AML MEETING
Ravenna, October 27, 2017

Evolving role of immunotherapy in acute myeloid leukemia

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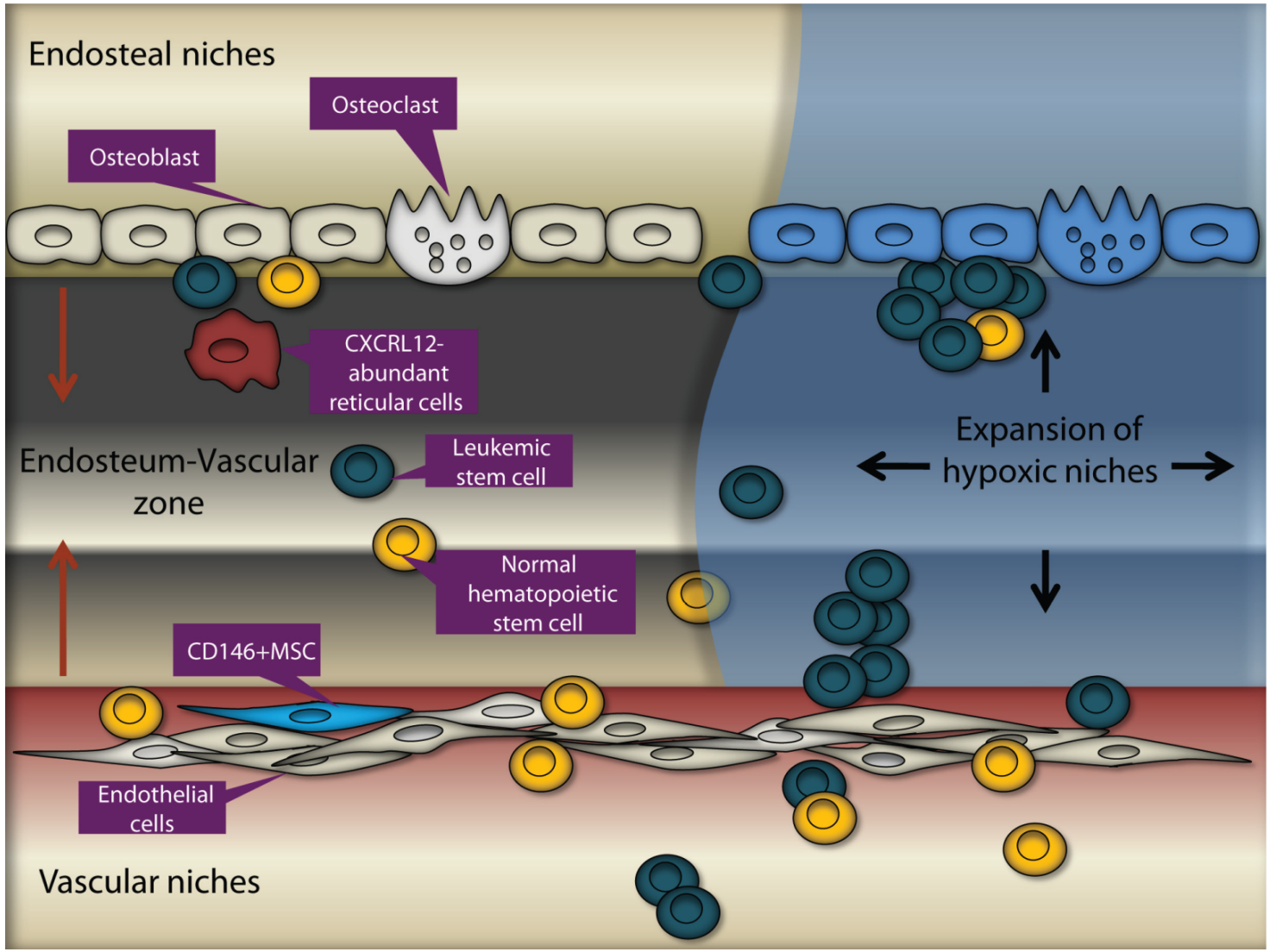
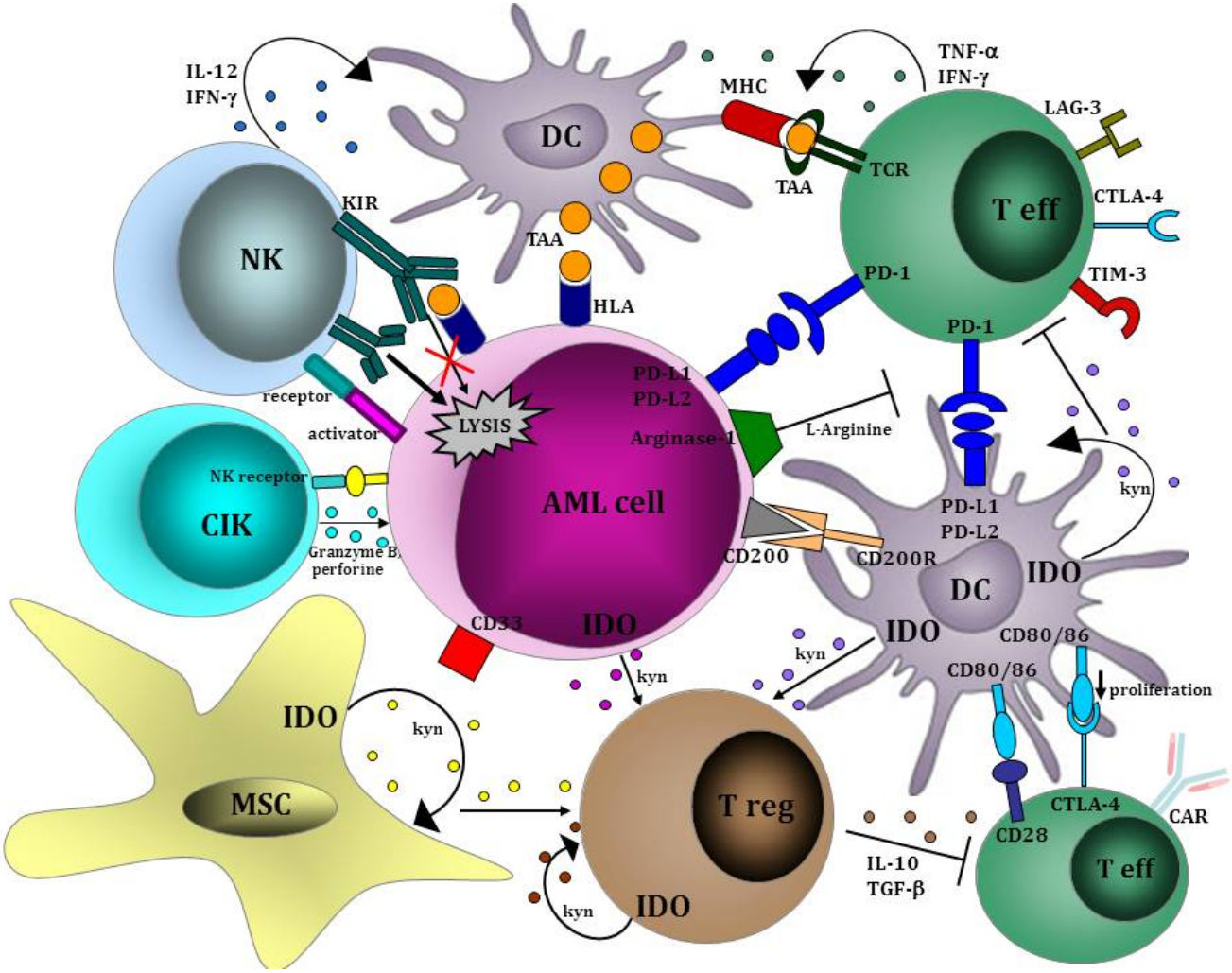
Leukemia Stem Cells and Microenvironment: Biology and Therapeutic Targeting



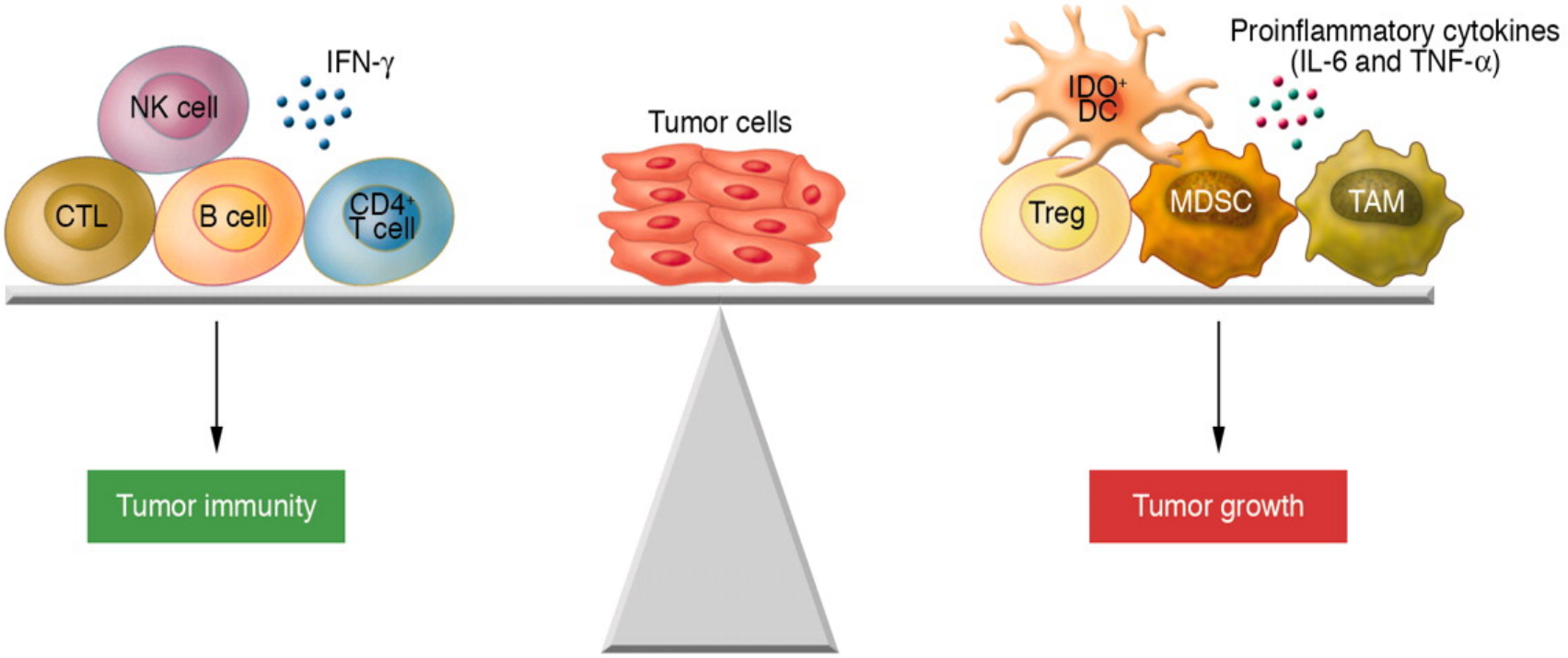
J Clin Oncol 29:591-599. 2011

AML and immunological microenvironment



Isidori & Curti, Cancer Res Frontiers, 2016

How to harness the immune system against cancer



Novel pathways as target for immunological therapies in AML

PATHWAY	THERAPEUTICAL ACTION	EFFECTS
PD-1/PD-L1	-mAb anti-PD-1 -mAb anti-PD-L1	- Increased T-cell cytotoxicity - Increased DC function as APCs
CD33	mAb anti-CD33	- AML cell lysis
CTLA-4	mAb anti-CTLA-4	- Increased T-cell cytotoxicity - Increased DC function as APCs
CD200	mAb anti-CD200	- Increased T/NK-cell cytotoxicity - Increased DC function as APCs
IDO	IDO1 inhibitor	- Prevention of T-cell tolerance
NK cells	adoptive cell therapy	- AML cell lysis
CAR-T cells	adoptive cell therapy	- AML cell lysis
Tregs	lymphodepletion therapy	- Prevention of T-cell tolerance
KIR	mAb anti-KIR	- AML cell lysis
Arginine	human recombinant arginase	- Prevention of immune tolerance
CIK cells	adoptive cell therapy	- AML cell lysis
TAAs (WT1, RHAMM..)	immunotherapy-peptide vaccines	- Specific AML cell lysis

Evolving immunological strategies to target AML cells

1) Antigen-targeted immunotherapies
   -Leukemia vaccines
   -Bispecific T-cell engangers (BiTes)
   -CAR T cells

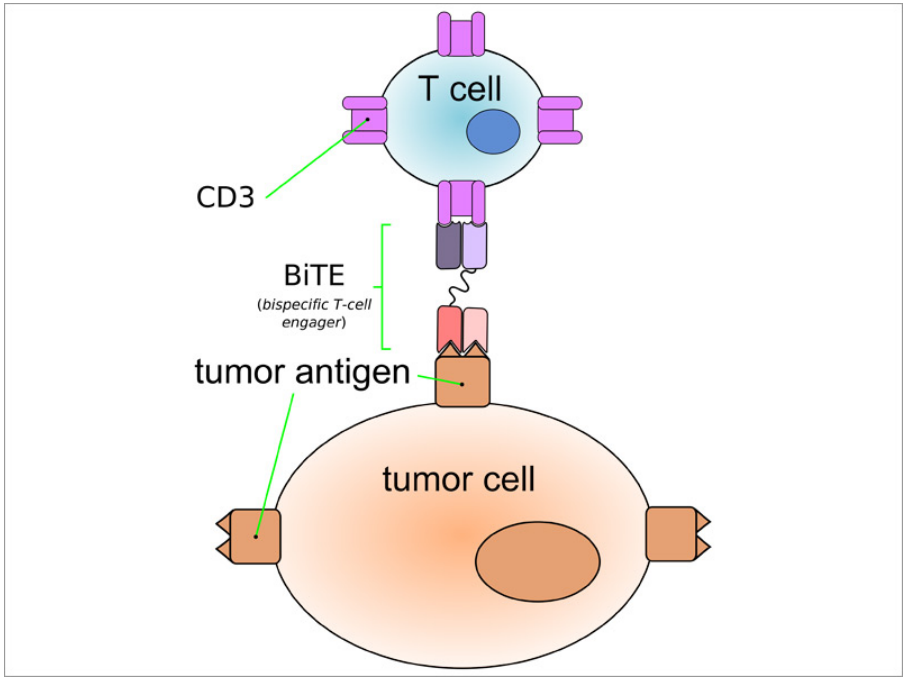
2) Immune checkpoint blockade

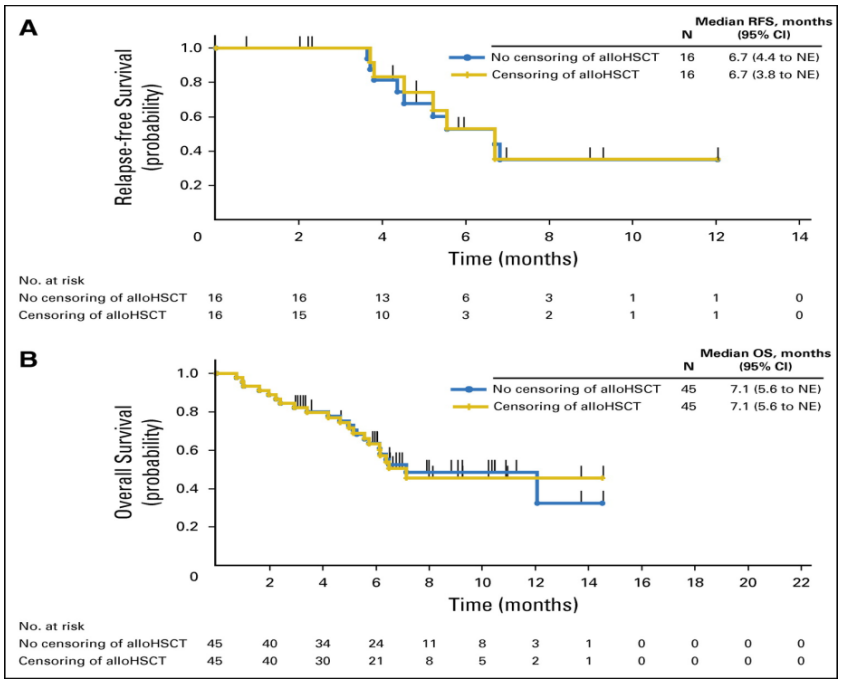
3) Inhibition of immunosuppressive factors

4) Cytokine therapies and adoptive transfer of NK cells

Bispecific T- cell engaging antibodies (BiTEs): biologic background

BiTEs monoclonal antibodies target, at the same time, a tumor antigen on cancer cells and the invariant epsilon subunit of CD3 in the T-cell receptor complex, thus enabling to effectively recruit polyclonal CD3+ T-cells in close proximity of target tumor cells irrespectively of their specificity

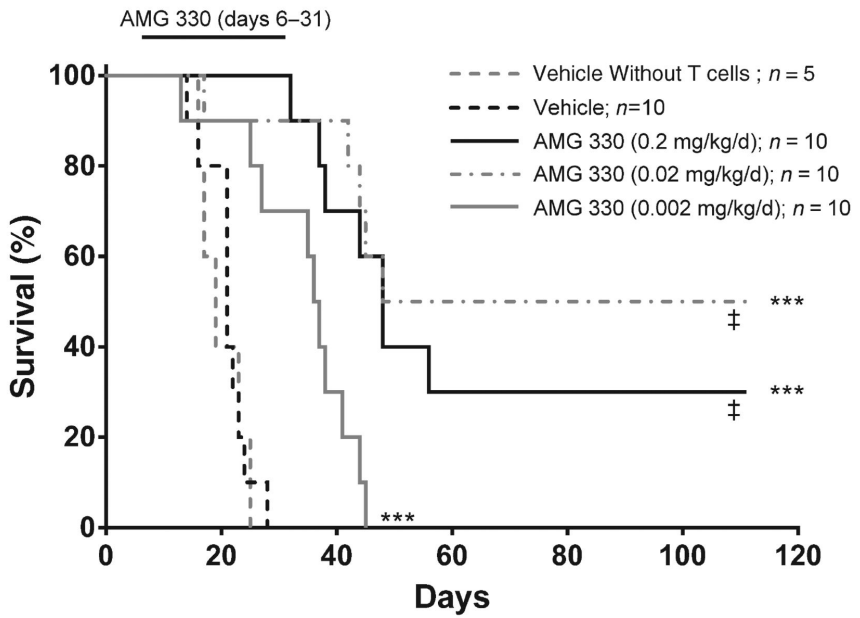




Martinelli and Topp, JCO, 2017

AMG330: preclinical studies and early clinical results

Antitumor activity of AMG 330 in a MOLM-13 xenograft model in NOD/ SCID mice.



Matthias Friedrich et al. Mol Cancer Ther 2014;13:1549-1557

A Phase 1 Study of AMG 330 in Subjects With Relapsed/Refractory AML (NCT02520427)

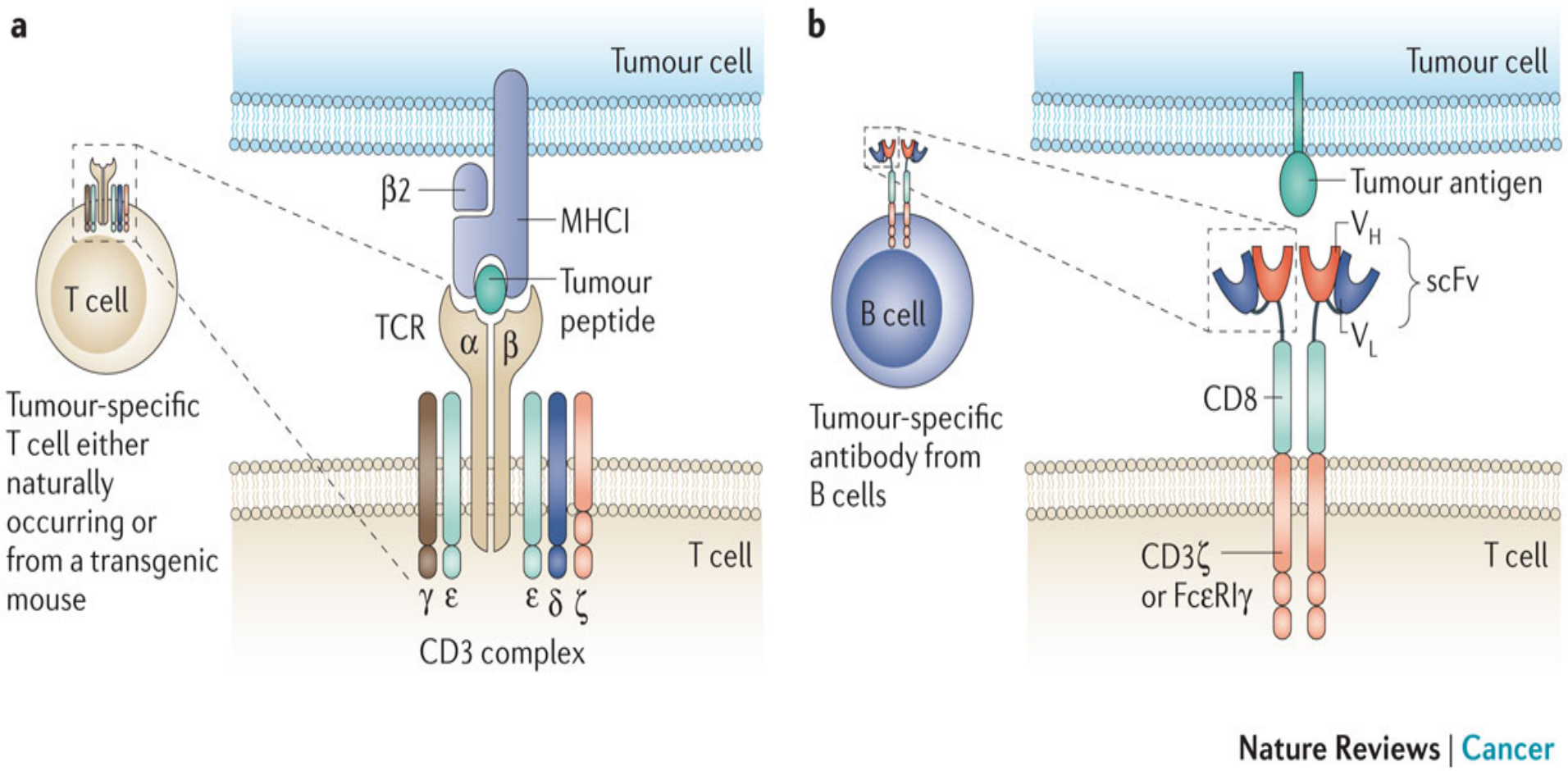
Primary Outcome Measures:

- Subject incidence of adverse events as a Measure of Safety
- Subject incidence of dose-limiting toxicities (DLTs) as a Measure of Safety

Secondary Outcome Measures:

- Incidence of anti-AMG 330 antibody formation
- Efficacy parameter: Response rate, duration of response time to progression, time to response
- Pharmacokinetic parameter

CAR T cells: biologic background



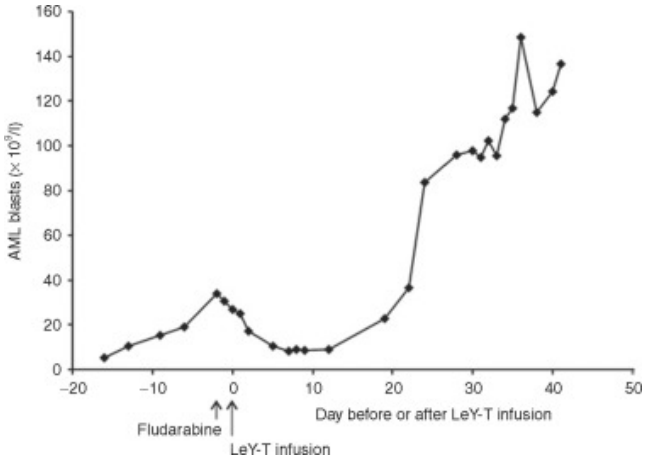
Nature Reviews | Cancer

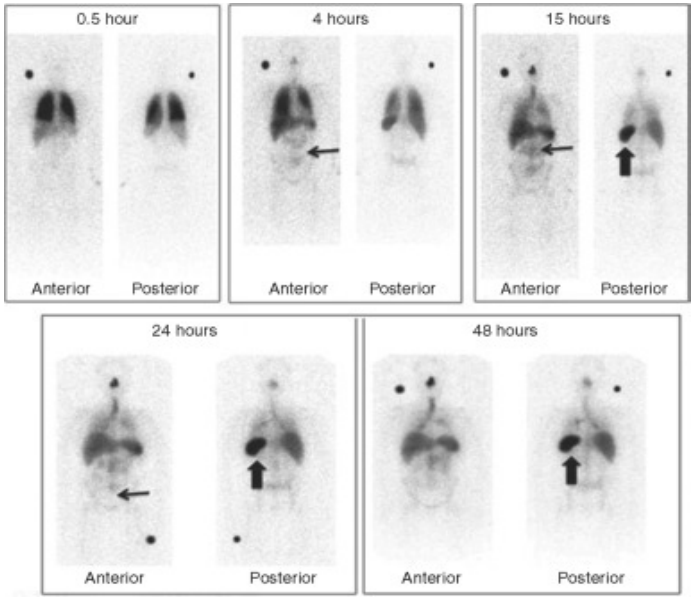
Possible targets for CAR T cells therapy in AML

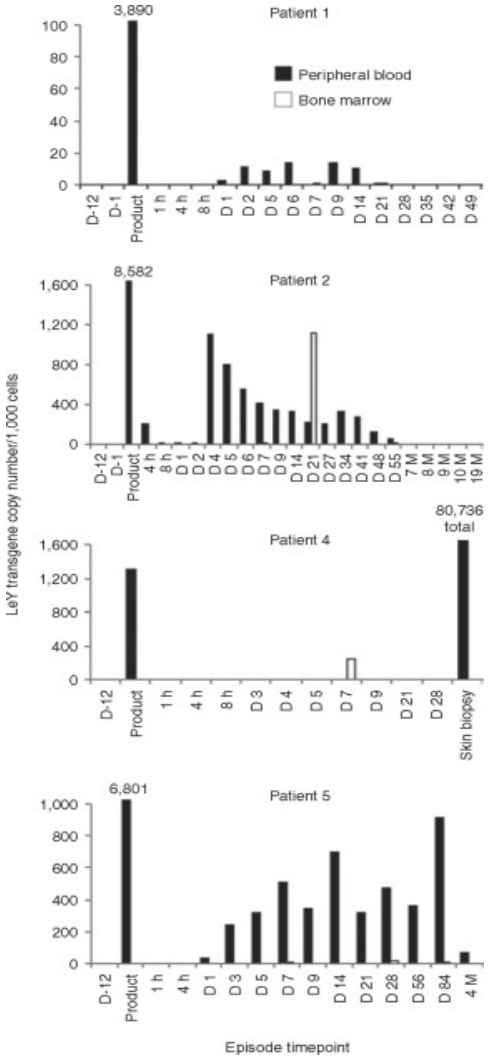
Target	Reference	Comments
CD123	Mardiros 2013 Gill 2014 Pizzitola 2014	Hematopoietic toxicity and possibly endothelial toxicity
CD33	Dutour 2012 Pizzitola 2014 Kenderian 2014	Hematopoietic toxicity and concern for hepatic toxicity
CD44v6	Casucci 2013	Concern for skin toxicity
FLT3	None	Neurologic tissue expression and hematopoietic toxicity
CD34	None	Endothelial expression and hematopoietic toxicity

Others: Lewis Y antigen, CD38, CD96, CD99, IL1RAP, NKG2D ligands.

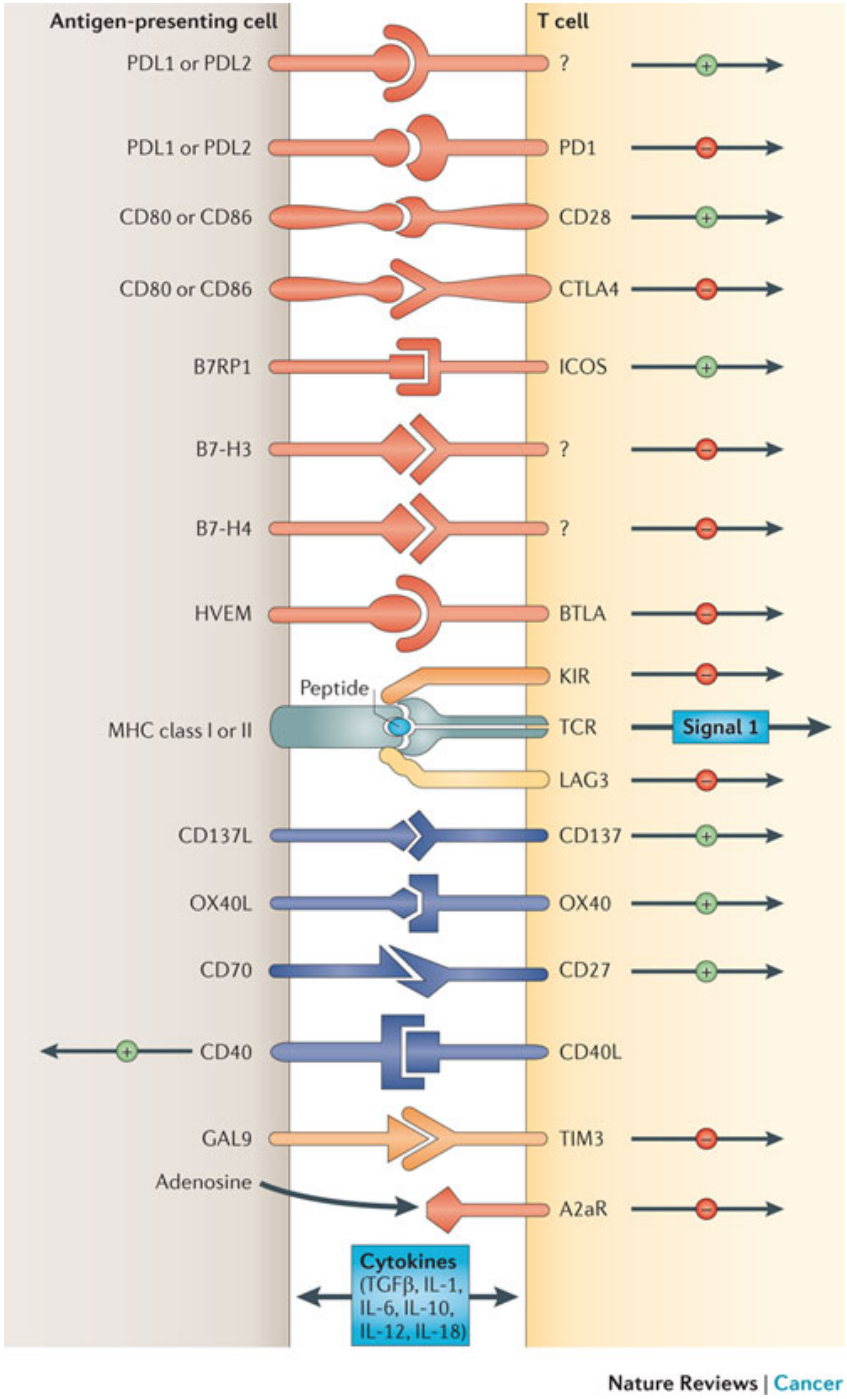
Persistence and Efficacy of Second Generation CAR T Cell Against the LeY Antigen in Acute Myeloid Leukemia







Ritchie DS et al. Molecular Therapy vol. 21 no. 11 nov. 2013



Multiple co-stimulatory and inhibitory interactions regulate T cell responses

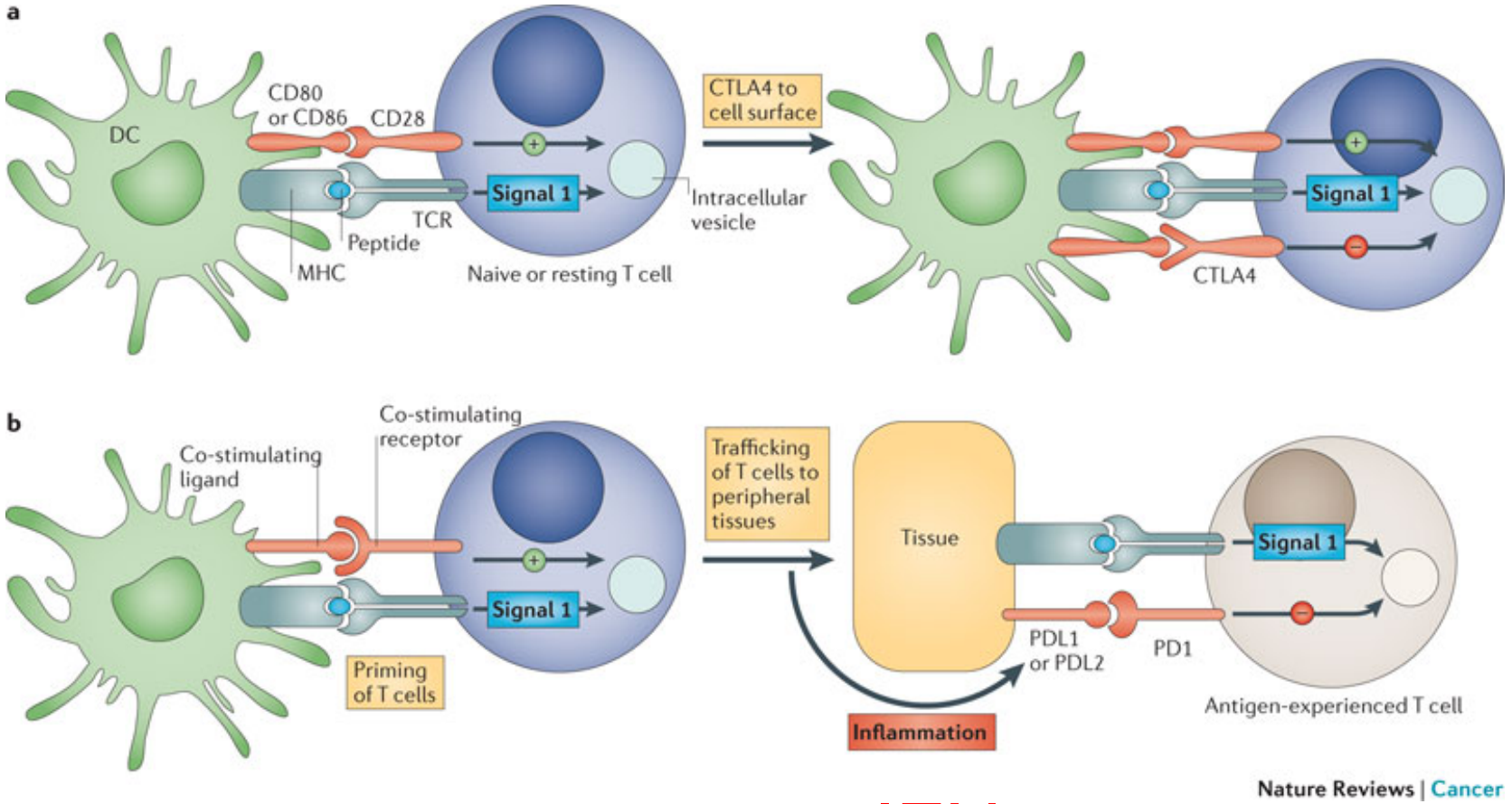
Ligand–receptor interactions between T cells and (APCs) can occur at the initiation of T cell responses in lymph nodes (where the major APCs are dendritic cells) or in peripheral tissues or tumours (where effector responses are regulated).

Drew M. Pardoll Nature Reviews Cancer 12, 252-264 (April 2012)

The blockade of immune checkpoints in cancer immunotherapy

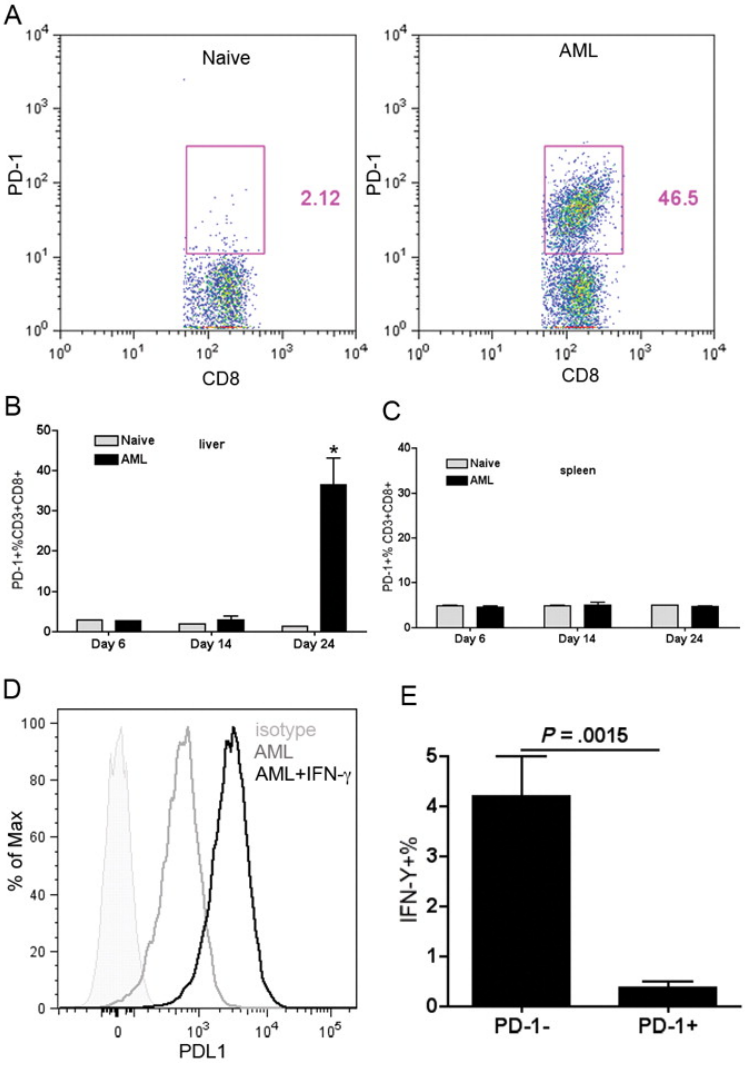
Drew M. Pardoll

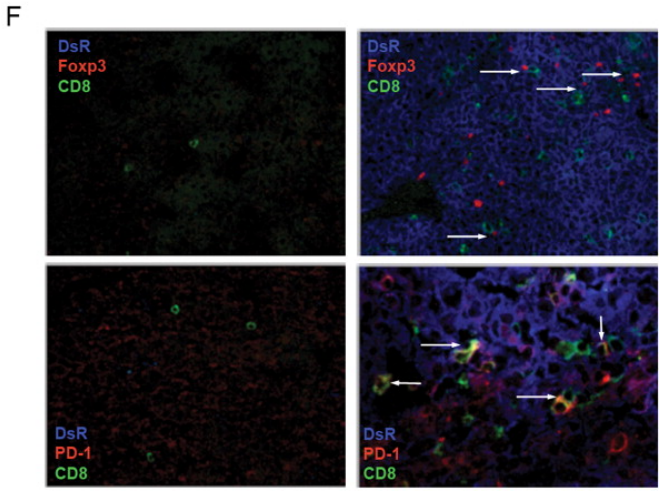
Nature Reviews Cancer 12, 252-264 (April 2012)



IFN-γ

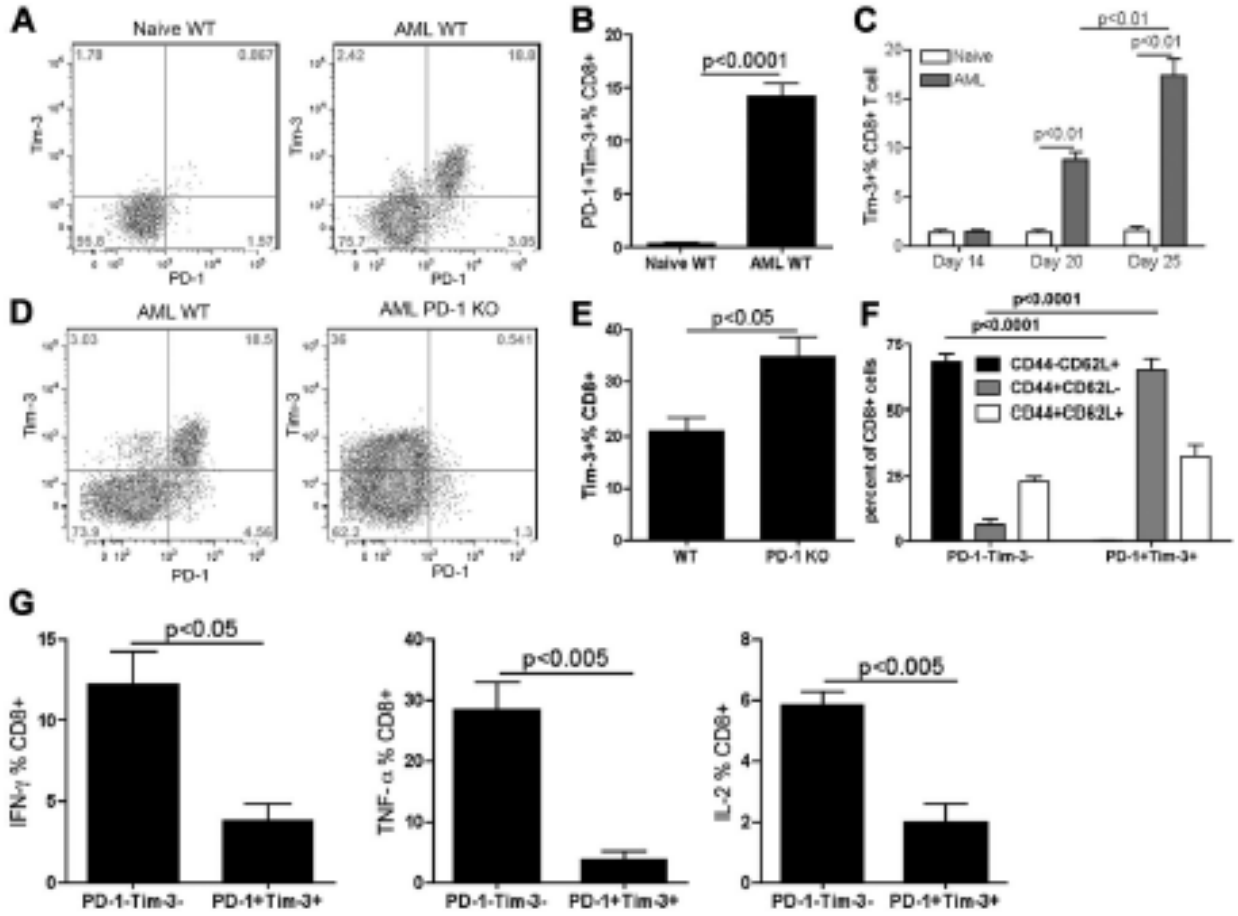
PD-1 expressing CD8+ T cells in the liver of AML-bearing mice displayed impaired function





Zhou Q et al. Blood 2010;116:2484-2493

Coexpression of TIM-3 and PD-1 identifies a CD8+T-cell exhaustion phenotype in mice with disseminated AML

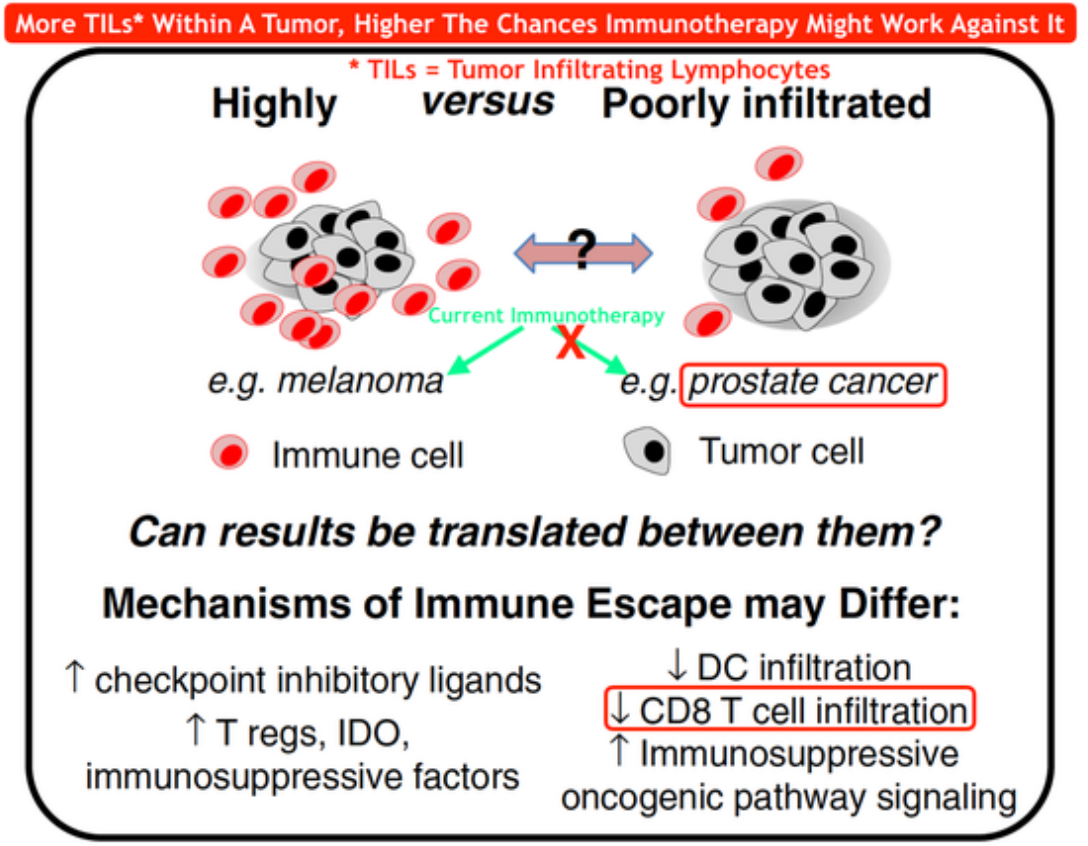


Zhou et al, Blood, 2016

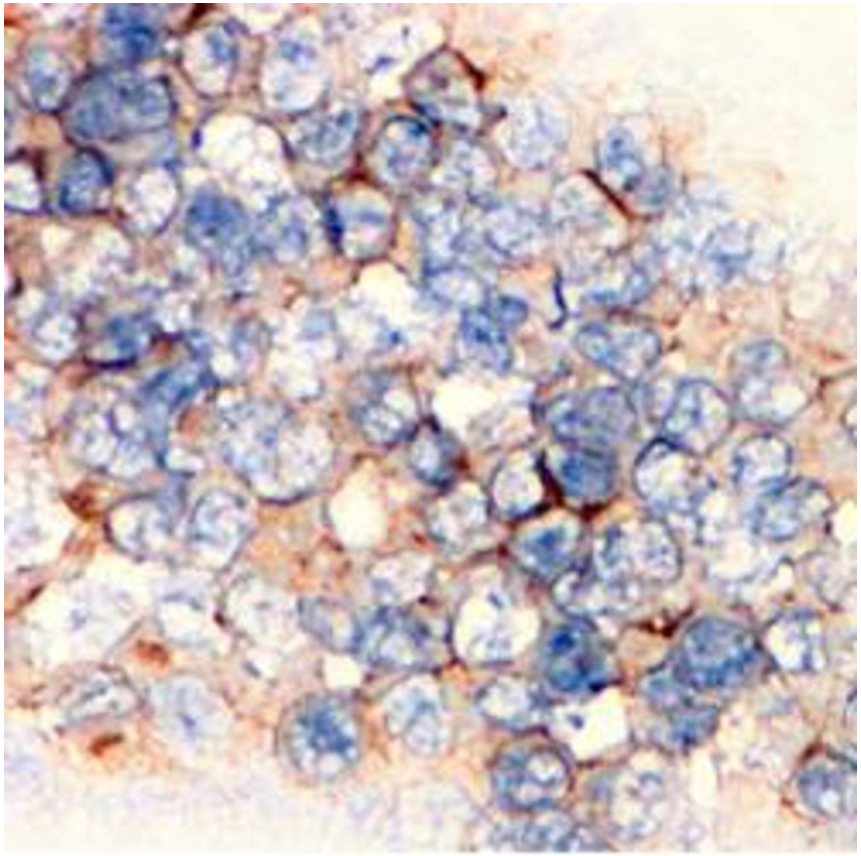
Immune checkpoint inhibitors for AML: on-going clinical trials

Study design	Phase	Code	Starting date
Anti-PD1 + DC AML vax	Phase 2	NCT01096602	March 2010
Ipilimimab in R/R MDS and AML with MRD	Phase 1	NCT017557639	December 2012
Ipilimumab or Nivolumab in relapsed HMs after SCT	Phase 1	NCT01822509	April 2013
Nivolumab in AML	Phase 1/2	NCT02464657	July 2015
Nivolumab in CR AML at high risk for relapse	Phase 2	NCT02532231	October 2015
Nivolumab in CR AML with MRD+	Phase 2	NCT02275533	May 2015
Nivolumab plus 5-azacytidine in R/R AML	Phase 2	NCT02397720	April 2015

Immune checkpoint inhibitors for AML: the question of leukemia lymphoid infiltrate



PD-L1 expression in MDS and AML cells is enhanced by HM agents



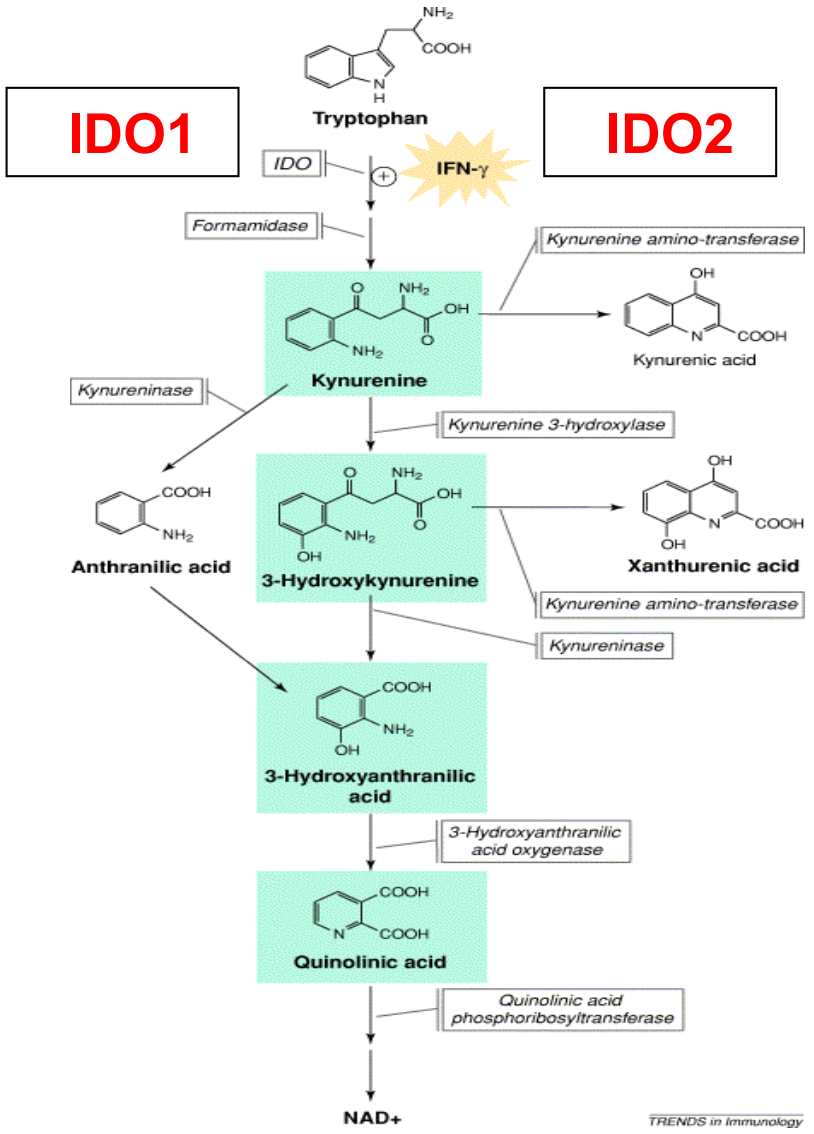
Exposure to decitabine resulted in demethylation of PD-L1 in AML cell lines, and the demethylation effect was also observed in HMAs treated MDS and AML patients

Expression of Immune Checkpoints PD-L1, PD-L2, PD-1 and CTLA4 Predict For Prognosis and Resistance To HAs In MDS

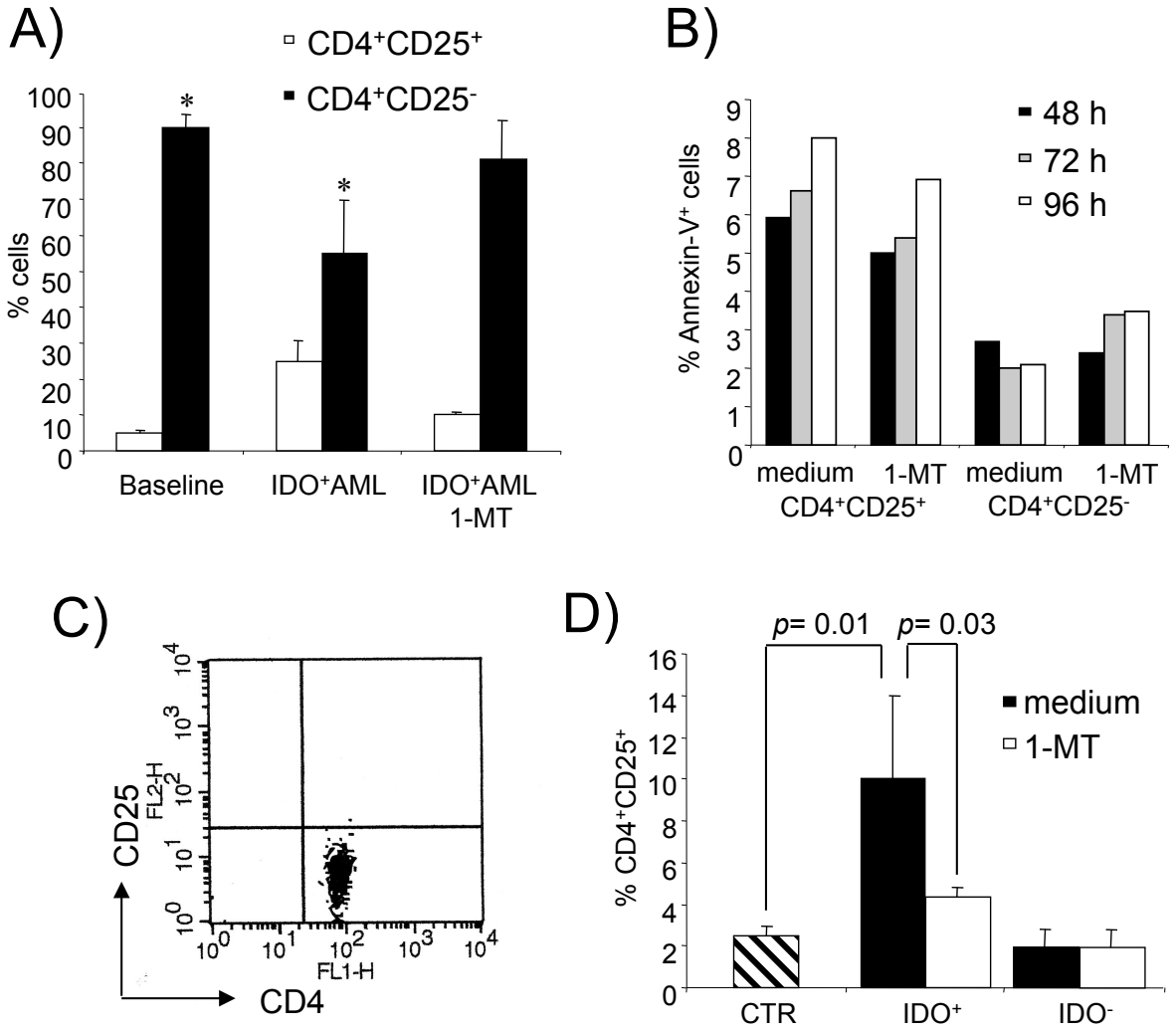
Carlos E. Bueso-Ramos et al. Blood 2013;122:2767

Inhibiting immune suppressive pathways: focus on IDO

- Indoleamine 2,3-dioxygenase (IDO) catalyzes the conversion of tryptophan into kynurenine
- Different cells, such as decidua cells, monocytes, regulatory DCs and mesenchymal stromal cells inhibit T-cell responses through IDO expression
- A wide variety of human tumors expresses IDO protein, which mediates immune tolerance

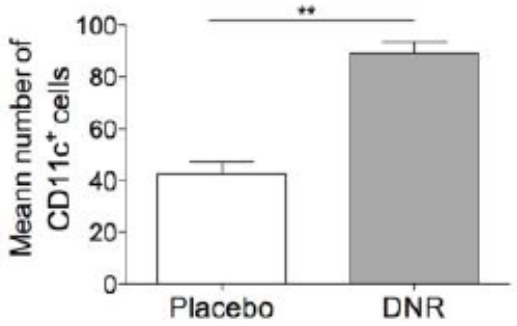


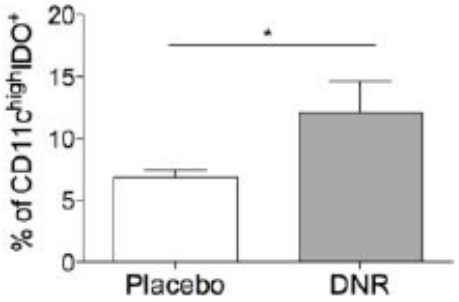
IDO$^+$ AML cells induce Tregs through the conversion of CD25$^-$ into CD25$^+$ CD4$^+$ FOXP3$^+$ T cells

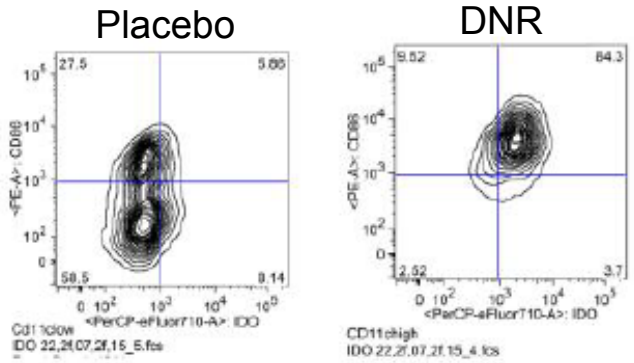


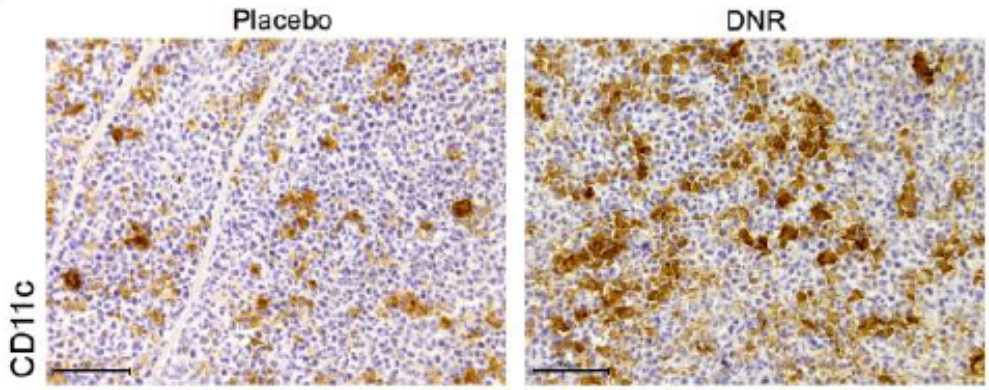
Curti et al. Blood, 2007; Blood, 2009; Haematologica, 2010

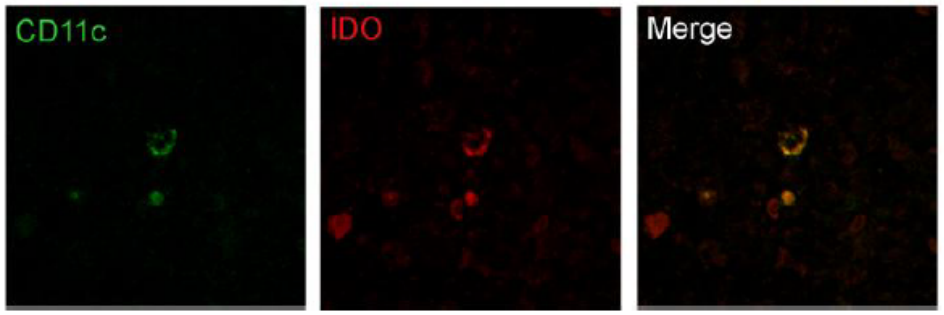
ATP release from chemotherapy-treated AML cells increases leukemia-infiltrating CD11c^{+}DC, expressing IDO1





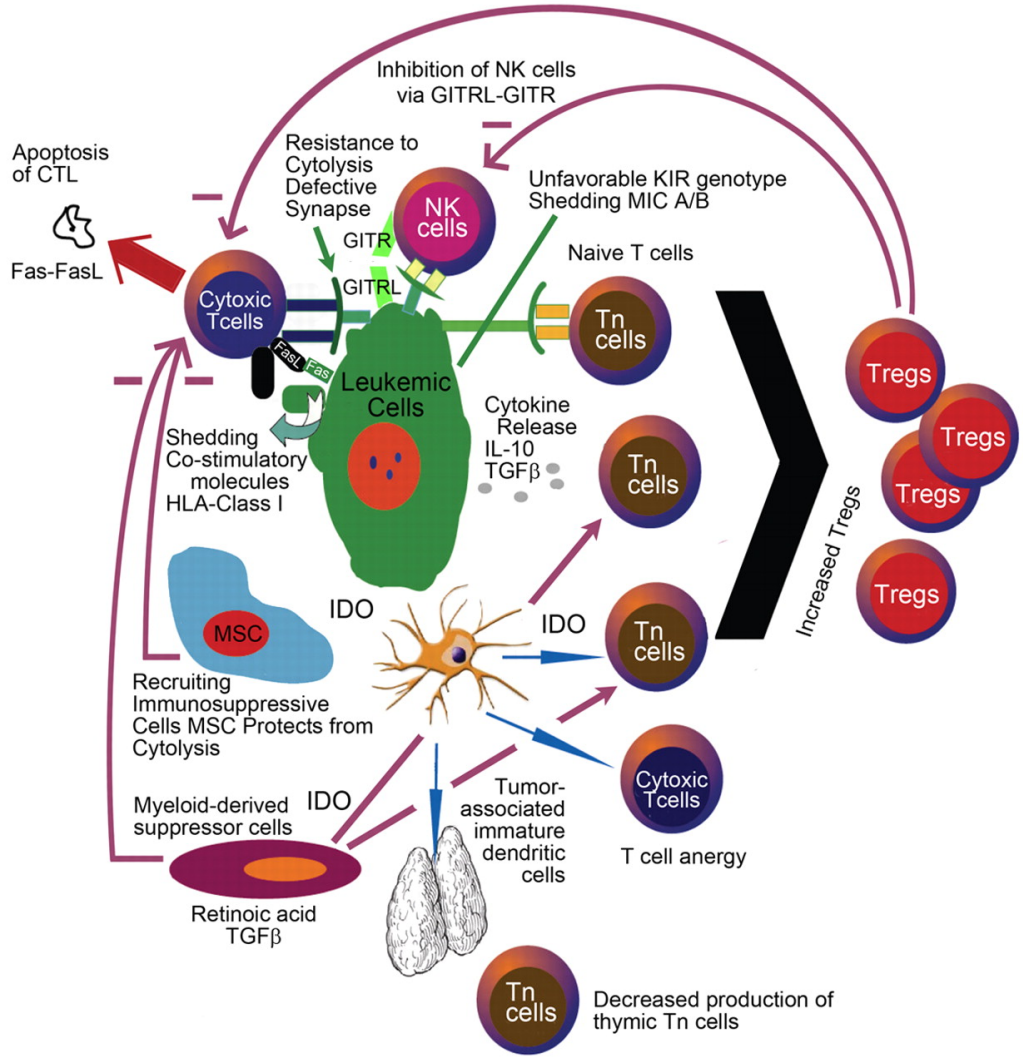






Lecciso M, Ocadlikova D et al, submitted

Tregs in AML: is it time for immunomodulation?



Ustun C et al. Blood 2011;118:5084-5095

IDO inhibitors: INCB024360 (Epacadostat)

A phase II study to determine the safety and efficacy of the oral inhibitor of indoleamine 2,3-dioxygenase (IDO) enzyme INCB024360 in patients with myelodysplastic syndrome and AML with 20-30% of marrow blasts

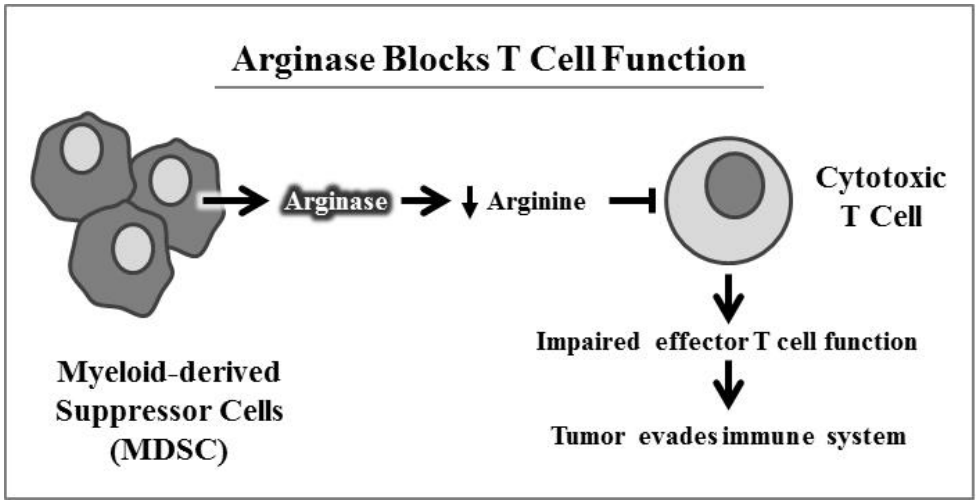
Primary endpoint: overall response
Secondary endpoints: 1) IDO suppression, 2) change in Treg and 3) the percentage of bone marrow MDSC change after treatment with INCB024360

Methods: All patients were treated with 600 mg oral twice a day for 16 weeks until progression or unless toxicity was evident.

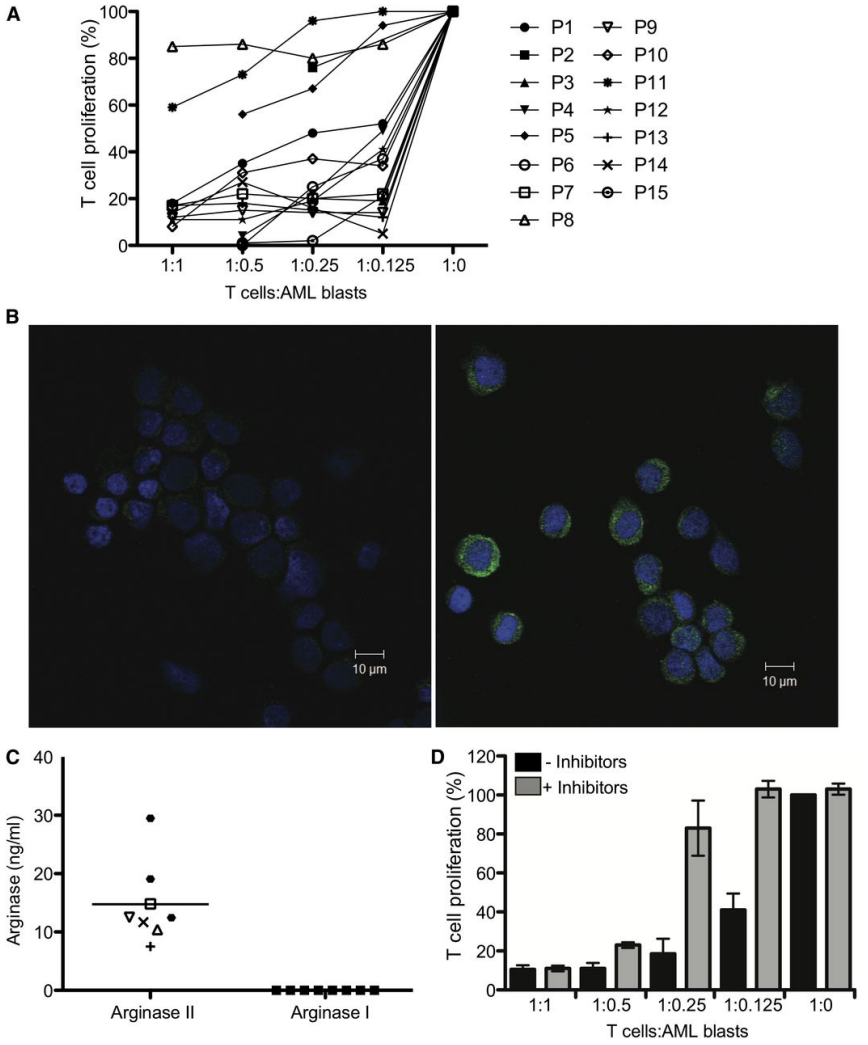
Results: 15 patients
SD (80%)
PD (20%)
No grade 3/4 events
Evidence of activity (laboratory)

Conclusions: well-tolerated. Significant activity. To be tested in combination

Arginine metabolism regulates the suppressive activity of AML blasts

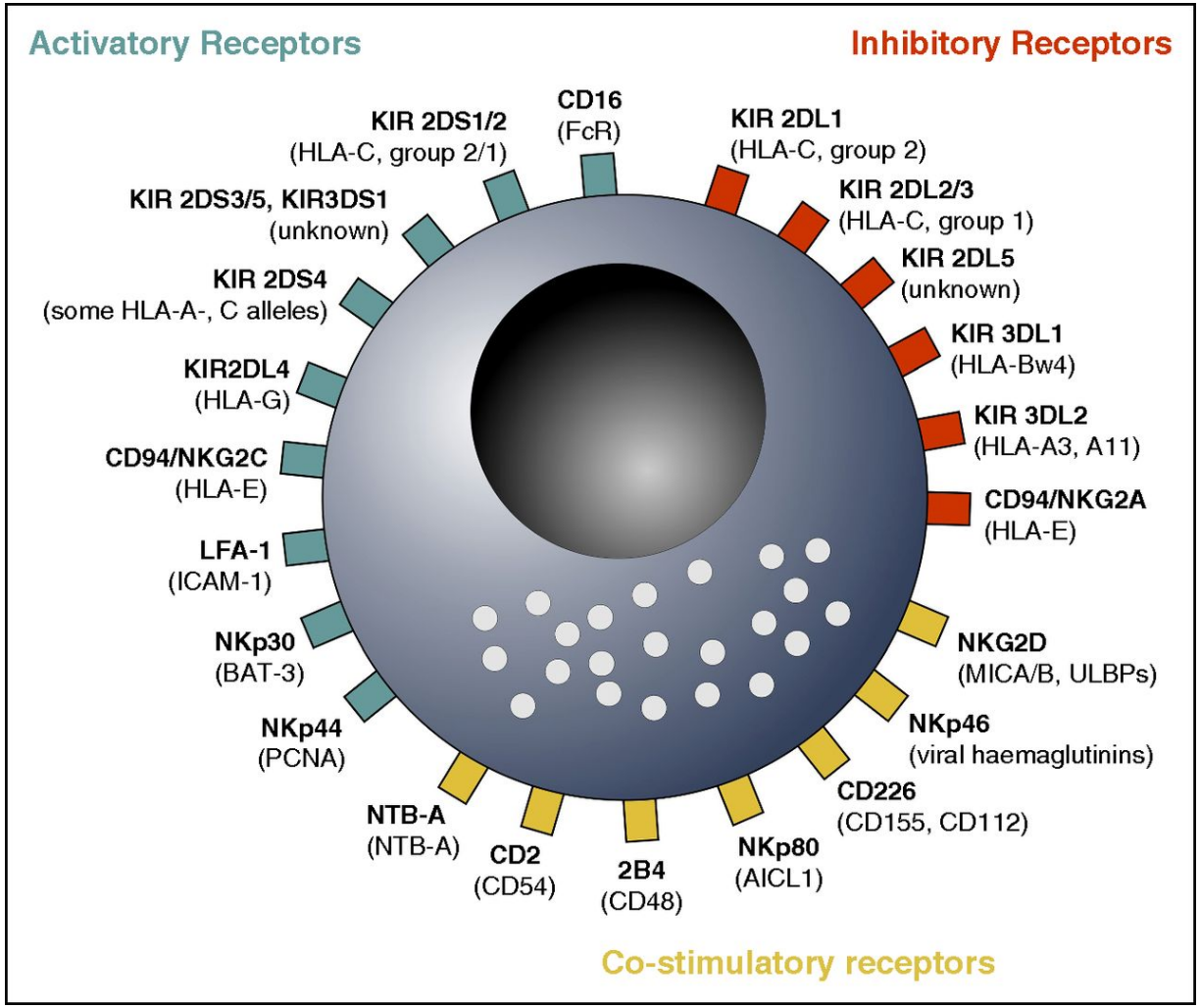


A Phase II Study of Arginine Deiminase (ADI-PEG20) in Relapsed/ Refractory or Poor-Risk Acute Myeloid Leukemia Patients



Francis Mussai et al. Blood 2013;122:749-758

NK cells “naturally” kill cell targets without prior sensitization

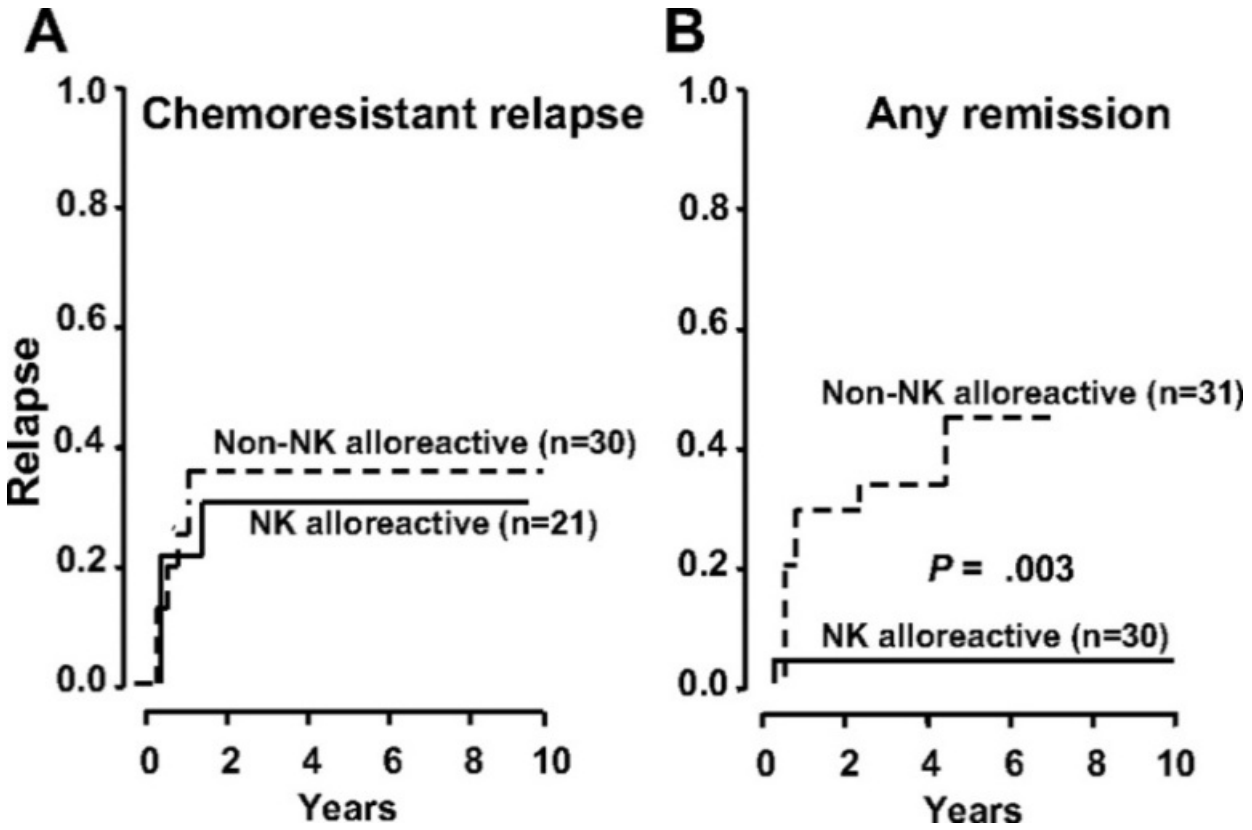


Handgretinger et al. Blood 2016

Killer Immunoglobulin-like Receptors (KIRs)

- Transmembrane proteins belonging to the Ig-SF with 2 or 3 extracellular Ig-like domains
- Specific for different alleles of MHC class I molecules (HLA-A, -B ,-C)
- Inhibitory KIR-receptors:
  - KIR2DL1 (97%) : receptor for HLA-C group 2
  - KIR2DL2/3 (100%): receptors for HLA-C group 1
  - KIR3DL1 (90%): receptor for HLA-Bw4

Clinical impact of KIR-L mismatch on relapse rate after haploSCT

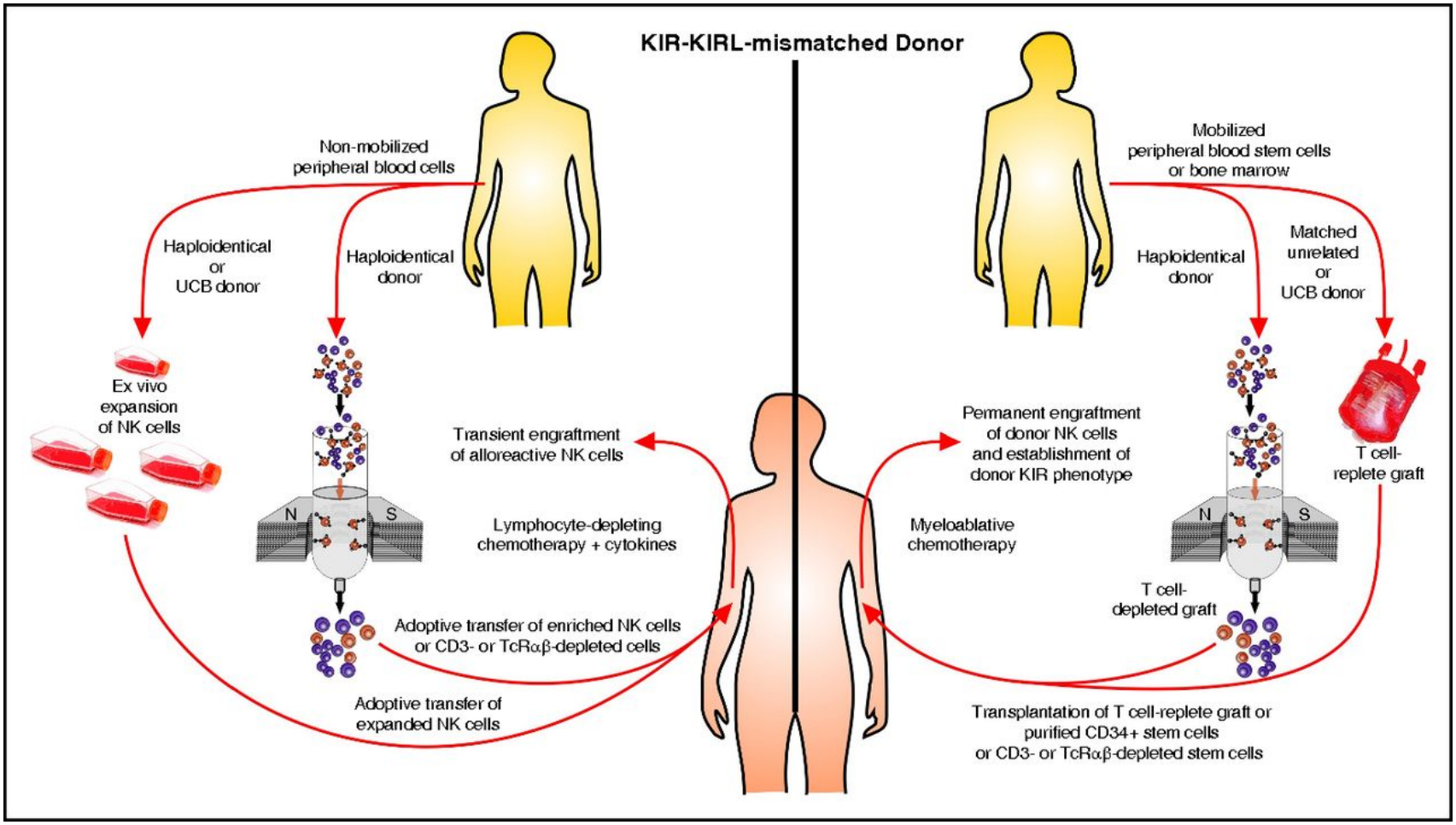


Ruggeri et al, Science 2002; Blood 2007

Clinical exploitation of alloreactive NK cells

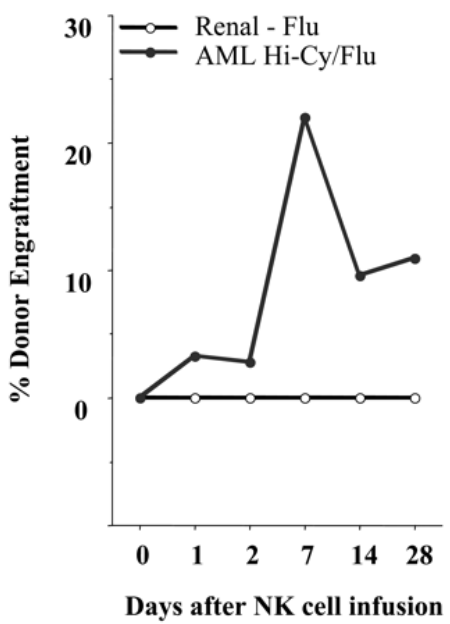
Adoptive immunotherapy

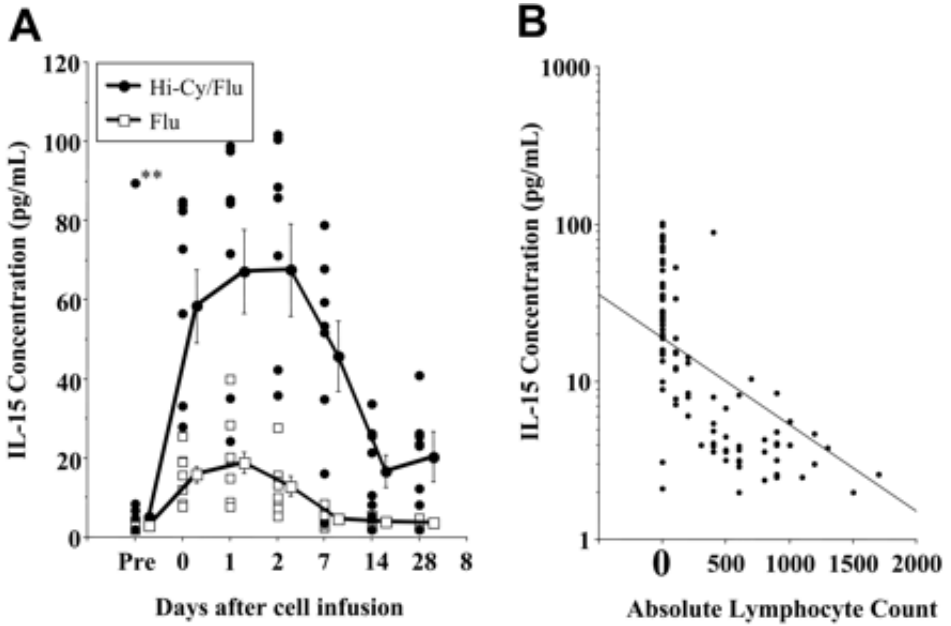
HSCT



Handgretinger et al. Blood 2016

Expansion of haploidentical NK cells after infusion into cancer patients

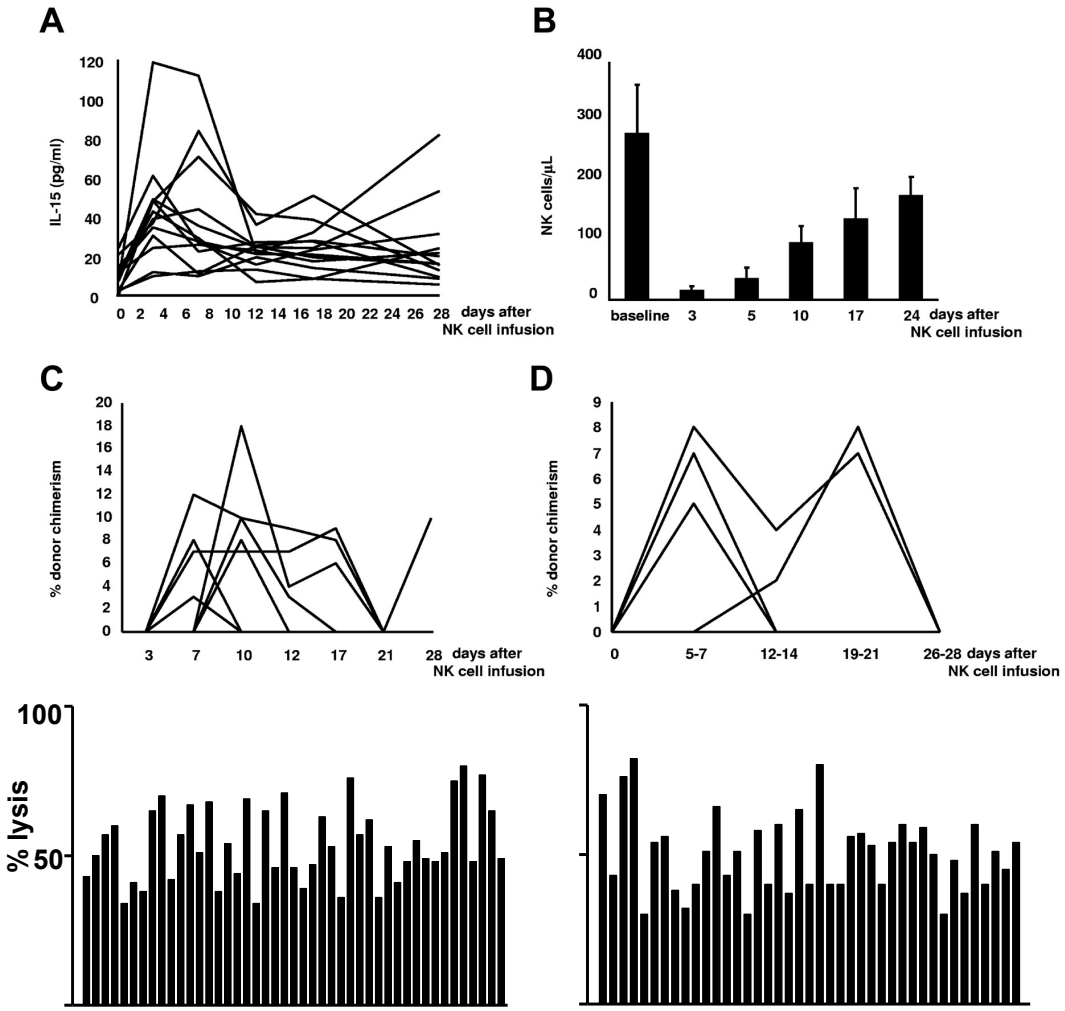




Five/19 poor-prognosis patients with AML achieved complete remission after infusion of partially purified haploidentical NK cells.

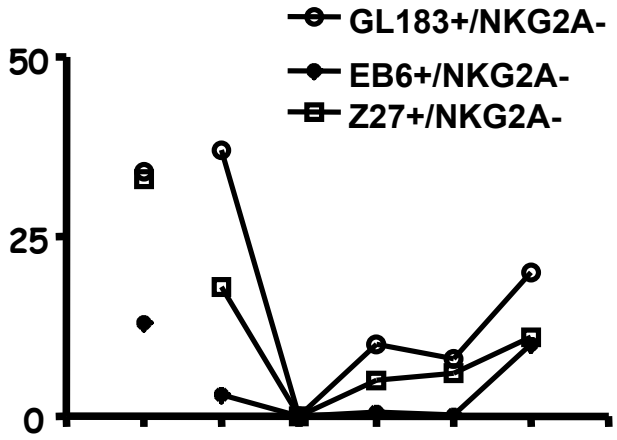
Miller et al. Blood 2005

Infused NK cells are alloreactive against AML

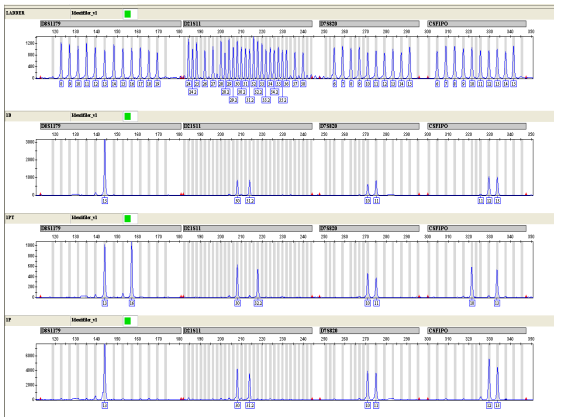


HLA-C1⁺ donor alloreactive
NK clones in C1 missing patients

HLA-C2⁺ donor alloreactive
NK clones in C2 missing patients

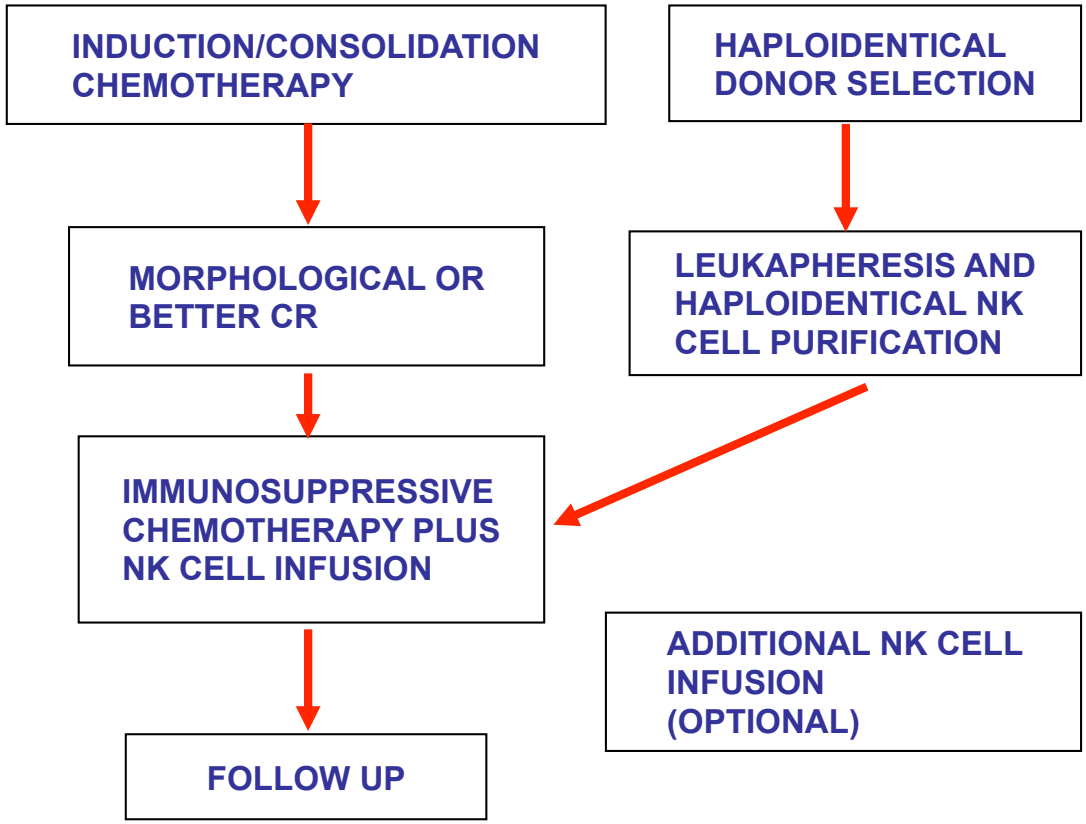


VNTR analysis



Curti et al. Blood 2011

Infusion of alloreactive NK cells into AML patients in CR



54 high risk AML patients were screened for the availability of one haploidentical KIR ligand mismatched donor

26 patients (48%) had one suitable donor.

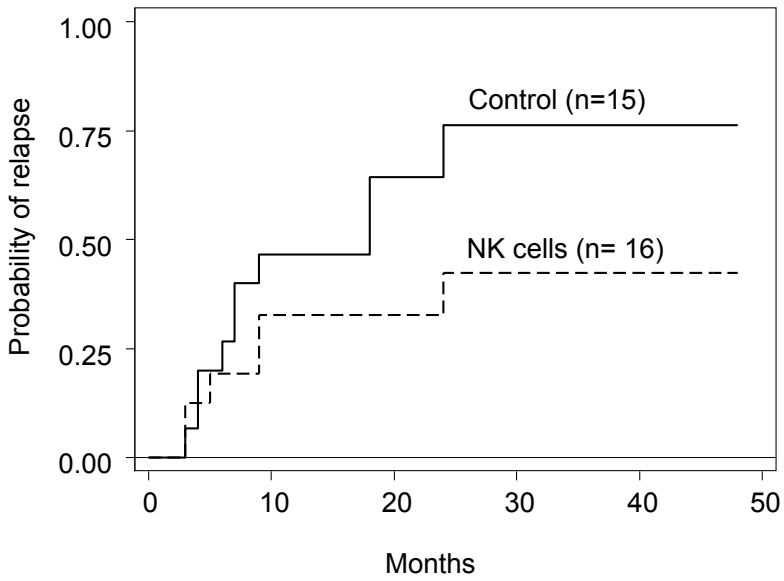
21 patients (38%) infused.

17 patients infused in CR

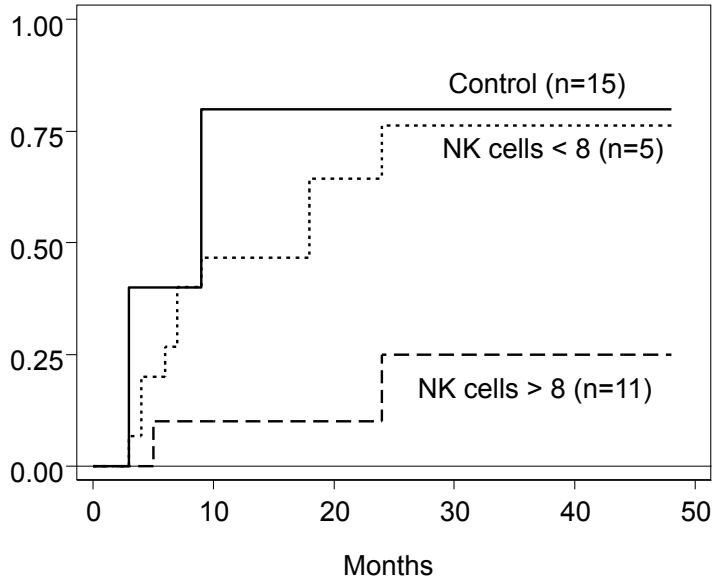
16 evaluable patients for clinical response

9 CR patients are disease-free after a median follow-up of 27 months

Larger NK alloreactivity is associated with reduced relapse



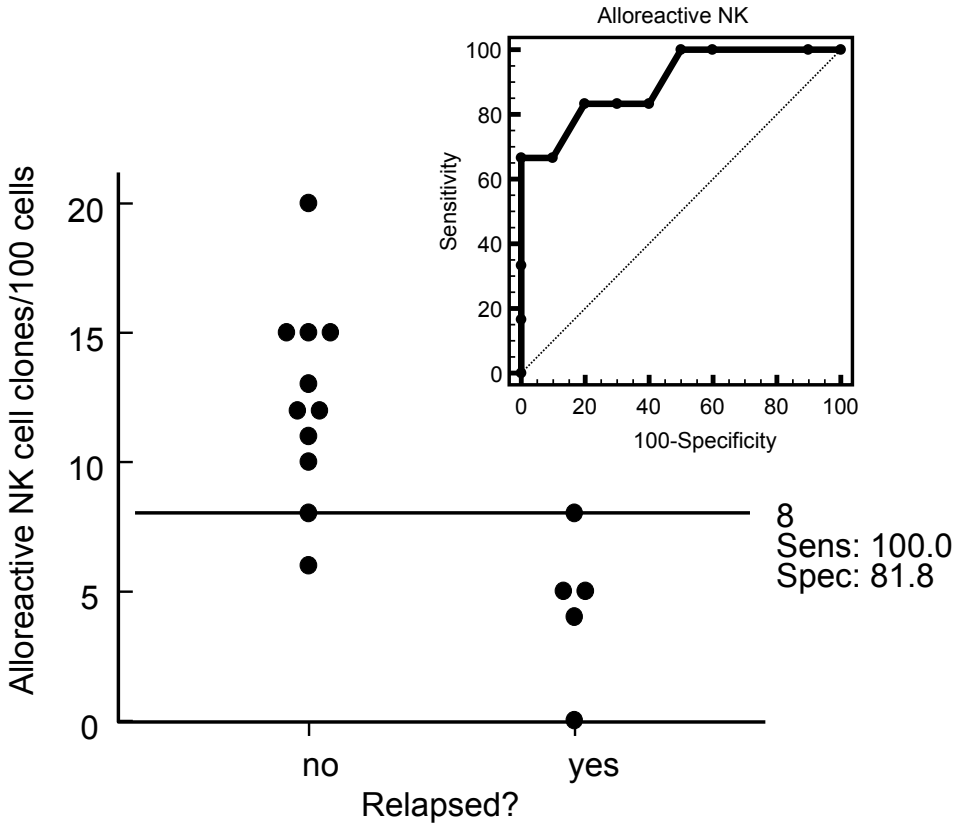
NK cells vs. control
HR 0.49 (95% 0.18-1.30)
P=0.138 Log Rank test

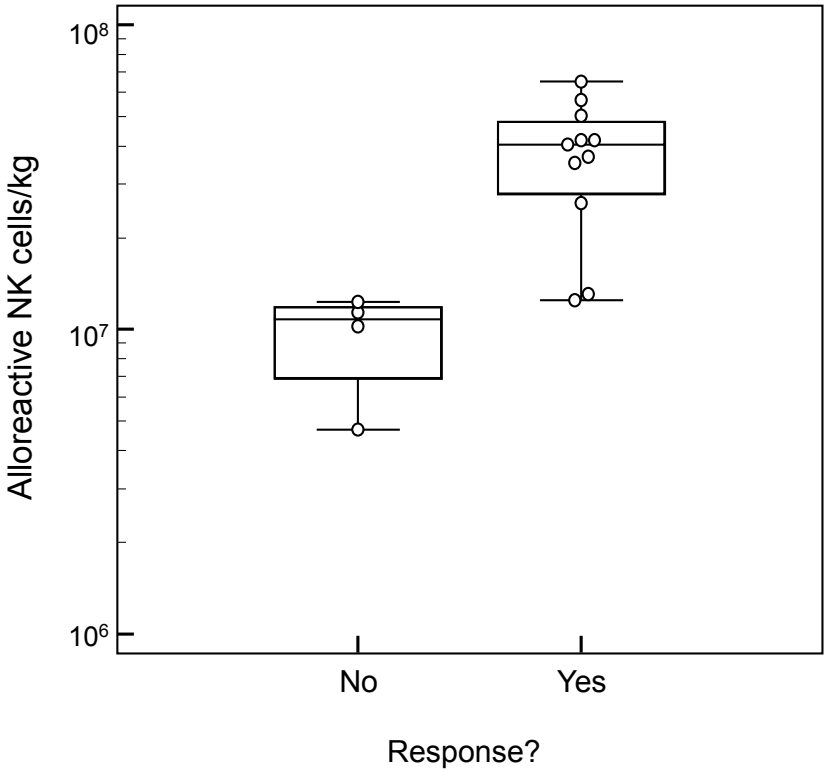


NK >8 vs. control
HR 0.15 (95% 0.03-0.70)
P=0.03 Log Rank test

Curti et al, Clin Cancer Res, 2016

A threshold of alloreactive NK cell clones is predictive for response





Curti et al, Clin Cancer Res, 2016

An algorythm for donor selection and cell processing based on NK functional dose

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

NK Cell Graft

NK Cells (10^6/kg)	T Cells (10^6/kg)	B Cells (10^6/kg)
38.7	ND	0.106
27.2	ND	1.700
31.1	ND	0.652
37.3	ND	0.148
80.9	ND	0.135
5.2	ND	0.007
7.3	ND	0.004
13.3	0.001	ND
47.7	ND	0.087
13.4	ND	0.082

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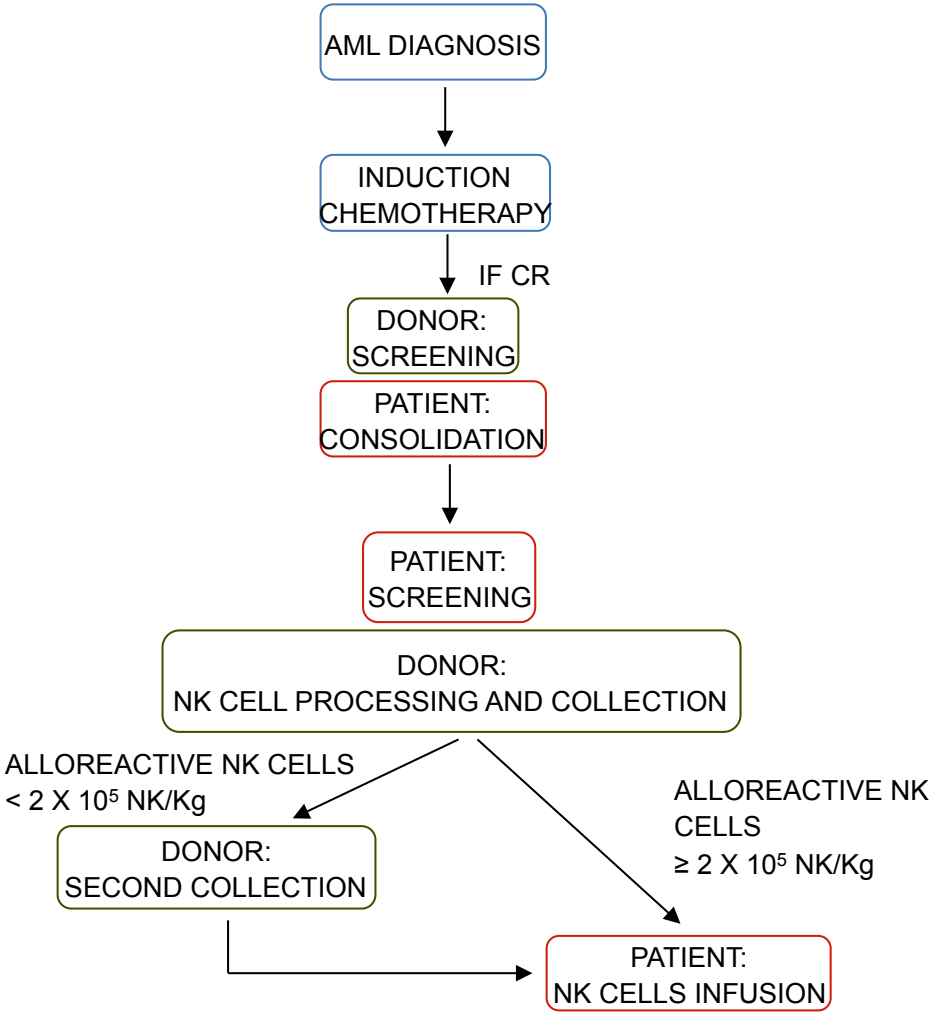
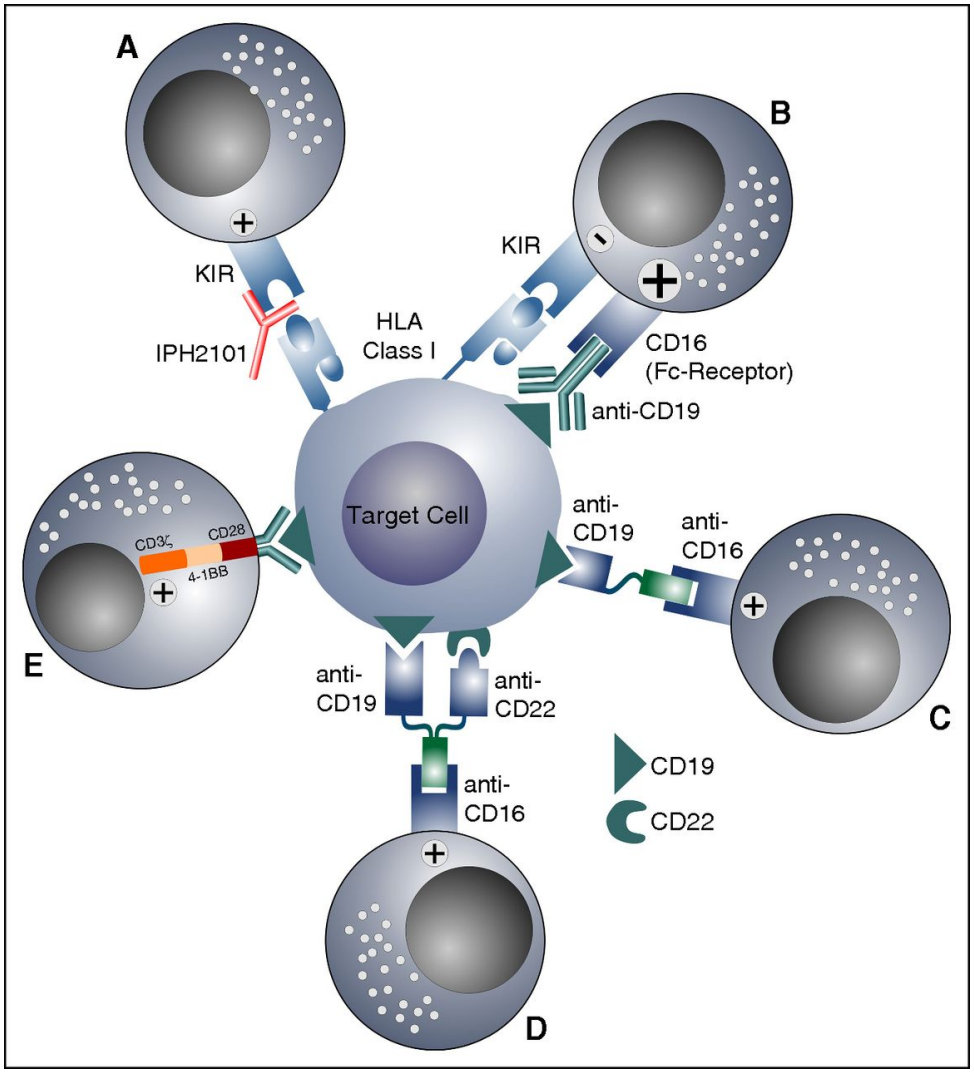


Lemoli & Curti, Exp Hematol, 2016;
Parisi et al, Frontiers in Immunology, 2017

Strategies to overcome the KIR-KIRL-mediated inhibition of NK cells



Handgretinger et al. Blood 2016

- Blocking KIR-KIRL interaction
- Activation of CD16 (Fc-receptor) on NK cells with an antibody directed against leukemic cells
- Bispecific and Trispecific killer engagers activate NK cells via the Fc-receptor against leukemia cells
- CAR-NK cells directed against leukemia antigens

Personal comments for discussion

- The results from early safety studies have clearly paved the way for designing a new generation of efficacy clinical studies exploring the real impact of novel immunological agents, including cell-therapies, in the management of AML

- However, biological issues still need full elucidation and clinical correlation

- The combination of immunotherapies with conventional anti-leukemia drugs, including chemotherapy and hypomethylating drugs, is promising to fully exploit the immunogenic potential of both strategies and tune their application

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