Nuove frontiere nella terapia delle malattie oncologiche ed oncoematologiche
Treviso, 20-21 Novembre 2015

Meccanismi di interazione tra il microambiente midollare, il sistema immune e le cellule leucemiche

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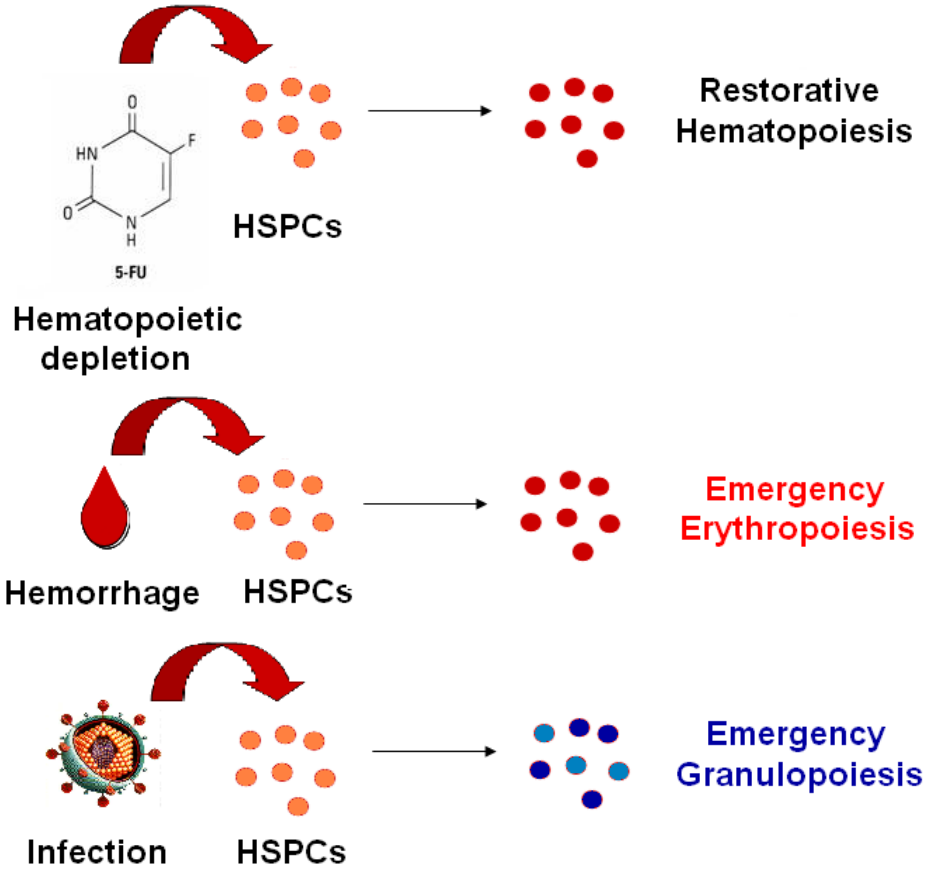
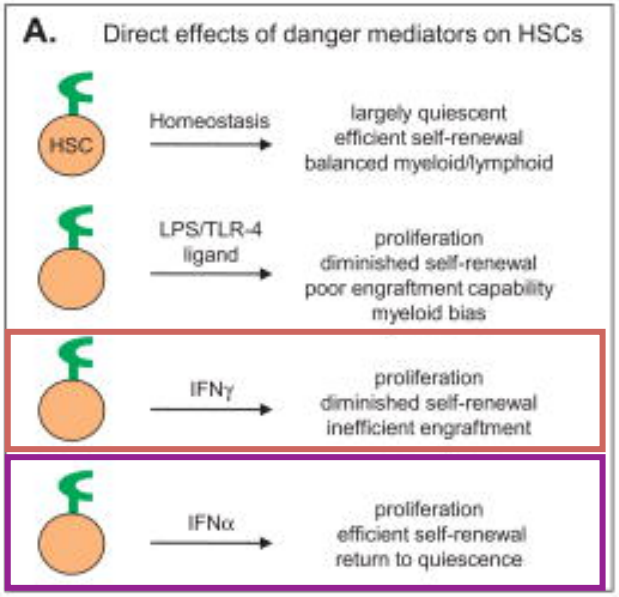
IRCCS A.O.U. S. Martino-IST, Genova

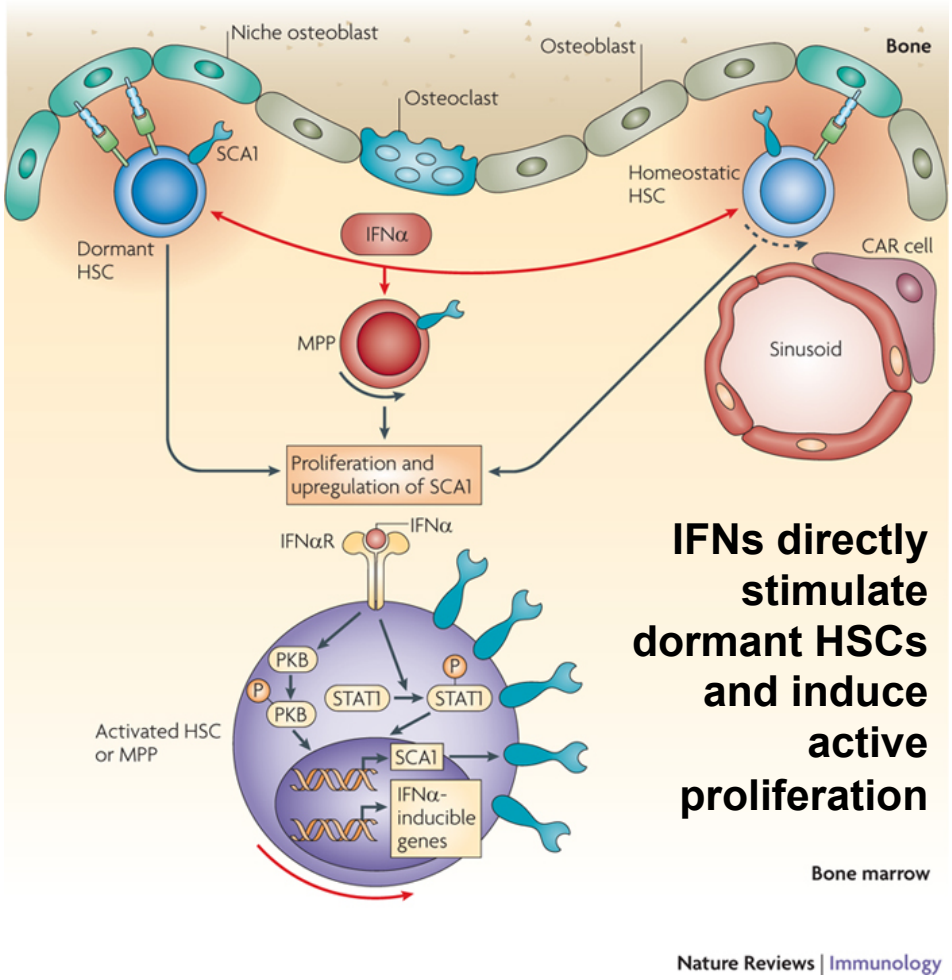
HSPC RESPONSE to HEMATOPOIETIC STRESS



HSPC response to Inflammatory Cytokines



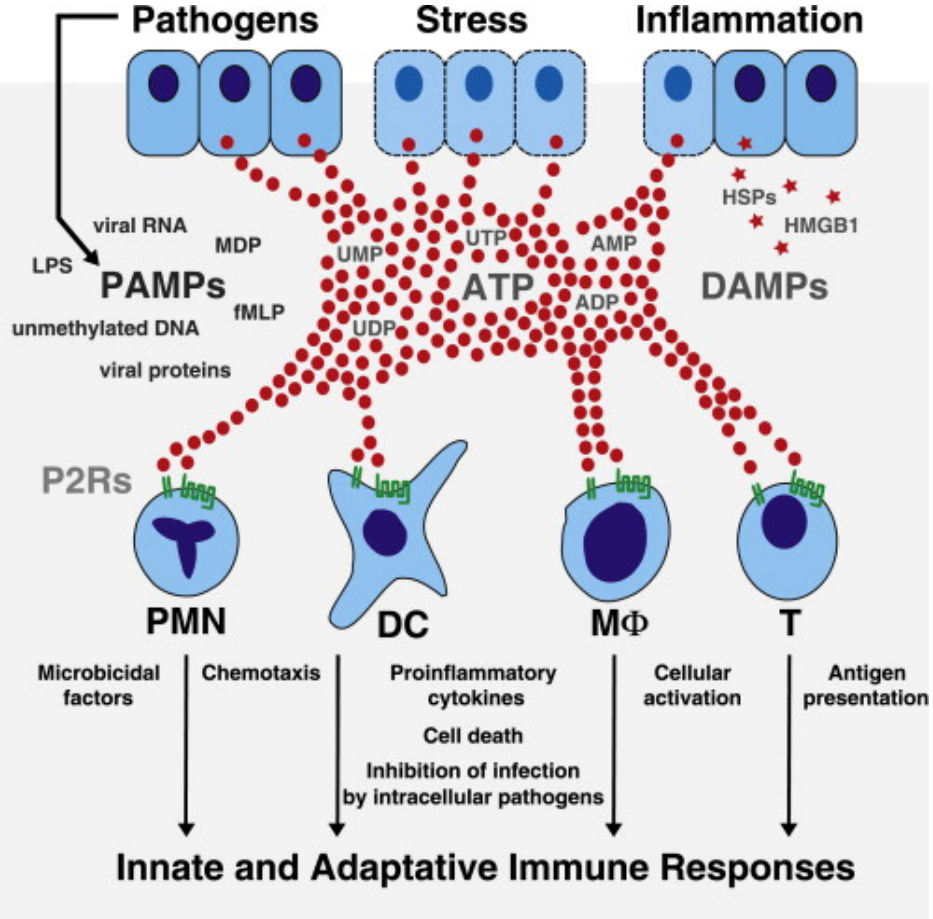
HSCs do not only respond to the depletion of PB cells: *the HSC compartment can react directly to inflammatory cytokines*



Essers MAG *et al. Nature* 2009
Trumpp A. *et al., Nat Rev Immunol* 2010

PAMPs, DAMPs, and Alarmins

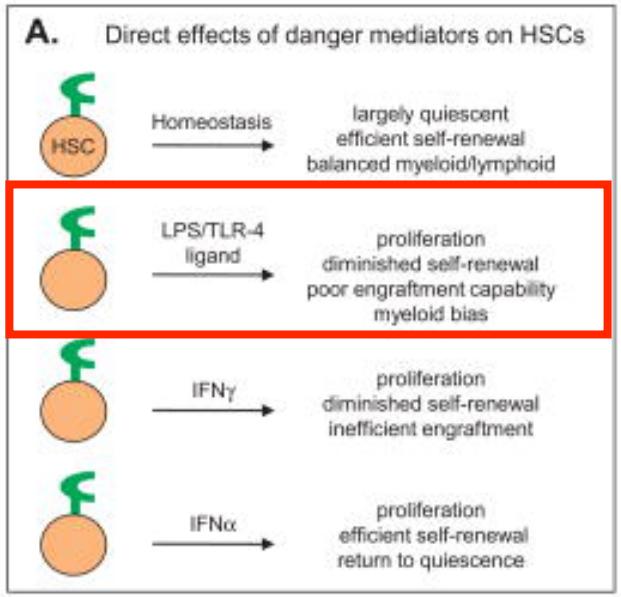
PRRs: Pathogen Related Receptors



P2Rs
TLRs
RIG-I-like Receptors
NOD-like Receptors
AIM2-like Receptors

Mostly expressed by immune cells (Monocytes, Macrophages, Neutrophils, and DCs)

HSPCs respond to Alarmins



Immunity 24, 801–812, June 2006 ©2006 Elsevier Inc. DOI 10.1016/j.immuni.2006.04.008

Toll-like Receptors on Hematopoietic Progenitor Cells Stimulate Innate Immune System Replenishment

Yoshinori Nagai,[1,3] Karla P. Garrett,[1] Shoichiro Ohta,[2] Uleng Bahrun,[2] Taku Kouro,[3] Shizuo Akira,[4] Kivoshi Takatsu.[3] and Paul W. Kincade[1,*]

The Journal of Immunology

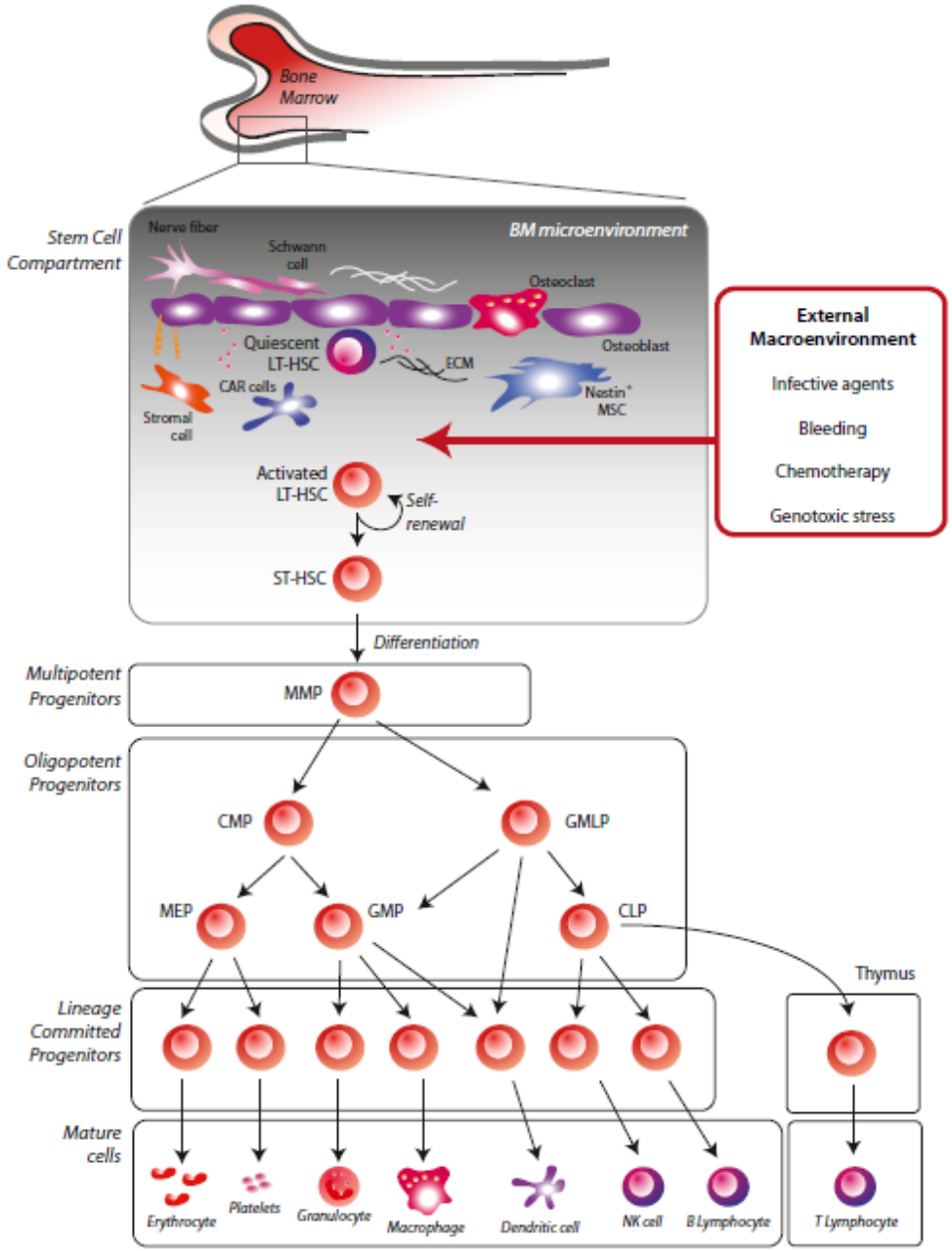
The Journal of Immunology, 2011, 186: 5367–5375.

Chronic Exposure to a TLR Ligand Injures Hematopoietic Stem Cells

Brandt L. Esplin,*,†,1 Tomoyuki Shimazu,*,1 Robert S. Welner,* Karla P. Garrett,* Lei Nie,* Qingzhao Zhang,* Mary Beth Humphrey,‡,§ Qi Yang,¶ Lisa A. Borghesi,¶ and Paul W. Kincade*

HSCs respond directly to stress- and pathogen-related molecules, sensing damage even before inflammatory cytokines are released

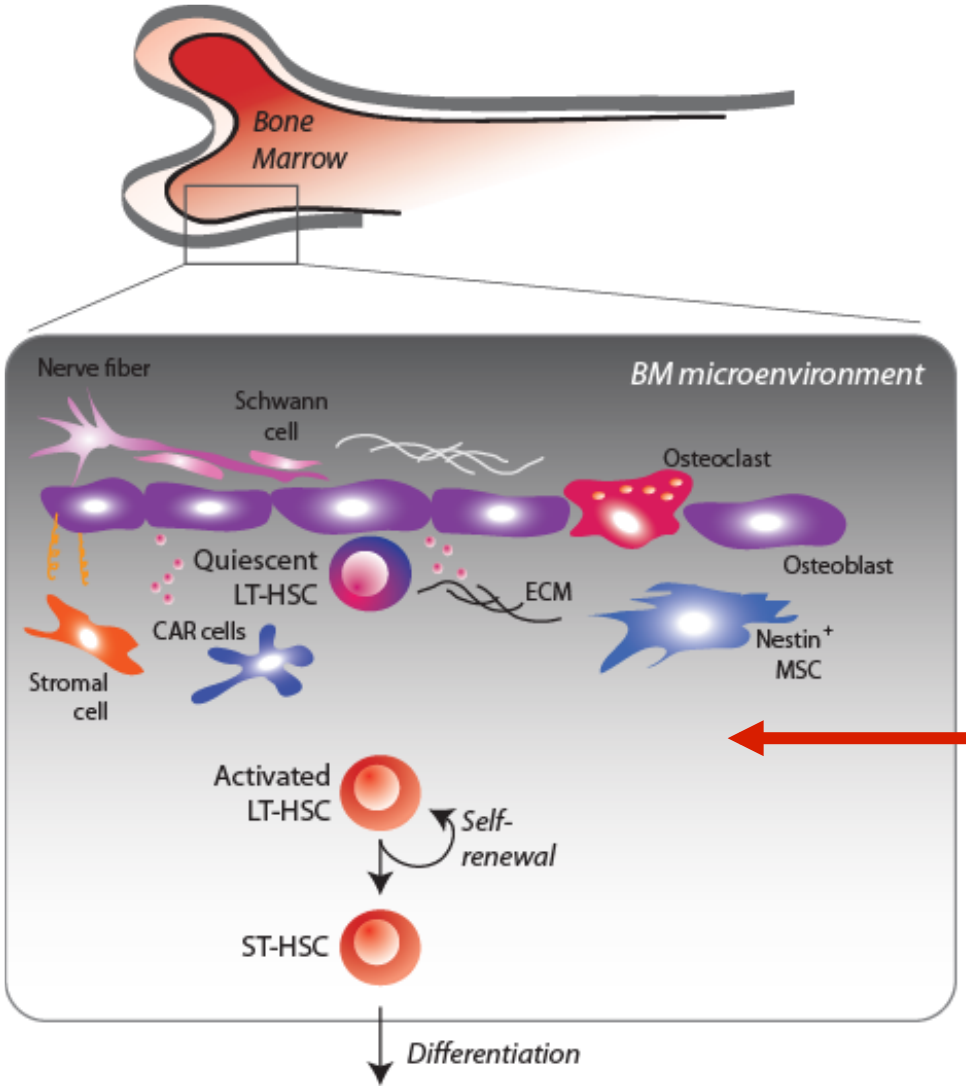
Hematopoietic Stem Cells (HSCs)



Rossi L. et al. *Cell Stem Cell* 2012

The network created by PAMPs, DAMPs, Alarmins, and PRRs **transversally affects the whole hematopoietic system**, from terminally differentiated cells, up to the compartment of immature HSCs

HSCs and BM niche: The end of a «splendid isolation»



Pro-inflammatory Factors/Alarmins

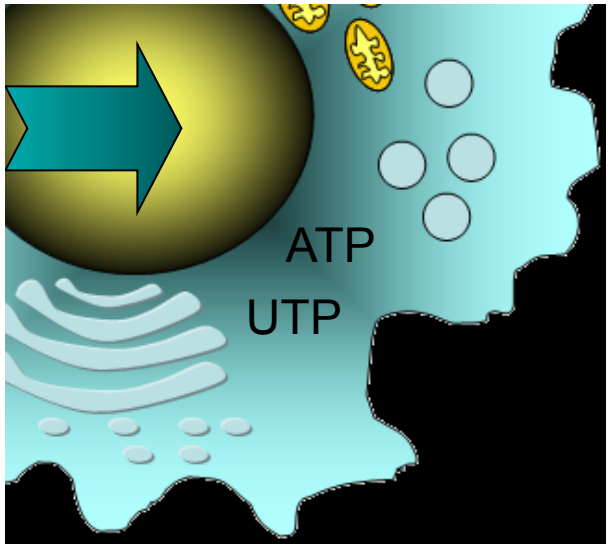
TNFα

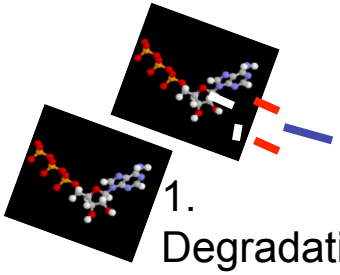
Interferons

Extracellular NTPs

TLR ligands

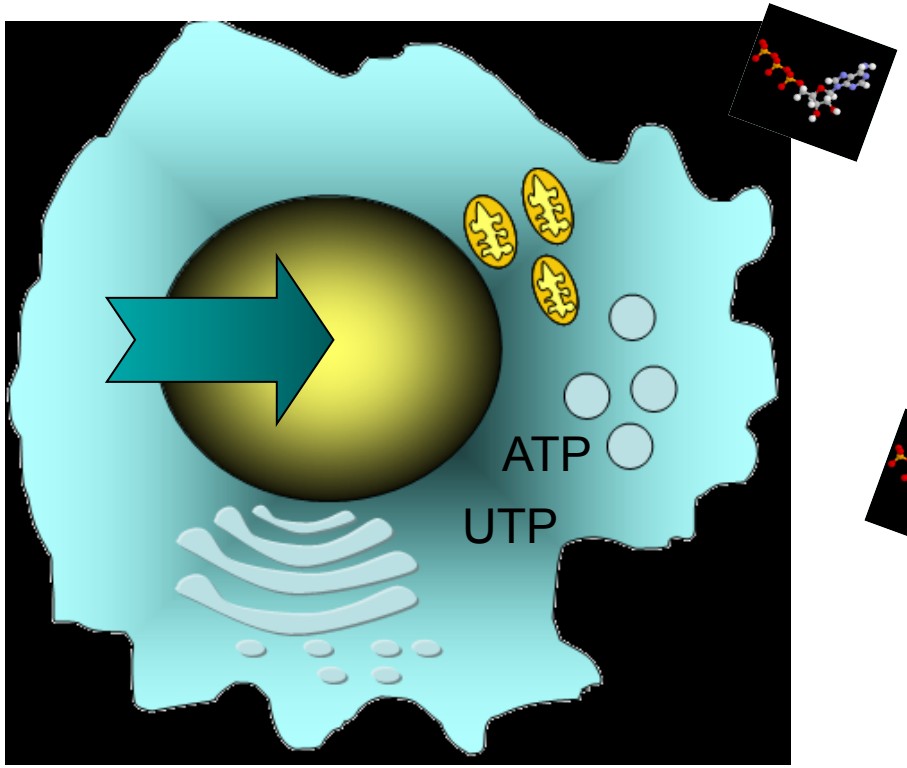
EXTRACELLULAR NUCELOTIDES

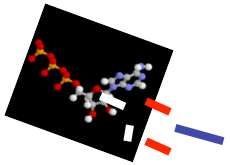




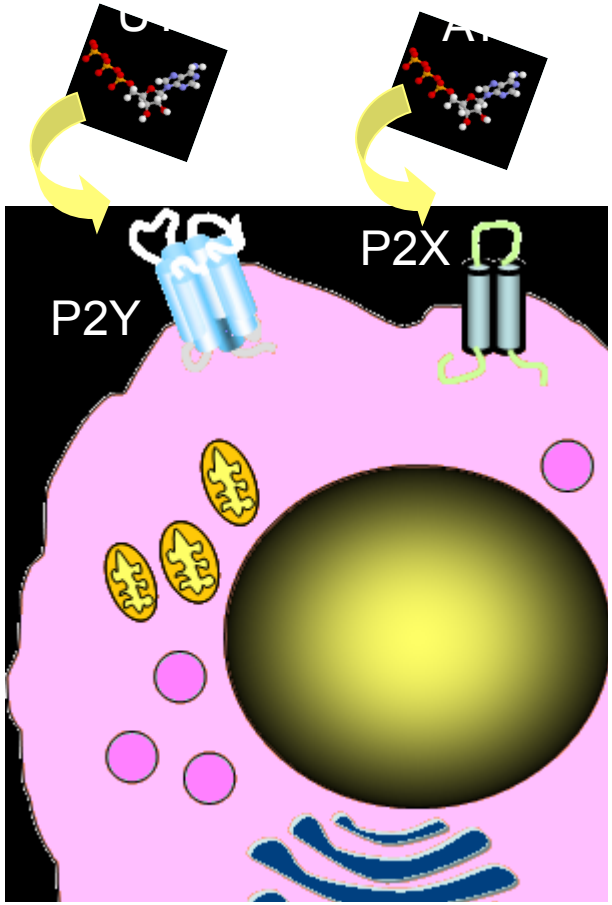
1.
Degradation

2.
P2 receptors
(P2Rs) activation

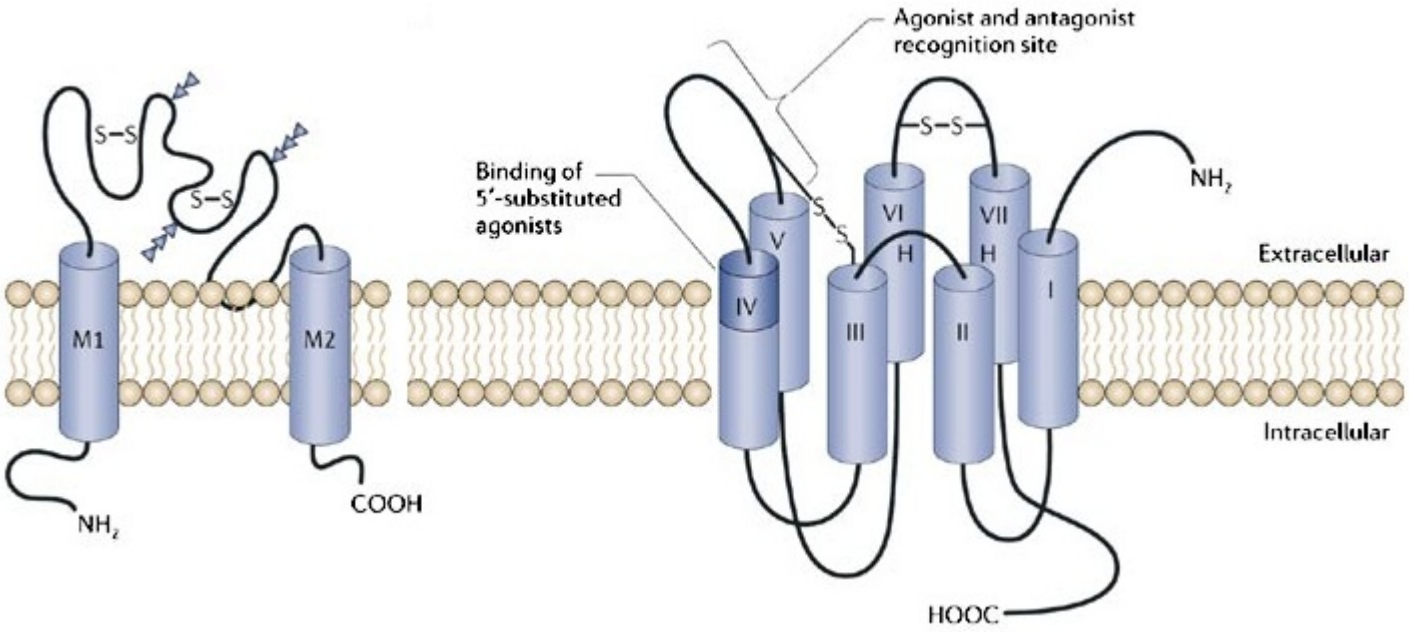




1.
Degradation



Purinergic Receptors



P2XR

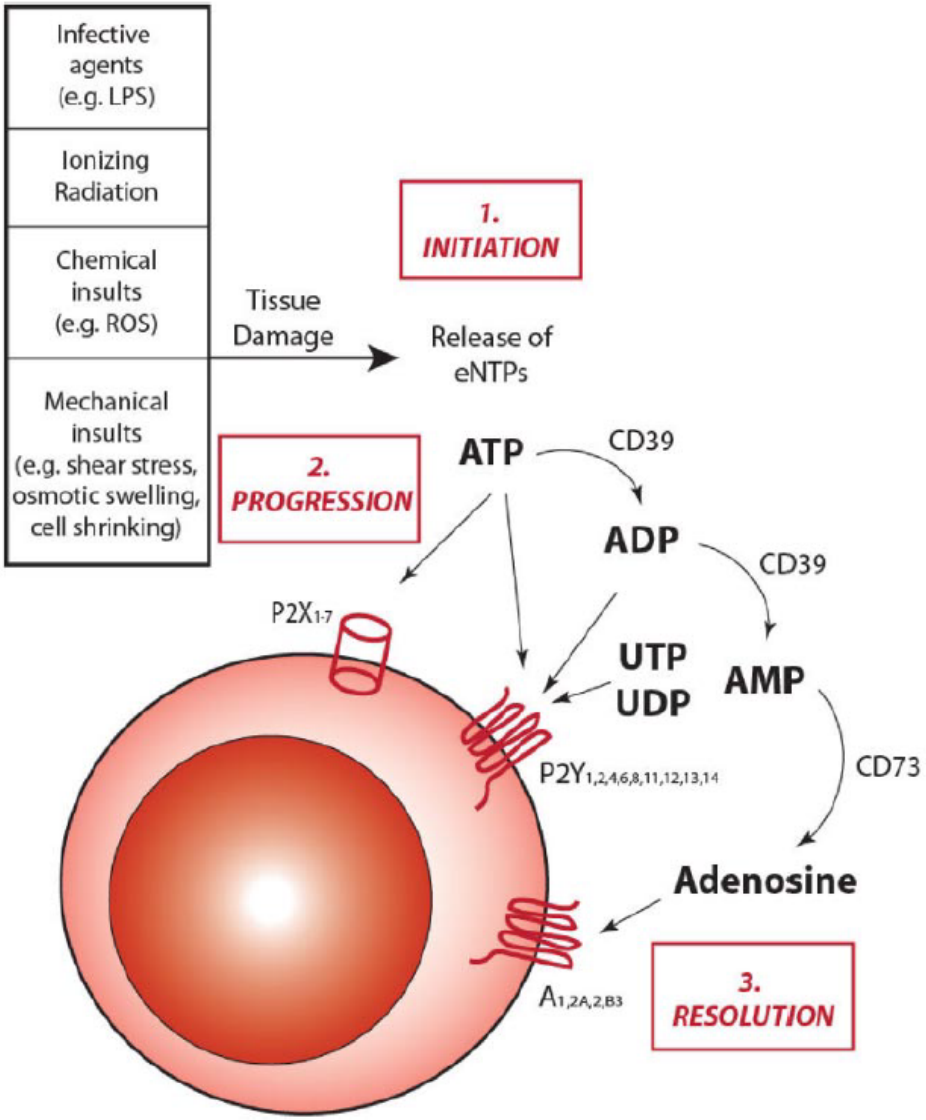
$P2X_1$ $P2X_2$ $P2X_3$ $P2X_4$
$P2X_5$ $P2X_6$ $P2X_7$

P2YR

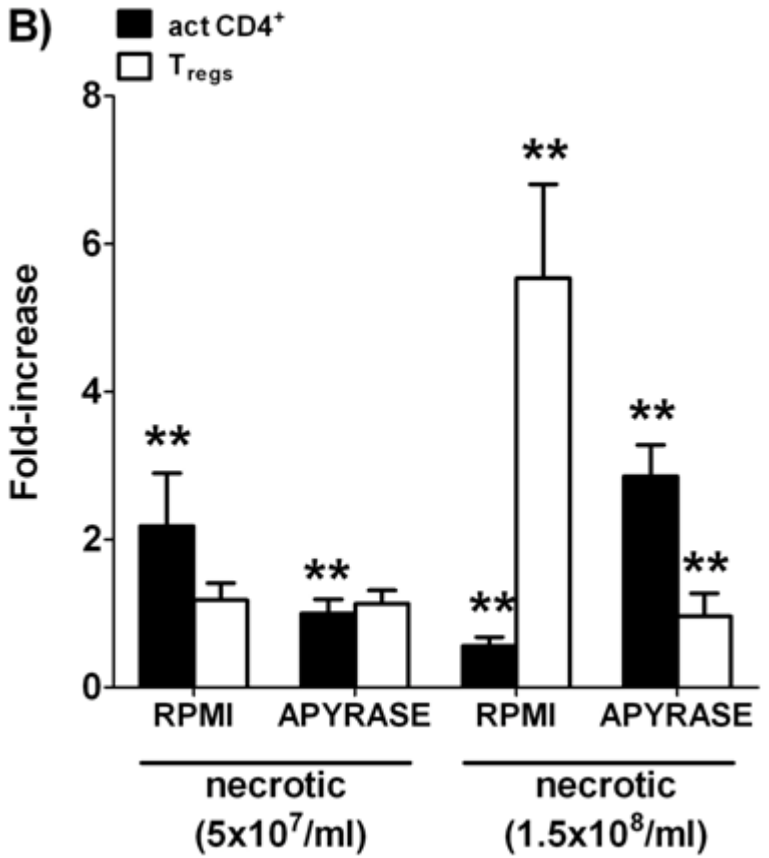
$P2Y_1$ $P2Y_2$ $P2Y_4$ $P2Y_6$
$P2Y_{11}$ $P2Y_{12}$ $P2Y_{13}$ $P2Y_{14}$

Nature Reviews Neuroscience 2006; modified

eNTPs and Inflammation

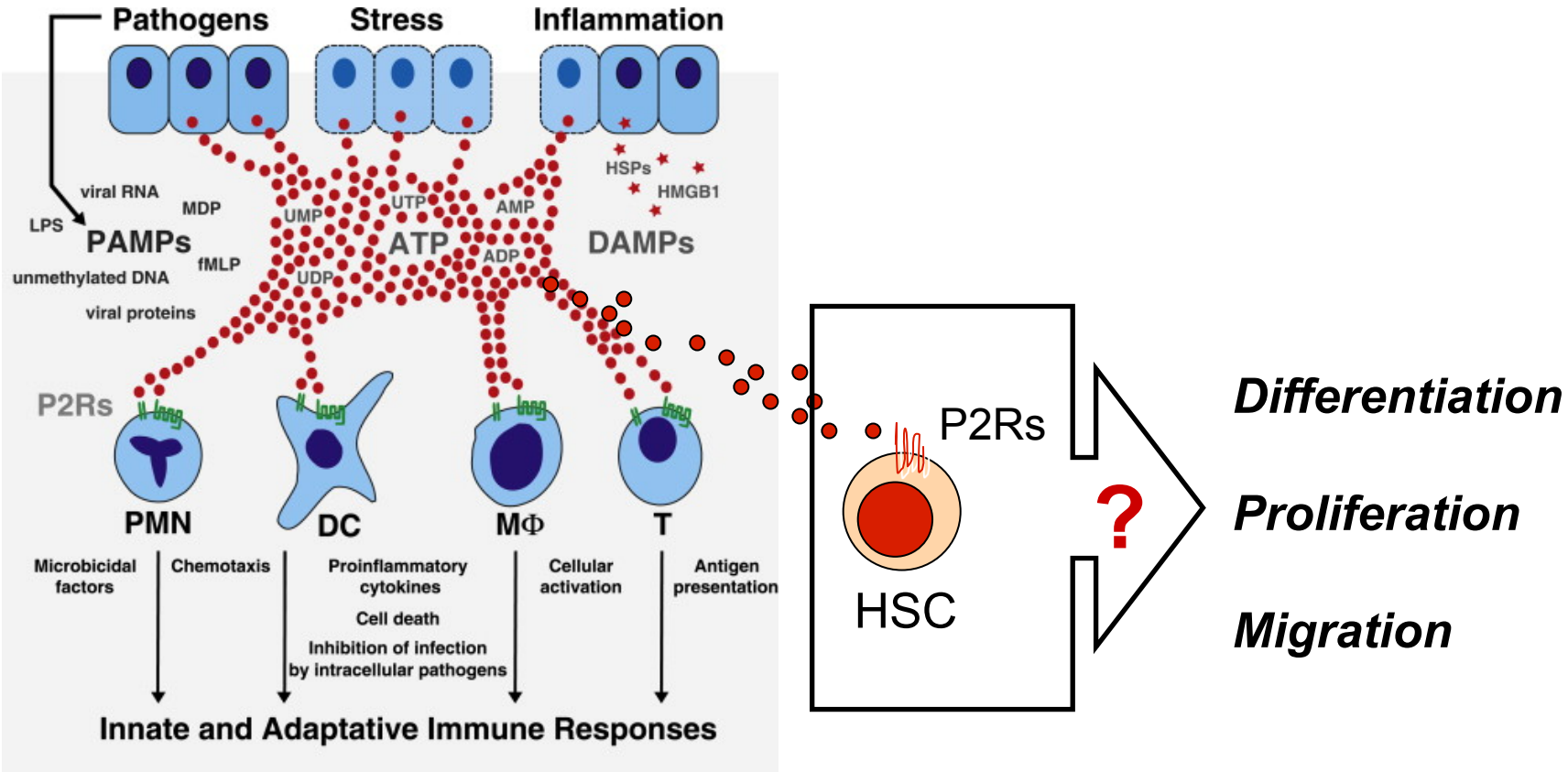


Extracellular nucleotides play a key role in *tuning* **inflammation**, **danger** and **tissue damage signals**



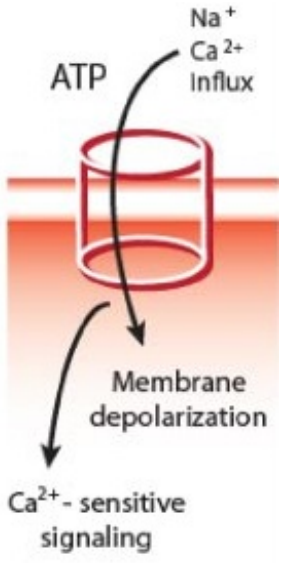
Trabanelli S et al., J Immunol 2012
Rossi L. et al. , Blood 2012

What role do eNTPs play in the HSC/LSC compartment?

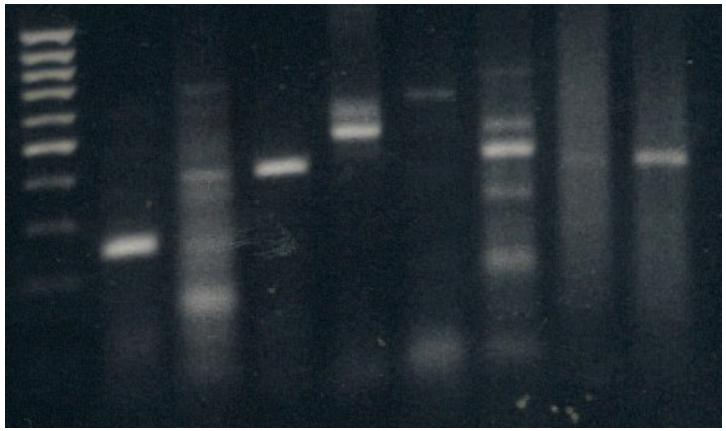


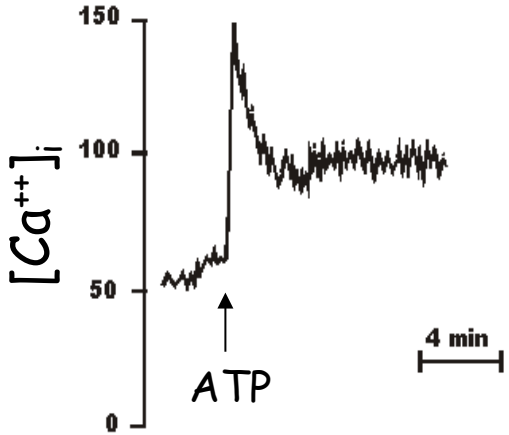
P2Rs AND PURINERGIC SIGNALLING: Expression Profile in CD34+ HSPCs

P2XRs

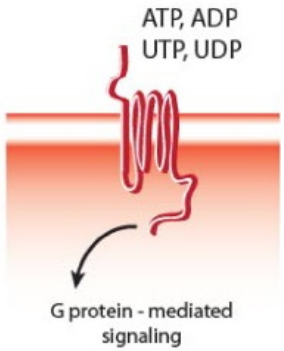


$P2X_1$ $P2X_2$ $P2X_3$ $P2X_4$ $P2X_5$ $P2X_6$ $P2X_7$ $P2X_7$

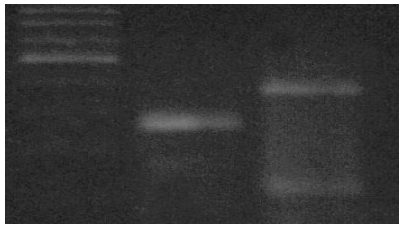


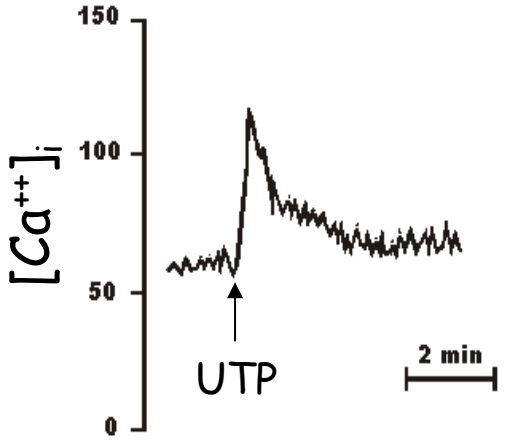


P2YRs



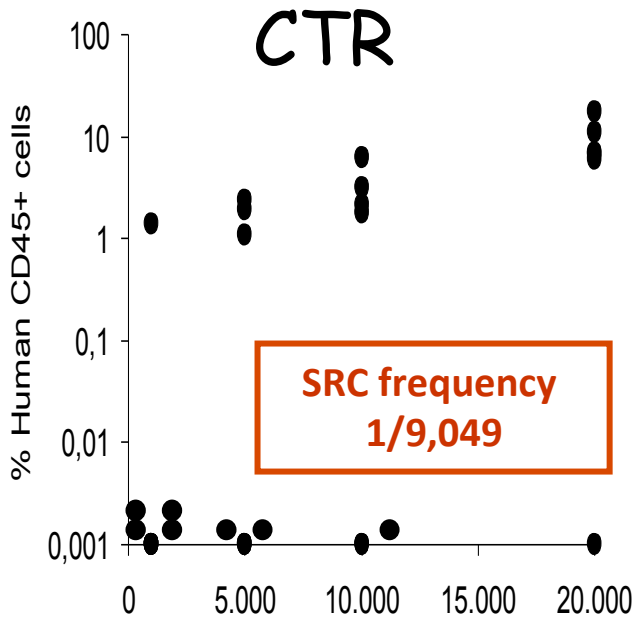
$P2Y_1$ $P2Y_2$

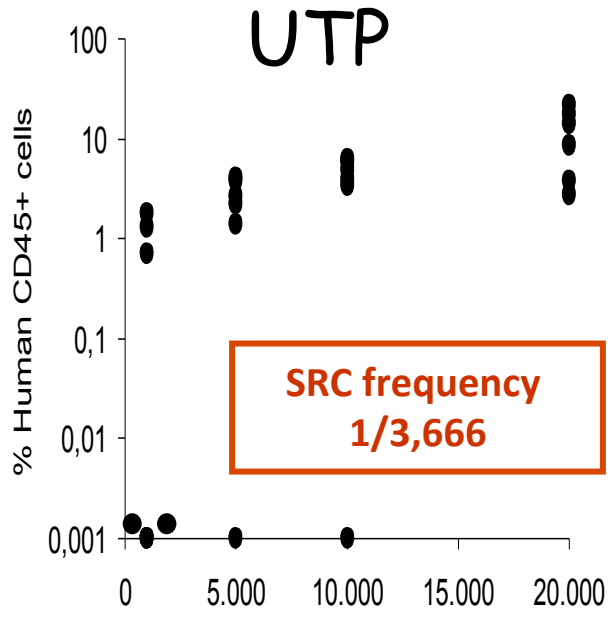




Lemoli RM et al. *Blood* 2004;
Rossi L et al. *Blood* 2012

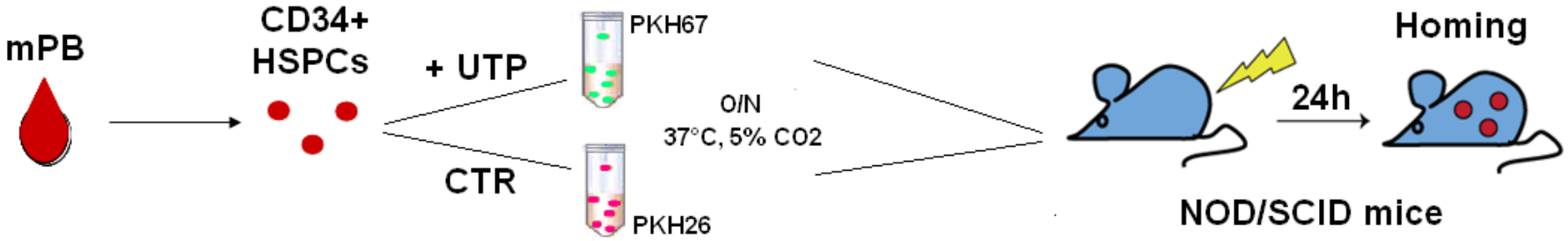
UTP expands SRCs in NOD/SCID mice

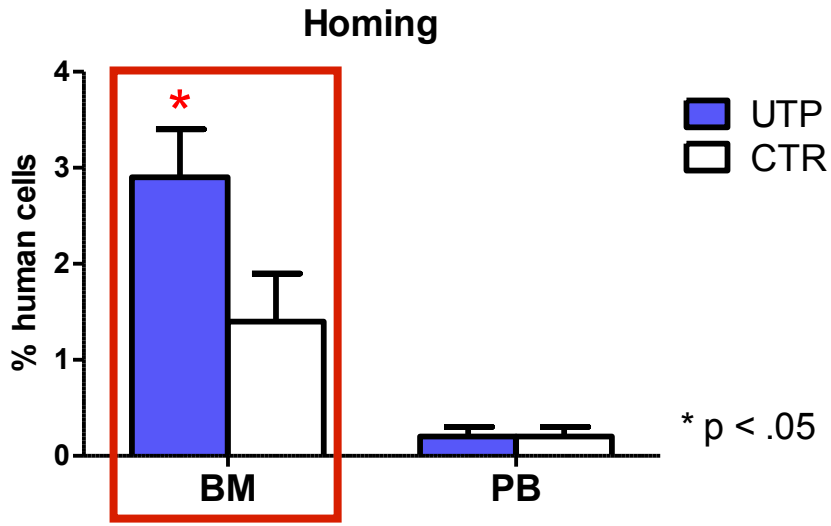




Lemoli RM et al. *Blood* 2004

Competitive repopulation assay in NOD/SCID mice



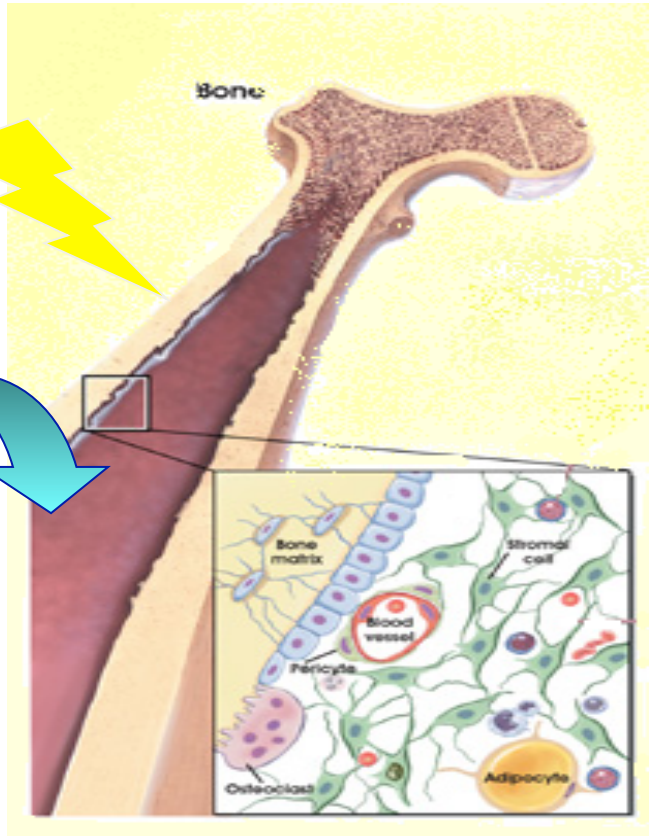


Rossi et al.; *Blood* 2007

HSC HOMING, PROLIFERATION AND FLOGOSIS

Radiation

CY, 5-FU



INFLAMMATORY FACTORS

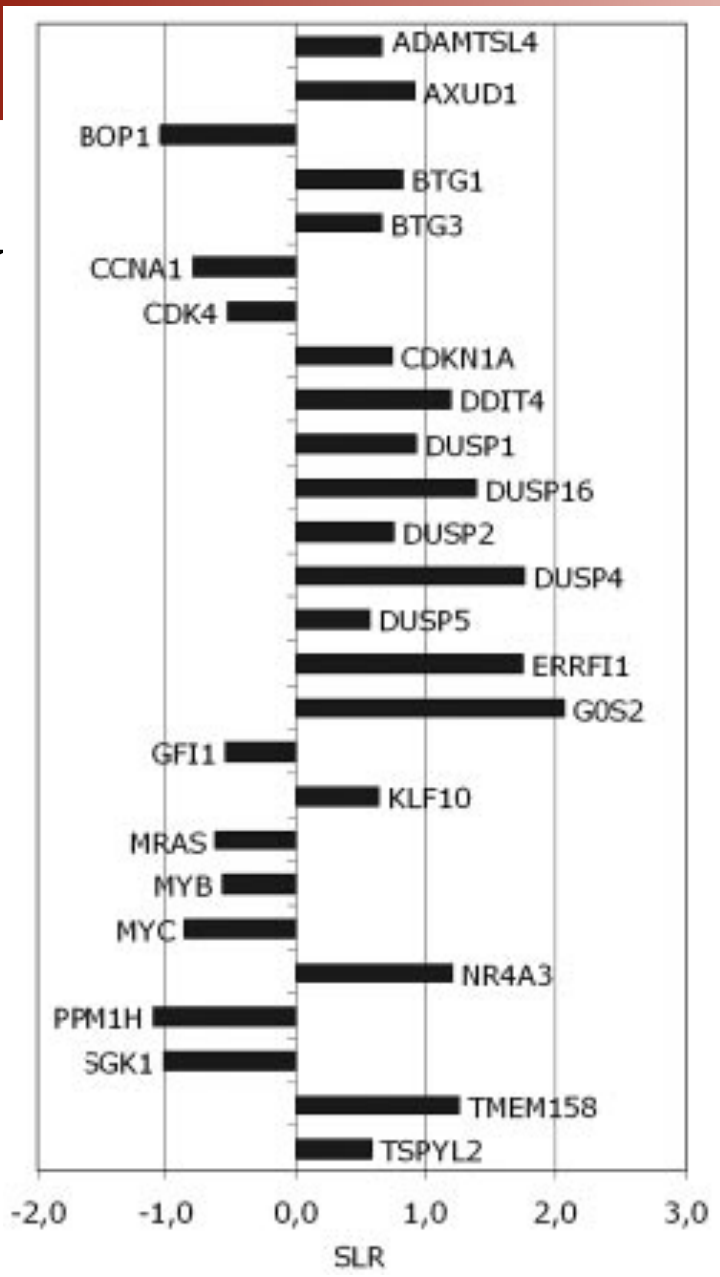
IL-1α IL-6
TNF-α
eNTPs

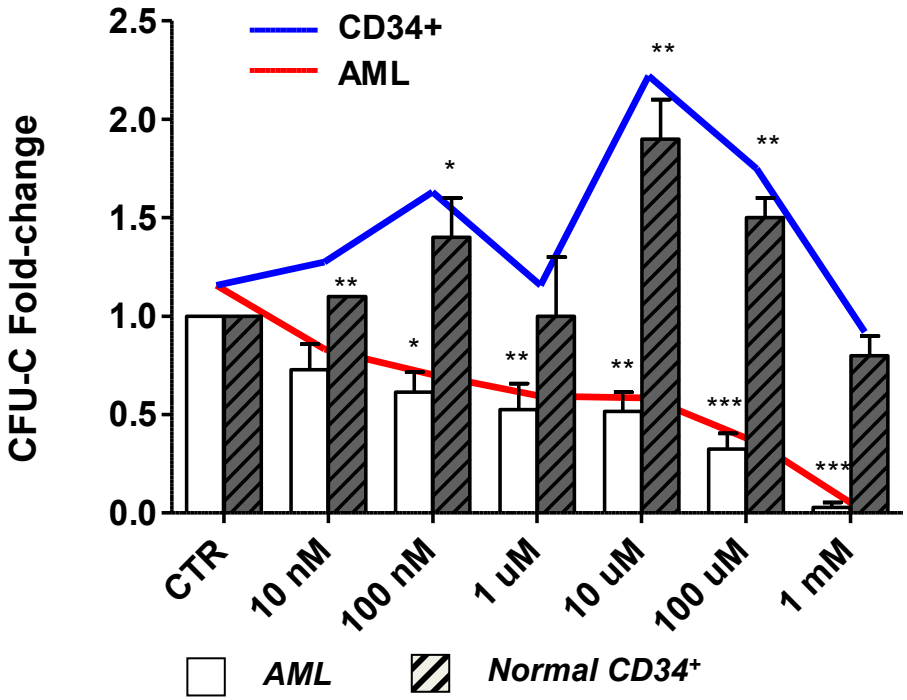
CHEMOTACTIC FACTORS

CXCL-12/CXCR4

Promotion of HSC homing, BM engraftment and proliferation

eNTPs inhibit AML cell proliferation

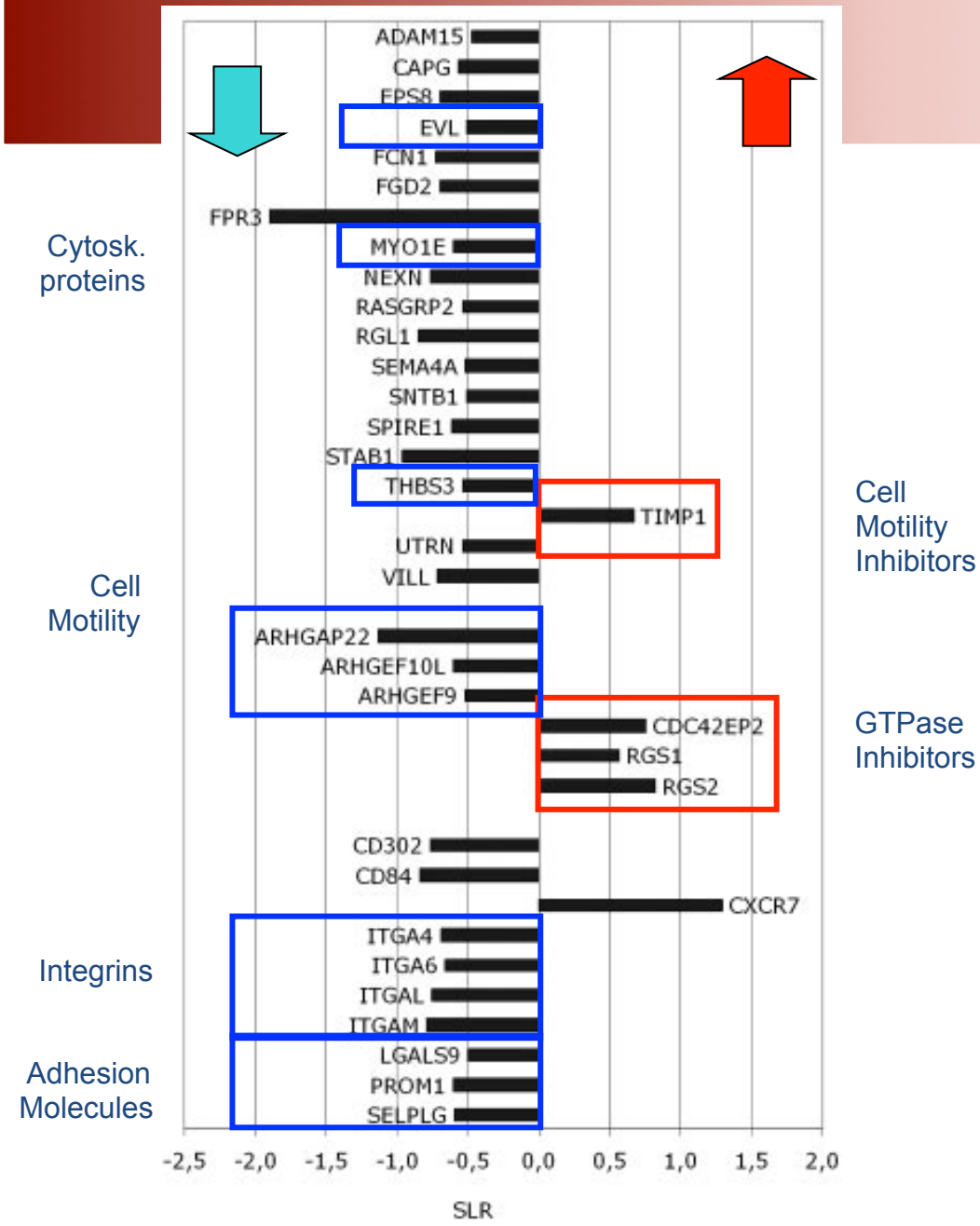


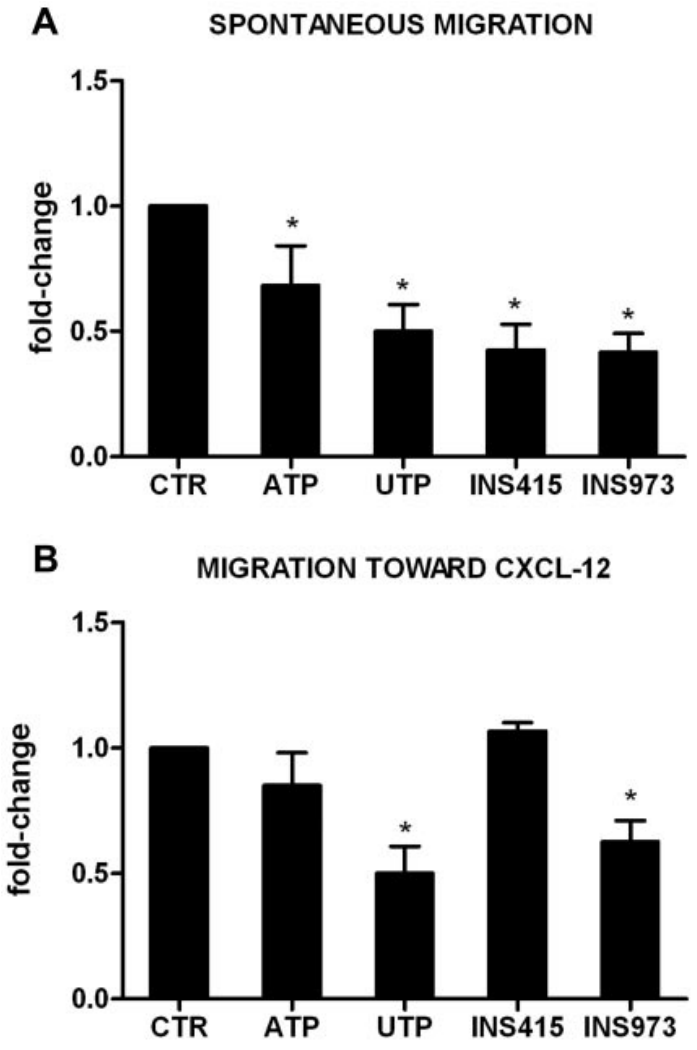


eATP increases CD34+ clonogenic potential,
however
it inhibits AML-derived CFU-L expansion

Salvestrini V et al, *Blood* 2012

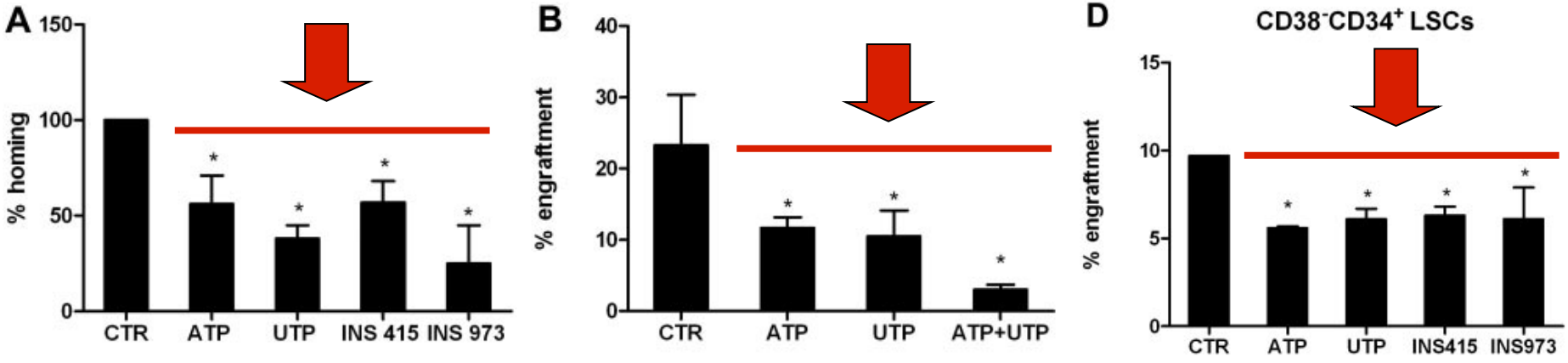
eNTPs inhibit AML cell migration





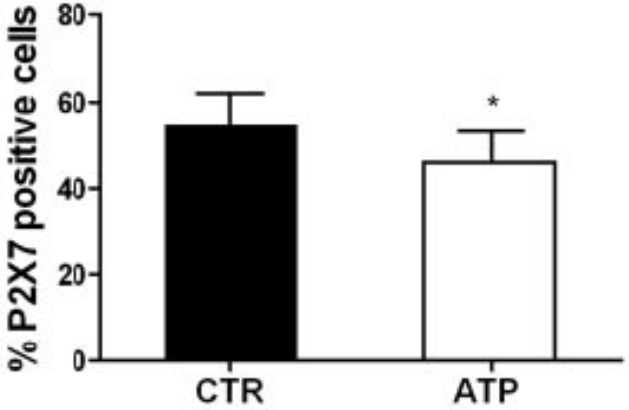
Salvestrini V et al, *Blood* 2012

Purinergic signaling inhibits AML cell Homing and Engraftment



eATP inhibits:

> AML cell homing
> AML cell engraftment
> AML LSC engraftment



Salvestrini V et al. *Blood* 2012

blood
2012 119: 217-226
doi:10.1182/blood-2011-07-370775 originally published online November 3, 2011

Purinergic signaling inhibits human acute myeloblastic leukemia cell proliferation, migration, and engraftment in immunodeficient mice

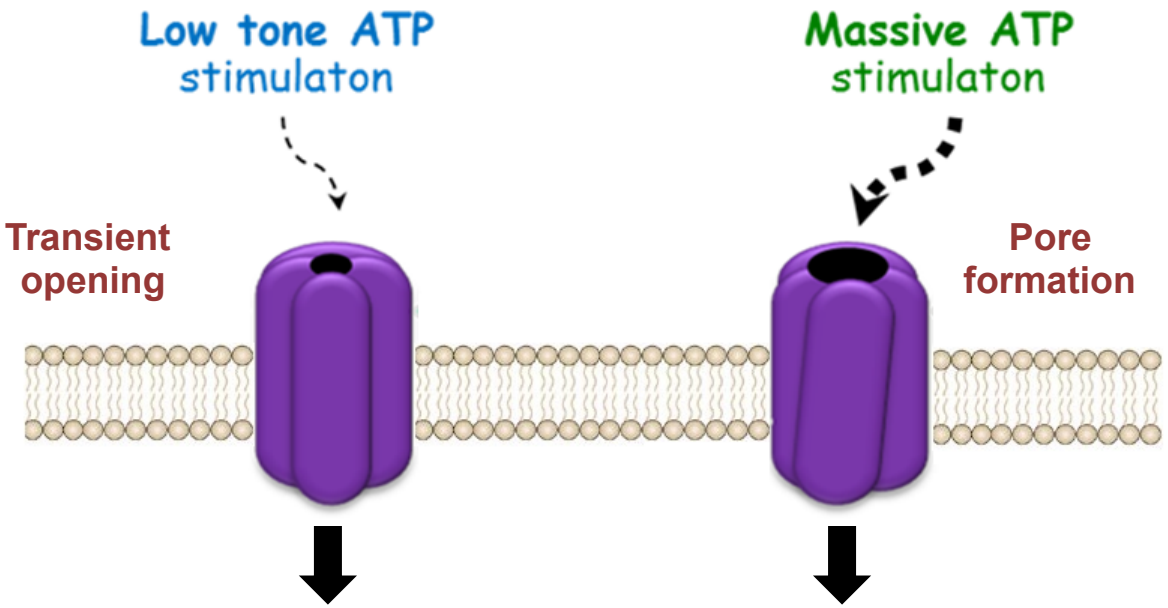
Valentina Salvestrini, Roberta Zini, Lara Rossi, Sara Gulinelli, Rossella Manfredini, Elisa Bianchi, Wanda Piacibello, Luisa Caione, Giorgia Migliardi, Maria Rosaria Ricciardi, Agostino Tafuri, Marco Romano, Simona Salati, Francesco Di Virgilio, Sergio Ferrari, Michele Baccarani, Davide Ferrari and Roberto M. Lemoli

Extracellular Nucleotides

Normal HSCs		Leukemic stem cells
↑	Proliferation	↓
↑	Migration	↓
↑	Homing	↓
↑	Engraftment	↓

Possible therapeutic use?

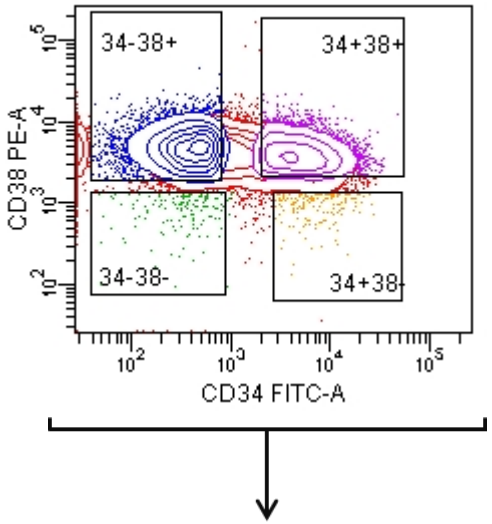
P2X7 receptor: “Death or life?”

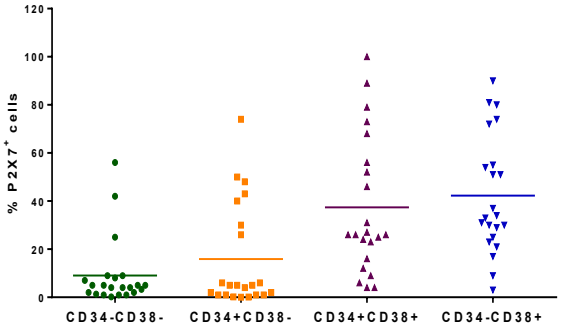


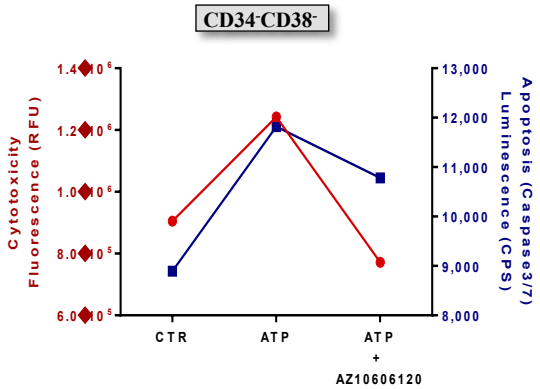
- Cell fusion
- ATP release
- **Proliferation**

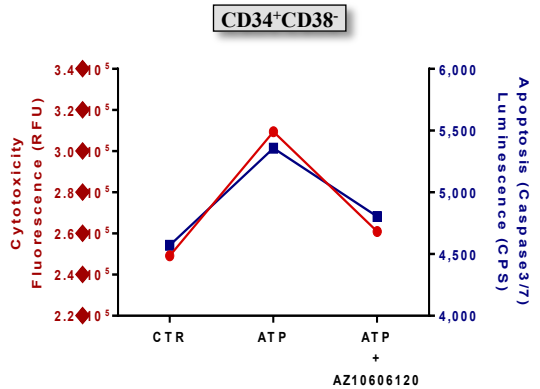
- Trascription factor activation
- Cytokine release
- **Cell death/ Apoptosis**

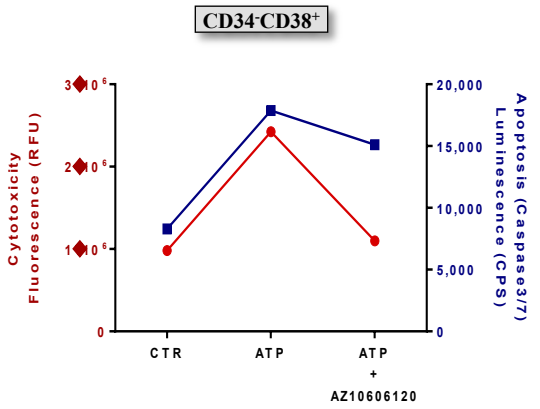
LSCs express P2X7 and its activation by ATP induces apoptosis of leukemic stem cell progenitors

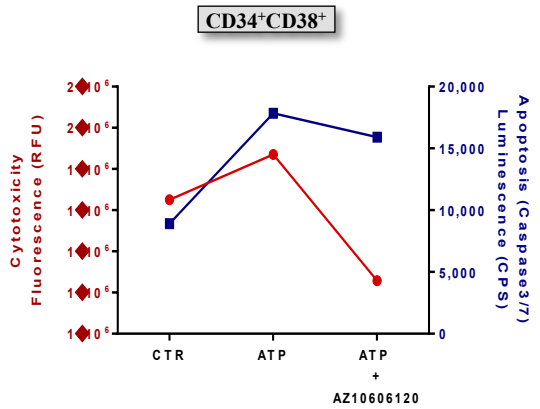




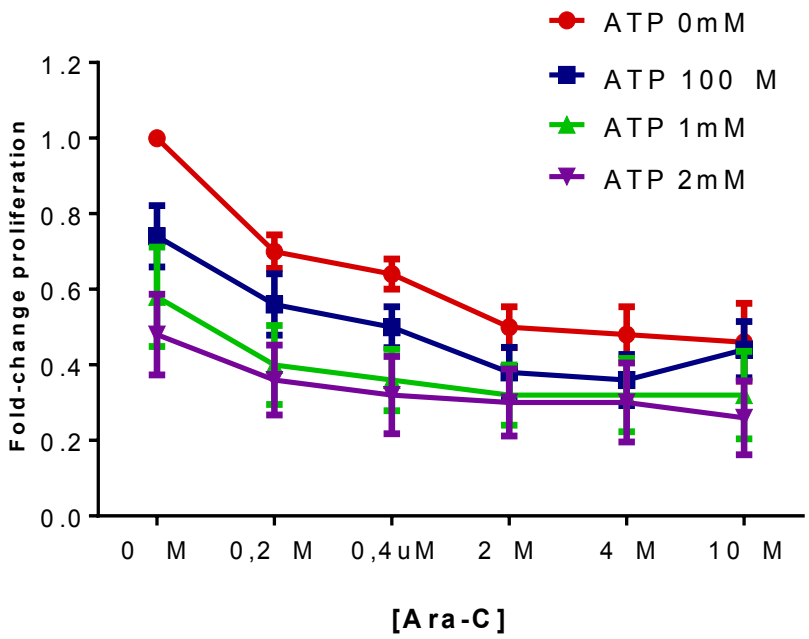


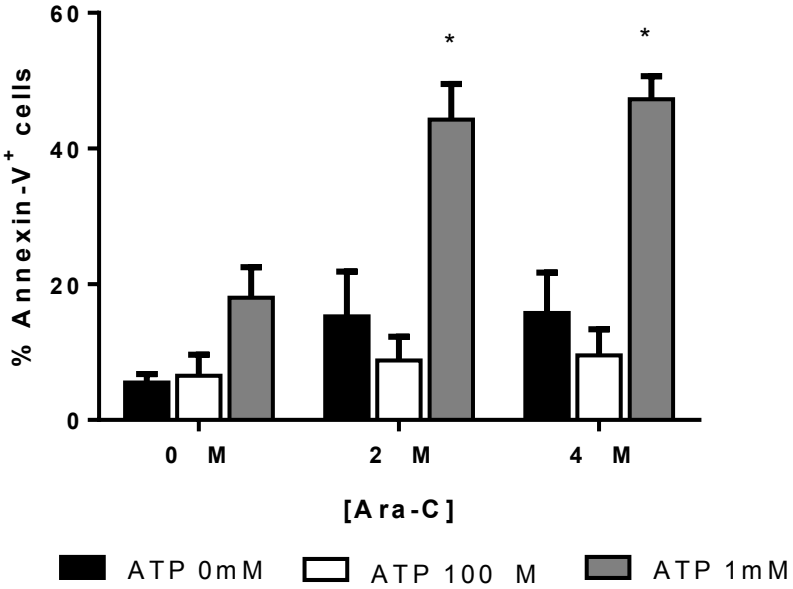




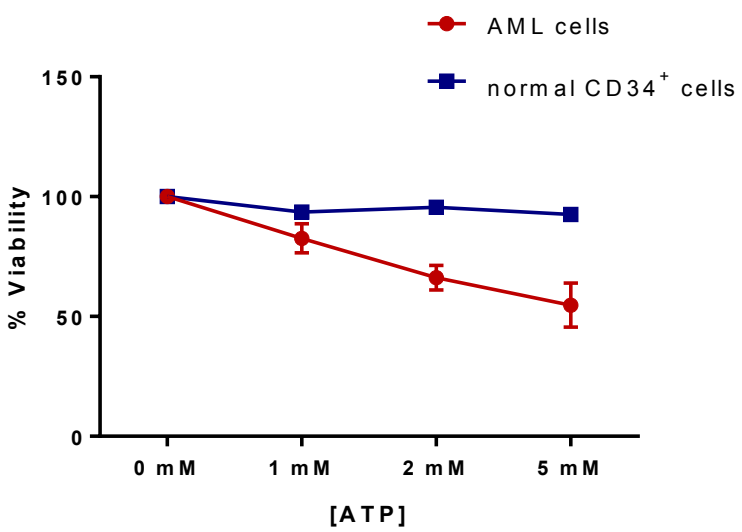


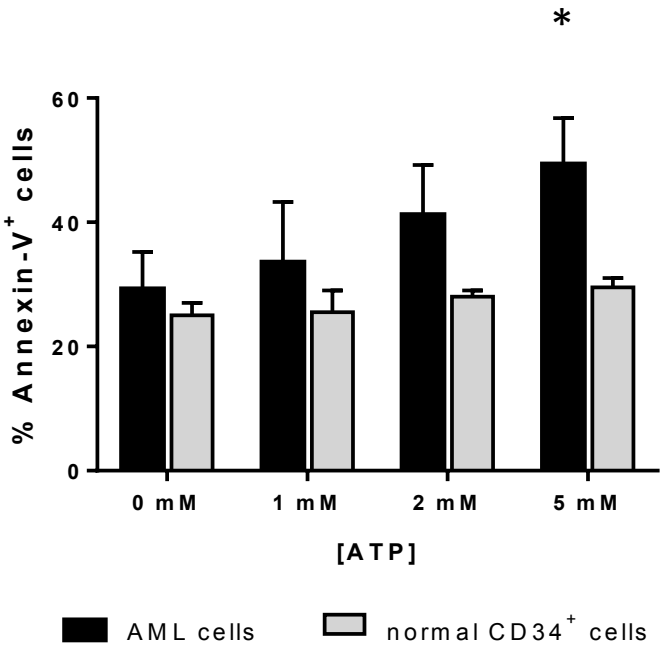
ATP potentiates the cytotoxic effect of antineoplastic drugs



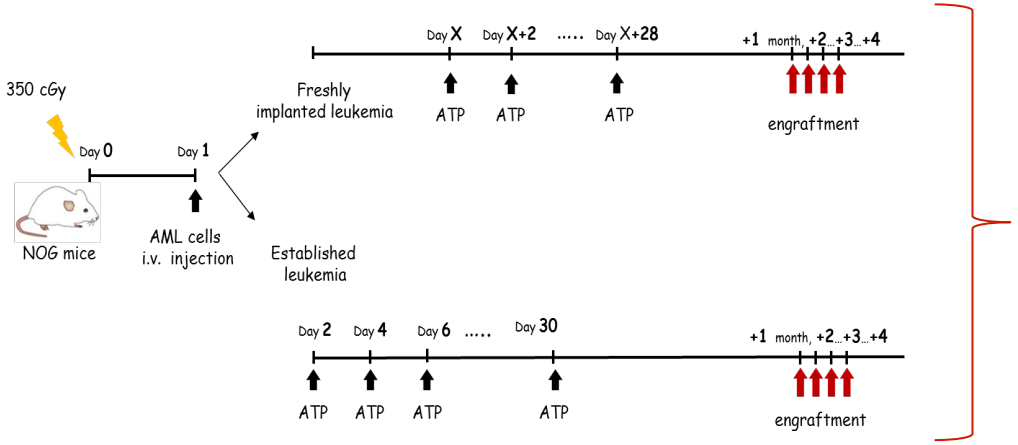


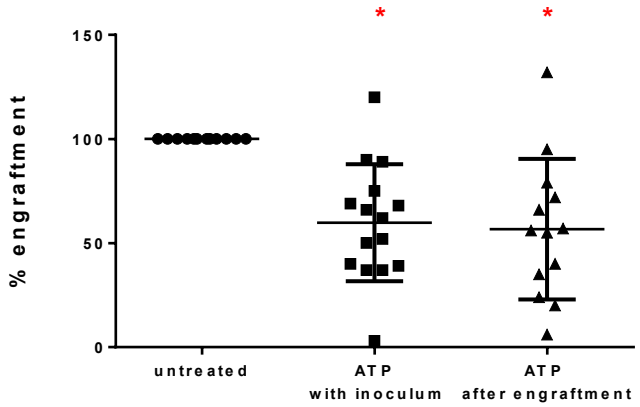
ATP exerts a direct cytotoxicity on AML cells but not on normal HSCs

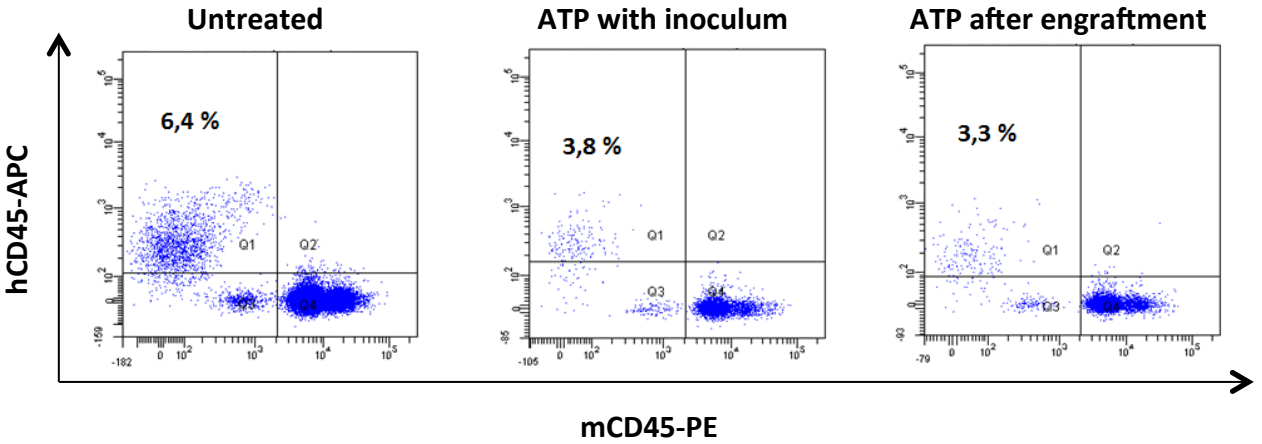




ATP inhibits leukemia cell growth in vivo



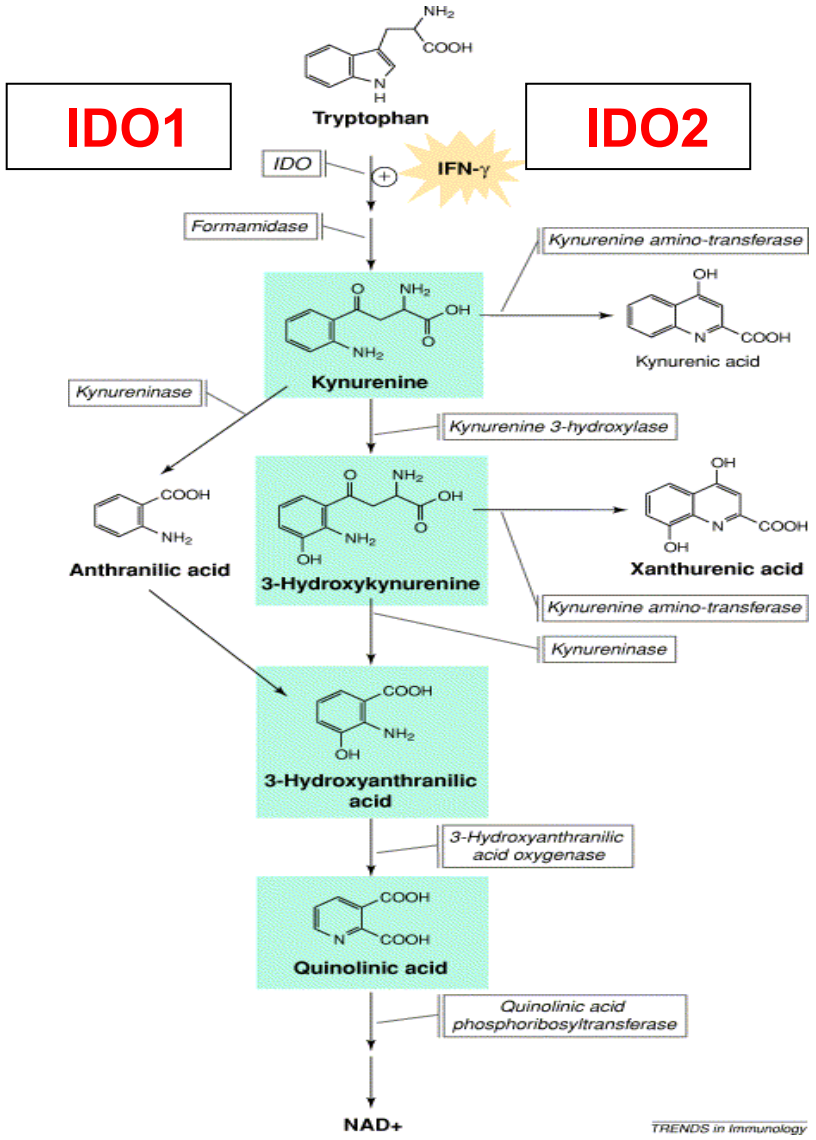




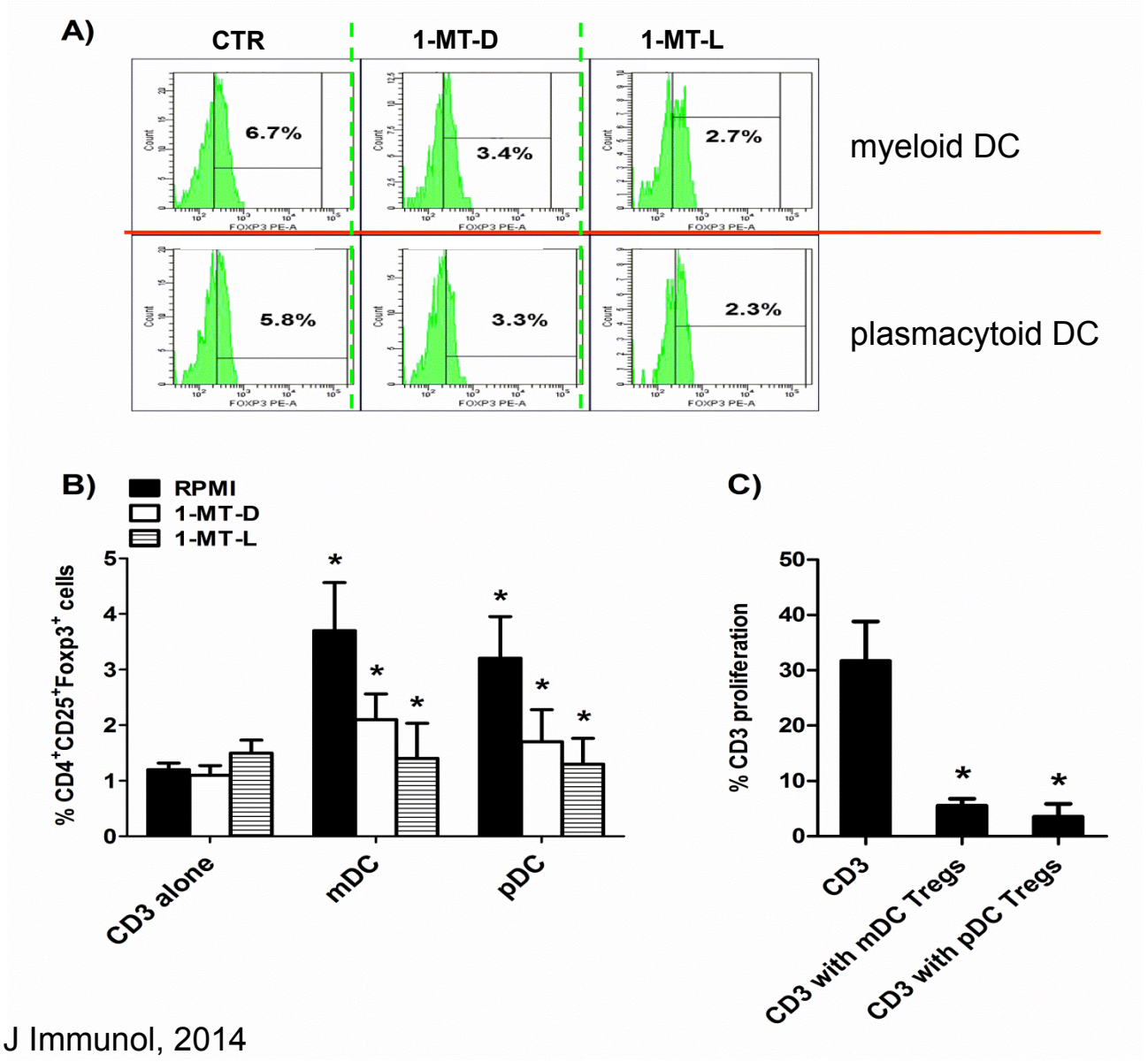
Indoleamine 2,3-dioxygenase (IDO) at the cross-road between inflammation and immunotolerance

- **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**



Dendritic cells induce Tregs by means of both IDO enzymes

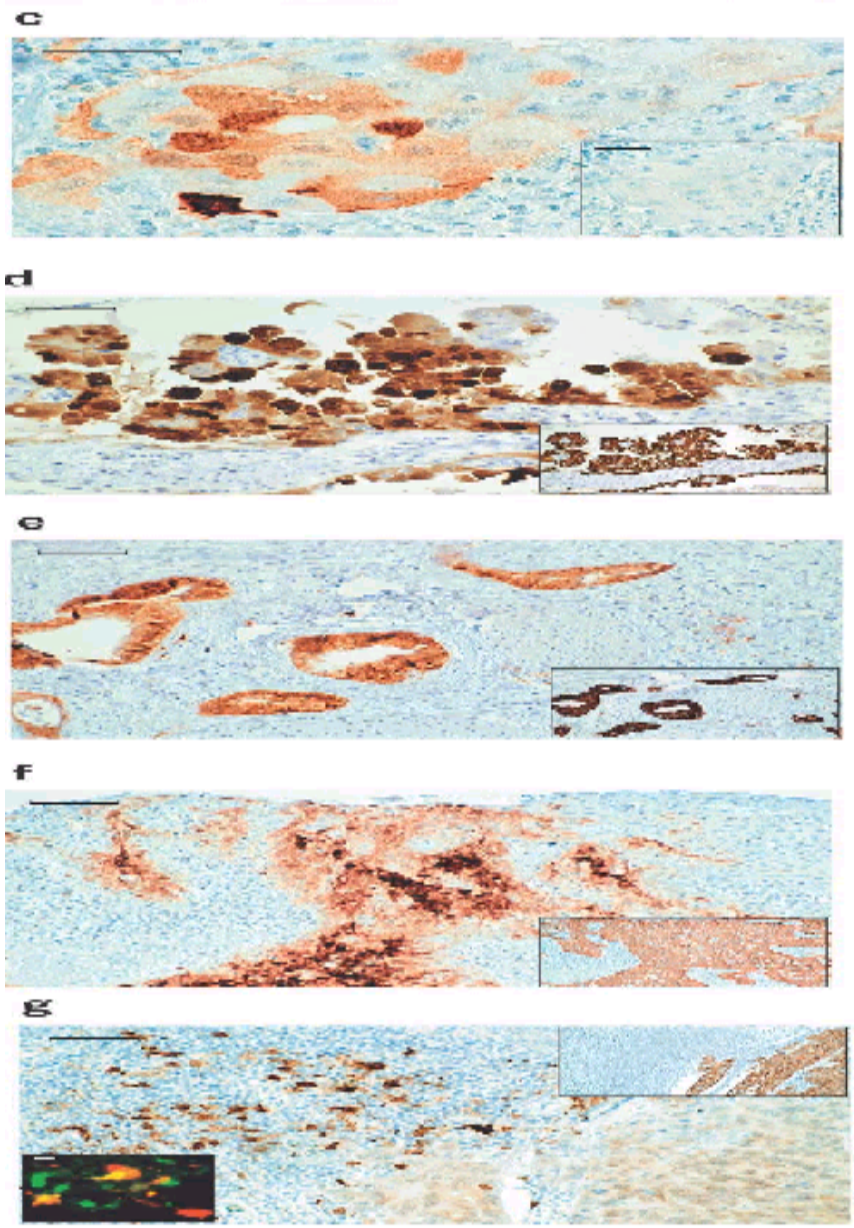


Trabanelli et al, J Immunol, 2014

MODULATION OF TRYPTOPHAN CATABOLISM BY TUMOR CELLS EXPRESSING IDO AS A STRATEGY OF IMMUNE EVASION

- A wide variety of human tumors expresses IDO protein, which mediates immune tolerance

- In humans, IDO over-expression correlates with poor prognosis (ovarian carcinoma, endometrial carcinoma, colon carcinoma)



Non small-cell lung cancer

Ovarian carcinoma

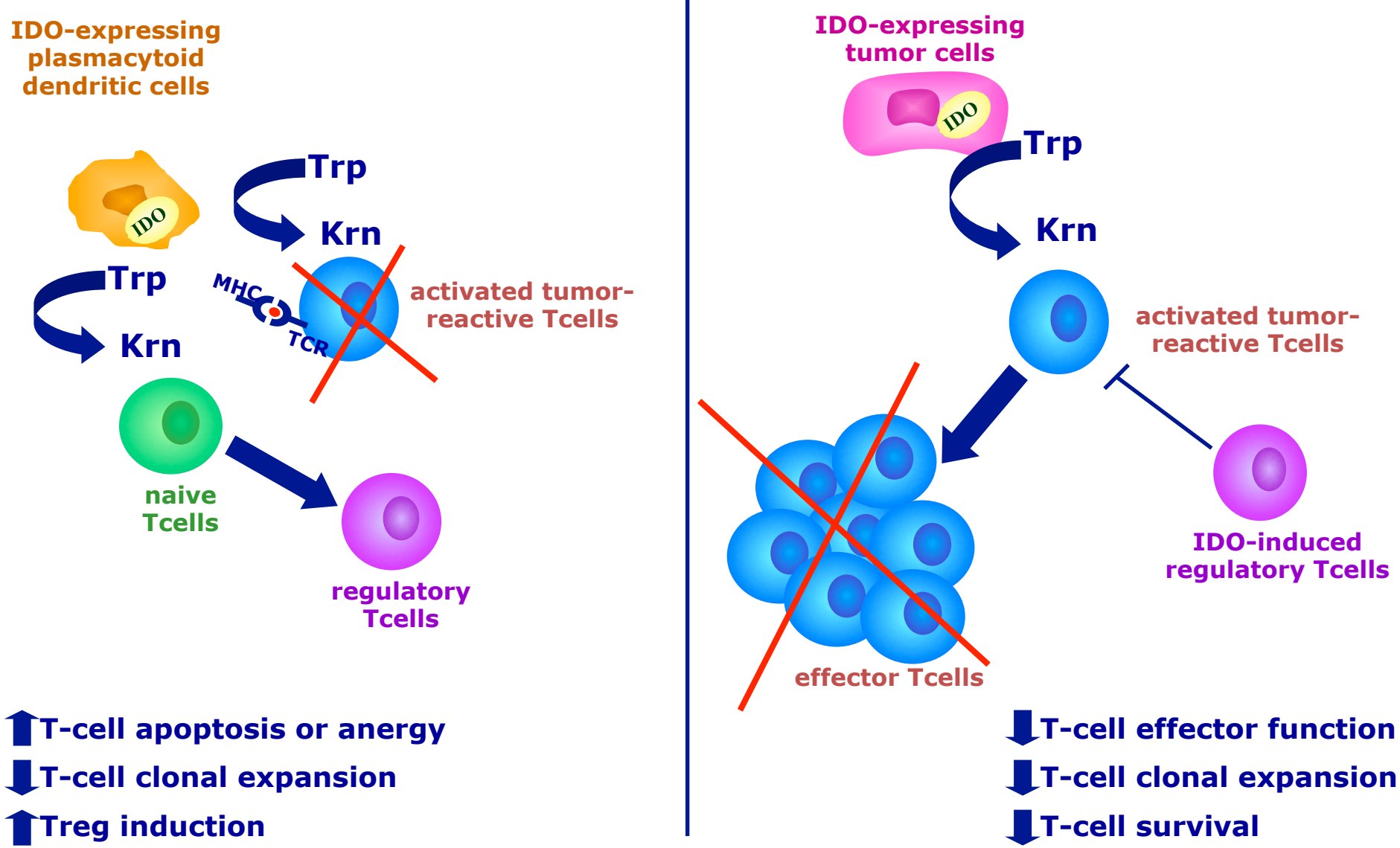
Pancreatic carcinoma

Head and Neck carcinoma

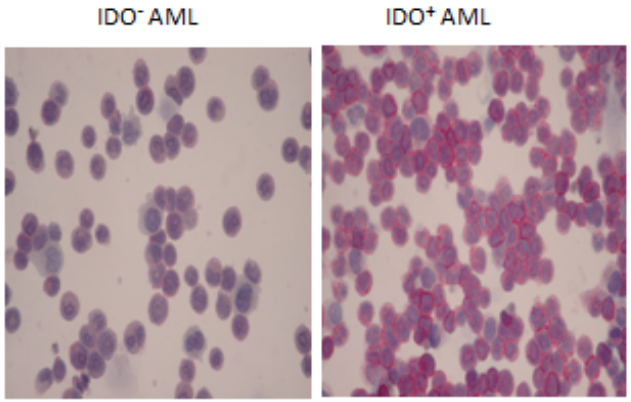
Colon carcinoma

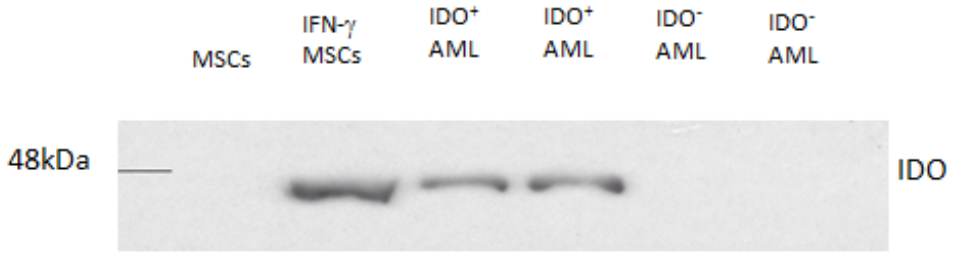
Uyttenhove et al, Nature Med, 2003

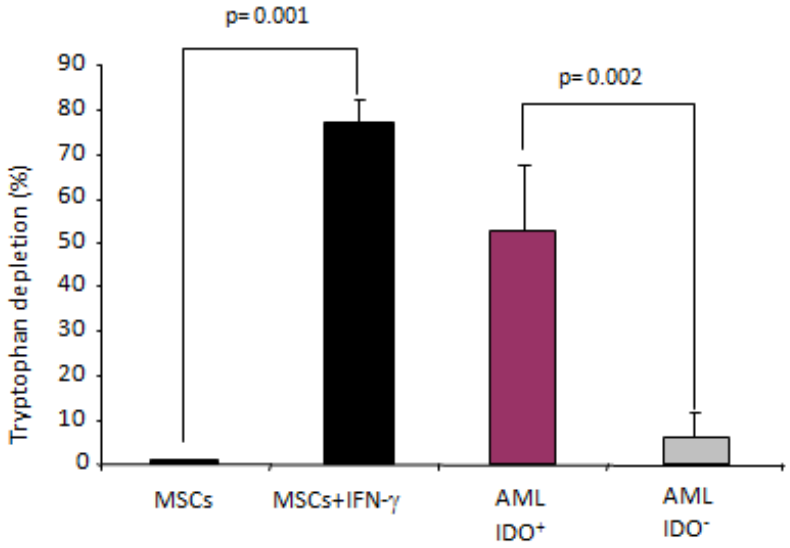
IDO protein is involved in the induction of immune tolerance to tumors

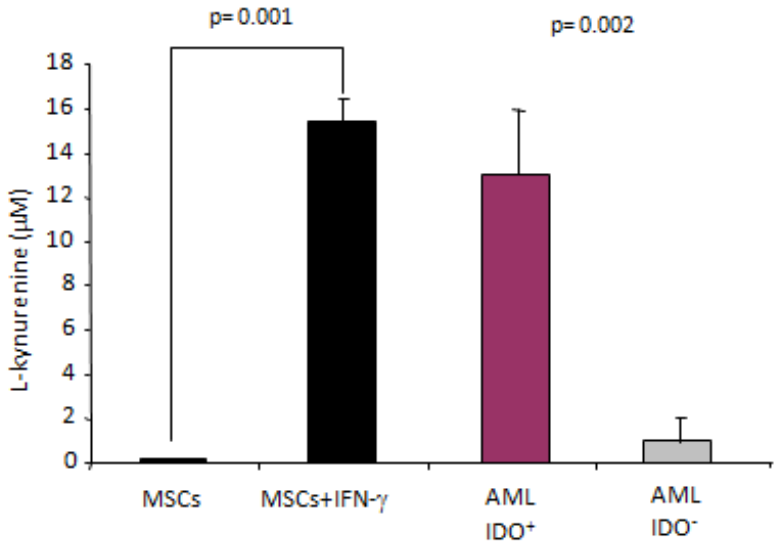


IDO is expressed and functionally active in AML



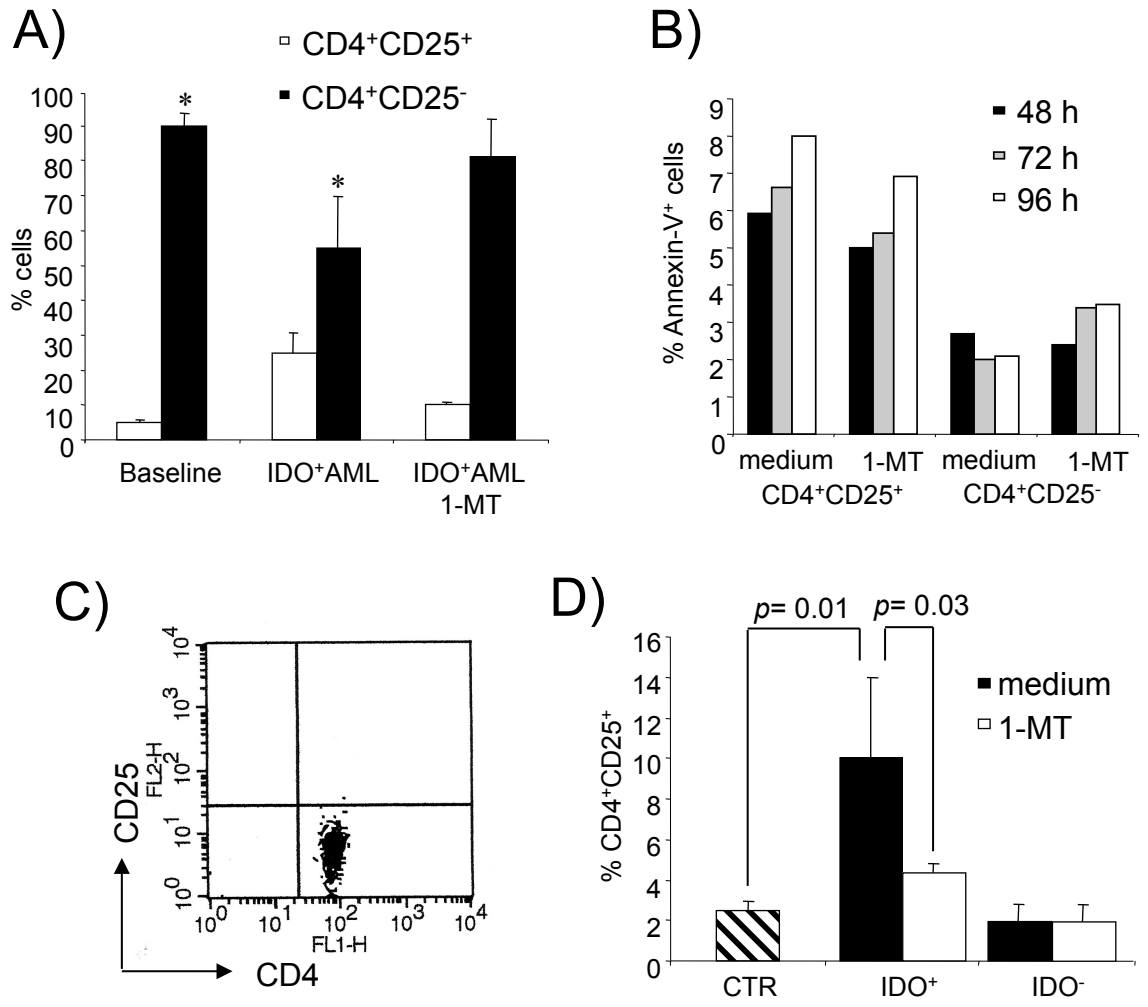






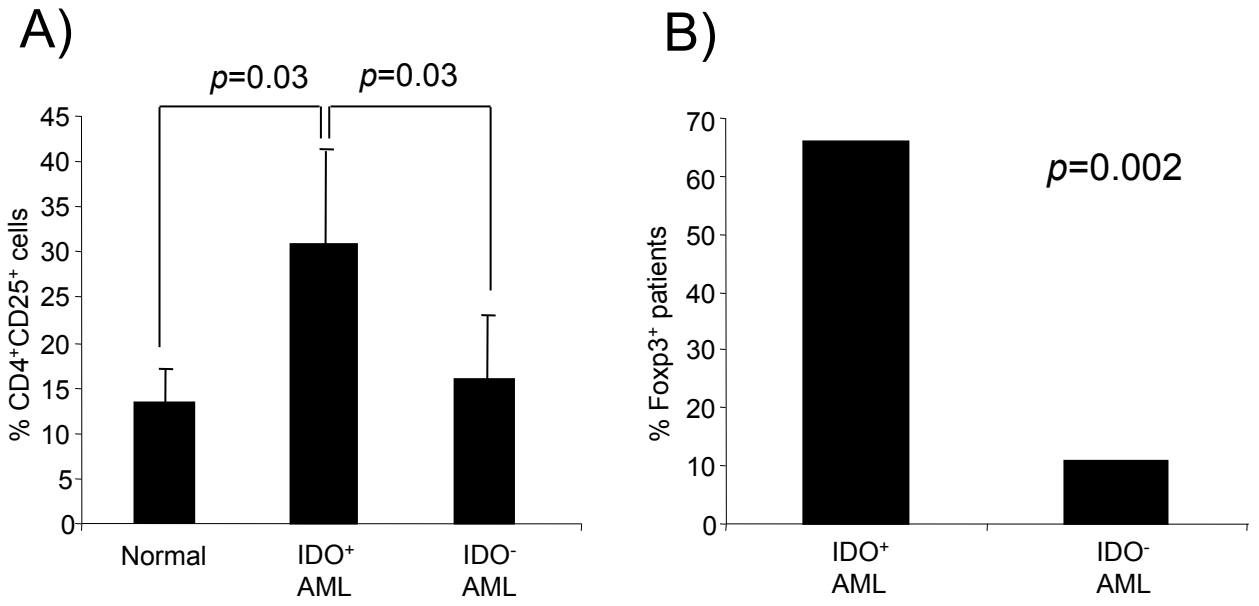
Curti et al, Leukemia, 2007

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



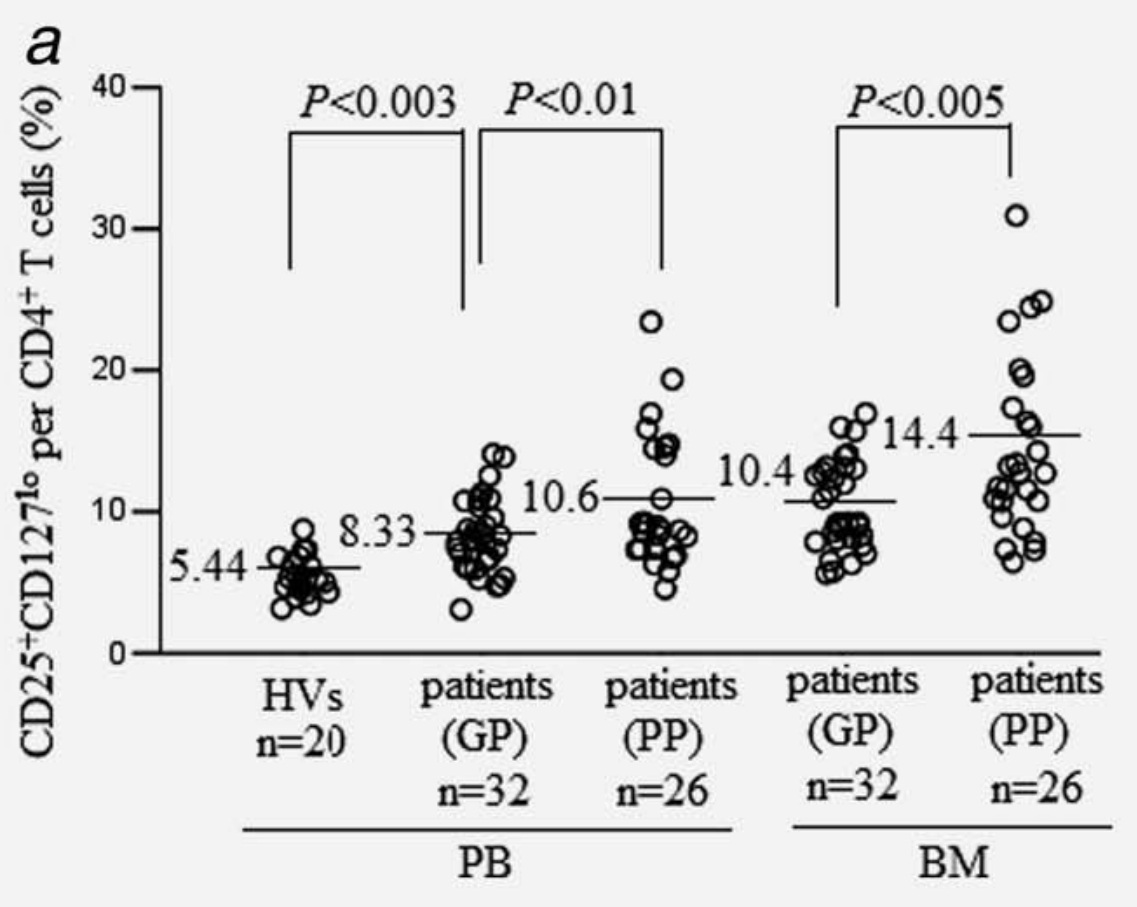
Curti et al. Blood, 2007; Blood, 2009; Haematologica, 2010

IDO$^+$ AML patients have an increased number of T regulatory cells in peripheral blood



Curti et al., Blood, 2007

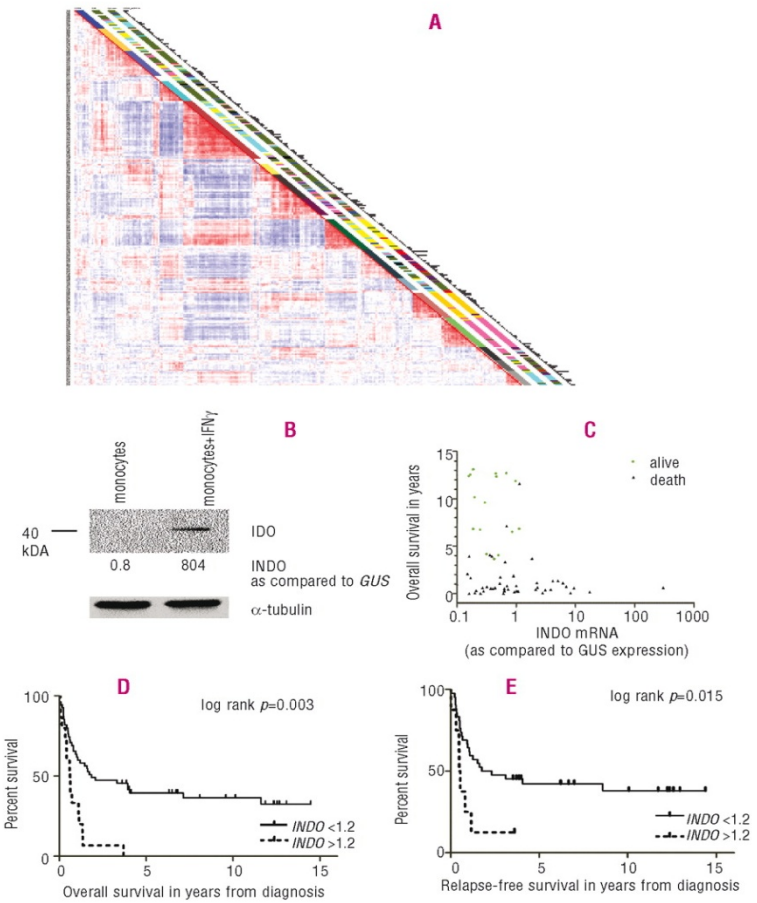
Elevated frequencies of Tregs are associated with poor prognosis in AML



Shenghui et al, Int J Cancer, 2011

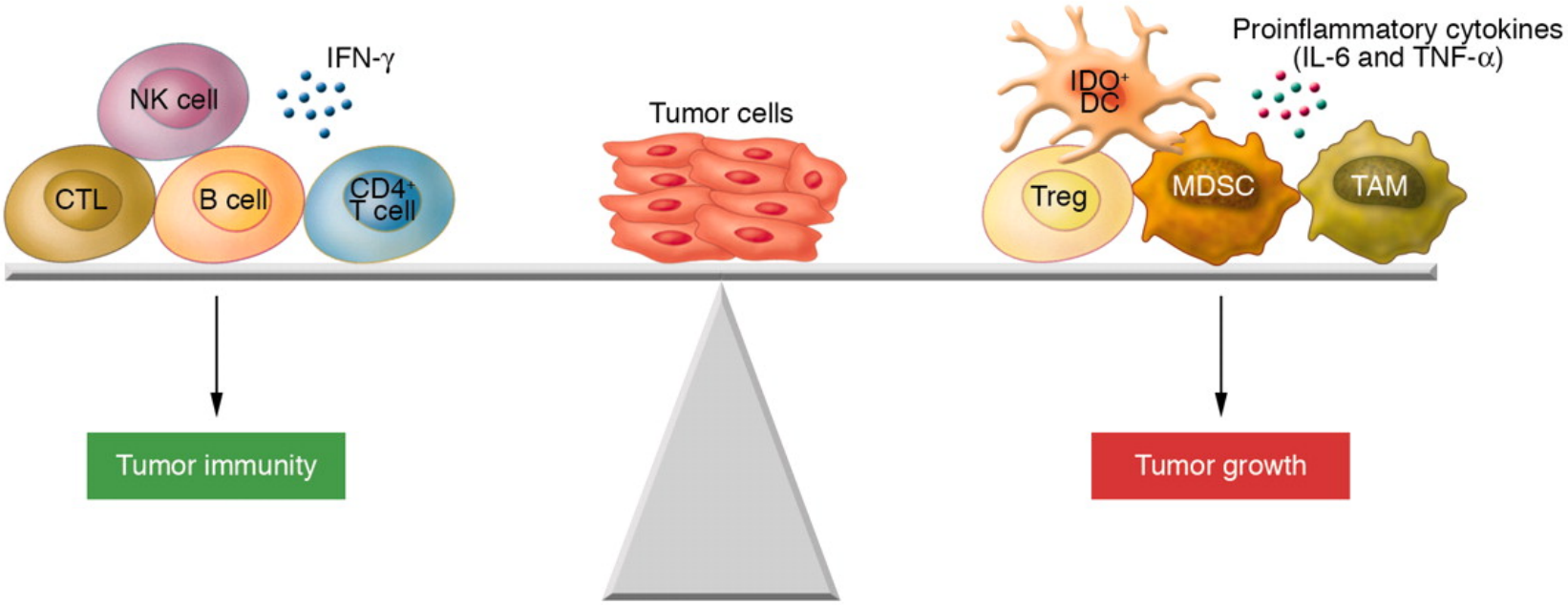
High *INDO* mRNA level in blasts of AML patients predicts poor clinical outcome

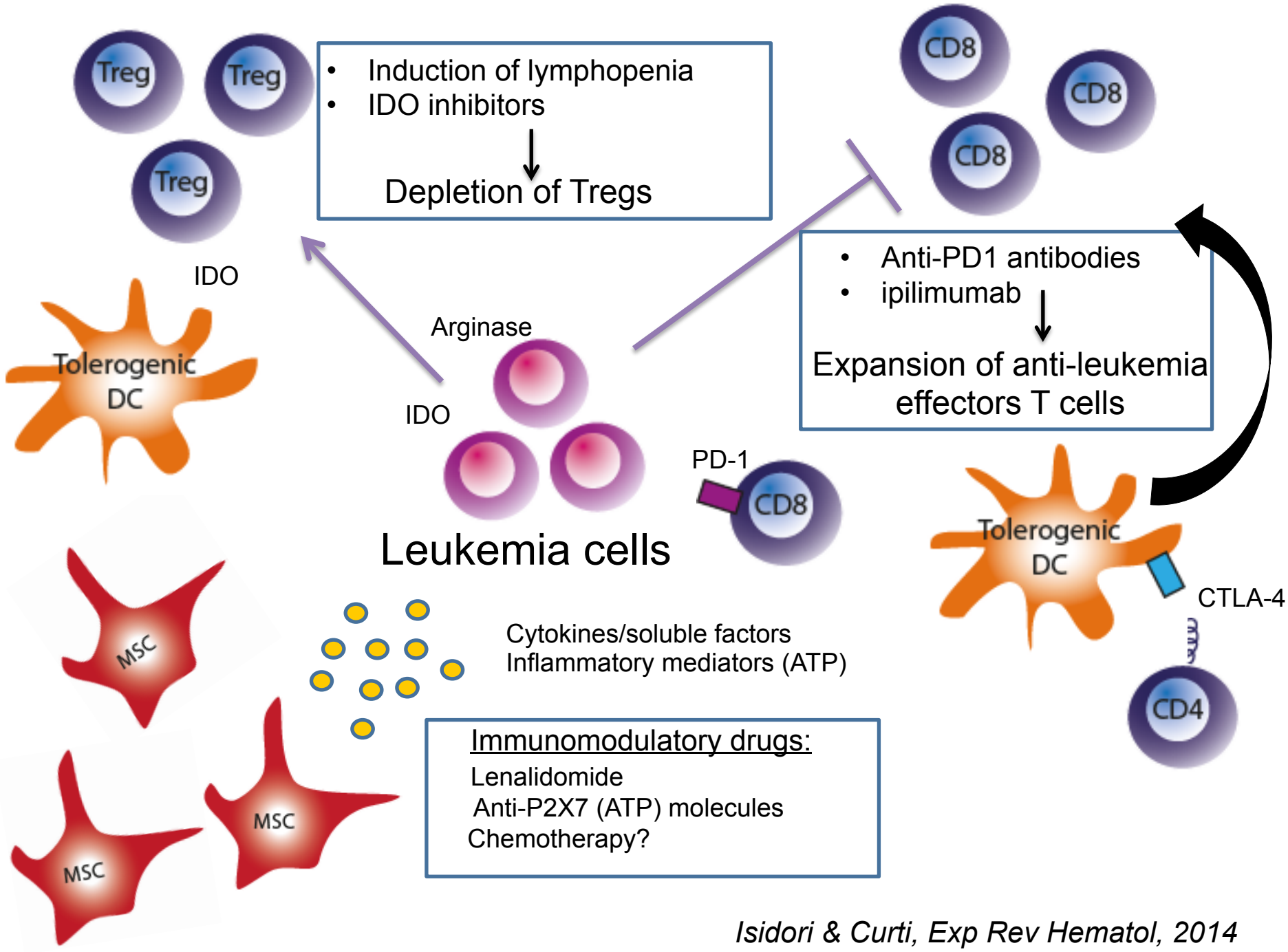
Type of analysis	*Gene-expression profiling*	*qPCR*
Number	286	71
Median age in years at diagnosis (range)	44 (15-78)	54 (16-75)
Median white blood cell count at diagnosis ($10\times^{9}$/L, range)	28 (0.3-582)	42 (0.4-282)
Complete remission rate, number (%)	203 (79.6)	55 (78)
Median overall survival in months (range)	13 (0-166)	14.4 (0.03-174)
Median relapse free survival in months (range)	11 (0-166)	16.6 (0.23-173)
FAB classification, number (%)		
AML M0	6 (2)	4 (6)
AML M1	63 (22)	9 (13)
AML M2	66 (23)	20 (19)
AML M3	19 (7)	3 (4)
AML M4	53 (19)	12 (17)
AML M5	65 (23)	20 (28)
AML M6	3 (1)	3 (4)
not determined	10 (3)	
Cytogenetic risk group, number (%)		
Favorable	57 (20)	4 (6)
Standard	176 (62)	40 (56)
Adverse	39 (14)	6(9)
No metaphasis	13 (4)	6 (9)
Not done		15 (21)
FLT3 status, number (%)		
FLT3 ITD pos	78 (27)	19 (27)
FLT3 ITD neg	207 (73)	52 (73)
FLT3 TKD pos	33 (12)	5 (7)
FLT3 TKD neg	252 (88)	66 (93)



Chamuleau, M. E.D. et al. Haematologica 2008;93:1894-1898

Harnessing the immune system to treat cancer





Isidori & Curti, Exp Rev Hematol, 2014

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Prof. Francesco Bertolini

University of Ferrara
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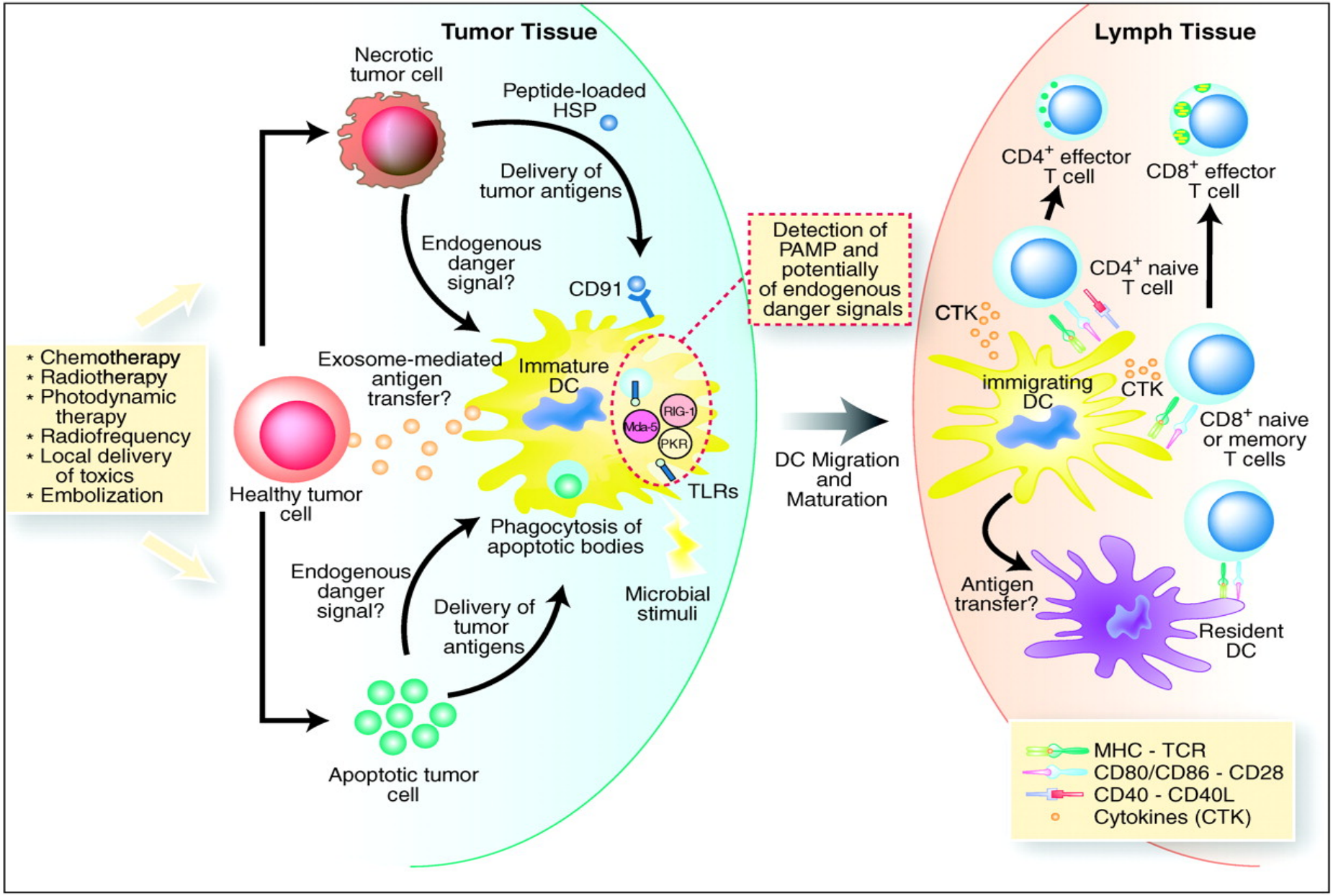


CCR Biology Behind

Melero I et al. Clin Cancer Res 2006;12:2385-2389

Clinical Cancer Research

CELLULAR STRESS RESPONSE
after chemotherapy

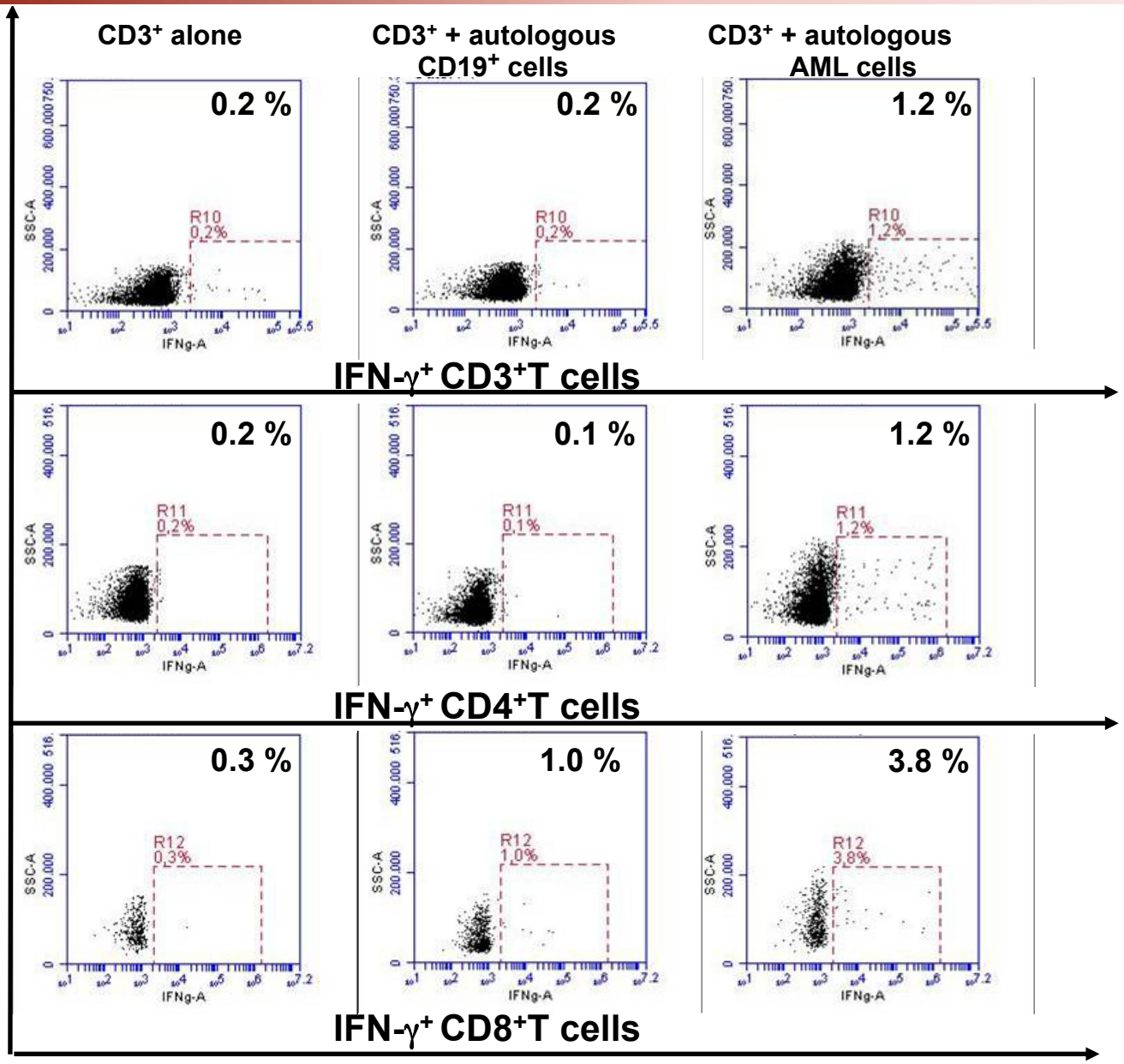
early

1. **Calreticulin (CRT)** from endoplasmatic reticulum (ER) to cell surface
   - induction of apoptosis via caspases
2. Cell surface exposure of **HSP-TA complexes (heat shock protein–tumor antigen)** = **"EAT - ME" signal** for DCs

late

3. **Autophagy-dependent** release of **ATP** (immune cells recruitment)
4. Release of pro-inflammatory **non-histone chromatin binding protein high mobility group box 1 (HMGB1)**
   - binds TLR4 on DCs (maturation) = **"FIND - ME" signal** for T cells

IFN-γ production by T-cells from AML patients after chemotherapy



n = 22

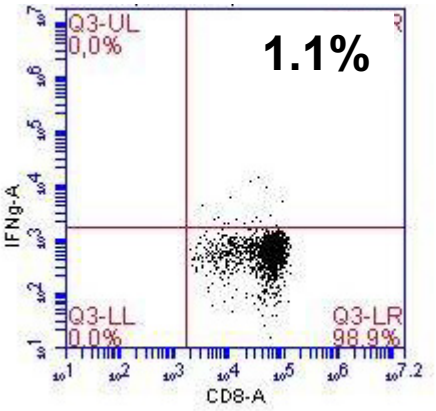
15/22 (68%) responding

7/22 (32%) non responding

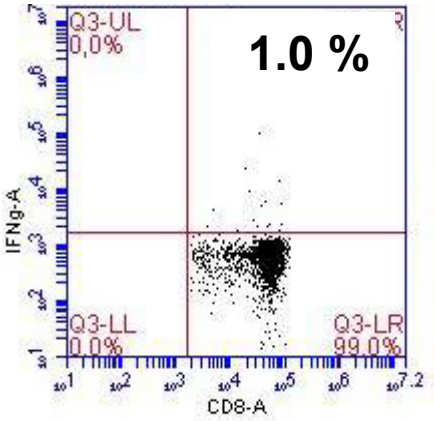
IFN-γ is mainly produced by CD8+ T cells

IFN-γ

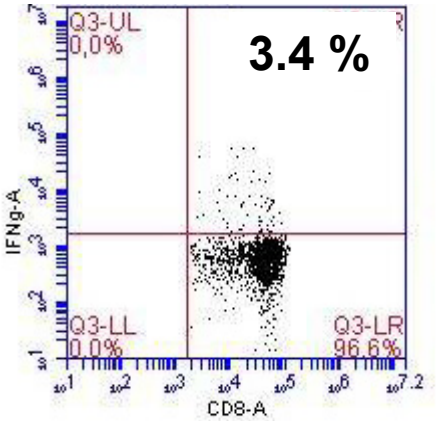
CD3+ alone



CD3+ + autologous CD19+ cells



CD3+ + autologous AML cells



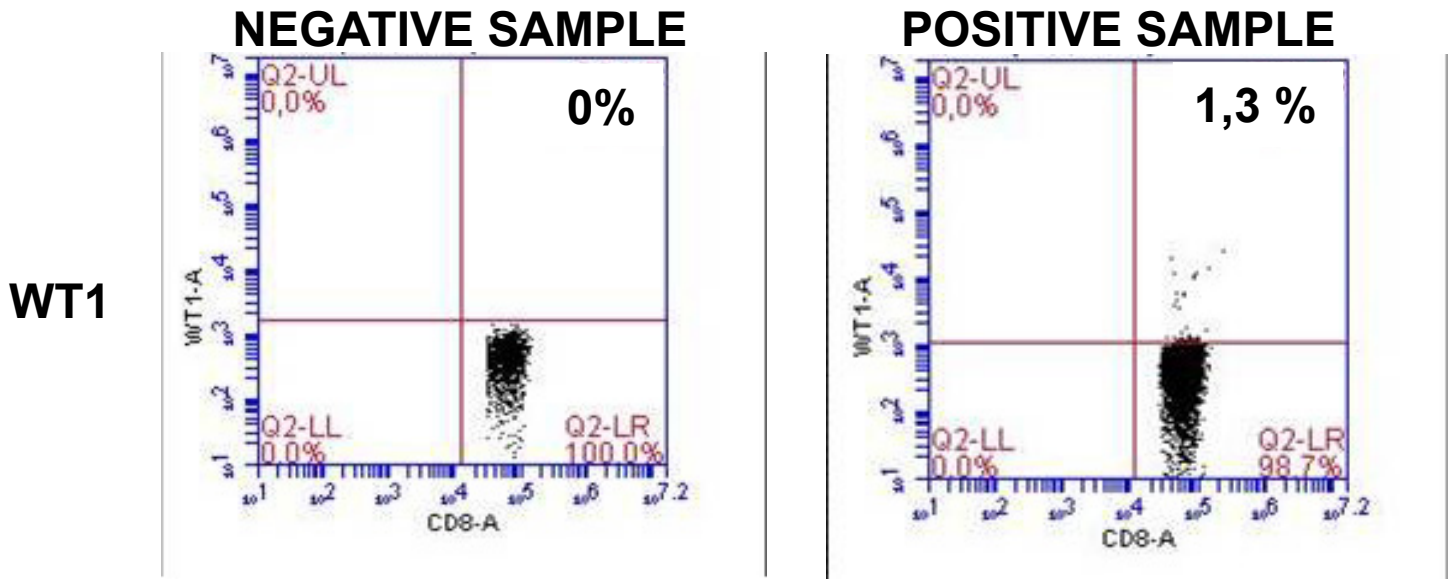
CD8+ T cells

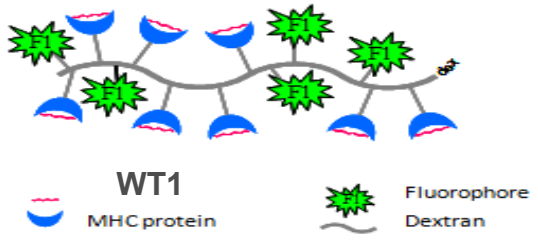
CD8+ T-cells specific for leukemia-associated antigen WT1 are detectable in PB after chemotherapy

*HLA-A*0201 positive patients; day 21 after chemotherapy*

NEGATIVE SAMPLE

POSITIVE SAMPLE

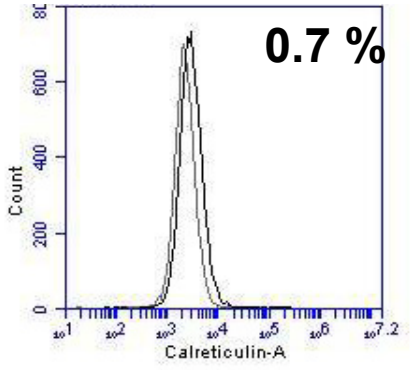




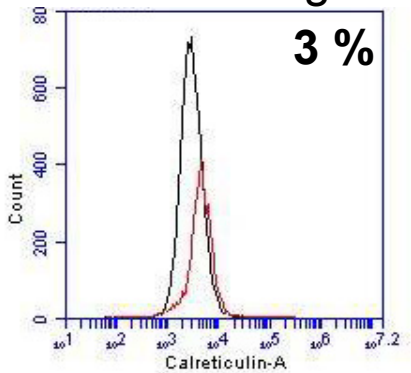
MHC dextramer antibody

Expression of Calreticulin (CRT) on AML cell surface after DNR treatment

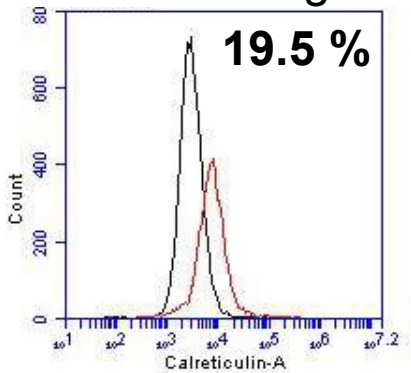
No treatment



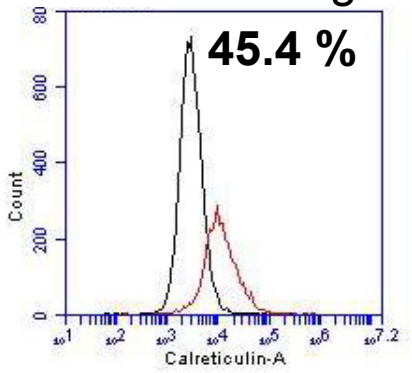
DNR 100 ng/ml



DNR 500 ng/ml



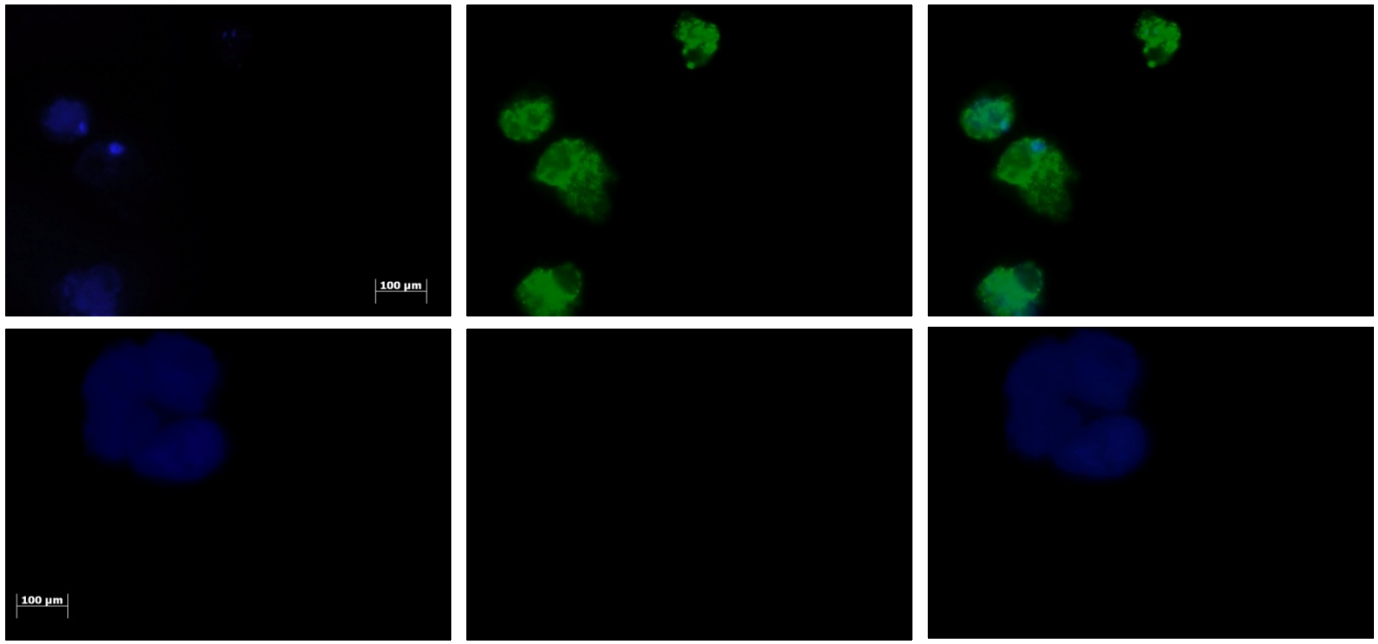
DNR 1000 ng/ml



CRT

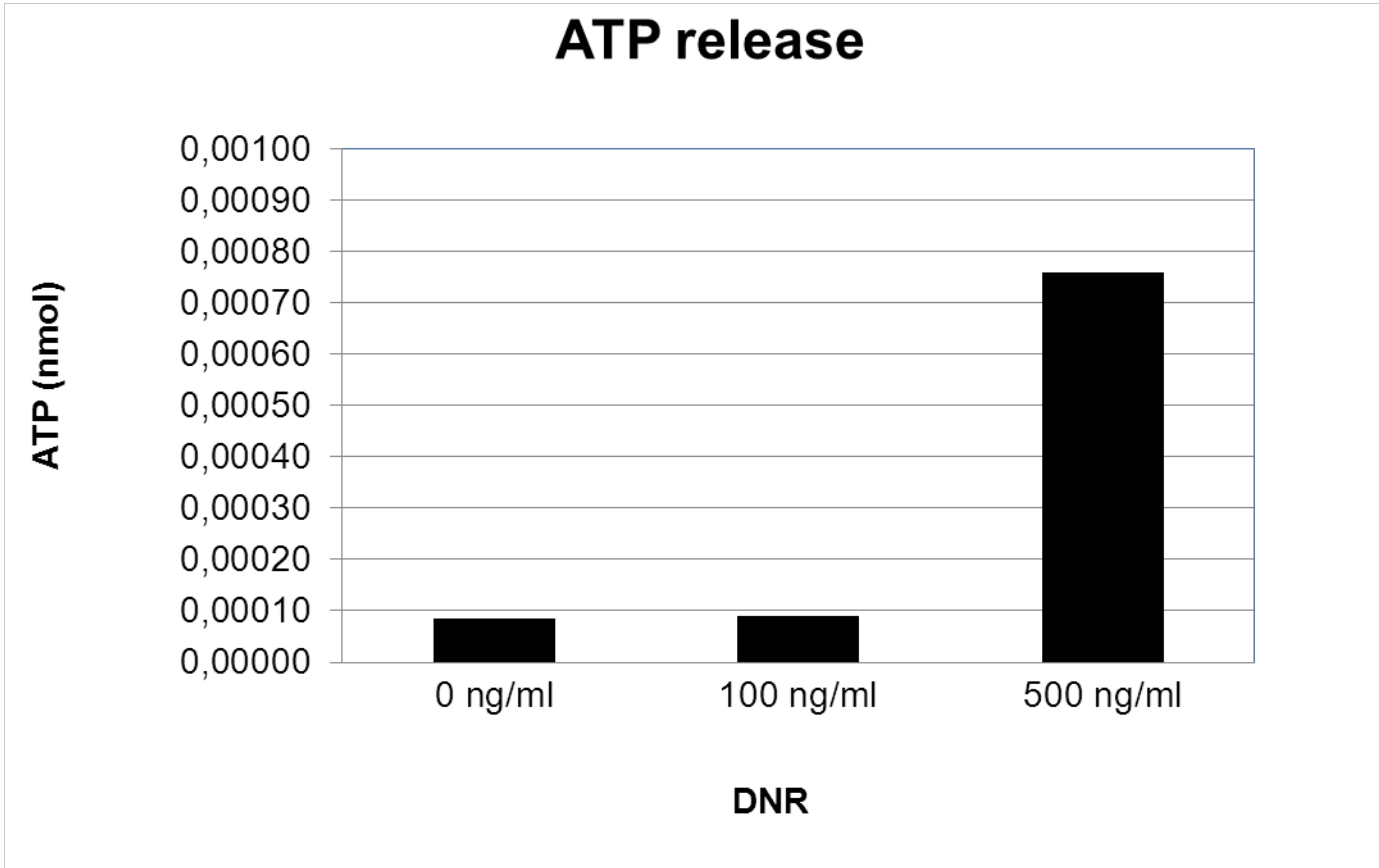
1. DNR-treated cells

2. No treated cells



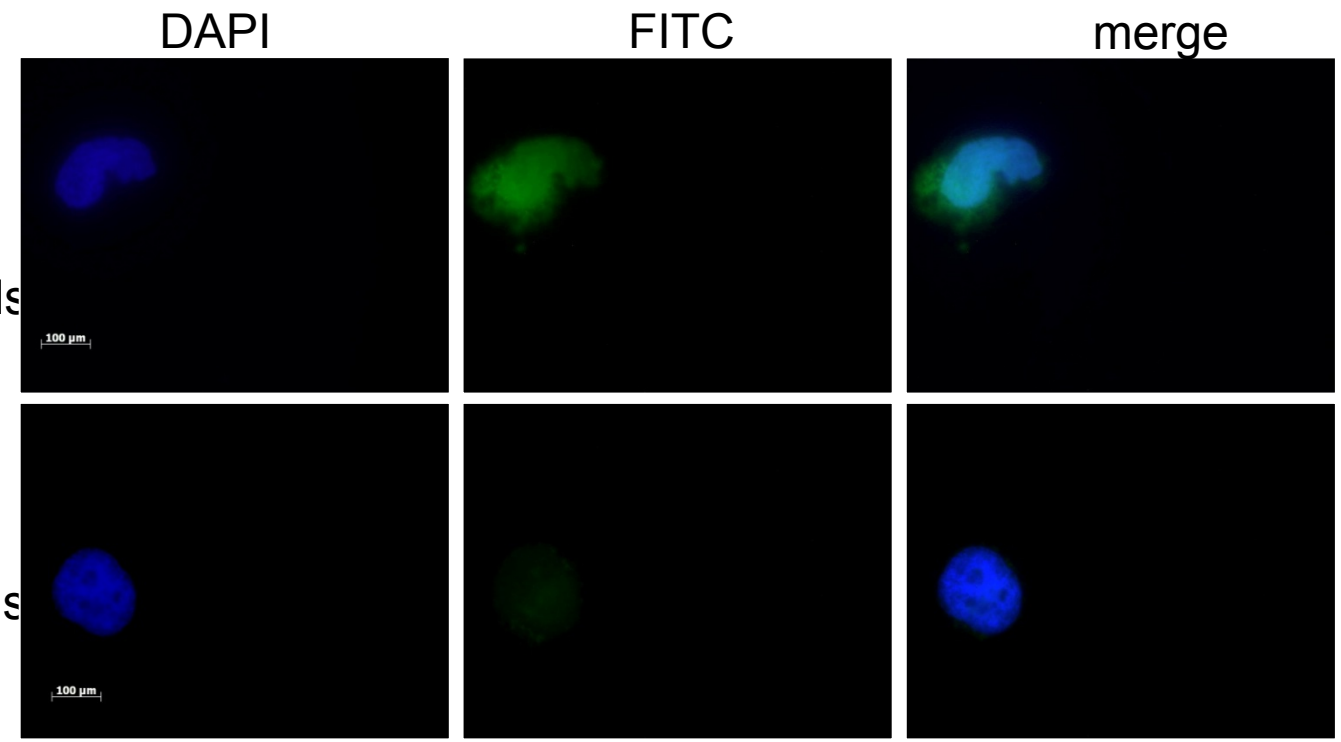
DAPI FITC merge

Release of ATP after DNR treatment (HL-60 cells)

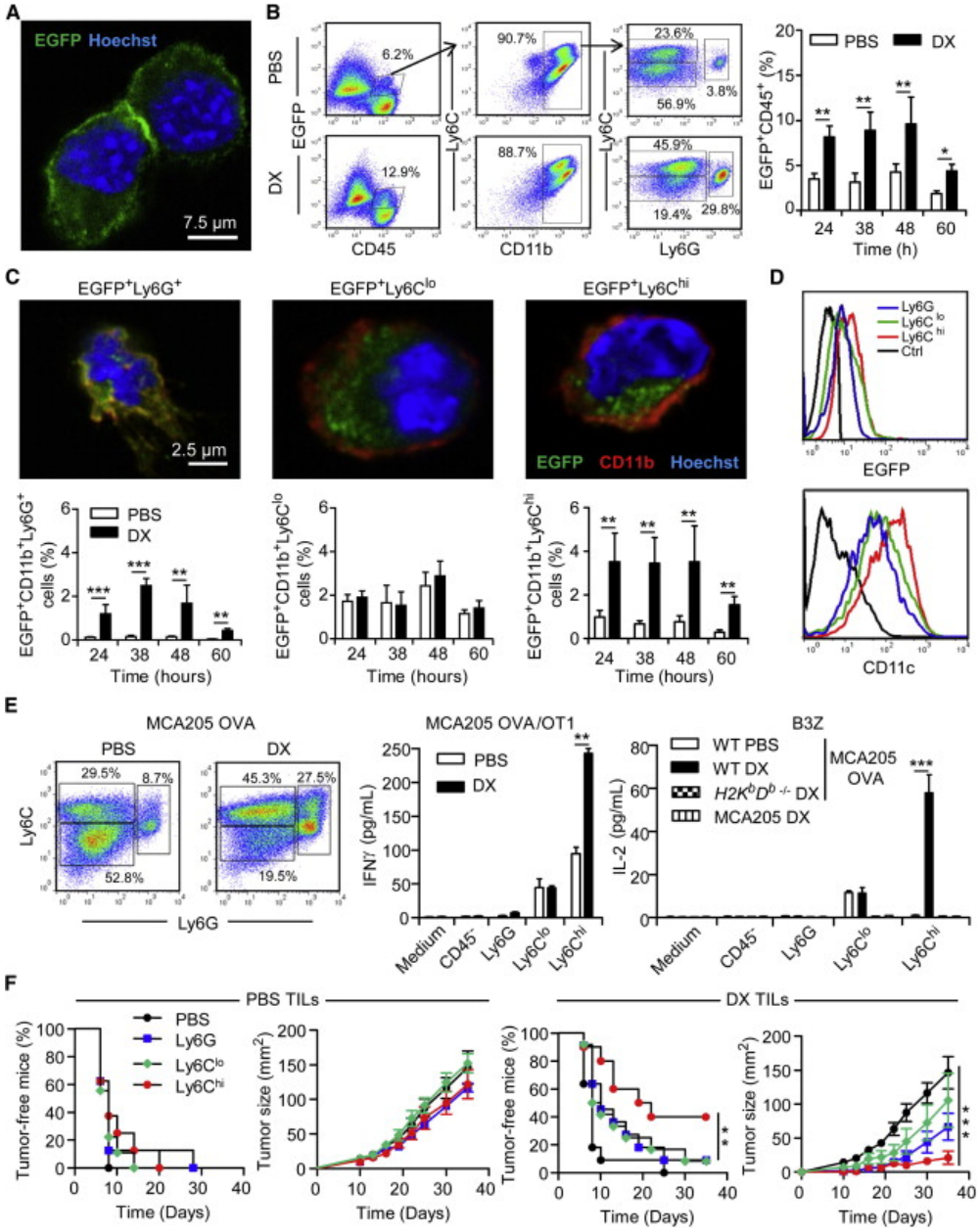


Expression of HMGB1 on AML cell surface after DNR treatment

HMGB1



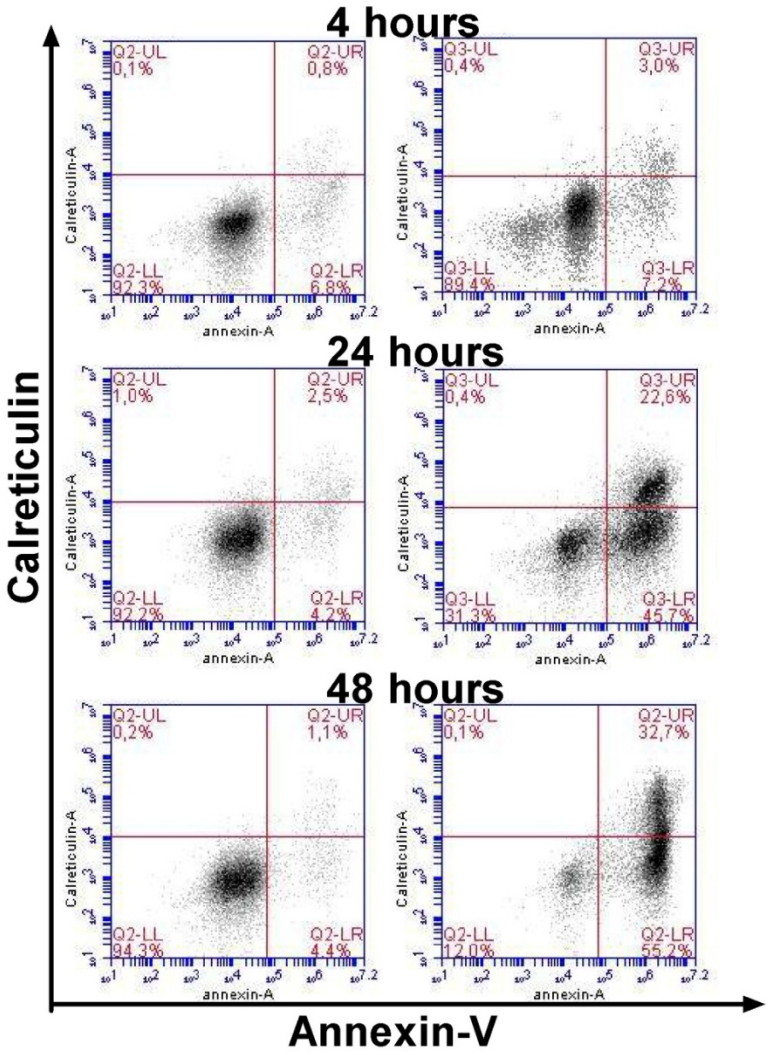
HL-60 cell line

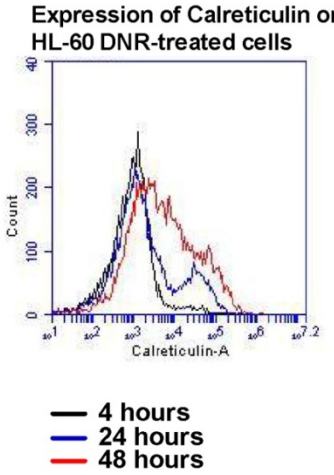


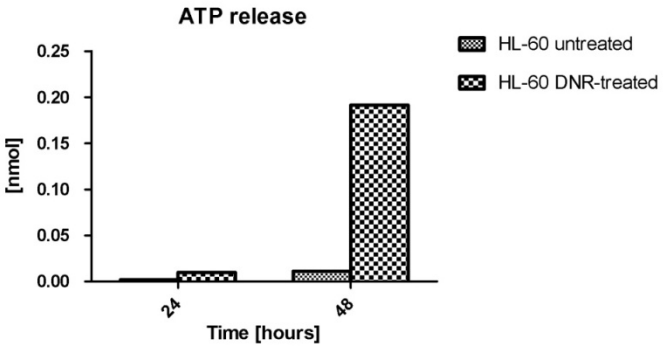
Anticancer Chemotherapy-induced Intratumoral Recruitment and Differentiation Of Antigen-Presenting Cells

Ma Y et al, Immunity, 2013

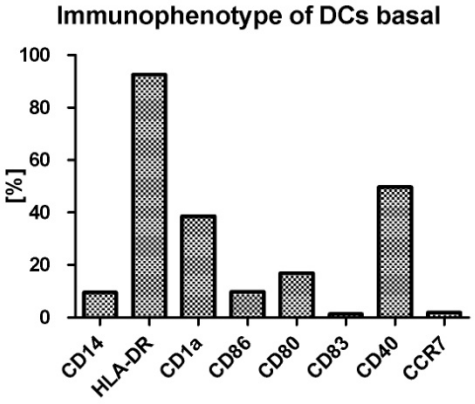
Expression of Calreticulin on untreated and DNR treated HL-60 cells

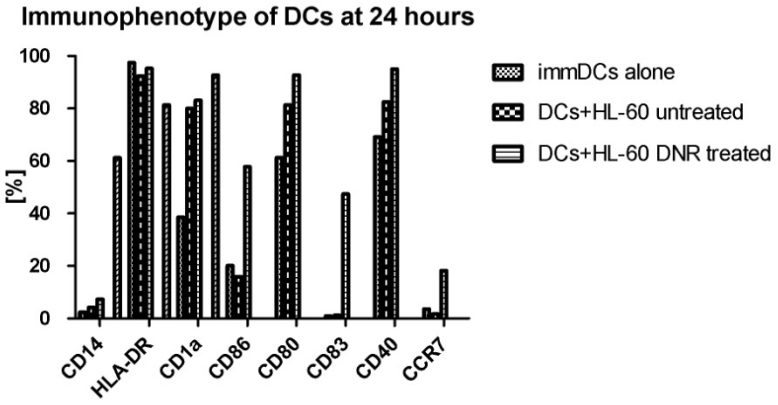


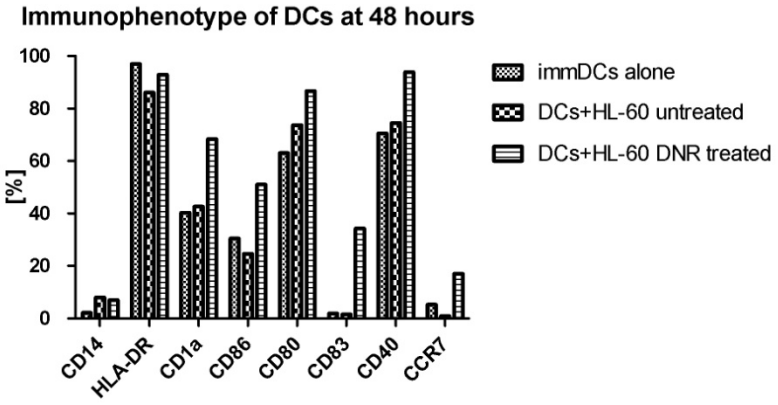




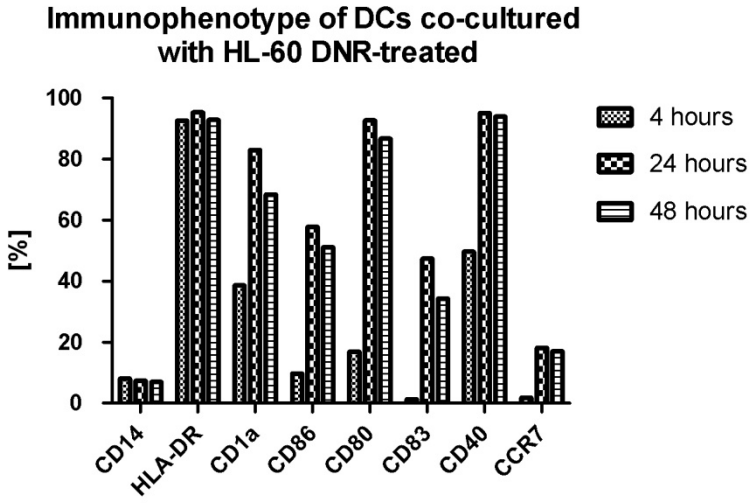
AML were treated with daunorubicin and then incubated with immature DCs. During co-culture with DCs, Calreticulin expression on AML cells and ATP release in the supernatant were evaluated. As shown, expression of CRT and ATP release were enhanced in treated AML samples and, as expected, segregates to apoptotic AML cells



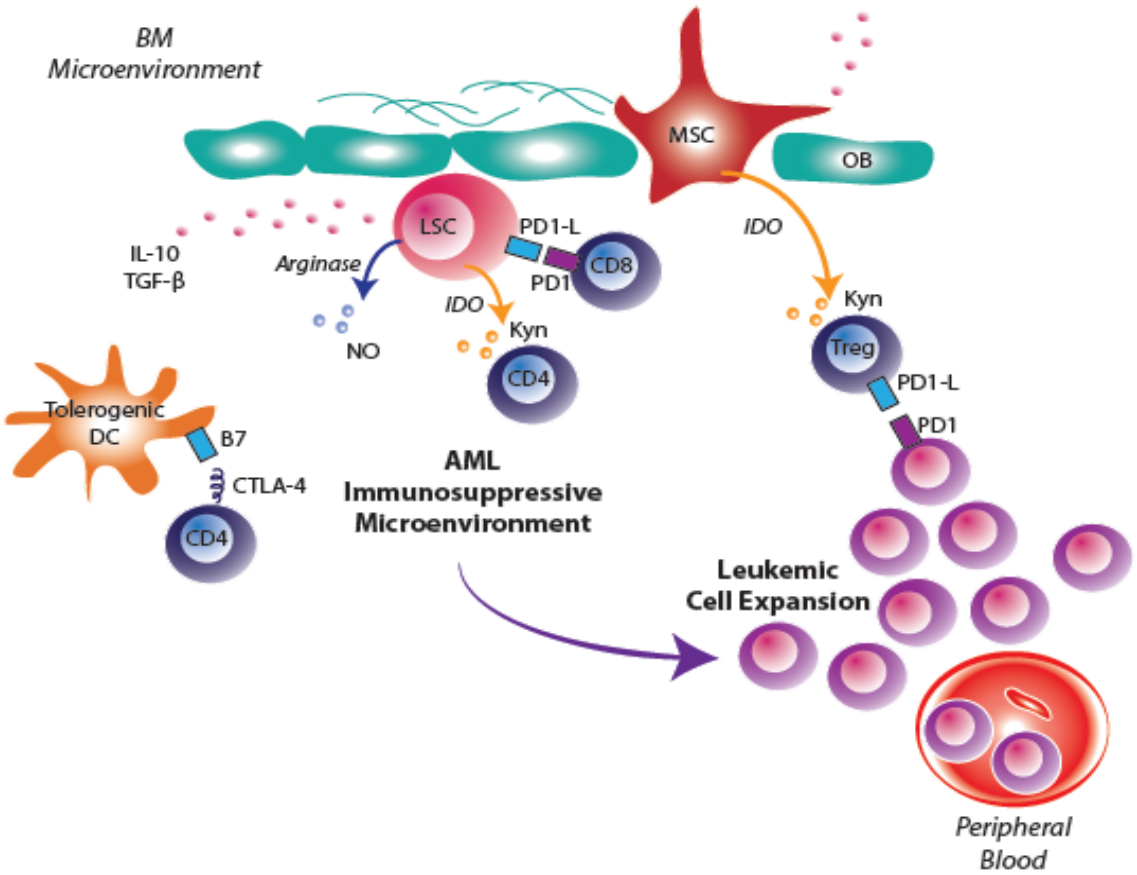




Pulsing of DCs with AML cells, previously treated with daunorubicin, significantly increases DC expression of maturation marker, such as CD86, CD83, CD40.

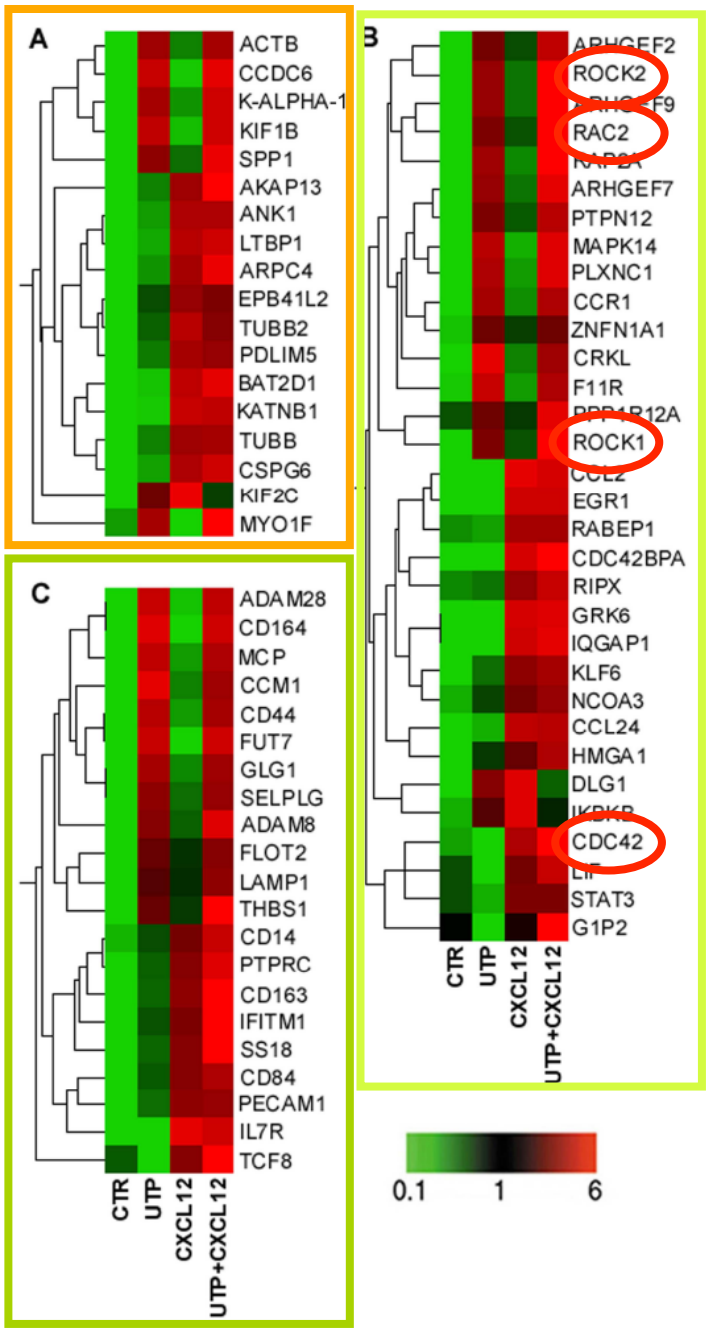


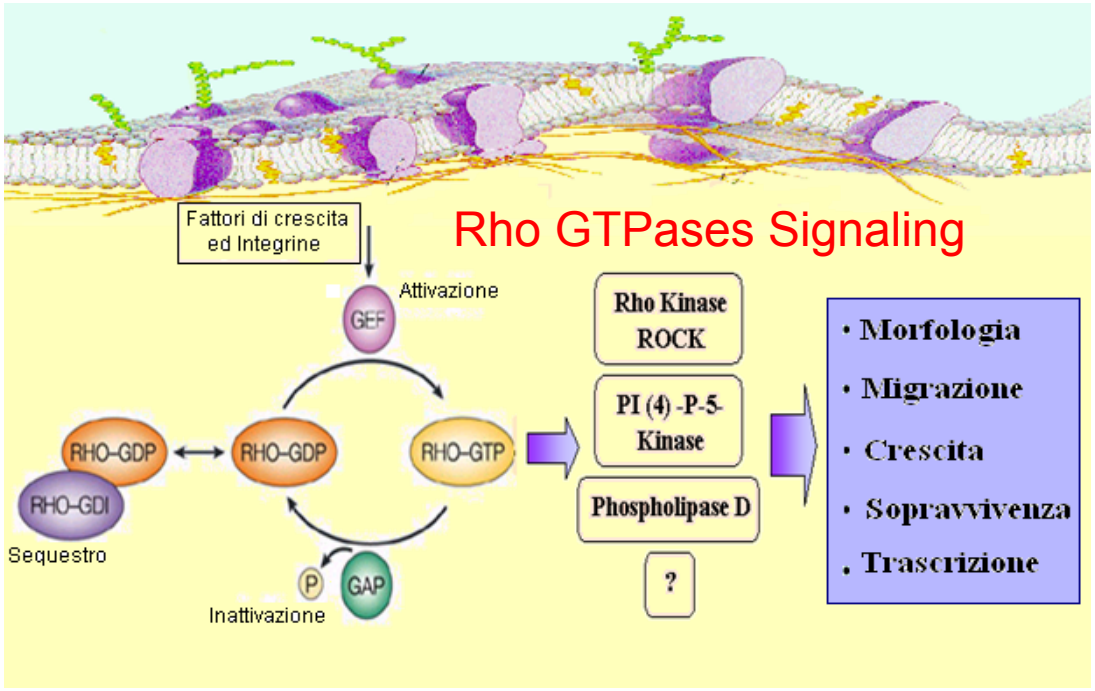
Bone Marrow Immunoregulatory Pathways: the immunosuppressive microenvironment in AML

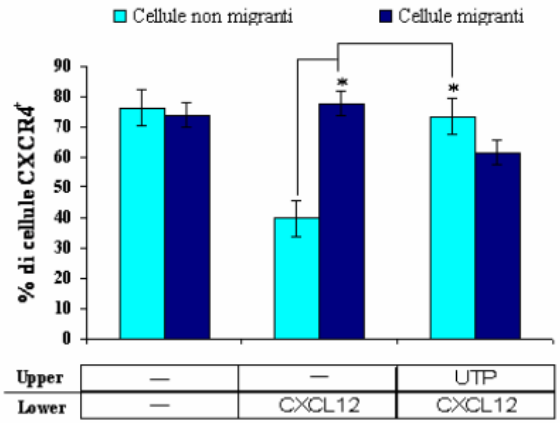


Isidori & Curti, Exp Rev Hematol, 2014

Gene Expression Profiling in UTP-treated HSPCs



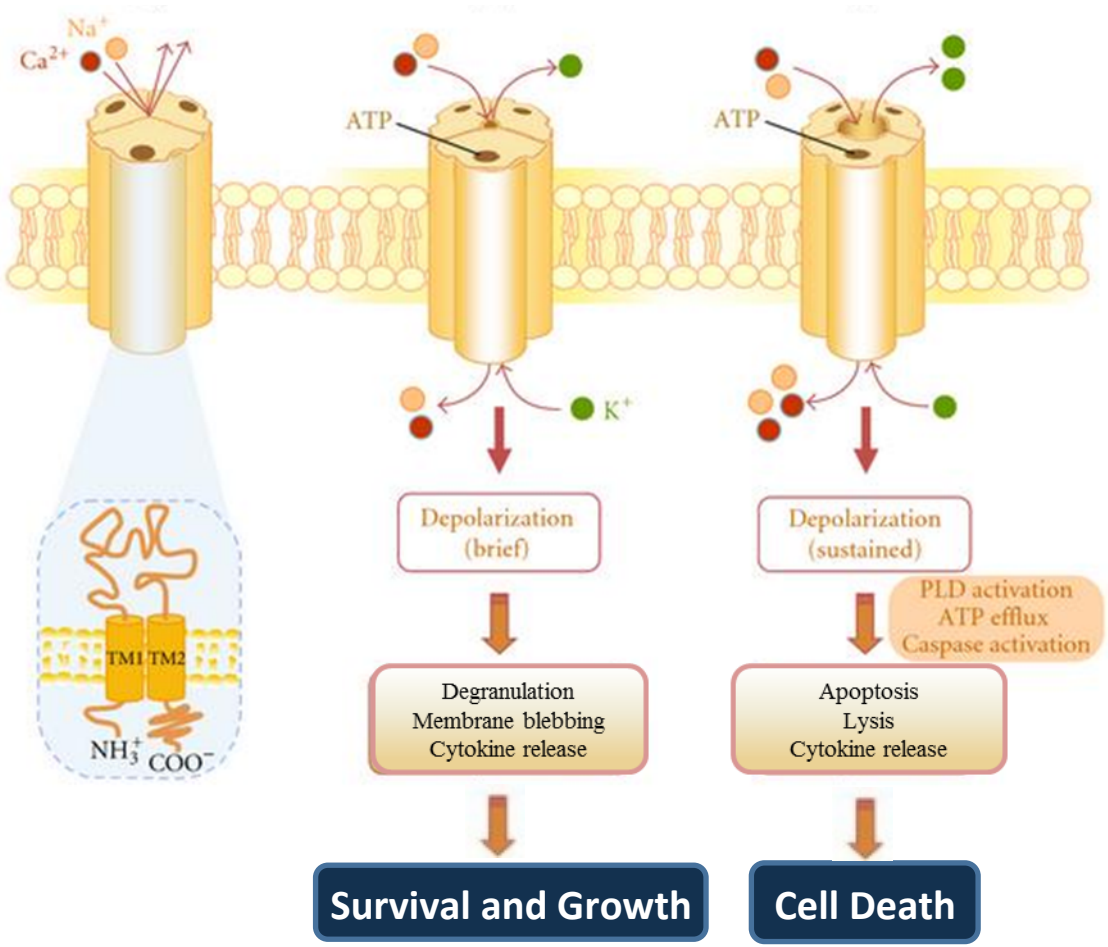




UTP treatment hinders CXCR4 *down-regulation* and *internalization* after CXCL12 binding

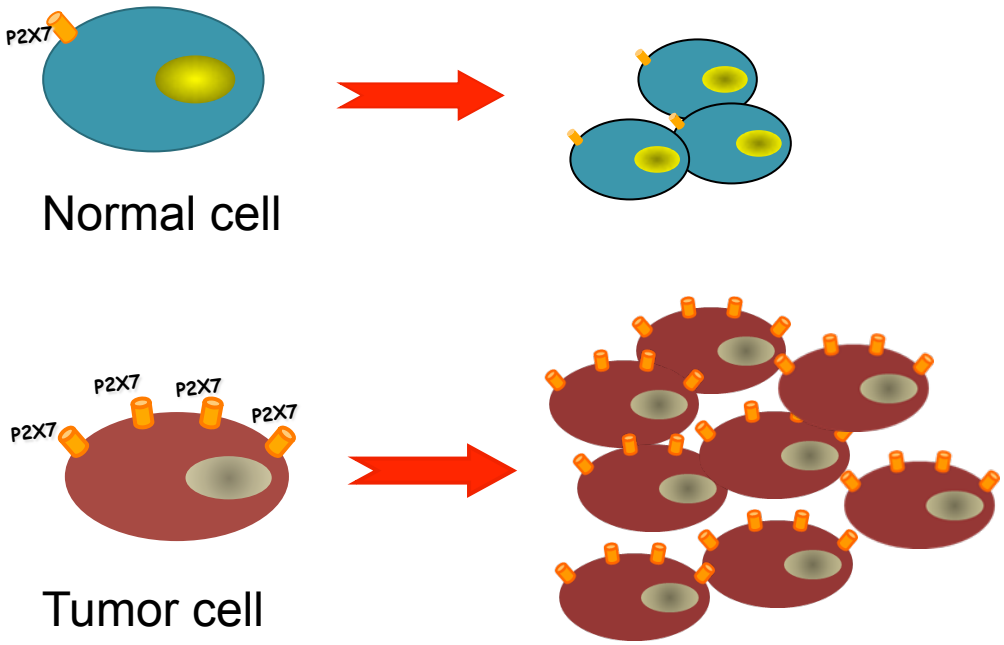
Rossi et al.; *Blood* 2007

P2X7 Receptor



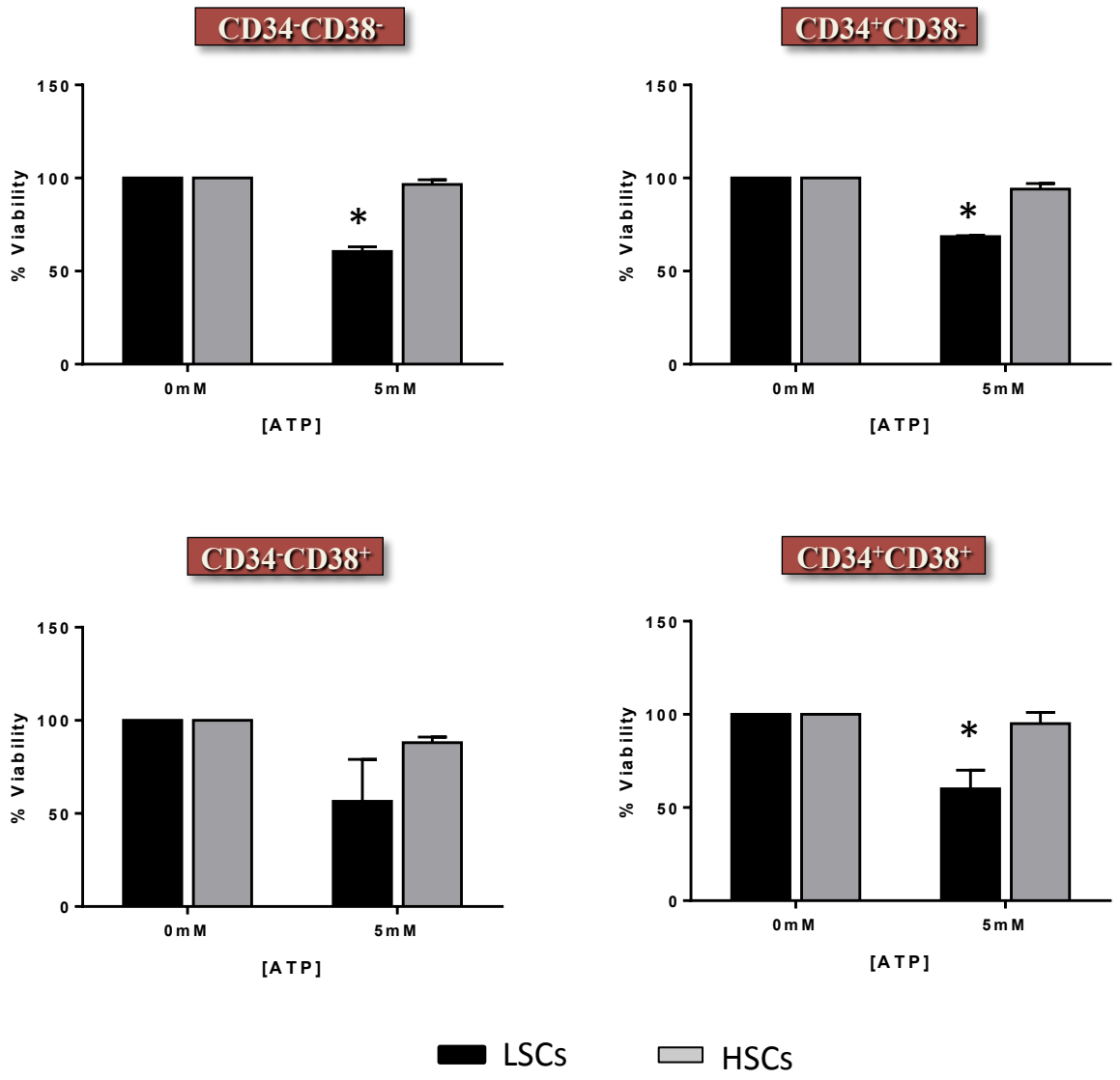
Skaper SD et al. *Cardiovasc Psychiatry Neurol* 2009. Modified

P2X7 receptor and cancer

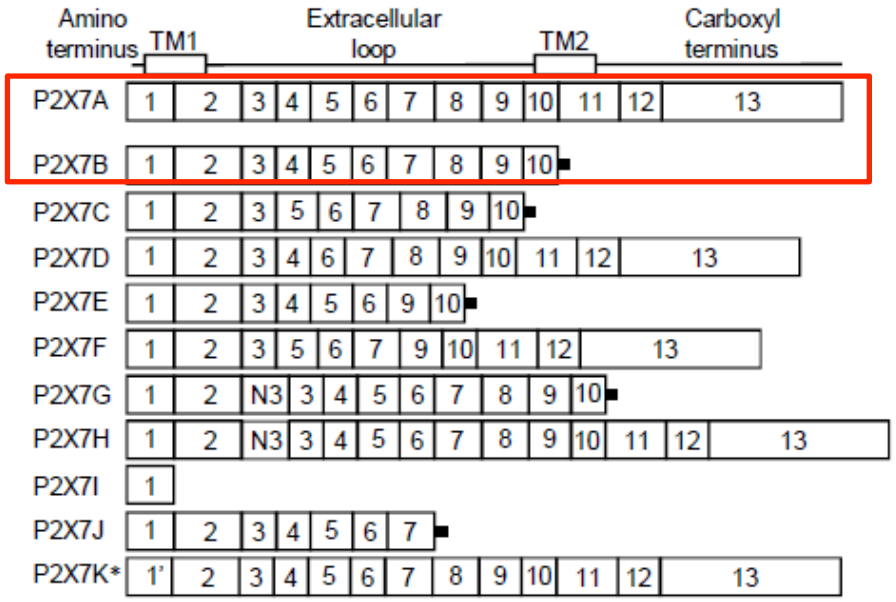


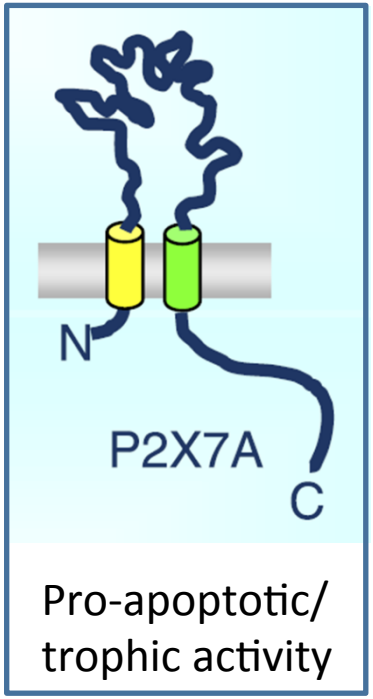
- ✓ P2X7 trophic stimulation supports cell proliferation
- ✓ Tumor cells over express P2X7
- ✓ $P2X7^{+}$ tumors show a more aggressive phenotype

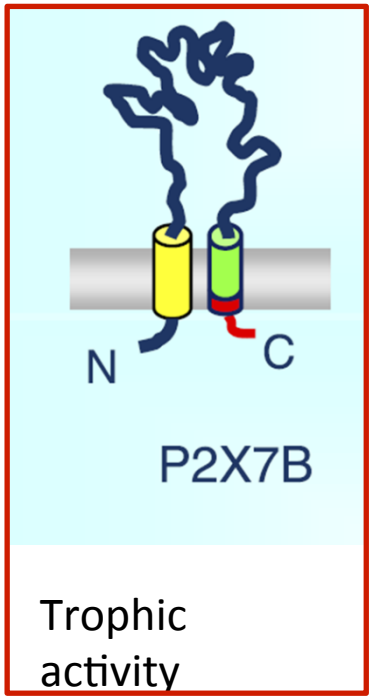
ATP treatment does not affect HSCs viability



P2X7 splice variants

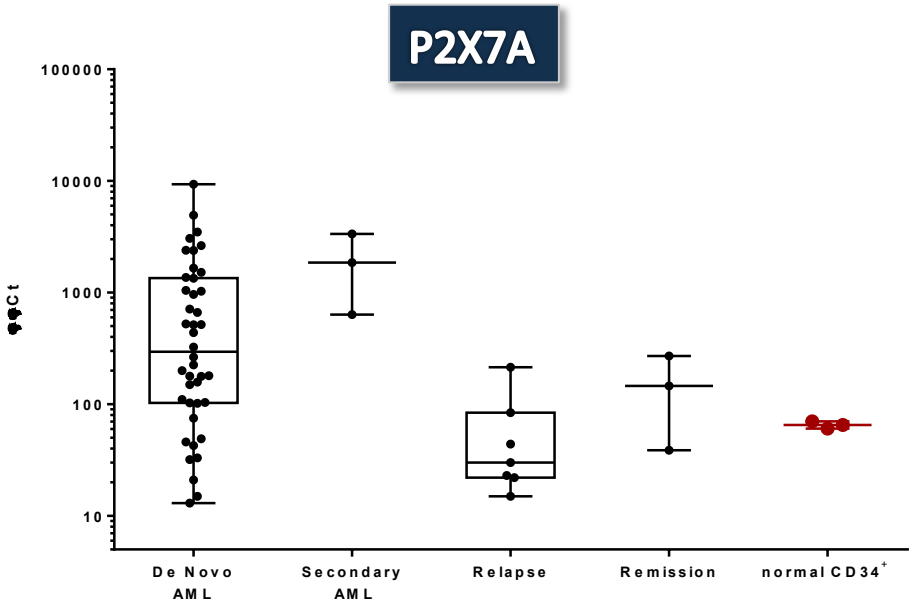


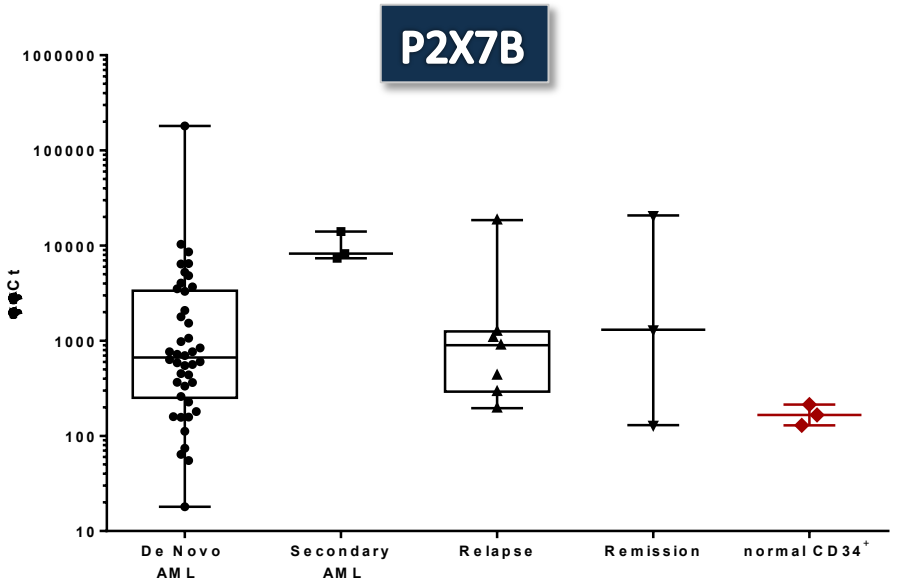




Sluyter R. et al. *Recent Pat DNA Gene Seq* 2011; Adinolfi E et al. *FASEB J* 2010

P2X7A and P2X7B characterization in AML patients





Conclusions

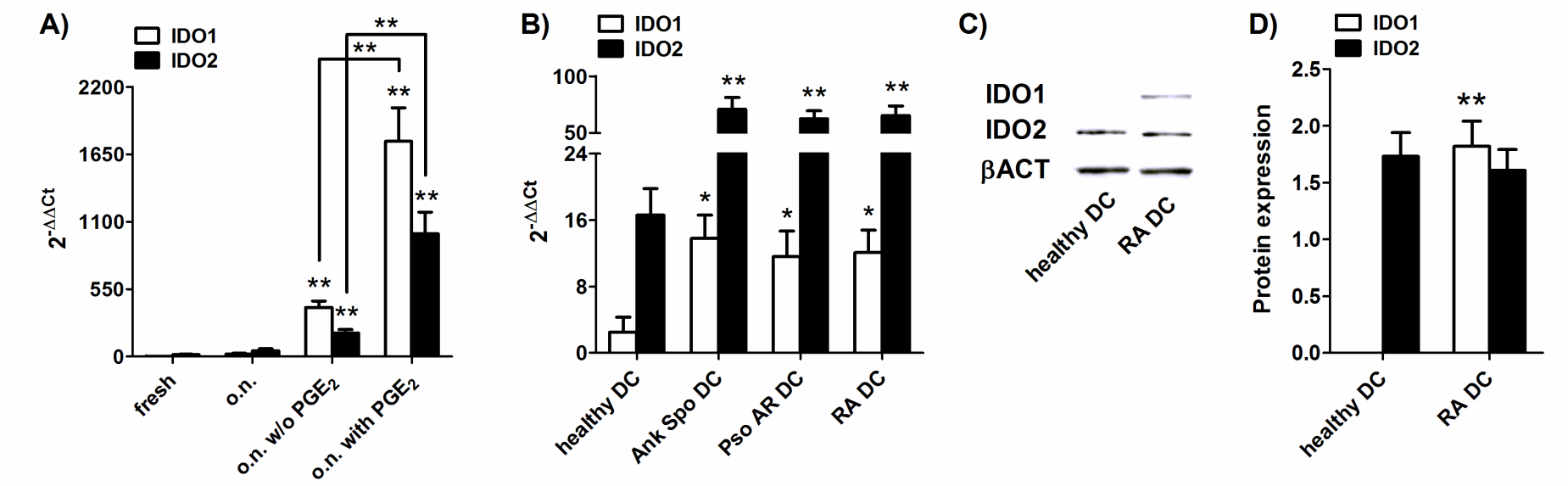
Disease relapse and toxicity of therapy represents the major limiting factors in AML treatment. Novel approaches that aim to reduce toxicity and to improve the efficacy of treatment are needed.

P2X7 stimulation by ATP has:

- direct and **selective toxicity** on leukemia cells and LSCs
- **low toxicity on HSCs**
- **synergistic effect** with anti-neoplastic drugs, allowing to reduce their dose

Good candidate for innovative therapy

IDO1 and IDO2 expression is differently regulated by inflammation

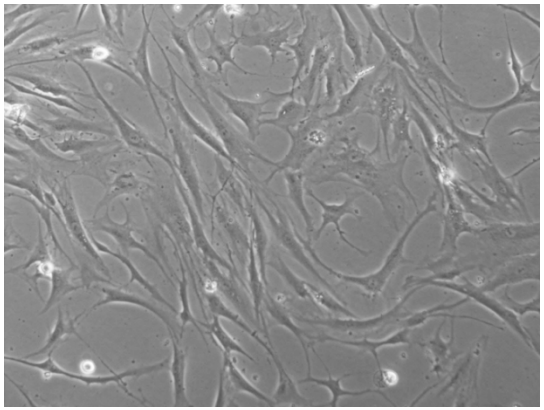


Trabanelli et al, J Immunol, 2014

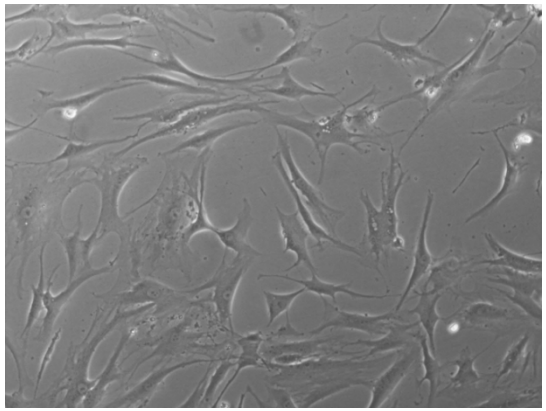
AML-MSC characterization

A. MORPHOLOGY

HD-MSCs



AML-MSCs



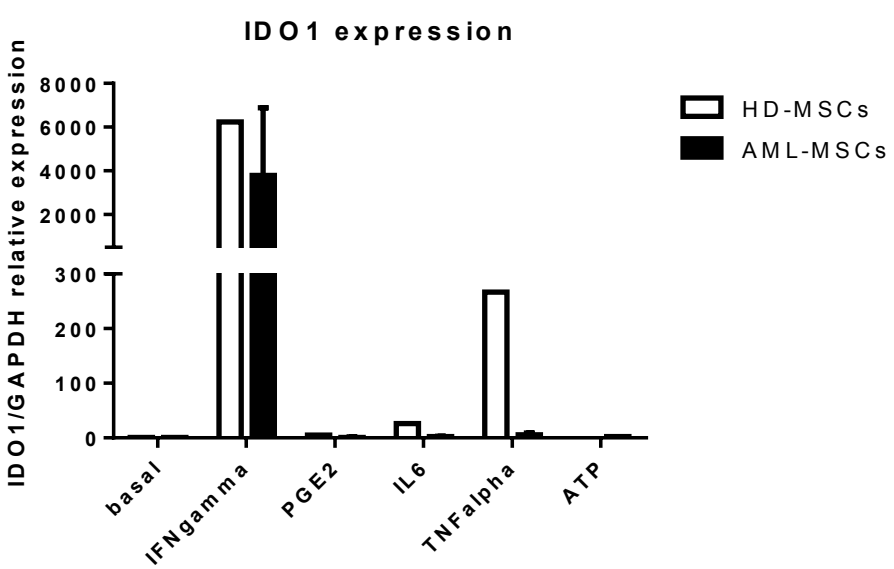
MSCs can be isolated and expanded from AML patients and show normal morphology

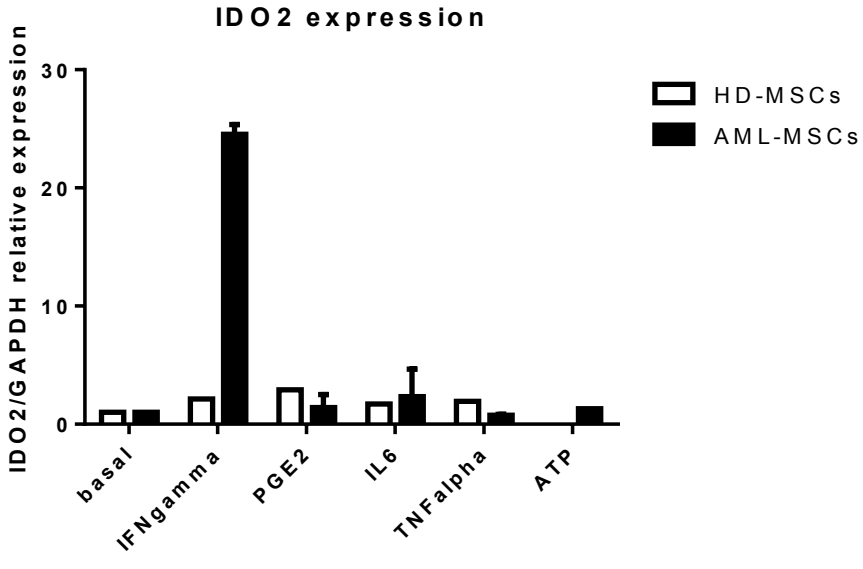
HD-MSCs: isolated from healthy donors

AML-MSCs: isolated from AML patients at diagnosis

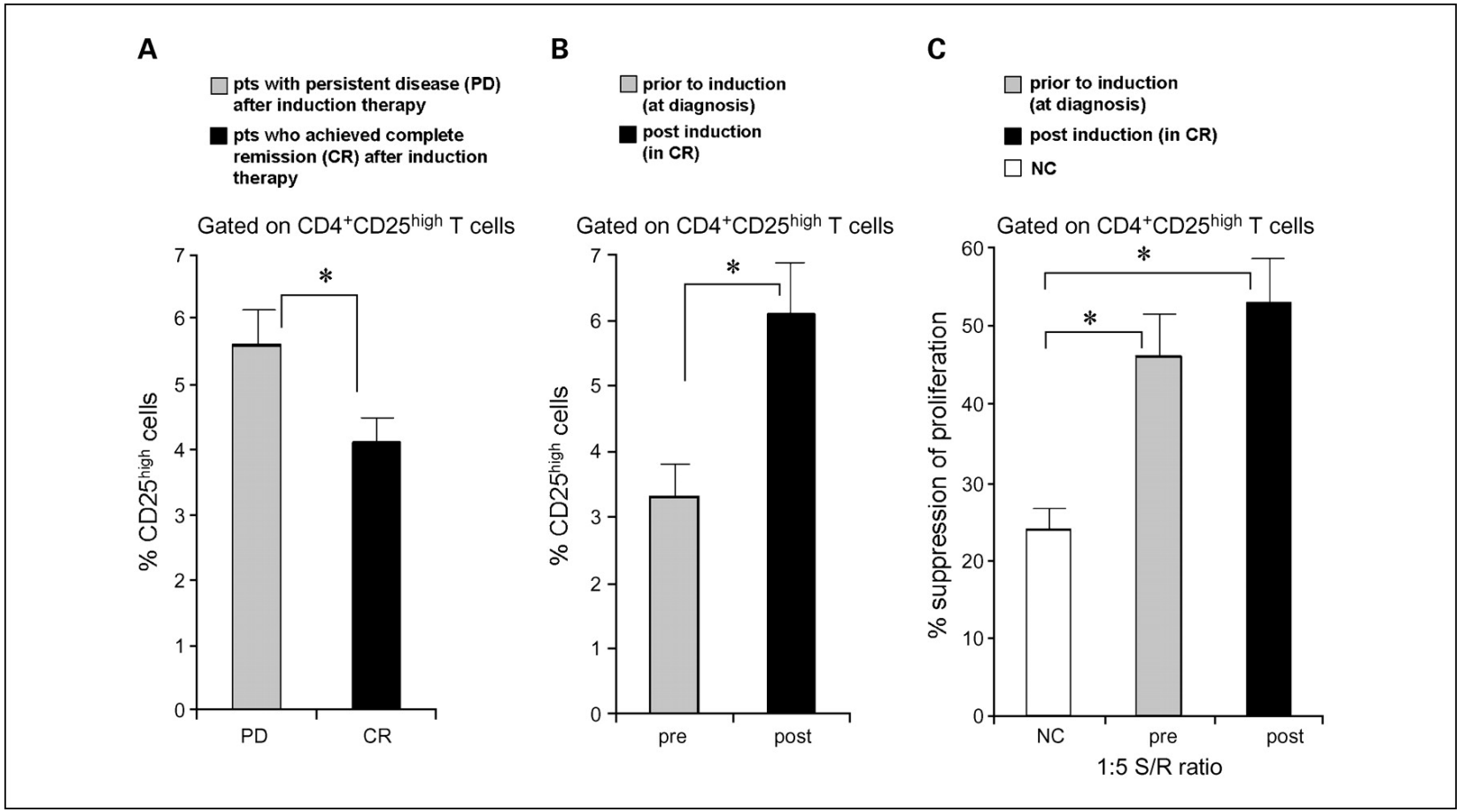
Tolerogenic pathway in AML-MSCs

IDO1/IDO2 EXPRESSION (mRNA)



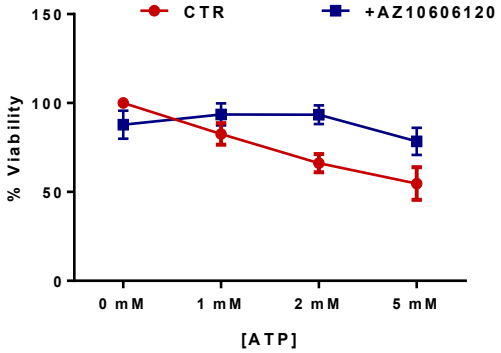


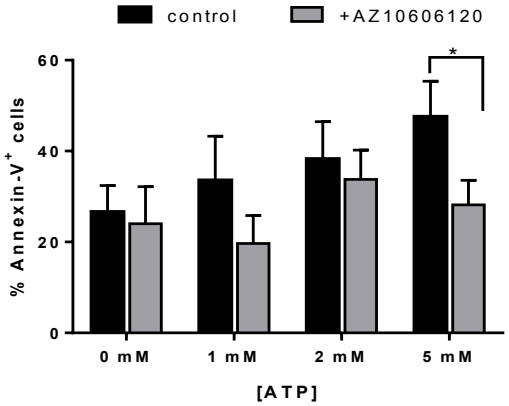
Frequency and suppressor function of Tregs in AML

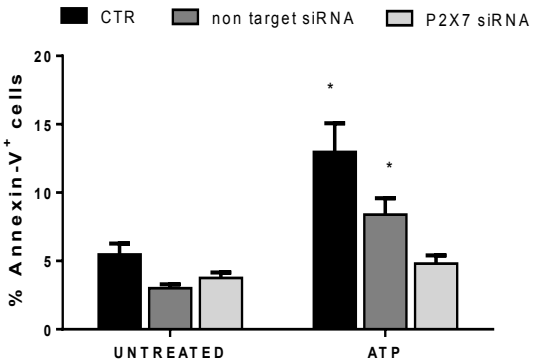


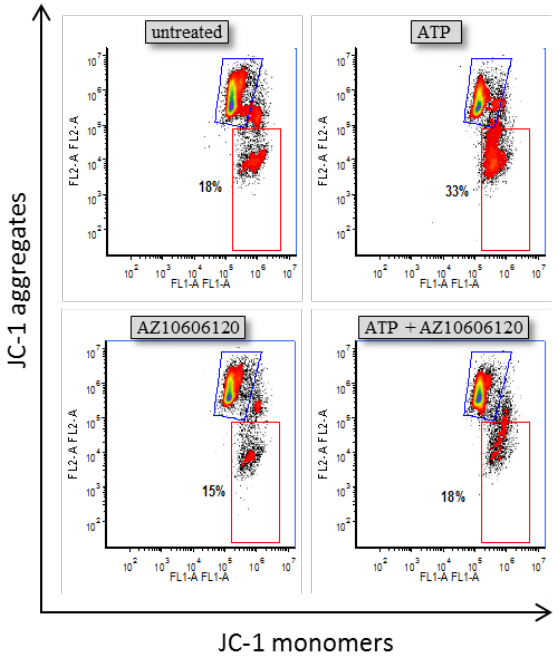
Szczepanski M J et al. Clin Cancer Res, 2009

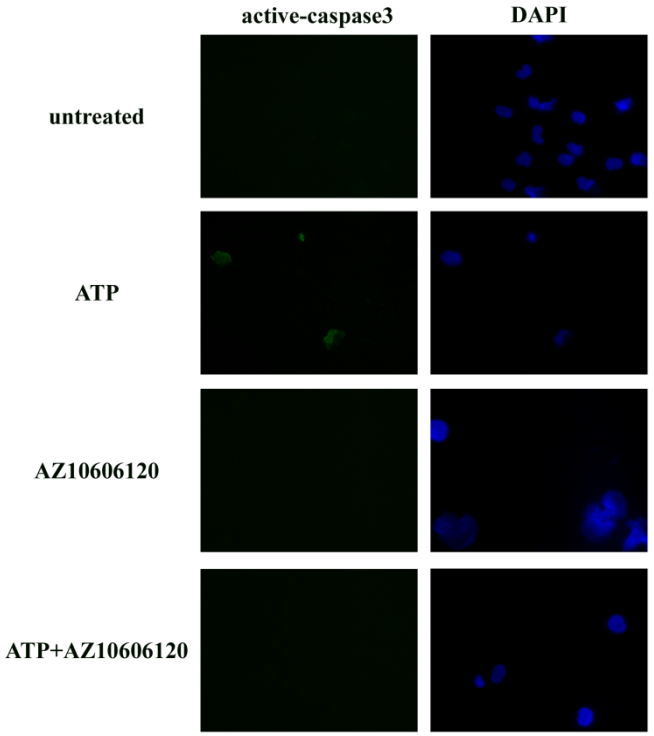
ATP induces apoptosis of AML cells via P2X7

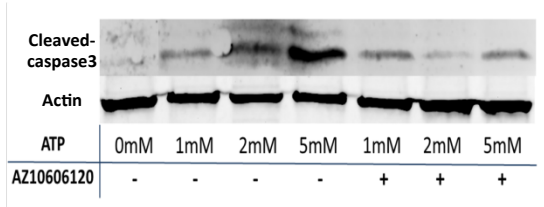












Tolerogenic pathway in AML-MSCs

IDO1/IDO2 EXPRESSION (protein)