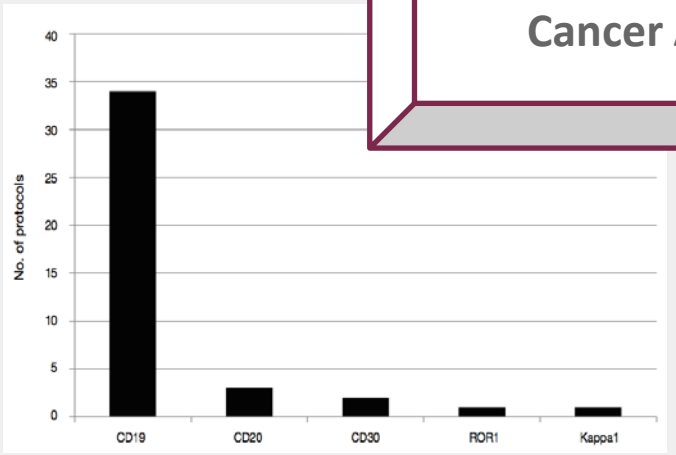
CARs: State of the science

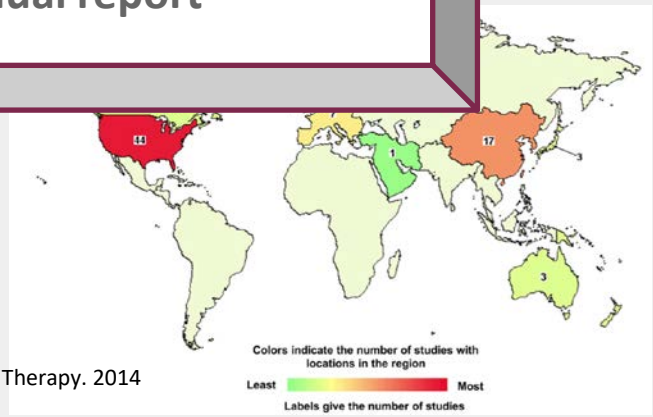




CAR T-cell immunotherapy earned the title "Advance of the Year" in the ASCO Clinical Cancer Advances 2018 annual report

atol Malig Rep.





Corrigan-Curay J., et al. Molecular Therapy. 2014

First two CAR-T cell medicines recommended for approval in EU

NOVARTIS

Novartis LAL <25 y: 475,000 $ / patient response) 408 m€
Soon in April that LAL in Adults and later NHL

NICE = £282,000 per patient (Sep 5, 2018) 317 m€

Kite
A GILEAD Company

Kite NHL DLBCL: 373,000 $ / patient

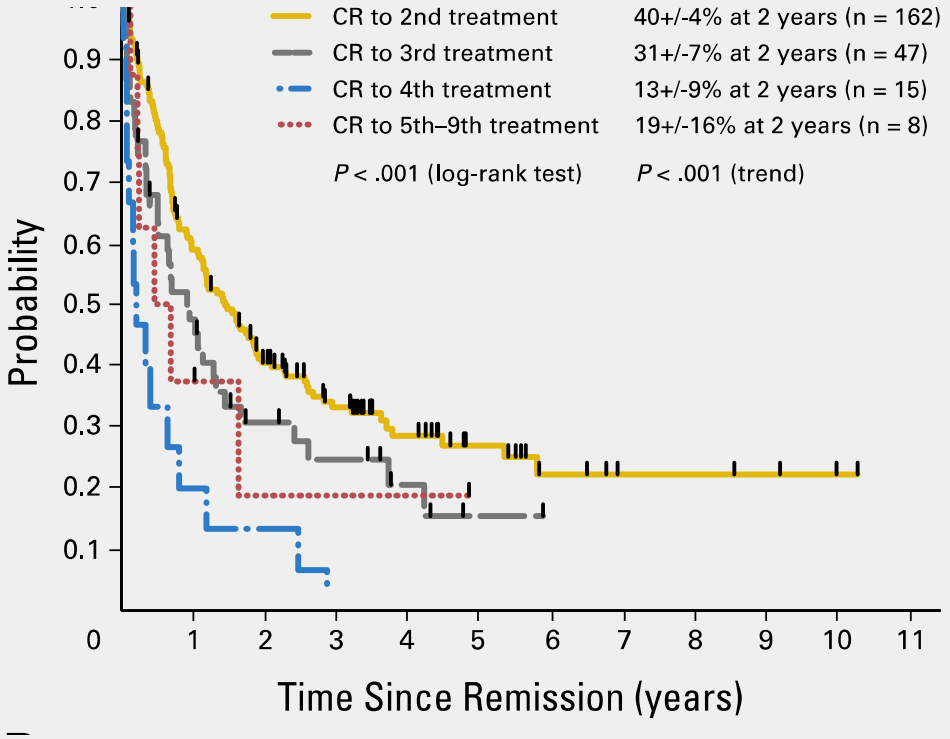
Pending Pr

http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2018/06/news_detail_002983.jsp&mid=WC0b01ac058004d5c1

Childhood leukemia

- By contrast with the steadily improved outcome of patients with newly diagnosed ALL, little progress has been made in the treatment of relapsed ALL

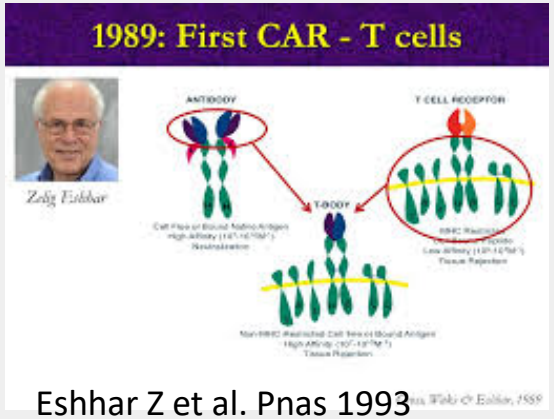
- r/r ALL: clofarabine+cy+etoposide. Toxic-related mortality 24%



Ko et al. J Clin Oncol 2010

CAR-T cells : “from the T-cell body approach to first class FDA approval”

The T-body approach



Eshhar Z et al. Pnas 1993

M. Sadelain

D. Campana

T-Body 1993

HER2-CAR 1994

GD2-CAR 2001

CD19-CAR 2003

CD28 CAR 2002

41BB CAR 2004

Kymriah, Novartis Pharmaceuticals
(Tisagenlecleucel)

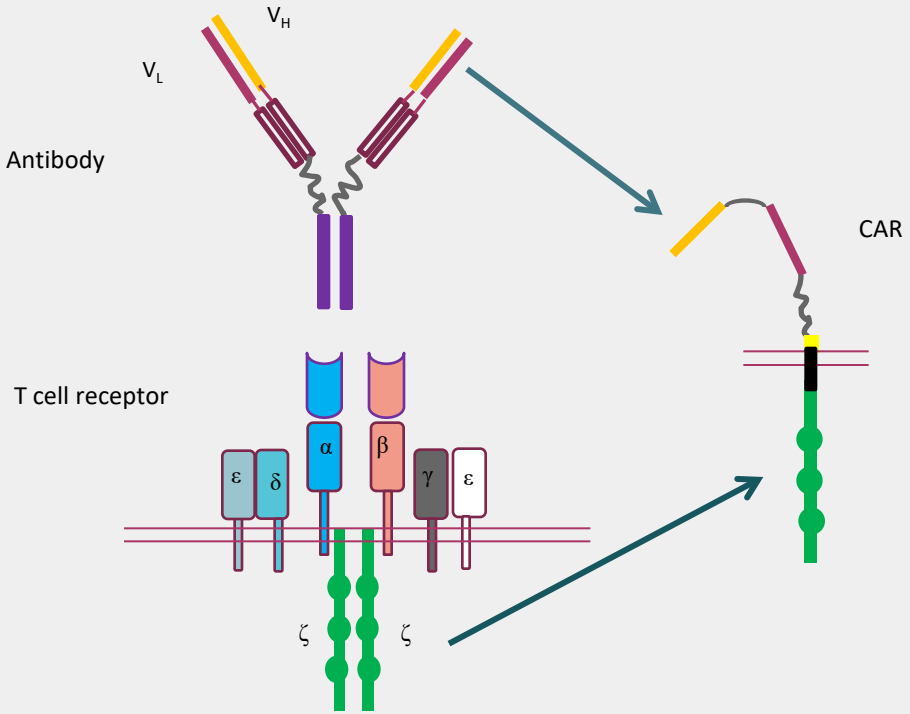
FDA approval
8/30/2017

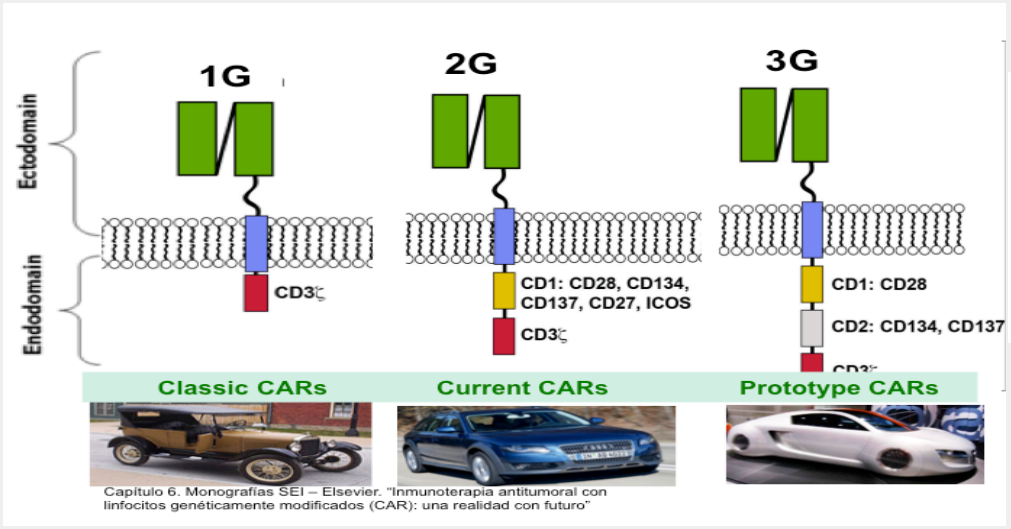
CAR-T cells : “from the T-cell body approach to first class FDA approval”

STRUCTURE AND FUNCTION

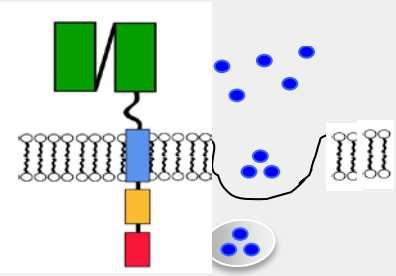
- Engineered receptor → redirect immune cells

  Phusion protein: Recognition Domain + Cytotoxic domain (CD3ζ) [+ costimulation (CD28/4-1BB)]





4G
(armored CARs)

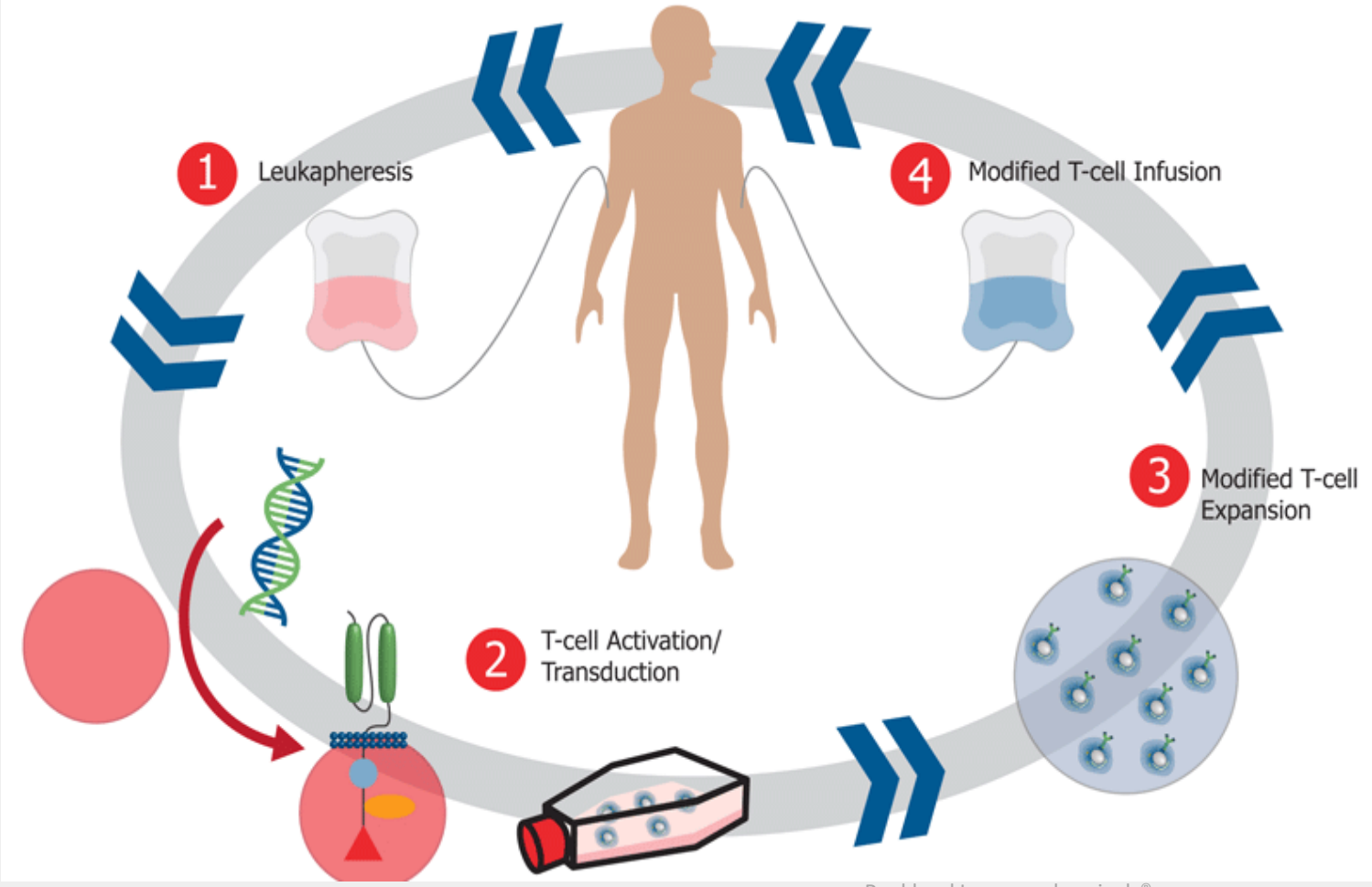


T cells Redirected for Universal Cytokine Killing

Adapted from:

B. Savoldo, G. Dotti / Immunology Letters 155 (2013) 40–42

CAR-T cell therapy

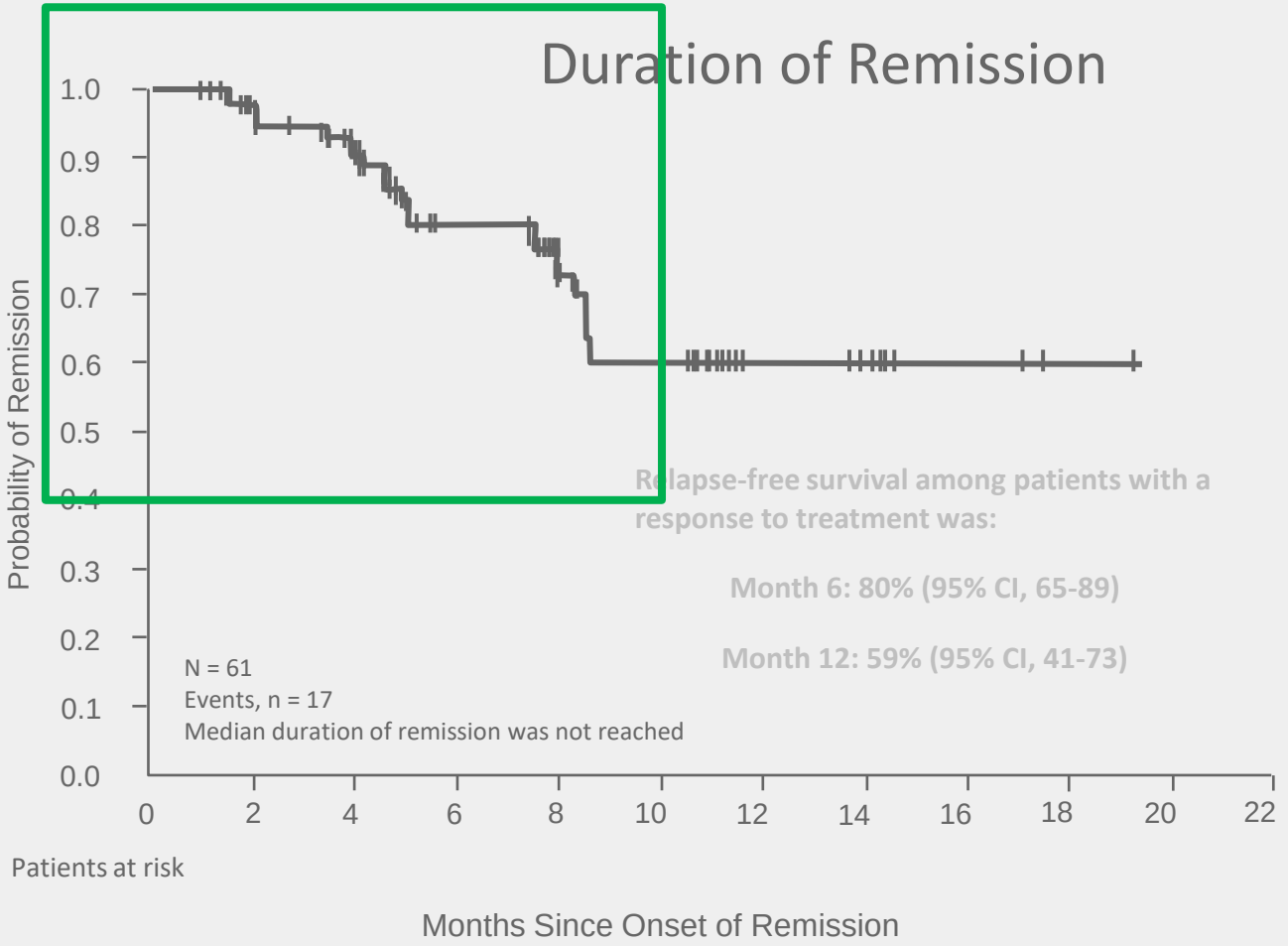


Rockland Immunochemicals®

CART19 Efficacy

75 patients who received a tisagenlecleucel infusion and had at least 3 months of follow-up:

- Overall remission rate was 81% (95% CI, 71 to 89)
  - 45 patients (60%) had complete remission
  - 16 (21%) had complete remission with incomplete hematologic recovery.
  - All of them were negative for minimal residual disease

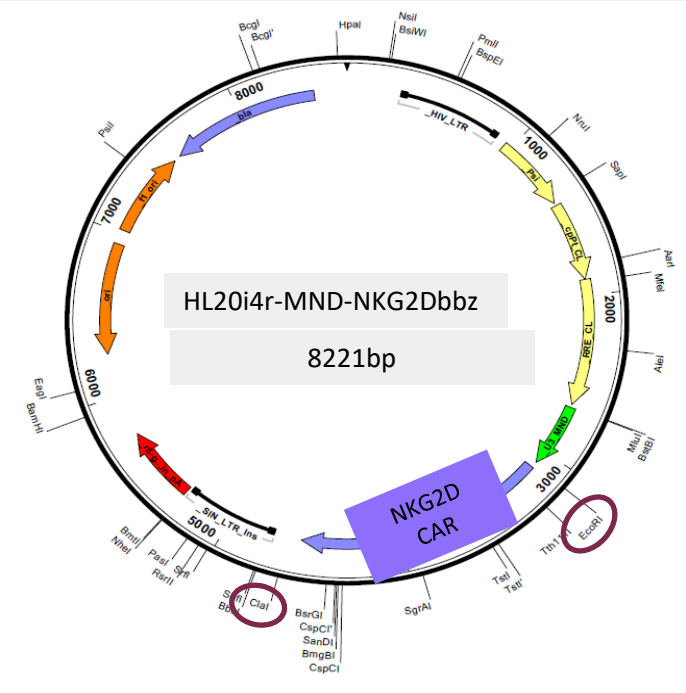


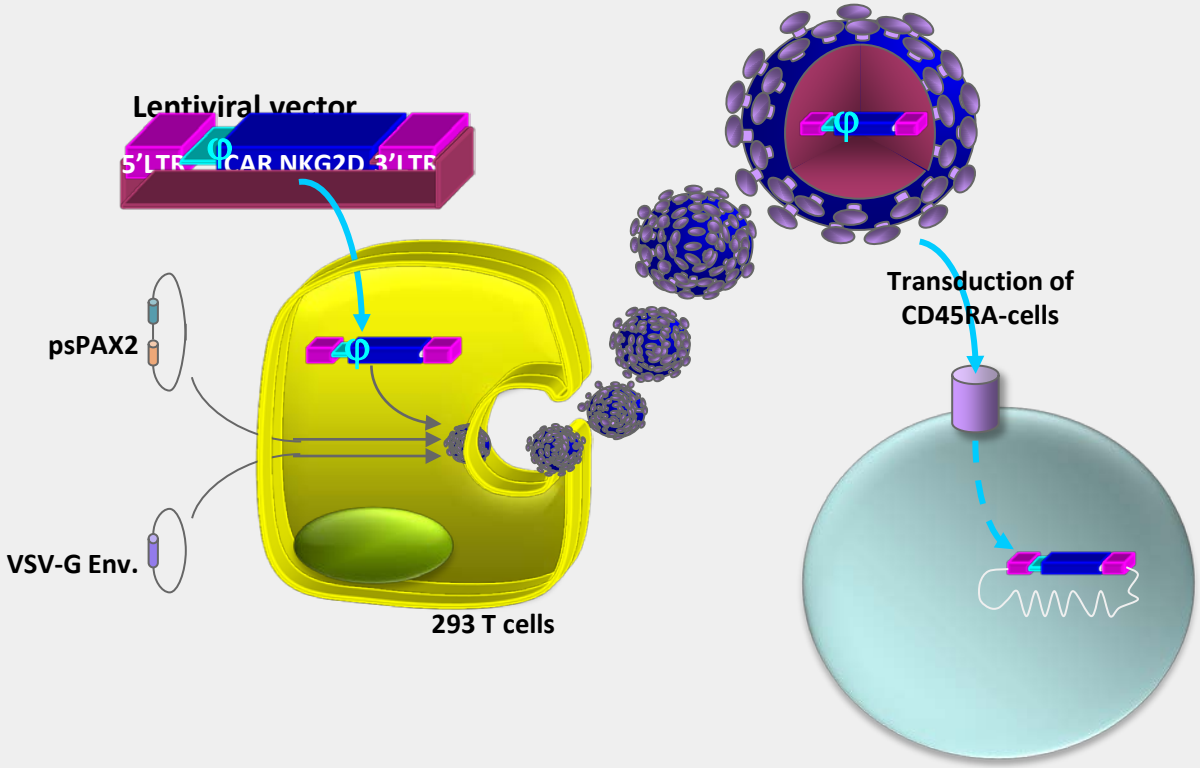
1 CD19+
15 CD19-
No CNS relapse

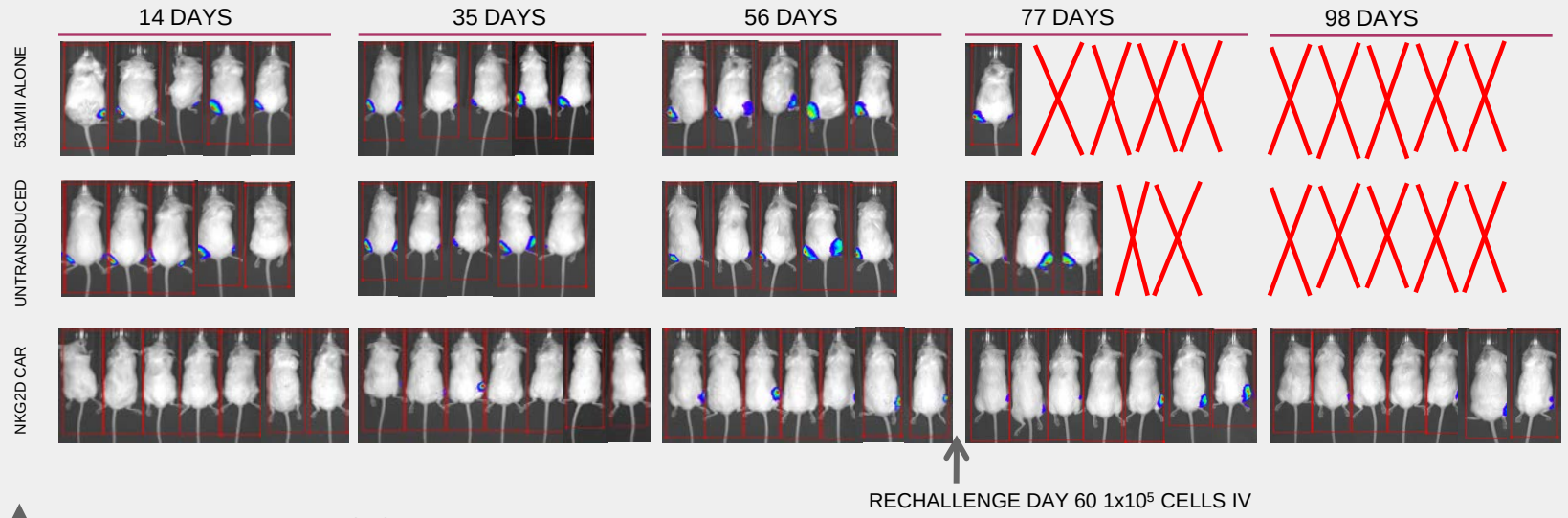
N Engl J Med 2018;378:439-48.

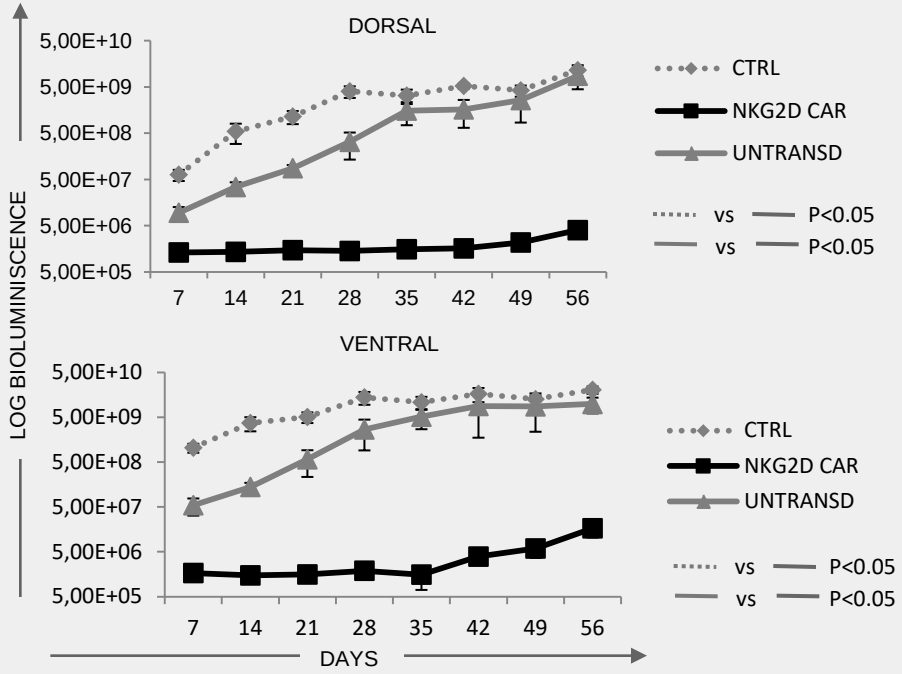
The T-NK approach: opportunity and innovation

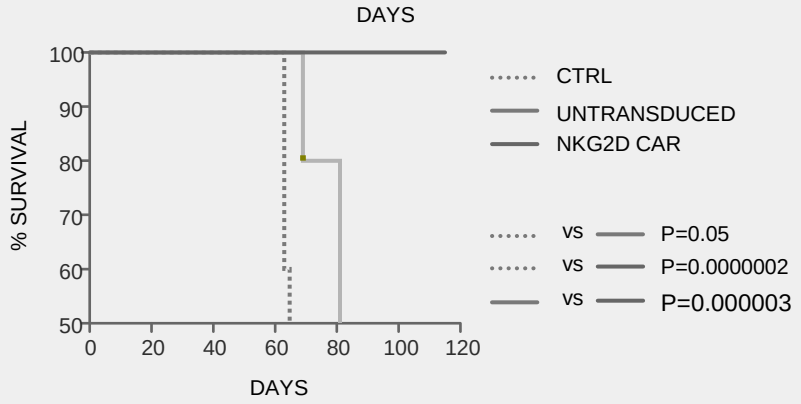
The T-NK approach



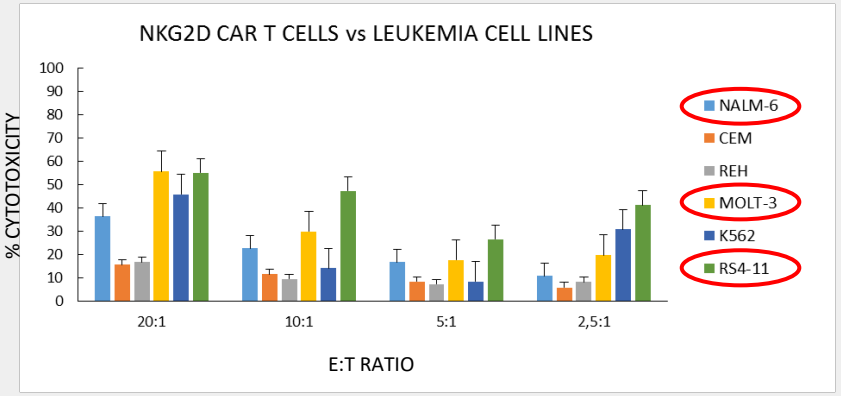


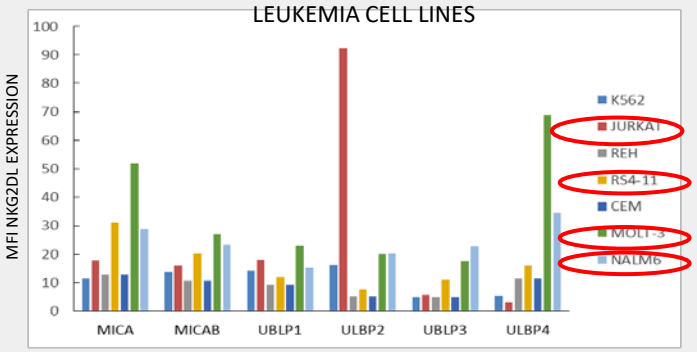




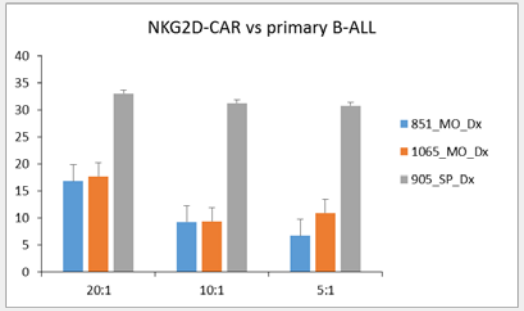


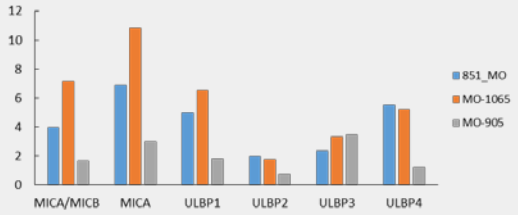
Fernandez et al. *Clin Can Res* 2016

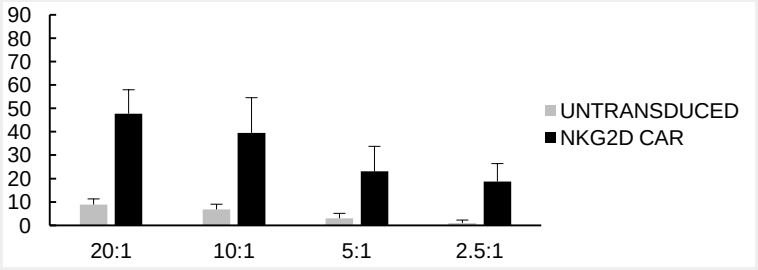


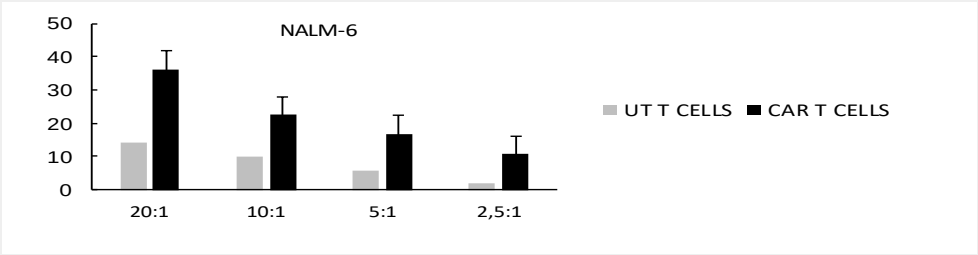


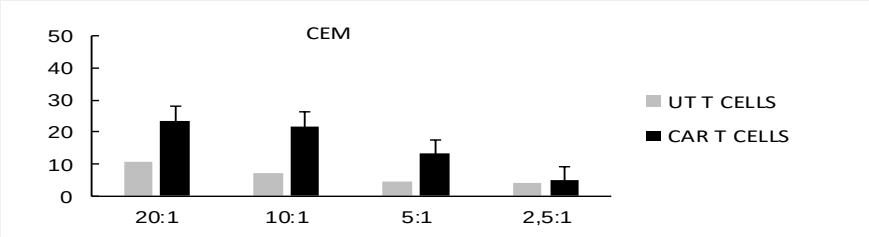
MOLT-3: T-ALL
RS 4-11: ALL t(4;11).
NALM-6: LYMPHOMA
K562: CML

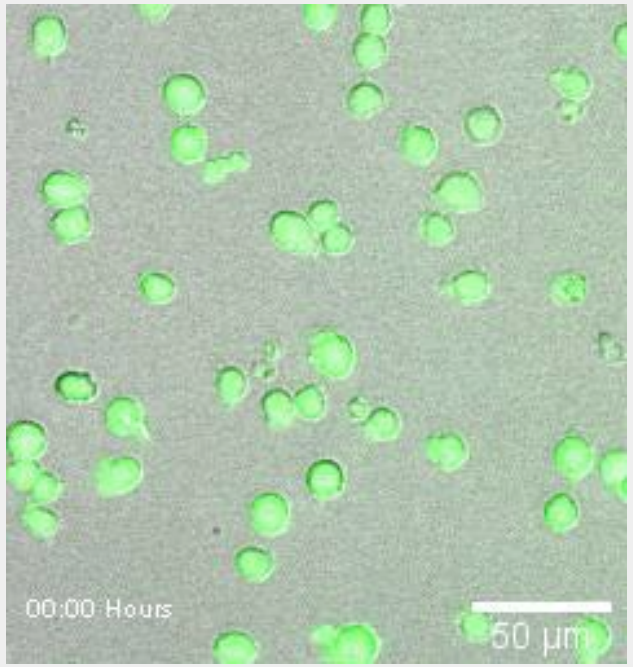


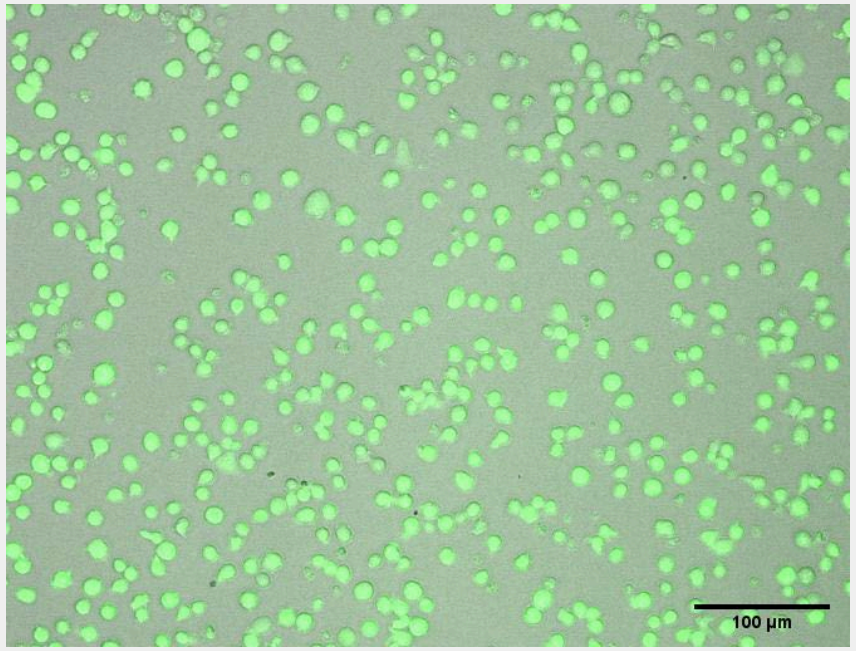


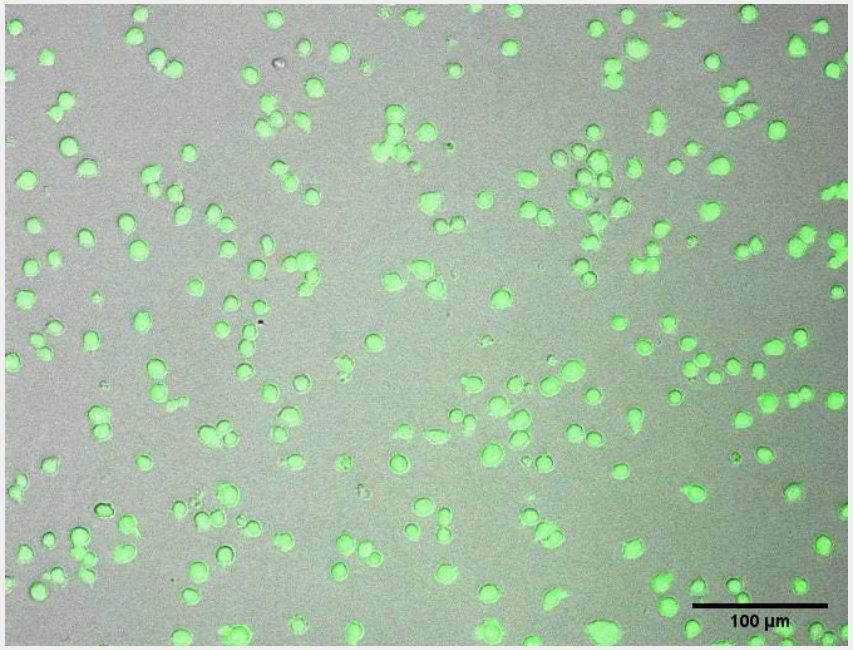

90
80
70
60
50
40
30
20
10
0
20:1
10:1
5:1
2.5:1
UNTRANSDUCED
NKG2D CAR


NALM-6
50
40
30
20
10
0
20:1
10:1
5:1
2,5:1
UT T CELLS
CAR T CELLS

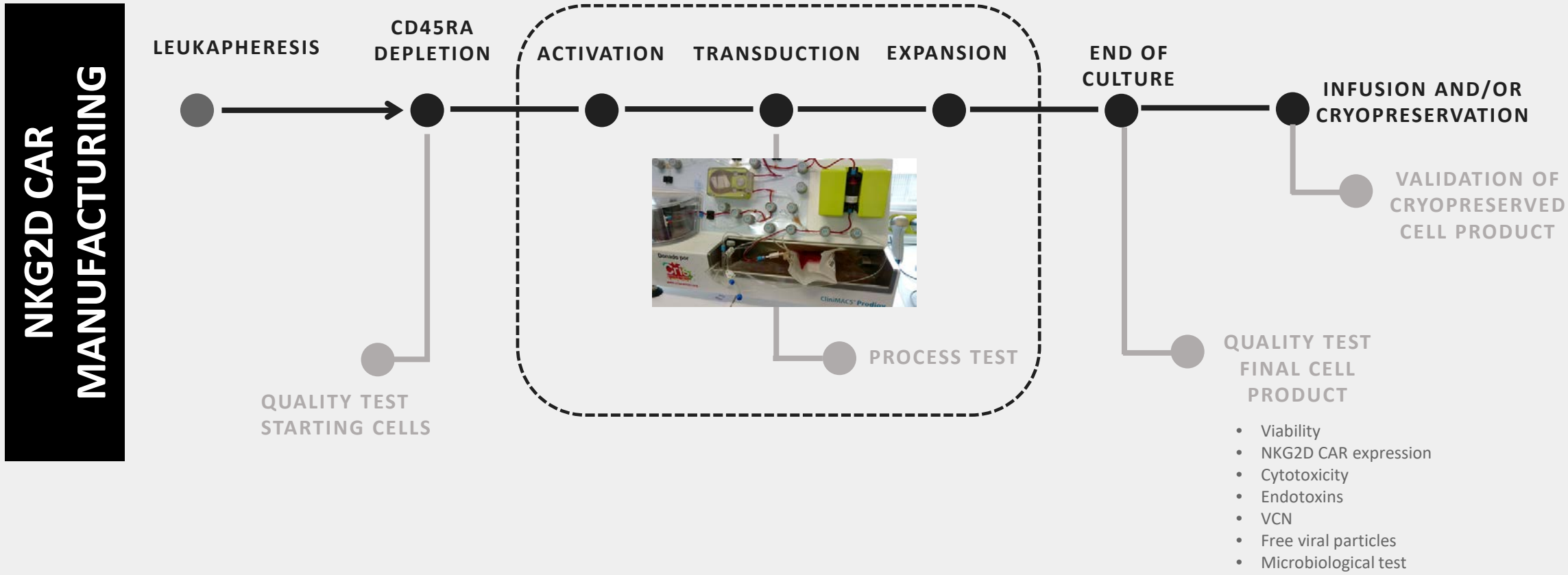

CEM
50
40
30
20
10
0
20:1
10:1
5:1
2,5:1
UT T CELLS
CAR T CELLS


00:00 Hours
50 µm


100 µm

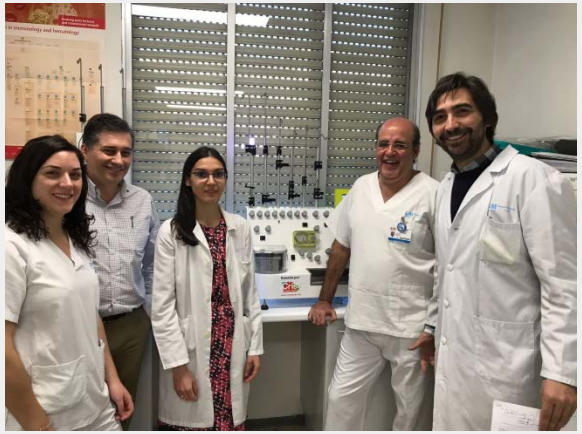

100 µm

Clinical grade automated production of NKG2D-CAR T cells



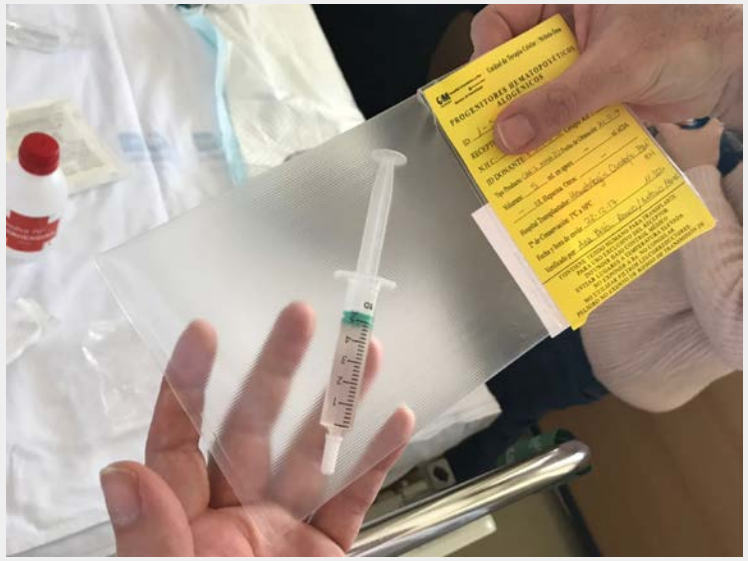
Characteristics of manufactured NKG2D-CAR T cells

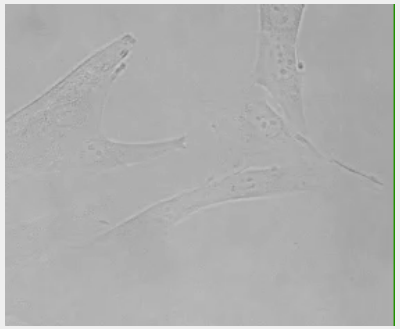
Test	Specification	1-565-2	1-518-3	1-595
Number of cells ($x10^6$)	N/A	2740	2140	1350
Cell viability	≥60%	65%	81.4%	82%
NKG2D expression	≥50%	55%	87.4%	91%
Potency	vs. Jurkat: ≥20% vs. 531MII: ≥20%	vs. Jurkat: 100% vs. 531MII: 20%	vs. Jurkat: 80% vs. 531MII: 42%	vs. Jurkat: N/A vs. 531MII: N/A
Mycoplasma/Sterility	Negative	Negative	Negative	Negative
Endotoxins	< 0.25 EU/ml	0.019 EU/ml	0.0035 EU/ml	0.01 EU/ml
Genome integrated copy/cell	≤ 5 copies/cell	3.62	12.32	2.43
Oncogenic gene expression (myc, tert)	No overexpression	No overexpression	No overexpression	Myc overexpression
Genetic stability	Normal CGH	Normal CGH	Normal CGH	Normal CGH



CliniMACS Prodigy (Miltenyi Biotec)


MAQUINA EN
FUNCIONAMIENTO
"NO TOCAR"





Instituto de Investigación Hospital Universitario La paz (IdiPAZ)

Centro Nacional de Investigaciones Oncológicas (CNIO)

Experimentación preclínica

Actividad anti tumoral de los linfocitos T de memoria CAR-NKG2D

Comunidad científica

Publicaciones

Fernández et al. Clin. Cancer Res. 2017 (D1)

Ud. Terapias Avanzadas (Hospital La Paz)

Producción celular

Células T memoria CAR-NKG2D a escala clínica

UNIDAD DE HEMATO-ONCOLOGÍA PEDIÁTRICA

TRATAMIENTOS PERSONALIZADOS

Pacientes

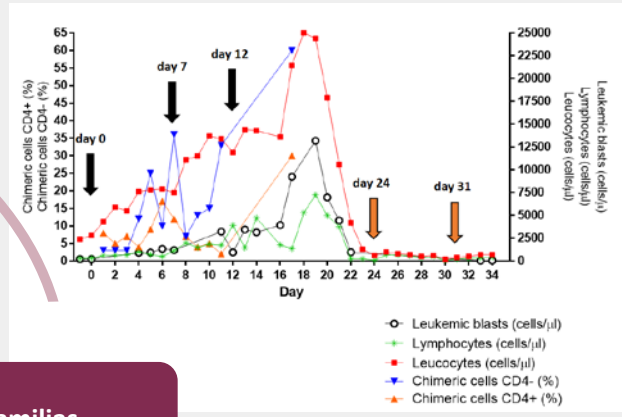
Leucemia refractaria tratamientos convencionales

Ud. Calidad Terapias avanzadas (Hospital La Paz)

Agencias reguladoras

IMPD, Guía de producción

Familias



Donantes sanos

Selección donante óptimo

1. Antecedentes de la inmunoterapia

2. Inmunoterapia con células NK

3. Inmunoterapia con células CAR-T

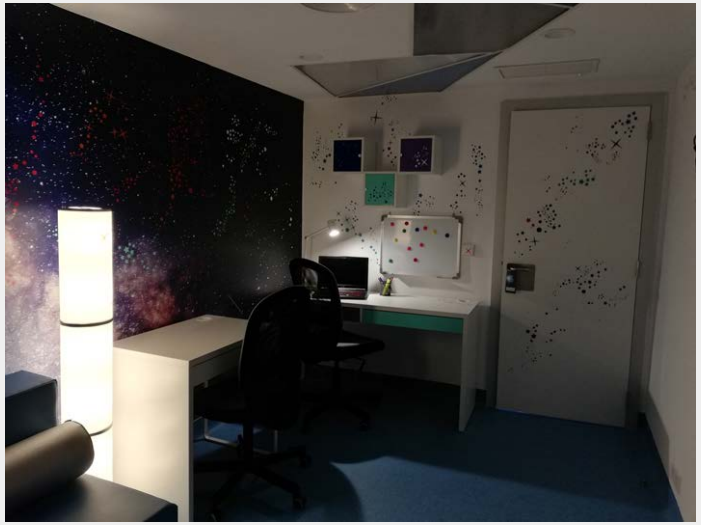
4. Transformación de nuestro hospital

HOSPITAL UNIVERSITARIO DE INVESTIGACIÓN LA PAZ

SaludMadrid
Hospital Universitario La Paz
Hospital de Cantoblanco
Hospital Carlos III

IdiPAZ
Instituto de Investigación
Hospital Universitario La Paz

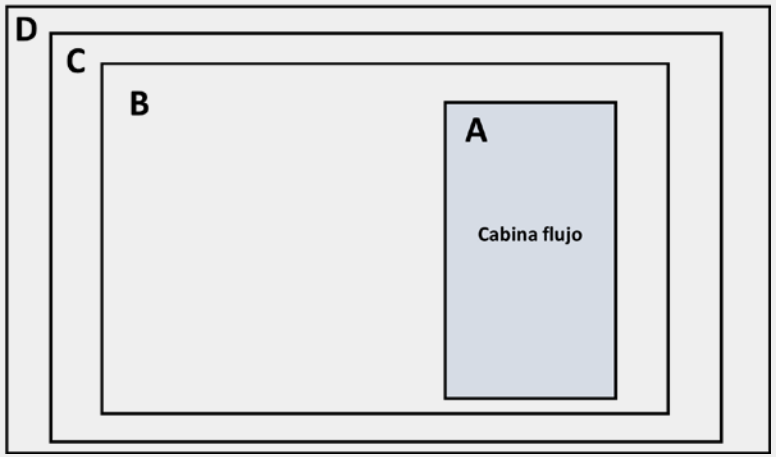
Cris Unit for Translational Research and Advanced Therapies

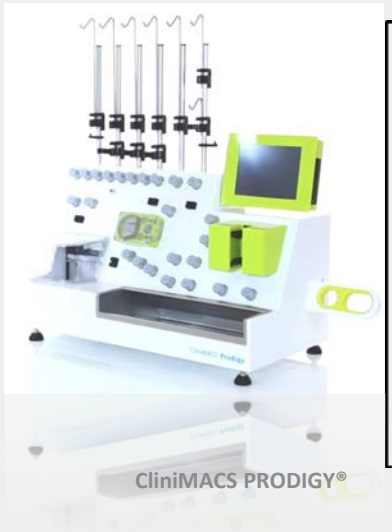


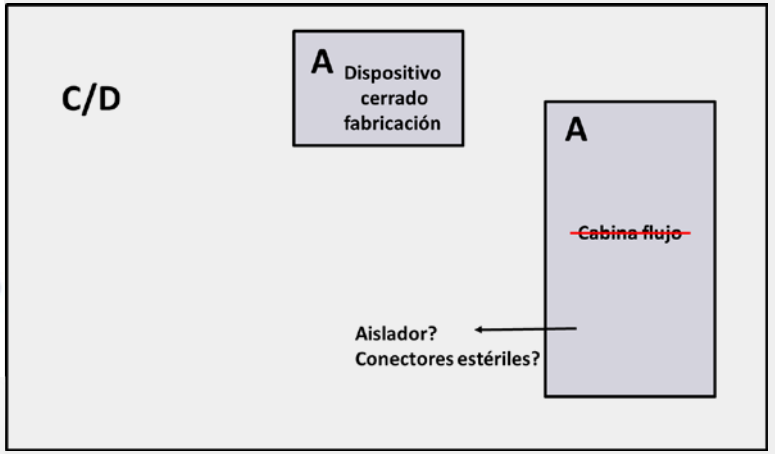
Cualificación

- Si la fabricación de CART cells fuese “manual” toda la manipulación se realizaría en grado A con un entorno B y las subsiguientes zonas de salida en gradiente descendente de clasificación (mayor complejidad a nivel de infraestructura, personal, etc).

- Para la fabricación de células CART en dispositivos cerrados éstos deben situarse en un entorno grado C o D. Las manipulaciones del producto o reactivos que van a ir en contacto con el mismo deben hacerse en grado A.







Inmunoterapia

Reconocimiento Ag tumoral

Ag específicos de tumor o asociados a tumor

Múltiples posibilidades

Múltiples dianas

Múltiples células

Consideraciones

Carga tumoral

Microbiota (virus)

Tumor dinámico en el tiempo

Buscar biomarcadores de respuesta

Muchas dianas pero desconocemos respuesta de los pacientes

Equipo multidisciplinar

Creación del grupo de Investigación Clínica Traslacional en Cáncer Infantil, Trasplante Hematopoyético y Terapia Celular

Departamento de Inmunidad Innata, Instituto de Investigación del Hospital Universiatrio La Paz (IdiPAZ)

Composición:

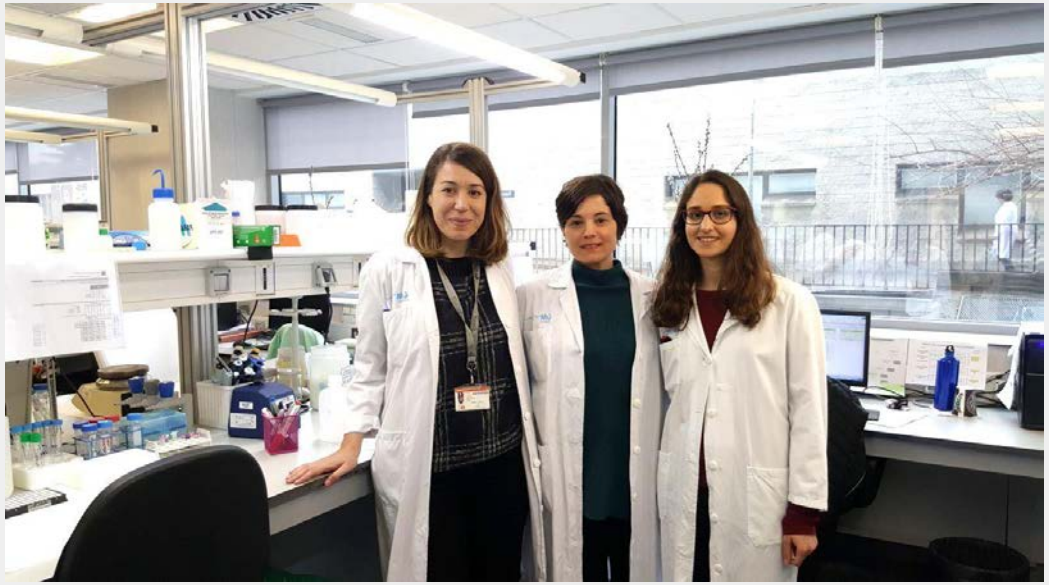
- Dr. Antonio Pérez Martínez (IP y facultativo hematooncología infantil)
- Dra. María Vela
- Jaime Valentín
- Pablo González
- Ariadna Brito

Creación del laboratorio de Hemato-Oncología pediátrica molecular

Sección 12, Instituto de Genética Médica y Molecular (INGEMM)

Composición:

- Dr. Antonio Pérez Martínez (IP y facultativo hemaotoncología infantil)
- Dra. Adela Escudero (Genetista)
- Dra. Leila Cardoso (Genetista/técnico)
- Beatriz Ruz (bioinformática)



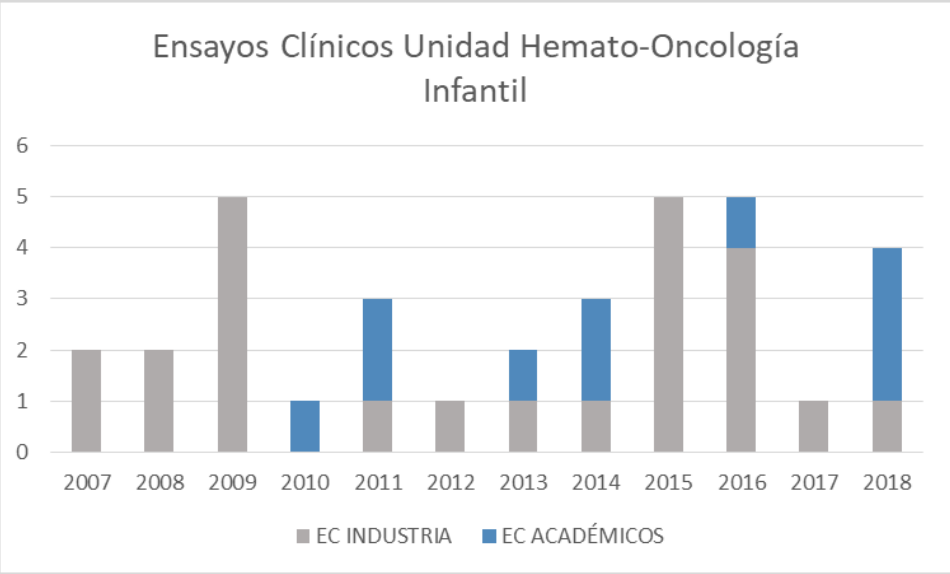
Creación de Unidad de Ensayos Clínicos en Oncohematología Infantil-UCICEC:

- 37 Ensayos Clínicos en total desde el año 2007 (industria y académicos)
- 15 Ensayos clínicos desde el 2015 en 10 de los cuales el investigador principal es el candidato (industria y académicos)

Composición:

- Dr. Antonio Pérez Martínez (IP y facultativo hemaotoncología infantil)
- Mario Muñoz
- Dra. Isabel Mirones Aguilar

BOCM número 234 2/10/2017



Acreditación JACIE (calidad) en el área clínica del Trasplante de Progenitores Hematopoyéticos

PLANIFICACIÓN
- Manual de calidad
- Organigrama programa trasplante
- Procedimientos generales
- Procedimientos normalizados

ACCIÓN
- Instalaciones y personal adecuado
- Formación inicial continuada
- Delimitación de las tareas del personal de su área especializada

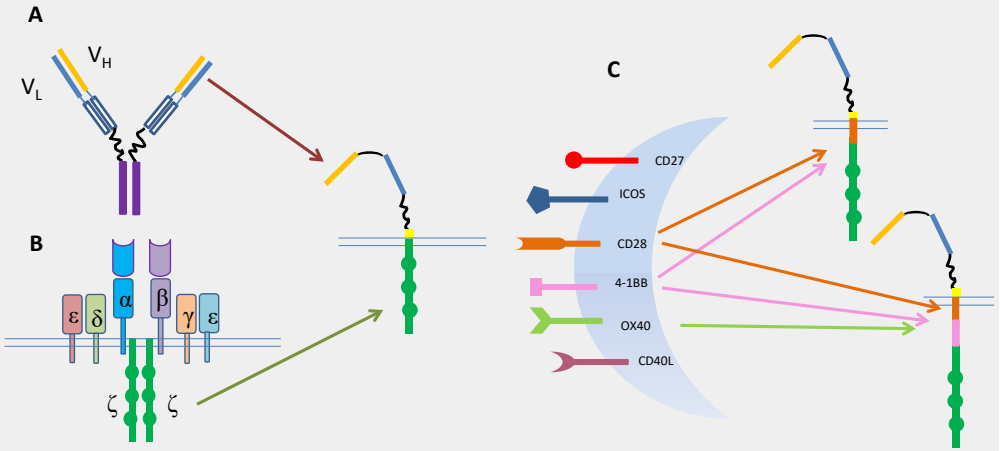
CORRECCIÓN
- Medidas correctoras
- Indicadores de Calidad
- Procedimientos
- Acuerdos con terceros

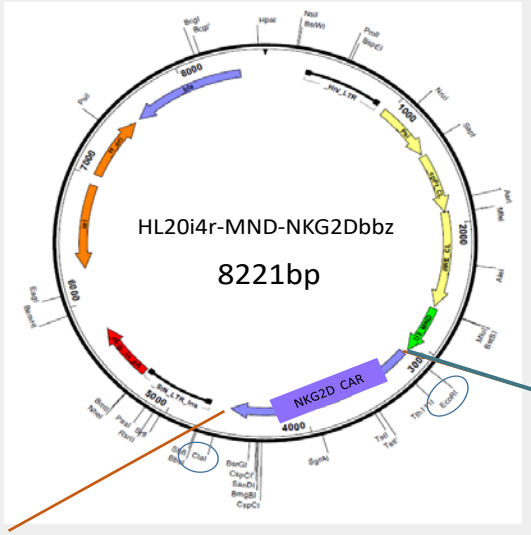
REVISIÓN (registros)
- Análisis resultados clínicos
- Auditorias internas
- Registro incidencias
- Indicadores de calidad

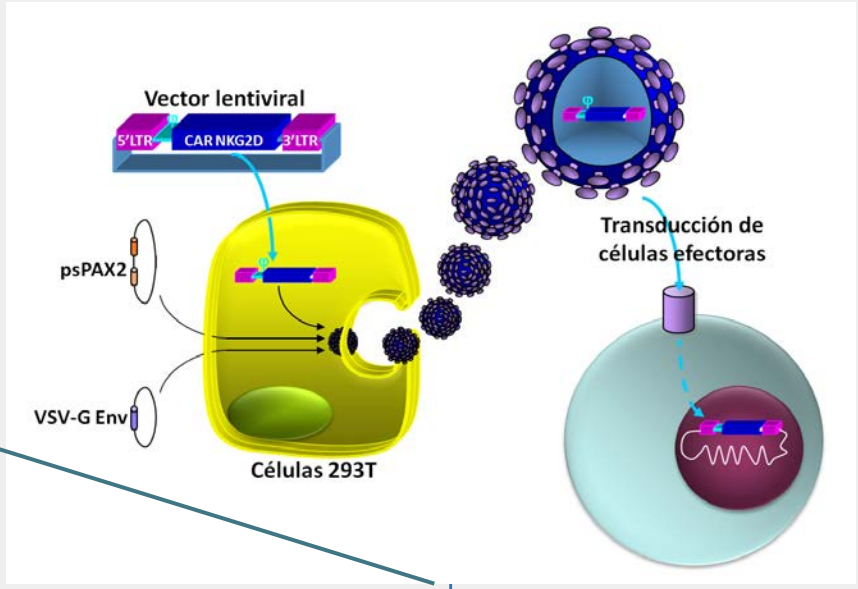
Composición:
- Dr. Antonio Pérez Martínez
- Alba Fernández-Arroyo
- Dra. Isabel Mirones Aguilar

Desarrollo de tratamientos:

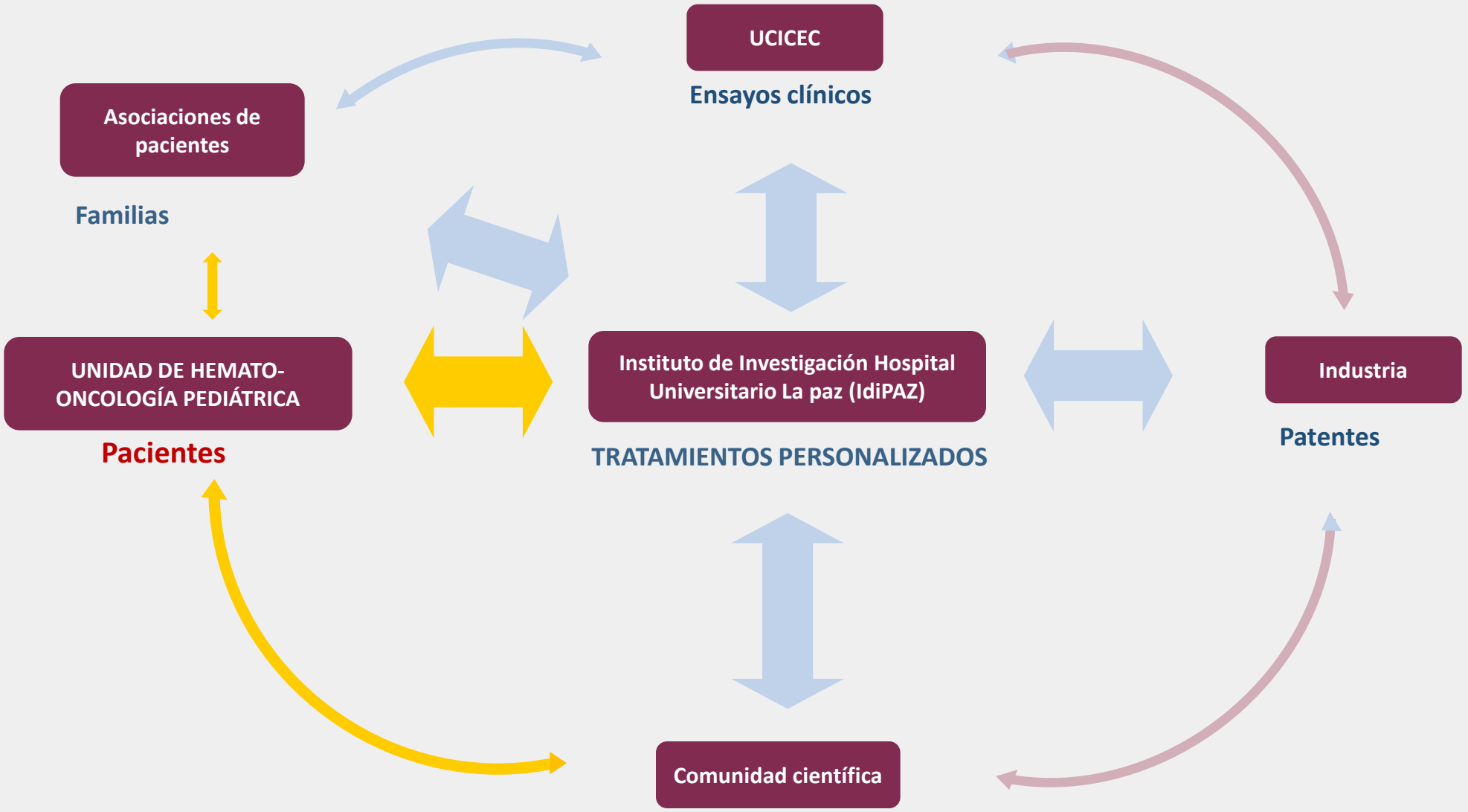
- **Oncología (osteosarcoma): Terapia celular con linfocitos T de memoria transducidos con CAR-NKG2D**
- Oncología (rabdomiosarcoma): Terapia combinada células NK y anticuerpos terapéuticos (αCXCR4)
- Terapia celular para la optimización del trasplante de órgano sólido (trasplante intestinal y renal)
- Optimización de los métodos de producción celular en condiciones GMP para el TPH y terapia celular







CD8 Sec señaliz	NKG2D dominio extracelular	CD8 dominio bisagra y transmembrana	4-1BB dominio de señalización	CD3ζ dominio de señalización


UCICEC
Ensayos clínicos
Asociaciones de pacientes
Familias
UNIDAD DE HEMATO-ONCOLOGÍA PEDIÁTRICA
Pacientes
Instituto de Investigación Hospital Universitario La paz (IdiPAZ)
TRATAMIENTOS PERSONALIZADOS
Industria
Patentes
Comunidad científica

Asociaciones de pacientes

Familias

La Sonrisa de Álex, FMPJ

UCICEC

Ensayos clínicos

EC SANKOMA

EudraCT 2016-003578- 42

PROTOCOLO DE ENSAYO CLÍNICO

Ensayo Clínico Fase I/II, multicéntrico, abier[illegible]de infusión de células NK activadas para el tratamiento de niños, adolescent[illegible] adultos jóvenes con sarcomas

Promotor: ANTONIO PÉREZ-MARTÍNEZ

Código de Protocolo: SANKOMA_2016

Código EudraCT: 2016-003578-42

Versión protocolo: versión 2 de 10 de julio de 2[illegible]

agencia española de medicamentos y productos sanitarios

UNIDAD DE HEMATO-ONCOLOGÍA PEDIÁTRICA

Pacientes

Sarcoma metastásico/refractario

Instituto de Investigación Hospital Universitario La paz (IdiPAZ)

TRATAMIENTOS PERSONALIZADOS

Actividad anti tumoral *in vivo* de la terapia combinada NK + αCXCR4

Industria

Bristol-Myers Squibb

Patentes

Bristol Myers Squibb and FIHULP:
U.S. Application No. 62/642,313

Comunidad científica

Publicaciones: **Vela et al. in preparation (Q1)**

Congresos: **ASCO2018, SIHOP2018, SNI2018, SETGyC2018, SEHOP2018**

Trial name	Title	Code	EudraCT	NCT
HNJ-NK-2009	TRASPLANTE DE PROGENITORES HEMATOPOYÉTICOS E INFUSIÓN DE CÉLULAS NK IL-15 EN TUMORES SÓLIDOS PEDIÁTRICOS REFRACTARIOS	HNJ-NK-01/2009	2009-010186-23	NCT01337544
HNJ-NKAES-2012	INFUSIÓN DE CÉLULAS NATURAL KILLER EN COMBINACIÓN CON QUIMIOTERAPIA EN PACIENTES PEDIÁTRICOS CON LEUCEMIA/LINFOMA T REFRACTARIA	HJN-NKAES-2012	2012-000054-63	NCT01944982
LYDIA	LANK-2: INMUNOTERAPIA CON CÉLULAS NATURAL KILLER ACTIVADAS Y EXPANDIDAS JUNTO CON QUIMIOTERAPIA DE RESCATE EN NIÑOS, ADOLESCENTES Y ADULTOS JÓVENES CON LEUCEMIA AGUDA EN RECAIDA O REFRACTARIEDAD	LANK-2	2012-005146-38	NCT02074657
LYDIA II	FASE II: INFUSION DE CÉLULAS NATURAL KILLER COMO TRATAMIENTO DE CONSOLIDACION EN NINOS Y ADOLESCENTES CON LEUCEMIA MIELOBLÁSTICA AGUDA	NKCell_LMA_2015	2015-001901-1	NCT02763475
SANKOMA	ENSAYO CLÍNICO FASE I/II, MULTICÉNTRICO, ABIERTO, DE INFUSIÓN DE CÉLULAS NATURAL KILLER ACTIVADAS PARA EL TRATAMIENTO DE NIÑOS, ADOLESCENTES Y ADULTOS JÓVENES CON SARCOMAS	SANKOMA_2016	2016-003578-42	No disponible
GABY	RECEPTOR ANTIGÉNICO QUIMÉRICO NKG2D PARA EL TRATAMIENTO DE PACIENTES PEDIÁTRICOS CON LEUCEMIA AGUDA Y LEUCEMIA MIELOMONOCÍTICA JUVENIL: VALIDACIÓN A ESCALA CLÍNICA Y PRIMER ESTUDIO DE SEGURIDAD EN PACIENTES	No disponible	No disponible	No disponible
PHINK	INFUSIÓN DE CÉLULAS NATURAL KILLER ALOREACTIVAS O ESTIMULADAS TRAS TRASPLANTE HAPLOIDÉNTICO DE PROGENITORES HEMATOPOYÉTICOS EN PACIENTES PEDIÁTRICOS CON NEOPLASIAS HEMATOLOGICAS	No disponible	No disponible	No disponible

" The cure of cancer starts with research"

ACKNOWLEDGEMENTS

RESEARCH (CNIO)

Adrián Fernández
Ale
Joa
Flo
Cor
Ani

RE

Ma
Jai
Pab

MC

Ad
Lei
Be
Pil

UC

Isab
Ma
Alb
Bel

PEDIATRIC HEMATO-ONCOLOGY

RAPY

Hospital Universitario La Paz
Hospital de Cantoblanco
Hospital Carlos III
SaludMadrid

IdiPAZ
Instituto de Investigación
Hospital Universitario La Paz

INGEMM

Cris
contra el cáncer

Inmunoterapia con células NK y CAR-T en el cáncer infantil

Antonio Pérez-Martínez[1,2,3,4]

[1]Translational Research in Pediatric Oncology, Hematopoietic Transplantation and Cell Therapy Unit, Hospital Universitario La Paz, Madrid (Spain), [2]Instituto de Genética Médica y Molecular (INGEMM), Hospital Universitario La Paz, Madrid (Spain), [3]Profesor Titular de Pediatría de la UAM, [4]Jefe de Servicio de Hemato-Oncología Pediátrica, Hospital Universitario La Paz, Madrid (Spain)