«Targettare il microambiente midollare: le vescicole extracellulari»

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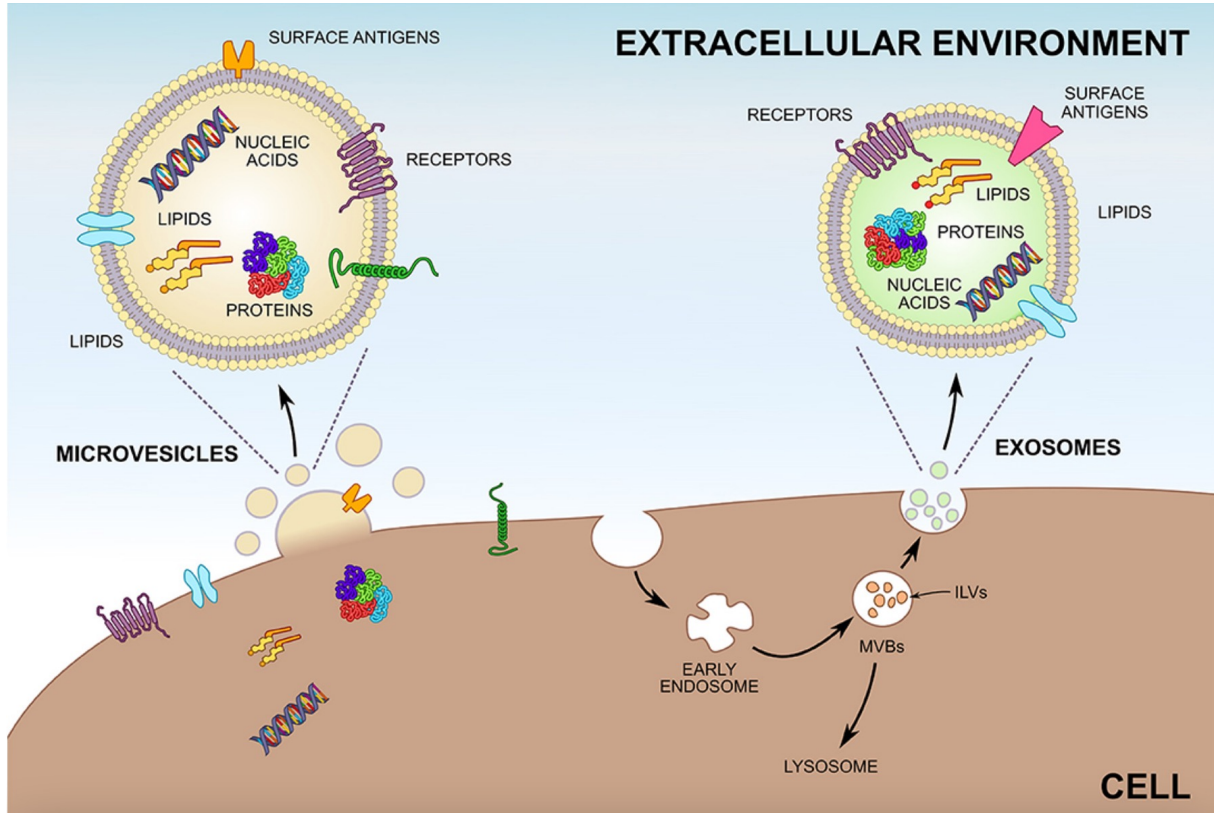
Disclosures of Name Surname

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

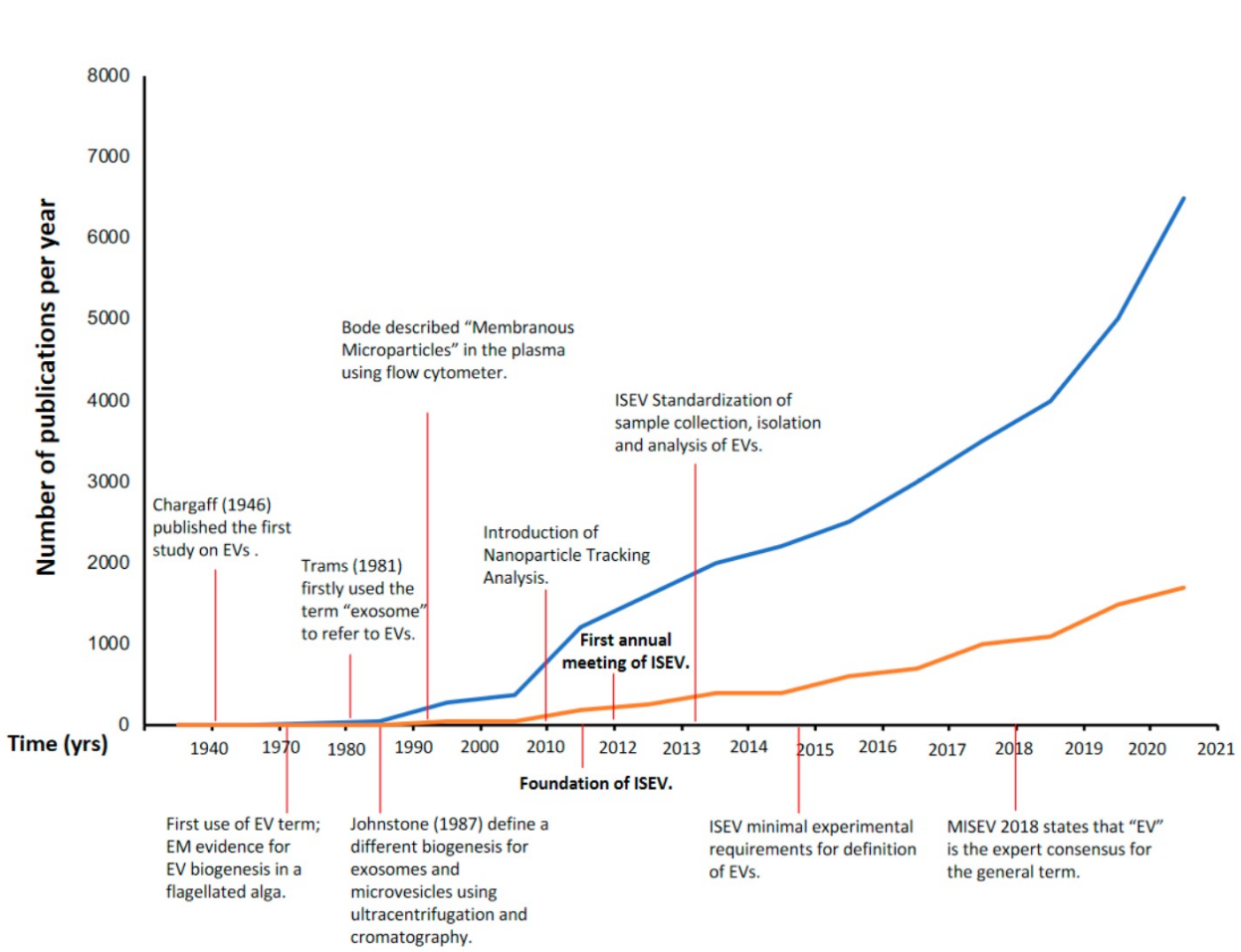
Introduction: What are Extracellular Vesicles (EVs)

- Lipid bilayer nano-sized vesicles containing various bioactive and cell-specific molecules, including DNA, microRNA (miRNA), proteins, and lipids;
- Can be released in the extracellular space from both normal and neoplastic cells;
- Can be isolated from both cell culture and body fluids;
- Major role in the intercellular communication.



Slomka A et al, Front. Immunol. 2018

Introduction: What are Extracellular Vesicles (EVs)



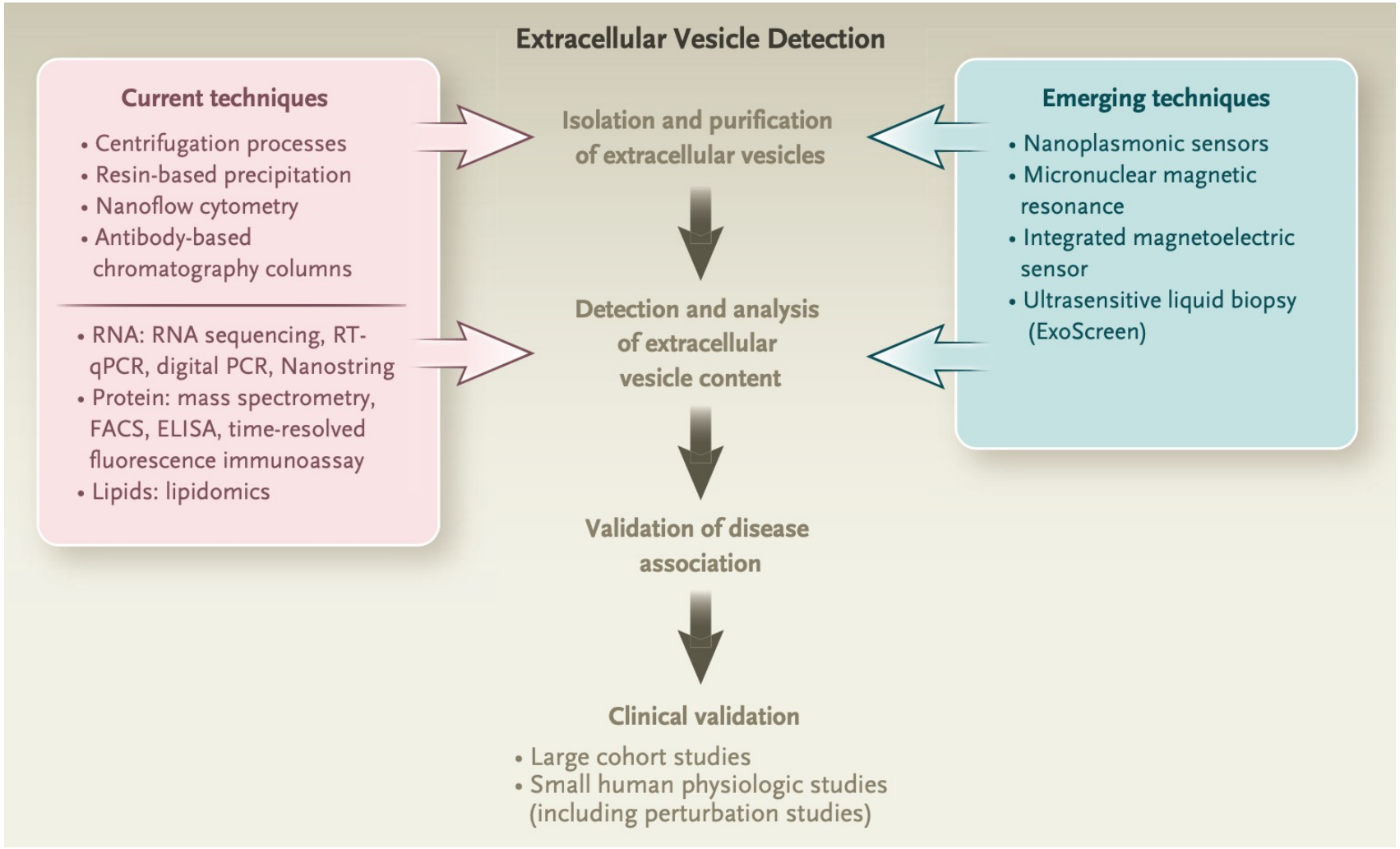
Bazzan E et al, Int J Mol Sci. 2021

EVs Classification According to The International Society of EVs (ISEV) 2018 Guidelines

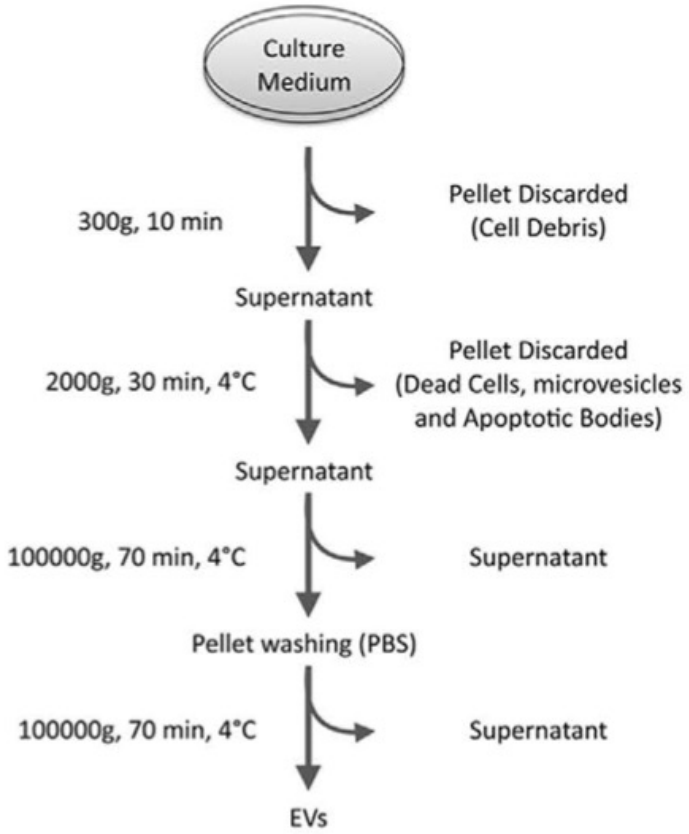
	Large EVs	***Small EVs***
Origin	Plasma membrane	Endosome
Size	>200 nm	< 200 nm
Sub-populations	Apoptotic bodies Large oncosomes Microvesicles	Exosomes

Théry C et al, J.Extracell Vesicles 2018

Proposed isolation methods



Shah et al, N Engl J Med 2018



Di Trapani M et al, Sci Rep 2016

Role in Cancer and Clinical Applications

PHYSICOCHEMICAL FEATURES:

Bioactive and stable
Tissue-direct delivery
Favourable cell up-take
Low toxicity
Favourable pharmacokinetics
Immunorecognition
Biocompatibility

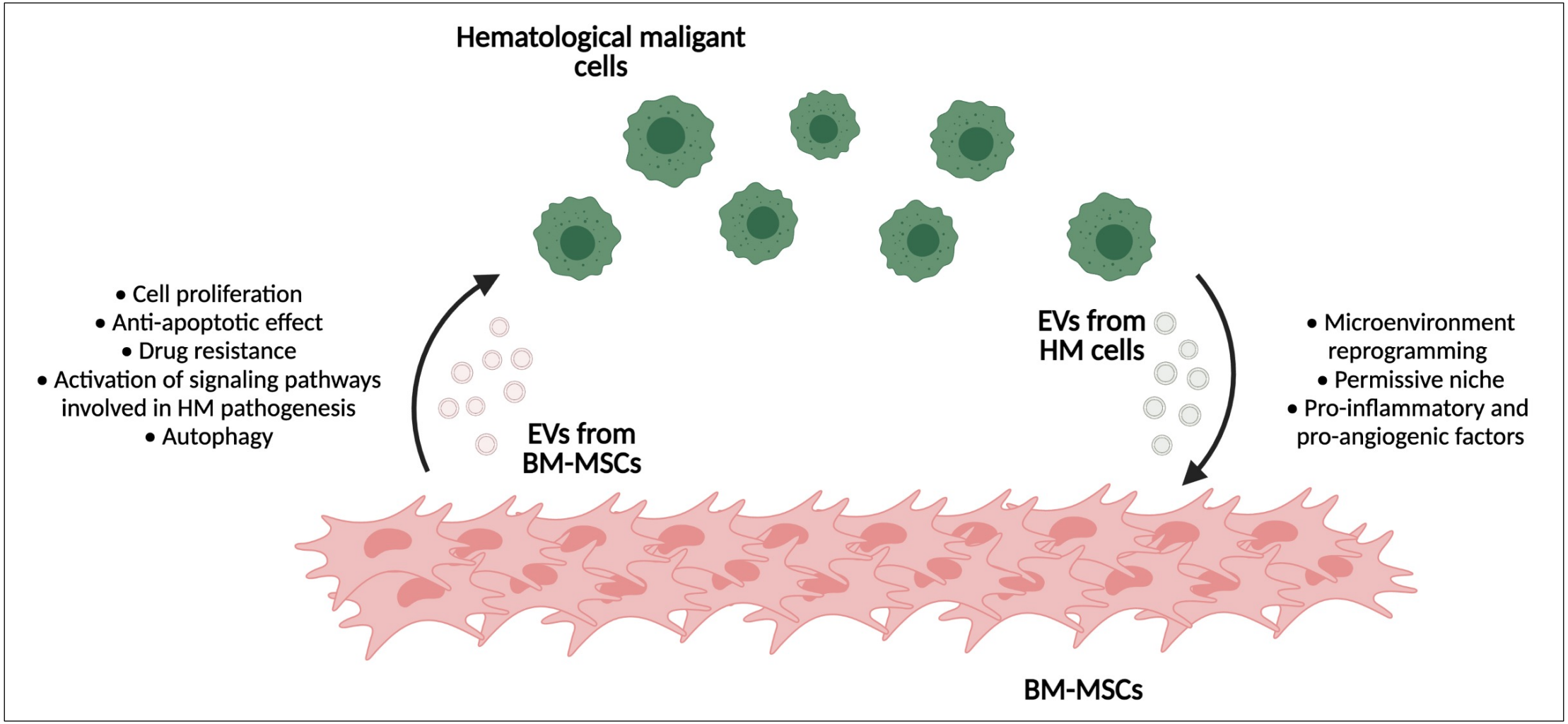
CLINICAL APPLICATIONS:

Biomarkers
Therapeutic targets
Therapeutic agents

ROLE IN CANCER:

Tumor support
Metastasis
Angiogenesis
Progression
Immune escape
Drug resistance

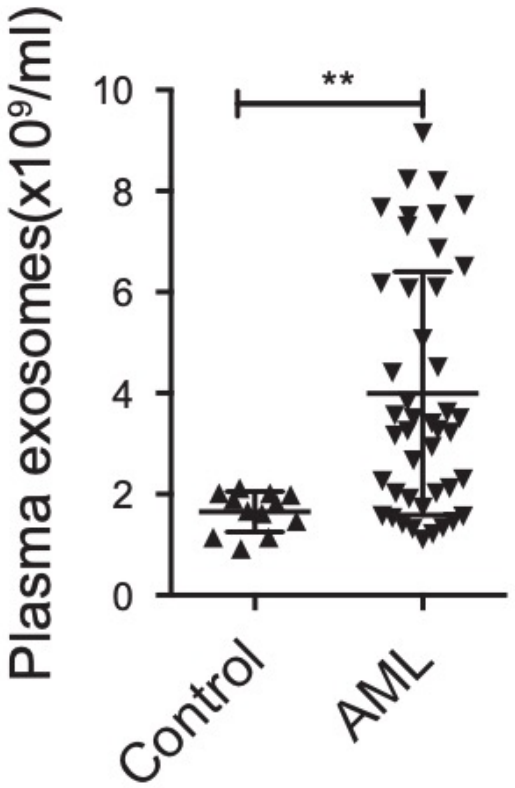
EV-based communication between microenvironment and cancer cells

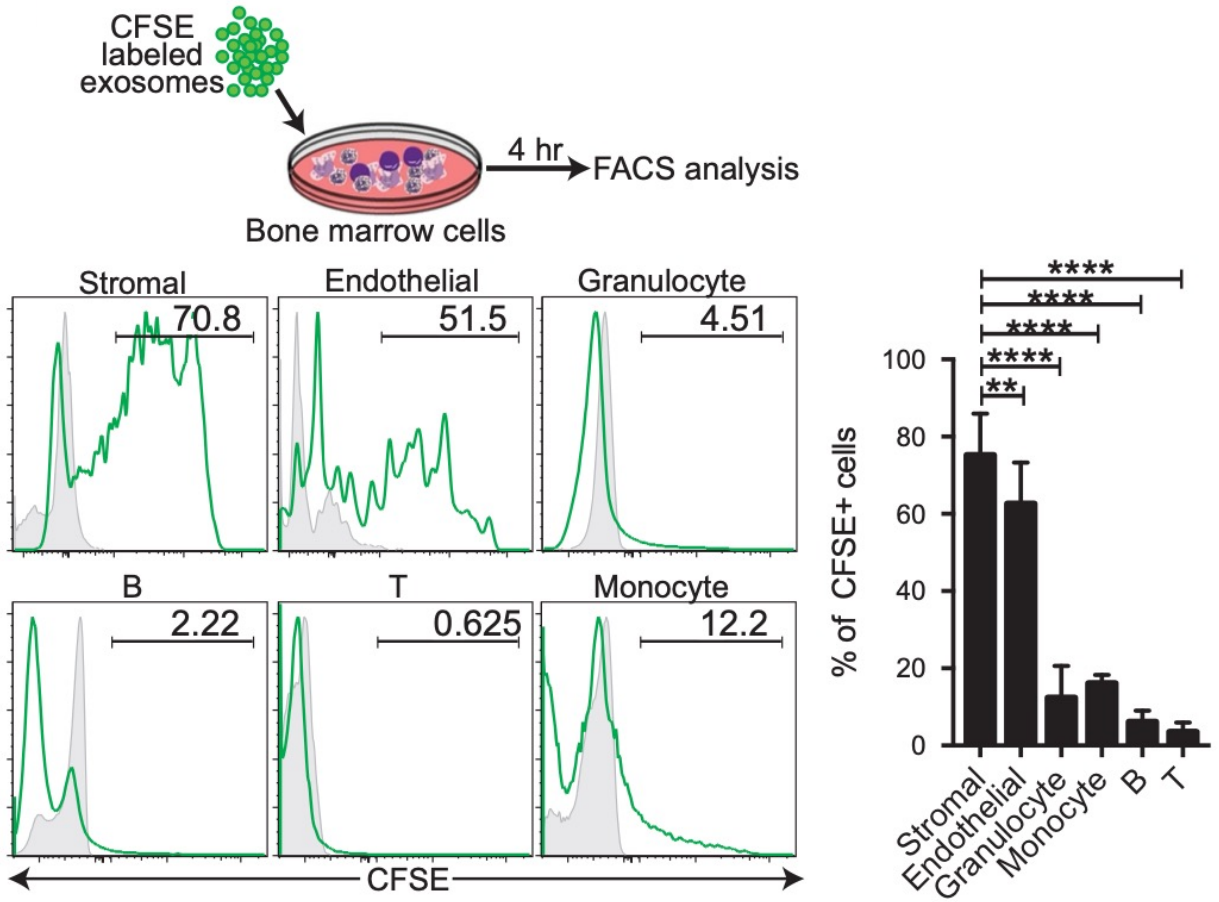


BM-MSCs = bone marrow mesenchymal stromal cells

Bazzoni R, Tanasi I, Krampera M, Stem Cells under revision

AML Small EVs on Microenvironment and Immunomodulation



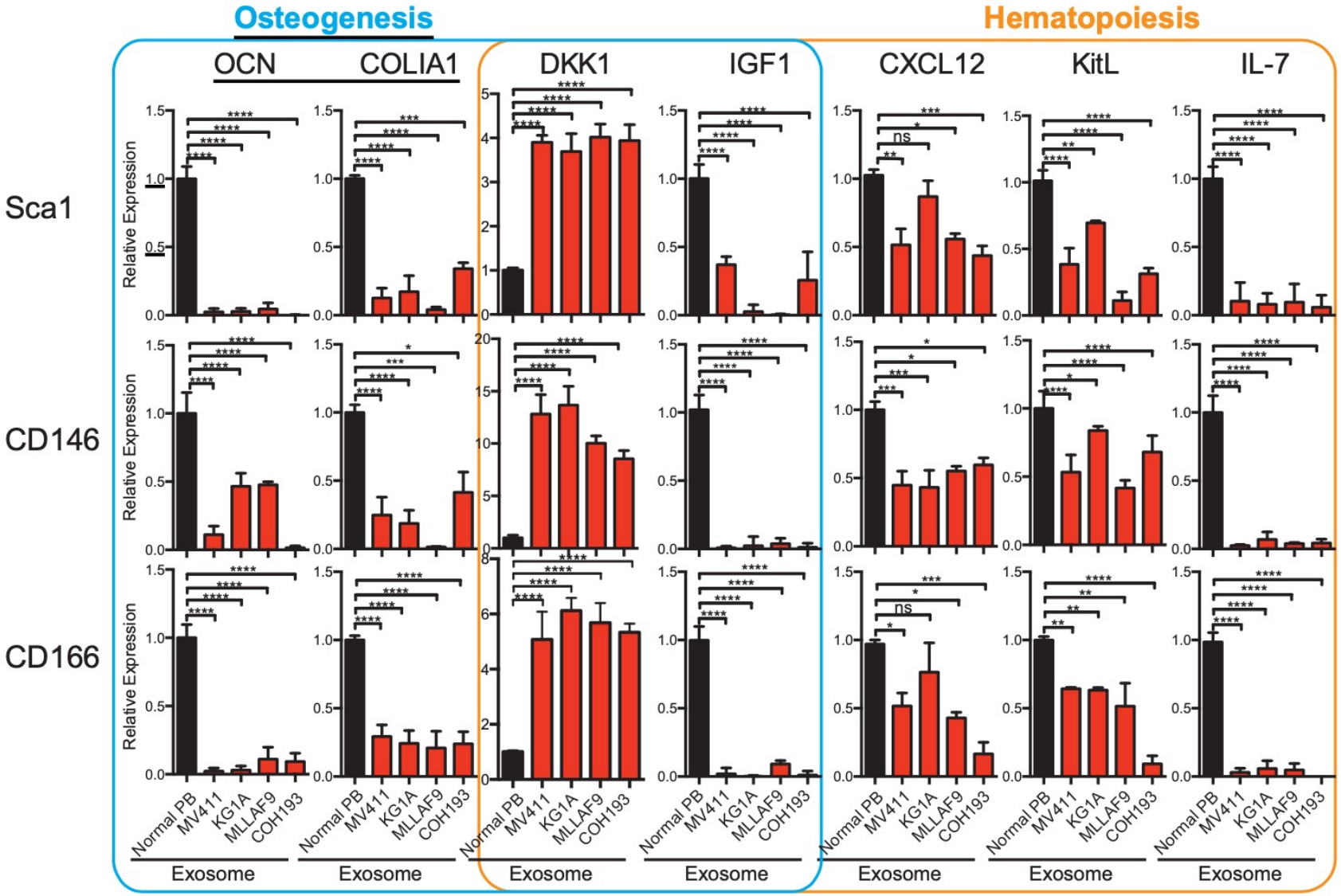


- BM stromal and endothelial fractions are preferential targets for AML-derived exosomes.

CTRL, n=12; de novo o secondary AML, n= 43

Kumar B et al, Leukemia 2018

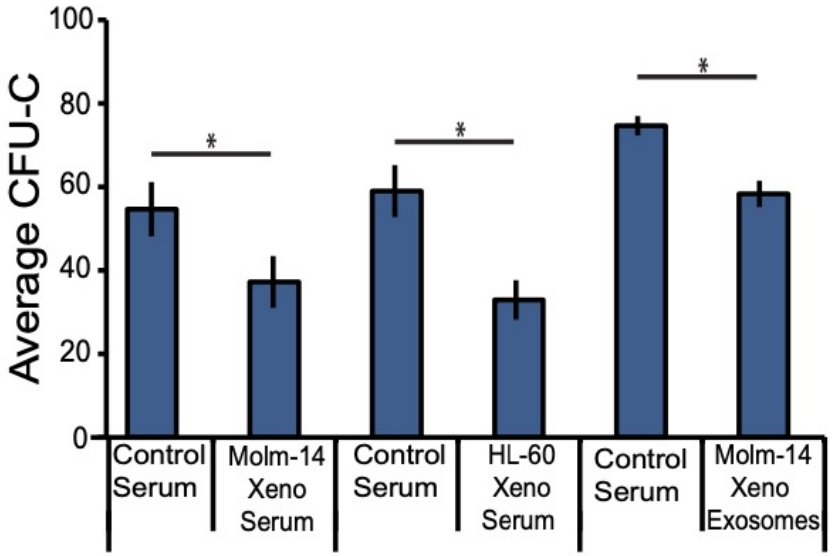
AML Small EVs on Microenvironment and Immunomodulation

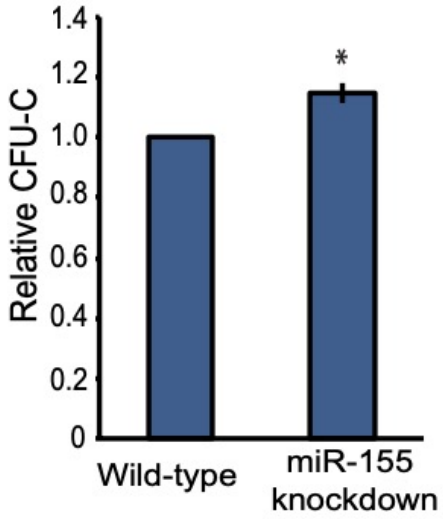


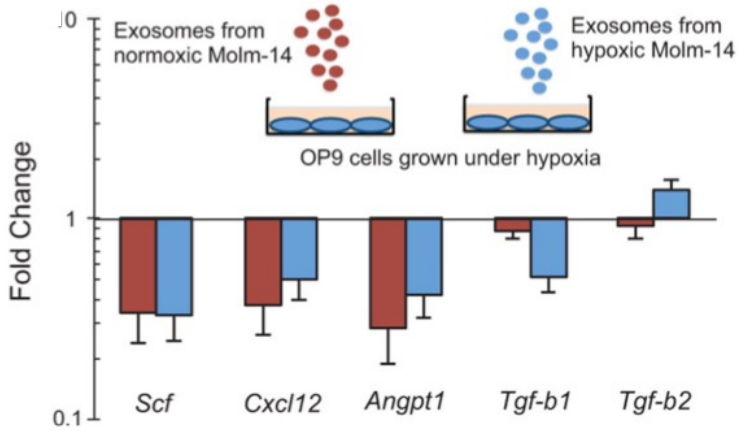
- AML-derived exosomes modulate gene expression in BM stroma and suppress osteogenic differentiation of mesenchymal stromal progenitors.

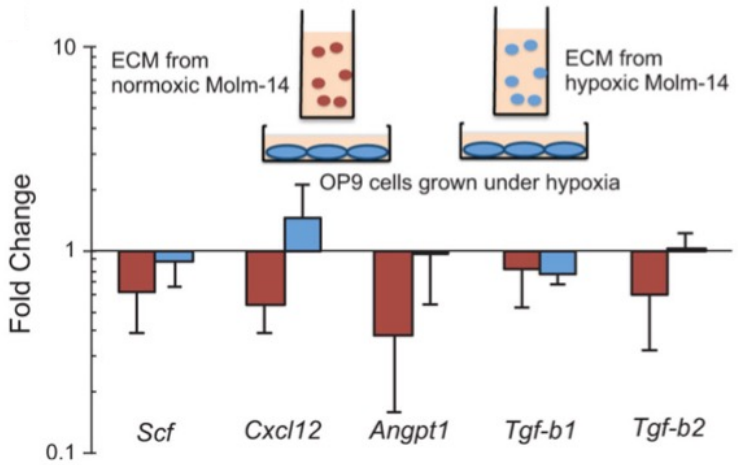
Kumar B et al, Leukemia 2018

AML Small EVs on Microenvironment and Immunomodulation





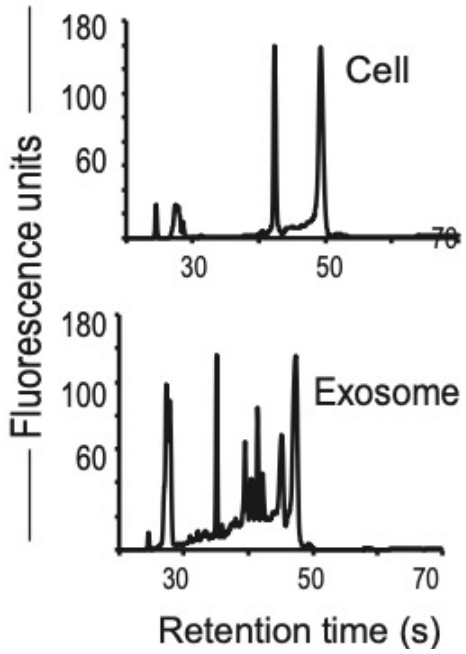




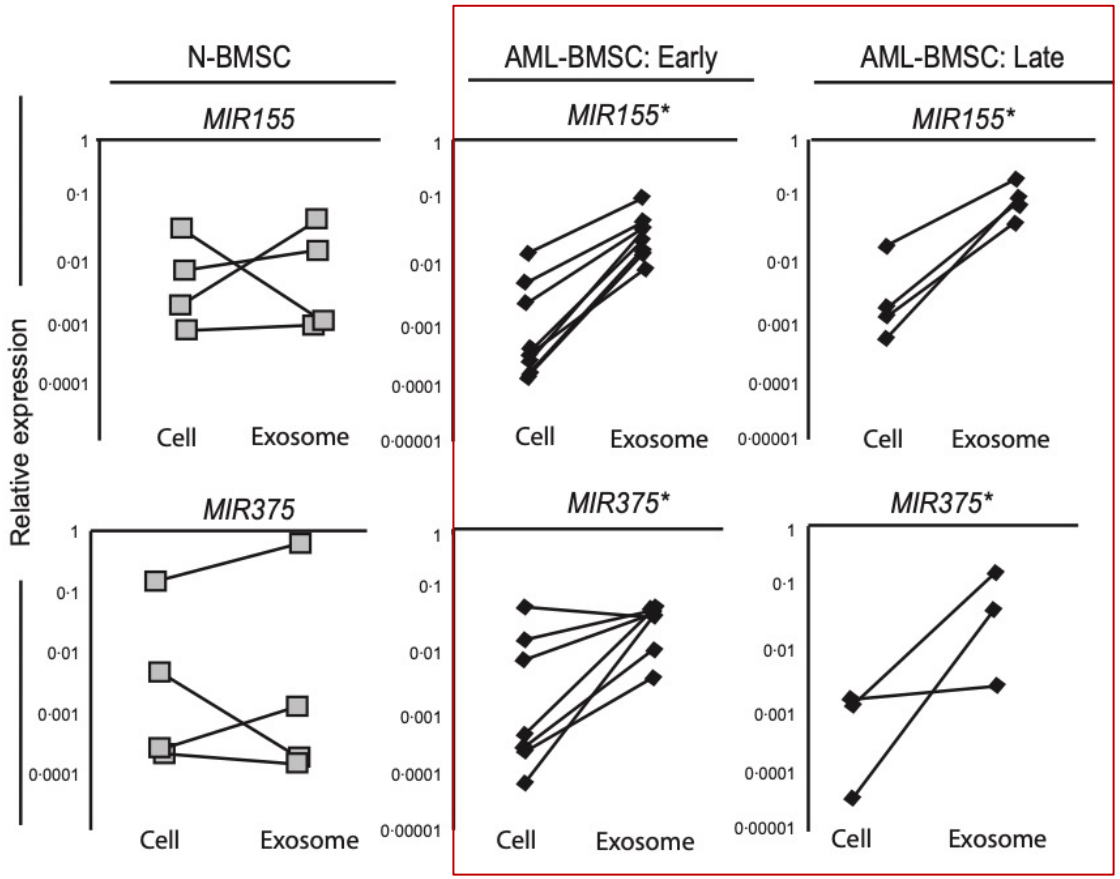
- Leukemia-derived exosomes are sufficient to induce systemic impairment of hematopoiesis;
- Exosome-delivered miRNAs down-regulate critical hematopoietic regulators.

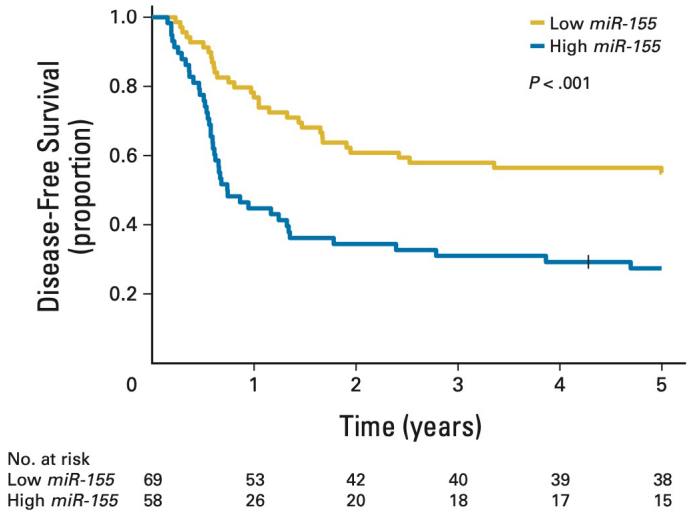
Hornick N et al, Cancer Exos 2016
Huan J et al, Leukemia 2015

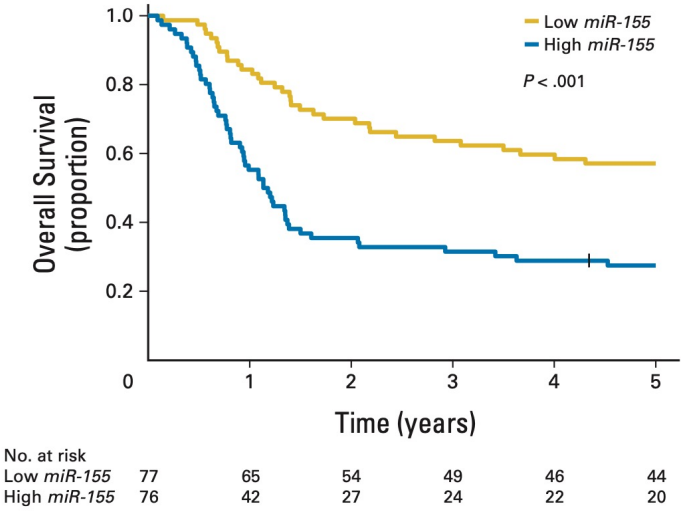
AML Small EVs on Microenvironment and Immunomodulation



N= normal; BMSC= Bone Marrow Mesenchymal Stromal Cells;

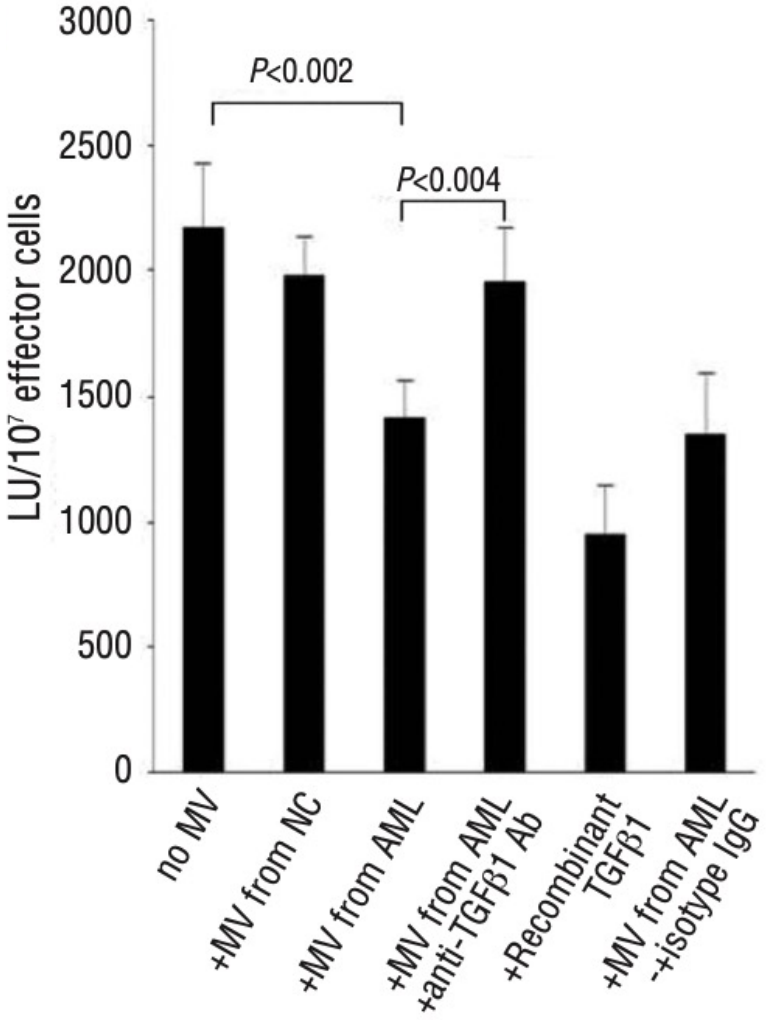




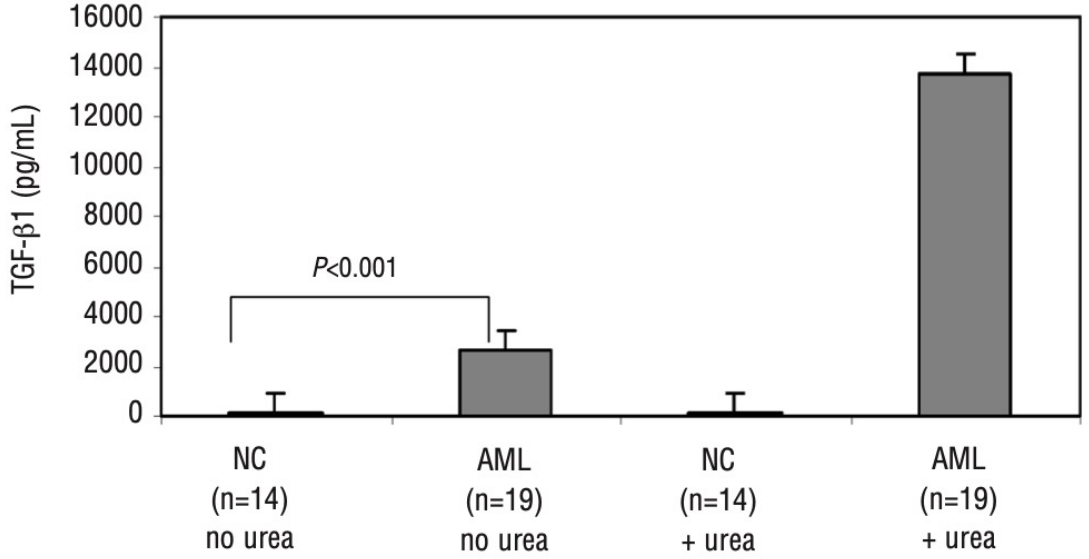


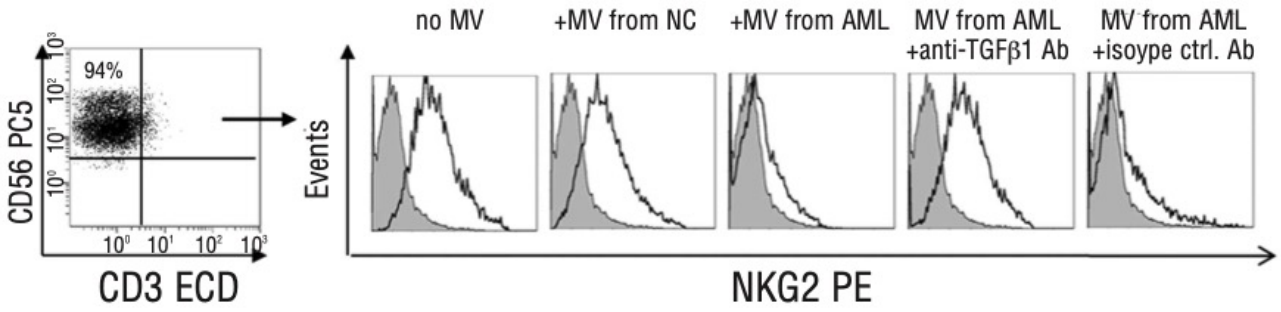
Viola S et al, Br J Hematology 2016
Marcucci G et al, JCO 2013

AML Small EVs on Microenvironment and Immunomodulation



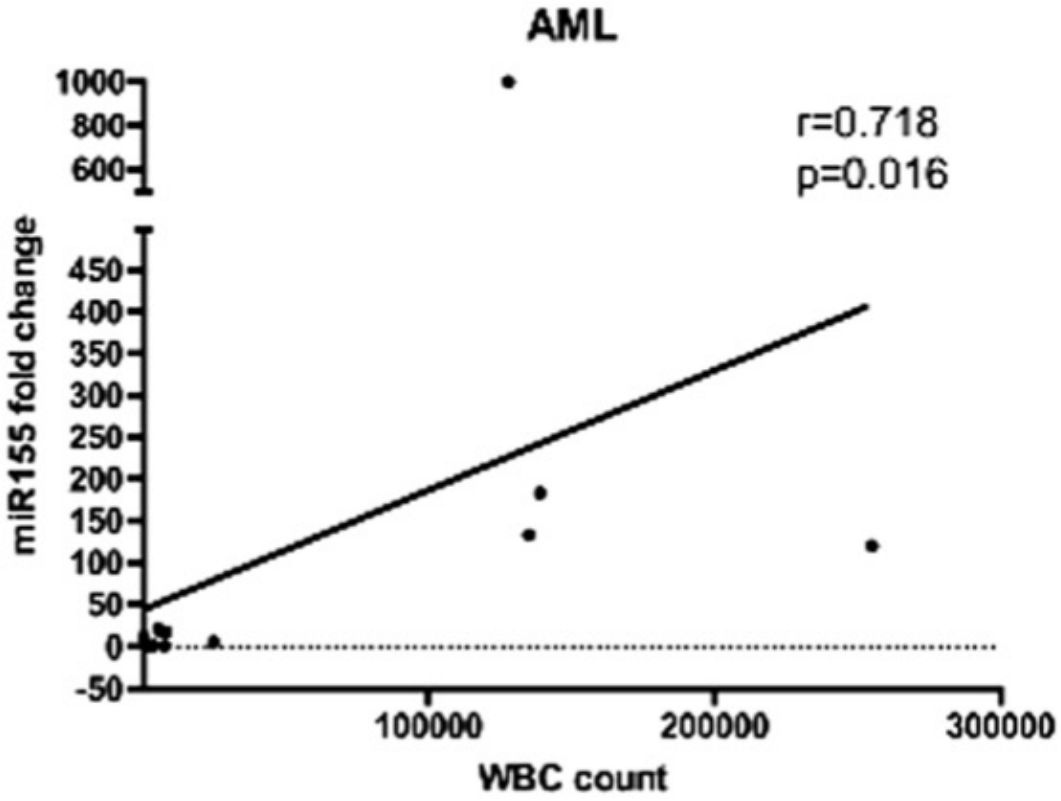
Szczepanski M et al, Haematologica 2011



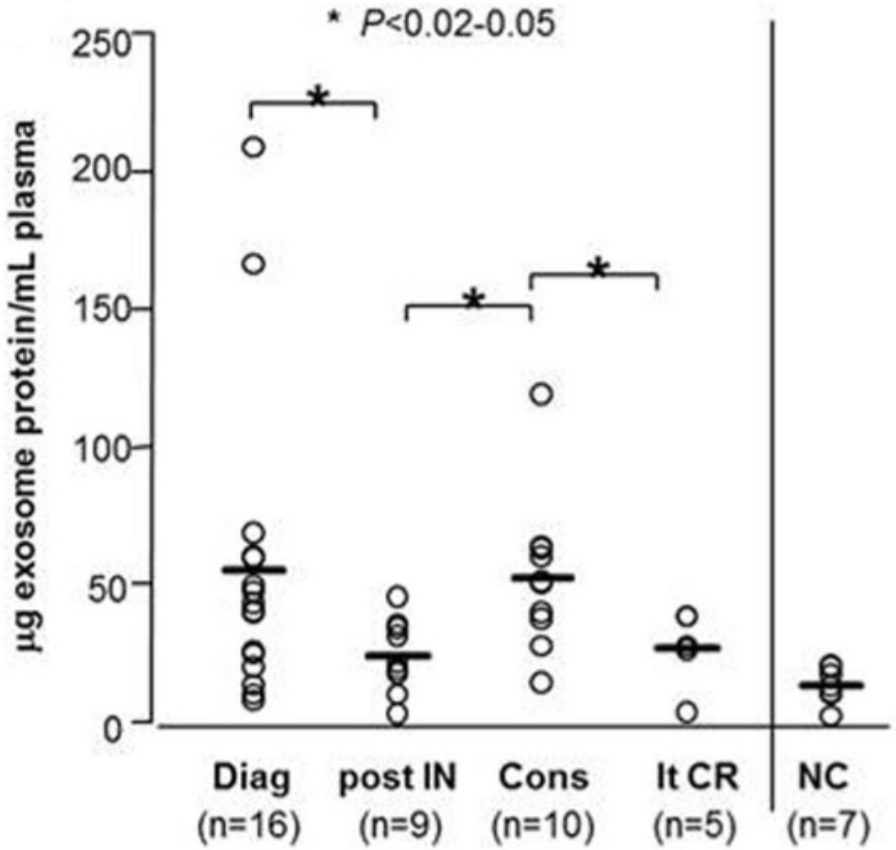


- EVs downregulate NKG2D expression via TGF-β1 and suppress NK cell activity in AML patients.

EVs as Disease Biomarkers

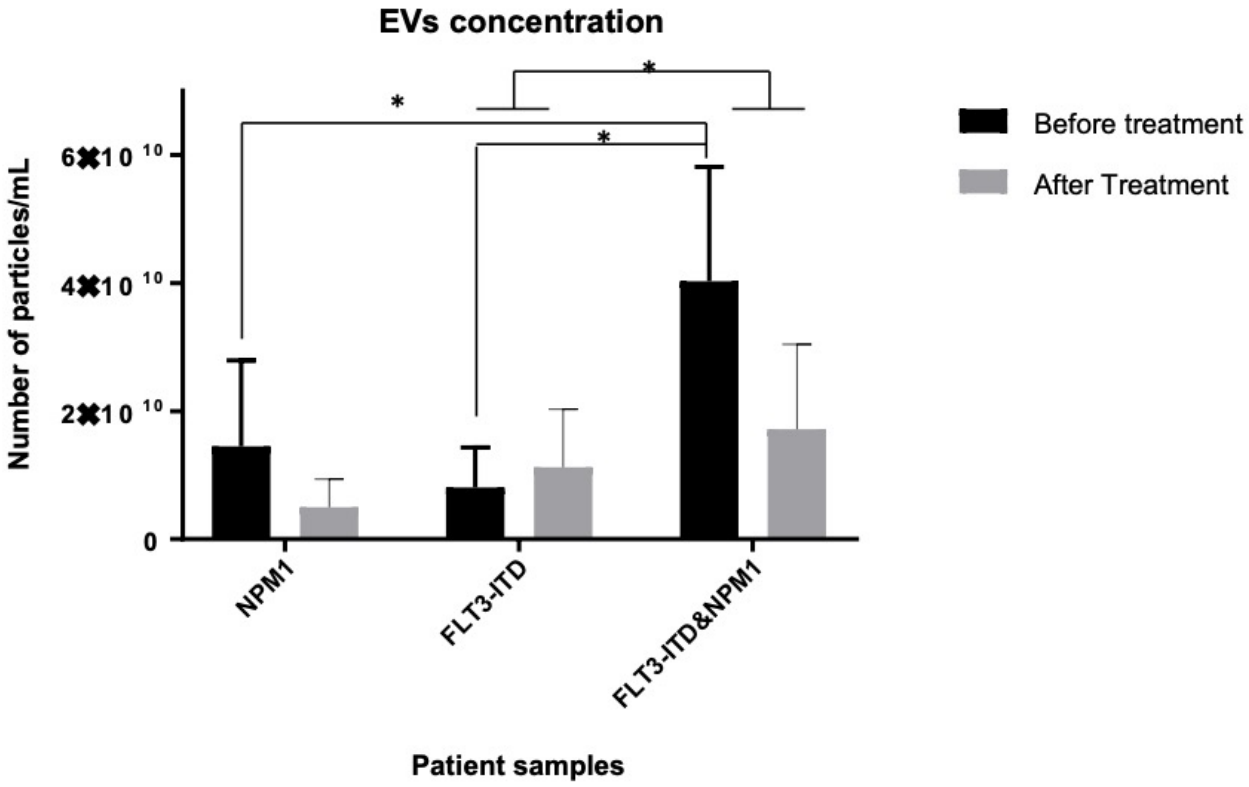


Caivano A et al, Cell Oncol. 2017



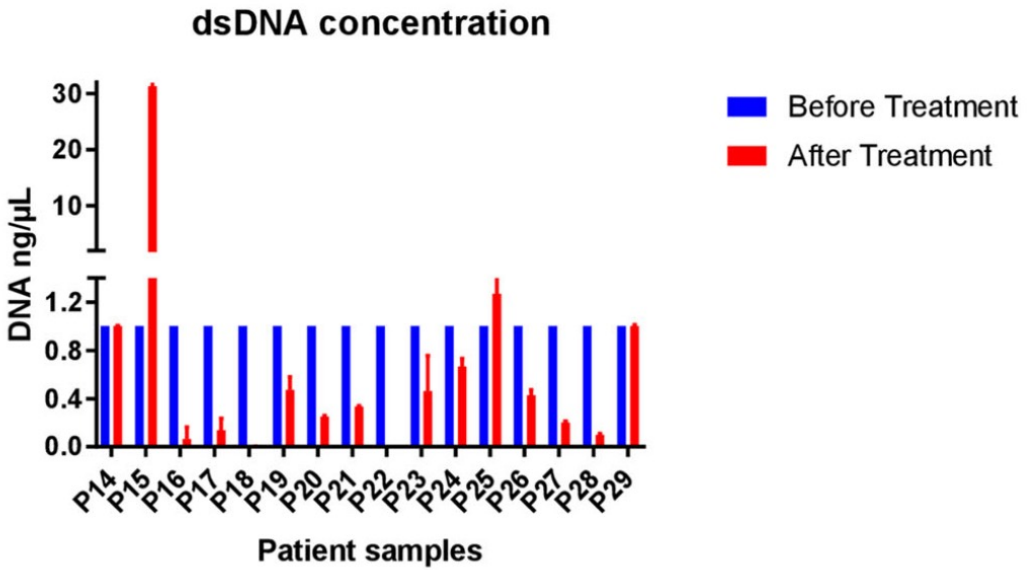
Hong C et al, Front Immunol.2014

EVs as Disease Biomarkers



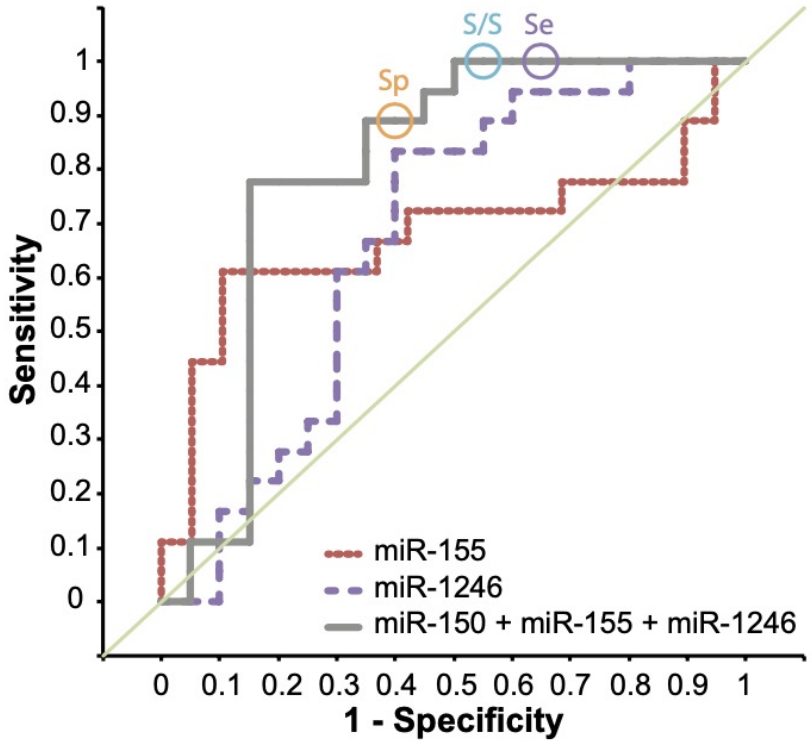
Kunz F et al, Ann Hematol 2019

Patient	Cancer type	Mutation in gDNA of AML cells	EV-DNA
P1	AML-M1	NPM1 FLT3/TKD WT1 GATA2 ETV6 (SNP) ZRSR2(SNP)	NPM1 FLT3/TKD WT1 GATA2 ETV6 (SNP) ZRSR2(SNP)
P2	AML-M2	FLT3/ITD RAD21 KIT EZH2	n.d n.d n.d n.d
P3	AML-M3	GATA2 (SNP) NOTCH1 (SNP)	GATA2 (SNP) NOTCH1 (SNP)
P4	AML-M4	NRAS KIT (SNP) PHF6 (SNP)	NRAS KIT (SNP) PHF6 (SNP)



Kontopoulou E et al, Ann Hematol 2020

EVs as Disease Biomarkers



For Specificity (Sp):

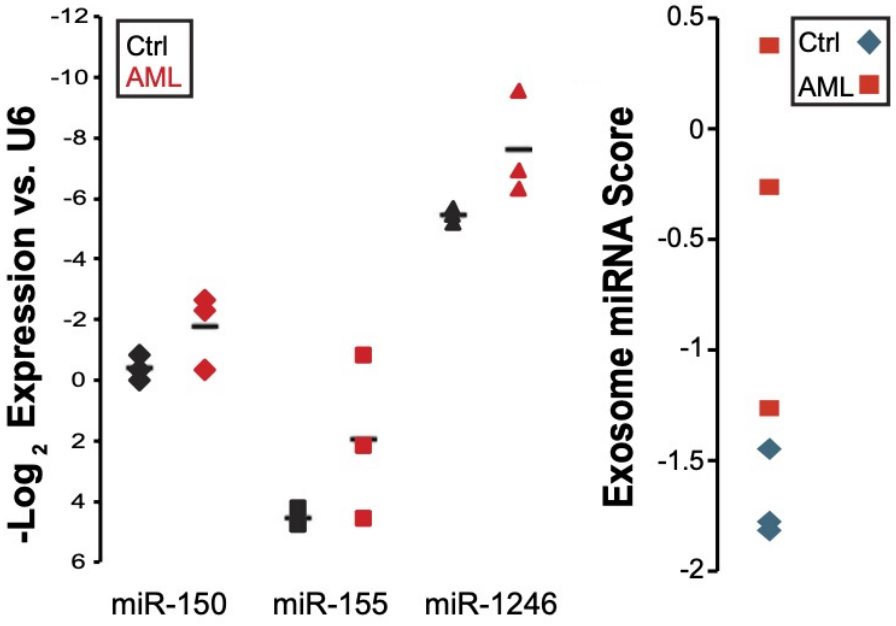
Cohort	Positive	Negative	%CD45+
CD34+	0/4	4/4	0-0.1%
pre-Tx	4/9	5/9	<0.1%
End of Tx	5/9	4/9	0.1-1.5%
1 wk Post-Tx	5/9	4/9	0.1-23.0%

Balance Sensitivity / Specificity (S/S):

Cohort	Positive	Negative	%CD45+
CD34+	1/4	3/4	0-0.1%
pre-Tx	5/9	4/9	<0.1%
End of Tx	8/9	1/9	0.1-1.5%
1 wk Post-Tx	8/9	1/9	0.1-23.0%

For Sensitivity (Se):

Cohort	Positive	Negative	%CD45+
CD34+	2/4	2/4	0-0.1%
pre-Tx	7/9	2/9	<0.1%
End of Tx	8/9	1/9	0.1-1.5%
1 wk Post-Tx	9/9	0/9	0.1-23.0%

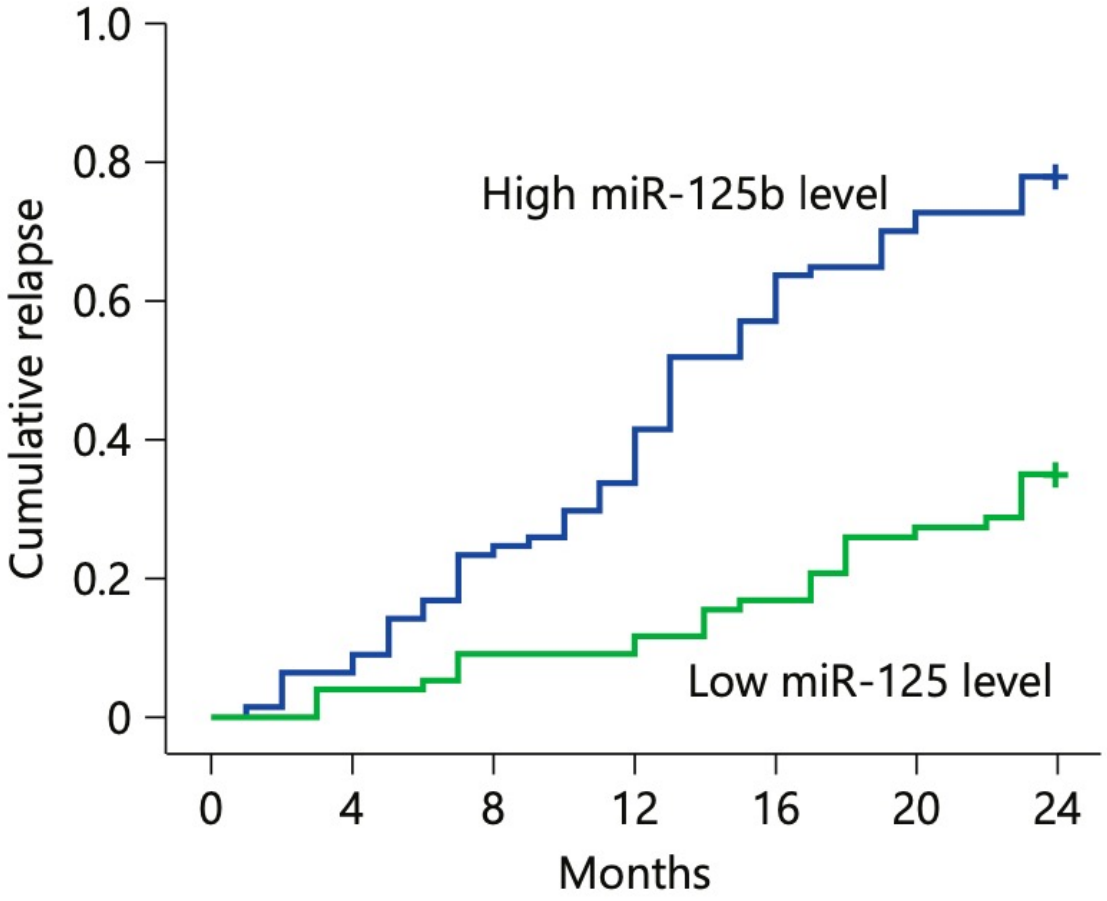


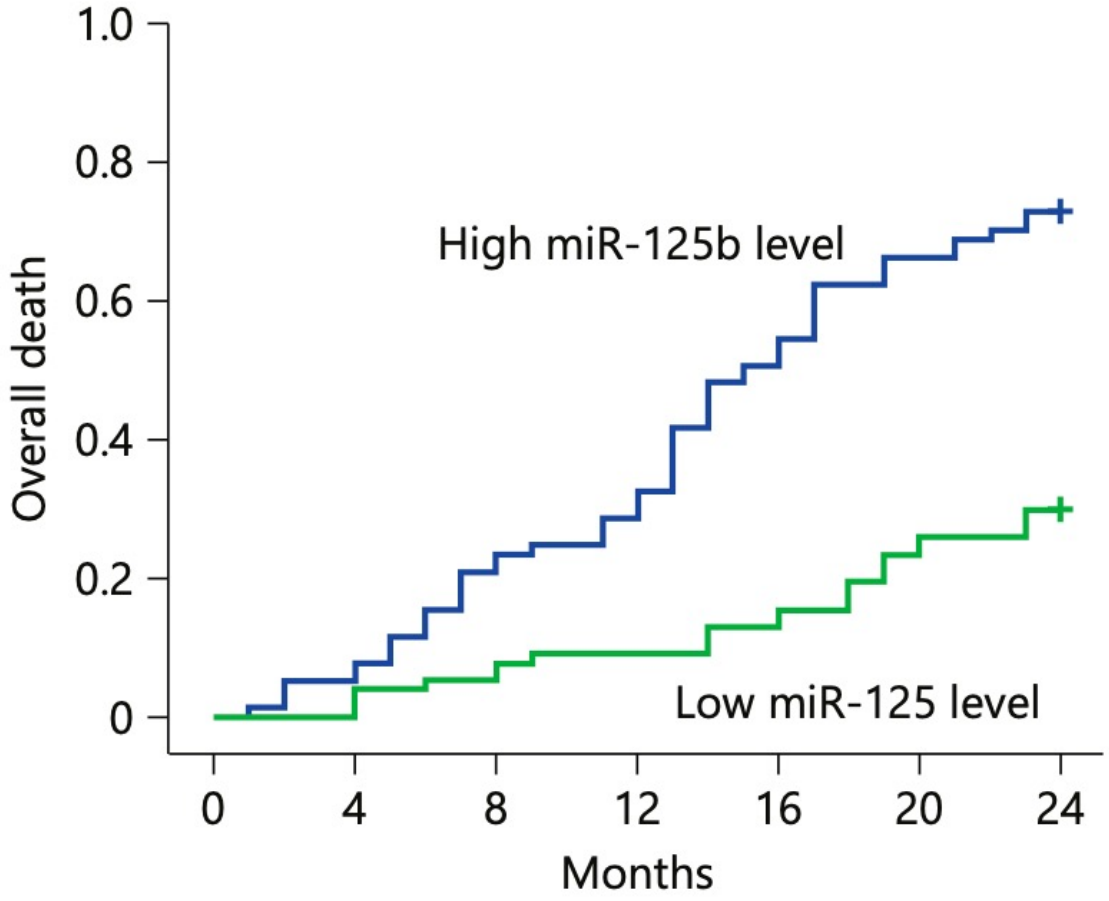
- Circulating exosomal miRNA can be detected at low marrow tumor burden and before reappearance of circulating blasts.

Hornick N et al, Sci Rep 2015

EVs as Disease Biomarkers

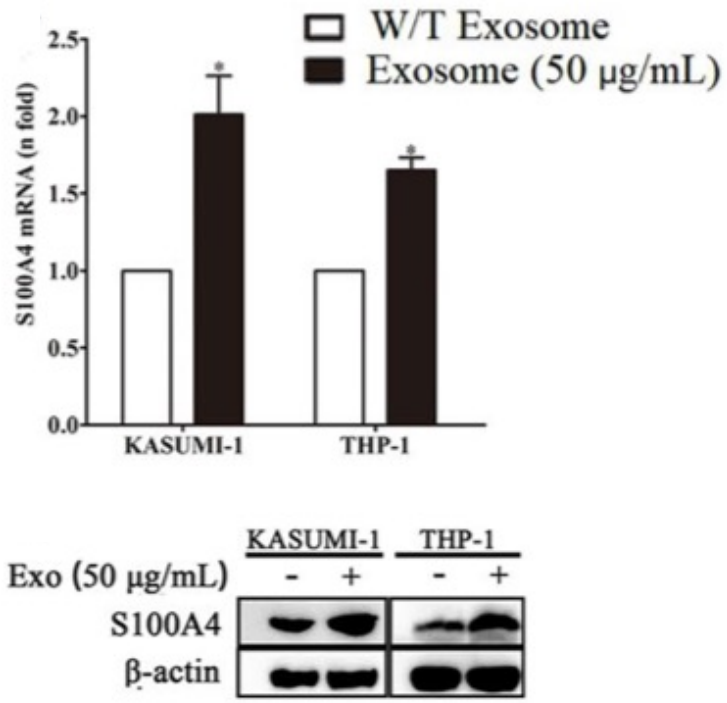
154 pts, INT-risk AML

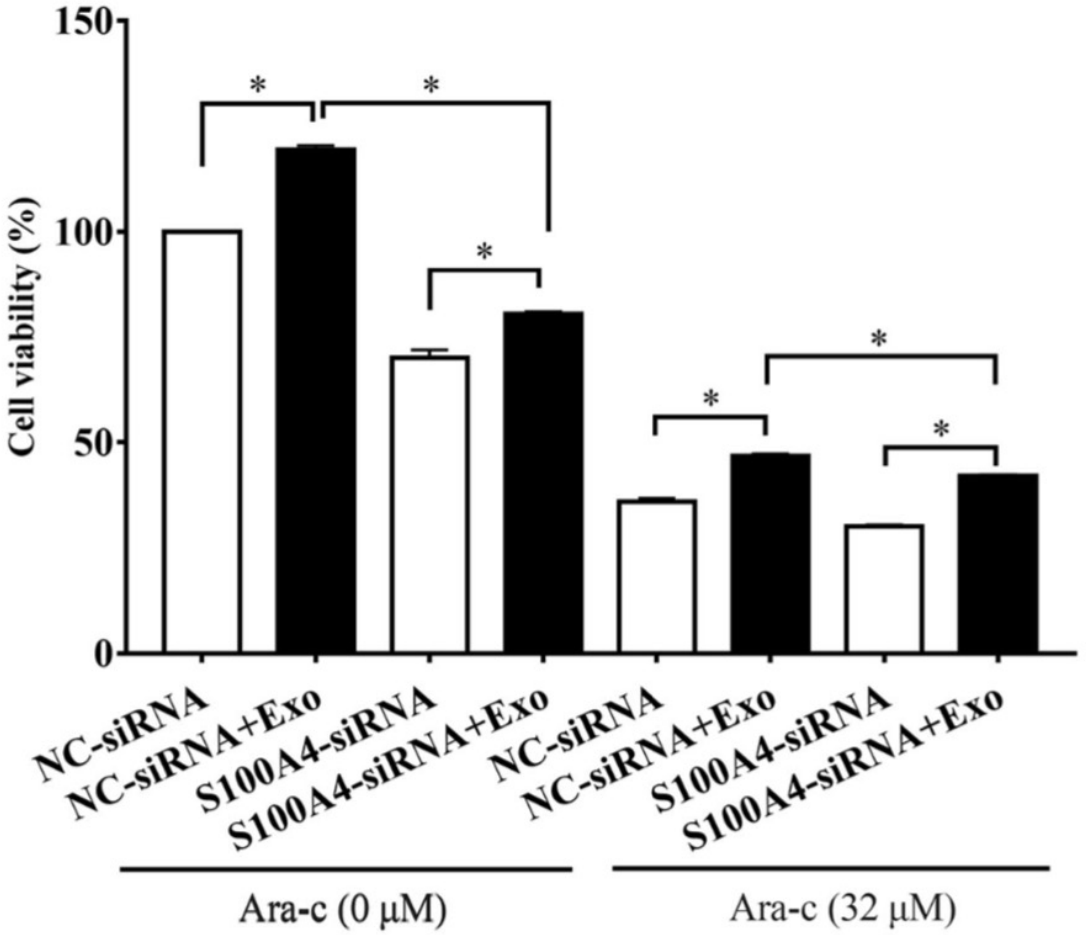




Jiang L et al, Acta Haematol 2018

EVs- Mediated Drug Resistance

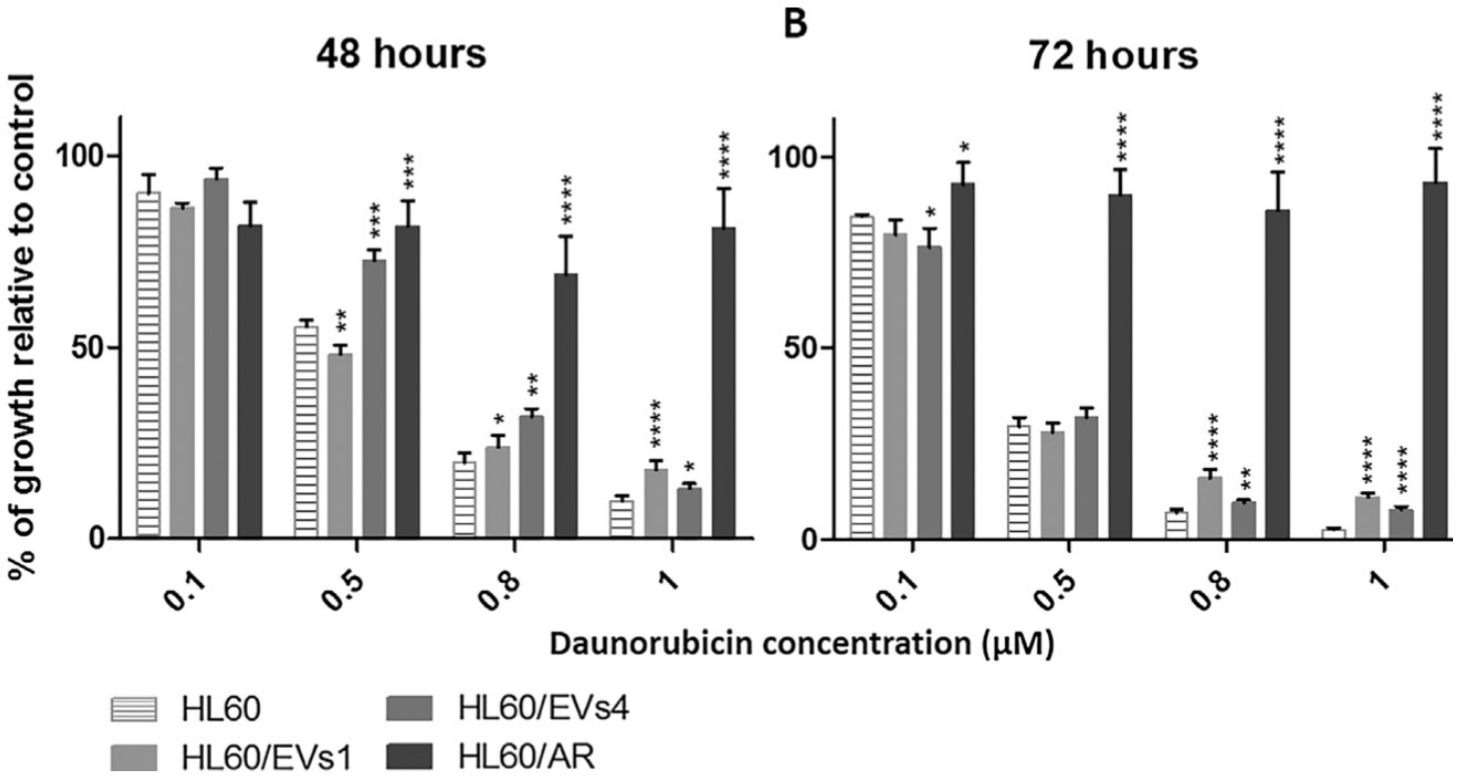


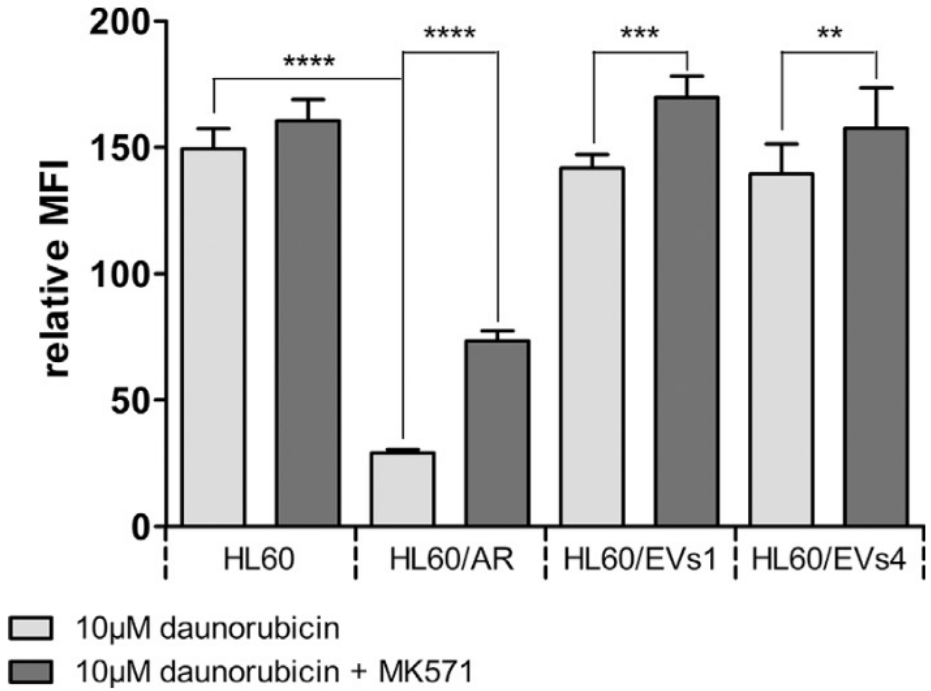


- BM-MSCs exosomes promote proliferation and chemoresistance of AML cells via upregulation of S100A4.

Lyu T et al, Exp Hematol Oncol 2021

EVs- Mediated Drug Resistance



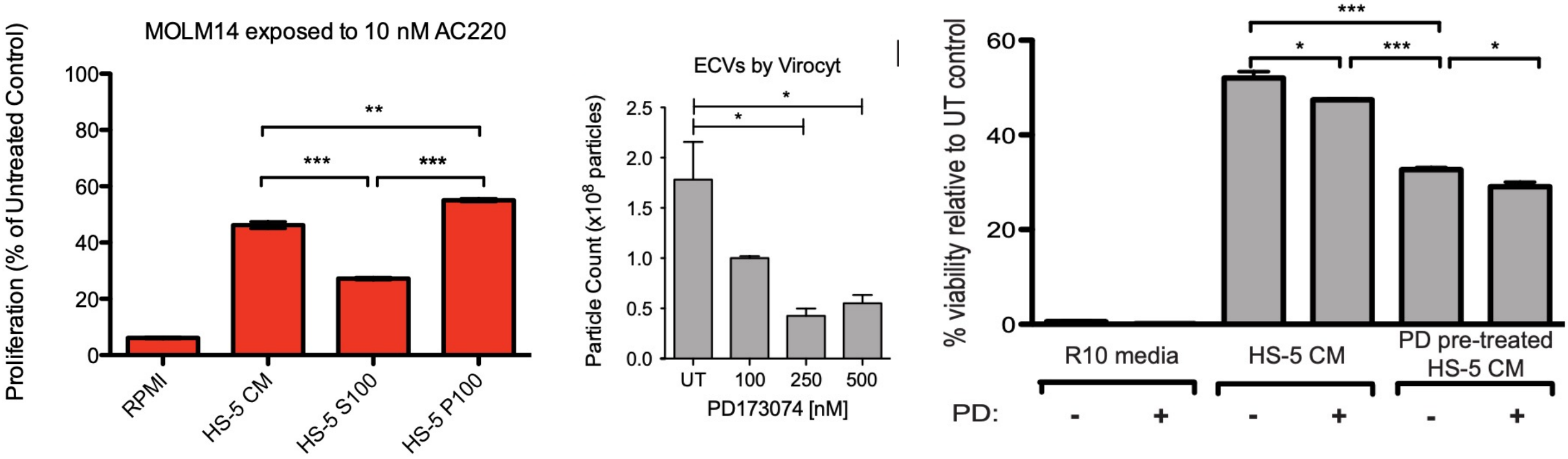


- Resistant leukemic cells may transfer their chemoresistance via EVs.

MRP-1= multidrug resistance pump
MK571= MRP-1 inhibitor

Bouvy C et al, Leukemia Research 2017

Therapeutic Potential Of Evs

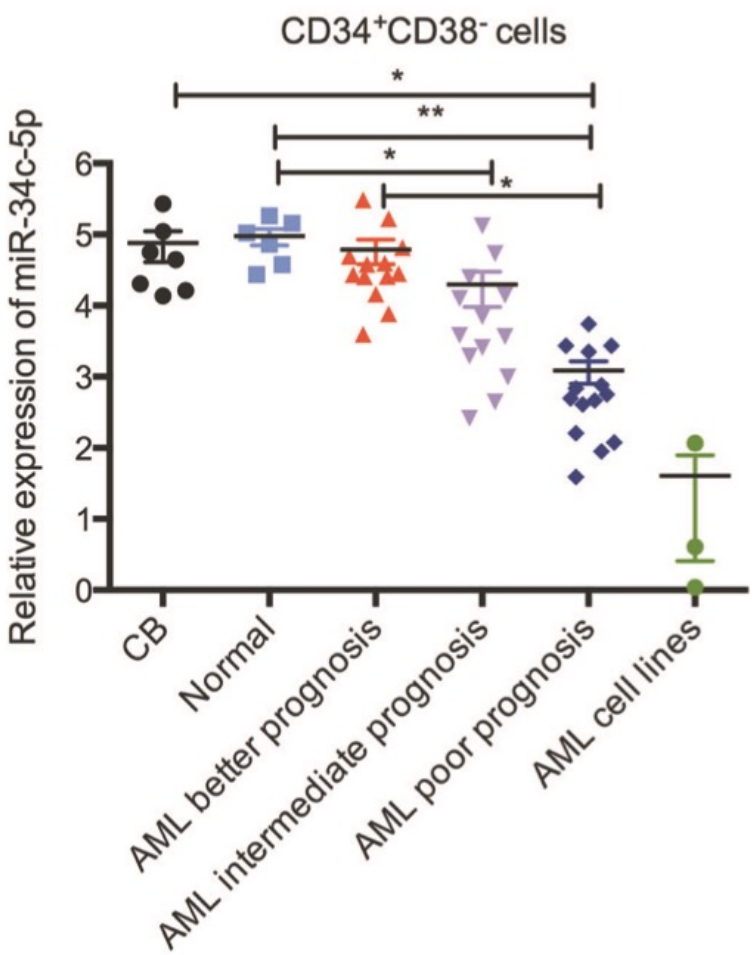


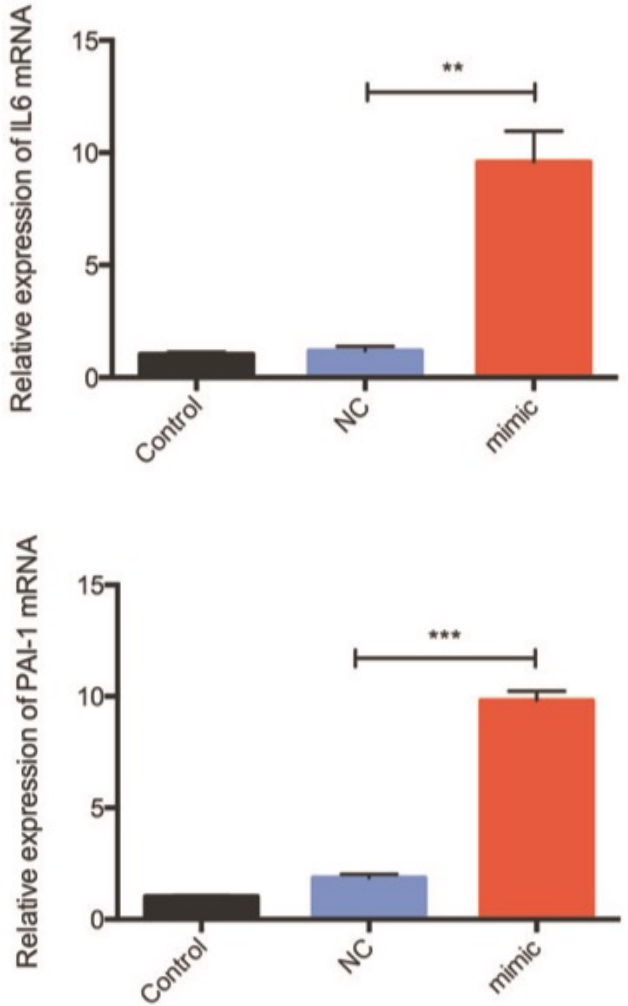
- FGFR inhibition significantly decreases secretion of FGF2-containing exosomes, resulting in less stromal protection of leukemia cells.

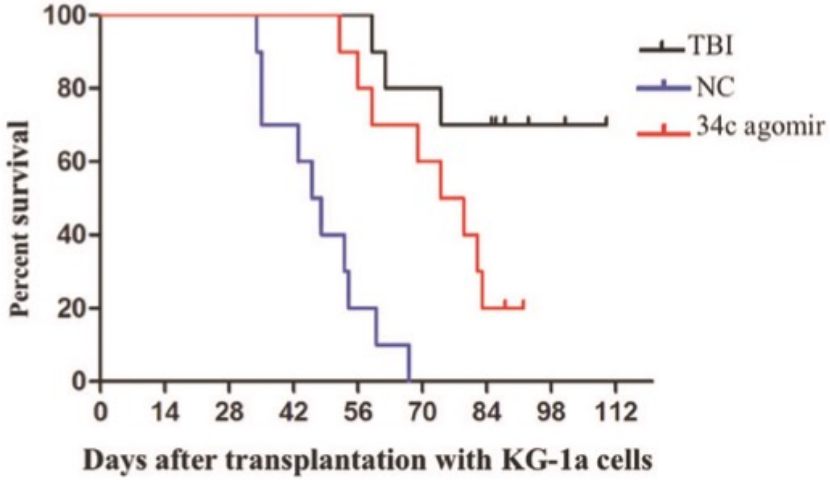
Javidi-Sharifi N et al, Elife 2019

PD (173074)= FGFR inhibitor

Therapeutic Potential Of Evs





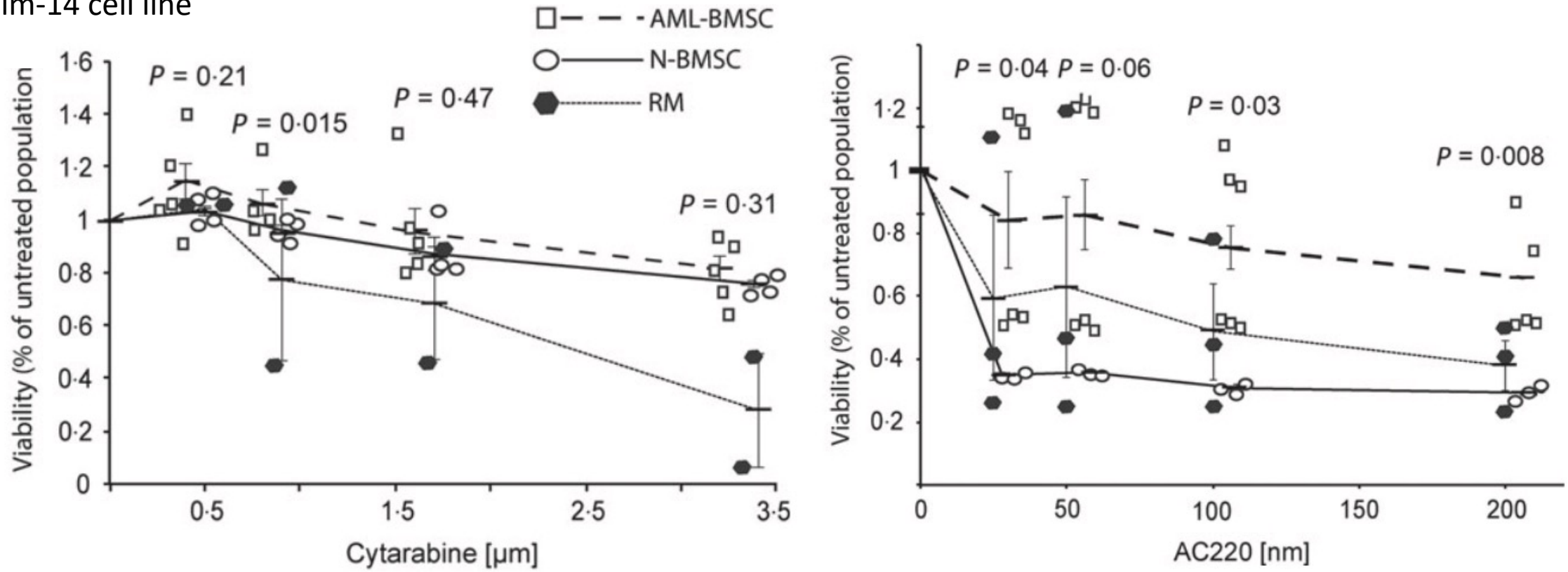


- Increased miR-34c-5p expression induce in vitro AML stem cell senescence and coul promote AML eradication.

Peng D et al, Leukemia 2018

Therapeutic Potential Of Evs

Molm-14 cell line



- In contrast to exosomes from AML patients, exosomes of healthy donors sensitized AML cells to FLT3 inhibitor.

Viola S et al, Br J Hematology 2016

Conclusions and future perspectives

- EVs represents one of the most important cell-to-cell communication mechanism and display a diagnostic, prognostic and therapeutic potential;
- The understanding of the leukemic microenvironment and exosomes interaction with AML blasts may led to the development of promising target therapy;
- Therapeutic strategies that interfere with the release of EVs and impair EV-mediated cell-to- cell communication could potentially be exploited in the future.

Acknowledgements

Grazie per l’attenzione.