Phenotipic Heterogeneity of Leukemias

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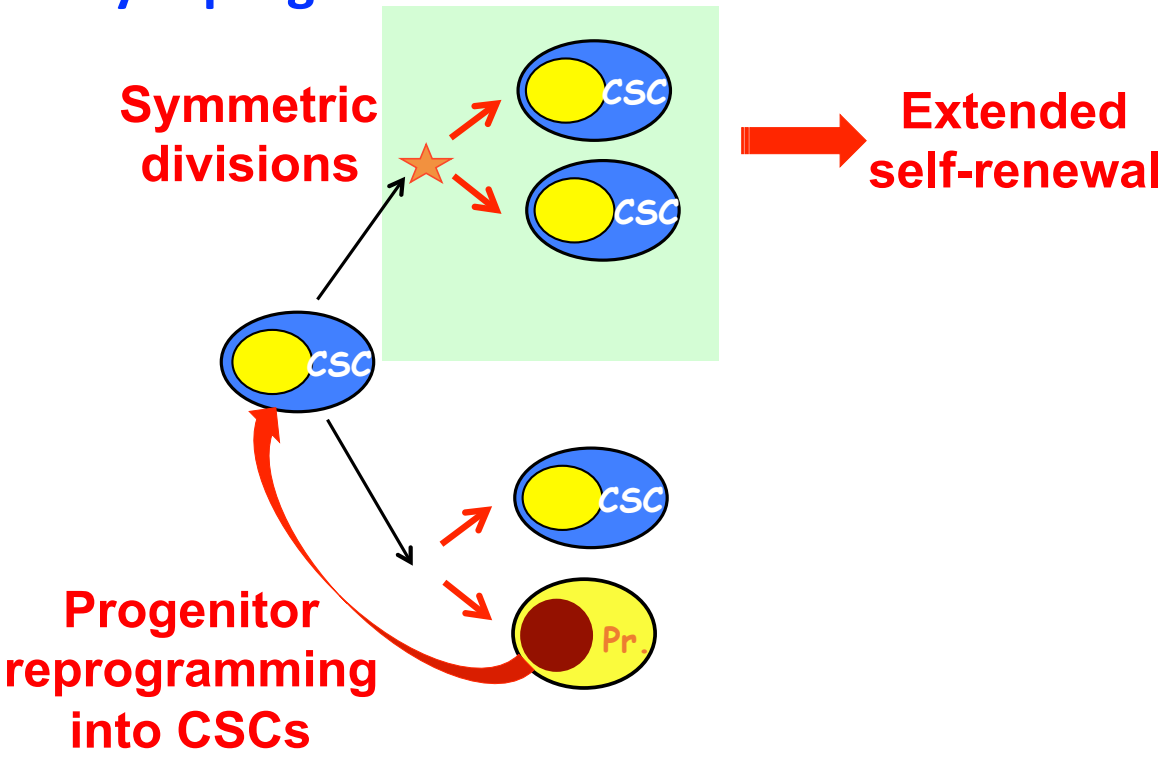
Rome, September 24th-27th 2017

Cancer Stem Cells (AMLs, Breast Cancer):

- Have unlimited self renewal potential
- Divide both asymmetrically and symmetrically
- Symmetric divisions prevail
- Progenitors are continuously reprogrammed into CSCs

Viale et al, Nature 2010
Cicalese et al, Cell 2011
Tomilov et al, Aging Cell 2011
Pece et al, Cell 2012
Pasi et al, Cell Death & Diff, 2012
Gambino et al, Aging Cell 2012
Insinga et al, PNAS 2013
Migliaccio et al, Aging Cell 2013

A Santoro, T Vlachou; under revision



Cancer Stem Cells (AMLs, Breast Cancer):

- **Altered self-renewal of CSCs is due to attenuated p53 signalling and activation of Myc**

Viale et al, Nature 2010
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Pasi et al, Cell Death & Diff, 2012
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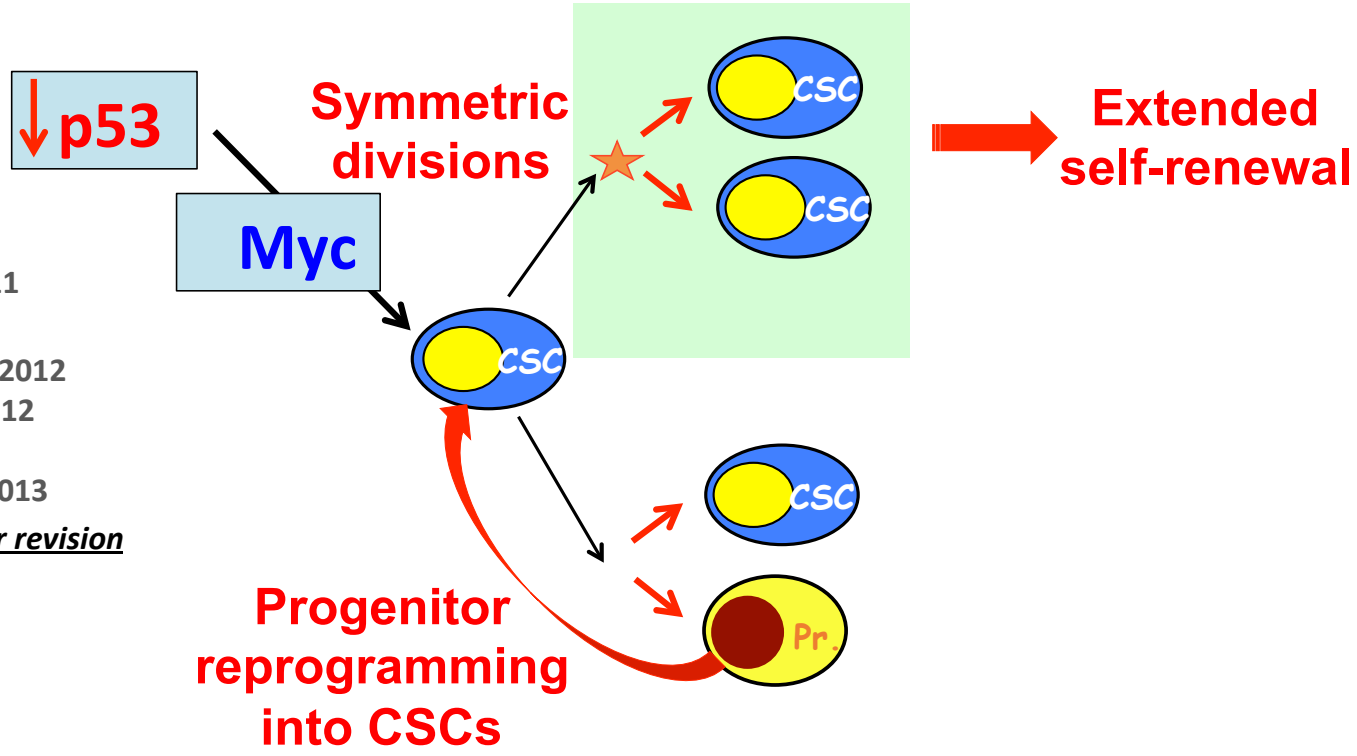
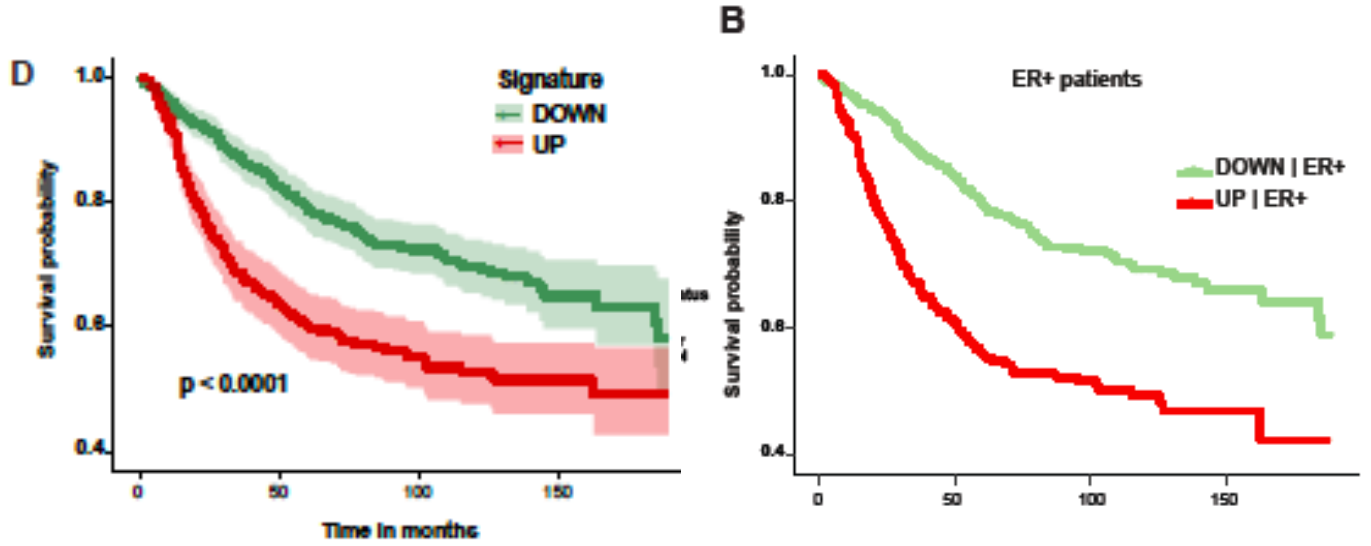


The p53:Myc expression signature is predictive of clinical outcome, independently of other known risk factors

(4 independent cohorts; 892 patients)



significant decrease in the disease-free survival of the UP cohort

Same power in ER+ patients

Symmetric divisions, progenitor reprogramming, extendend self-renewal:

- **Maintenance and continuous expansion of the pool of Cancer Stem Cells:**

Asymmetric Divisions:

- **Maintenance of biological heterogeneity**

Loss of p53 and Myc activation:

- **General mechanism of self-renewal de-regulation in CSCs**

Viale et al, Nature 2010
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Tomilov et al, Aging Cell 2011
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Pasi et al, Cell Death & Diff, 2012
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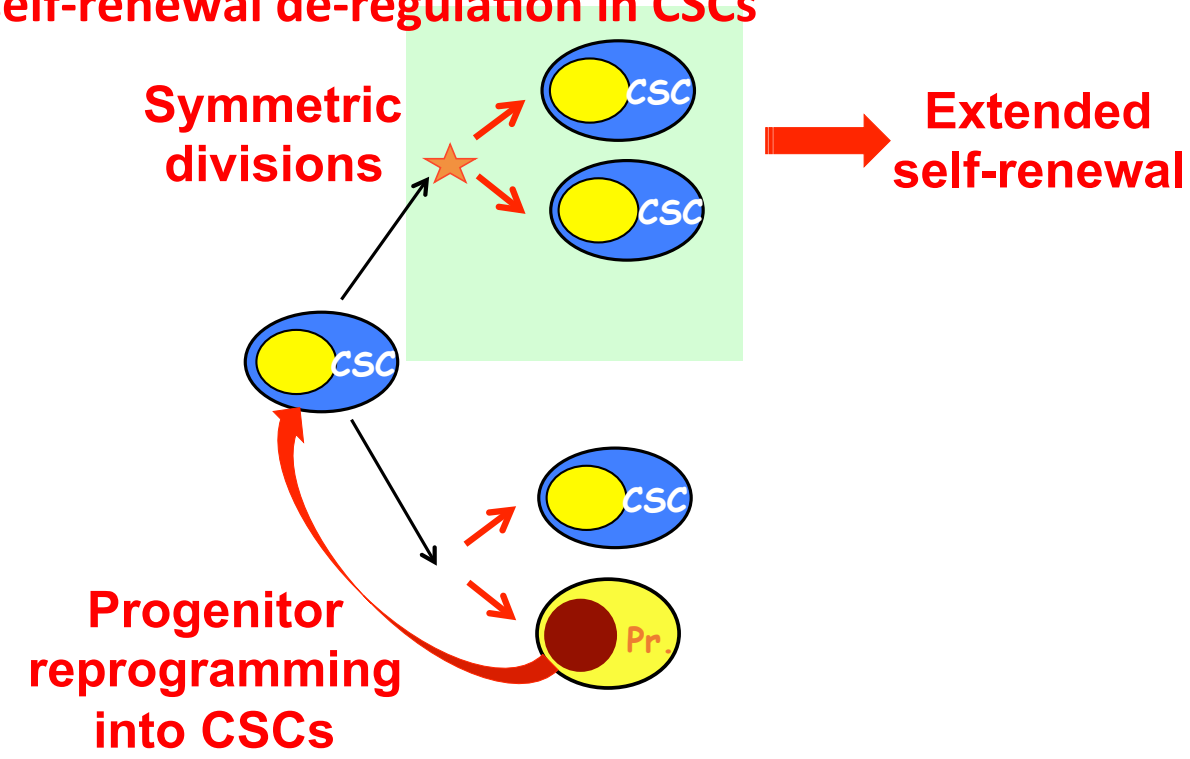
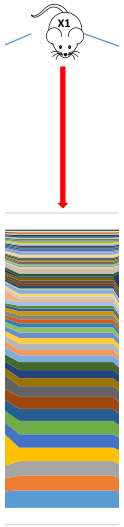


High Clonal Heterogeneity within the pool of LSCs

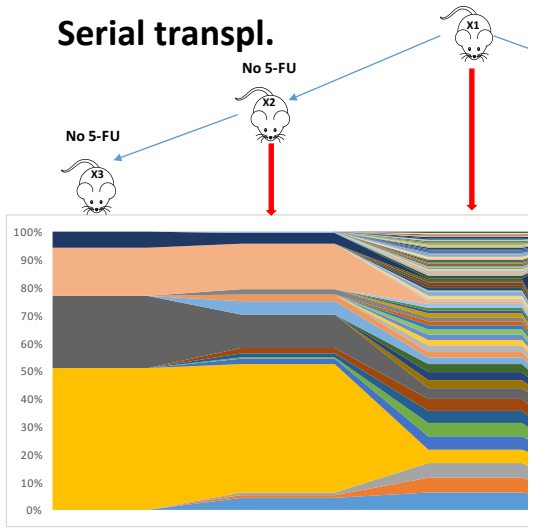
Human AMLs grown
in immunodeficient mice (PDX)



- **Hundreds of LSCs with heterogenous growth potential *in vivo***
- **Strong clonal selection during leukemia growth (serial passaging)**
- **The process of clonal selection can be perturbed by environmental signals (5-FU)**

Andrea Cammarata, unpublished

High Clonal Heterogeneity within the pool of LSCs



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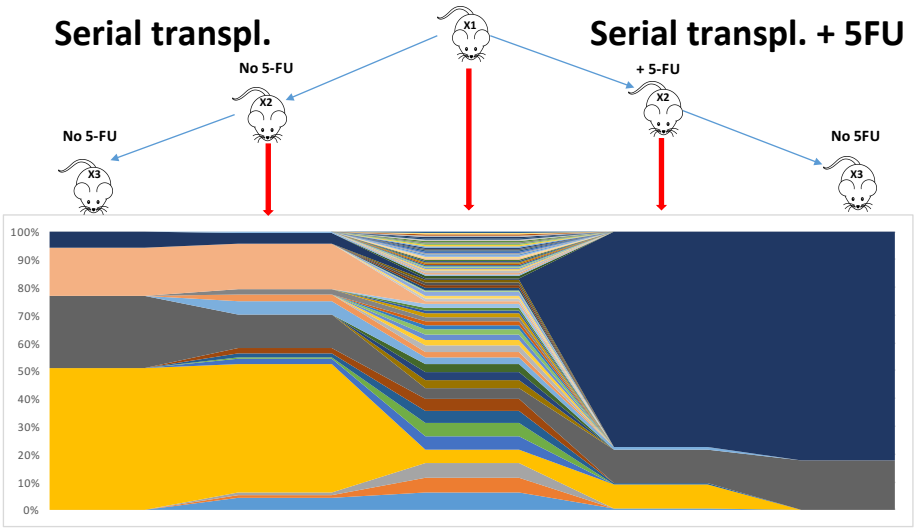

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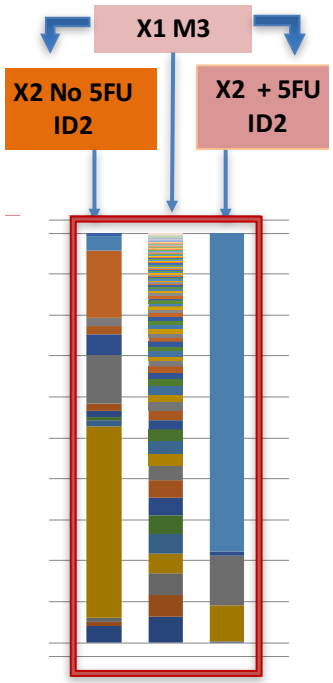
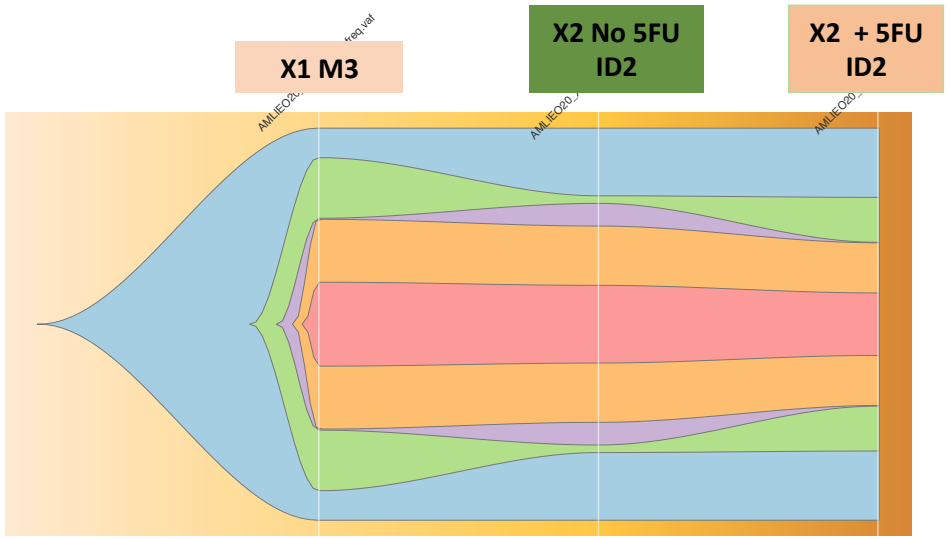
Andrea Cammarata, unpublished

Limited genetic heterogeneity within the pool of LSCs





Biological heterogeneity exceeds genetic heterogeneity by one order of magnitude

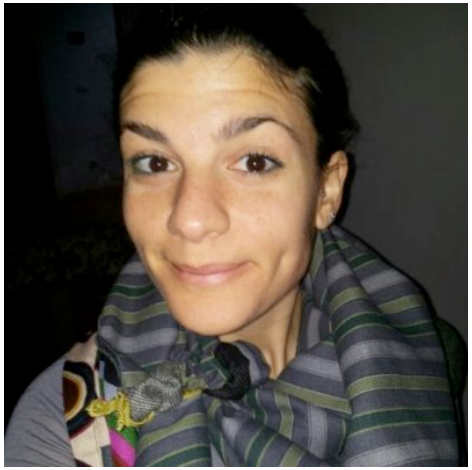
Thalia Vlachou, unpublished

- **How biological heterogeneity is generated?**
- **Is it generated by phenotypic adaptation of Leukemia Stem Cells to (micro)environmental signals?**
- **How genetic and non-genetic (epigenetic) mechanisms interact in the selection of best-fitted cancer phenotypes by environmental cues?**

Model systems:

- Macrophage-activated CD4+ cells
- Obesity
- Nutrient deprivation

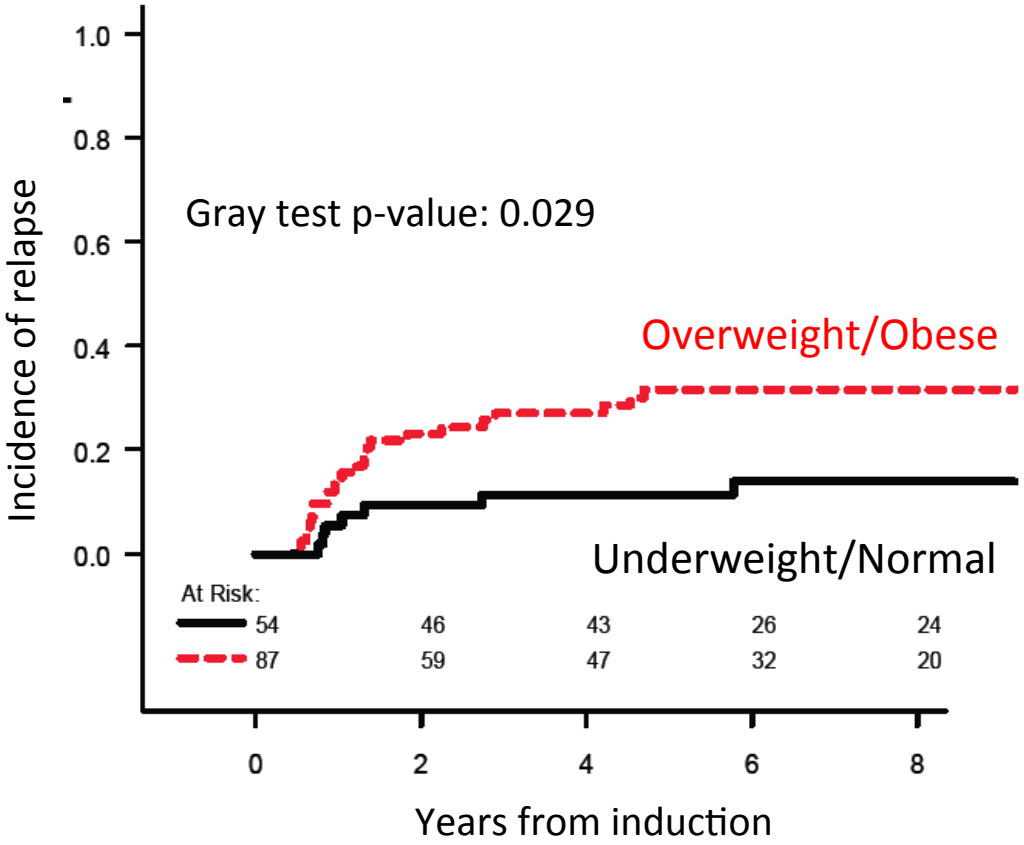
Effects of obesity
on the self-renewal of Leukemia Stem Cells



Anna Giulia Sannarico, PhD

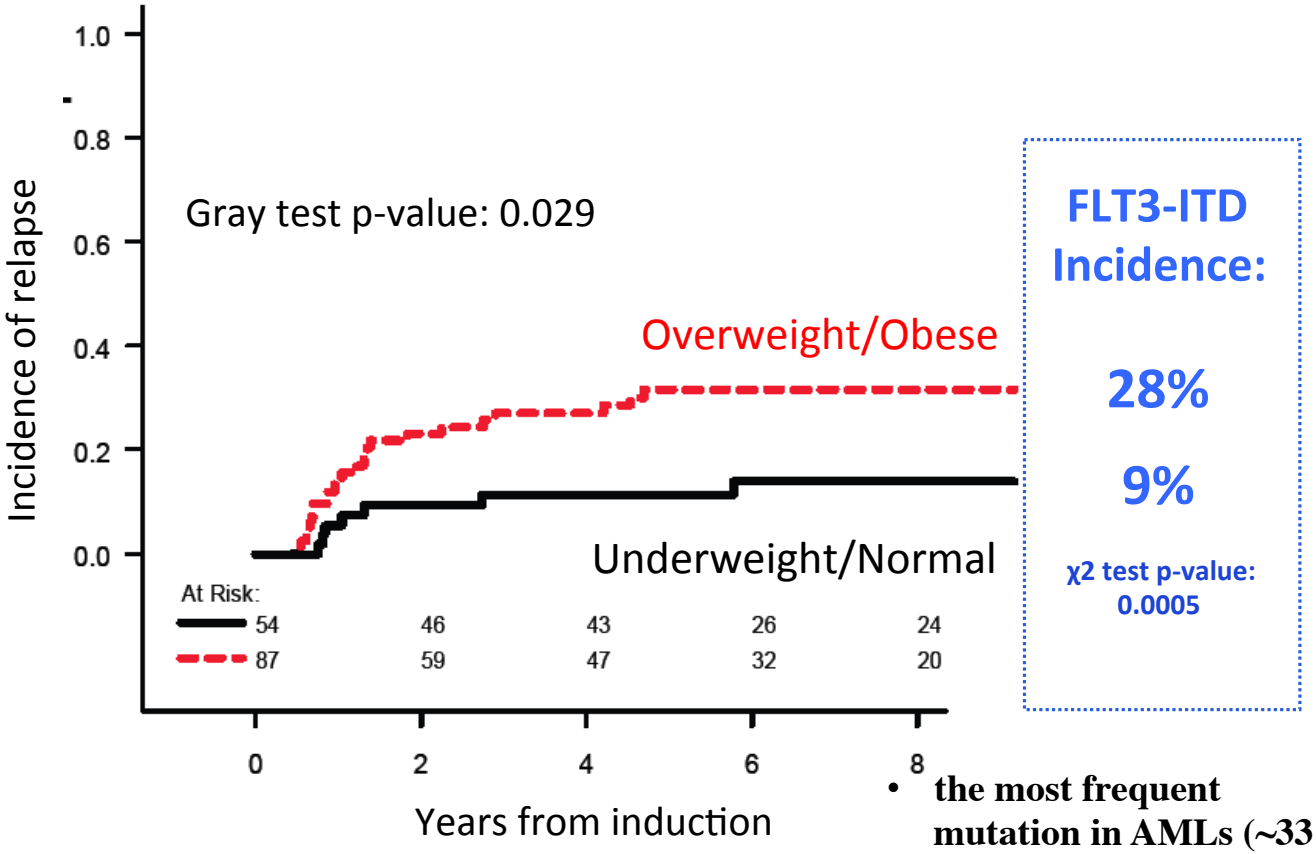
Luca Mazzarella
MD-PhD

Body-mass index (BMI) correlates with worse prognosis in the Acute Promyelocytic Leukemia (APL) subtype of AMLs



Breccia et al., Blood 2012

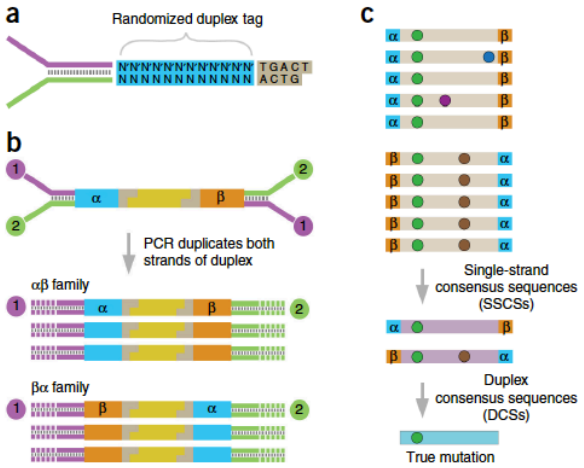
Higher incidence of FLT3-ITD mutations in the obese APL-patients



- **the most frequent mutation in AMLs (~33%)**
- **linked to bad prognosis**

Driver mutations in AMLs are frequently found in rare subclones, including FLT3

Duplex Sequencing



(Schmitt MW et al., PNAS 2012; Kennedy SR et al., Nature Protocols 2014)

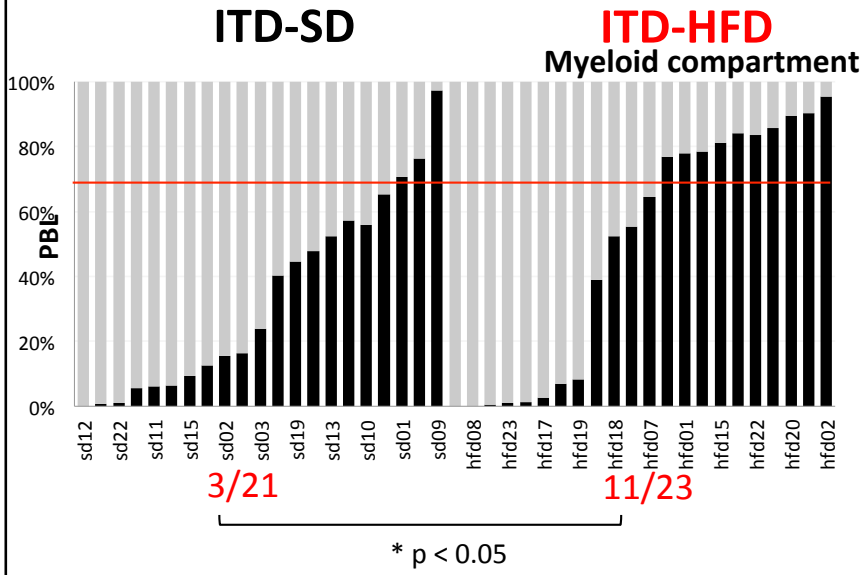
VAF: 0,001%-5%

	number of mutations per gene		
Mutated genes	UD6	UD5	TO1 primary
NRAS	1	/	/
FLT3	1	1	/
IDH2*	3	1	/
TP53§	7	2	3
BRD4*	2	1	/
U2AF2*	1	1	/
DNMT3A	1	/	/
ASXL1	1	3	2
TET2	3	7	/
KIT	2	/	/
EZH2	1	3	/
GNAQ	1	/	/
JAK2	2	5	/
AXIN1	/	1	/
CEBPA	/	1	/
RUNX1	/	1	1
EGFR	/	1	/
PER1	/	/	2
TOTAL	26	28	8

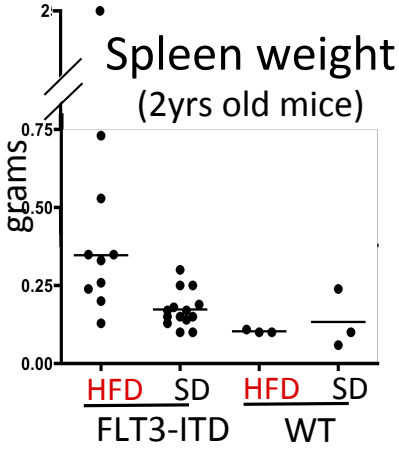
* exact same mutation identified in both patients in UD6 and UD5

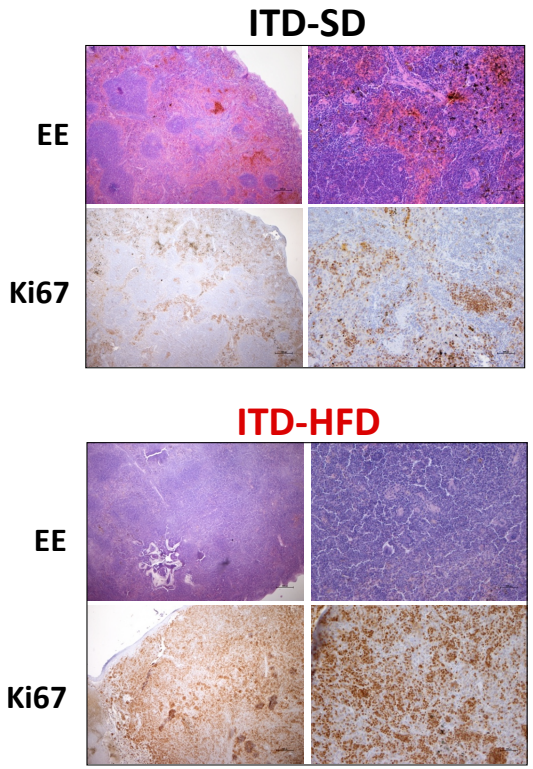
§ exact same mutation identified in both patients in UD6, UD5 and TO1

Obesity confers a competitive advantage to the FLT3-ITD BM
And increases disease aggressiveness of FLT3-ITD transgenic mice



Competitive BM transplantation

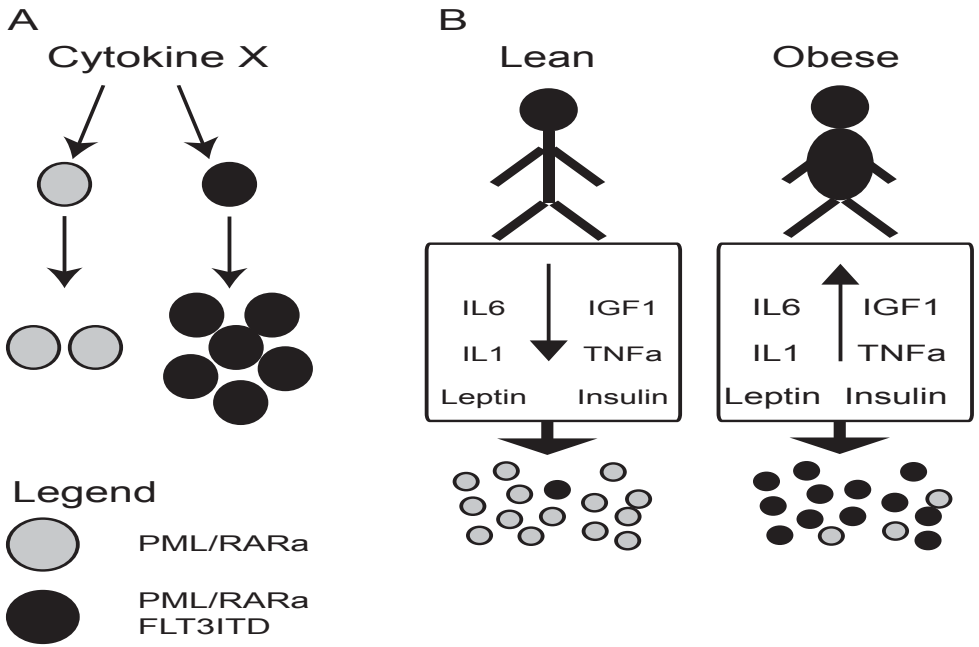




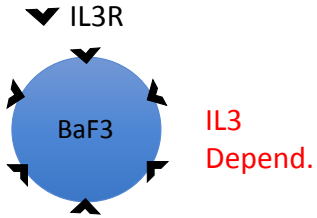
Spleen

FLT3-ITD mice from G. Gilliland

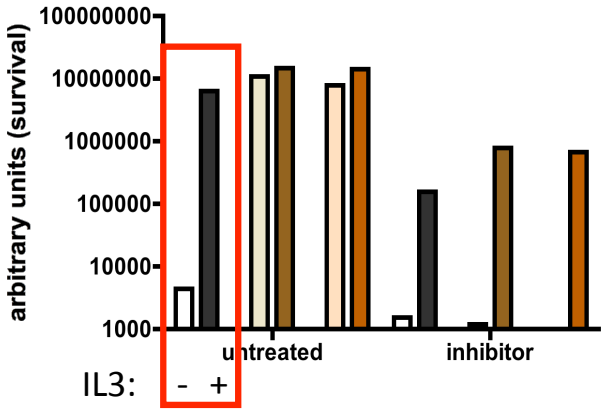
Obesity might select FLT3-ITD mutations through adipose-tissue - secreted adipokines.



The oncogenic potential of the FLT3-ITD mutation depends on Insulin/IGF1 signalling



IL3
Depend.



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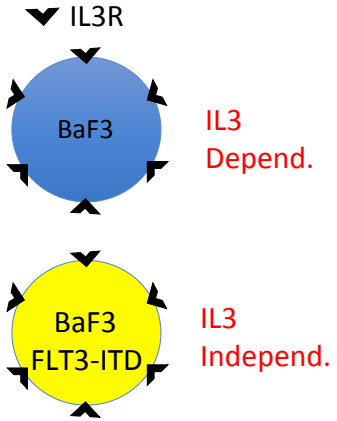

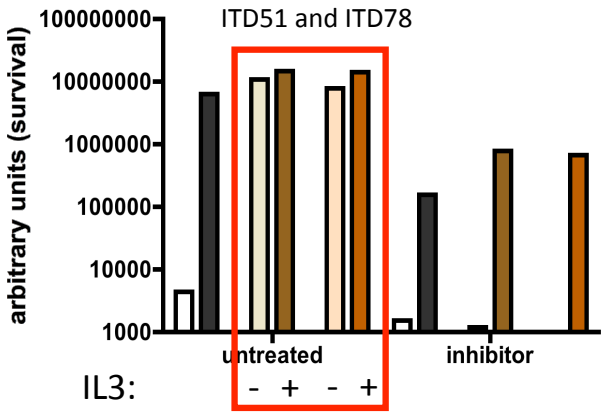

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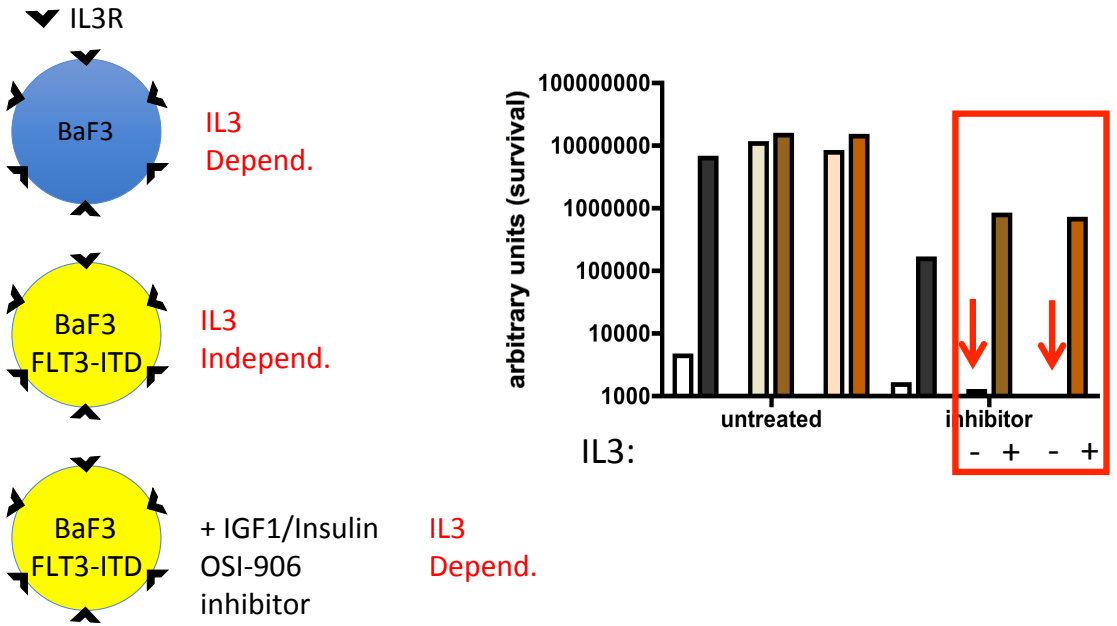
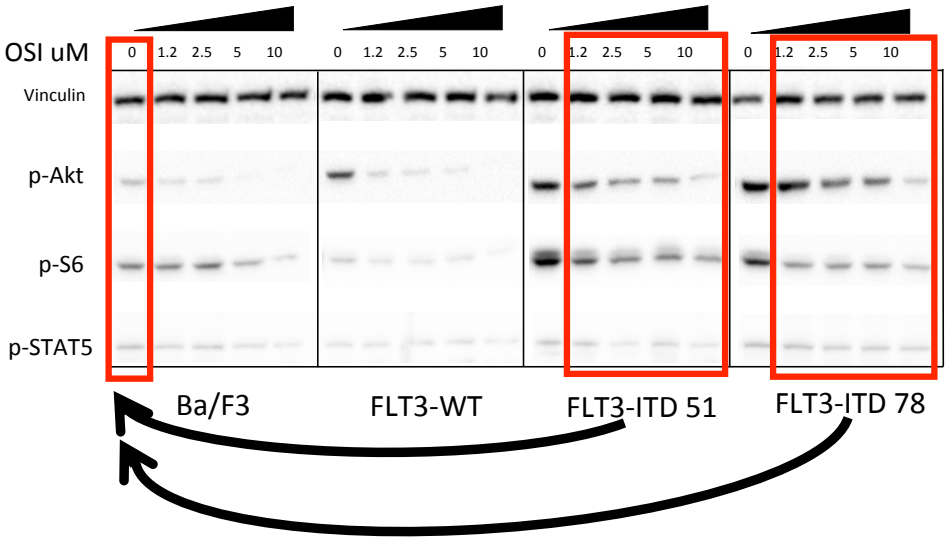
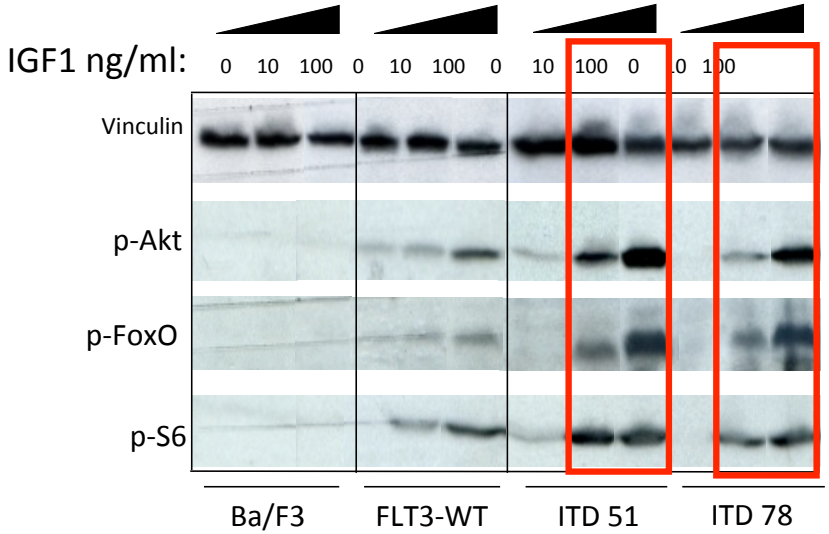


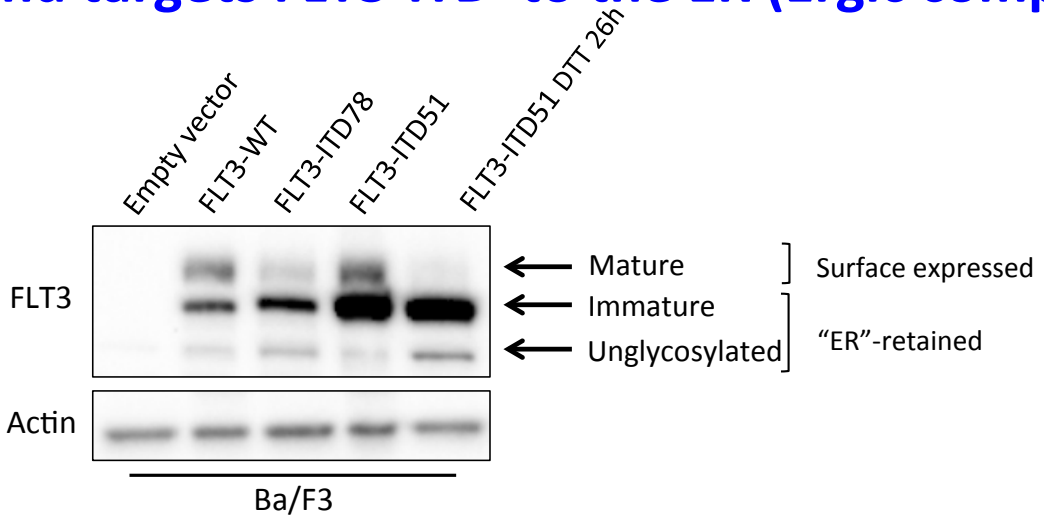
The oncogenic signaling of the FLT3-ITD mutation depends on Insulin/IGF1 signalling

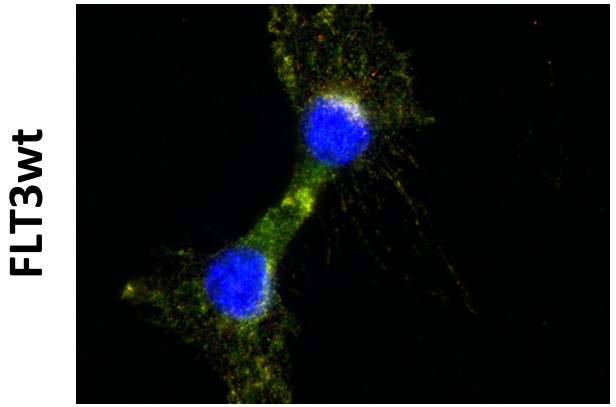


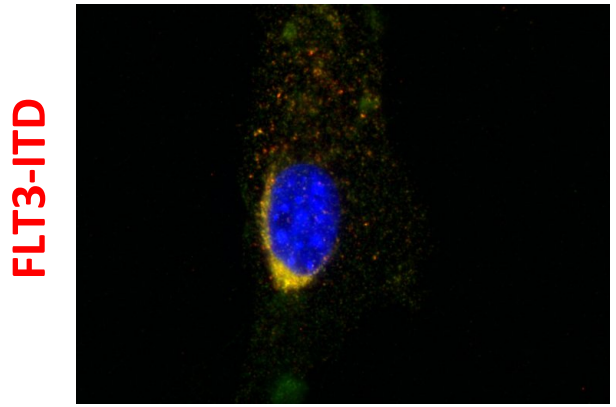
IGF1 potentiates the signaling potential of FLT3-ITD



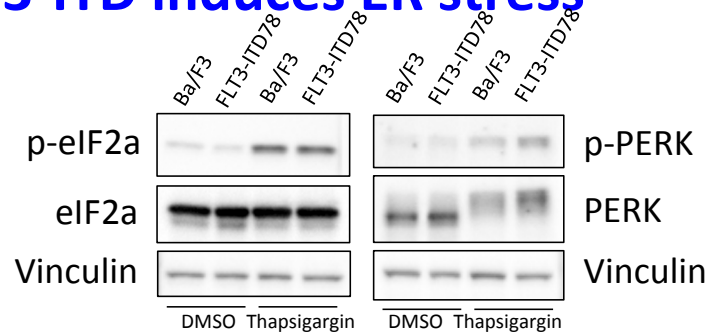
The ITD mutation increases the immature, non-glycosylated form of FLT3, and targets FLT3-ITD to the ER (Ergic compartment)





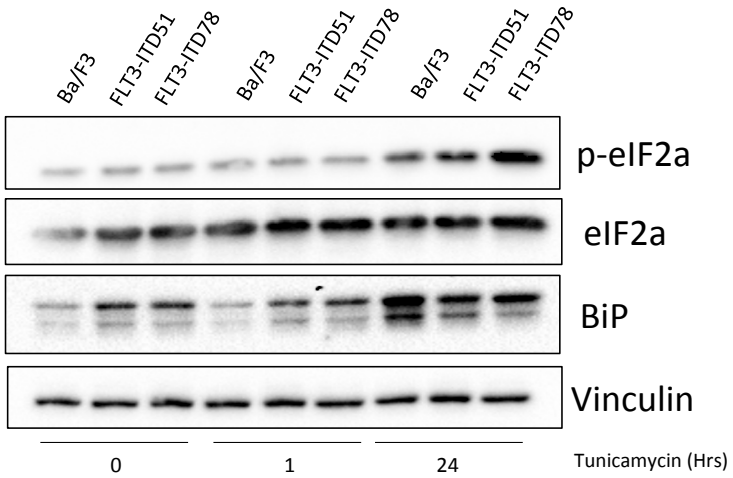


FLT3-ITD induces ER stress



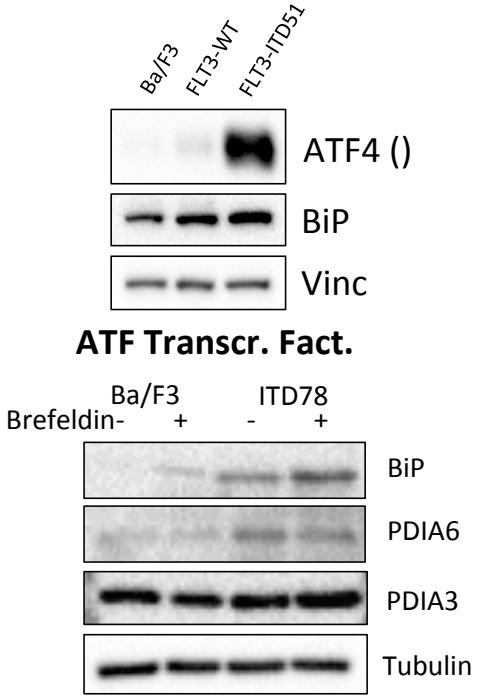
stress-sensing kinase PERK

FLT3-ITD induces a sustained UPR response



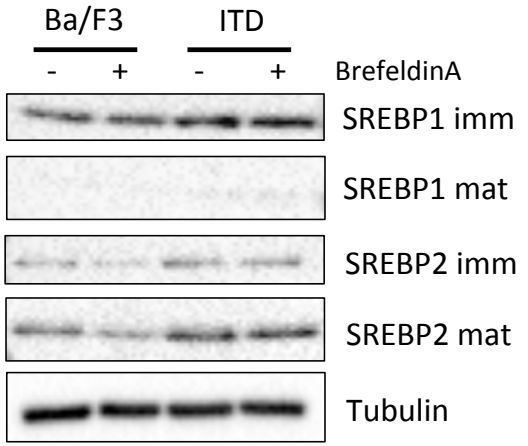
Translational initiation factor 2a (eIF2a) (cap-dependent protein translation)

Translational Reprogramming



PDIA3/6 disulphide isomerases the BiP ER-chaperone

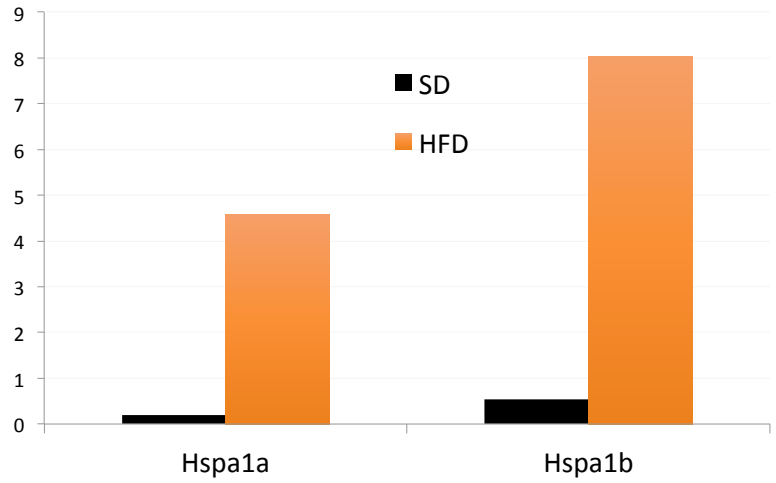
Transcriptional Reprogramming



SREBP2 transcription factor (sterol regulatory element-binding protein)

Colesterol Reprogramming

Obesity increases the UPR response to FLT3-ITD induced ER stress



Chaperone synthesis

RNAseq on FLT3-HO mice

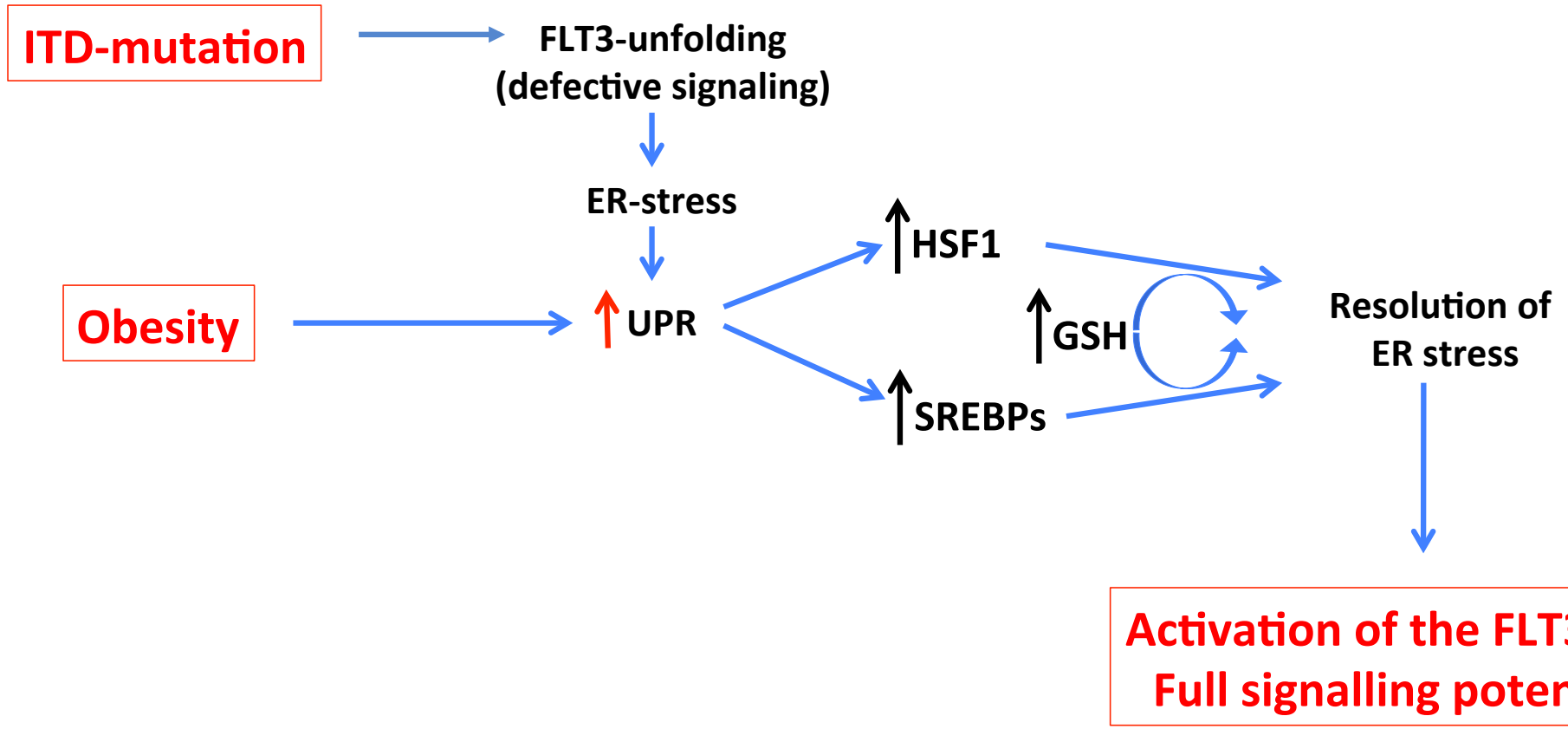
Gene	SD (TPM)	HFD (TPM)
Sqle	38,56	46,76
Hmgcr	48,20	65,97
Hmgcs1	27,43	35,89
Srebf2	120,62	147,23

RNAseq on SD mice

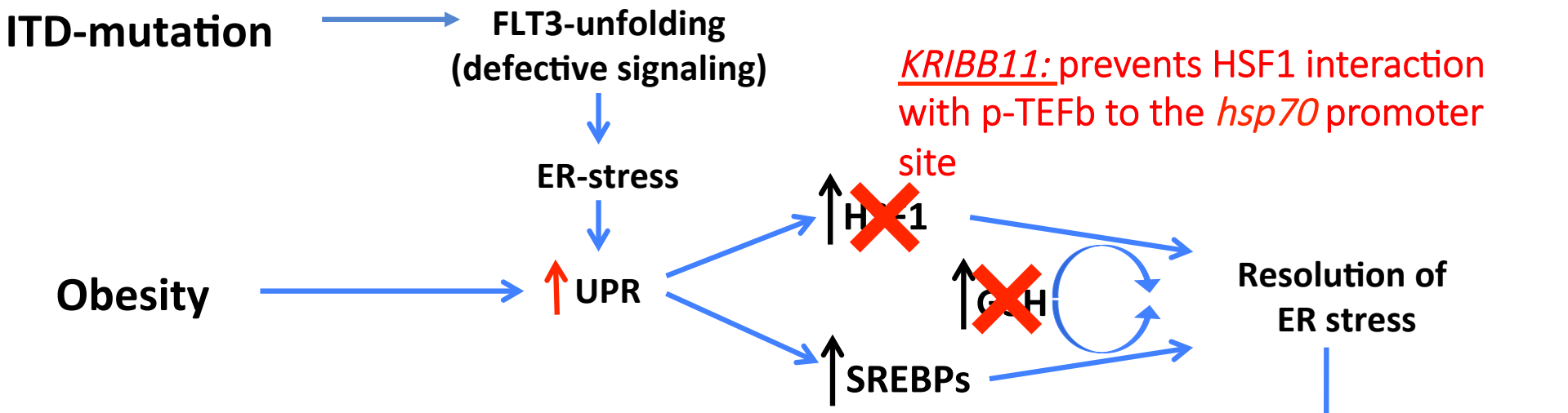
Gene	WT (TPM)	FLT3 (TPM)
Me2	52,16	83,99
Sqle	23,71	38,56
Hmgcs1	21,84	27,43
Scd2	93,40	188,13
Fasn	53,06	80,04
Slc16a1	24,29	50,39
Srebf2	124,44	120,62

Cholesterol metabolism

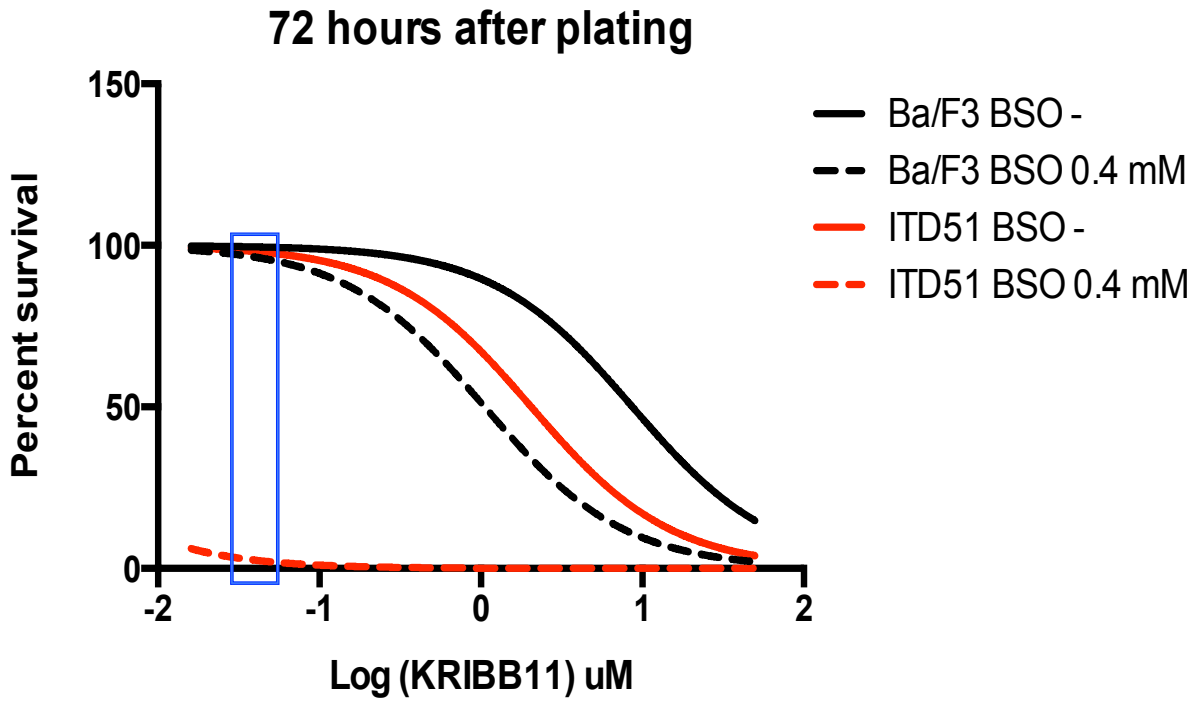
Obesity favors resolution of ER-stress induced by the ITD mutation (UPR response) and activates the full oncogenic potential of FLT3-ITD



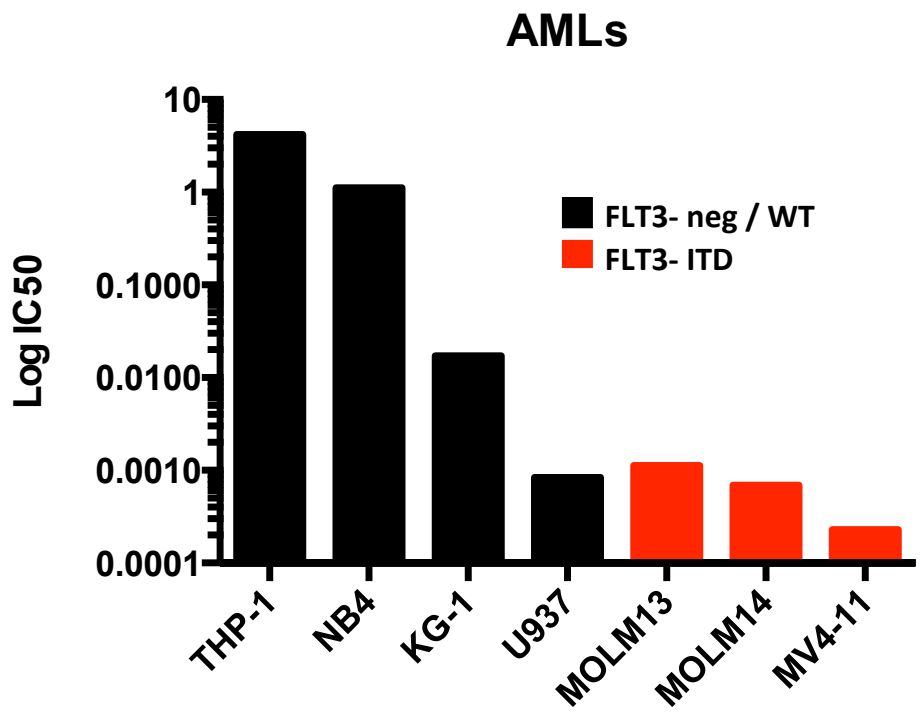
Pharmacological targeting of the UPR response



Modest effects of BSO or KRIBB1 single-agents; >95% cell death upon combination



FLT3-ITD sensitizes AMLs to BSO-KRIBB11 combined treatment



Conclusions

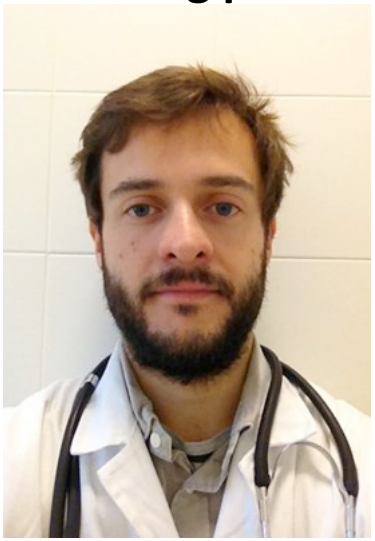
- **Obesity activates the oncogenic potential of FLT3-ITD by releasing FLT3-ITD – induced ER stress (through insulin/IGF1 signaling)**

- **This adaptive response to FLT3-ITD – induced ER stress creates selective vulnerabilities of FLT3-ITD AMLs, unraveled by inhibition of chaperone activity or GSH depletion**

Effects of nutrient deprivation on Leukemia Stem Cells

- The ***tumor micro-environment*** is characterized by a chronic state of nutrient and oxygen deprivation

- ***Nutrient scarcity*** is among the critical environmental conditions driving phenotypic plasticity

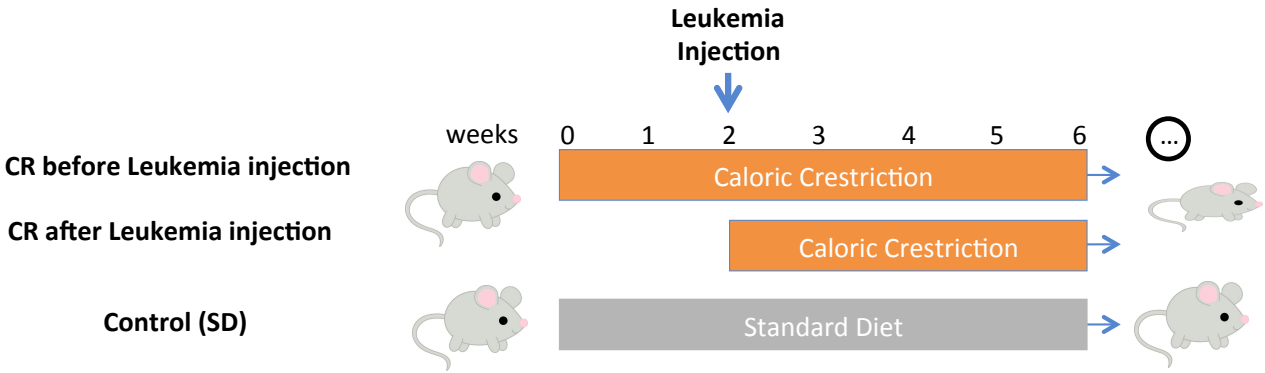


Luca Mazzarella, MD-PhD

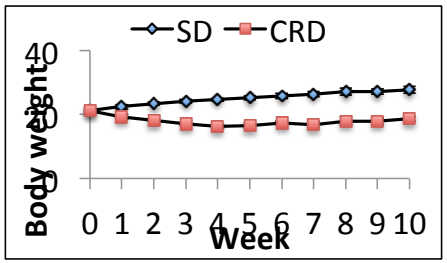
Rani Pallavi, PhD

Saverio-Minucci's Group

Caloric restriction (CR) as model system to study nutrient deprivation

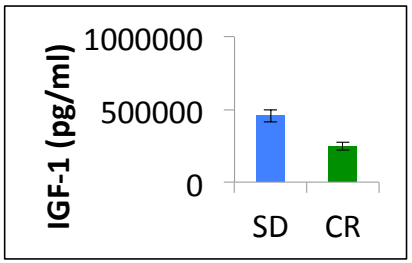


Change in Body Mass

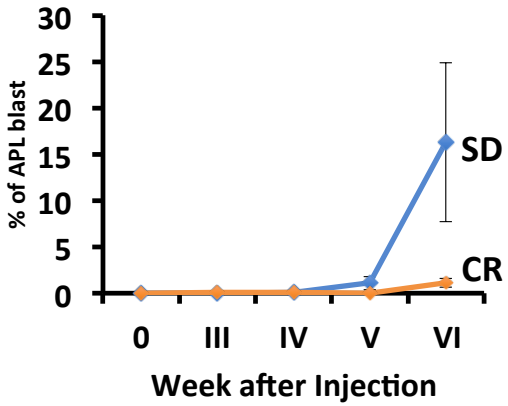


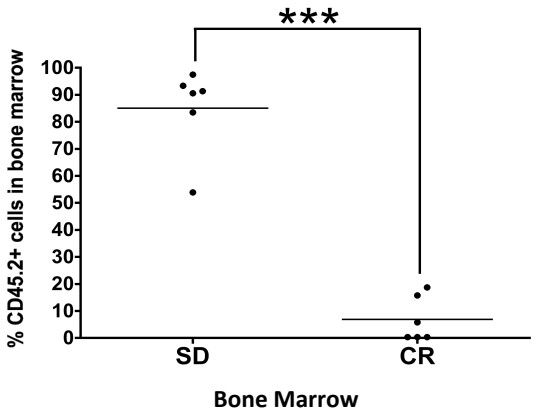
~15% body-weight reduction in the CR group

Circulating IGF-1

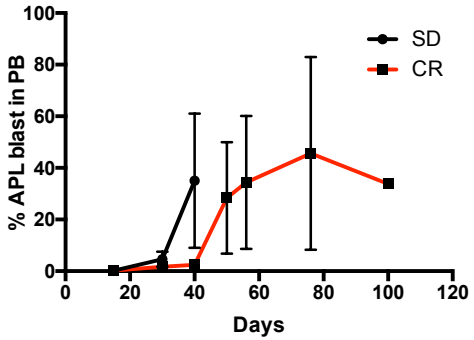


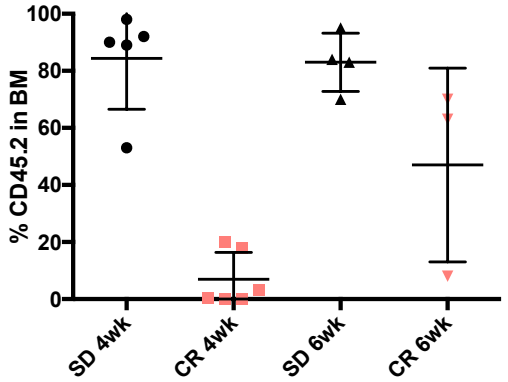
Early time points:
CR markedly reduces leukemic burden



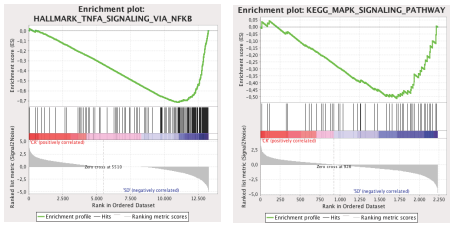


Later time points:
CR-treated Leukemias re-expand leading to leukemia associated mouse death



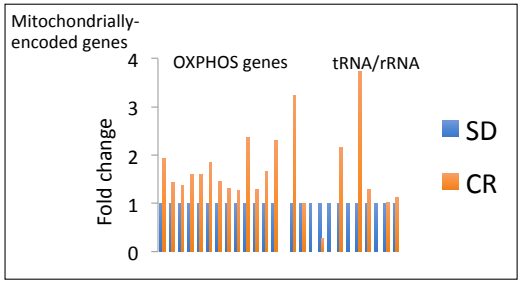


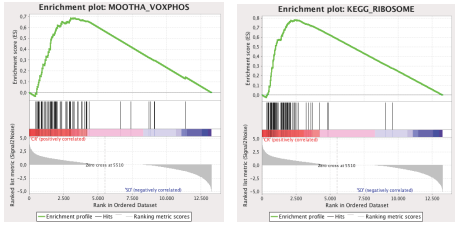
Transcriptional rewiring in CR



Depleted in CR

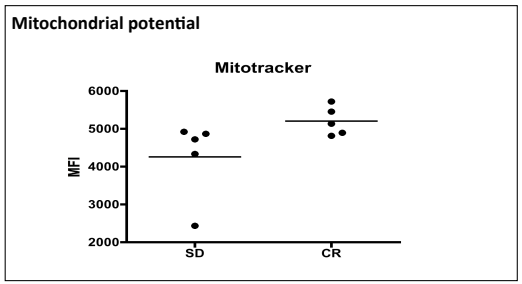
KEGG_NEUROACTIVE_LIGAND_RECEPTOR_INTERACTION
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION
KEGG_MAPK_SIGNALING_PATHWAY
KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY
KEGG_PHOSPHATIDYLINOSITOL_SIGNALING_SYSTEM
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY





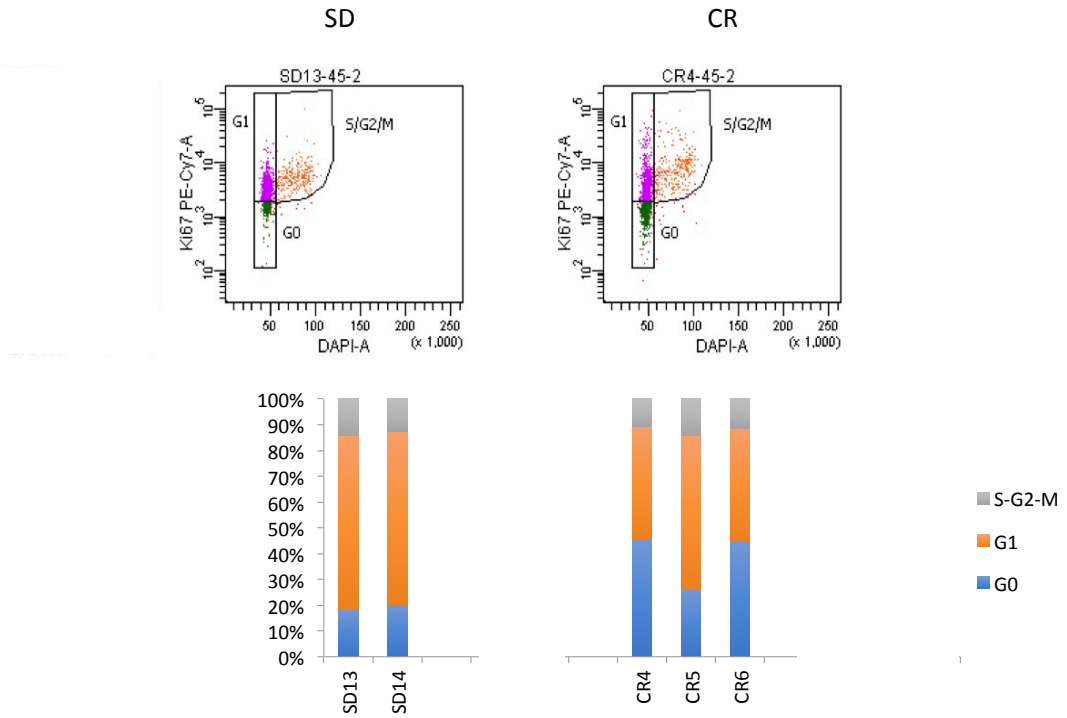
Enriched in CR

KEGG_PPAR_SIGNALING_PATHWAY
KEGG_PARKINSONS_DISEASE
KEGG_SPLICEOSOME
KEGG_ANTIGEN_PROCESSING_AND_PRESENTATION
KEGG_RNA_DEGRADATION
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS
KEGG_PYRIMIDINE_METABOLISM
KEGG_HUNTINGTONS_DISEASE
KEGG_OXIDATIVE_PHOSPHORYLATION
KEGG_RNA_POLYMERASE
KEGG_PROTEASOME
KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION
KEGG_CELL_ADHESION_MOLECULES_CAMS
KEGG_RIBOSOME
KEGG_ALZHEIMERS_DISEASE
KEGG_CITRATE_CYCLE_TCA_CYCLE

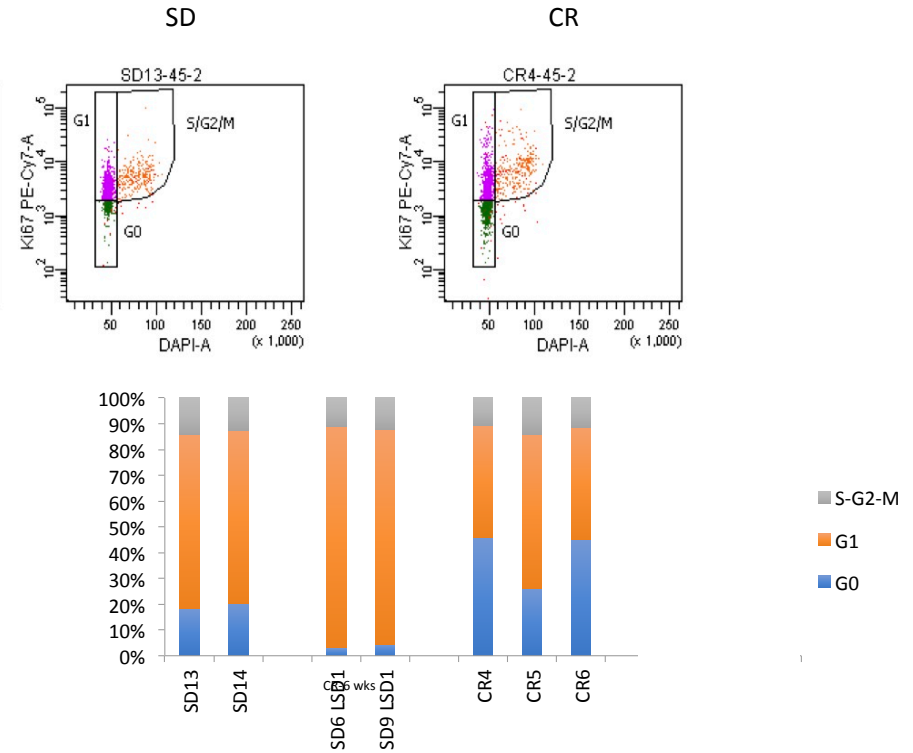


Increased transcription of oxydative phosphorylation genes and oxydative metabolism

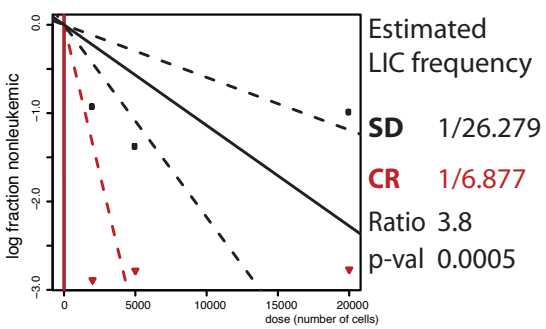
CR-treated leukemias initially slow down their cell cycle (3 weeks)



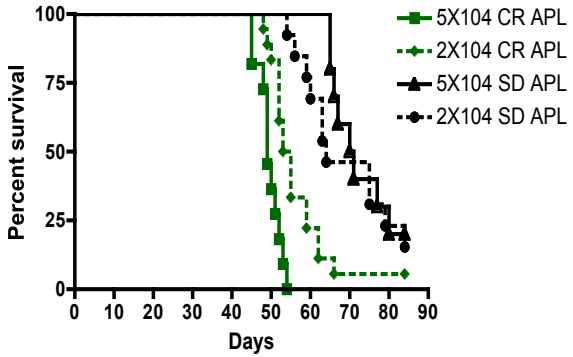
And then resume normal (or accelerated) proliferation



CR increases frequency and “aggressiveness” of LICs

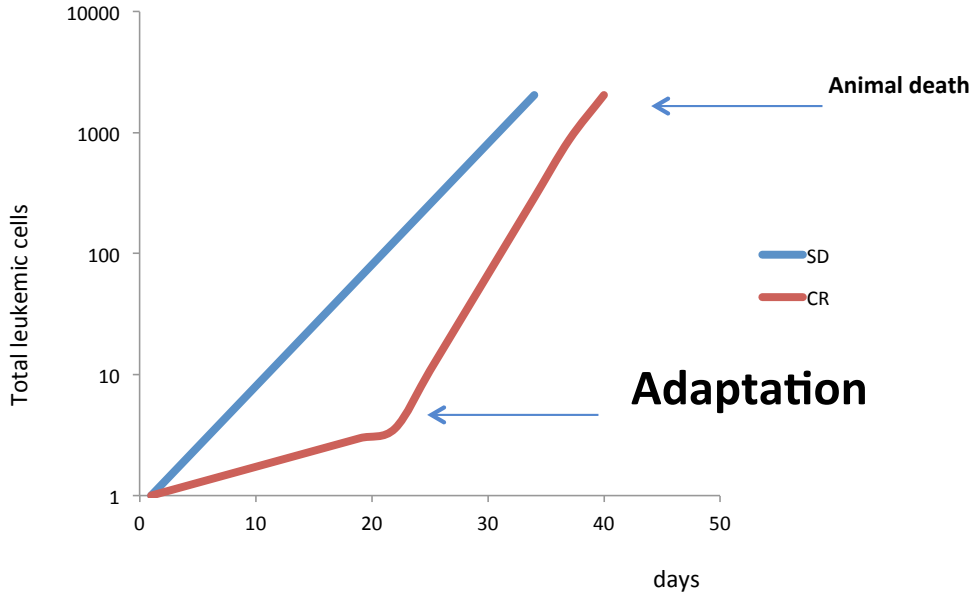


Frequency

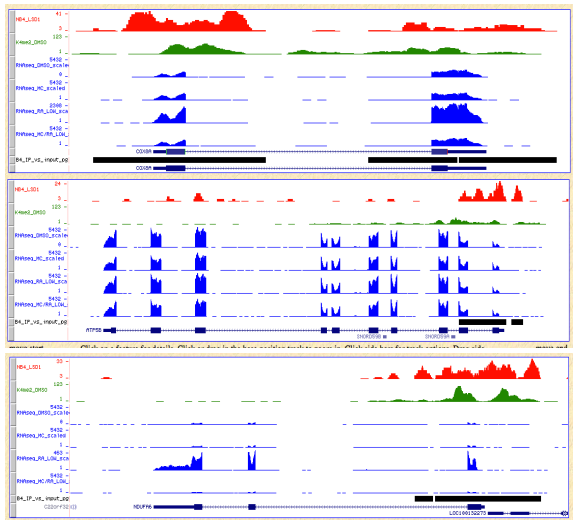


Leukemogenic potential after transplantation (equal numbers)

Adaptation of leukemia stem cells to Caloric Restriction

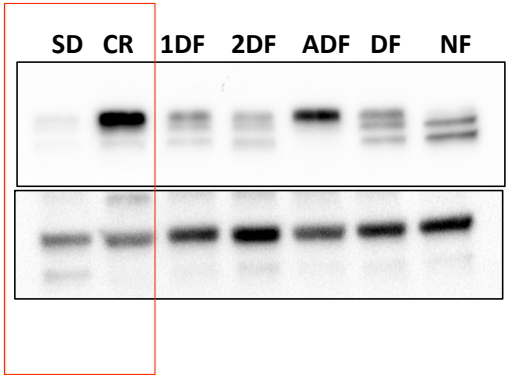


The LSD1 Lysine demethylase is involved in the adaptive response to CR



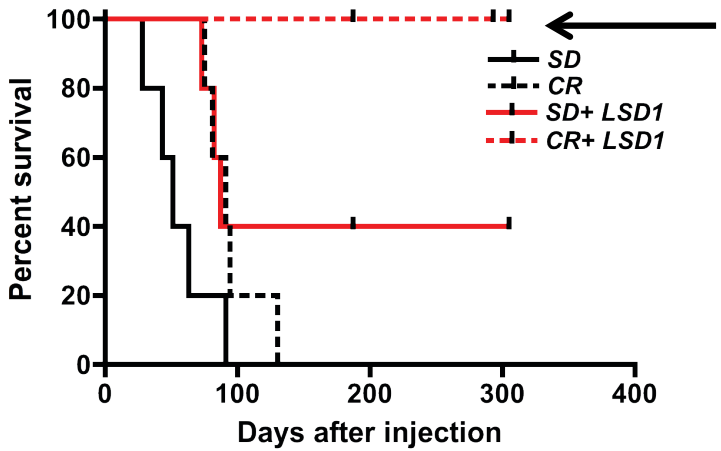
3T3-L1 cells day 7		Complex I		Complex II		Complex III		Complex IV		Complex V	
		Ndufa6	Ndufa3	Sdha	Sdhb	Uqcrc1	Uqcrc2	Cox6a1	Cox8a	Atp5b	Atp5l
ChIP-seq	Ctrl										
	LSD1										
	Refseq										
RNA-seq	FC day 7	2.83	2.89	2.21	4.56	3.86	3.38	3.09	3.59	3.68	2.54

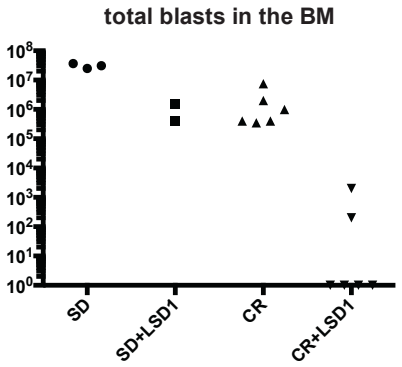
Many OXPHOS genes are targets of LSD1

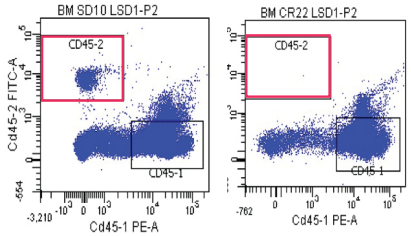


CR or Fasting increases levels of LSD1 in leukemia cells in vivo

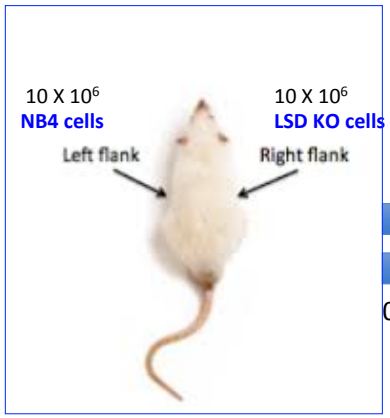
CR+LSD1 inhibition leads to disease eradication



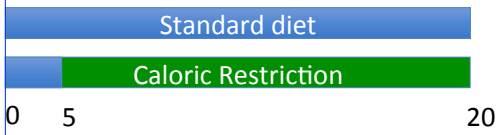


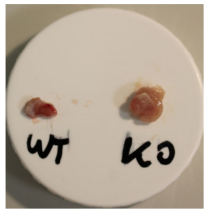


Genetic evidence for co-operation between LSD1 inhibition and Caloric Restriction

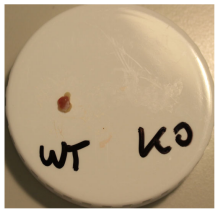


NB4 cells (human APL cell line)
NB4 LSD1KO cells (Saverio Minucci's Lab)

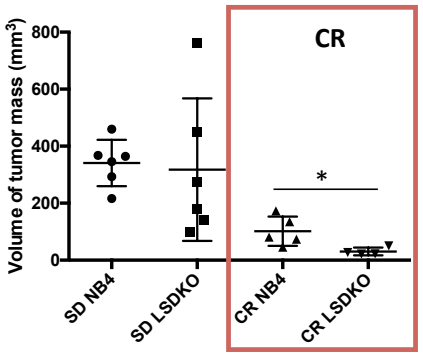


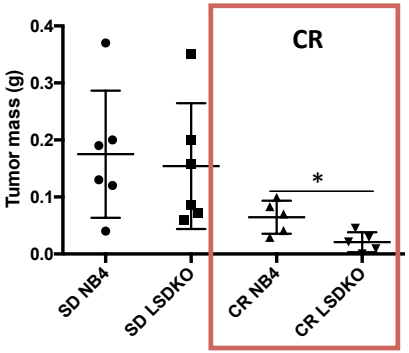


SD

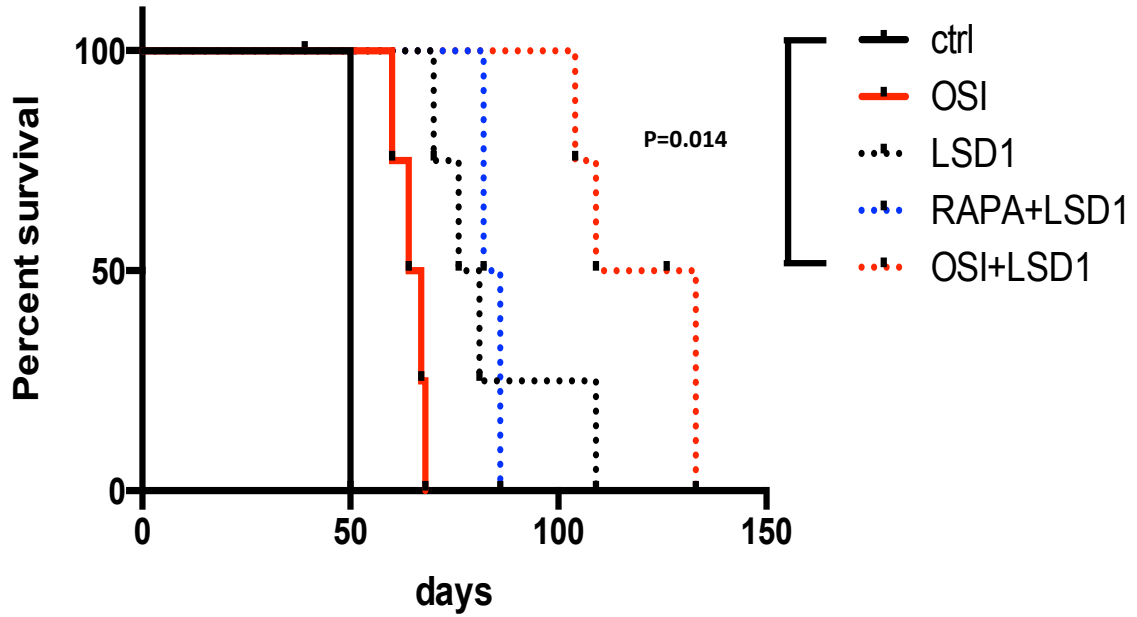


CR

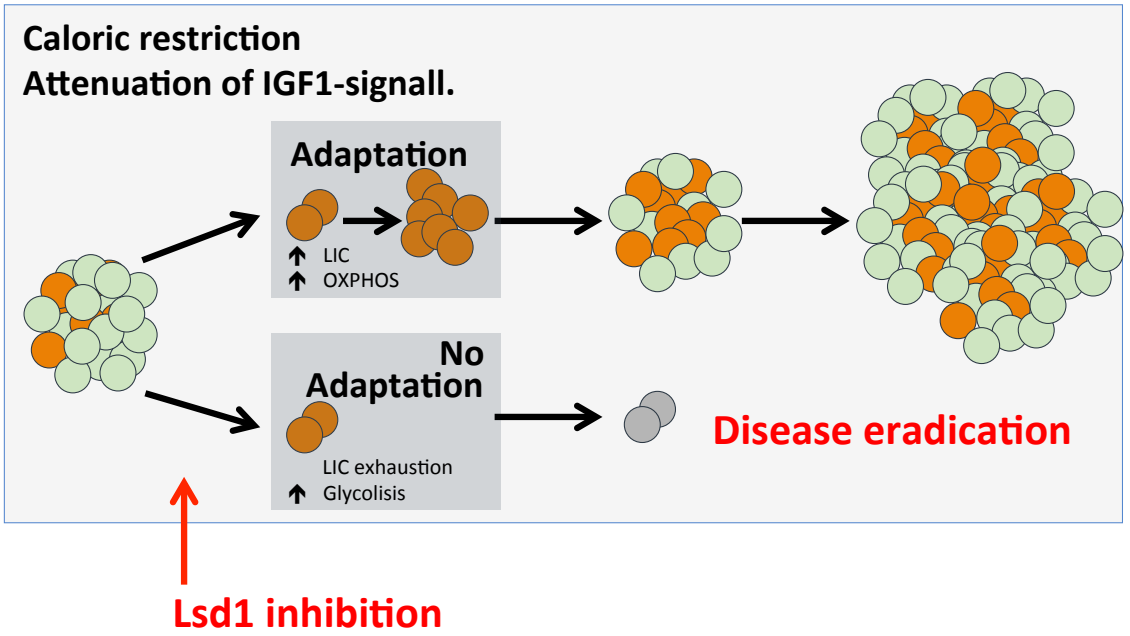




The insulin/IGF1R inhibitor OSI-906, but not Rapamycin, mimics nutrient deprivation and synergizes with the LSD1 inhibitor



Inhibition of phenotypic adaptation (to nutrient deprivation) eradicates leukemias



Working hypotheses

Phenotypic (non-genetic) adaptation to the changing tumor micro-environment:

- **is critical for tumor development**
- **is influenced by the specific genetic make-up of each tumor**
- **can be exploited to develop innovative anti-cancer strategies.**