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**Deciphering the crosstalk between stroma and AML cells:
an *in vitro* model to uncover the mechanisms underlying
extramedullary progression in acute myeloid leukemia**

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Disclosures of Giorgia Silvestrini

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
I have no conflicts of interest to disclose							

Extramedullary Acute Myeloid Leukemia (EM-AML): identikit



Rare complication of AML, reported in about 10% of cases. It could be newly diagnosed, with or without BM involvement^{1,2}

The molecular mechanisms underlying the development of EM-AML are not yet fully understood...



It can be also manifestation of disease relapse after allogeneic hematopoietic cell transplantation

EM-AML?

Leukemic cells reach secondary sites through the activation of an EMT-like process³



Most commonly involved sites are the skin, lymph nodes and the nervous system (CNS)

Identification of signaling pathways involved in the migration and invasive ability of blasts, promoting leukemia progression³

¹Shallis RM, *et al.* Blood Rev. 2021

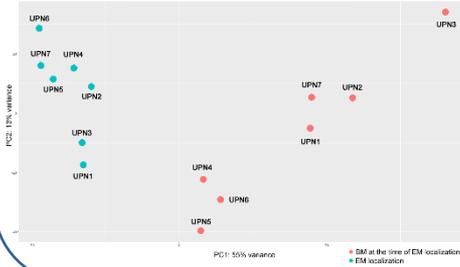
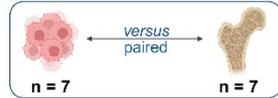
²Mohammadiasl J, *et al.* J Cancer Metastasis Treat. 2016

³Ottone T and Silvestrini G, *et al.* Leukemia. 2023

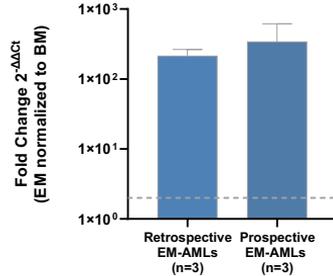
Transcriptomic and functional characterization of EM spreading

Principal Component Analysis & Gene Expression Analysis³

EM localization vs BM

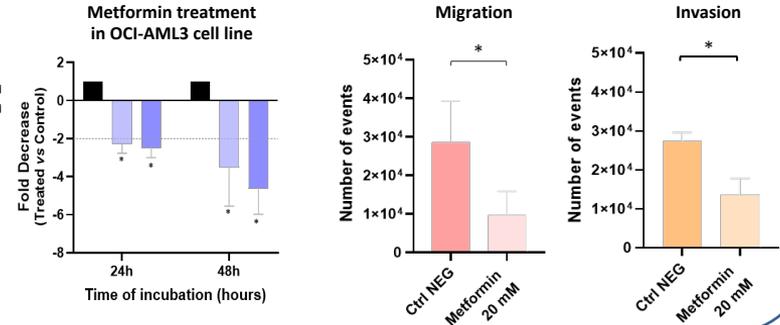


TWIST1 is significantly upregulated in patients complicated by an EM localization



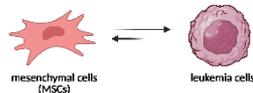
Molecular and *in vitro* findings³

Treatment with metformin, which impairs wider EM-interaction pathways, decreases both migration and invasion



→ No differences between BM samples from AMLs and from EM-AMLs

Does the BM niche play any role in triggering the capability of leukemic blasts to colonize secondary sites?

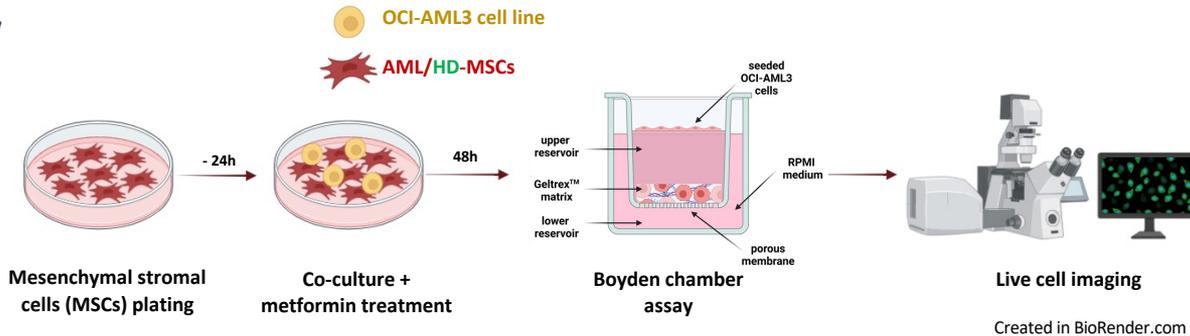


MSCs can promote the hallmarks of cancer such as **increased proliferation**, **cancer stemness**, drug resistance, EMT, invasion and metastasis, **immune evasion**, **angiogenesis**, and **resistance to apoptosis**^{4,5}

Study aims

- Aims**
- To demonstrate that MSCs may affect the **metastatic potential** of leukemic cells
 - To investigate the **cross-talk** between MSCs and leukemic cells

Workflow

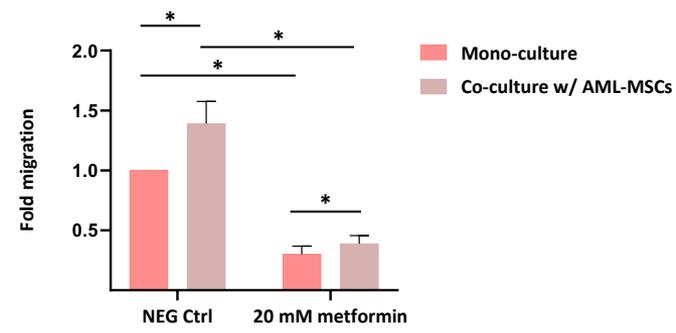


Direct contact co-culture with **AML-MSCs** boosts the metastatic potential of OCI-AML3 cells

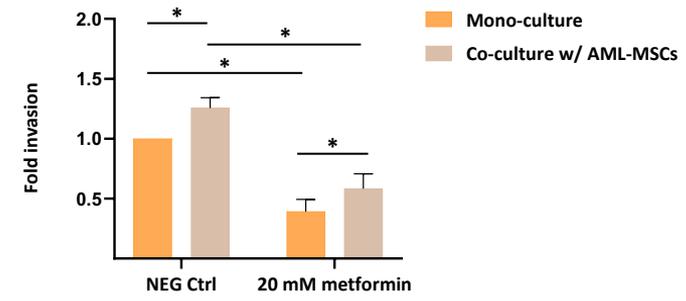
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AML-MSCs

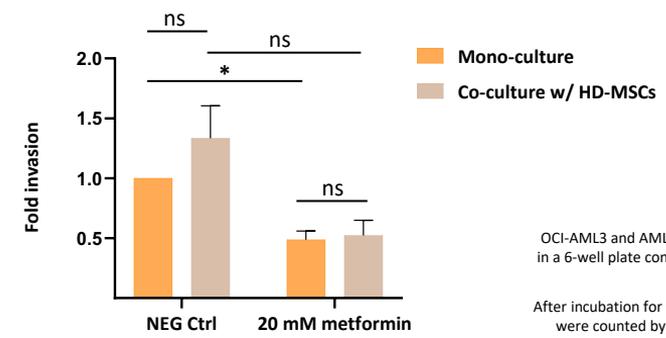
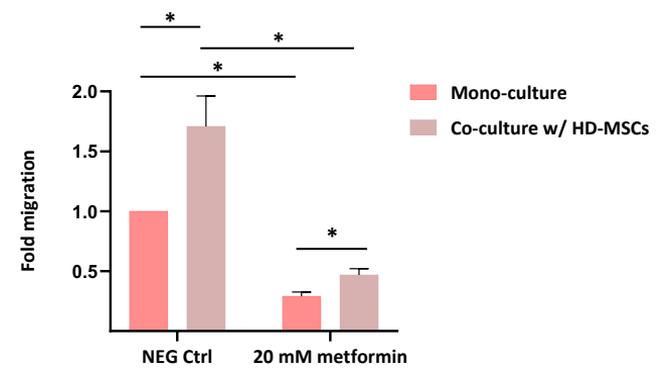
Migration assay



Invasion assay



HD-MSCs

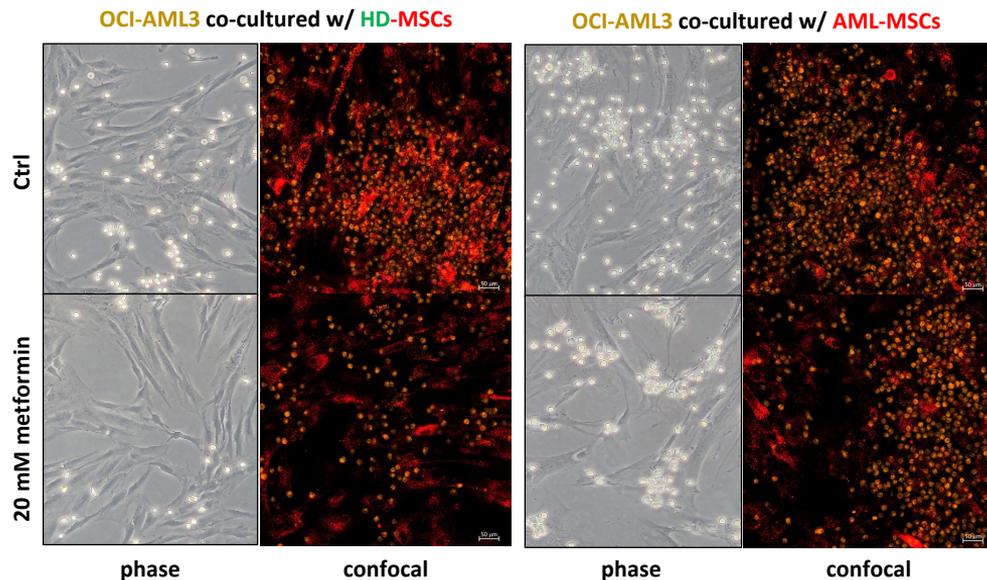


OCI-AML3 and AML-MSCs or HD-MSCs cells were seeded in a 6-well plate containing RPMI medium with 20% FBS and cultured for 48h

After incubation for 4 hours at 37°C, transmigrated cells were counted by flow cytometry, for 5 minutes after labeling with anti-CD45 human monoclonal antibody

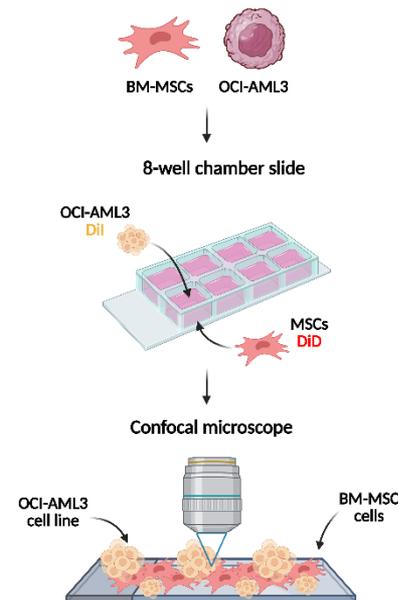
Leukemic cells take deep contact with MSCs: is that true?

... initial observation



experimental design

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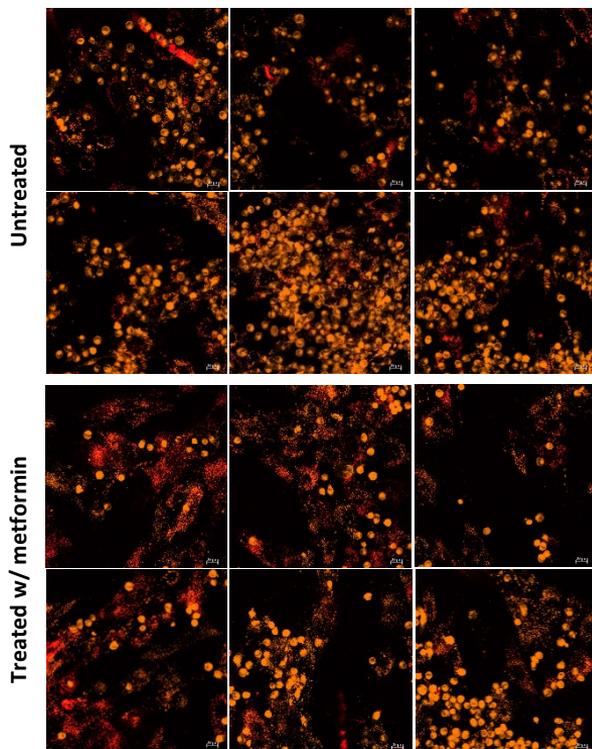


The images were acquired using the ZEISS LSM 900 confocal microscope
The images show an exemplary replicate of the experiment.
Images were acquired with a x10 objective.

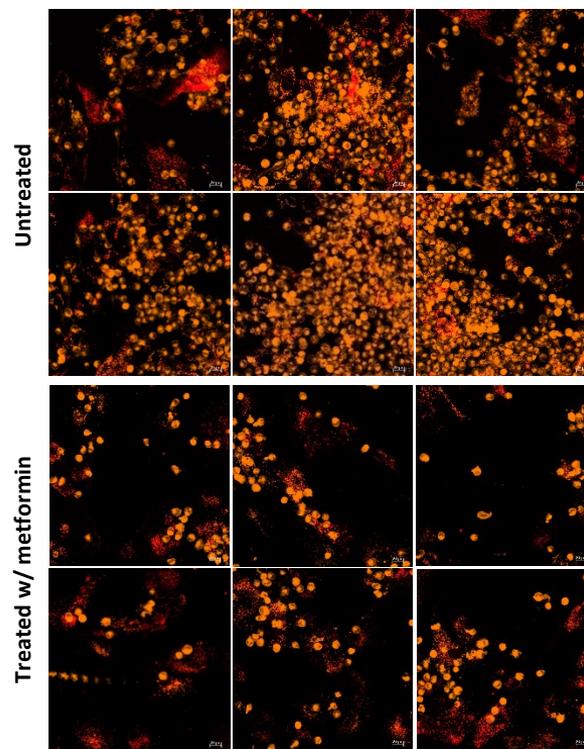
High-resolution confocal microscopy highlights cell-to-cell adhesion dynamics

OCI-AML3 co-cultured w/ HD-MSCs

HD/AML-MSC-DID 647
OCI-AML3-Dil 555



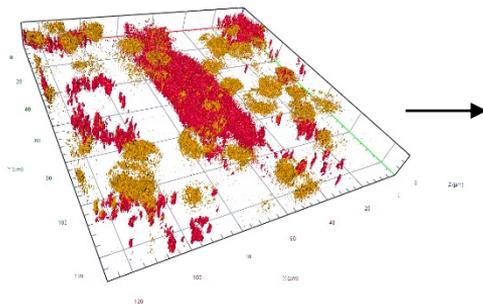
OCI-AML3 co-cultured w/ AML-MSCs



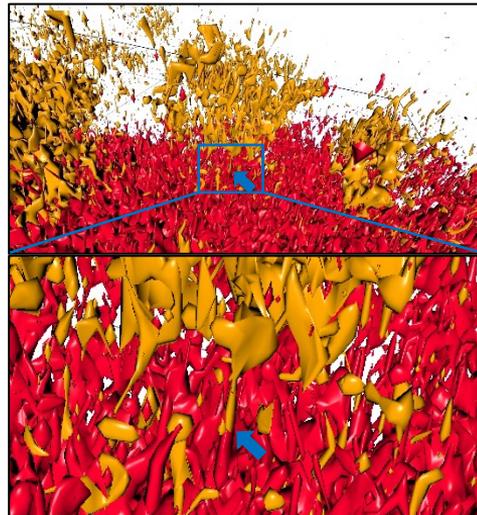
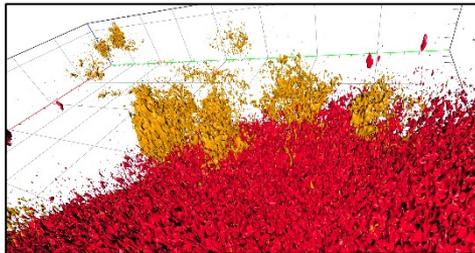
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OCI-AML3 cells take deep contact with MSCs and exchange membrane dyes

MSCs
OCI-AML3

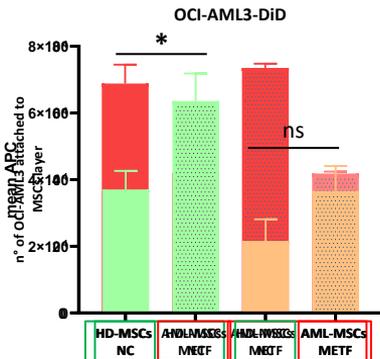
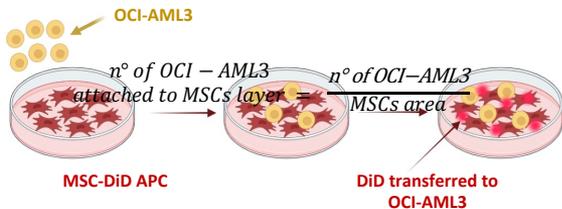


Snapshot 3D image of OCI-AML3 co-cultured with MSCs



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Flow cytometry analysis of floating OCI-AML3



The OCI-AML3 cell protrusions exhibit the same lipophilic tracer used for MSCs staining

Preferential adhesion of OCI-AML3 cells to AML-MSCs interactions and the exchange of vital dyes between leukemic and stromal cells

ImageJ software was used for cell counting and to determine MSCs area

Data are the means \pm SEM of three independent experiments (* $P < 0.05$; ns.: not significant)

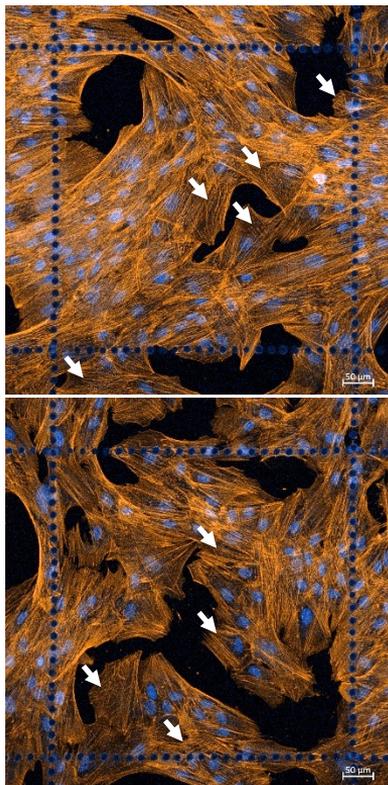


MSCs preserve cytoskeletal integrity when co-cultured with leukemic cells

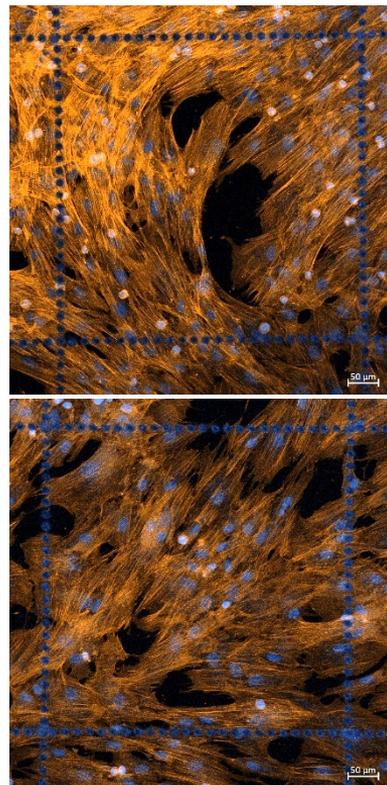
- MSCs nuclei (Hoechst)
- MSCs cytoskeletal filaments (phalloidin)

Metformin induces specific alterations in the cytoskeleton of cultured and treated alone MSCs

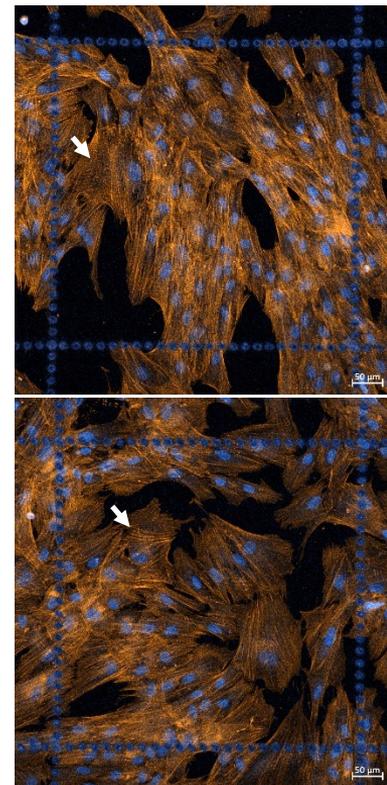
HD-MSC mono-culture w/metf
AML-MSC mono-culture w/metf



HD-MSC co-culture w/ untreated OCI-AML3
AML-MSC co-culture w/ untreated OCI-AML3



HD-MSC co-culture w/ treated OCI-AML3
AML-MSC co-culture w/ treated OCI-AML3



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White arrows point to cytoskeletal fragmentation.

Images were acquired with a $\times 20$ objective. Scale bar 50 μm .
Selected images refer to quadrants representative of the entire experiment.

Conclusions

- ❖ **AML**-MSCs actively support migration and invasion of OCI-AML3 as compared to **HD**-MSCs and counteract the effect of metformin treatment.
- ❖ OCI-AML3 cells interact more tightly with **AML**-MSCs than **HD**-MSCs. This close physical interplay of **AML**-MSCs w/OCI-AML3 allows AML cells to migrate and invade the ECM-like coating.
- ❖ MSCs treated with metformin undergo cytoskeletal breakage. The co-culture system of OCI-AML3 with both **HD**-MSCs and **AML**-MSCs mitigates the impact of metformin treatment on stromal cells, preserving the integrity of their actin cytoskeleton.

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