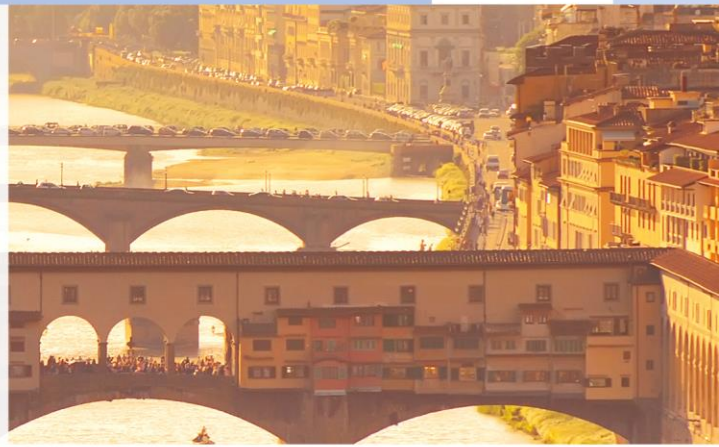
8° WORKSHOP IN EMATOLOGIA TRASLAZIONALE

DELLA SOCIETÀ ITALIANA DI EMATOLOGIA SPERIMENTALE

Firenze - Auditorium CTO - A.O.U. Careggi, 22-23 giugno 2023



Expression profiling of extramedullary acute myeloid leukemia suggests involvement of epithelial-mesenchymal transition pathways

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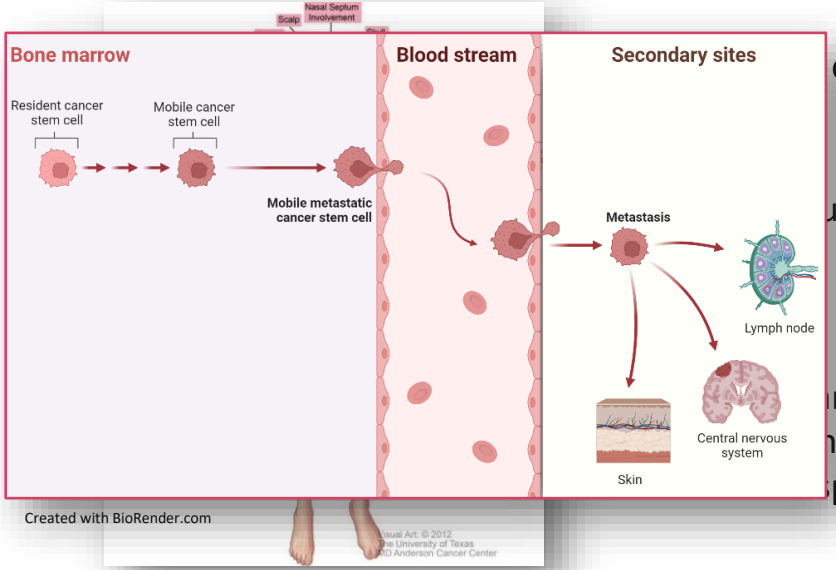
I have no conflicts of interest to disclose

Extramedullary Acute Myeloid Leukemia (EM-AML)

EM-AML, defined “myeloid sarcoma” (MS) in the WHO classification, is an extramedullary mass of myeloid blasts with or without maturation that affect the tissue architecture.[1-3]



complication of AML, reported in about 10% of cases

- The exact mechanisms underlying the development of MS are unclear

uld be newly diagnosed, with or without bone marrow involvement

- Most commonly involved sites are the skin, lymph nodes and the nervous system

n be also manifestation of disease relapse after notherapy or post-allogenic hematopoietic cell splantation

[1]Shallis RM, Gale RP, Lazarus HM, et al. Myeloid sarcoma, chloroma, or extramedullary acute myeloid leukemia tumor: A tale of misnomers, controversy and the unresolved. *Blood Rev.* 2021;47:100773.
[2]Kahali B, Kahali B. Myeloid Sarcoma: The Other Side of Acute Leukemia. *Hematol. - Latest Res. Clin. Adv.* 2018
[3]Loscocco GG, Vannucchi AM. Myeloid sarcoma: more and less than a distinct entity. *Ann Hematol.* 2023 Jun 7.

Study Aims

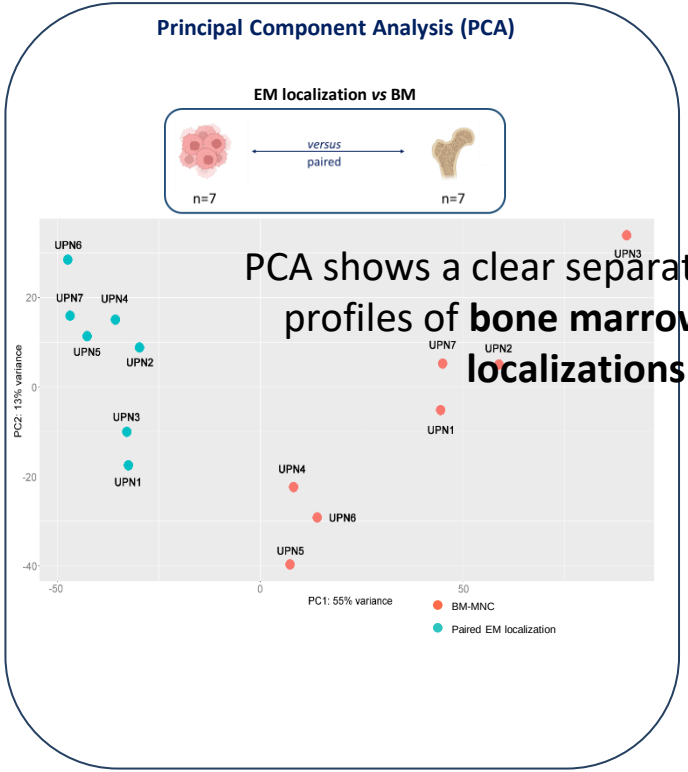
Clinical and biological features of AML study cohort

To shed light on novel predictive markers of extramedullary spread

Cohorts		UPN	Gender	Age (yrs)	EM-localization features		Bone-marrow features at the time of EM-localization			
					Tissue	AML phase	Blasts %	Karyotype	Mutated genes*	Immunophenotype
	Retrospective	UPN1	M	56	Skin	Relapse	95	del7q	*FLT3-TKD*	CD45+, MPO+, CD13+, CD33+, CD34+, CD38+, CD177+, HLA-DR-
		UPN2	F	53	Lymph node	Relapse	8	del(12)(p13)	*NPM1*	CD45+, CD117+, CD34+, CD13+, HLA-DR-, CD33+, CD15-
		UPN3	F	47	Skin	Relapse	73	Normal Karyotype	*NPM1, FLT3-ITD*	CD45+, CD33+, CD14+, CD64+, CD13+, HLA-DR+, CD4+, CD56+, MPO+, TDT+
		UPN4	M	55	Bone	Diagnosis	<5	Normal Karyotype	None	CD45+, MPO+, CD33+, CD64+, CD15+, HLA-DR+, CD34+, CD117+, CD4+/-, CD7+/-, CD13-
		UPN5	M	71	Lymph node	Diagnosis	20	50, XY, +2, +5, +8 [X4]	*NPM1*	CD45+, CD33+, CD64+, CD13+, HLA-DR+, CD56+, CD34-, CD117-, CD3-, CD7-
		UPN6	M	60	Skin	Diagnosis	<5	t(8;13)(p11;q12)	None	CD45+, MPO+, CD34+, CD117+, CD13+, CD15+, CD33+, CD56+/-
		UPN7	M	72	Skin	Relapse	46	48, XY, 2+mar[10], XY[5]	None	CD45+, CD34+/-, CD33+, CD117+, CD64+, HLA-DR+, CD4+, CD56+, CD13-
		UPN8	M	71	Skin	Relapse	48	Normal Karyotype	*IDH1*	CD45+, CD34+, CD33+, CD117+, CD13+, HLA-DR+, CD4+, CD15-, CD56-
		UPN9	F	75	Bone	Relapse	18	47, XX, +8	None	CD45+, CD34+, CD33+, CD117+, CD13+, HLA-DR3+, MPO+
	Prospective	UPN14	M	69	Bone	Diagnosis	87	Normal Karyotype	*NPM1, FLT3-ITD, IDH2*	CD45+, CD33+, CD117+, CD13+, CD4+, CD56-, CD64-
		UPN15	M	72	Lymph node	Relapse	12	Normal Karyotype	None	N.A.
		UPN16	F	74	Skin	Relapse	57	46XY,del(1)(q42),del(2)(q31),del(7)(q22),t(8;21(q22;q22)der(11)t(11;?),+15	*RUNX1/RUNX1T1, IDH1*	CD45+, CD33+, CD34+, HLA-DR+, CD13+, CD117-, CD15-

*including RUNX1/RUNX1T1, CBFB/MYH11, BCR/ABL1, DEK/NUP214, NPM1, FLT3 (ITD/TKD)

Expression profiling by RNA-Seq: differential gene expression analysis

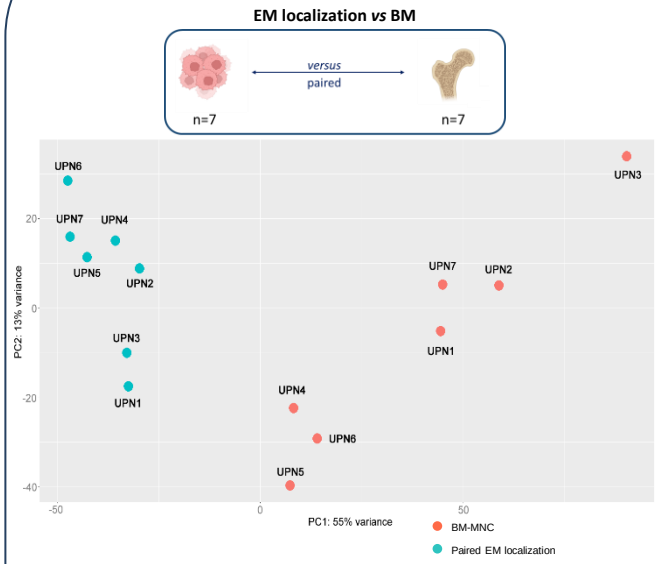


PCA shows a clear separation of the expression profiles of **bone marrow** *versus* **secondary localizations** samples

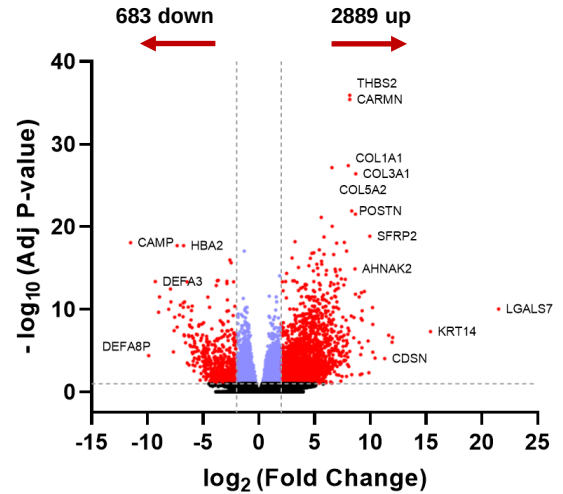
HiSeq

Expression profiling by RNA-Seq: differential gene expression analysis

Principal Component Analysis (PCA)



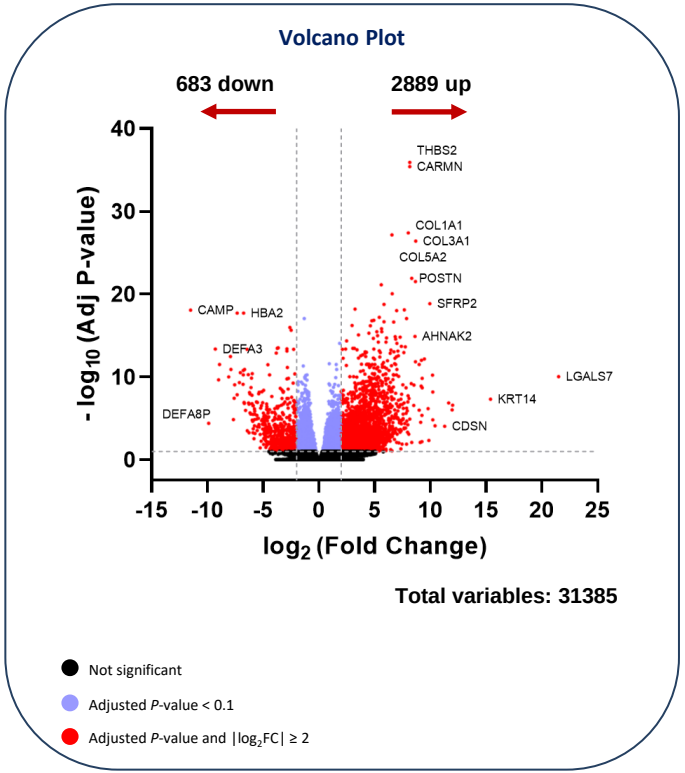
Volcano Plot

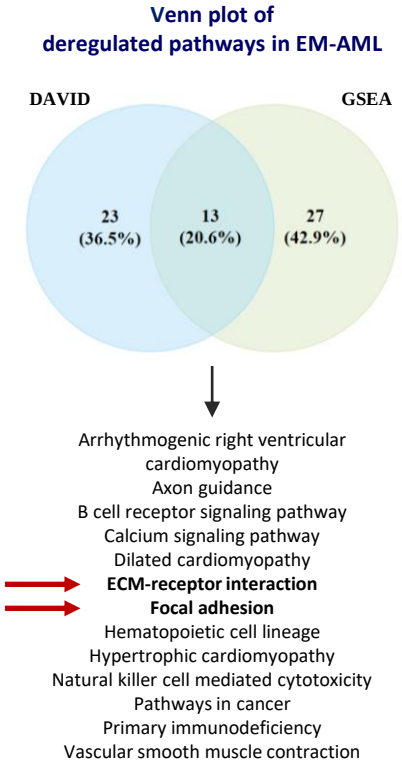


Total variables: 31385

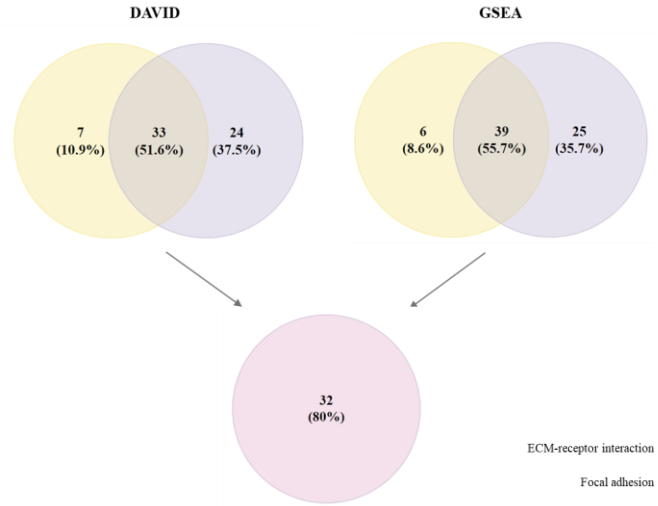
- Not significant
- Adjusted *P*-value < 0.1
- Adjusted *P*-value and $|\log_2 FC| \geq 2$

Expression profiling by RNA-Seq: KEGG pathway enrichment analysis





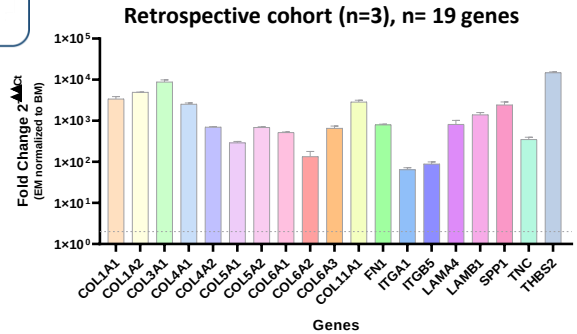
32 common upregulated genes (80%) linked to:
- ✓ ECM-receptor interaction
- ✓ Focal adhesion

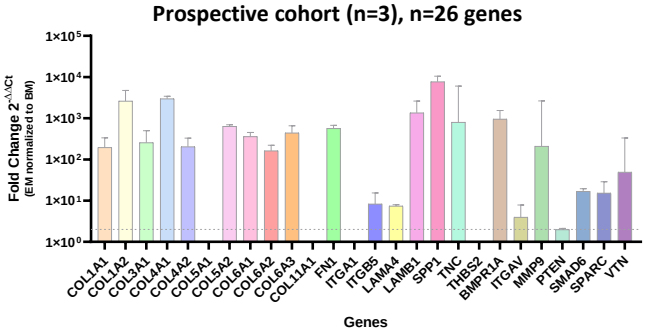


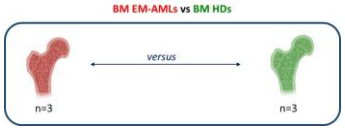
DAVID: P-value < 0.01 and FDR < 0.01
GSEA: P-value < 0.01 and FDR < 0.25
|log2FC|>2

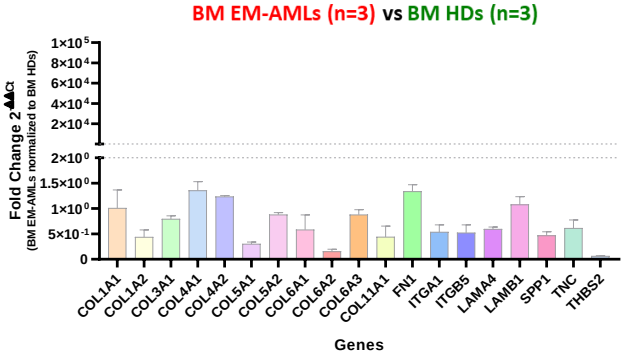
*The pathways are sorted alphabetically by pathway name.

qRT-PCR validation of dysregulated genes confirms their upregulation in EM sites







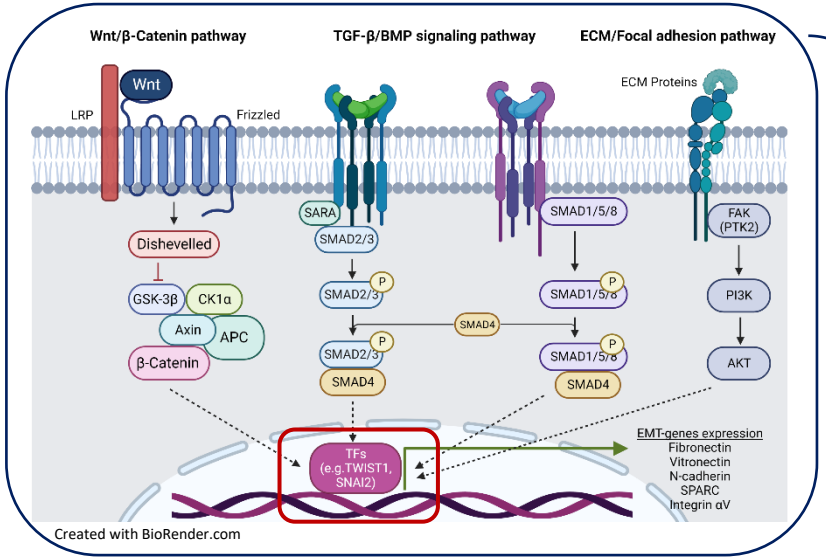


NO differential expression in cancer invasion pathway related genes

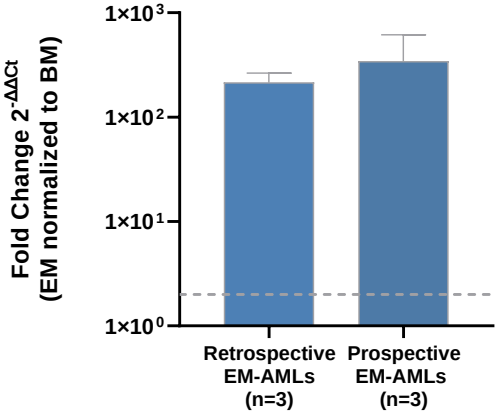
This indicates that EM-AML cells probably acquire specific capabilities

Fold Change = $2^{-\Delta\Delta Ct}$; $2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct\ EM\text{-}AML\ -\ \Delta Ct\ BM)}$;
Fold Change = $2^{-\Delta\Delta Ct}$; $2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct\ BM\ EM\text{-}AMLs\ -\ \Delta Ct\ BM\ HDs)}$;
ΔCt = Ct test gene – Ct housekeeping gene;
Housekeeping gene = B2M

ECM-receptor interaction and Focal adhesion pathways may cooperate to induce extramedullary colonization of leukemic blasts



TWIST1 is significantly upregulated both in the retrospective and prospective cohort of patients complicated by an extramedullary localization



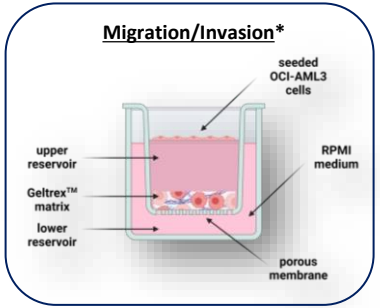
Fold Change = $2^{-\Delta\Delta Ct}$; $2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct\ EM\text{-}AML\ -\ \Delta Ct\ BM)}$;
ΔCt = Ct test gene – Ct housekeeping gene;
Housekeeping gene = B2M

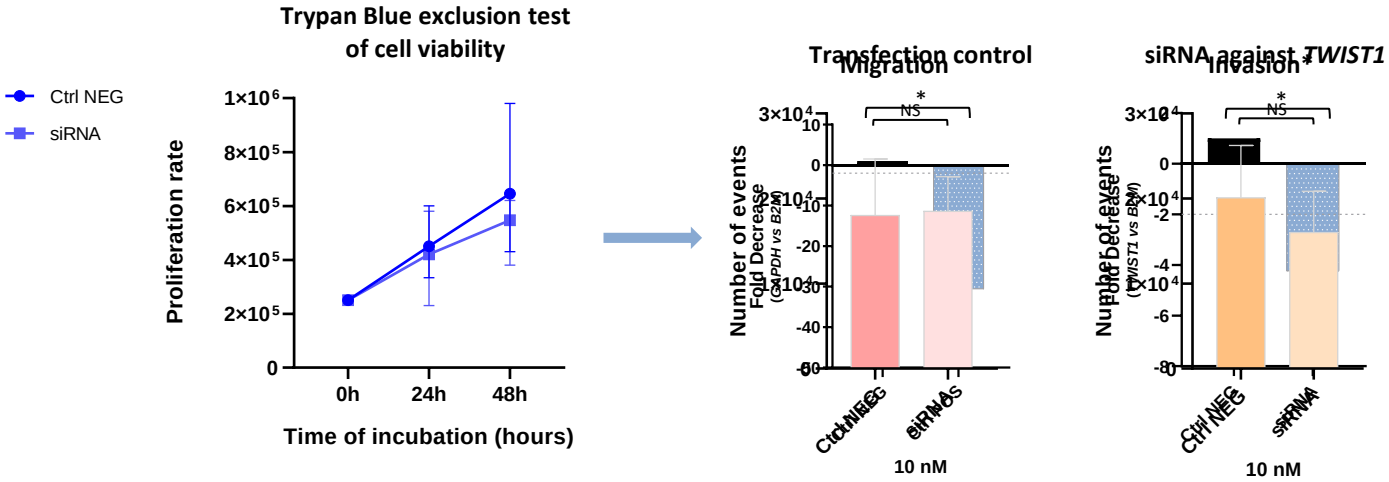
Modulation of the metastasis-promoting gene *TWIST1* by siRNA

1 *In vitro* cell treatment for 48h

2 *TWIST1* mRNA evaluation by qRT-PCR

3 Functional studies by Boyden Chamber Assay





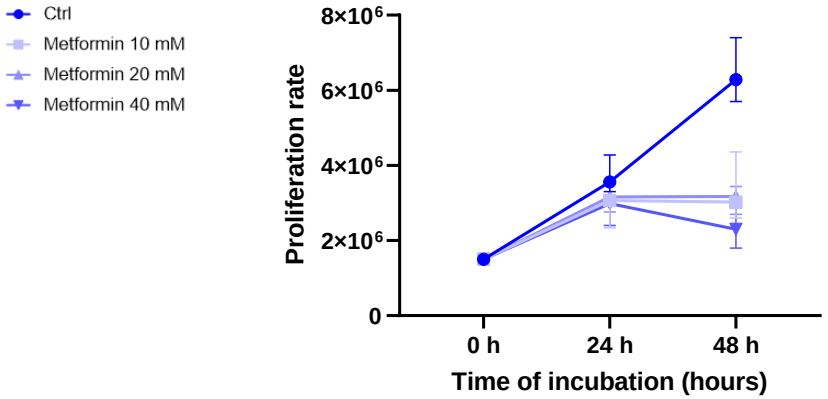
*For the invasion assay, the chamber was coated with Geltrex™ matrix, to mimic the extracellular matrix.

|FC| > 2
Fold Change = $2^{-\Delta\Delta Ct}$; $2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct\ Treated\ -\ \Delta Ct\ Control)}$;
ΔCt = Ct test gene – Ct housekeeping gene;
Housekeeping gene = B2M

Wilcoxon matched-pairs test
Data are presented as the median with range of three independent experiments
NS: not significant

In vitro metformin treatment affects expression of *TWIST1* and other EMT-related genes

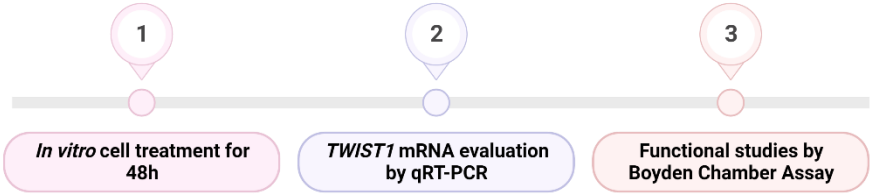
[4]**Metformin**, used in combination with chemotherapeutic agents, **reduces tumor progression in various cancer types**.
In clinical trials it is used as a ***TWIST1* inhibitor**.

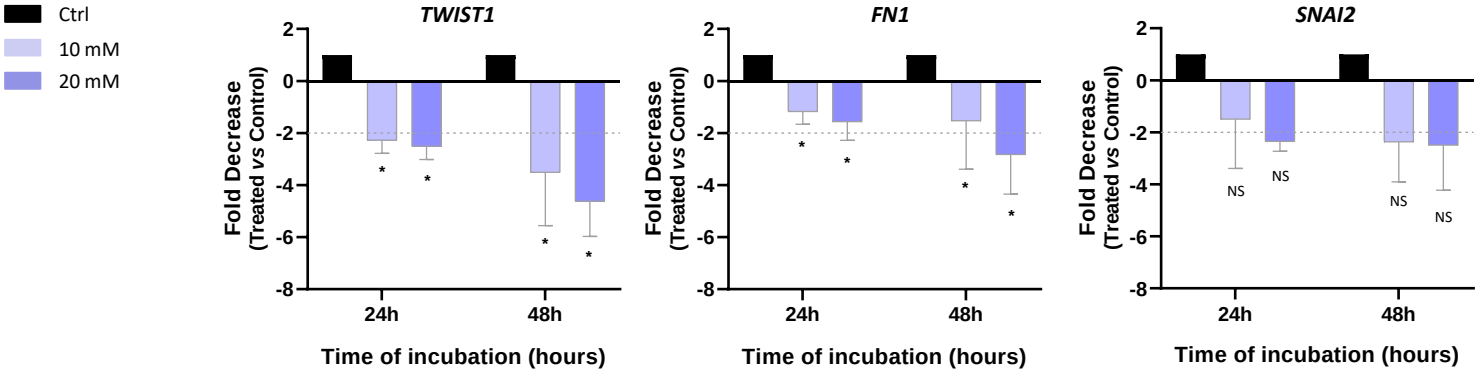


The effect of the drug changes in a dose- and time-dependent fashion

[4]Zhang N, Ng AS, Cai S, Li Q, Yang L, Kerr D. Novel therapeutic strategies: targeting epithelial-mesenchymal transition in colorectal cancer. Lancet Oncol. 2021 Aug;22(8):e358-e368

In vitro metformin treatment affects expression of *TWIST1* and other EMT-related genes



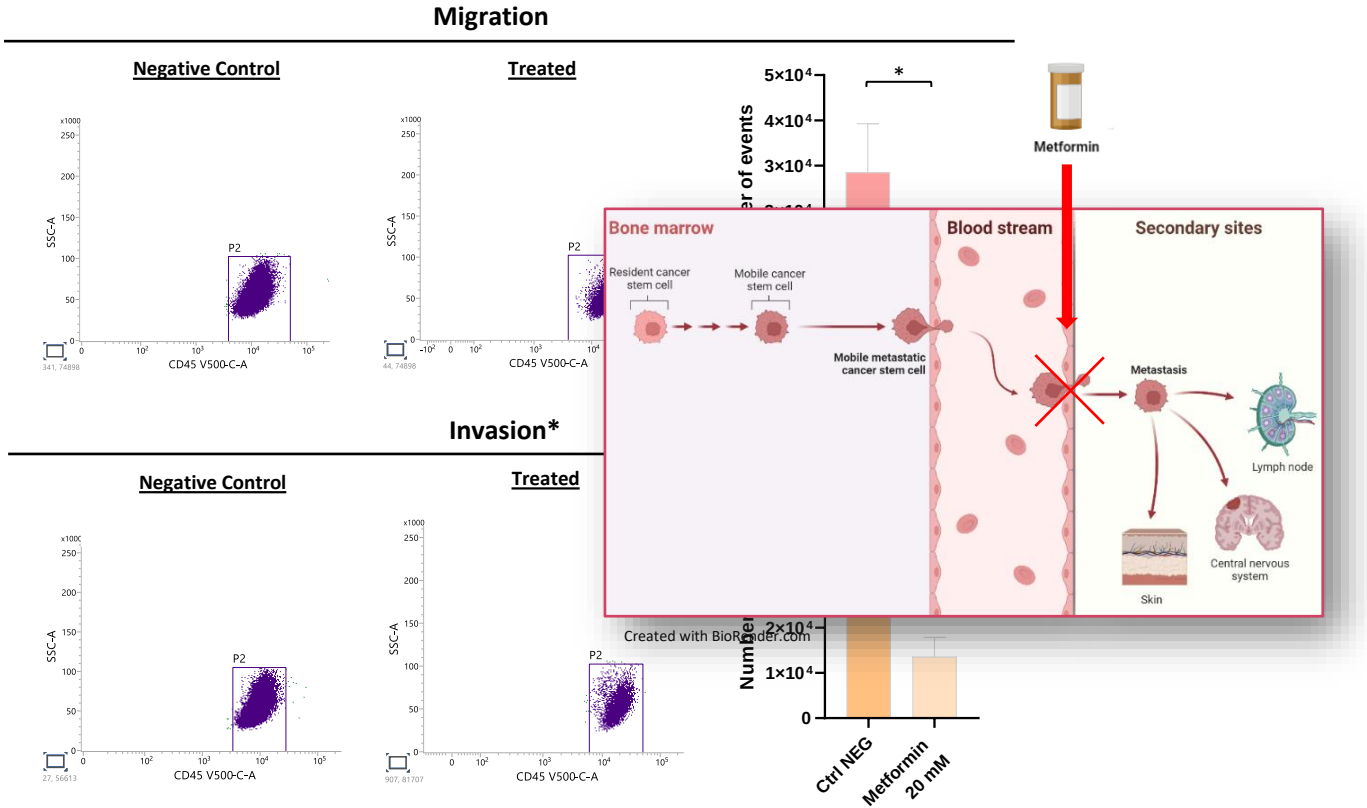


The black bar represents the negative control, while the bars with increasing intensity of blue represent the scaling up doses of metformin.

|FC| > 2
Fold Change = $2^{-\Delta\Delta Ct}$; $2^{-\Delta\Delta Ct} = 2^{-(\Delta Ct\ Treated\ -\ \Delta Ct\ Control)}$;
ΔCt = Ct test gene – Ct housekeeping gene;
Housekeeping gene = B2M

Wilcoxon matched-pairs test
Data are presented as the median with range of three independent experiments
*: *P*-value < 0.05; NS: not significant

Metformin treatment impairs both migration and invasion



*For the invasion assay, the chamber was coated with Geltrex™ matrix, to mimic the extracellular matrix.

Wilcoxon matched-pairs test
Data are presented as the median with range of three independent experiments

Conclusions

- The expression profile of extramedullary localizations is significantly different from that of paired bone marrow samples
- The invasion and metastatization pathways are enriched in extramedullary localizations
- *In vitro* treatment of OCI-AML3 cells with metformin reduces the invasion and migration potential of AML cells

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