

CALCRL (Calcitonin Receptor-Like Receptor) :

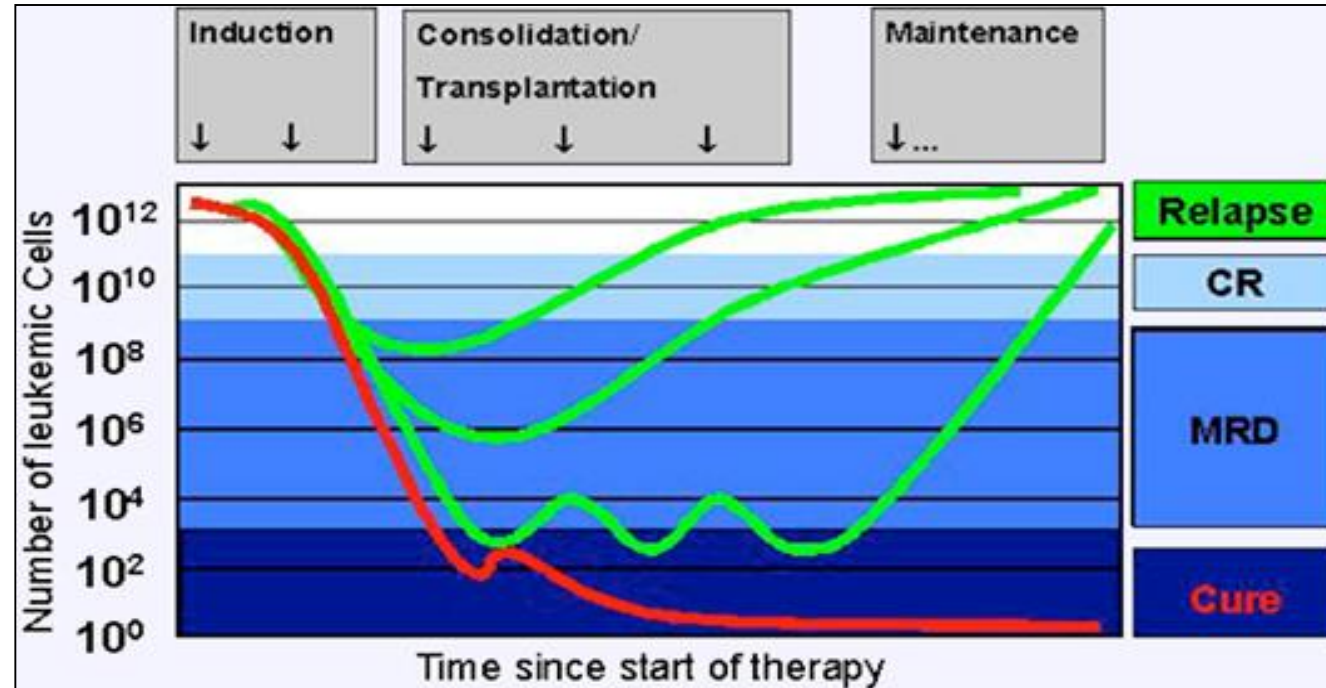
**A novel target of relapse-initiating leukemic stem cells
in acute myeloid leukemia**

Jean-Emmanuel Sarry

Team METAML – METabolism and drug resistance in Acute Myeloid Leukemia

Cancer Research Center of Toulouse

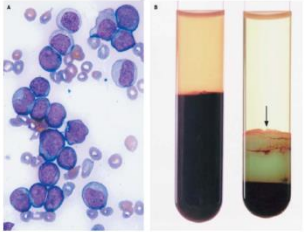
Relapses and drug resistance in cancer



Relapse: regrowth of **tumor-regenerating drug-resistant cells** following initial clinical benefit (complete remission and prolonged stable disease)

Drug resistance: arises from **genetic and non-genetic** mechanisms induced through the selective pressures imposed by therapy

Mechanisms-Of-Action of intensive chemotherapy in acute myeloid leukemia



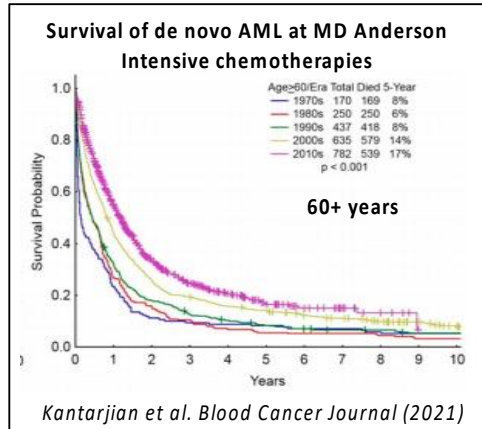
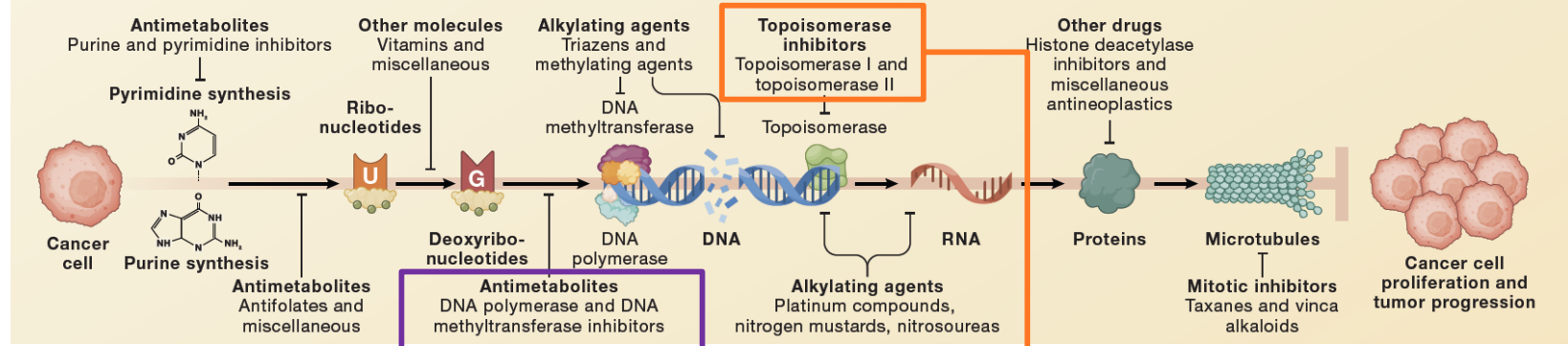
SnapShot

Cancer chemotherapy

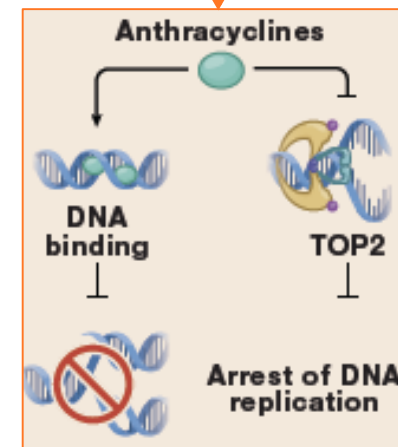
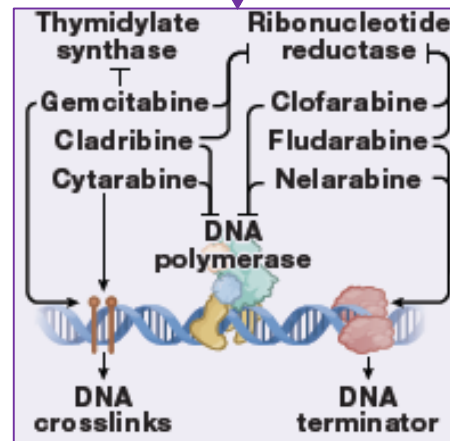
Luca Falzone¹, Roberto Bordonaro², and Massimo Libra¹

¹Department of Biomedical and Biotechnological Sciences, University of Catania, 95123 Catania, Italy; ²Oncological Department, Garibaldi Hospital, 95126 Catania, Italy

Cell
2023



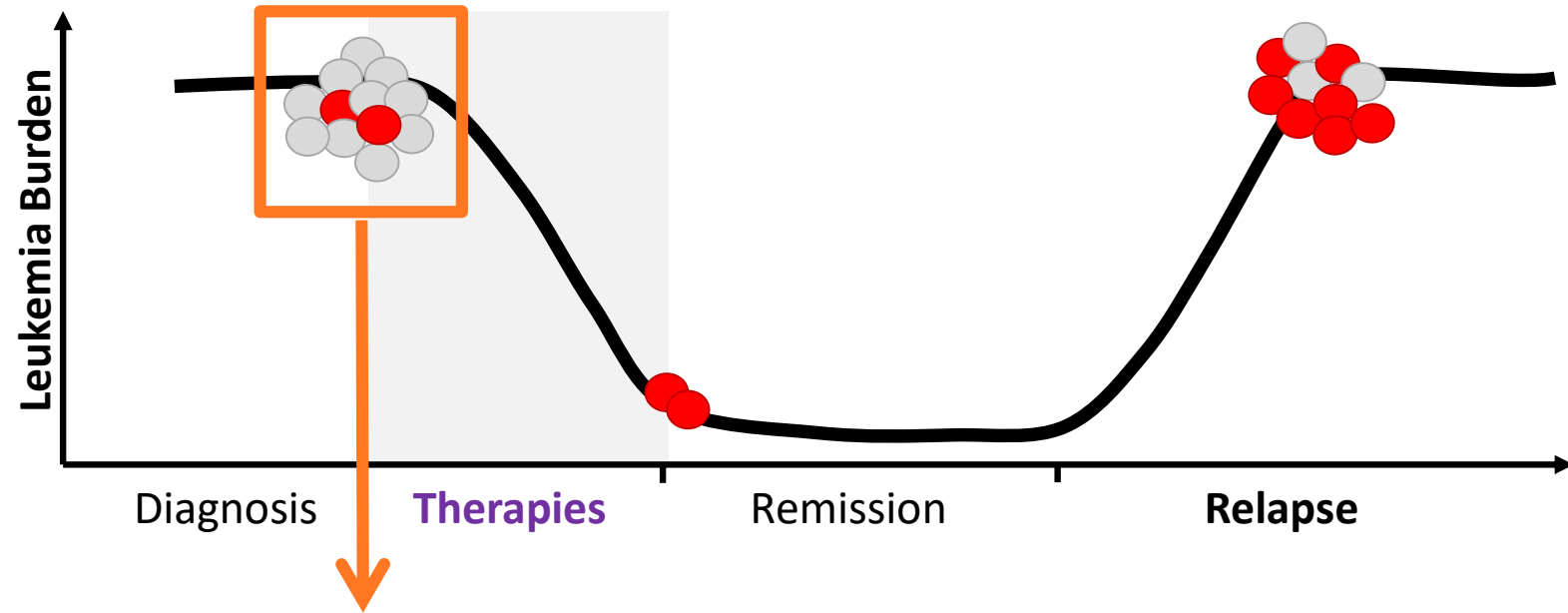
Cytarabine
AraC



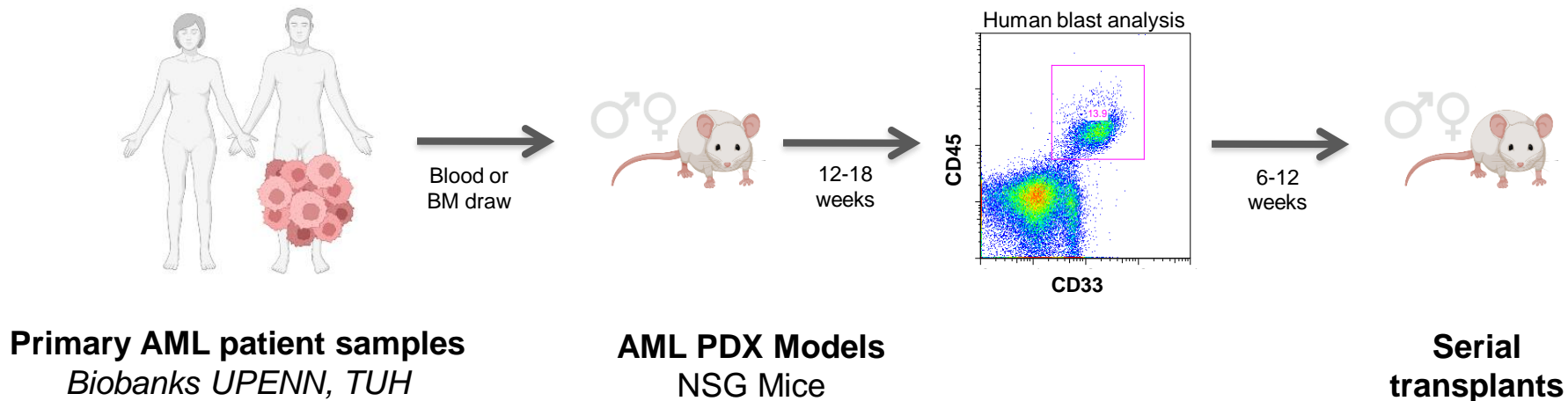
Idarubicine
IDA

> Killing cycling (through S-phase)/proliferating cells and sparing non-cycling cells

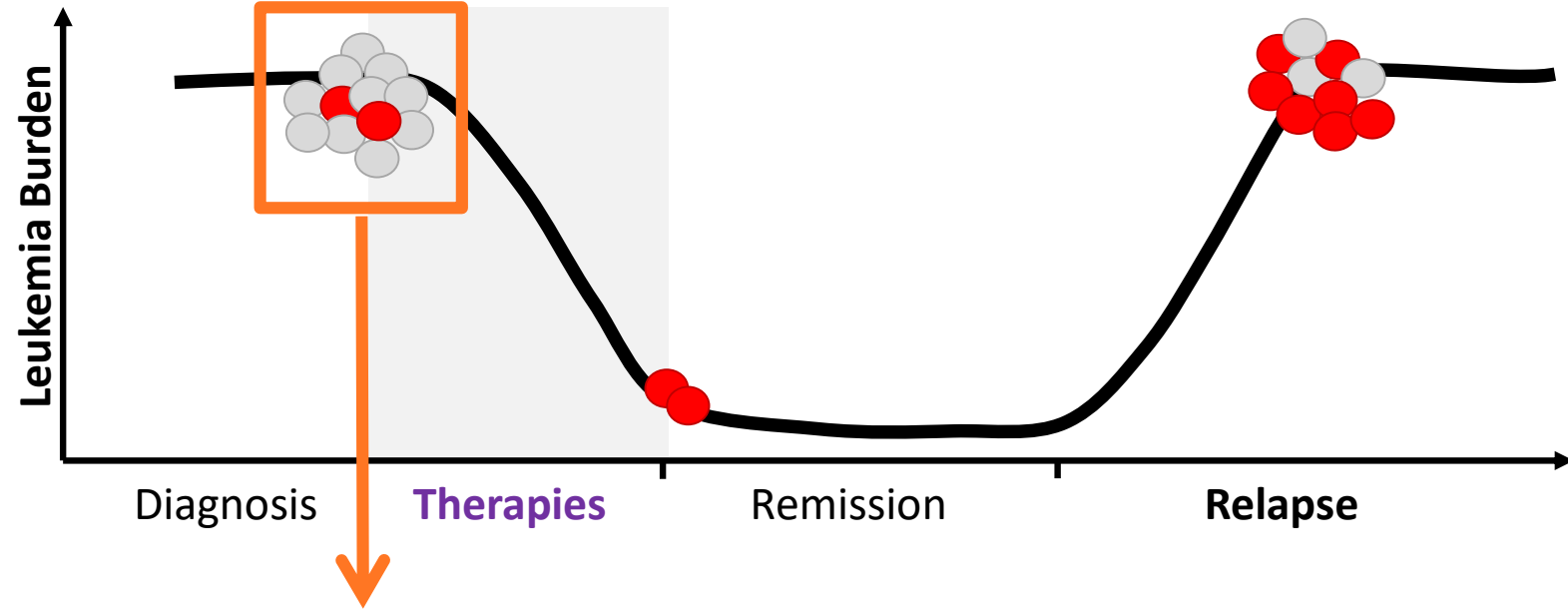
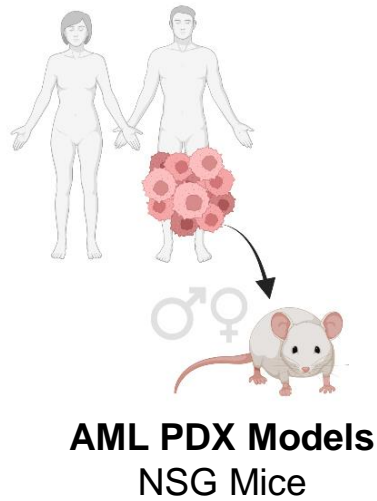
Relapses, drug resistance and **cancer stem cells**



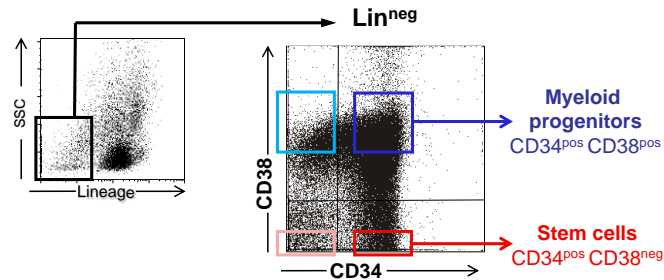
AML stem cells (LSCs) are functionally defined as **SCID leukemia-initiating cells (SL-ICs)**, recapitulate the myeloid leukemia, and propagate the disease with their capacity of self-renewal



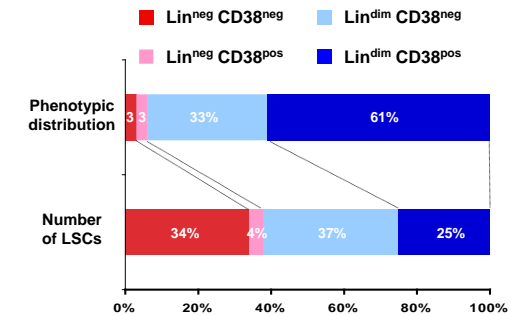
Relapses, drug resistance and **cancer stem cells**



LSCs are enriched (but NOT restricted to) in the immature **CD34+ CD38-** compartment: **phenotypically heterogeneous** however each LSC subset can recapitulate the phenotypic diversity of the primary sample



Fractions	Number of cells injected					LSC Frequency one LSC in
	10 000	50 000	100 000	500 000	1 000 000	
unfractionated	-	1/4	0/5	7/11	6/6	430 240
Lin ^{dim} CD38+	-	-	0/4	2/4	5/5	1 023 815
Lin ^{dim} CD38-	-	0/4	1/4	5/7	4/4	372 259
Lin- CD38+	-	0/2	1/4	2/3	5/5	345 807
Lin- CD38-	1/4	4/4	3/4	8/8	-	38 030



Diverse strategies to study leukemic stem cell in AML as of 2010/2011

**Phenotypical heterogeneity
of therapy-naive LSCs
(diagnosis)**

Plasticity model



° **Craig Jordan's lab:**

Metabolism (ROS^{LOW})-based cell sorting/definition

° **John Dick's lab:**

Transcriptional programs and gene signatures
cycling/primed/quiescent LSCs

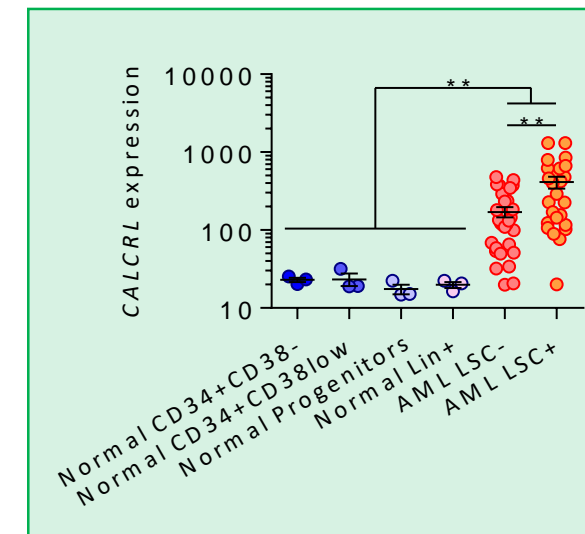
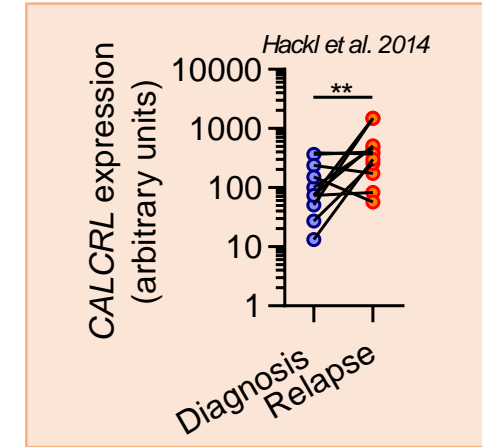
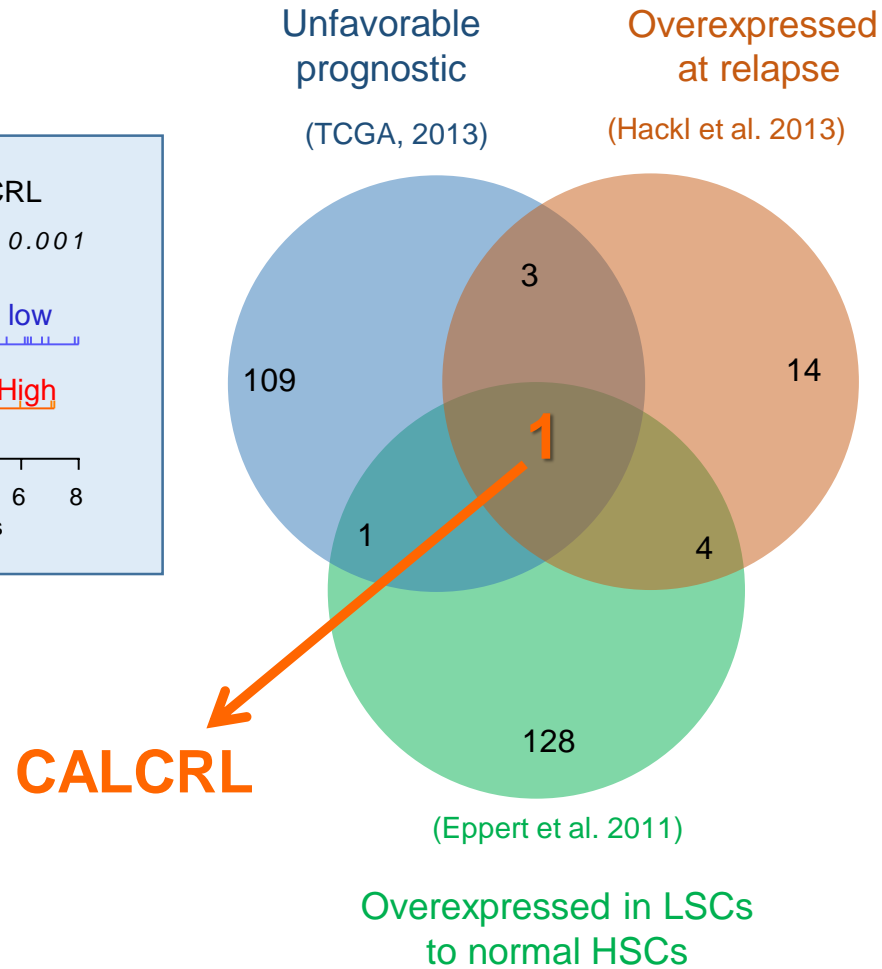
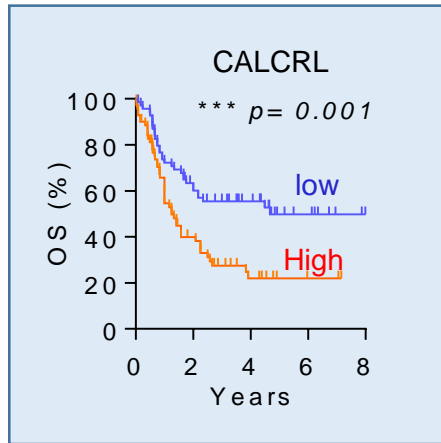
° **Our lab:**

1-Novel stem cell marker discovery

**2-*In vivo* MRD composition and functions:
stressed/persisting/resistant/residual LSCs**

Goardon et al. Cancer Cell. 2011
Eppert et al. Nat Med. 2011
Sarry et al. JCI 2011
Taussig et al. 2010

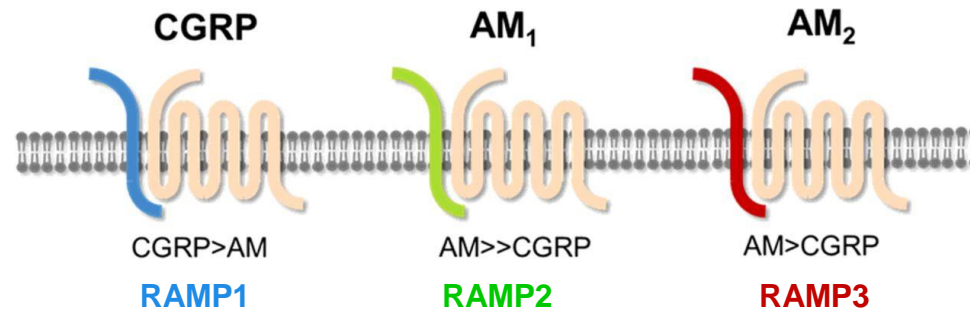
Identification of adrenomedullin receptor CALCRL as a novel LSC-associated poor prognostic marker in AML



Adrenomedullin receptor CALCRL

Encoded for a G Protein-Coupled Receptor related to the calcitonin receptor.

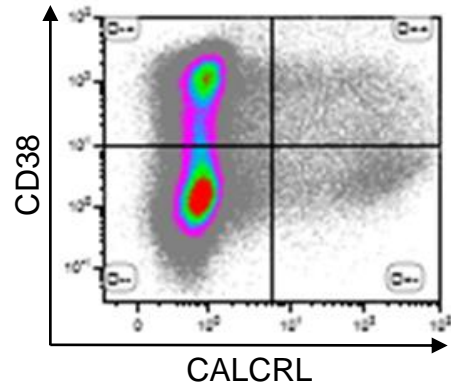
CALCRL ligands: Adrenomedullin (ADM), Calcitonin gene-related peptide (CGRP)



ADM

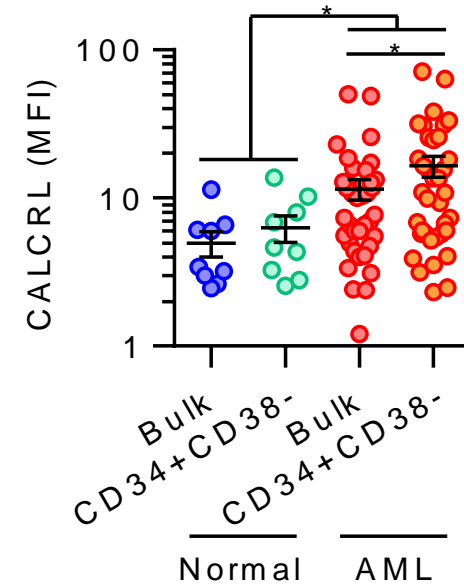
- Pro-angiogenic factor increased and secreted in response to hypoxia
- Inflammatory peptide induced by NRC3A1
- Overexpressed in several cancers (multiple myeloma, prostate cancer ...)

CALCRL expression in AML is associated with an immature phenotype

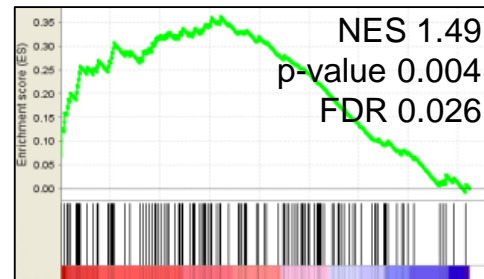


10 healthy donors
34 AML patients

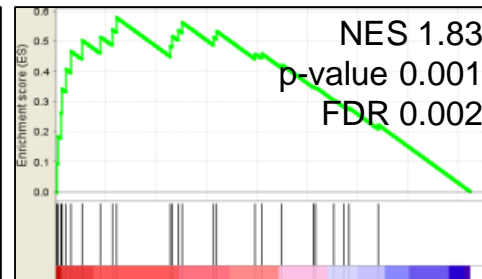
F Vergez, E Saland, ML Nicolau-Travers



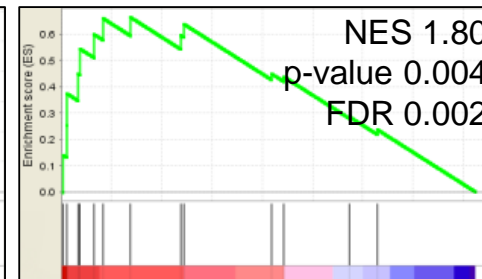
Eppert_LSC vs HSC
Nature Med 2010



Gentles_LSC UP
JAMA 2010



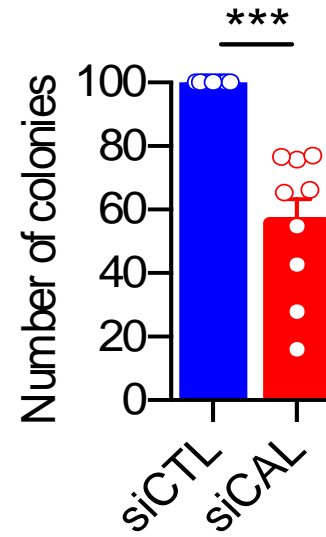
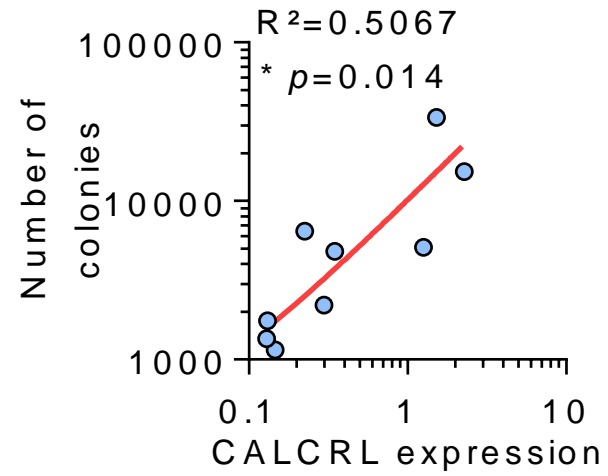
Ng_LSC-17
Nature 2016



CALCRL_HIGH CALCRL_LOW CALCRL_HIGH CALCRL_LOW CALCRL_HIGH CALCRL_LOW

AML patient transcriptomes from TCGA

CALCRL expression in AML is associated with high clonogenicity



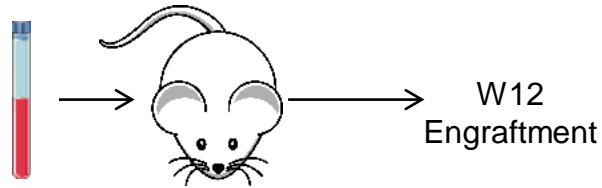
9 primary AML patient specimens

CALCRL silencing impaired LSCs in PDX of AML

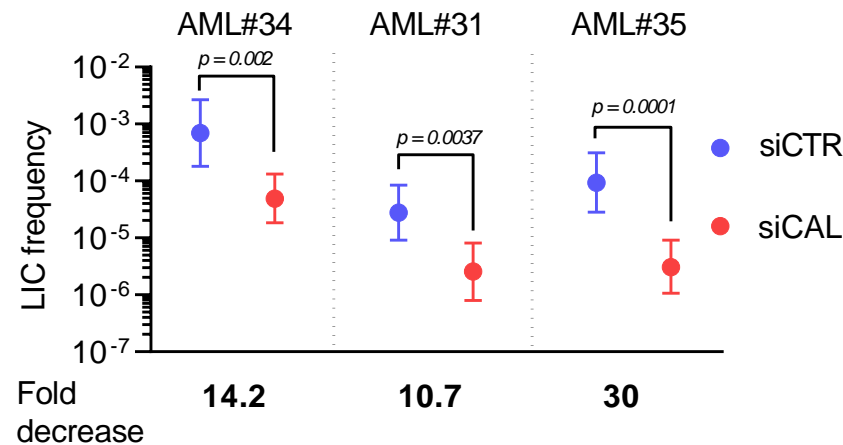
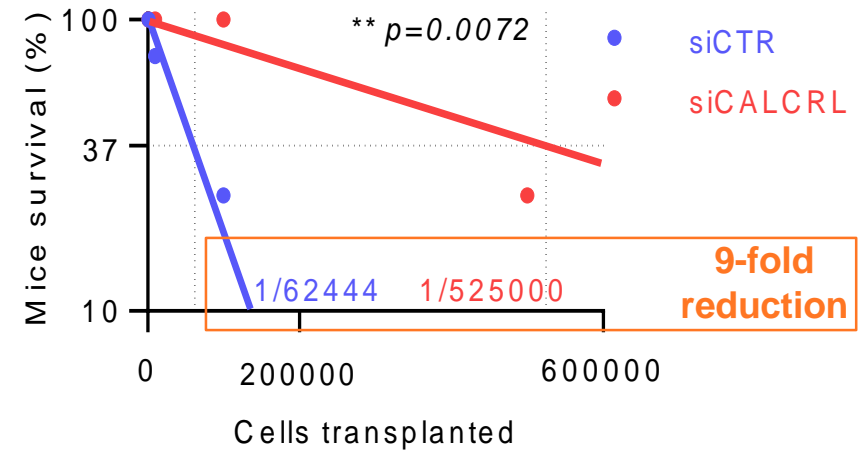
Primary AML samples

siCTR vs siCALCRL

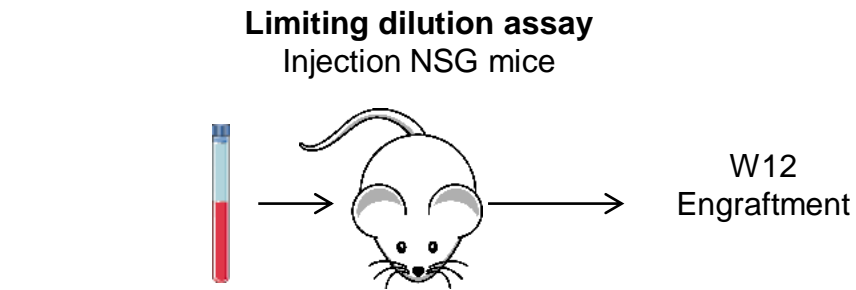
Limiting dilution assay
Injection NSG mice



AML#34		
Cell dose	siCTR	siCAL
25x10 ⁴	4/4	4/4
5x10 ⁴	4/4	4/4
1x10 ⁴	4/4	1/4
1x10 ³	2/4	0/4
LIC frequency	1/1,424	1/20,203
p-value	0.002	



Reduction of LSCs frequency in mice engrafted with CALCRL^{neg} cells



Primary AML samples :

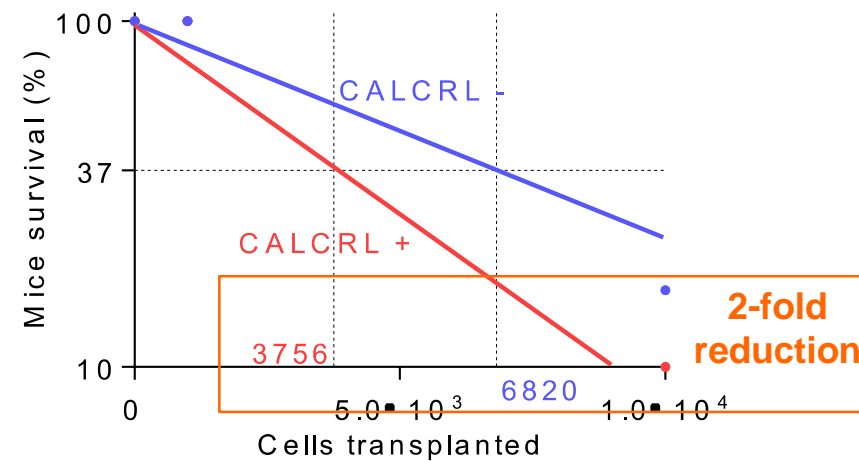
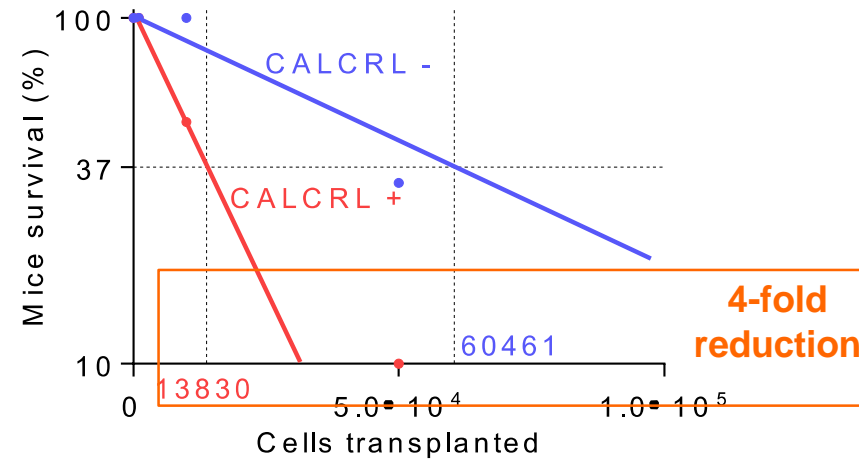
sorted/purified

CALCRL **neg/low** cells

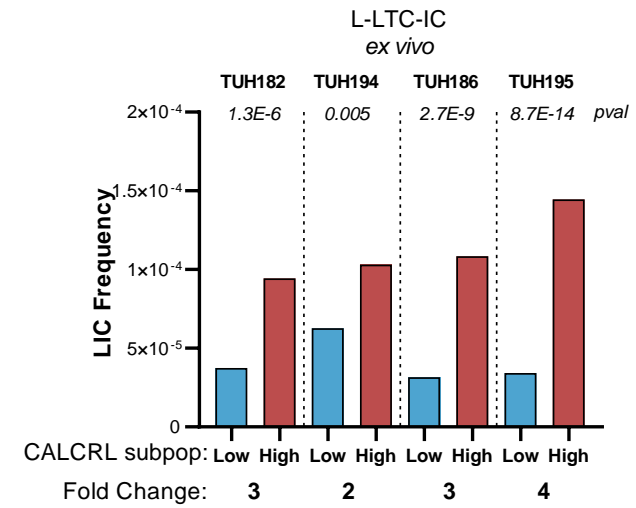
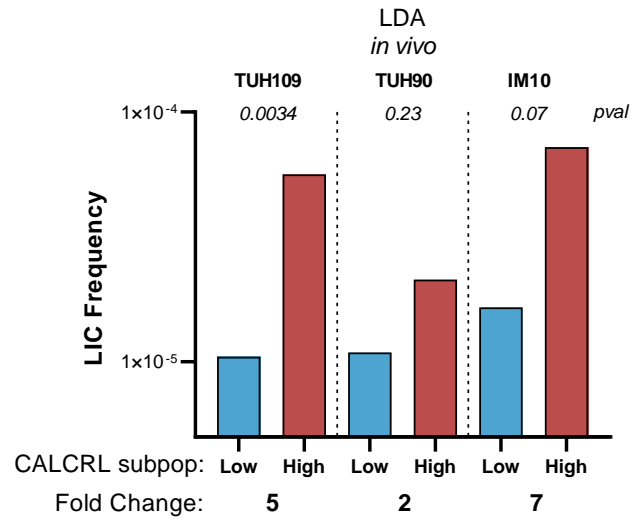
vs

sorted/purified

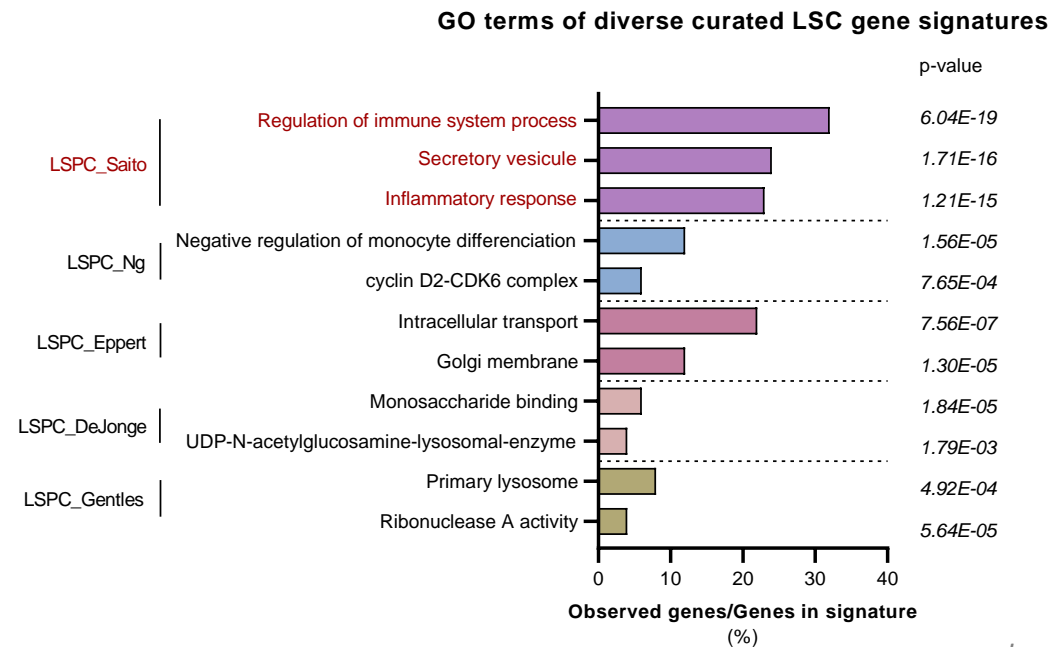
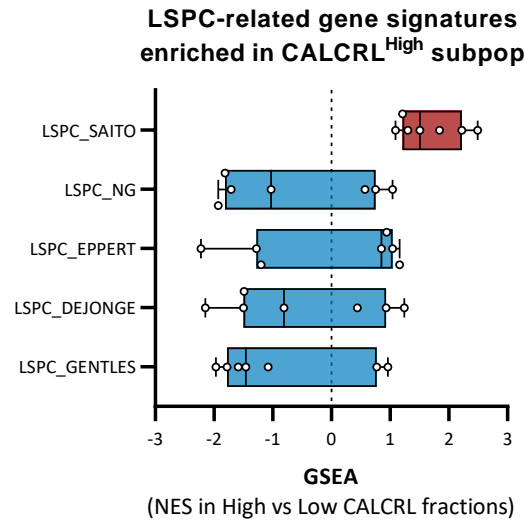
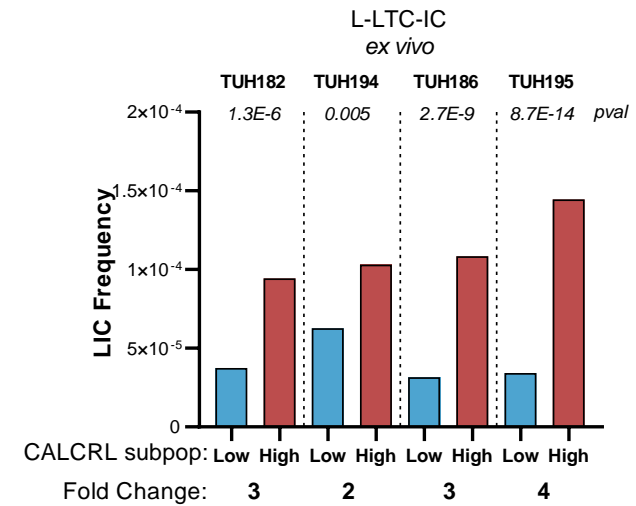
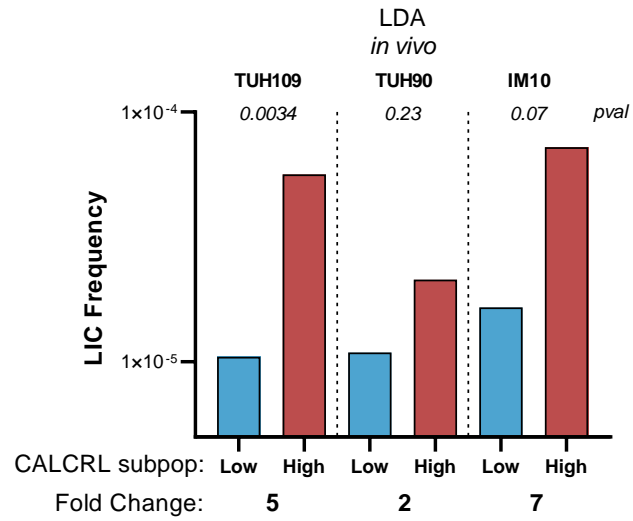
CALCRL **pos/high** cells



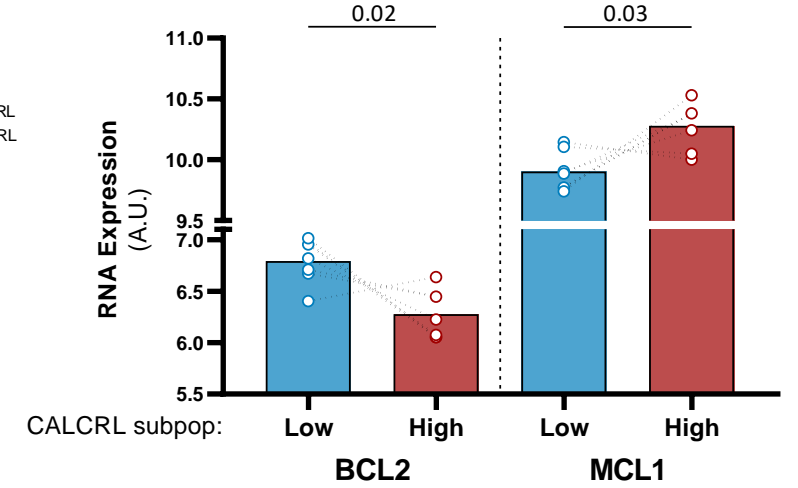
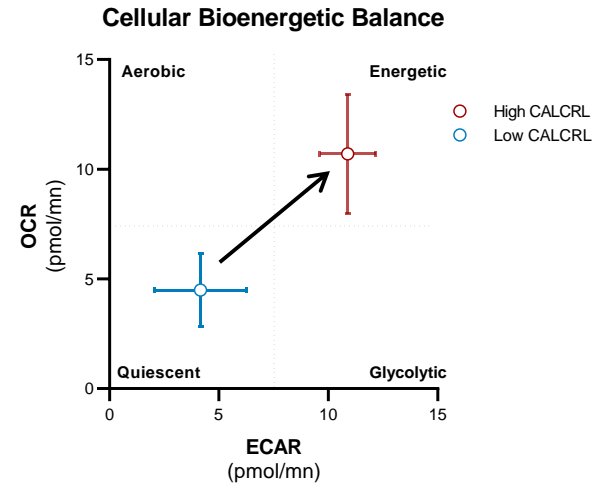
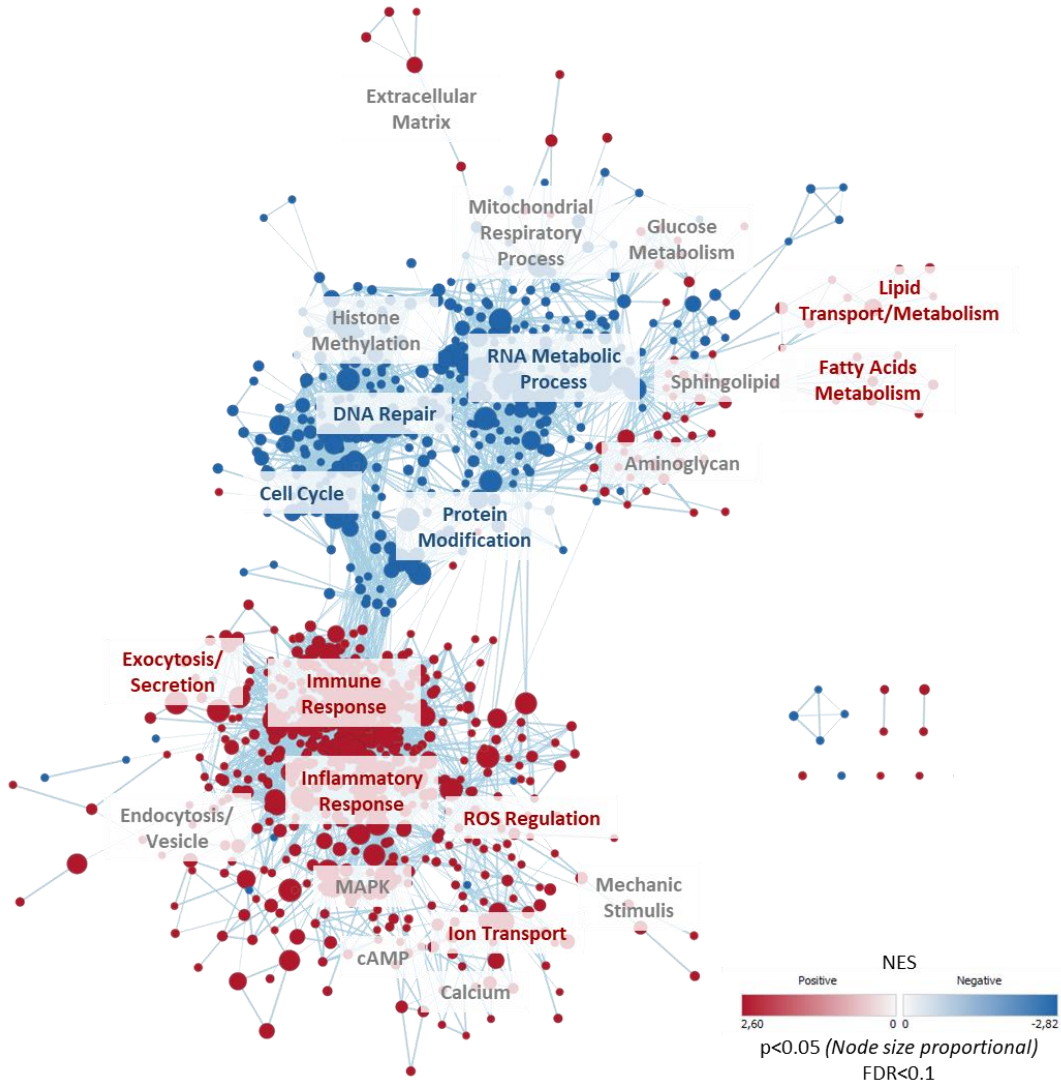
CALCRL^{high} cell population is enriched in leukemic stem cells *in vitro* and *in vivo*



CALCRL^{high} cell population is enriched in leukemic stem cells *in vitro* and *in vivo*

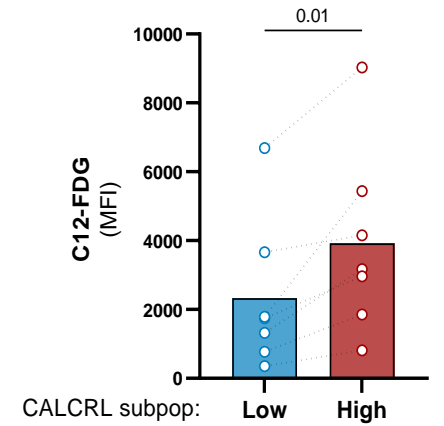
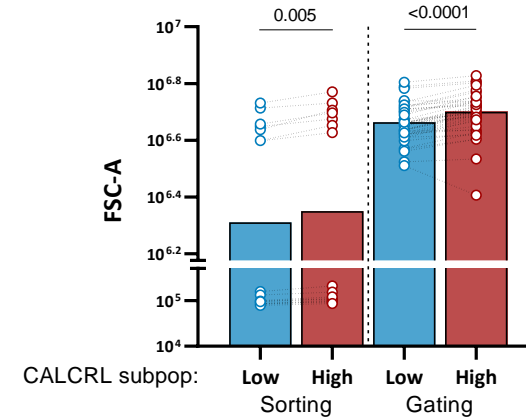
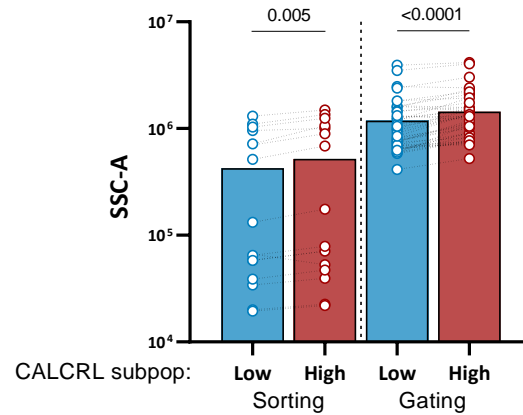
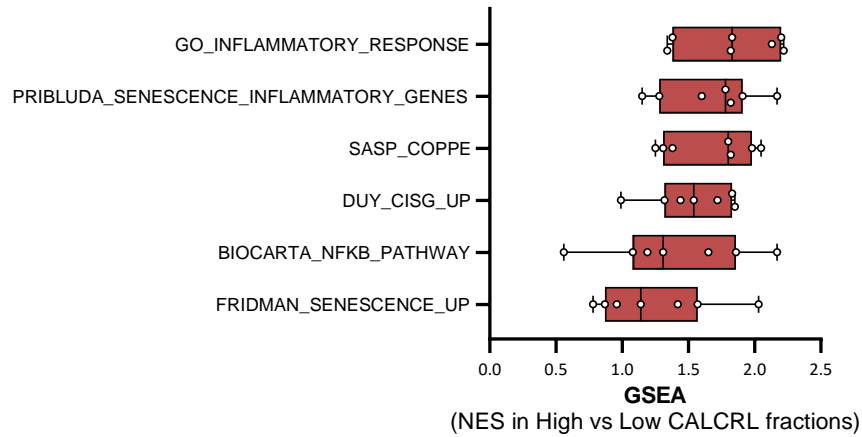


CALCRL^{high} cell population is enriched in leukemic stem cells *in vitro* and *in vivo*

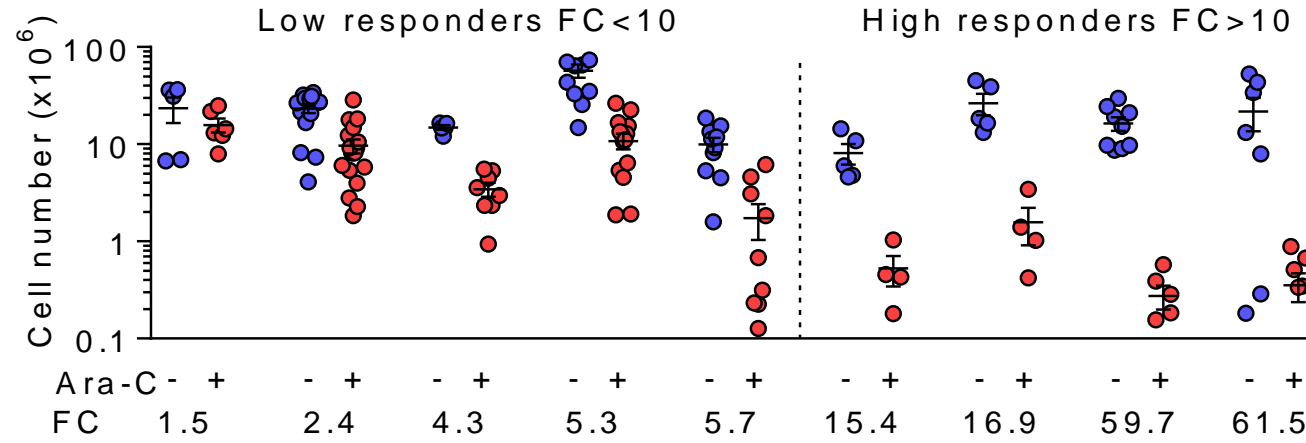
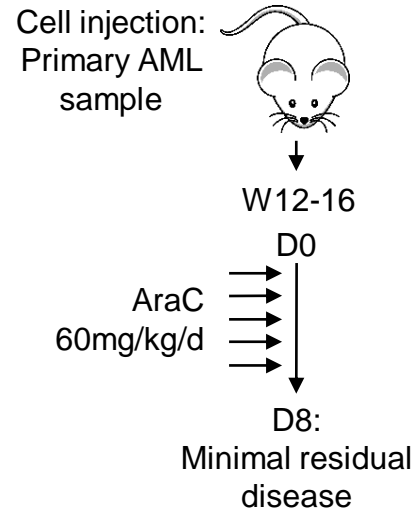


Inflammatory and senescent-like phenotype is enriched in CALCRL^{high} LSCs

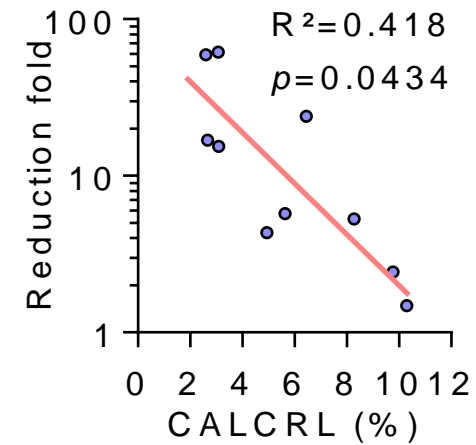
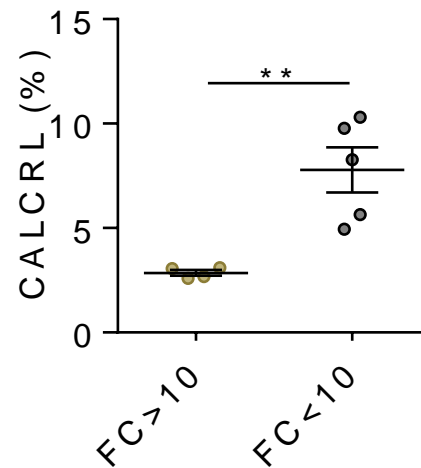
Inflammatory-related gene signatures enriched in CALCRL^{high} cells



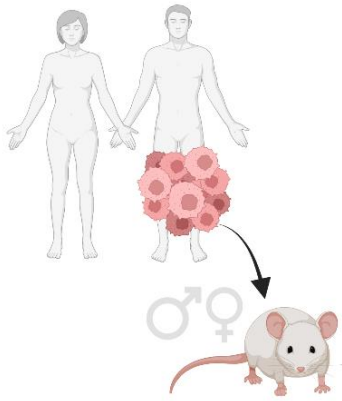
CALCRL level predicts response to chemotherapy in PDX



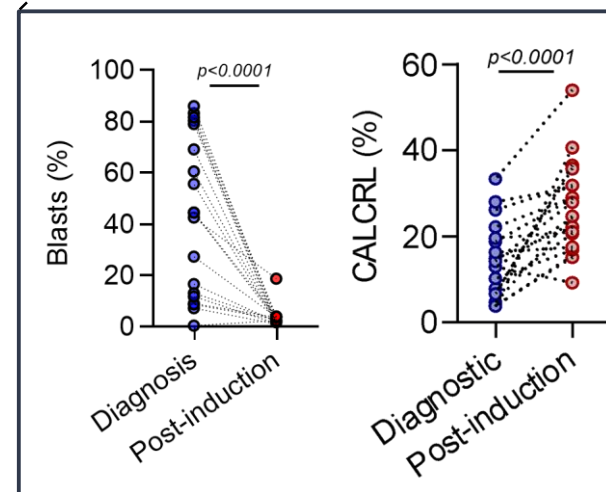
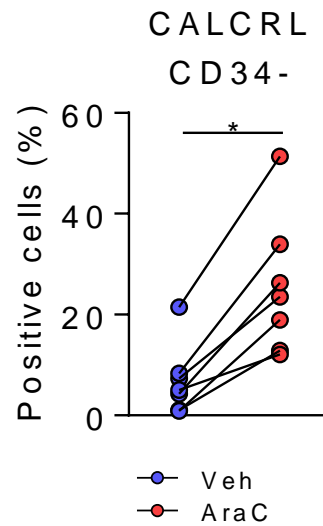
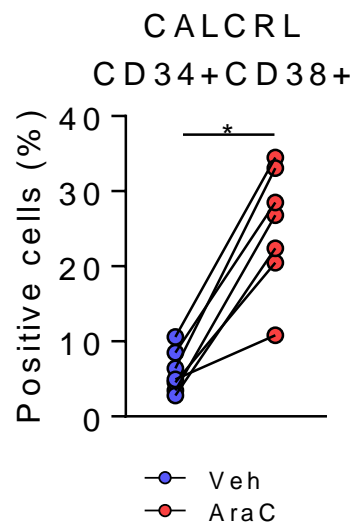
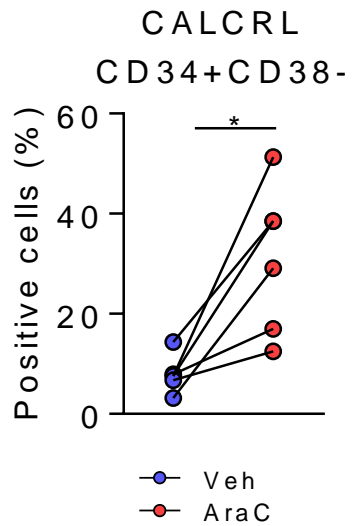
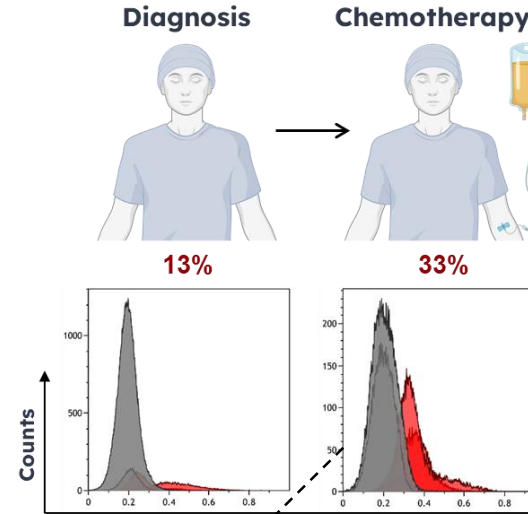
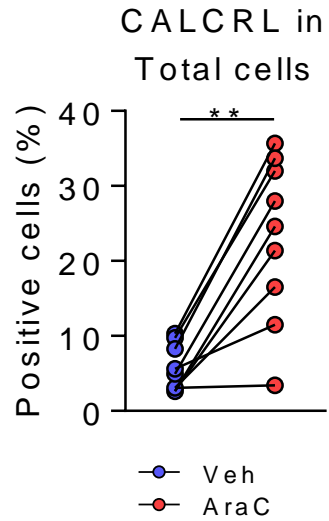
9 primary AML
patient specimens



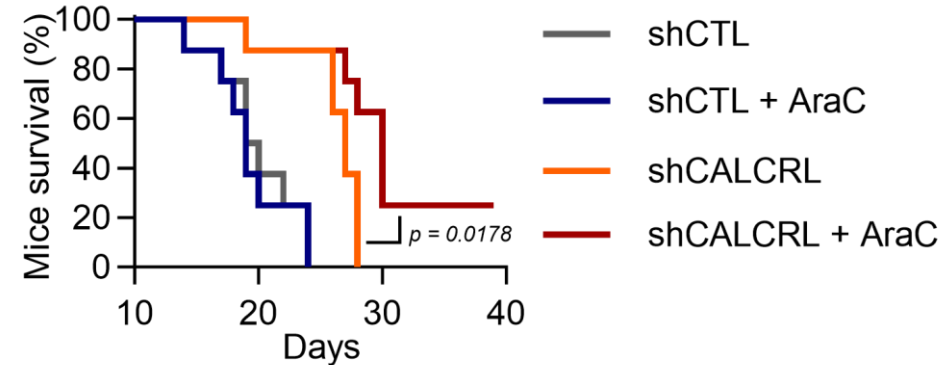
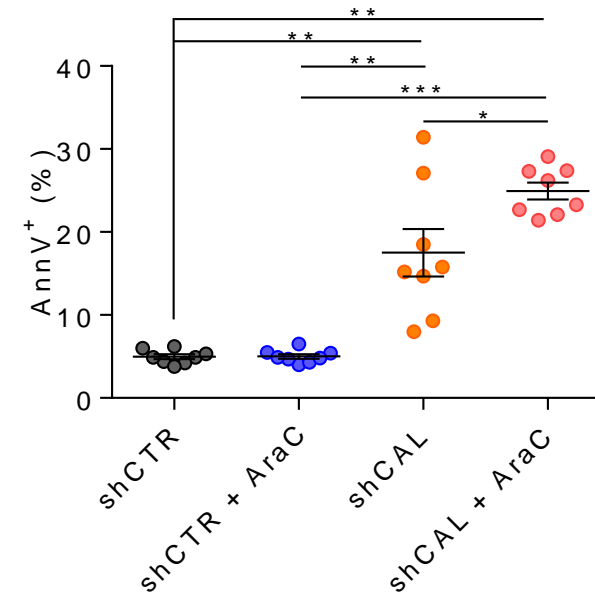
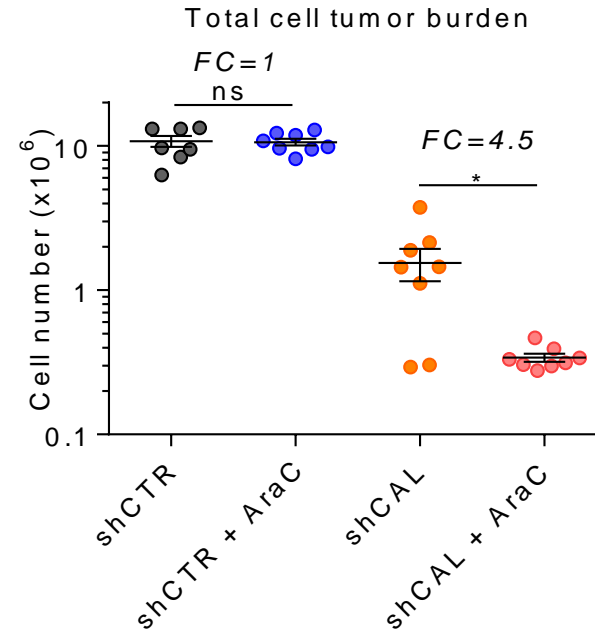
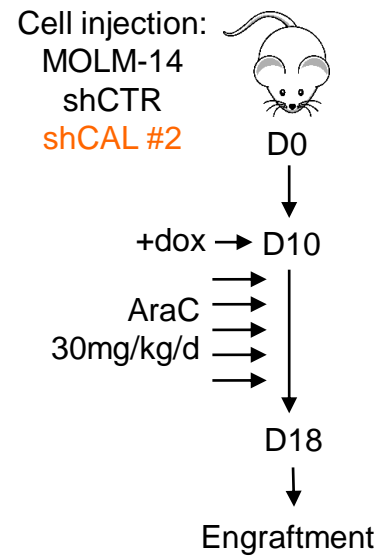
CALCRL is enriched in AraC residual disease *in vivo*



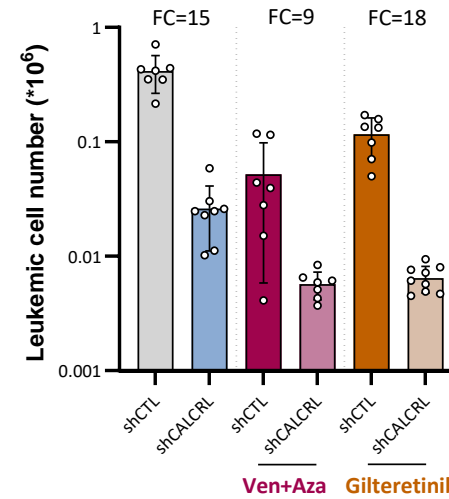
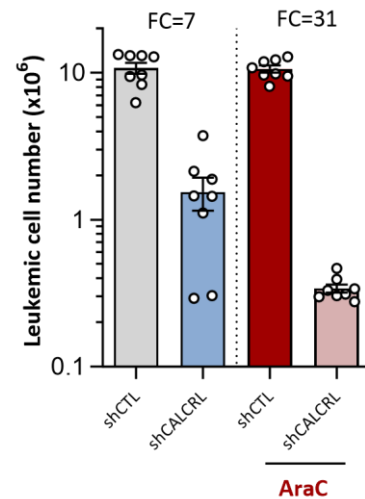
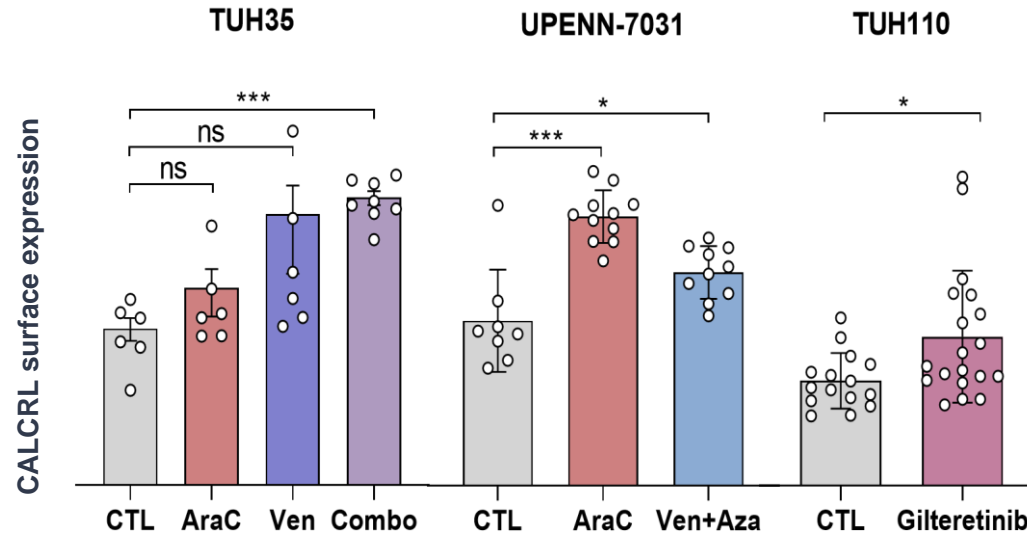
9 diverse
PDX models



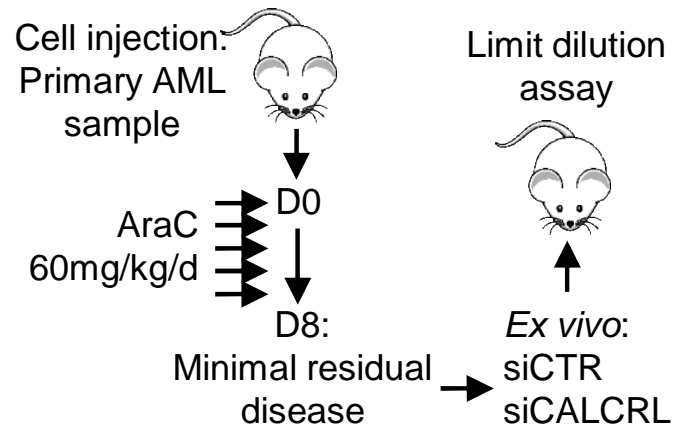
CALCRL knockdown improves response to cytarabine *in vivo* in highly resistant/refractory MOLM14 CLDX model



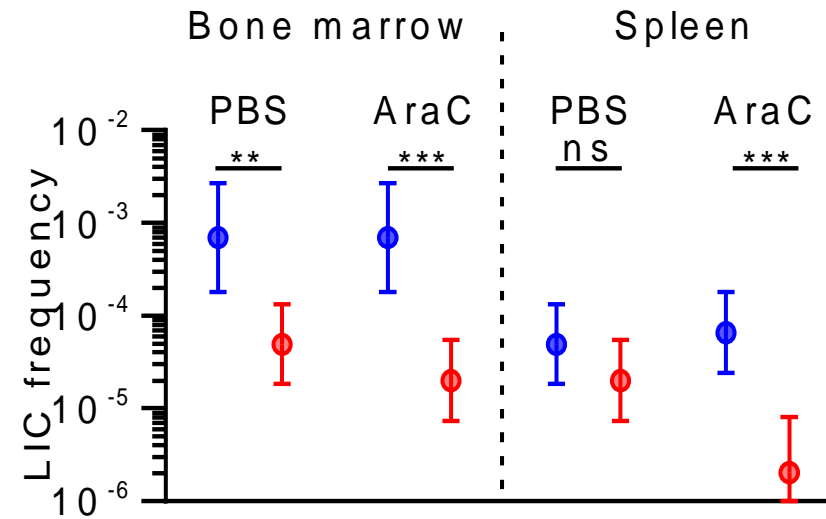
In vivo CALCRL is overexpressed after any treatment and its invalidation sensitizes to chemo and targeted therapies



CALCRL sensitized AML stem cells to cytarabine *in vivo* in PDX model



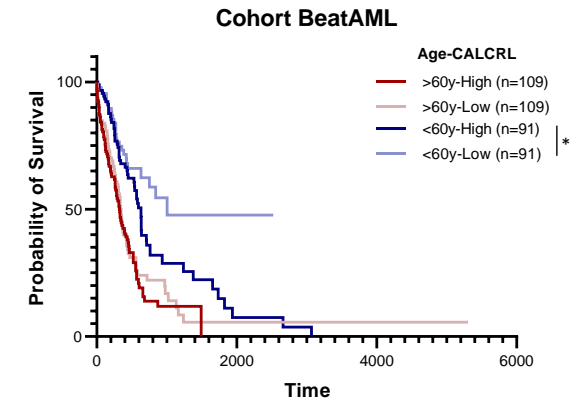
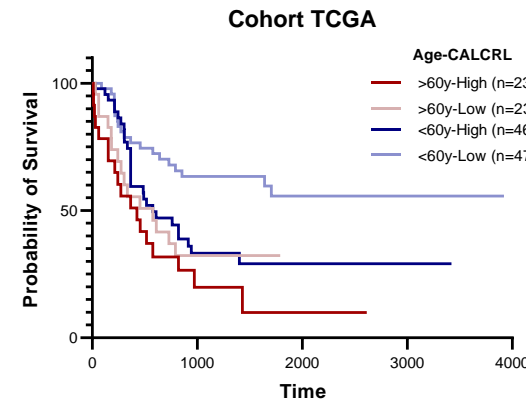
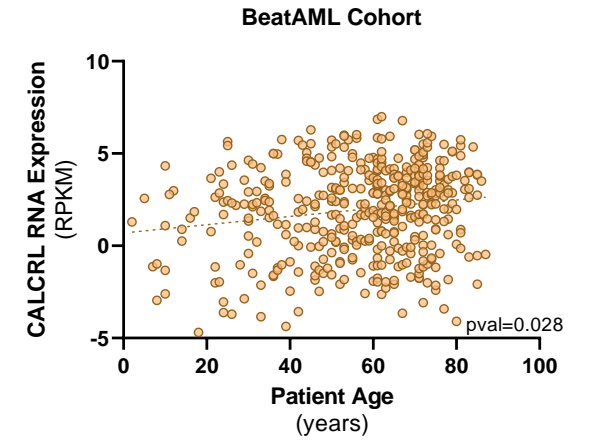
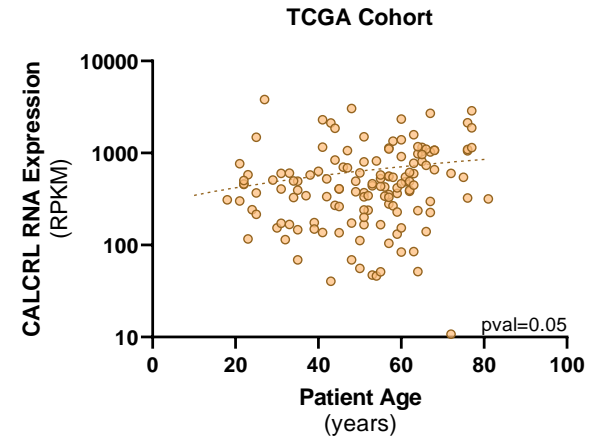
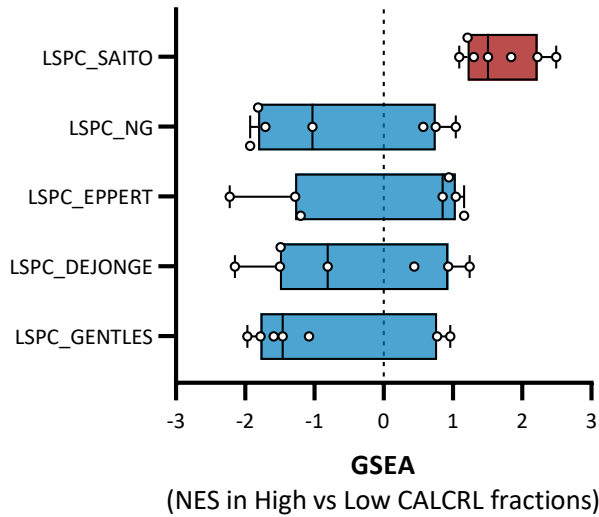
1 PDX – 80 mice



siCALCRL	-	+	-	+	-	+	-	+
Fold reduction								
		14.2		35.1		2.5		32.1

CALCRL level might predict the biological age of LSCs in AML patients ?

LSPC-related gene signatures enriched in CALCRL^{High} subpop



	LSPC Gene Signatures				
	Saito et al.	Ng et al.	DeJonge et al.	Gentles et al.	Eppert et al.
Median age	66.5	61.6	55	51	50

Summary

- ADM-CALCRL-RAMP2 axis is activated in AML
- CALCRL is overexpressed in the immature CD34+CD38- compartment
- CALCRL knockdown impaired AML growth *in vitro* and *in vivo*, and LSCs *in vivo*

- CALCRL expression predicted response to chemotherapy in PDX models
- CALCRL knockdown sensitized AML to chemotherapy and targeted therapies
- CALCRL could better define the residual/resistant population with inflamaging phenotype

- Antibody for diagnosis and open to collaborate for multicentric prospective study with LSC/MRD flow cytometry (PI. François Vergez, TUH)

Acknowledgements

Current members

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Fanny Granat
Anais Grignon
Nathan Guiraud
Alexis Hucteau
Latifa Jarrou
Carine Joffre
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Goncalo Pomba
Nathaniel Polley
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Estelle Saland
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Hélène Boutzen
Fabienne de Toni
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Coralie Caryon
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Justine Bertrand-Michel
Fabien Jourdan
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From France and Abroad

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M. Carroll, S. Scotland (UPenn)

M. Konopleva, C. DiNardo (Houston)
A. Wei (Melbourne)



Groupe de travail français “MitoAML: from bench to patients”

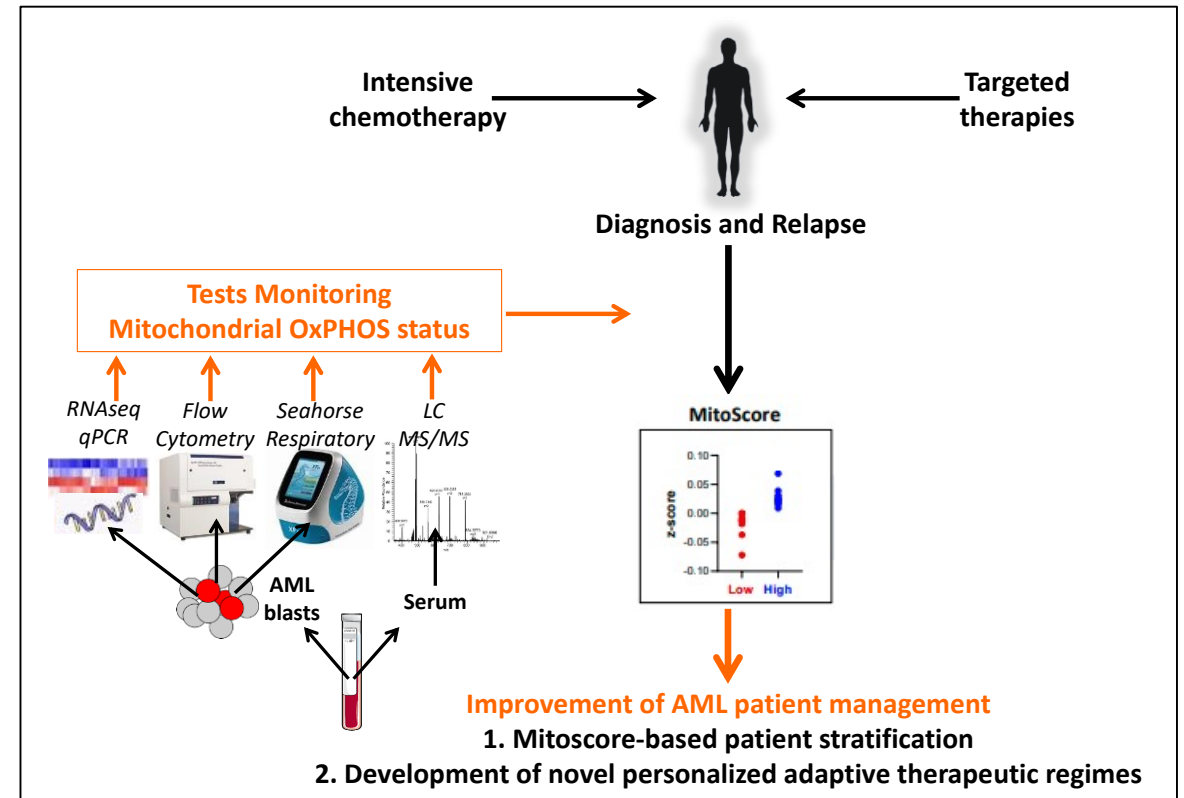
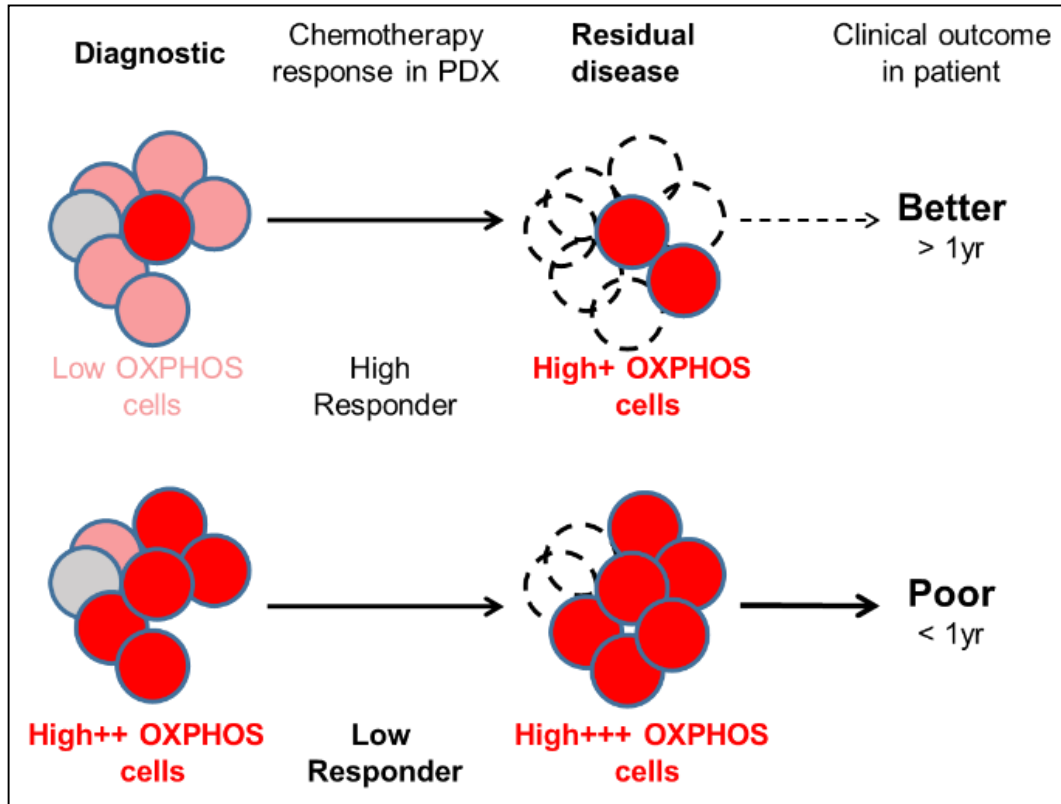


Jean-Emmanuel Sarry, Jérôme Kluza
Julie Mondet, Pascal Mossuz
Nicolas Chapuis, Didier Bouscary, Olivier Herault

Et tous les volontaires cliniciens et chercheurs intéressés sont les bienvenus

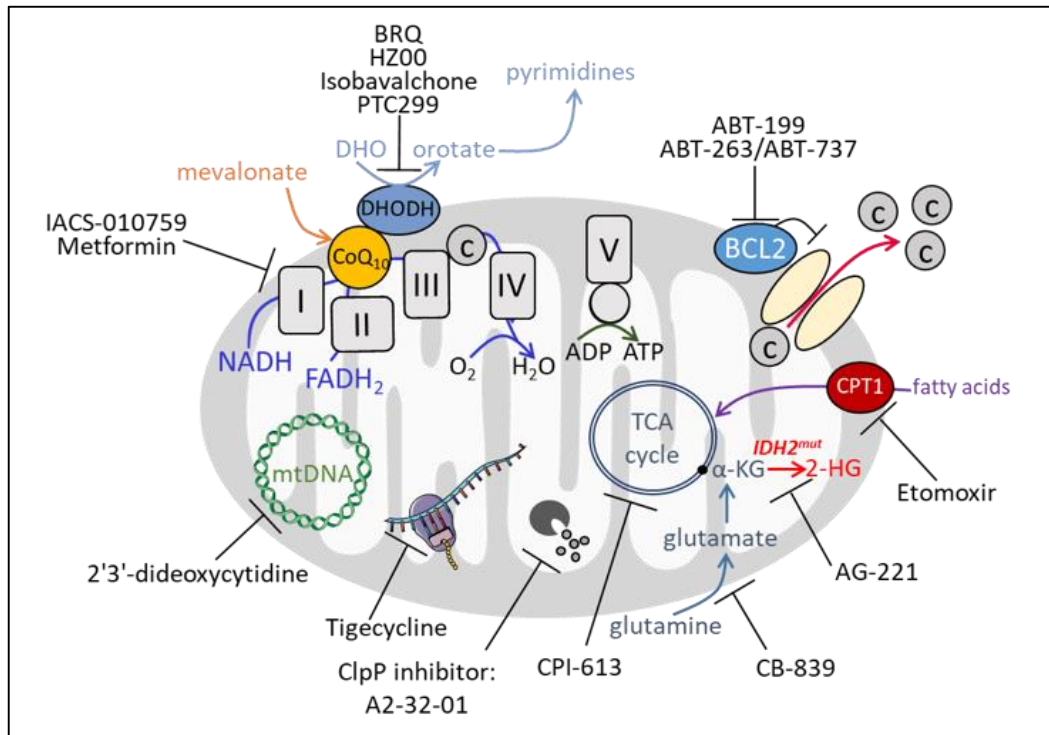
To support diverse clinical perspectives:

> **Revisiting stratification and decision-making tree of AML patients by combining cytogenetics with metabolic and mitochondrial biomarkers: Mito-Score with RNA-/Seahorse/LC-MS-mitoDNA-based approaches**

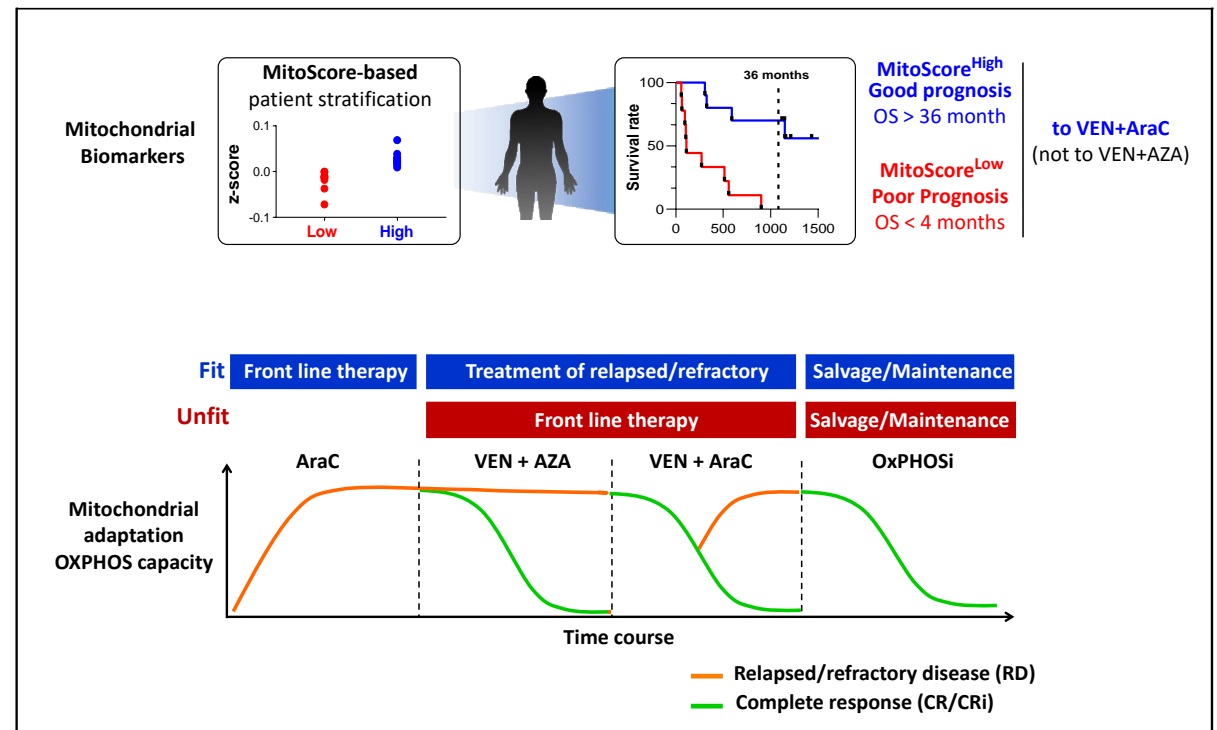


To support diverse clinical perspectives:

> **Developing innovative therapeutic solutions** inhibiting ANY aspect of mitochondrial OxPHOS metabolism to circumvents adaptive resistance to drugs and to enhance the sensitivity of AML cells to chemotherapy or currently approved targeted therapies/combinations



Stuani *et al.* BMC Biol. 2019; Bosc *et al.* Cell Metab. 2017



Bosc *et al.* Nature Cancer. 2021