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AML and the Bone Marrow Microenvironment

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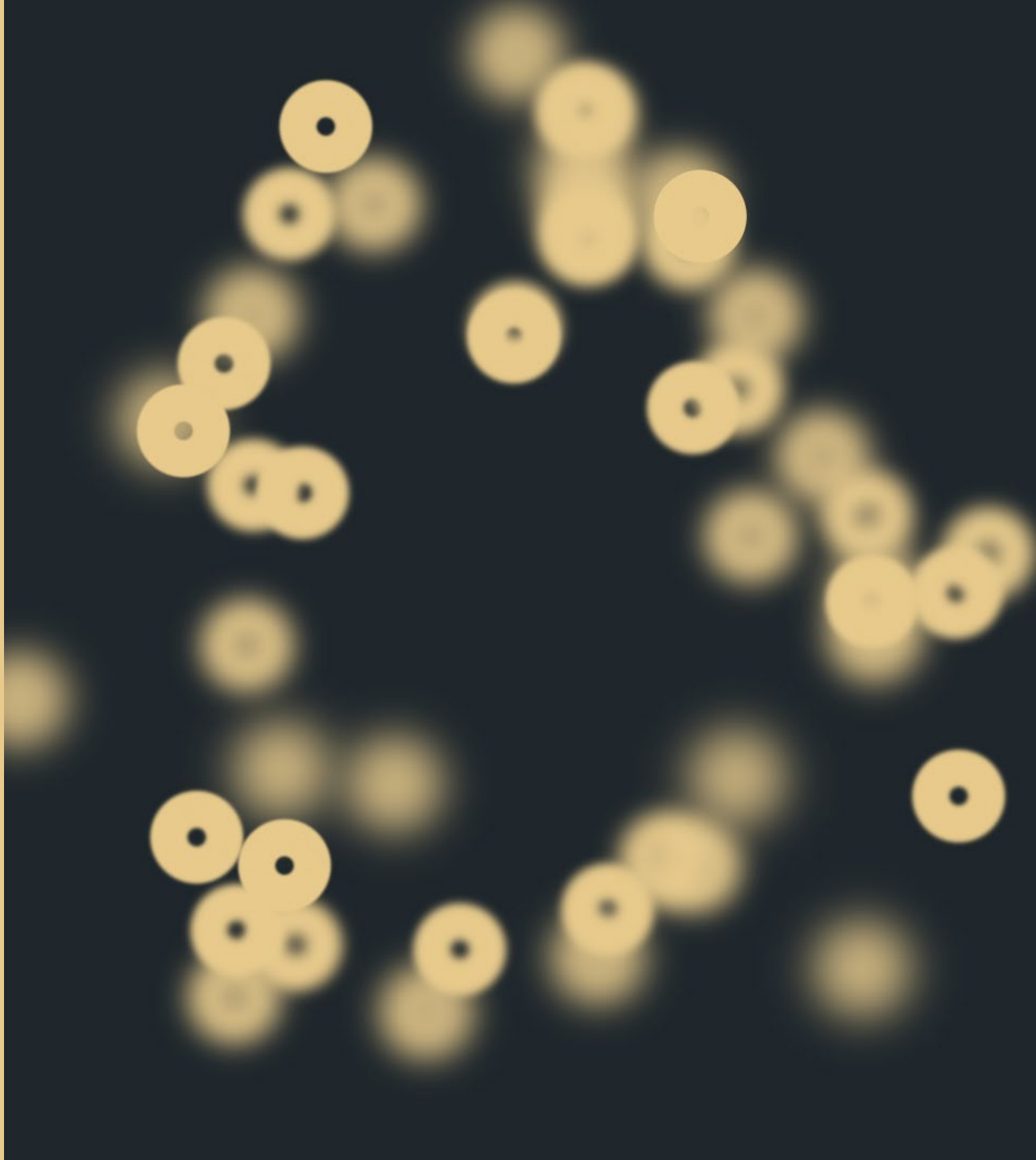
The Francis Crick Institute, London, UK

Berlin, Germany
April 25-26, 2025

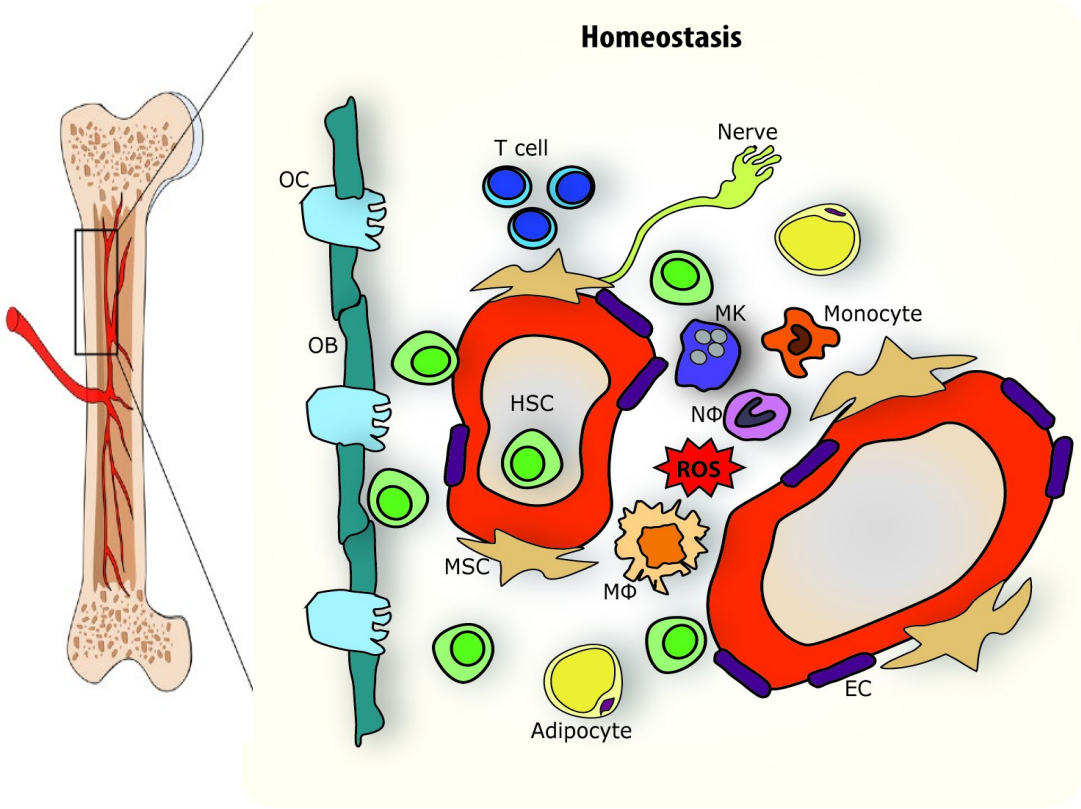


DISCLOSURES OF COMMERCIAL SUPPORT

- Nothing to declare

Bone Marrow Environment (BME): Complex ecosystem

- AML-IC could be maintained *ex vivo* via co-culture with MSC
(Griessinger E et al., Stem Cell Trans Med, 2014; Griessinger et al. Cancer Res, 2016)



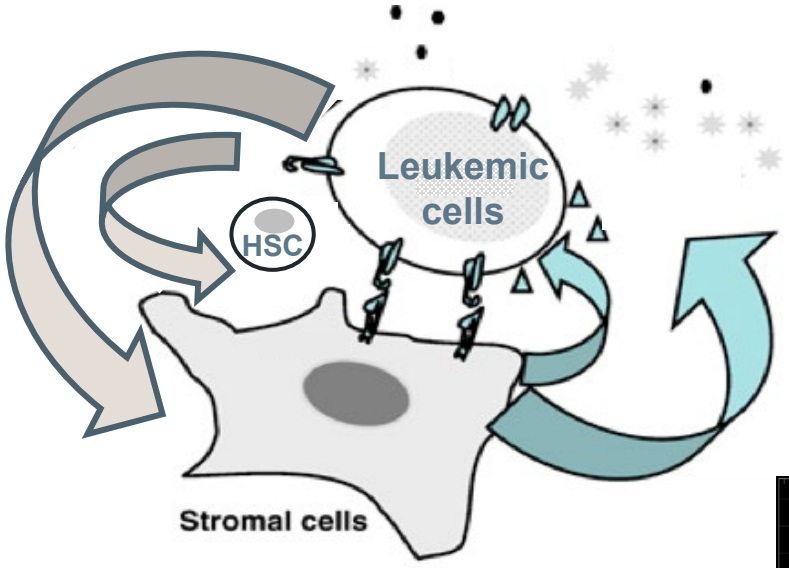
- How malignant cells interact with the BME ?

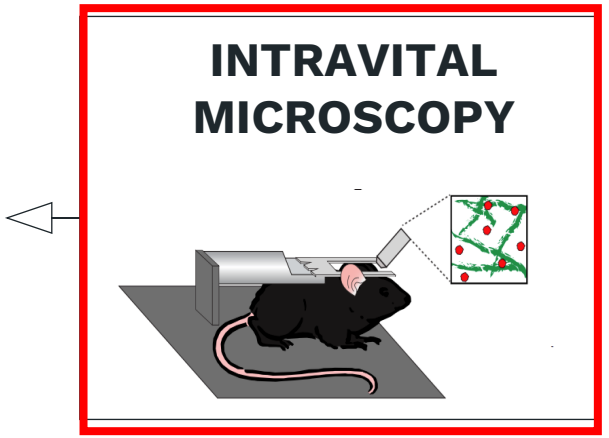
- What are BME changes during leukaemic evolution: from CH to MDS to AML ?

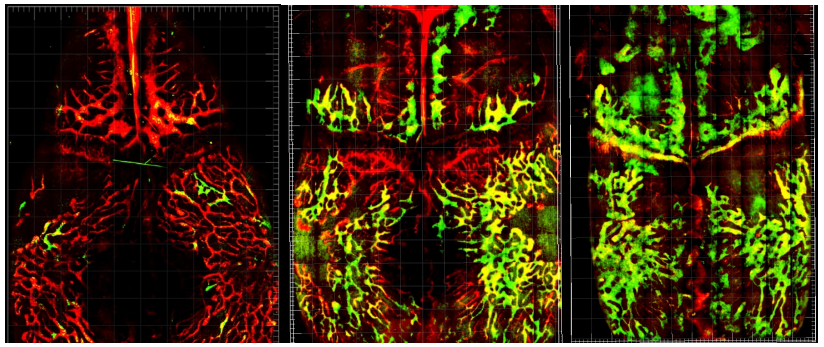
- How is the BME involved in chemoresistance of LSC ?

- Can cytotoxic drugs impede BME and potentially promote AML dev/relapse?

Functional cross-talk between AML and the BM microenvironment

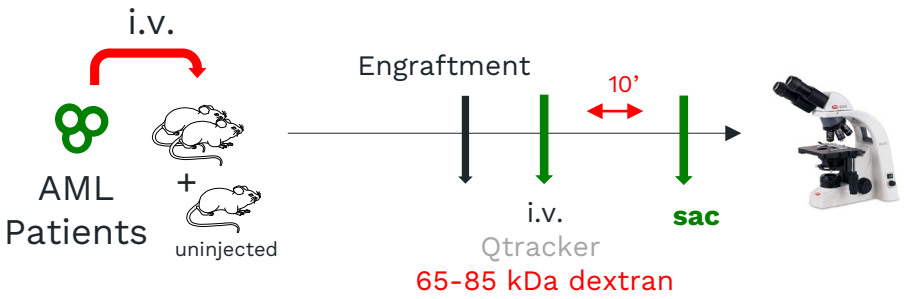


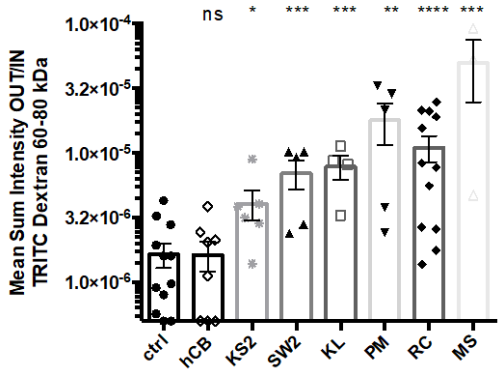


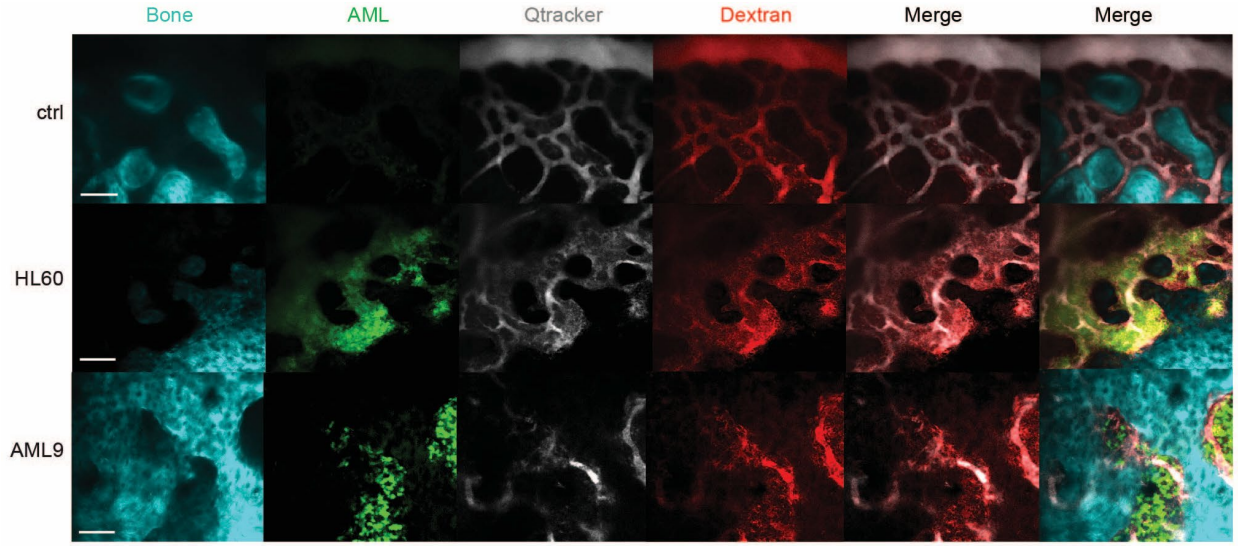


4. AML and the BM niche.

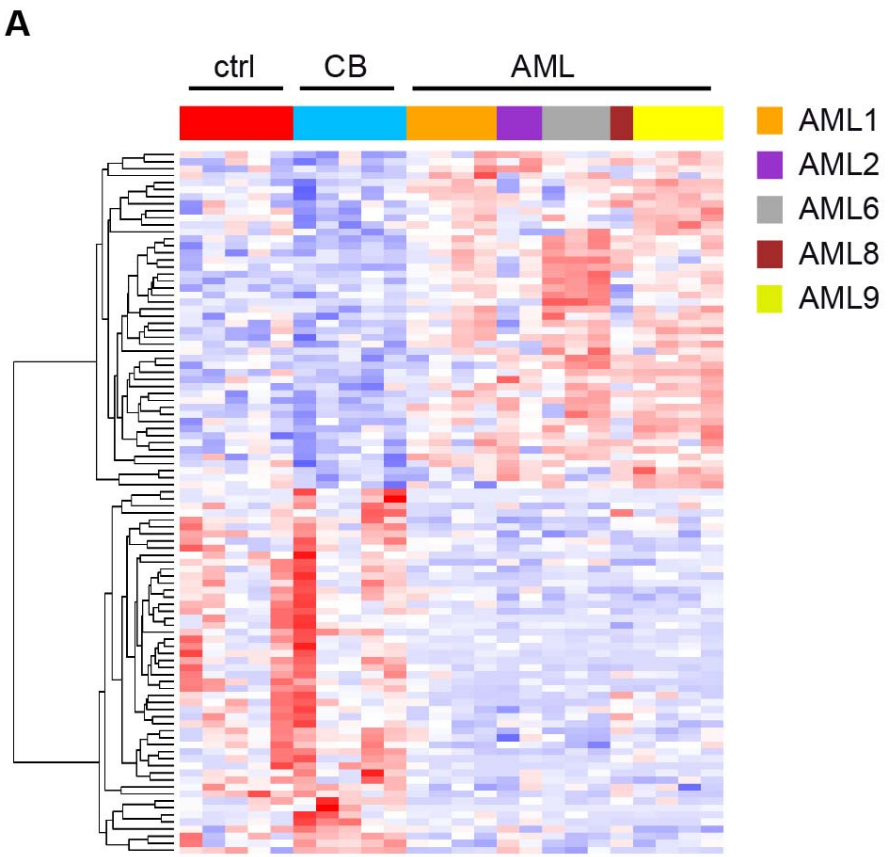
AML-induced toxicity on vessel permeability

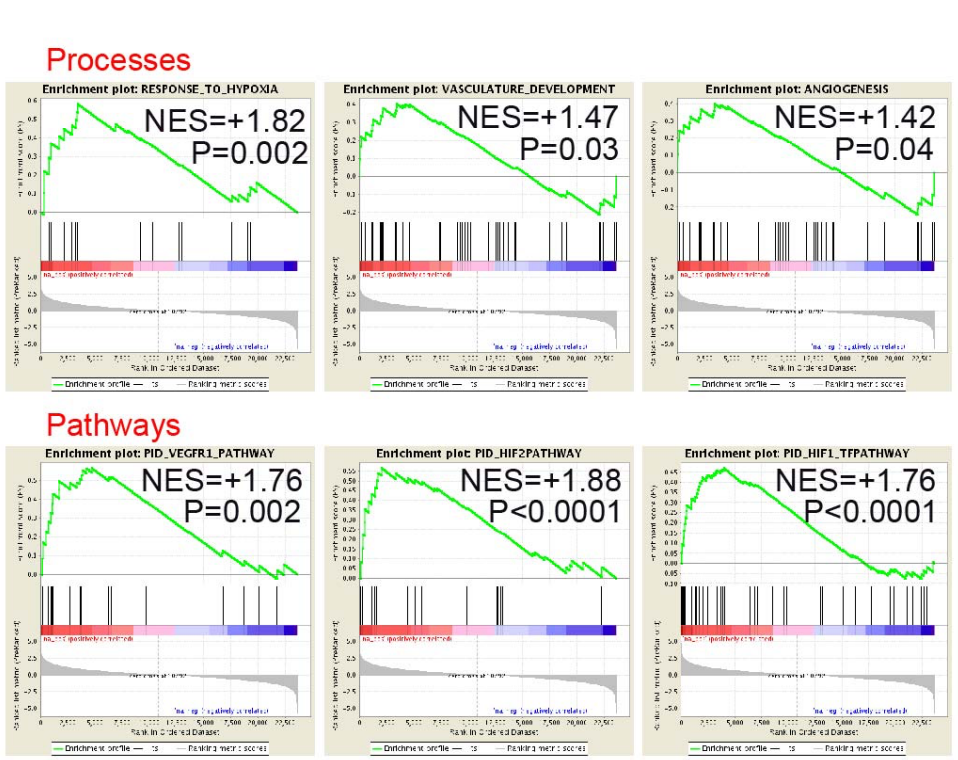




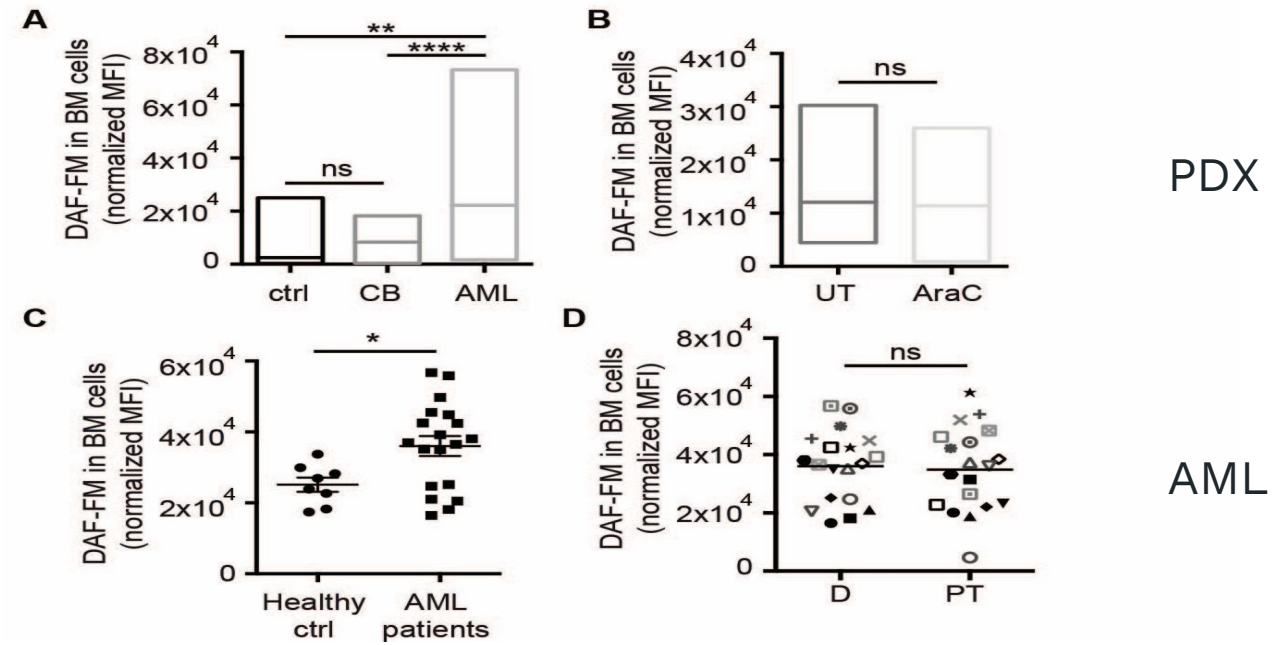


Endothelial cells in contact with AML have altered vascular development, angiogenesis, increase in VEGF-R and HIFs pathways



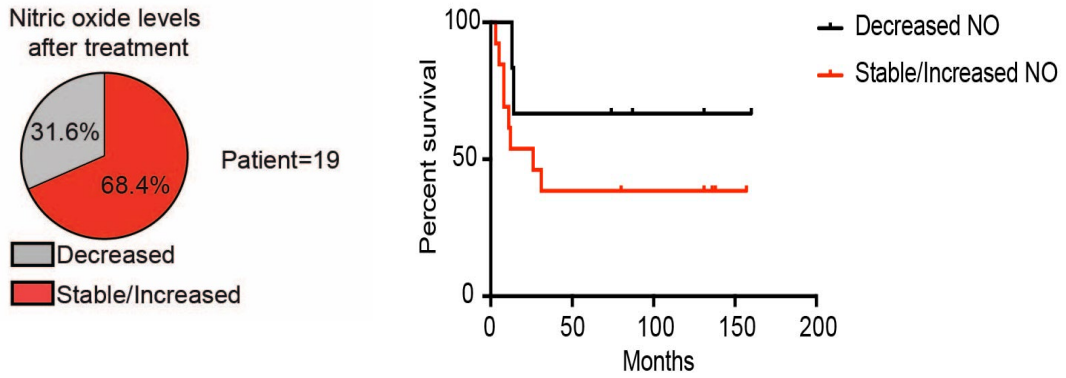


Upregulation of nitric oxide (NO) pathway



PDX model

AML patients



Passaro D et al. Cancer Cell, 2018

Summary I

- We found several abnormalities in the vascular architecture and function in patient-derived xenografts (PDX), i.e. vascular leakiness.
- We identified an increase in nitric oxide (NO) as major mediator of this phenotype in PDX and in patient-derived BM biopsies.
- Moreover, induction chemotherapy failed to restore normal vascular permeability and NO levels.
- Strikingly, inhibition of NO production reduced vascular permeability, and significantly improved treatment response in PDX.

Passaro D et al. Cancer Cell, 2018

Does the drugs treatment impact the bone marrow microenvironment?

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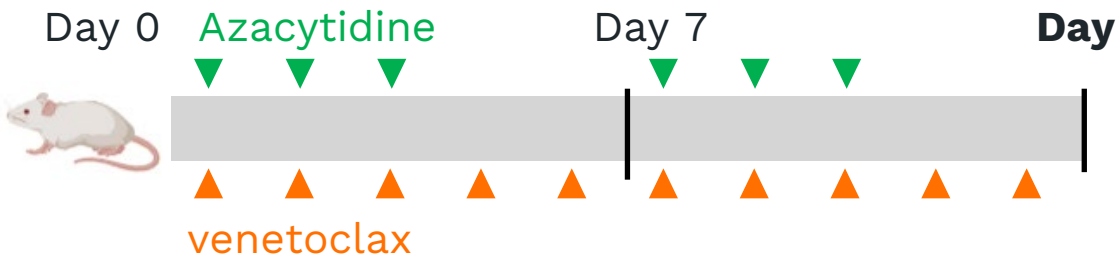
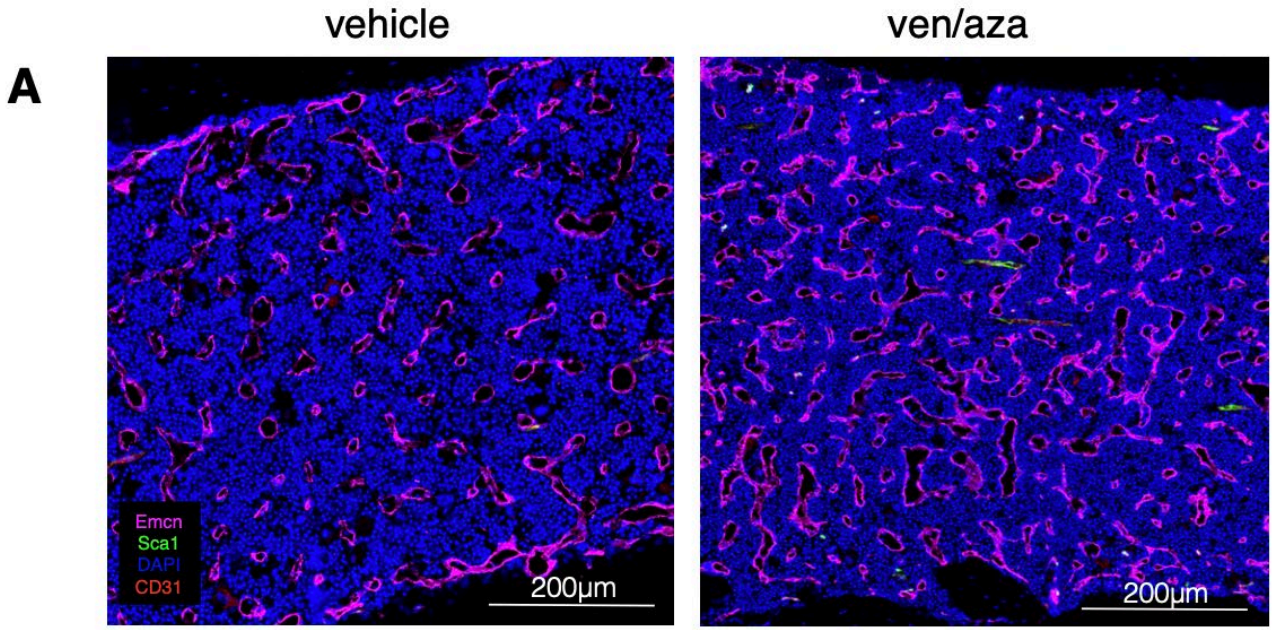
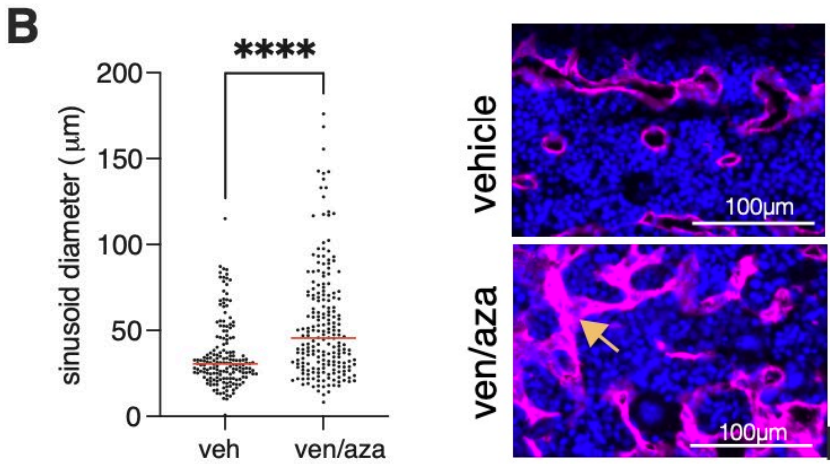
Ven/aza treatment induces remodeling of the vascular niche

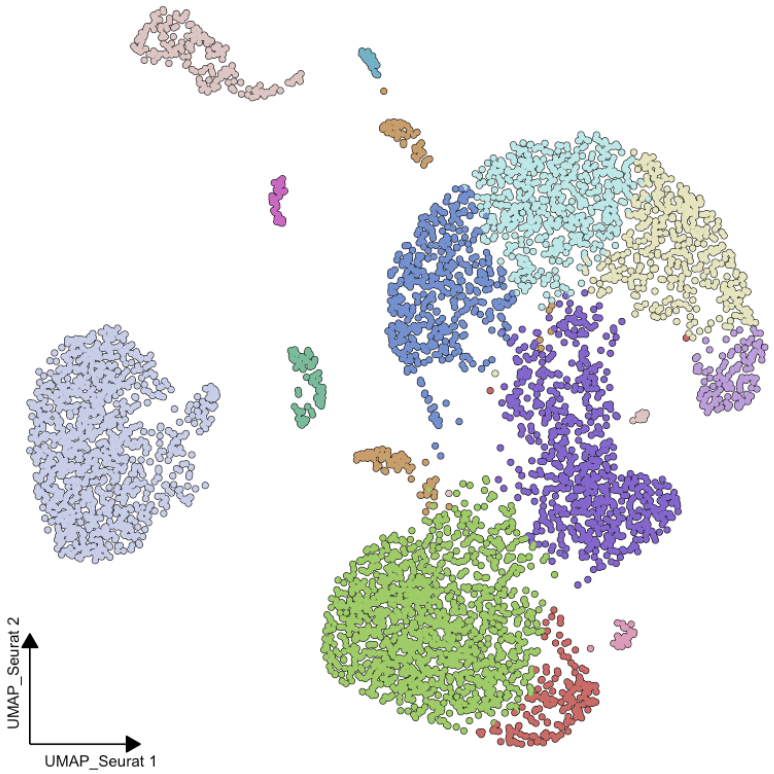


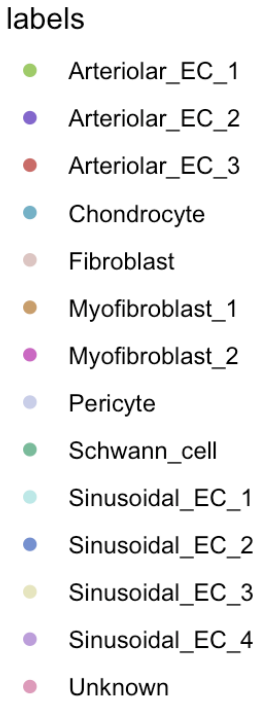
cull mice for:
- Immunofluorescent imaging
- Flow cytometry analysis
- scRNAseq

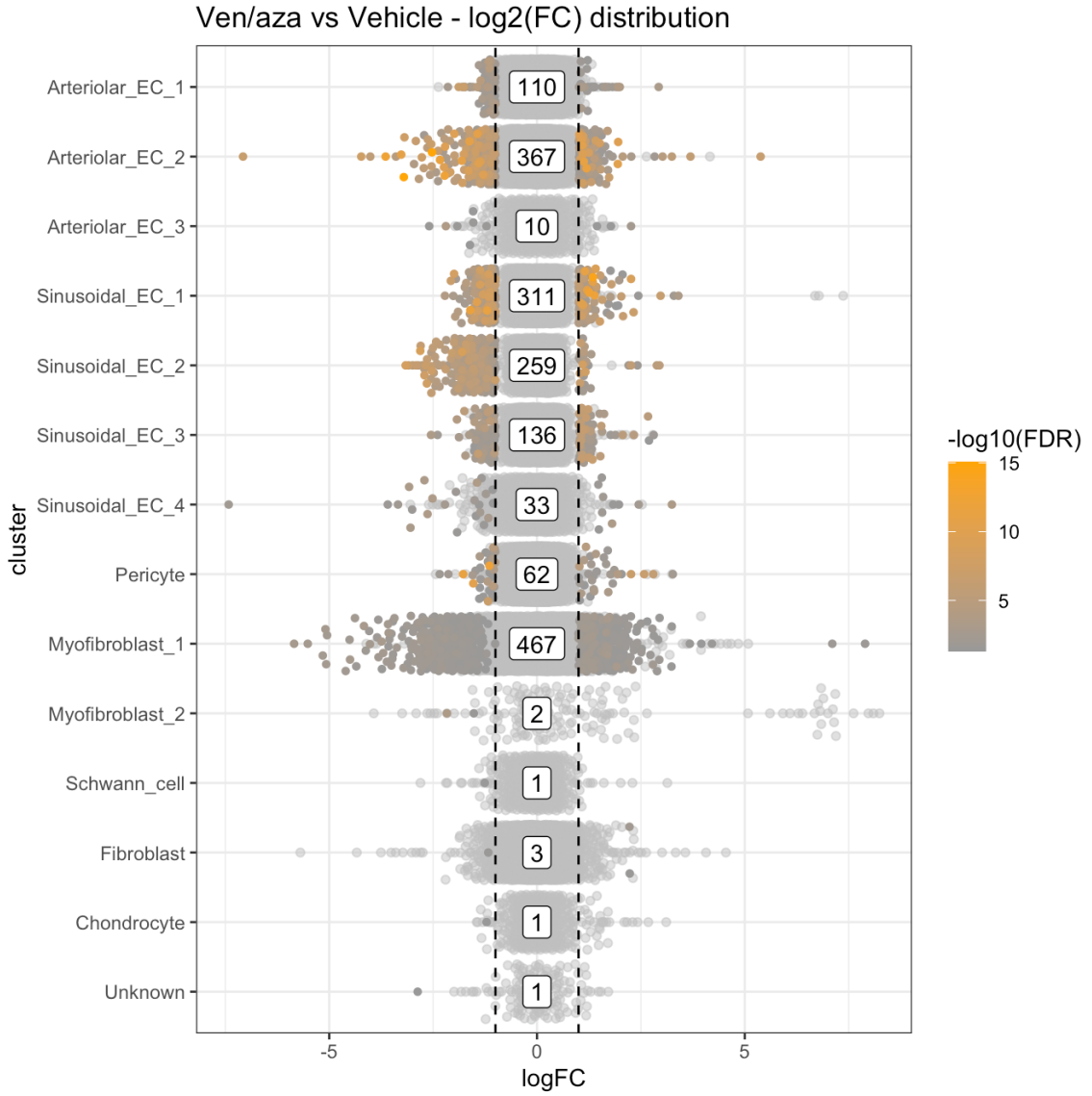




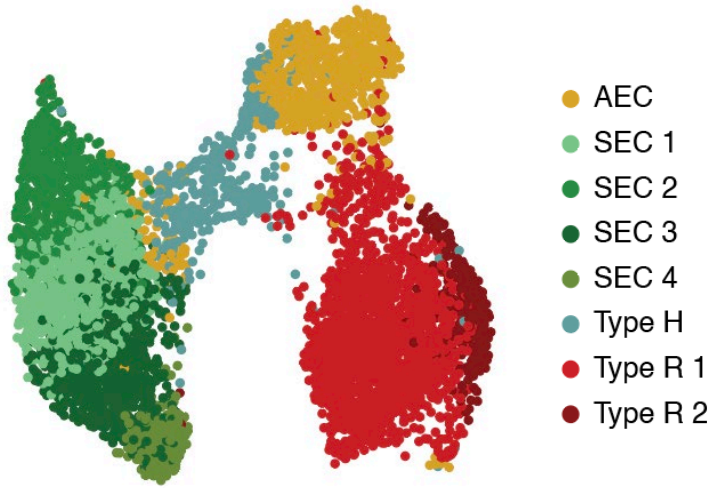
Ven/aza induces transcriptional reprogramming of ECs



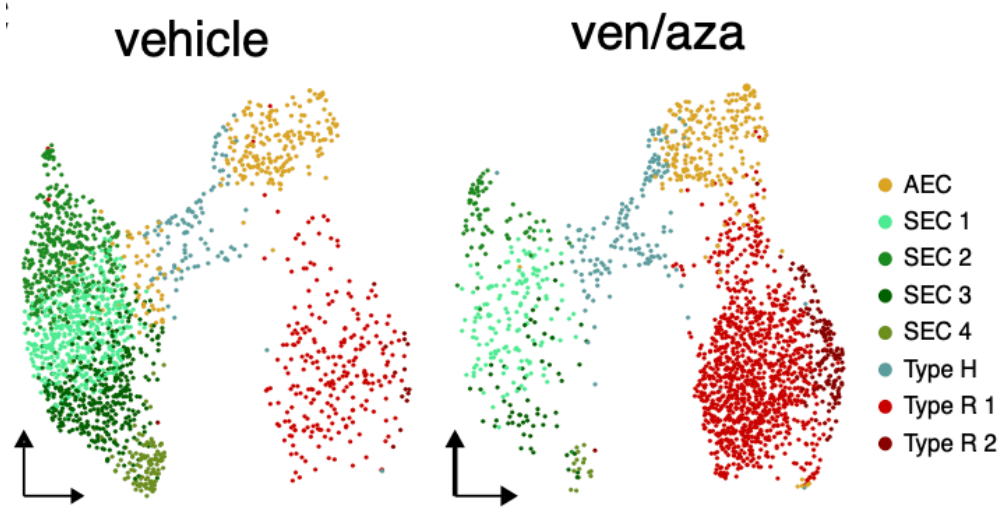


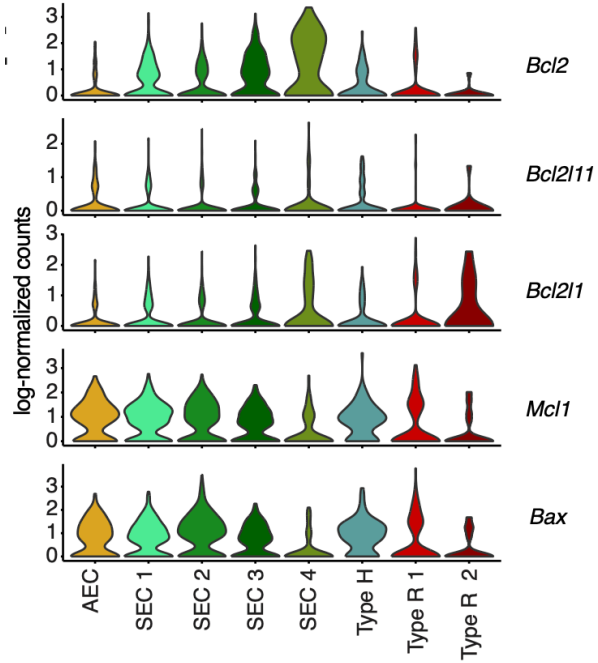


Transcriptional reprogramming in the ECs in response to ven/aza



SECs: Sca1^{low}, PDPN^{+}, VEGFR3^{+}
AECs: Sca1^{high}, PDPN^{-}, VEGFR3^{-}
Type H: CD31^{high}/Emcn^{high}
metaphysis capillary ECs - "**mpECs**"
(Kusumbe et al. 2014)
Type R: remodelling-associated capillaries - "**rECs**" (Mohanakrishnan et al. 2024)





Expansion of novel Type R vessels in response to ven/aza

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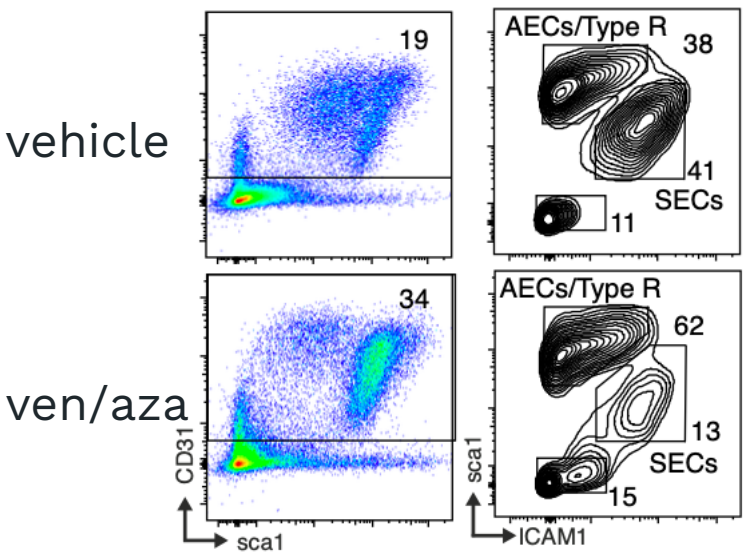
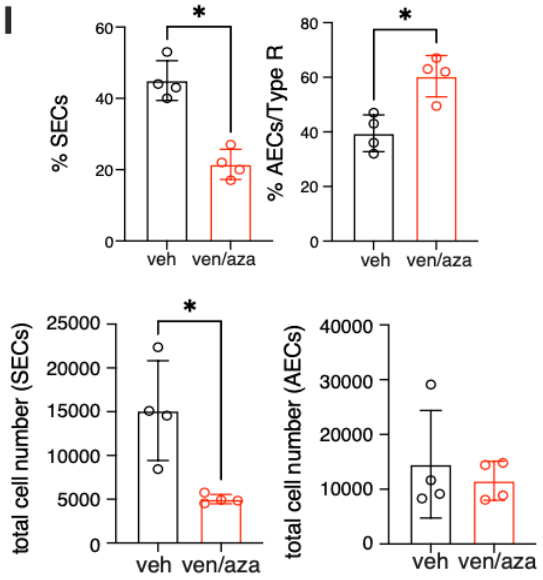
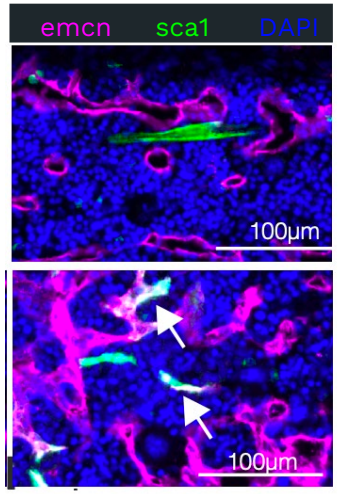
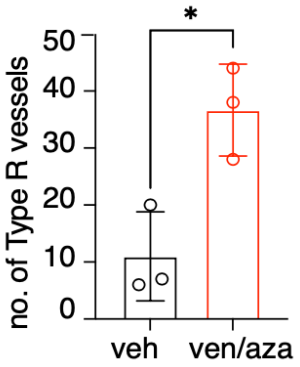
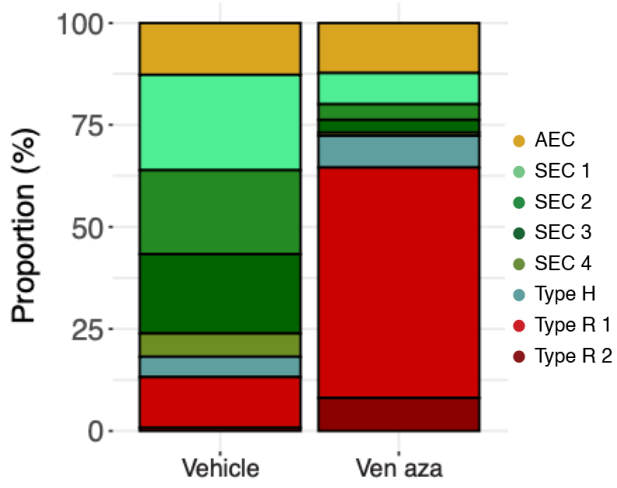
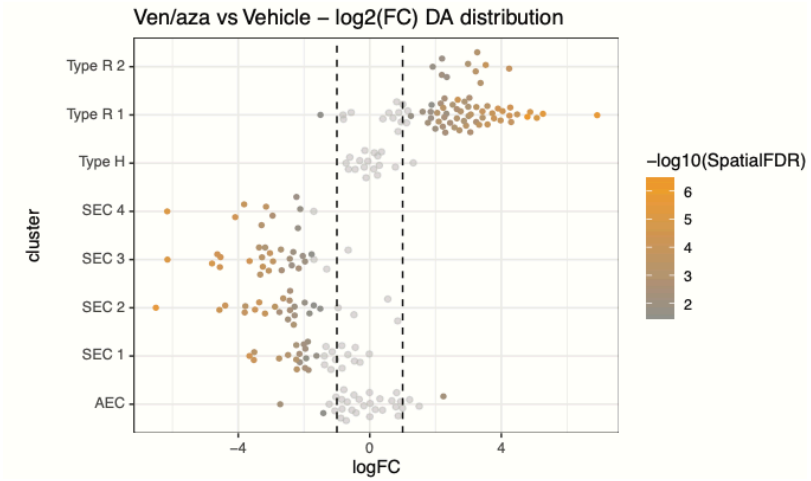


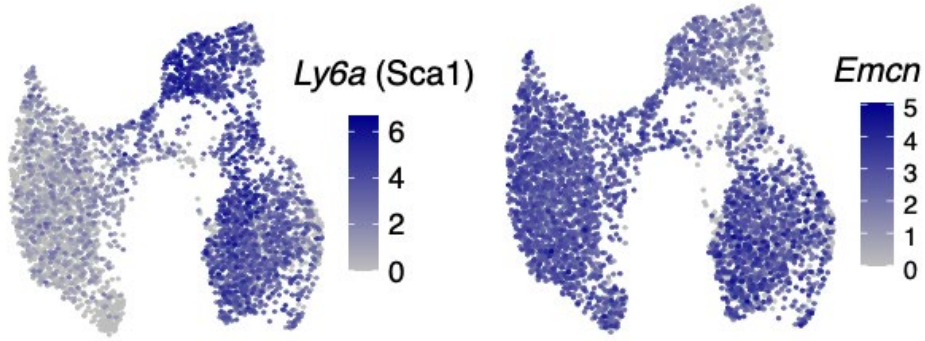




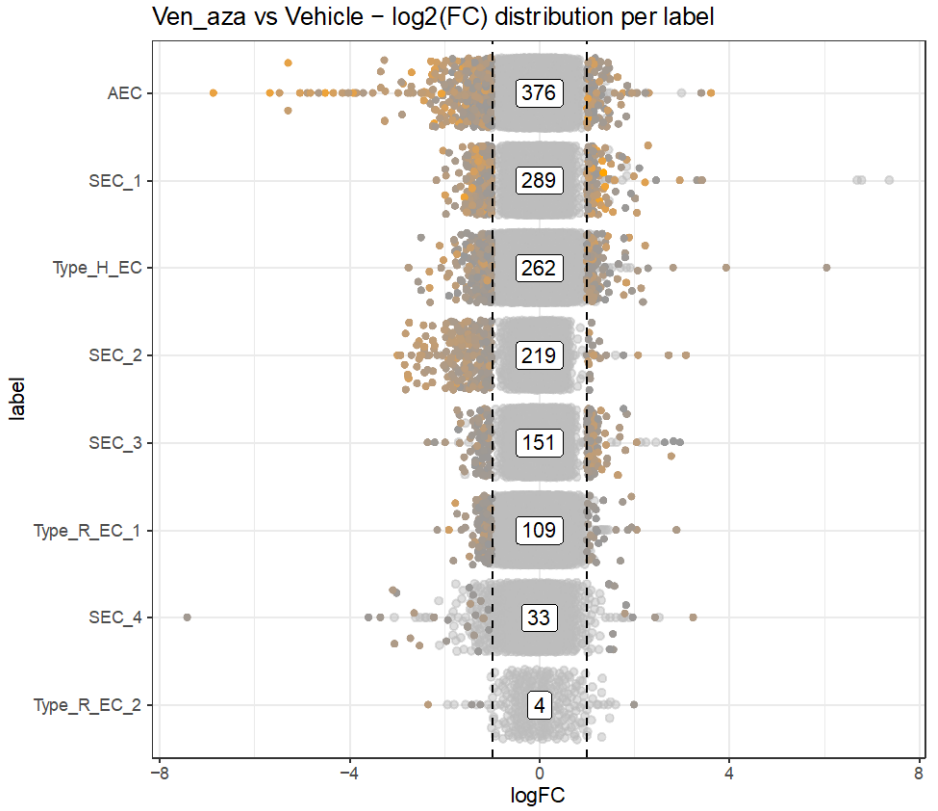


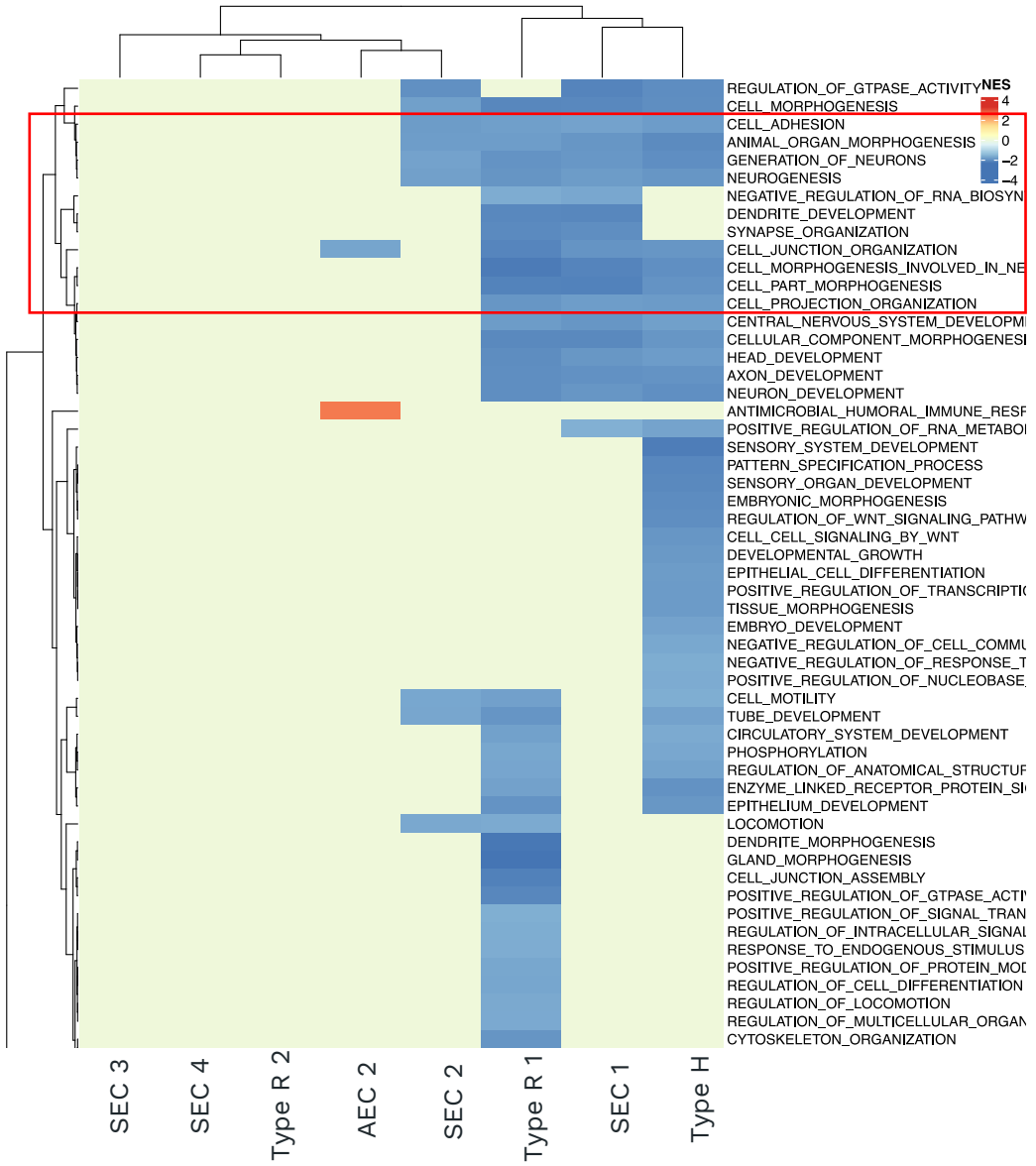






Significant downregulation of cell adhesion/cell junction pathways





Dilation of sinusoids is reversed after three weeks post treatment

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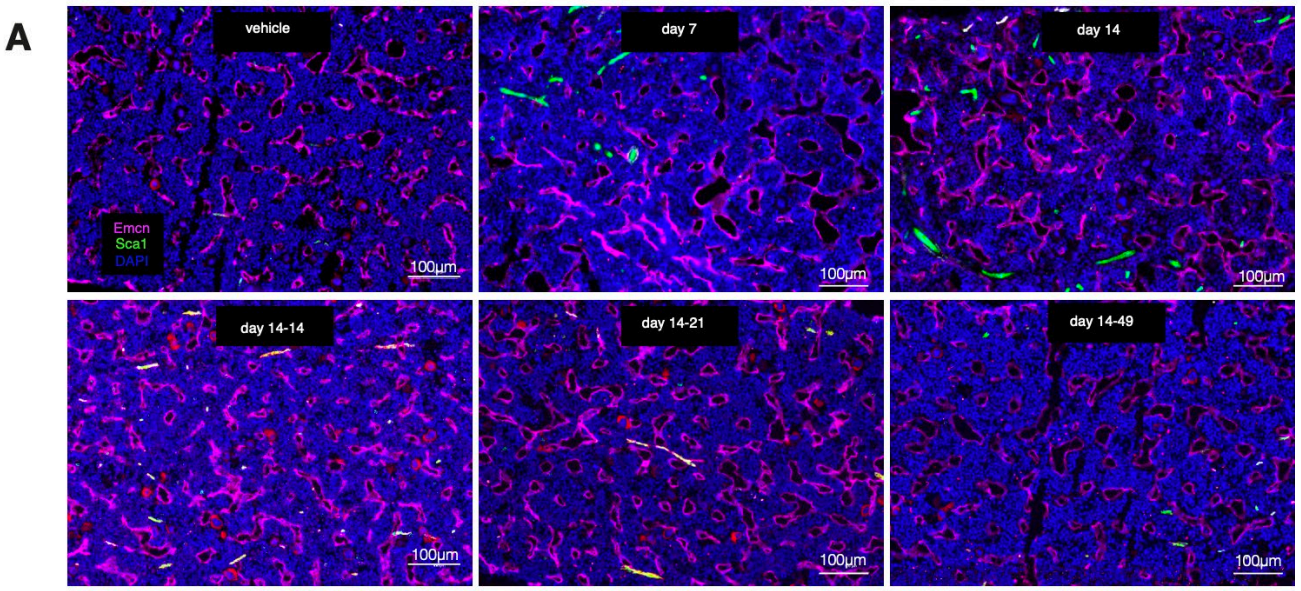

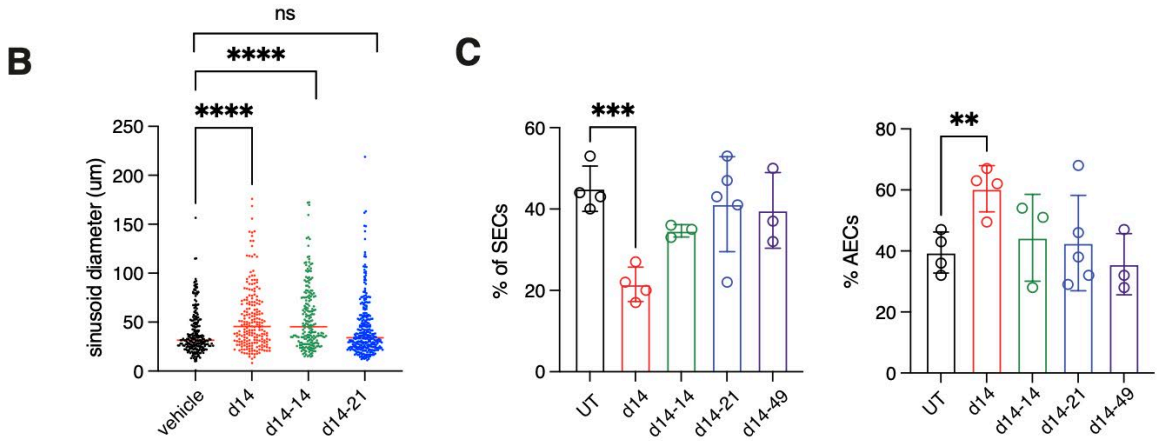

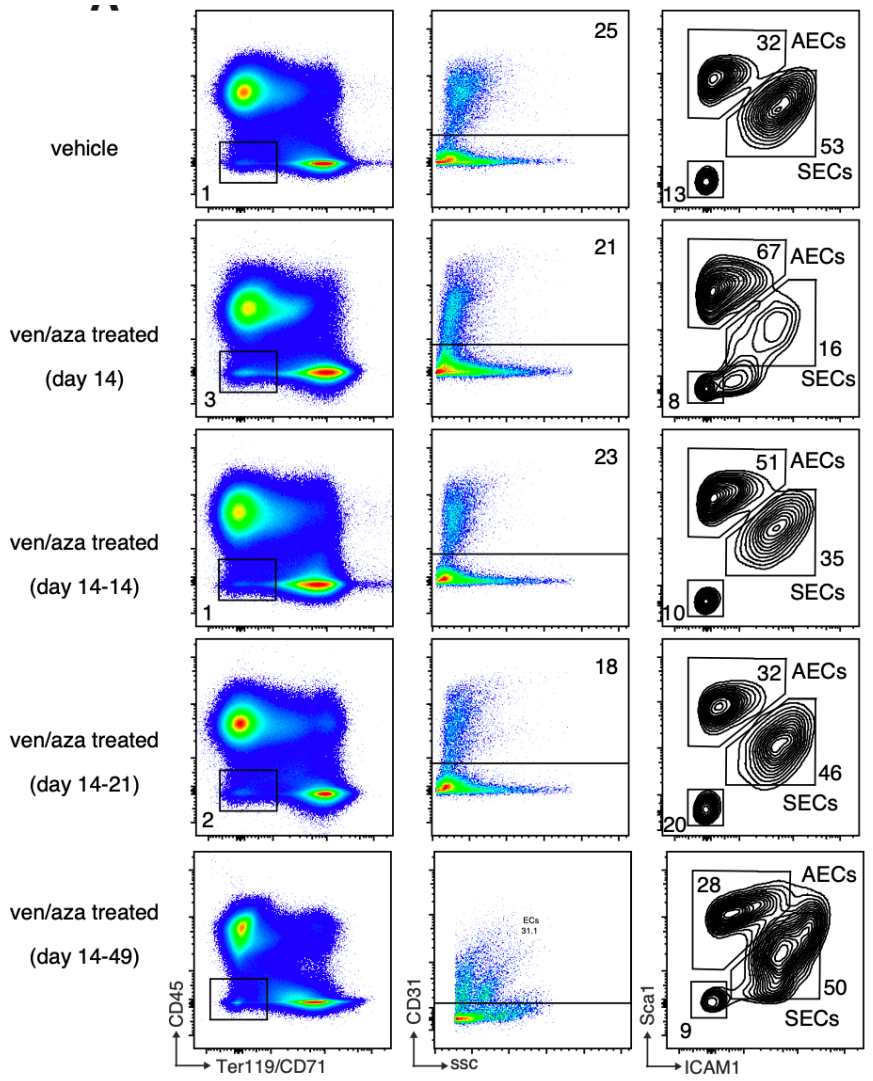

Cell type composition is partially recovered 7 weeks post therapy

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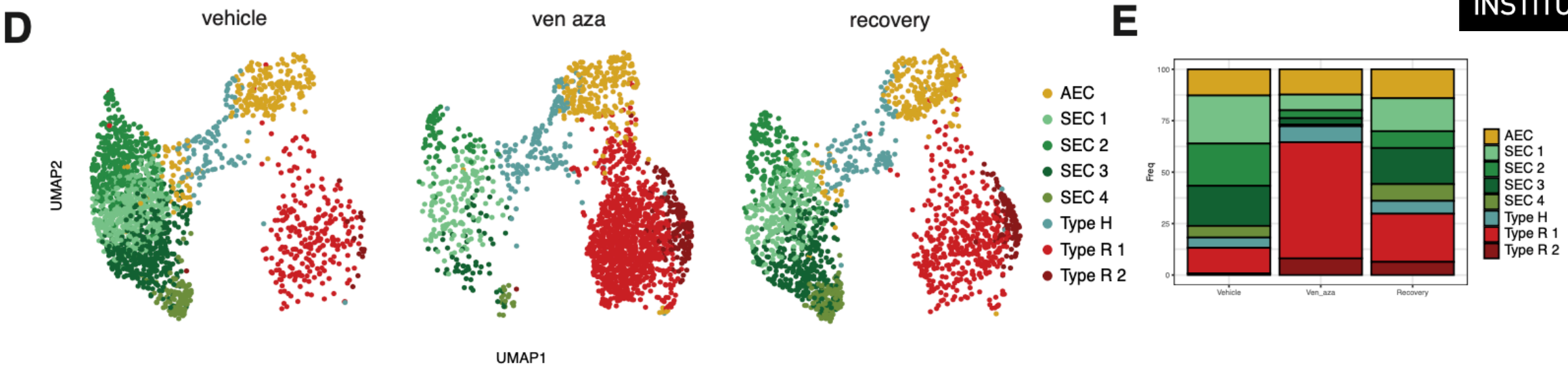

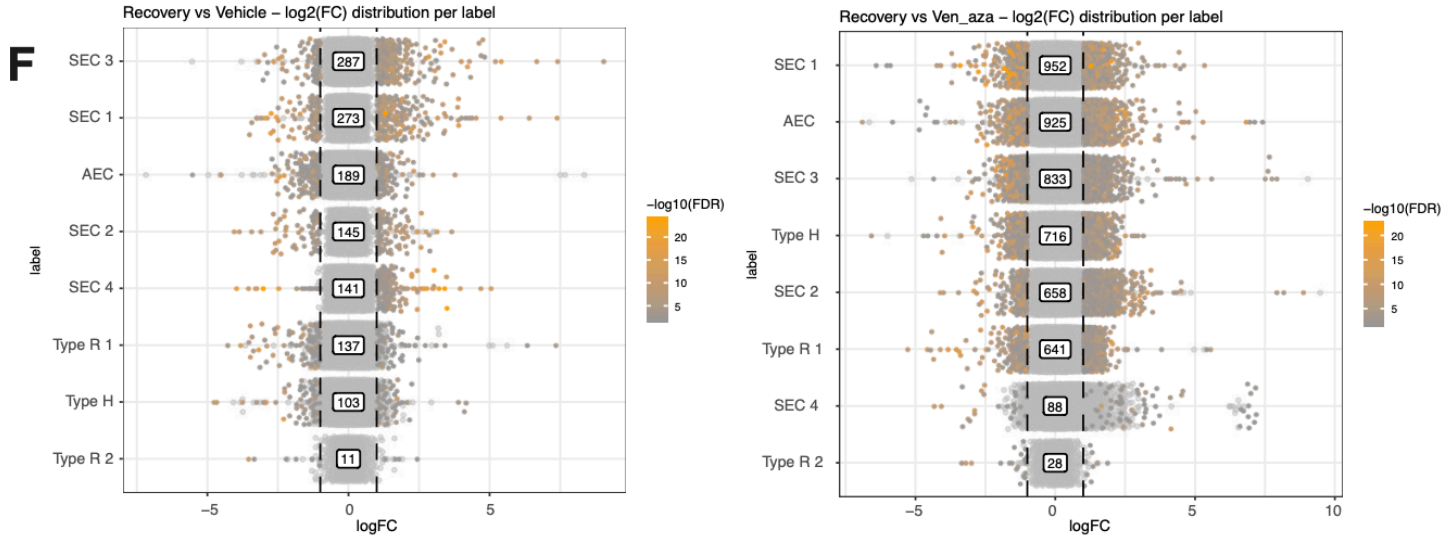

Partial recovery
at the transcriptional level

Summary II:

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- Venetoclax and azacitidine combinational therapy selectively targets the bone marrow vascular network
- SECs express highest Bcl2 amongst all vascular niche cell types and are most sensitive to therapy
- Damage to the vasculature is partially reversed after three weeks off treatment

Can cytotoxic drugs impede BME and potentially promote AML dev/relapse?

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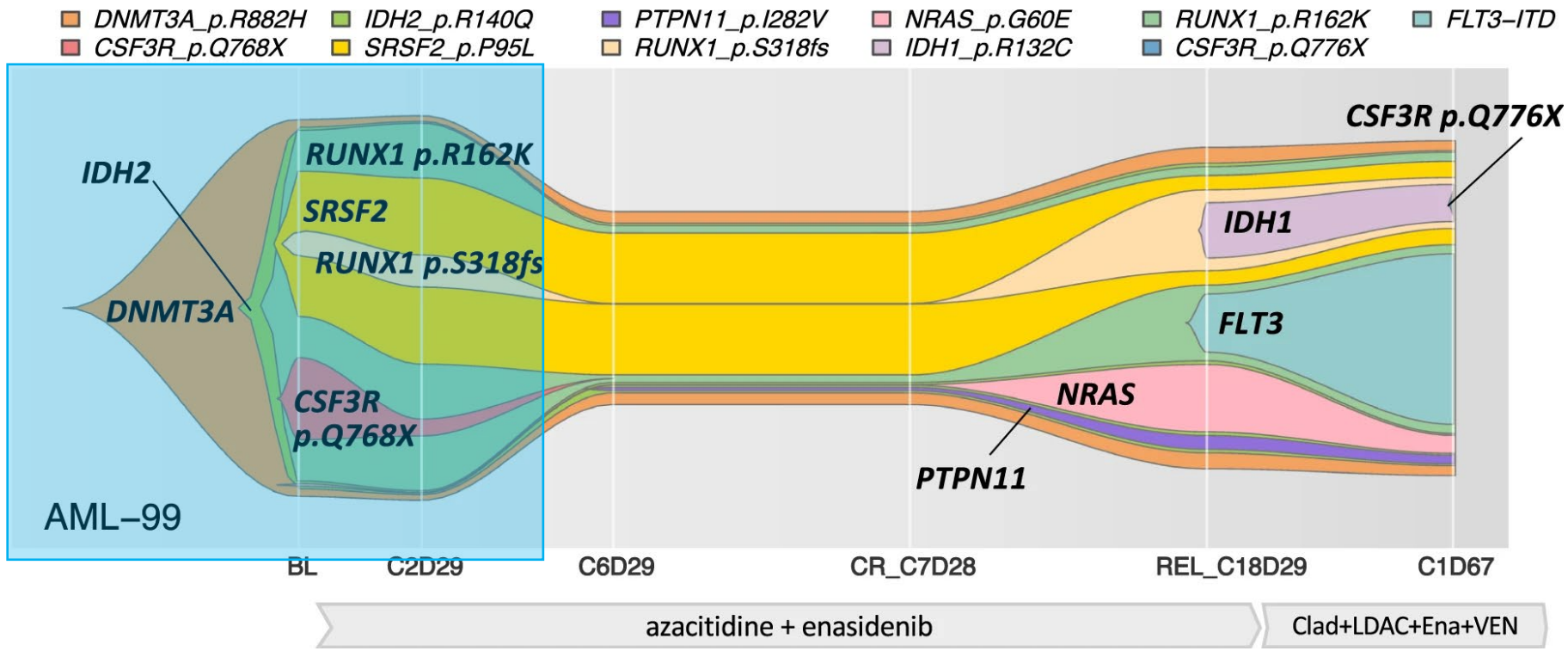
Adapted from Morita et al Nature Comms. 2020



What role does the BME play at each of these stages of disease?

Could different subclones present in an AML patients communicate with the BME differently ?

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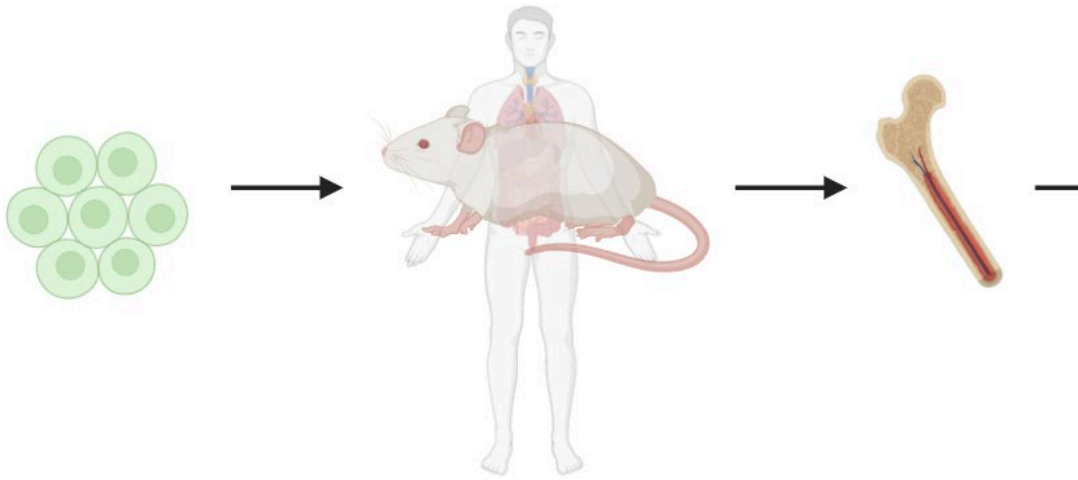
AML1

Mutation	VAF (%)
CUX1	13.5
DNMT3A	44.1
FLT3-ITD	7
IDH1	47.3
NF1	4.5
RUNX1	16.05
STAG2	16.67

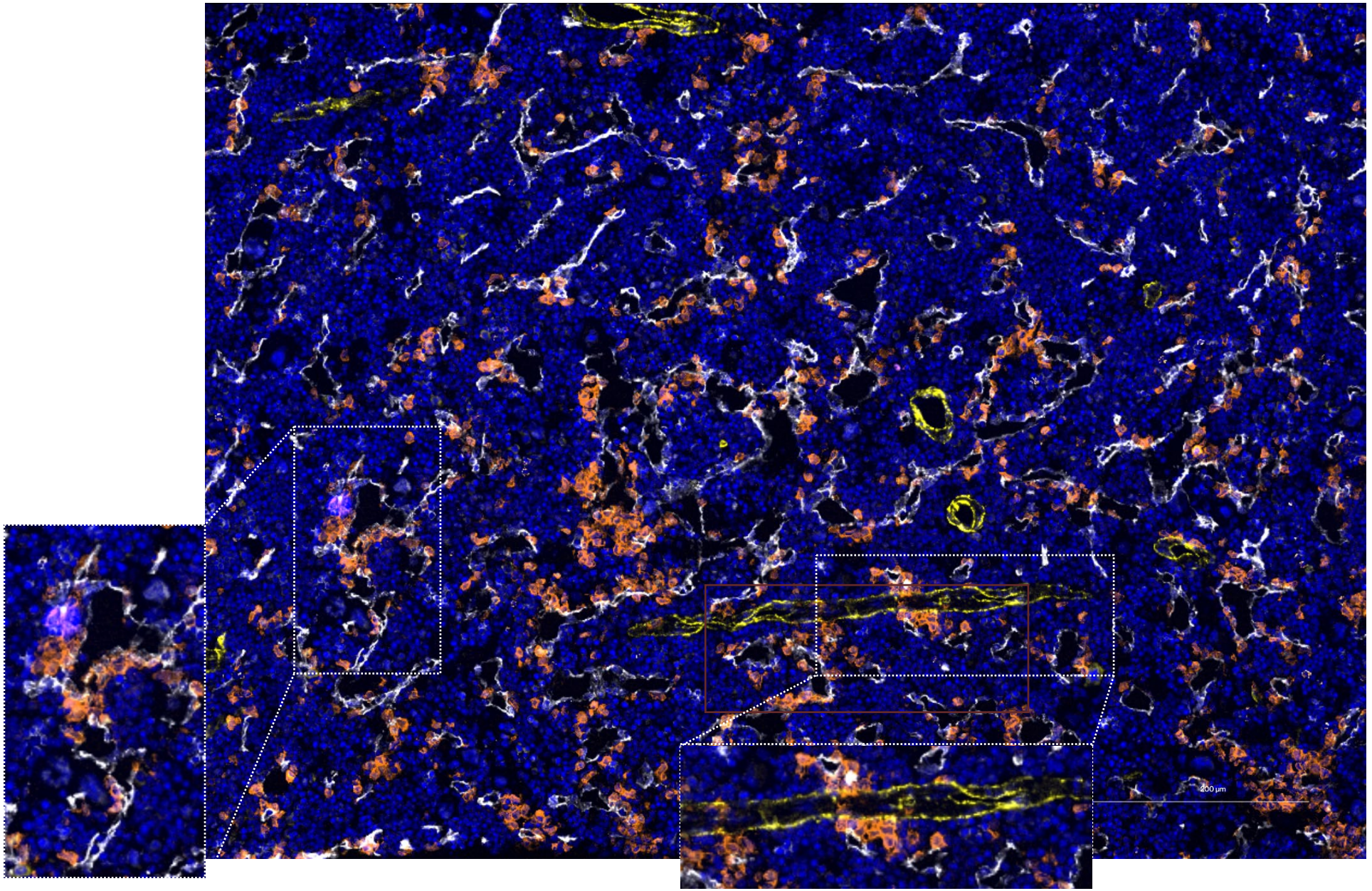


CODEX Multiplex imaging
scRNAseq

- NSG-SGM3 kit $^{w41/w41}$ humanized mice allows high engraftment of patient derived cells without irradiation

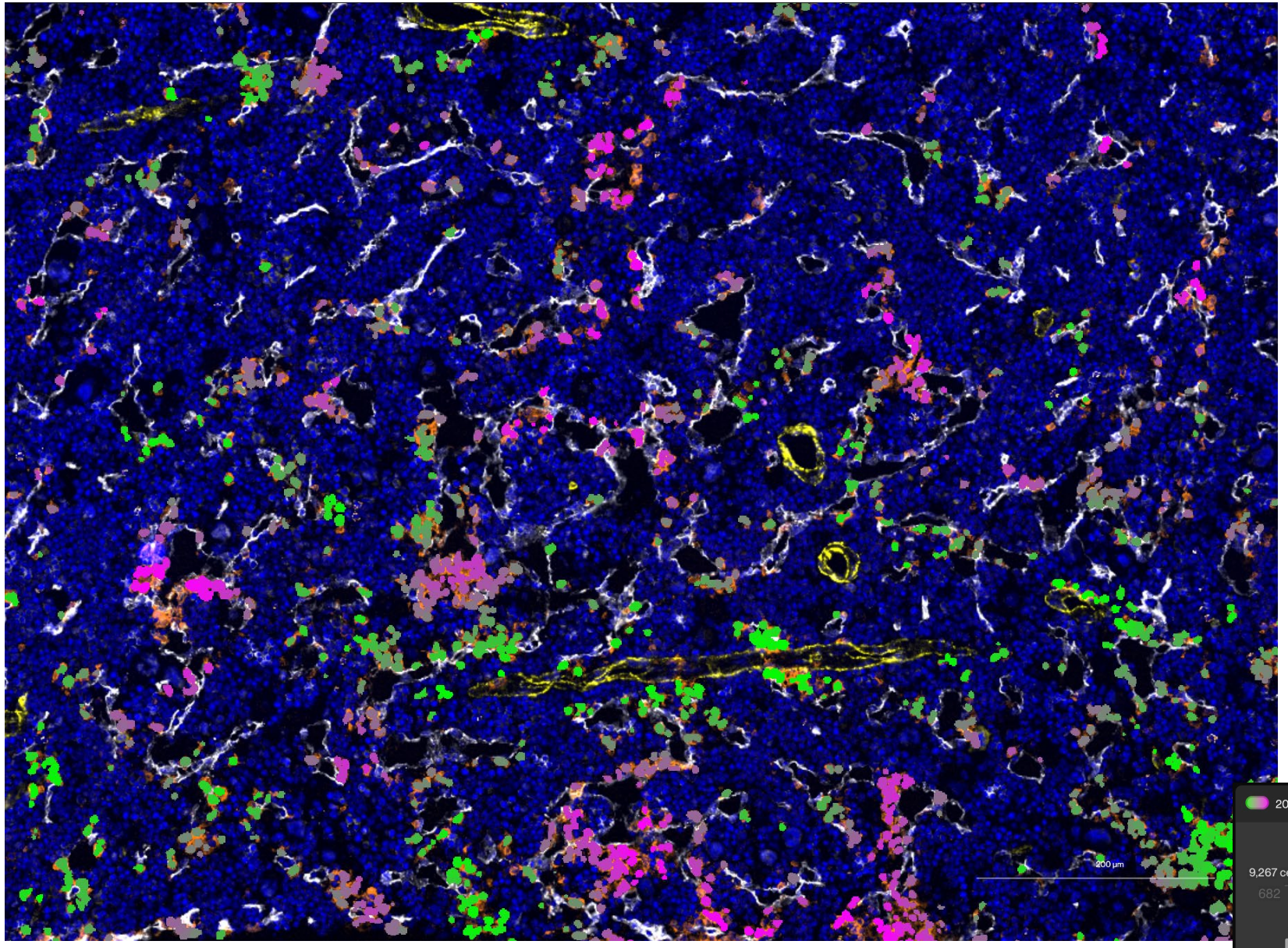
- mCD45-,hCD45-,Ter119-,CD71- cells were FACs sorted for scRNAseq analysis of the BMN cells

AML blasts localize to either the SECs or AECs



Emcn
hCD45
Sma1
DAPI

AML cells localize to either the SECs or AECs



FACs based approach in the isolation of distinct AML subclones

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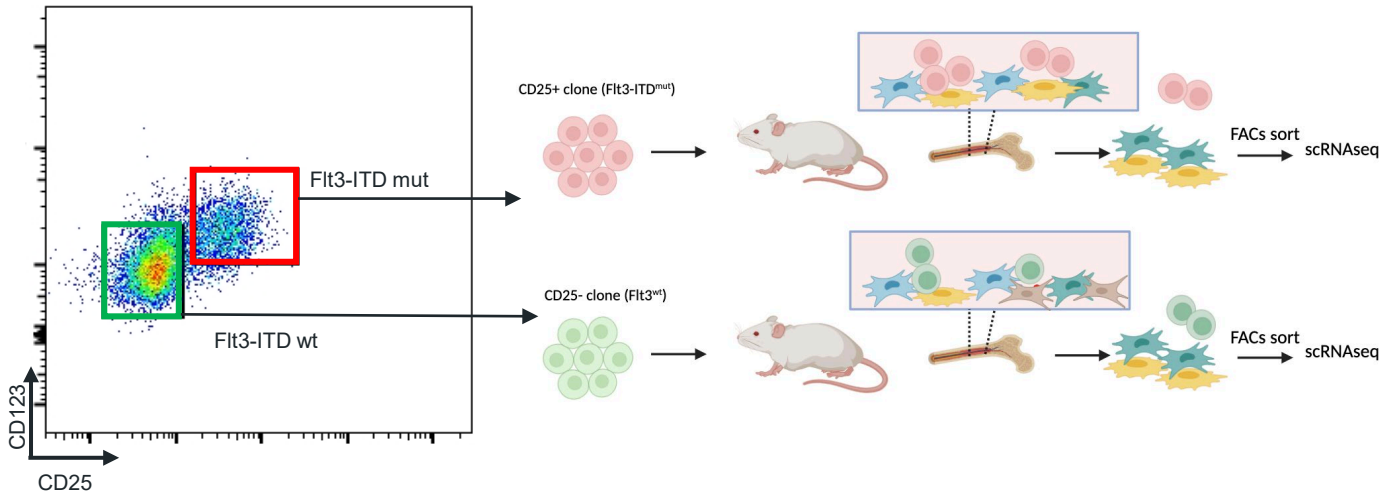
Marker	fluorophore
CD34	FITC
CD38	BUV737
CD45ra	APC-e780
ILRaP	AF350
CD97	PE
CD82	PE-Cy7
CD135	BV711
CD93	BUV661
CD117	APC
CD45	PacO
CD200	BV650
CD25	BV421
CD33	PE-Cy5
viability	Sytox blue

de Boer et al. Cancer Cell 2018

Mutation	VAF (%)	CD25+	CD25-
CUX1	13.5	ns*	ns*
DNMT3A	44.1	45%	45%
FLT3-ITD	7	36%	0
IDH1	47.3	50%	50%
NF1	4.5	ns*	ns*
RUNX1	16.05	ns*	ns*
STAG2	16.67	ns*	ns*



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Significant differential gene expression in the presence of AML

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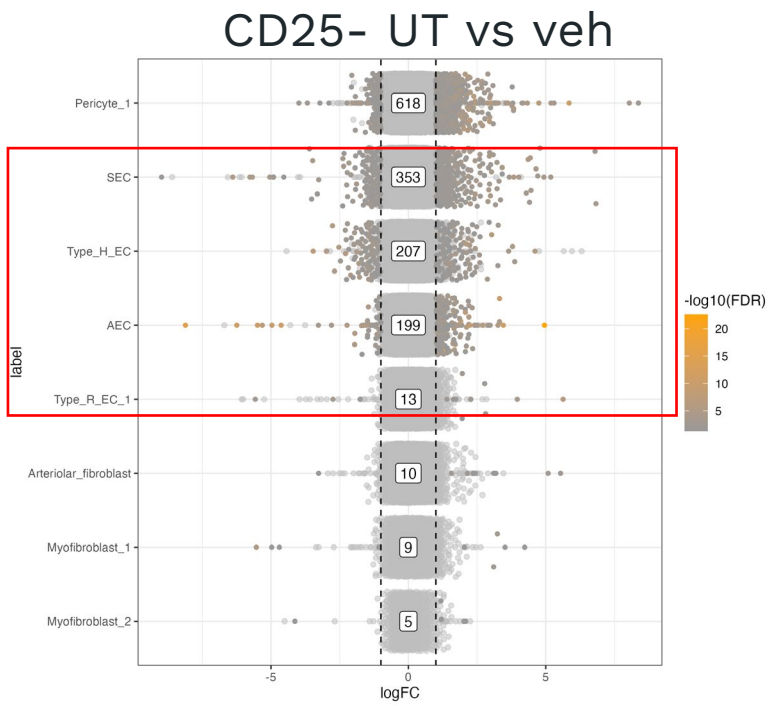

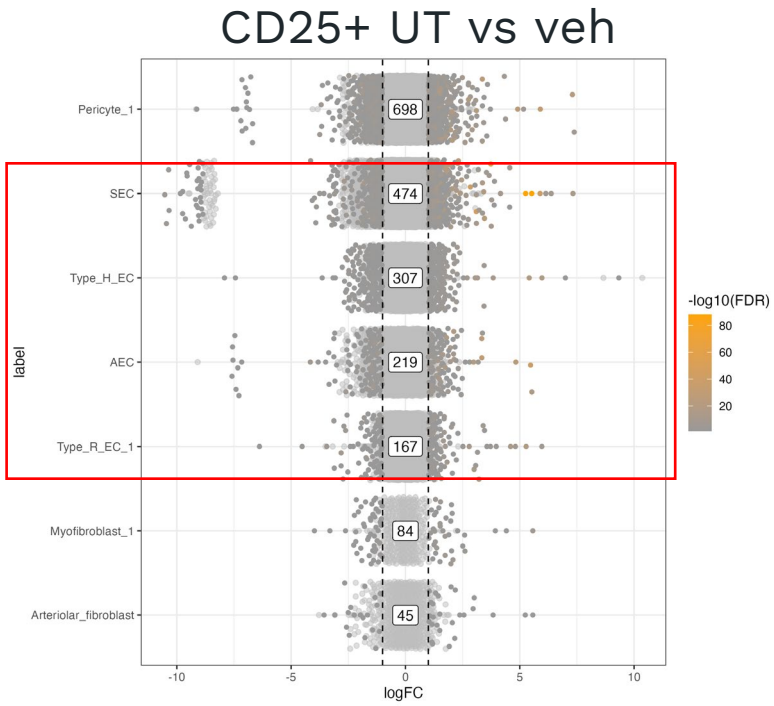

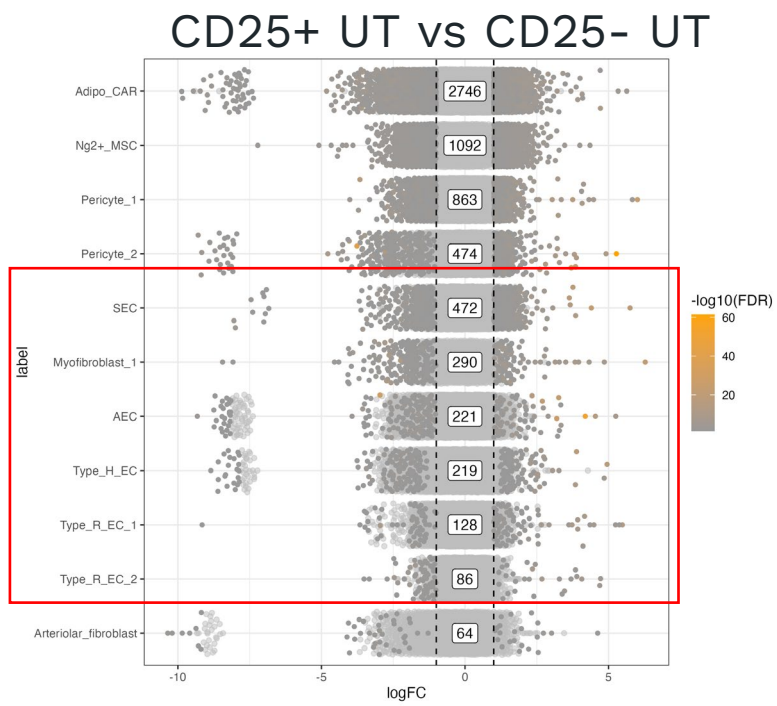

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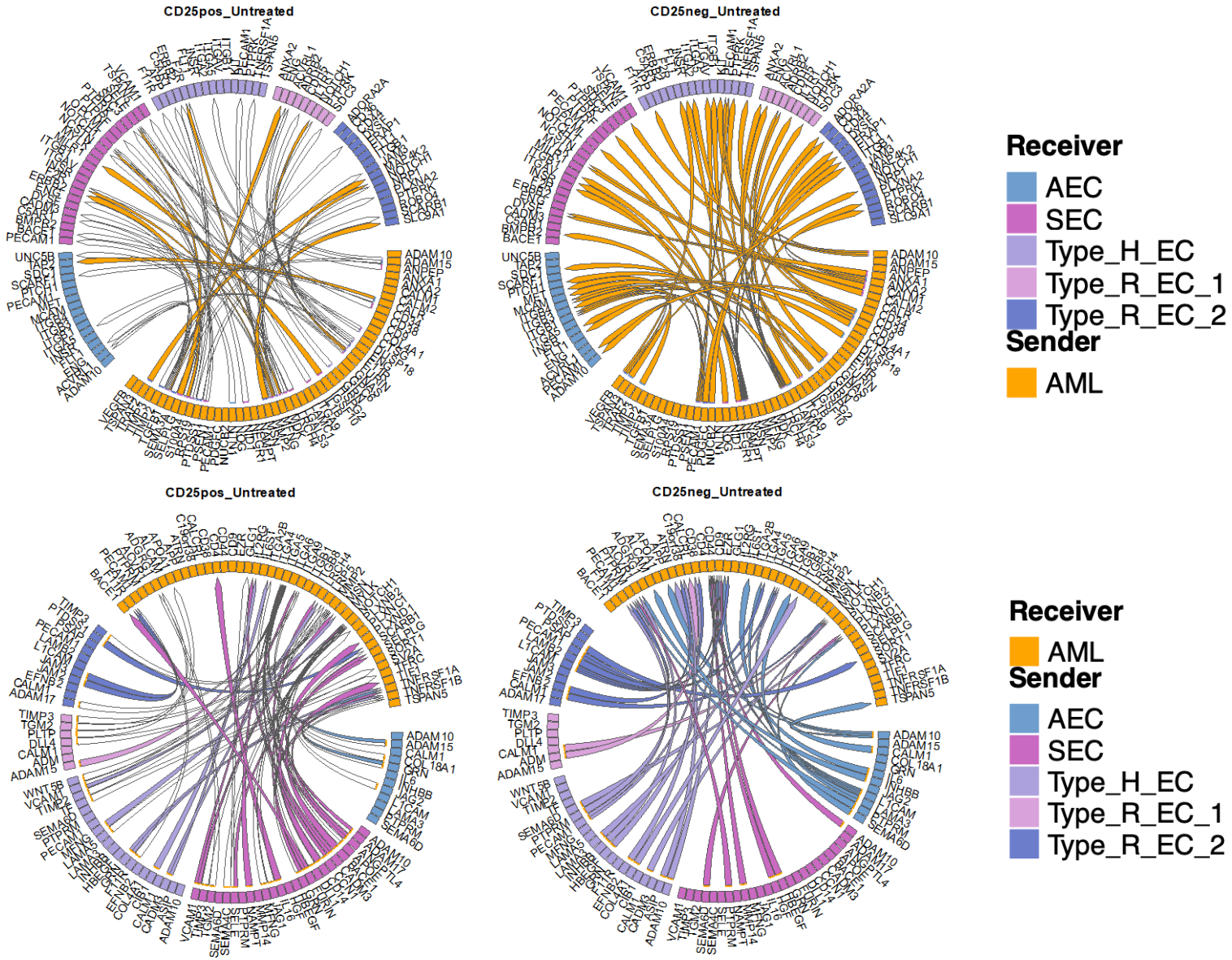
Differential preference in EC interaction between subclones



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AML1 contains a FLT3-ITD ven/aza resistant subclone

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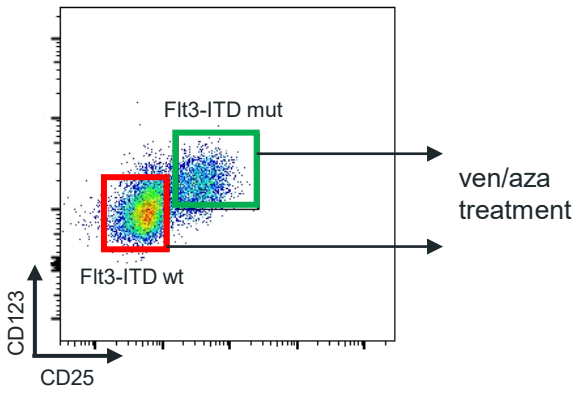
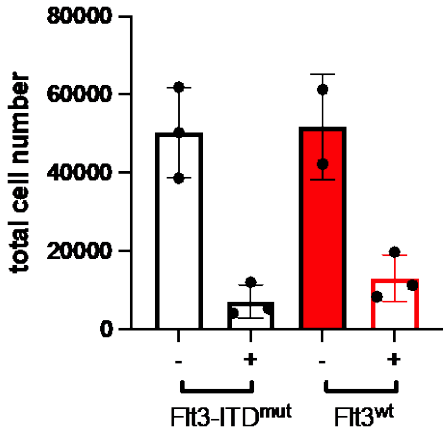
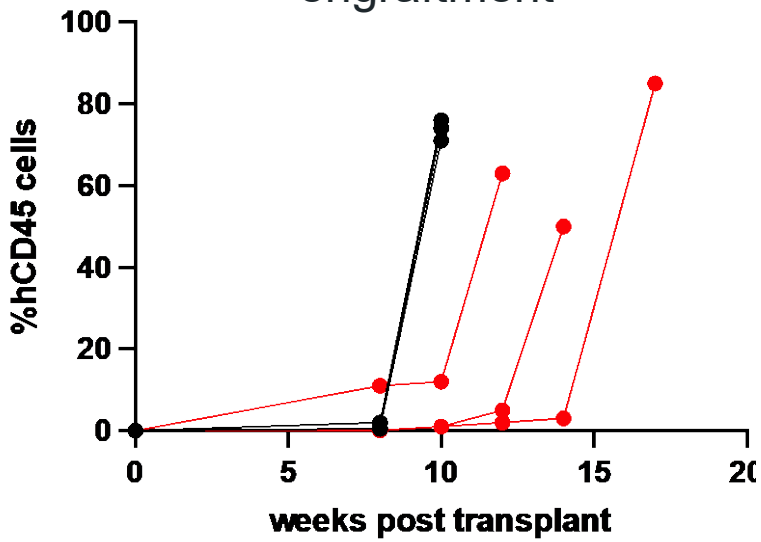


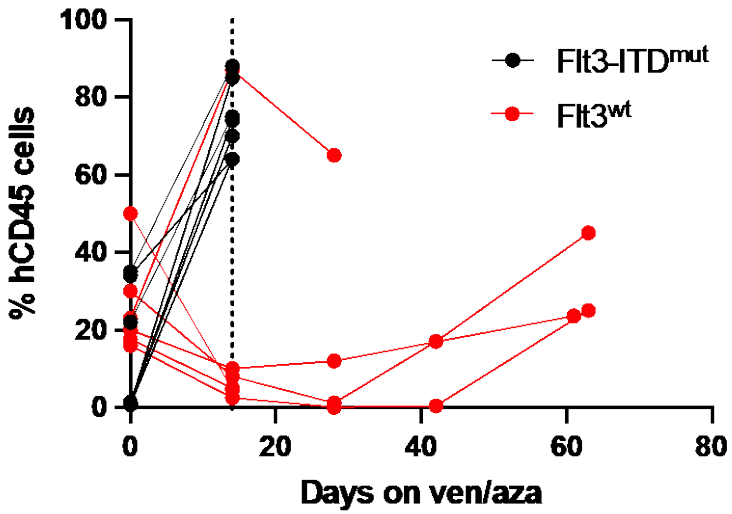
ex vivo



engraftment



ven/aza sensitivity



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Summary and future directions:

- AML1 contains two distinct genetic subclones that can be separated by the expression of CD25.
- CD25+ subclone is resistant to ven/aza whereas CD25- subclone is sensitive
- We show that CD25+ subclone communicate more with SECs and CD25- subclone with AEC
- Does cross-talk between SECs and the CD25+ subclone contribute to resistance to ven/aza therapy?
- What interactions between the CD25+ and SECs are present following ven/aza treatment?

ACKNOWLEDGMENTS

Thanks for your attention !!

Haematopoietic Stem Cell Lab

Syed Mian
Linda Ariza-McNaughton
Alessandra Ferrelli
Katherine Sturgess
Despoina Papazoglou
Fatihah Mohamad Nor
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Fernando Anjos Afonso
Helene Foissner
Kadidja Habel
Eric Jong
Amirtha Pryia Ramesh
Giuseppe D'Agostino

Manuel Garcia-Albornoz
Marion Piganeau
Aneesh Sharma
Alexander Waclawiczek
Diana Passaro
Jenny Huang
Anna Song
Henry Wood

Scientific Technical Platforms
Flow cytometry
Advanced sequencing
Bioinformatic/Biostatistic

Clinical collaborators:

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Prof. Matteo Della Porta
Dr Shahram Kordasti
Dr Tata Nageswara Rao
Dr Jiri Mayer
Dr Catherine Cargo
Prof. Ghulam Mufti
Prof Michaela Fontenay

Funded by the European Union
European Research Council
Synergy grant

Questions related to t-AML?

1. Is the bone marrow microenvironment (BMME) the hidden catalyst in malignant haematopoiesis?

2. Genome-wide DNA damage in HSCs after cytotoxic therapies are driving the initiation and progression to t-MN: What about the long-term toxicities/effects of the BMME?