Giornate Ematologiche Vicentine
Vicenza, 10-12 2016

Adoptive immunotherapy with haploidentical alloreactive NK cells for the treatment of Minimal Residual Disease in elderly patients with Acute Myeloid Leukemia

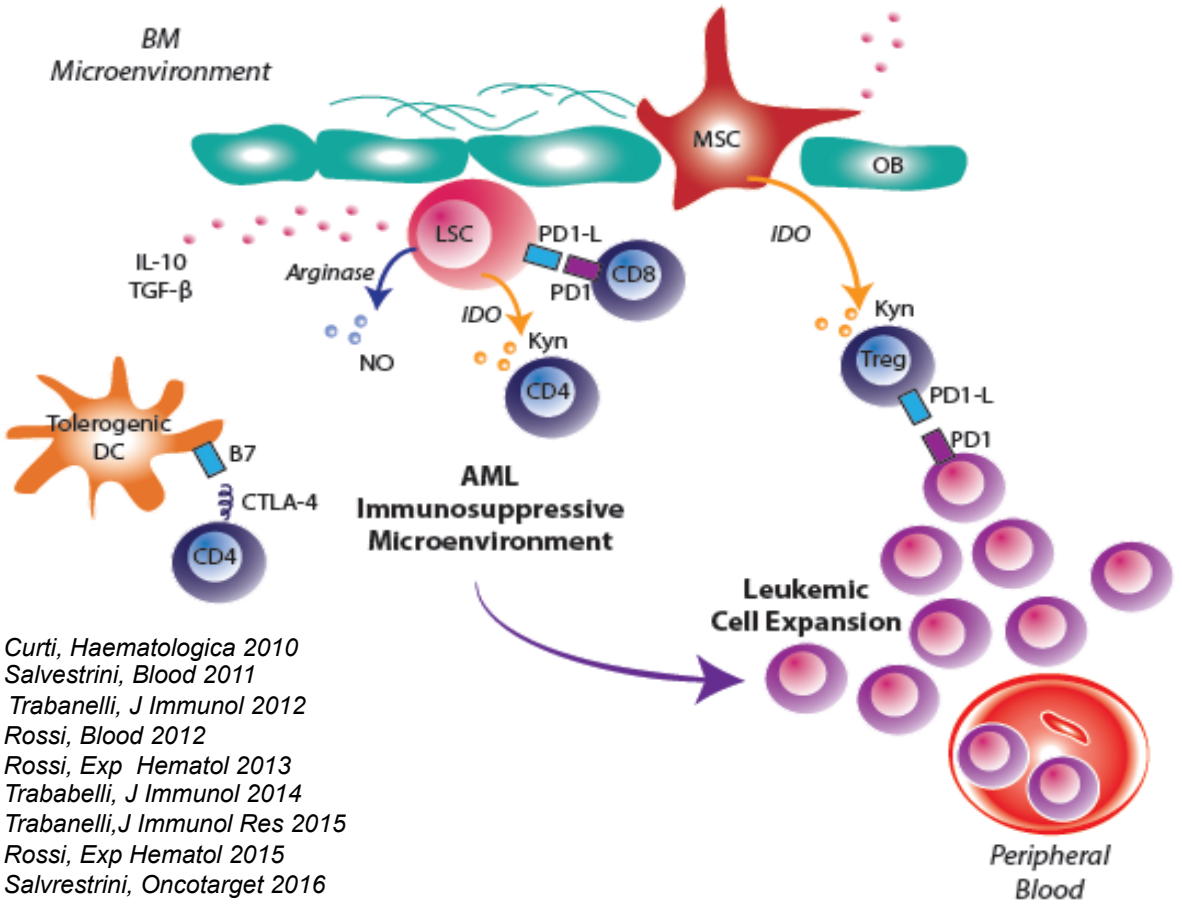
Roberto M. Lemoli

Clinic of Hematology, Department of Internal Medicine (DiMI),

University of Genoa

IRCCS A.O.U. S. Martino-IST, Genoa, Italy

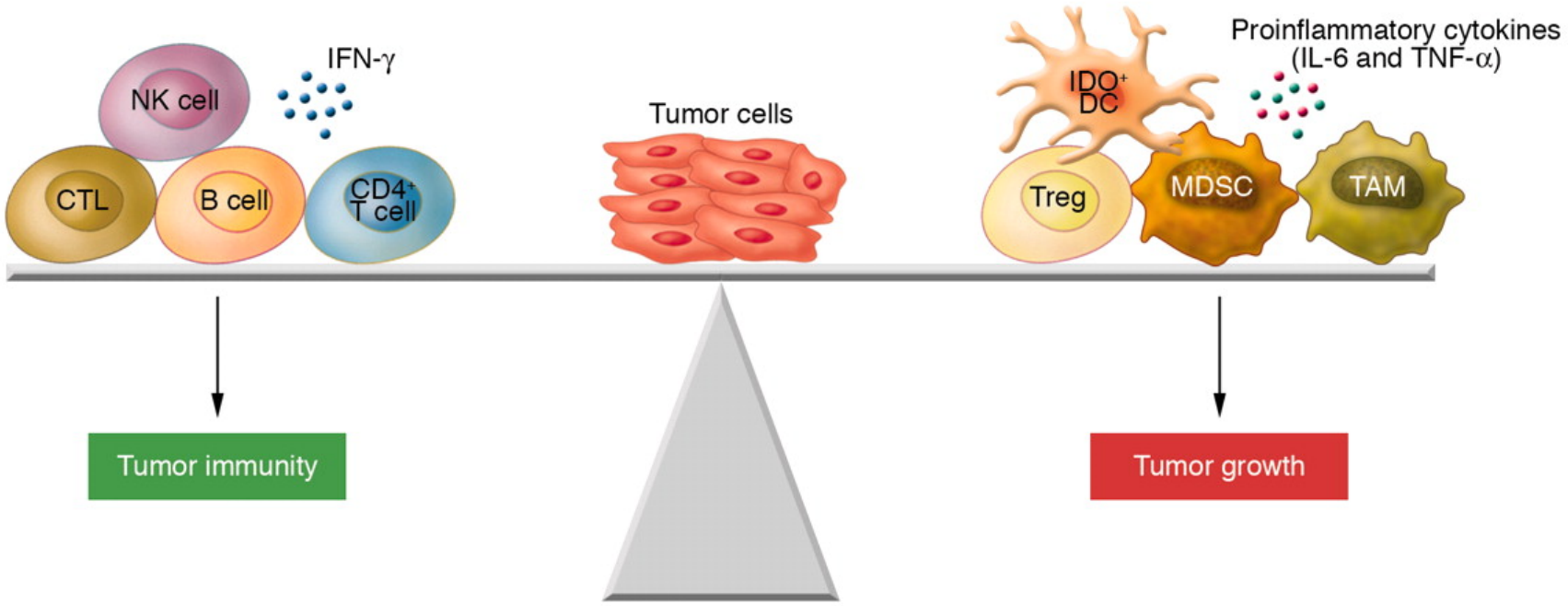
AML and immunological microenvironment



Lemoli, Blood 2004
Curti, Exp Hematol 2005
Curti, Leukemia 2007
Curti, Blood 2007
Rossi, Blood 2007
Curti, Blood 2009
Isidori, Exp Rev Hematol 2014

Curti, Haematologica 2010
Salvestrini, Blood 2011
Trabanelli, J Immunol 2012
Rossi, Blood 2012
Rossi, Exp Hematol 2013
Trababelli, J Immunol 2014
Trabanelli,J Immunol Res 2015
Rossi, Exp Hematol 2015
Salvrestrini, Oncotarget 2016

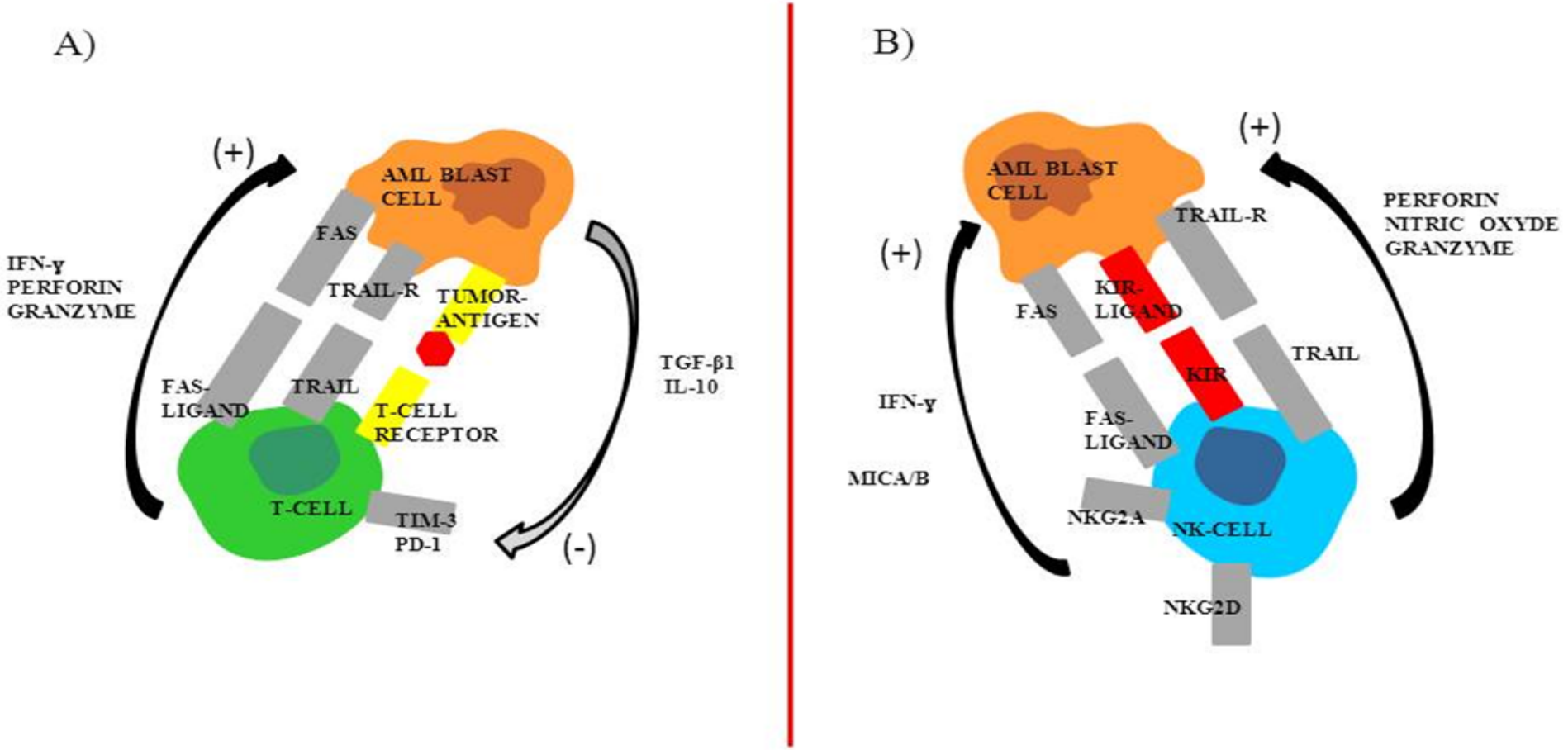
Harnessing the immune system to treat leukemia



J. Clin. Invest. **117**:1130-1136 (2007)

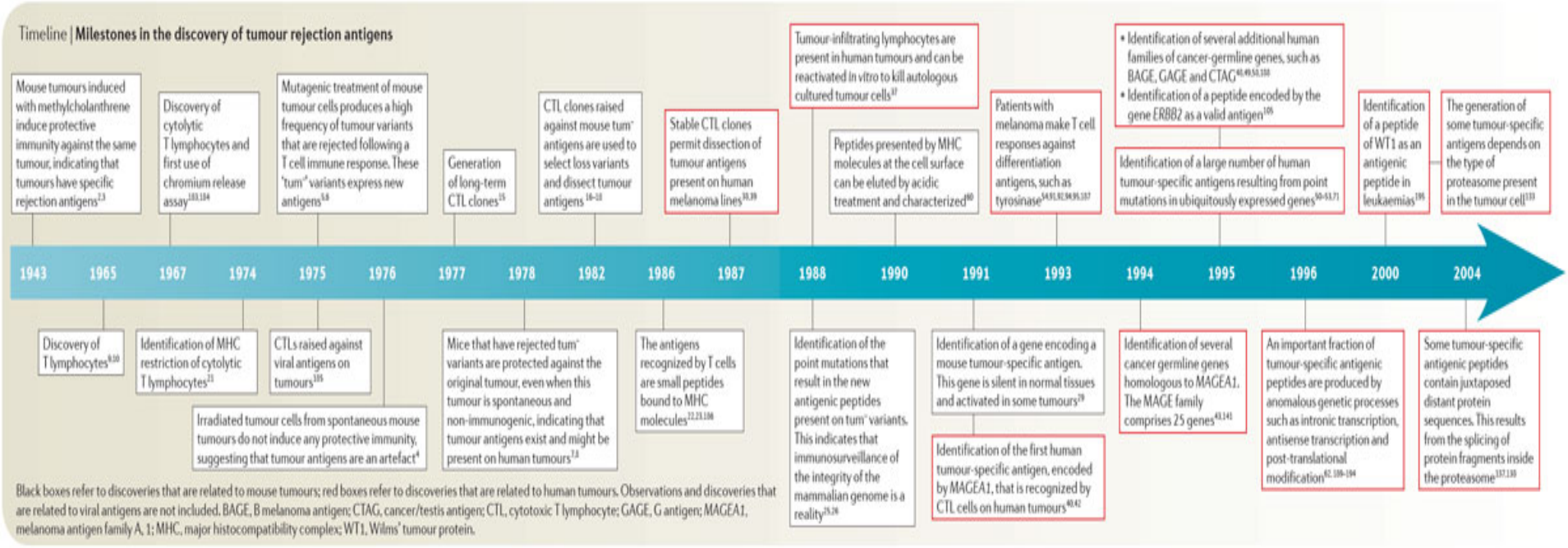
Differential mechanisms of tumor cell recognition

Fig. 1



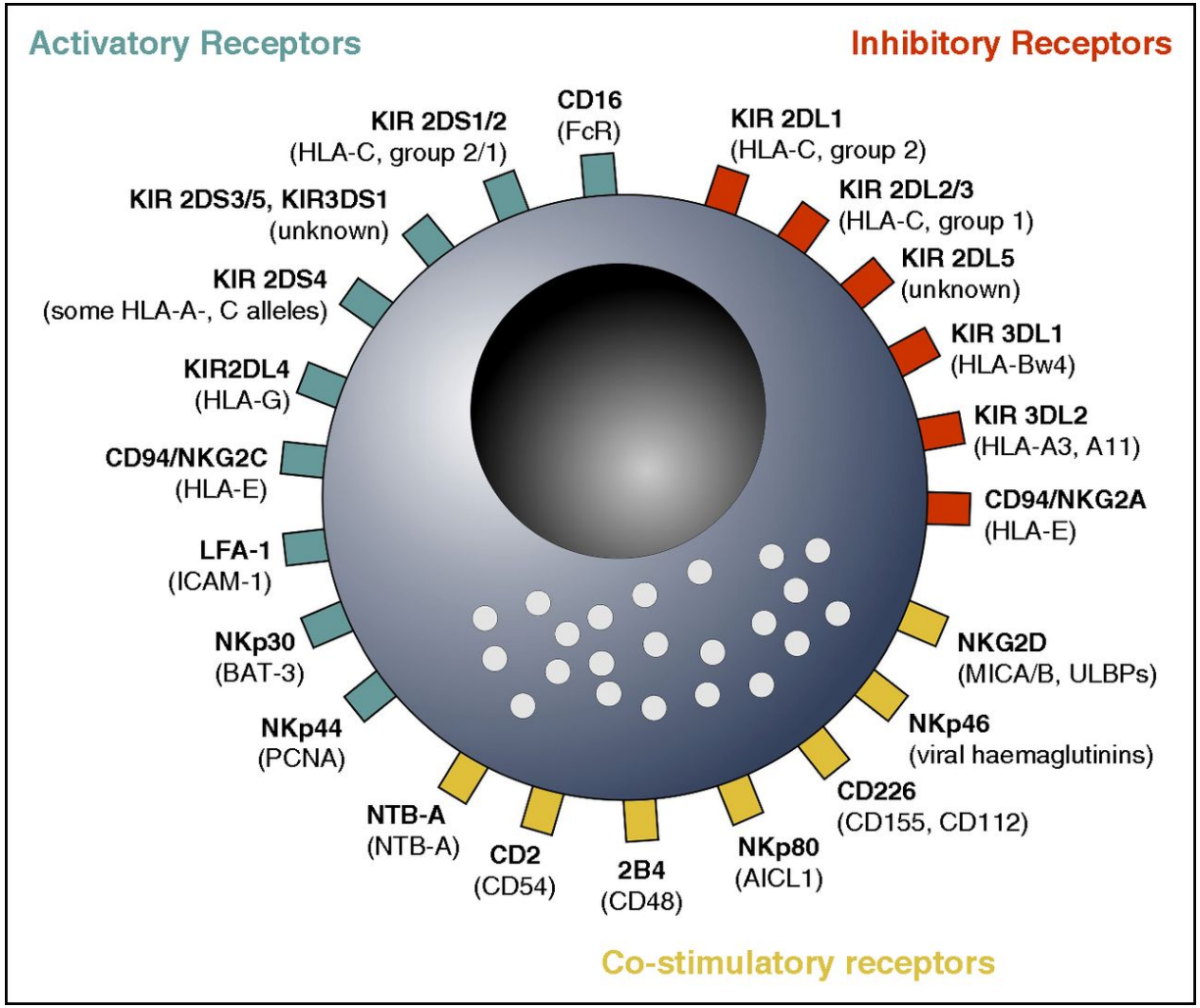
Lemoli et al, Exp Hematol 2016

Tumour antigens and T lymphocytes: "*croce e delizia*" for immunologists



Coulie P & Boon T, Nature Reviews Cancer, 2014

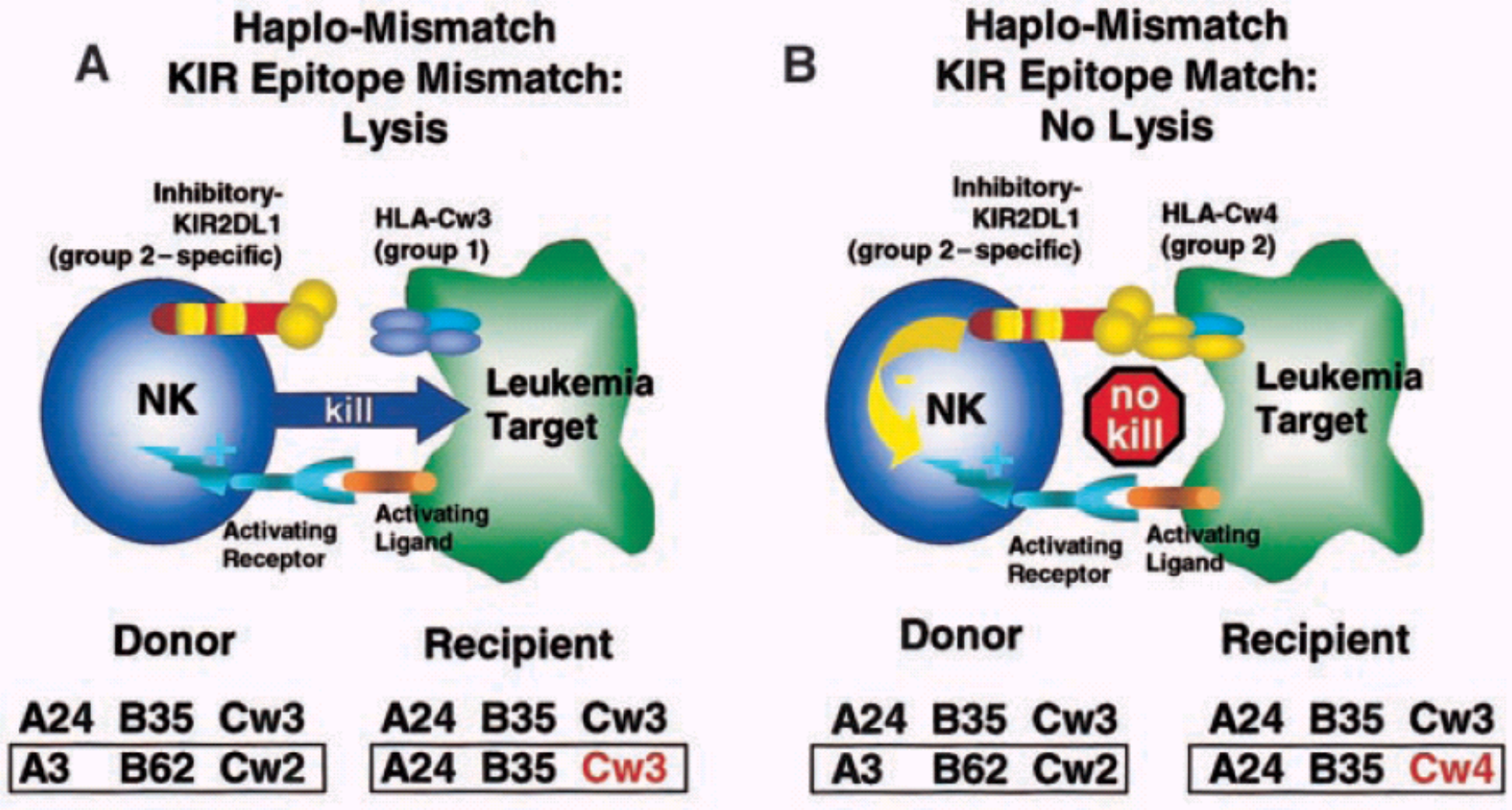
NK cells “naturally” kill cell targets without prior sensitization



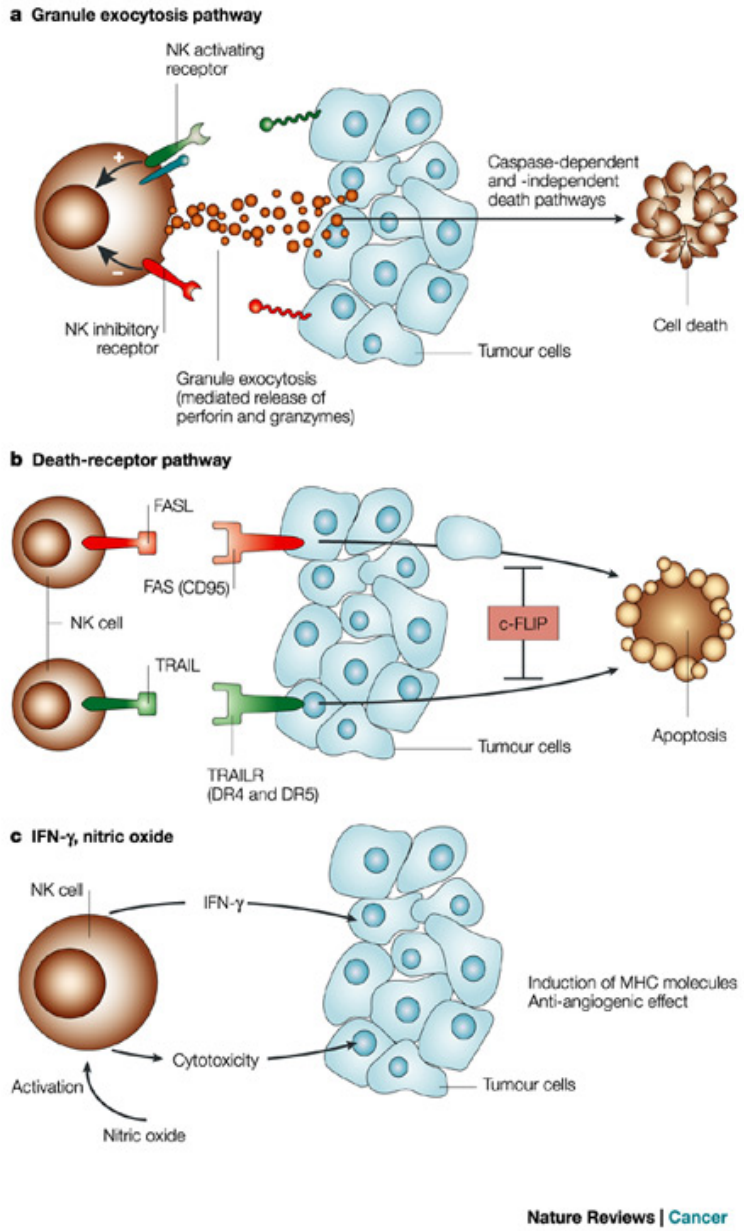
blood

Handgretinger et al. Blood 2016;127:3341-3349

The “allo NK” haplo transplant: The «missing self» hypothesis



Farag S et al, Blood 2004



Cytotoxic effects of NK cells on tumor cells

- Granule exocytosis via activating and inhibitory receptors (perforin and granzyme)
- Death receptor pathways (FAS-FASL; TRAIL-TRAILR)
- Soluble factors and small molecules (cytokines and NO)

Science

AAAS

Effectiveness of Donor Natural Killer Cell Alloreactivity in Mismatched Hematopoietic Transplants

Loredana Ruggeri, *et al.*

Science **295**, 2097 (2002);

DOI: 10.1126/science.1068440

blood

2007 110: 433-440

Prepublished online Mar 19, 2007;

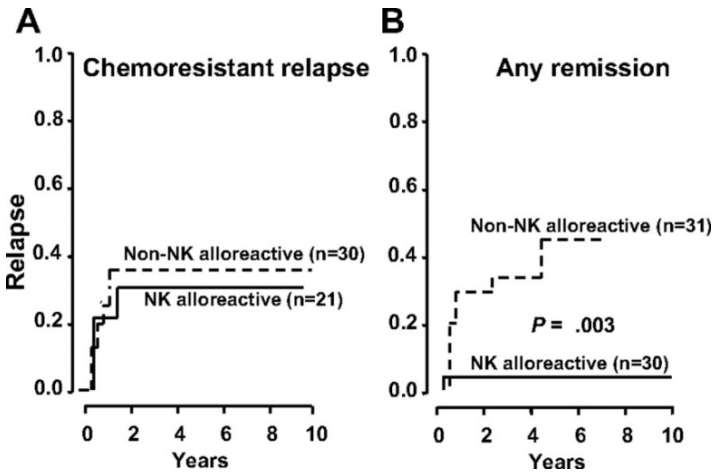
doi:10.1182/blood-2006-07-036567

Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value.

Loredana Ruggeri, Antonella Mancusi, Marusca Capanni, Elena Urbani, Alessandra Carotti, Teresa Aloisi, Martin Stern, Daniela Pende, Katia Perruccio, Emanuela Burchielli, Fabiana Topini, Erika Bianchi, Franco Aversa, Massimo F. Martelli and Andrea Velardi

Data from haploidentical T-cell depleted transplantation suggested that KIR mismatch with tumor MHC may significantly impact on tumor cell killing, particularly in AML .

High risk AML patients receiving haploidentical T-cell depleted transplant with a KIR-ligand mismatch in the graft-versus-host (GVDH) direction had a relapse rate of 0% compared to KIR-ligand matched patients who had a relapse rate of 75%.



blood

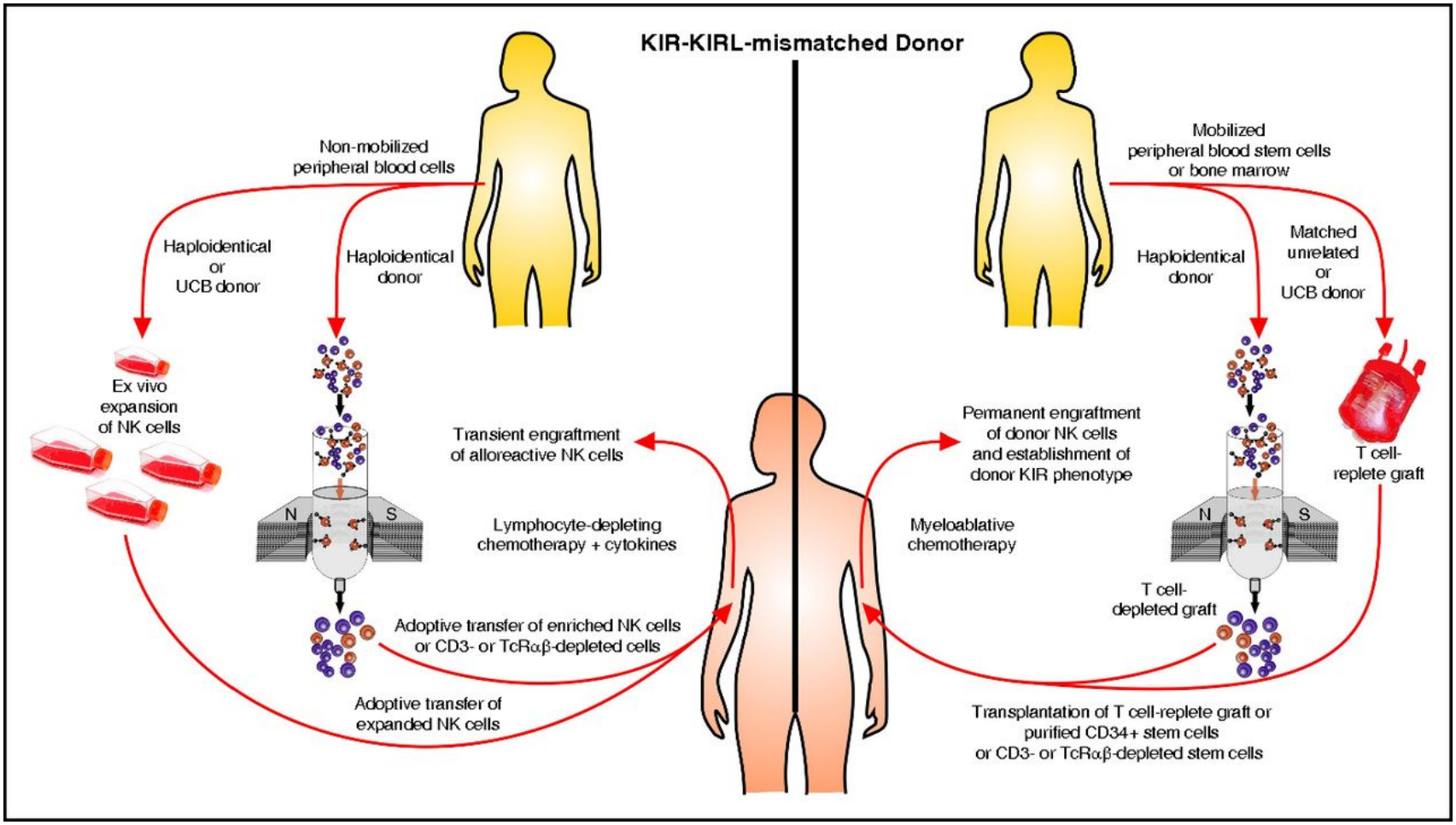
Anti-leukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: evaluation of the functional role of activating KIR and redefinition of inhibitory KIR specificity

Pende D et al, Blood, 113; 3119-3129; 2009

Clinical exploitation of alloreactive NK cells

Adoptive immunotherapy

HSCT



blood

Handgretinger et al. Blood 2016;127:3341-3349

blood

2005 105: 3051-3057
Prepublished online Jan 4, 2005;
doi:10.1182/blood-2004-07-2974

Successful adoptive transfer and in vivo expansion of human haploidentical NK cells in patients with cancer

Jeffrey S. Miller, Yvette Soignier, Angela Panoskaltsis-Mortari, Sarah A. McNearney, Gong H. Yun, Susan K. Fautsch, David McKenna, Chap Le, Todd E. Defor, Linda J. Burns, Paul J. Orchard, Bruce R. Blazar, John E. Wagner, Arne Slungaard, Daniel J. Weisdorf, Ian J. Okazaki and Philip B. McGlave

Five/19 poor-prognosis patients with AML achieved complete remission after infusion of partially purified haploidentical NK cells.

VOLUME 28 · NUMBER 6 · FEBRUARY 20 2010

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

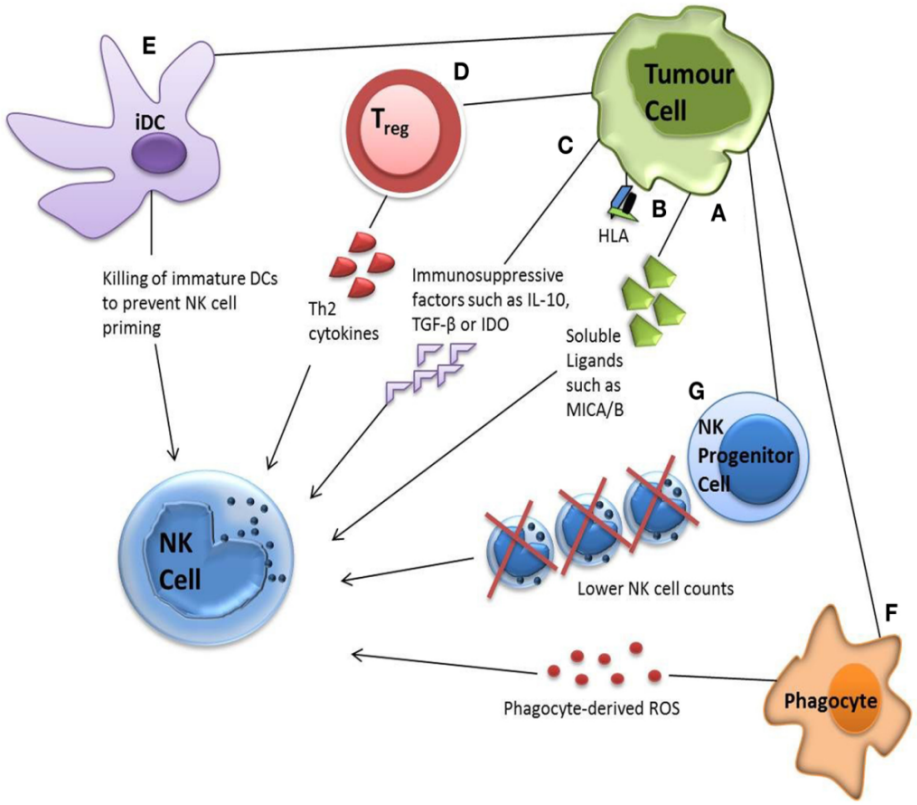
NKAML: A Pilot Study to Determine the Safety and Feasibility of Haploidentical Natural Killer Cell Transplantation in Childhood Acute Myeloid Leukemia

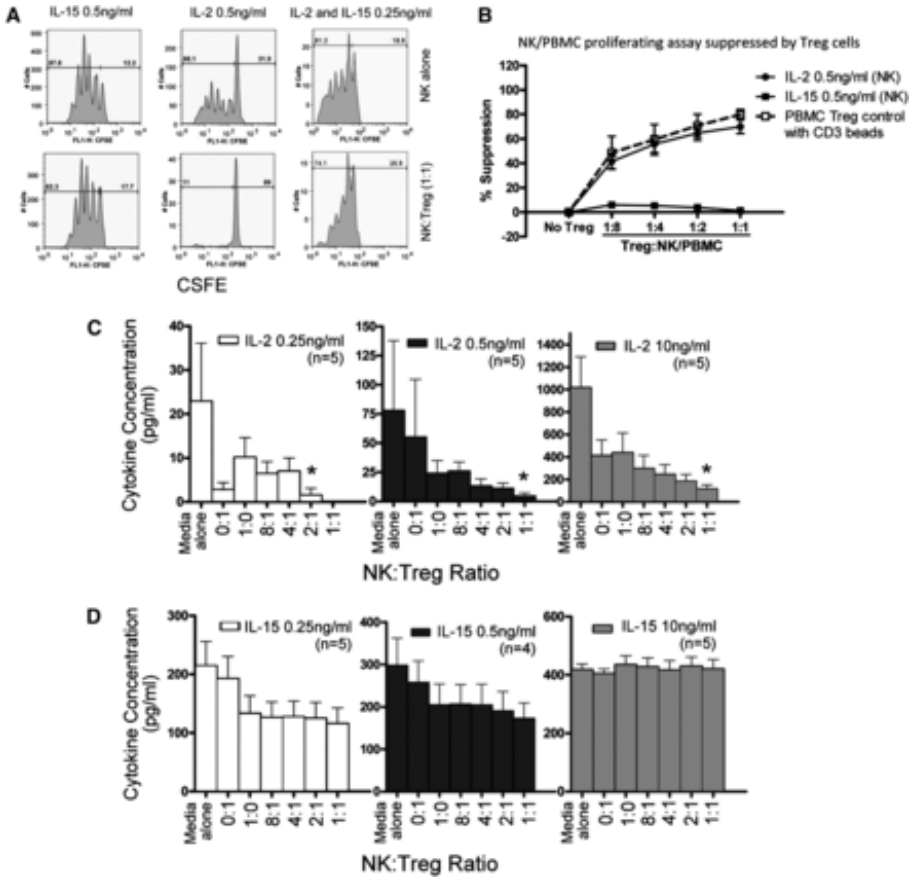
Jeffrey E. Rubnitz, Hiroto Inaba, Raul C. Ribeiro, Stanley Pounds, Barbara Rooney, Teresa Bell, Ching-Hon Pui, and Wing Leung

Ten AML patients (0.7 to 21 years old) in first CR received cyclophosphamide (60 mg/kg on day –7) and fludarabine (25 mg/m2/d on days –6 through –2), followed by KIR-L mismatched NK cells (median, 29 x 106/kg NK cells) and six doses of interleukin-2 (1 million U/m2). With a median follow-up time of 964 days (range, 569 to 1,162 days), all patients remain in remission. The 2-year event-free survival estimate was 100% (95% CI, 63.1% to 100%).

JOURNAL OF CLINICAL ONCOLOGY

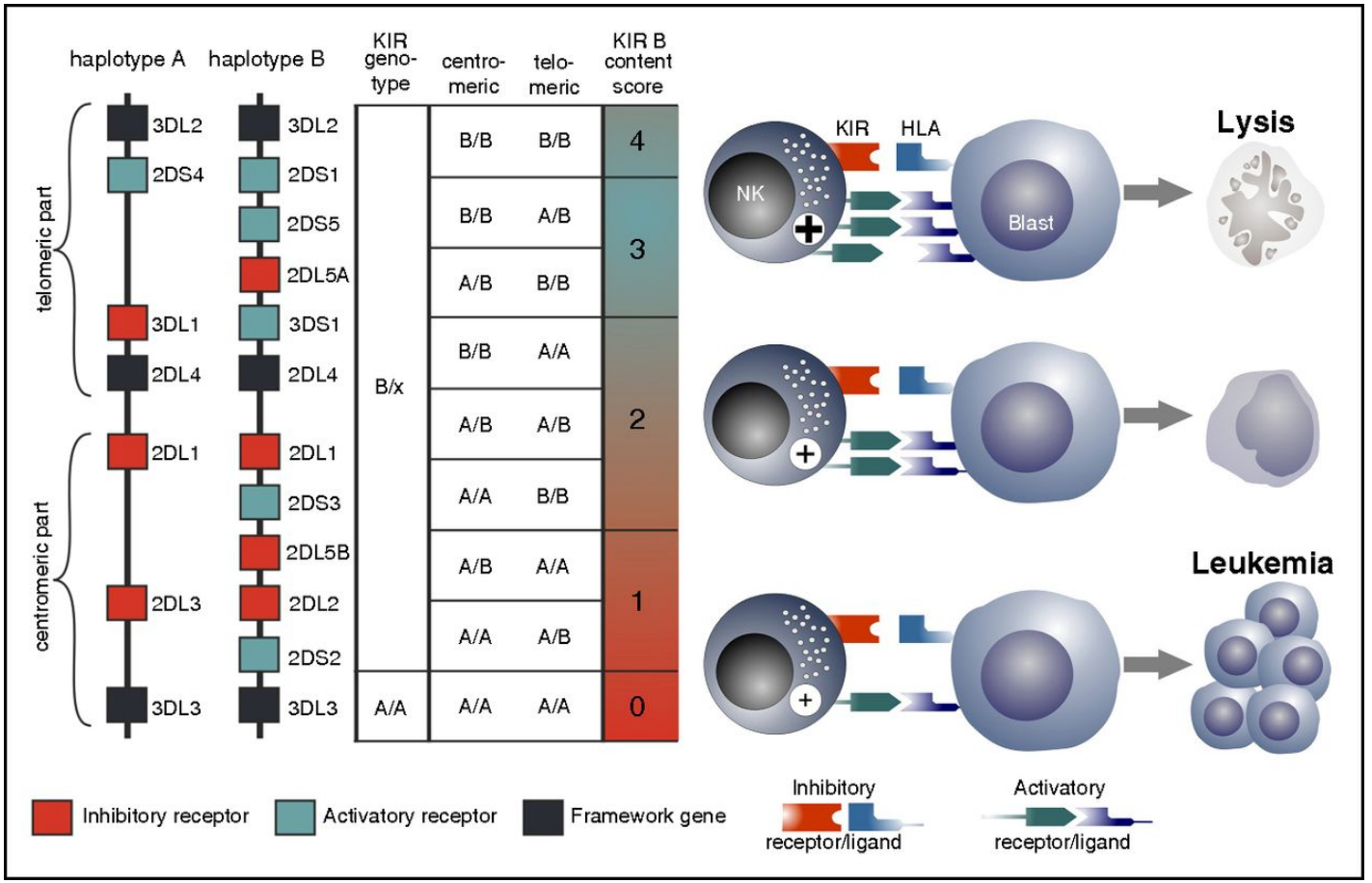
Patient-derived factors on alloreactive NK immunotherapy: the role of Tregs





Bachanova et al, Blood, 2014

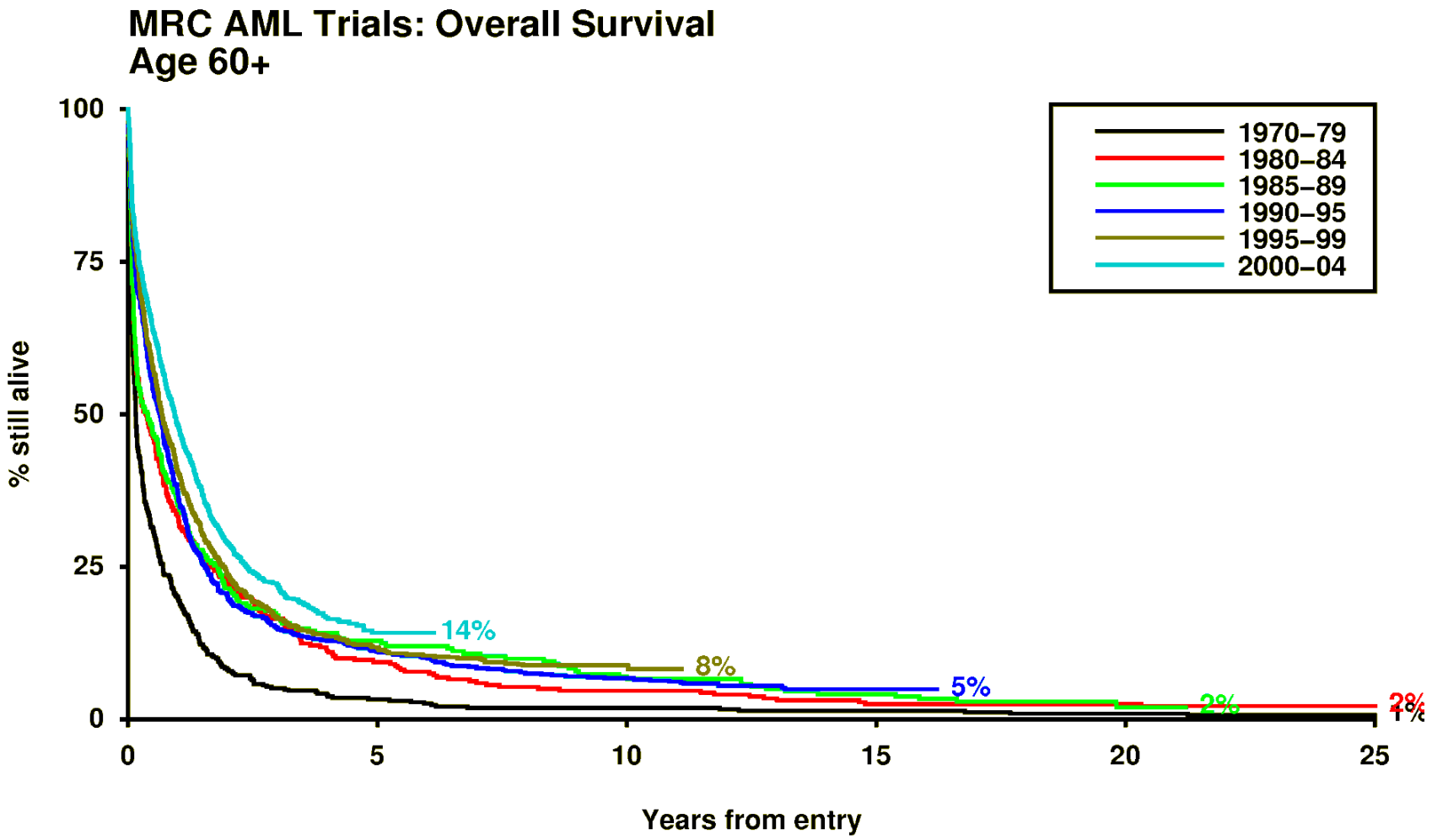
Defining the optimal donor: KIR-L mismatch plus activating KIRs



blood

Handgretinger et al. Blood 2016;127:3341-3349

MRC Trials for Older Patients >60 years (n=3541)



Study Design- Eligibility criteria

1) High risk AML patients with age greater than 18 years with assessable disease, not eligible for stem cell transplantation

2) a suitable haploidentical KIR L-mismatched donor (HLA class I typing and KIR genotyping)

Five patients with active disease were preliminarily enrolled in the safety/feasibility phase of the study

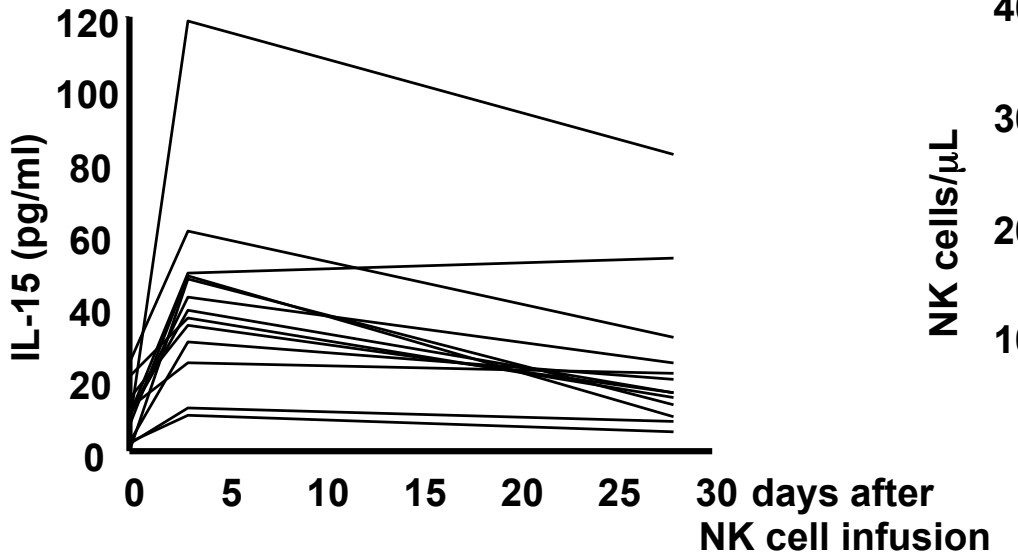
blood

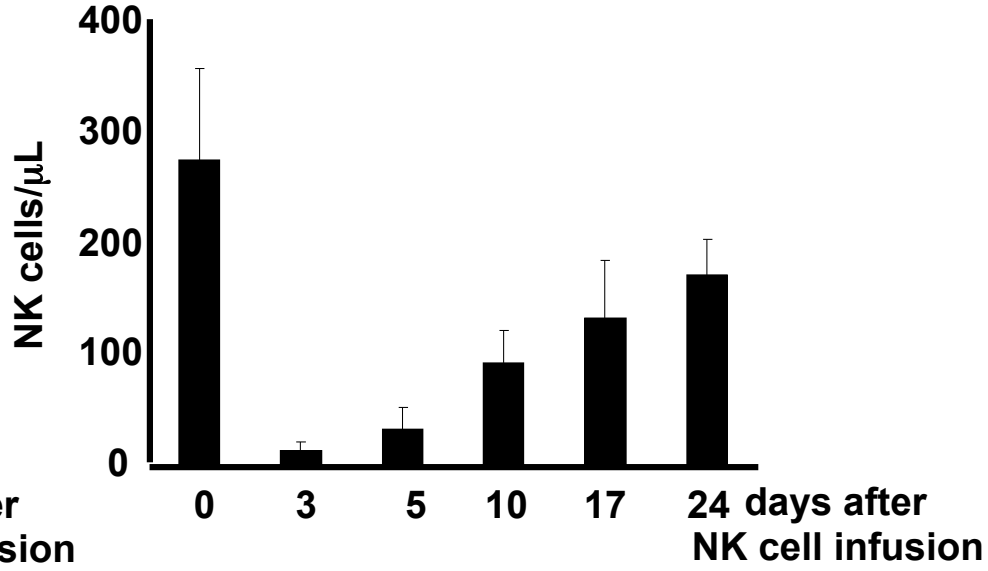
BLOOD, 22 SEPTEMBER 2011 • VOLUME 118, NUMBER 12

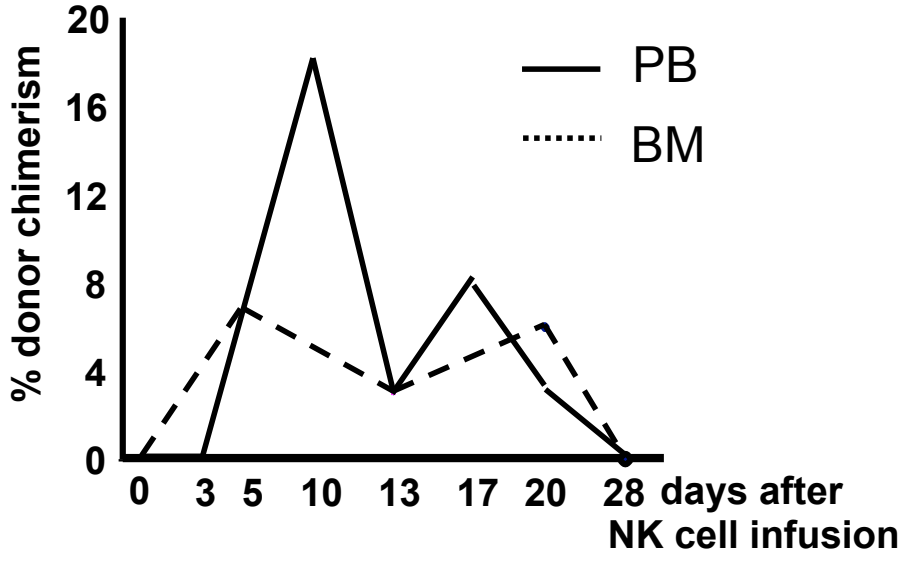
Successful transfer of alloreactive haploidentical KIR ligand-mismatched natural killer cells after infusion in elderly high risk acute myeloid leukemia patients

Antonio Curti,[1] Loredana Ruggeri,[2] Alessandra D'Addio,[3] Andrea Bontadini,[4] Elisa Dan,[1] Maria Rosa Motta,[1] Sara Trabanelli,[1] Valeria Giudice,[4] Elena Urbani,[2] Giovanni Martinelli,[1] Stefania Paolini,[1] Fiorenza Fruet,[4] Alessandro Isidori,[5] Sarah Parisi,[1] Giuseppe Bandini,[1] Michele Baccarani,[1] Andrea Velardi,[2] and Roberto M. Lemoli[1]

[1]Institute of Hematology, Department of Hematology and Oncological Sciences "L. and A. Seràgnoli," University of Bologna, S. Orsola-Malpighi Hospital, Bologna, Italy; [2]Division of Hematology and Clinical Immunology, Department of Clinical and Experimental Medicine, University of Perugia, Ospedale Santa Maria della Misericordia, Perugia, Italy; [3]Azienda Istituti Ospitalieri, Hematology Unit, Cremona, Italy; [4]Immunohematology Service and Blood Bank, S. Orsola-Malpighi Hospital, Bologna, Italy; and [5]Hematology and Hematopoietic Stem Cell Transplant Centre, San Salvatore Hospital, Pesaro, Italy

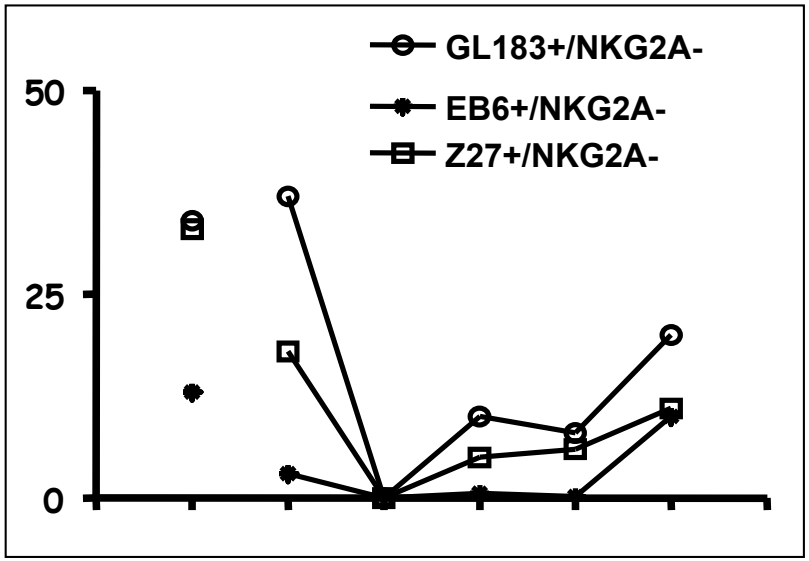




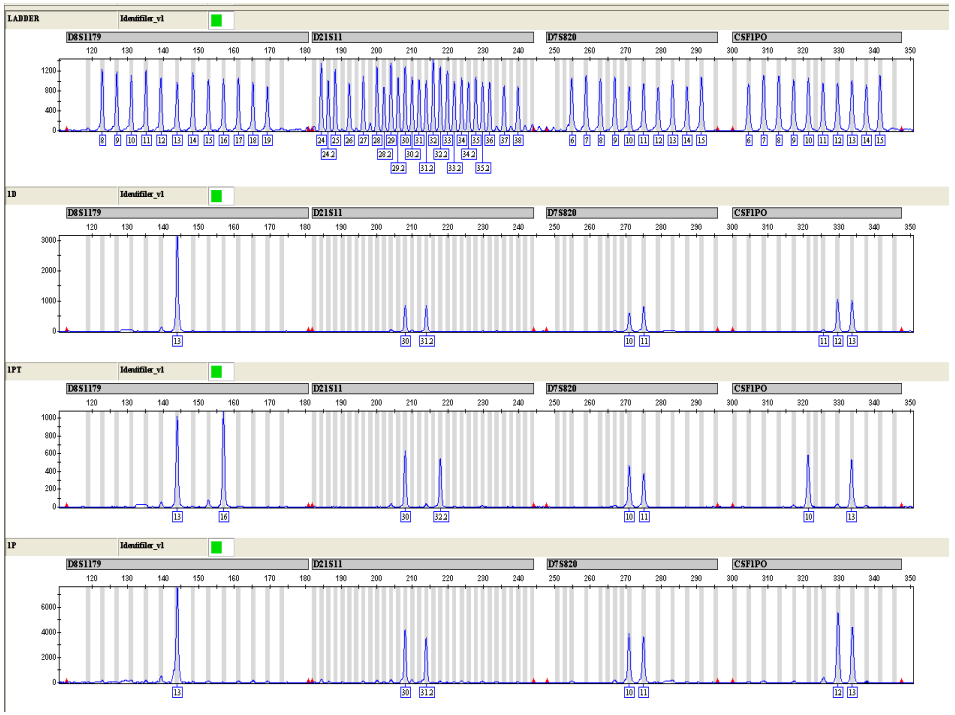


Curti et al, Blood 2011

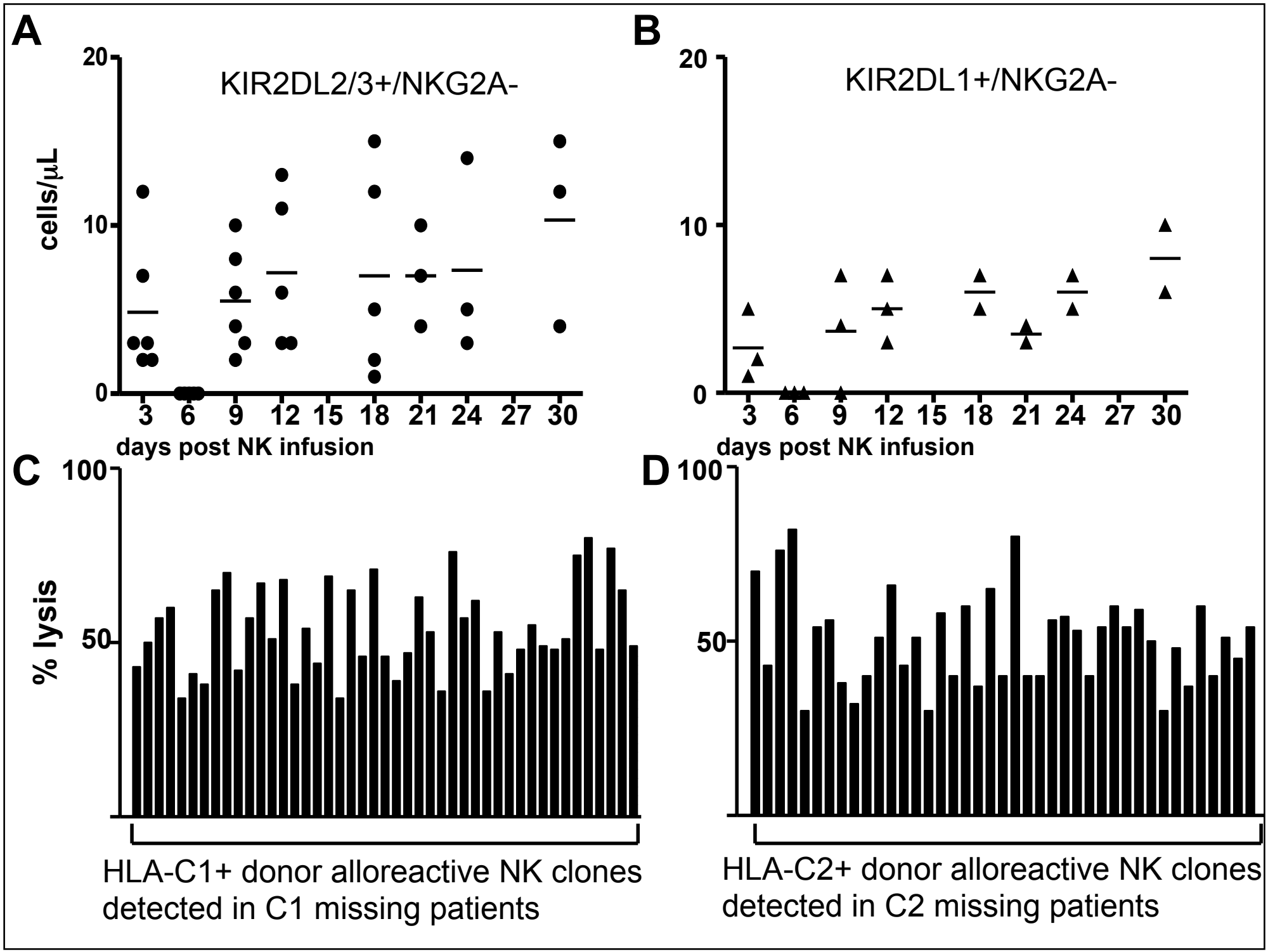
Detection of alloreactive KIR^{+}/NKG2A^{-} NK cells after haploidentical NK cell infusion



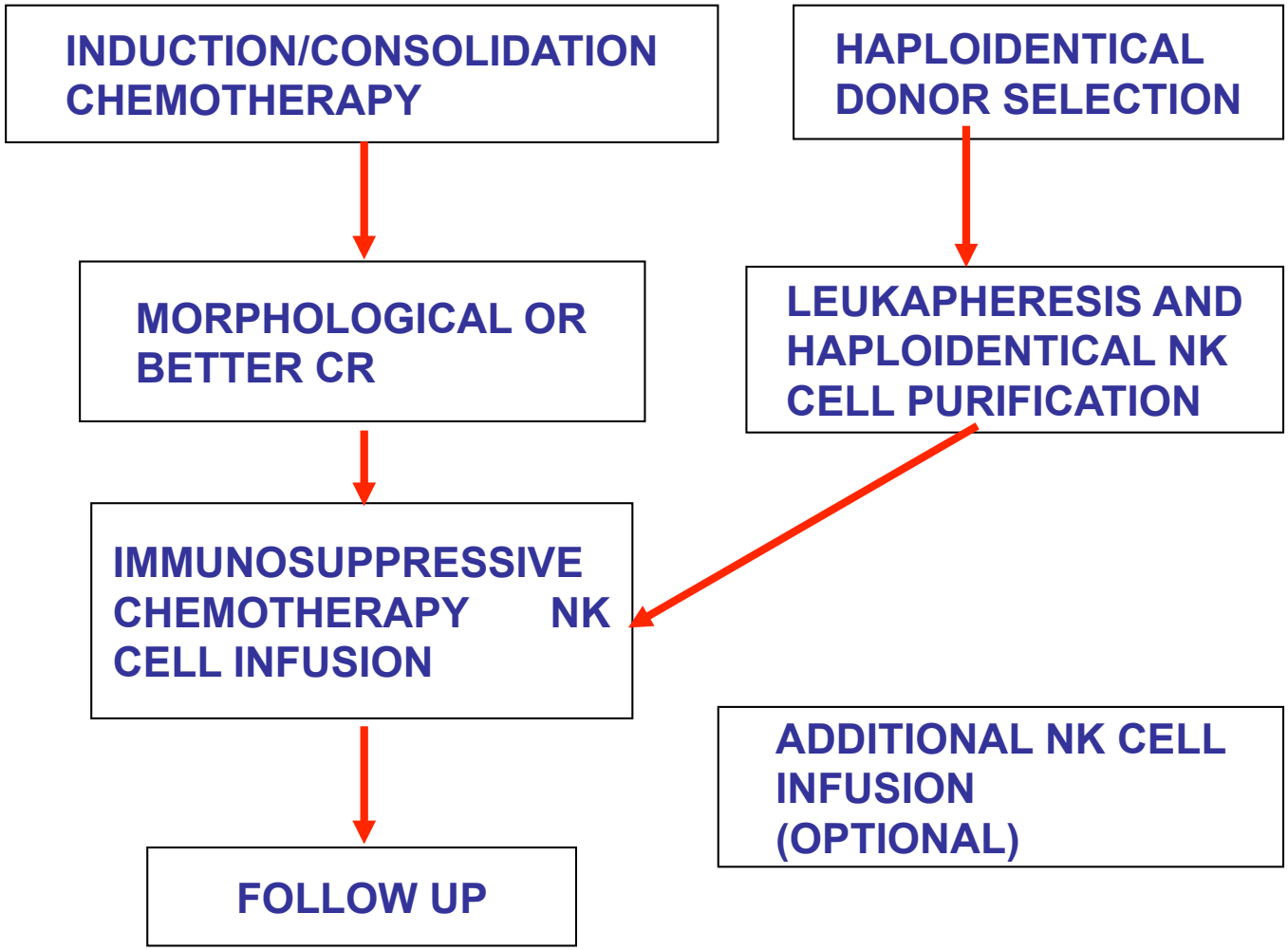
VNTR analysis



Curti et al., Blood, 2011


A
KIR2DL2/3+/NKG2A-
cells/µL
20
10
0
3 6 9 12 15 18 21 24 27 30
days post NK infusion
B
KIR2DL1+/NKG2A-
20
10
0
3 6 9 12 15 18 21 24 27 30
days post NK infusion
C
% lysis
100
50
HLA-C1+ donor alloreactive NK clones
detected in C1 missing patients
D
100
50
HLA-C2+ donor alloreactive NK clones
detected in C2 missing patients

Phase II trial: Study Design



17 patients	Median
Age (yrs)	65 (51-73)
Sex (M/F)	9/8
WBC>30x10^9/L	6/17 (35%)
Secondary AML	3/17 (18%)
Cytogenetics:	
Favorable (t8;21;inv16)	2/17 (12%)
Intermediate (normal; -Y)	13/17 (76%)
Unfavorable (other than favorable and intermediate)	2/17 (12%)
Genotype:	
NPM+/FLT3-	1/17 (6%)
NPM+/FLT3+	0/17 (0%)
NPM-/FLT3-	13/17 (76%)
NPM-/FLT3+	3/17 (18%)

Immunosuppressive Regimen

- **FLUDARABINE (Flu) 25 mg/m^2/day for 5 days (from day –7 to -3).**
- **CYCLOPHOSPHAMIDE (Cy) 4 g/m^2 (day -2).**

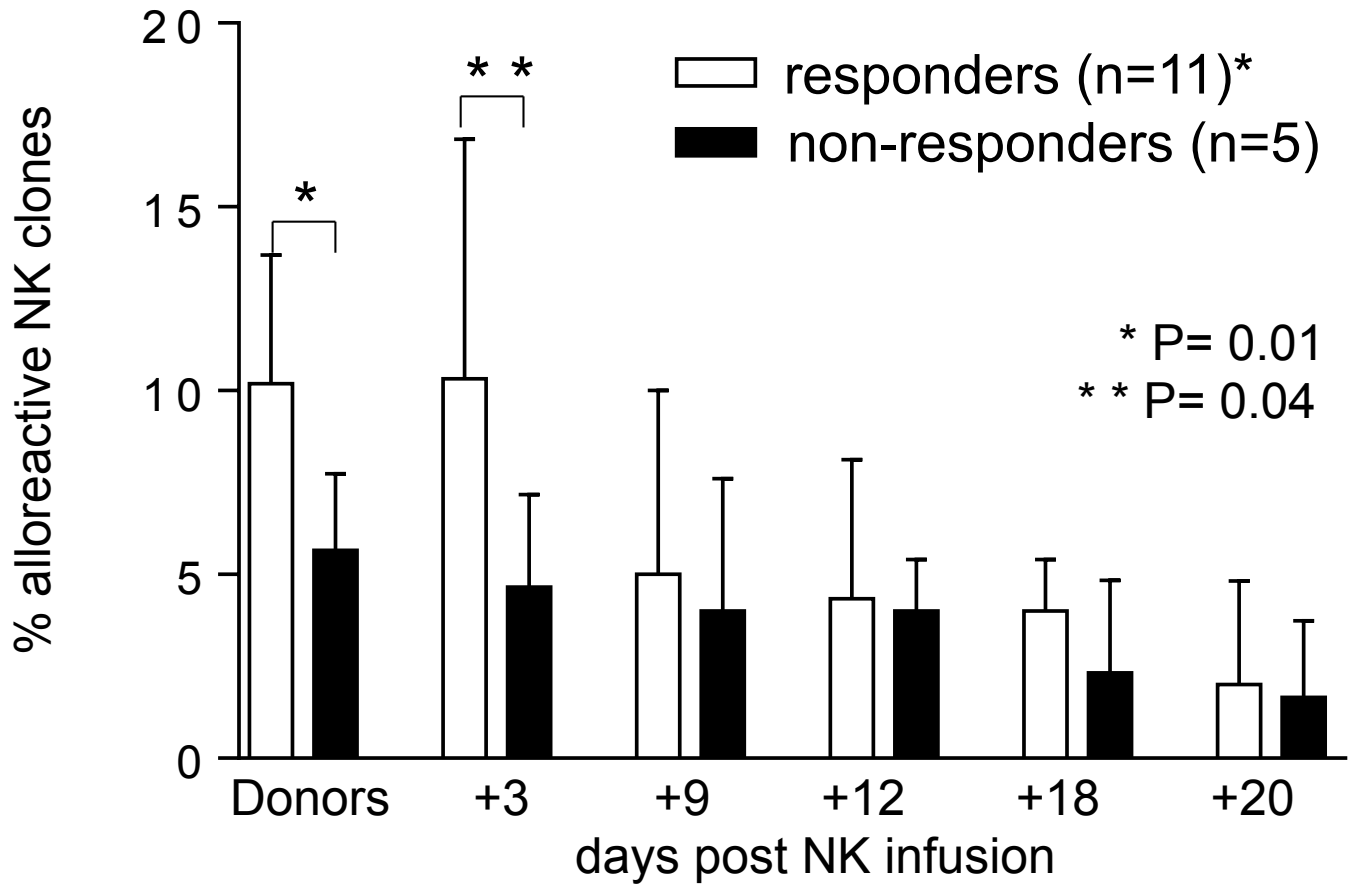
After 2 days from the administration of Cy, patients proceed to NK cell infusion (day 0). No GVHD prophylaxis is used as GVHD is not anticipated. IL-2 (10x10^6 IU/day, 3 times weekly) is administered sc for 2 weeks (6 doses total) after NK cell infusion.

Median time from CR to NK cell therapy = 5.5 months (range 4-9).

Patients characteristics, response to NK cell infusion and follow-up

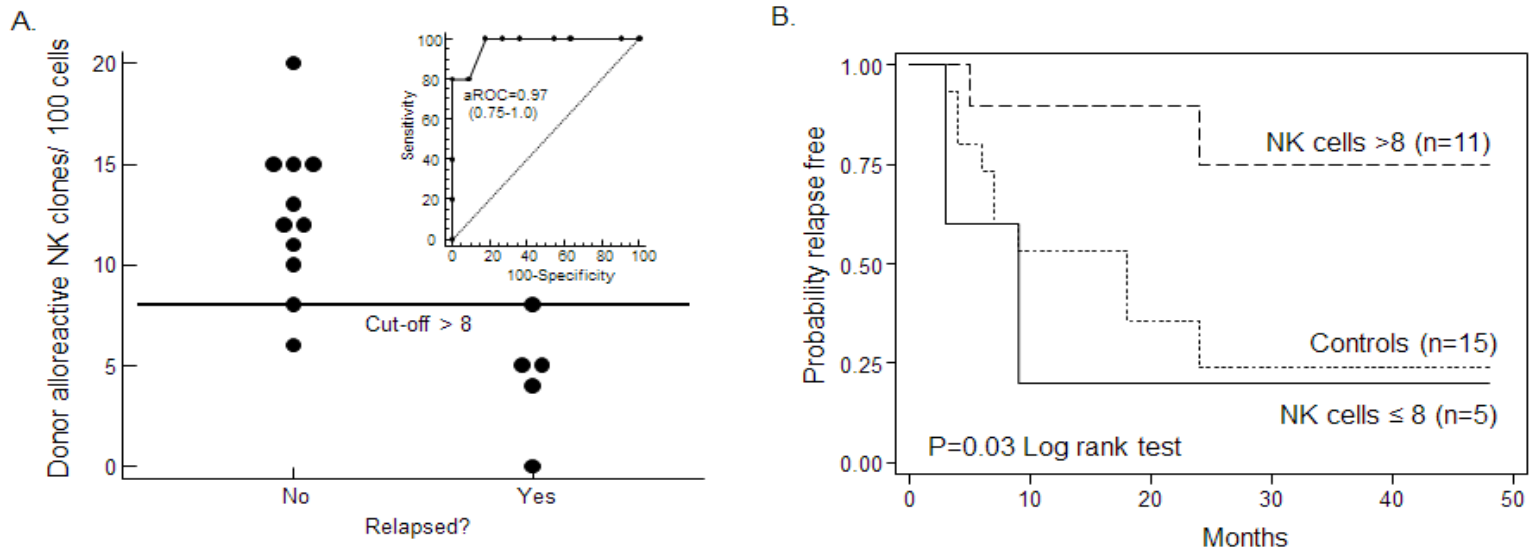
patient	age	sex	FAB	WBC	kariotype	AML type	disease status before NK infusion	response	folow-up (months)
1) D.E.R	63	M	M4	7.360	complex	de novo	morphological CR	CR	**CR(45)**
2) F.A	72	F	M1	1.170	+4;+8	de novo	morphological CR	CR	**CR(43)**
3) T.A	70	F	M5	58.600	XX	de novo	morphological CR	NE	dead(1)
4) D.F.S	73	M	M5	75.000	XY	de novo	morphological CR	CR	**CR(81)**
5) M.A	58	M	M4	74.800	XY	de novo	morphological CR	relapse(3)	dead (4)
6) V.V	58	F	M1	4.320	XX	de novo	morphological CR	CR	**CR(78)**
7) Z.G	64	M	M1	25.000	XY	de novo	morphological CR	relapse(5)	dead(6)
8) R.C.	53	F	M1	4.100	-7;+8	de novo	molecular relapse	CR	relapse(9)/ IICR(36)
9) P.R.	67	M	M0	2.700	XY	de novo	morphological CR	relapse(24)	dead (30)
10) D.P.C.	58	F	M1	5.800	inv16	de novo	persistent MRD+	CR	relapse(9)/II NK/ dead
11) D.D.	61	M	n.a.	2.900	XY	secondary	morphological CR	relapse (51)	CR (5-Aza)
12) V.A	72	M	n.a.	3.000	XY	secondary	morphological CR	CR	**CR(24)**
13) S.D	68	F	n.a.	59.000	XX	de novo	morphological CR	CR	**CR(23)**
14) C.A	61	M	n.a.	2.500	del(12)	secondary	morphological CR	Relapse (3)	Reinduction
15) V.L	62	F	M1	1.270	t(11)	de novo	morphological CR	CR	**CR(11)**
16) R.E.	64	F	M4	27.400	inv(16)	de novo	persistent MRD+	CR	**CR(9)**
17) N.A.	65	M	M0	189.500	XY	de novo	morphological CR	CR	**CR(6)**

Larger alloreactive NK cell repertoires are associated with reduced relapse rate



Curti et al., Clin Cancer Res 2016

Larger alloreactive NK cell repertoires are associated with reduced relapse rate



Curti et al., Clin Cancer Res 2016

Cell processing data according to clinical response

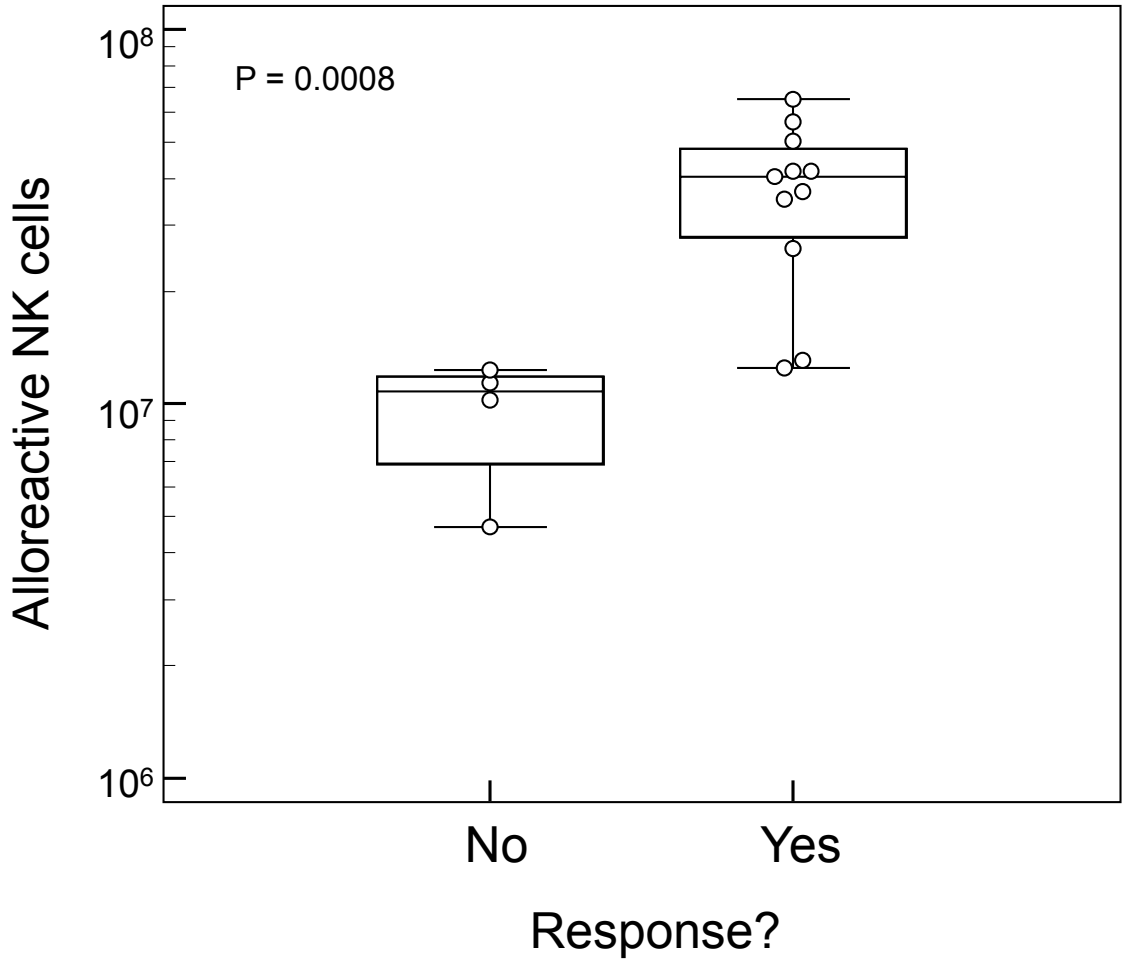
UPN	NK CELLS				T CELLS		
	PURITY	RECOVERY	COLLECTED (x 10^6/Kg)	INFUSED (x 10^6/Kg)	T-CELL LOG DEPLETION	COLLECTED (x 10^5/Kg)	INFUSED (x 10^5/Kg)
6	79,7	53.05	10.72	4.0	3.04	1579	1
7	96,8	57.78	17.1	4.75	3.11	1690	0.11
11	94,9	31.82	42.5	2.74	2.53	1671.13	1
12	94,9	45.61	21.55	2.51	2.6	1577.01	1
1	95,8	32.77	8.42	3.1	2.84	580.27	1
2	92,8	56.48	28.29	4.14	6.94	2167.93	3,1
4	95	54.97	24.29	5.53	3.41	1266.23	1
15	99,2	42.84	10.64	5.1	4.05	632.67	0.215
5	98,1	50.28	19.56	5	3.67	1880.48	0,1
16	92,9	51.8	29.3	5	2.33	1785.47	0.41
17	97,3	63.51	14.2	5	3.07	1698.27	0.255
Median (range)	94.3 (79.7-99.2)	49.2 (31.82-63.51)	20.6 (8.42-42.5)	4.3 (2.51-5.53)	3.4 (6.94-2.33)	1503 (580.27-2167.93)	0.84 (0.1-3.1)

Responders

UPN	NK CELLS				T CELLS		
	PURITY	RECOVERY	COLLECTED (x 10^6/Kg)	INFUSED (x 10^6/Kg)	T-CELL LOG DEPLETION	COLLECTED (x 10^5Kg)	INFUSED (x 10^5/Kg)
8	92.4	65.4	3.8	1.81	4.52	882.39	0.05
9	97.2	60.83	24.1	2.05	2.71	1013.13	1
10	99.2	35.95	11.98	3.89	4.3	1740.51	0.08
13	90.6	54.41	28.96	1.29	2.15	1726.27	1
14	99.1	63.29	26.04	5	6.86	1005.37	0.1
Median (range)	95.7 (90.6-99.2)	55.9 (35.95-65.4)	18.98 (3.8-28.96)	2.8 (1.29-5)	4.1 (6.86—2.15)	1273.53 (882.39-1740.51)	0.45 (0.05-1)

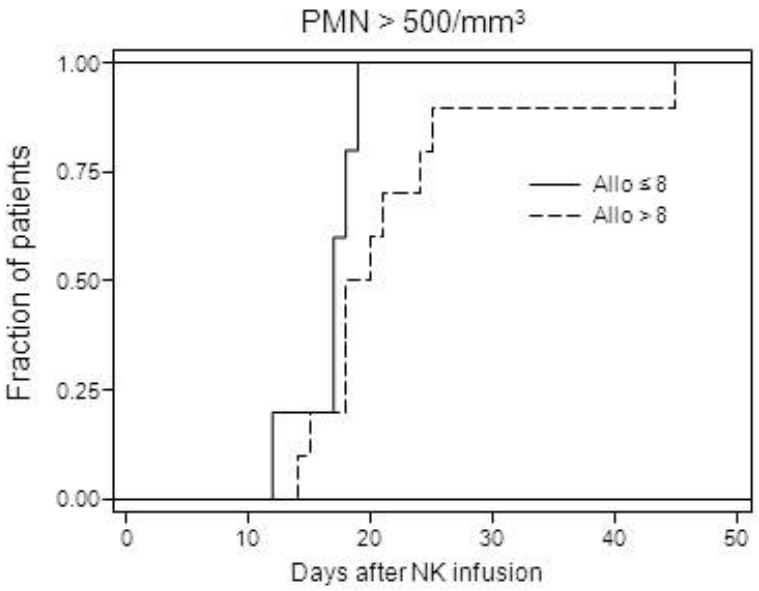
NON-responders

Impact of the absolute number of infused alloreactive NK cells on clinical response

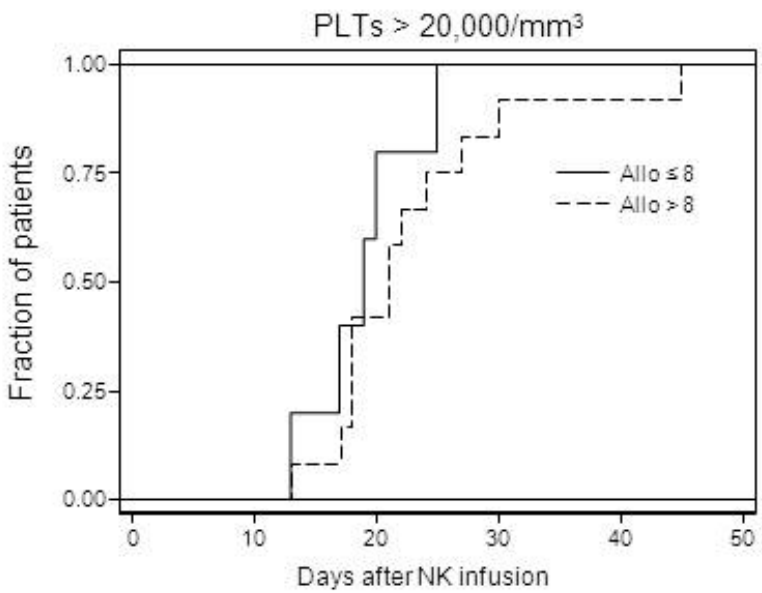


Curti et al., Clin Cancer Res 2016

Hematological Recovery according to donor alloreactivity



Allo ≤ 8: mean 16.6 (14.5-18.7)
Allo > 8: mean 17.8 (16.7-18.9)



Allo ≤ 8: mean 18.8 (15.3-22.4)
Allo > 8: mean 20.6 (18.5-22.7)

Curti et al., Clin Cancer Res 2016

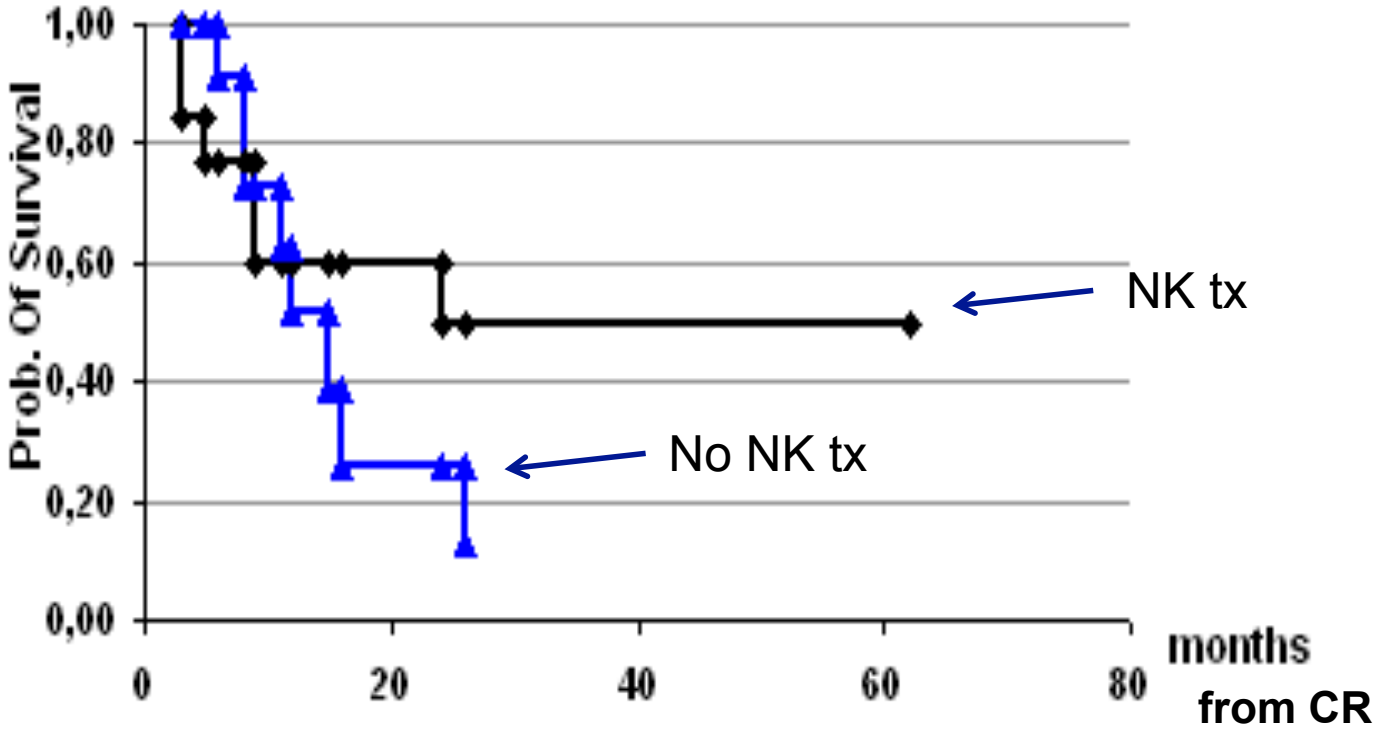
Conclusions

- Infusion of purified NK cells is feasible in elderly AML patients as post-CR consolidation strategy
- At the clinical level, 9/16 CR patients are disease-free after a median follow-up of 27 months, without any additional treatment. Two of the relapsed patients had a prolonged CR phase without concomitant anti-leukemia treatment.
- The infusion of higher number of alloreactive NK cells is associated with prolonged disease-free survival. The number of donor alloreactive NK cell clones may be used as a predictive biomarker for better clinical outcome

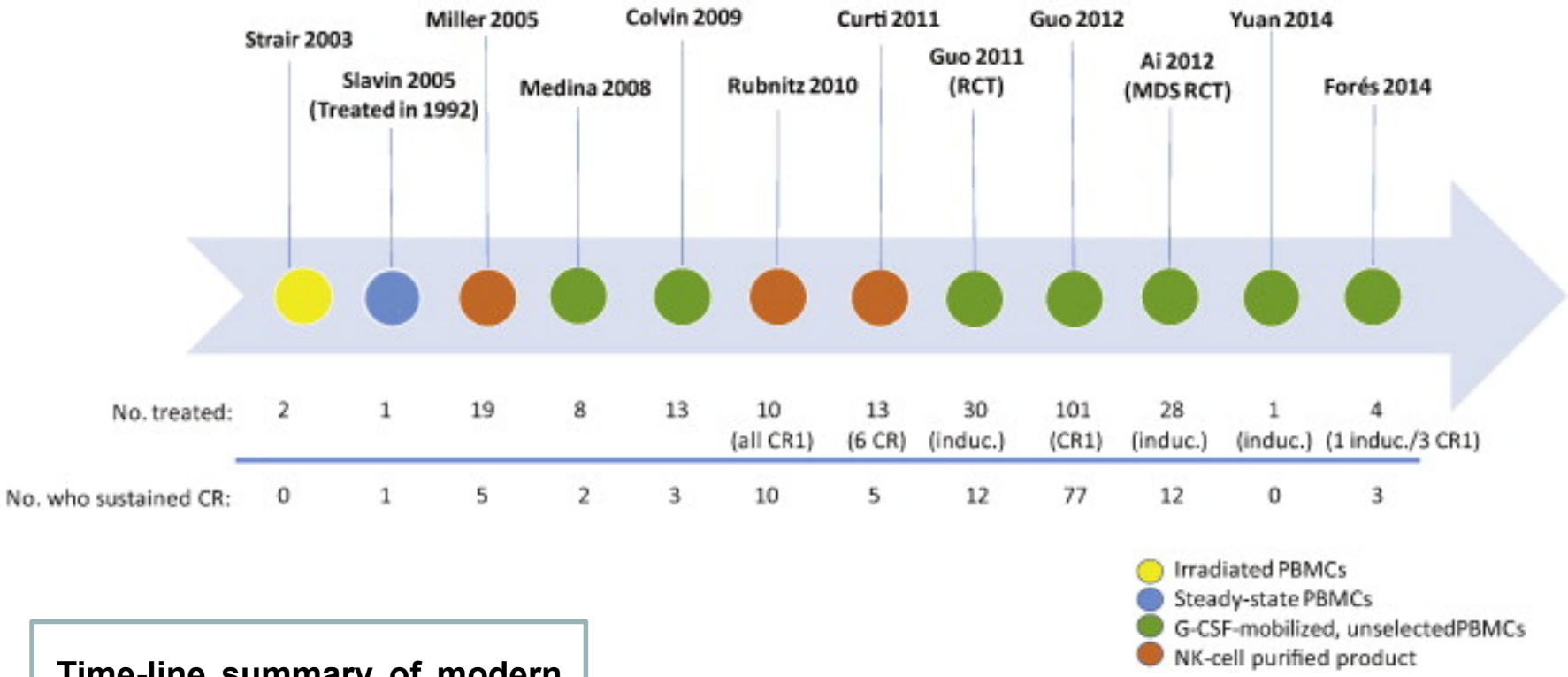
Curti et al, Clin Cancer Res 2016

RISK FACTORS	NK PATIENTS	CHEMOTHERAPY-ONLY PATIENTS
Age (yrs)	65 (51-73)	62 (50-68)
Sex (M/F)	8/6	3/8
WBC>30x10^9/L	4/14 (28%)	3/11 (25%)
Secondary AML	3/14 (21%)	2/11 (25%)
Cytogenetics:		
Favorable (t8;21;inv16)	1/14 (7.5%)	1/12 (8.3%)
Intermediate (normal; -Y)	11/14 (78%)	9/12 (75%)
Unfavorable (other than favorable and intermediate)	2/14 (14%)	2/12 (16.6%)

Overall survival of elderly AML patients in CR according to NK cell treatment



Harnessing the power of alloreactivity without triggering GvHD: how non-engrafting alloreactive cellular therapy might change the landscape of acute myeloid leukemia treatment



Time-line summary of modern clinical trials of non-engrafting alloreactive cell therapy for AML and MDS

Krakow et al, Blood Reviews, Volume 28, Issue 6, 2014, 249–261

Title: Multicenter phase II clinical study of adoptive immunotherapy with alloreactive NK cells as consolidation strategy for elderly acute myeloid leukemia patients

Type of study: multicenter, Phase II

Partecipating Centers: Bologna, Genova, Perugia

Time for enrollment: 36 months

Supporting Agency: Ministry of Health: Bando Ricerca Finalizzata 2013-codice RF-2013-02355949

Eligibility criteria

- **Adult AML patients in CR with at least morphologic CR, not eligible for SCT and with an haploidentical KIR-L mismatched donor, will be included.**
- Patients with low-risk AML in molecular CR will be excluded.
- Patients with active infections, abnormal renal, cardiac, pulmonary and hepatic function and poor performance score will be excluded.
- **Using a genetic randomization through a 'donor' vs 'no donor' approach, patients will undergo NK cell infusion (ARM 1; 40 pts) or followed up without treatment (ARM 2; 40 pts).**

Cell collection and infusion: The concept of «functional cell dose»

- Donors will undergo leukapheresis for isolation of mononuclear cells, which will then be incubated with NK-cell separation CliniMACS system

- Based on our previous studies and considering the median numbers of NK cells collected and reinfused in responding patients and the absolute number of alloreactive NK cell clones in this patient population, we set a **minimum cell dose of $20x10^6$ total NK cells collected/Kg.**

- After leukapheresis, assessment of donor NK cell repertoires by limiting dilution cloning, cytotoxicity assays and immunophenotype will be performed to assess the "**functional" target cell dose of $20x10^6$ with a minimum number of 12 $x10^6$ $CD56^+CD3^-$ NK cell clones.** Two leukapheresis products (1 month apart) could be processed to achieve the cellular target.

Lemoli et al., Exp Hematol 2016

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Institute of Hematology
"L. & A. Seràgnoli",
University of Bologna

A.Curti

M.Baccarani

E. Dan

M.R Motta

S. Paolini

V. Salvestrini

S. Rizzi

D. Ocadlikova

Dept. Hematology,

University of Perugia

L. Ruggeri

A.Velardi

Immunogenetics
Laboratory

S. Orsola Hospital-
Bologna

V. Giudice

T. Fruet

A. Bontadini