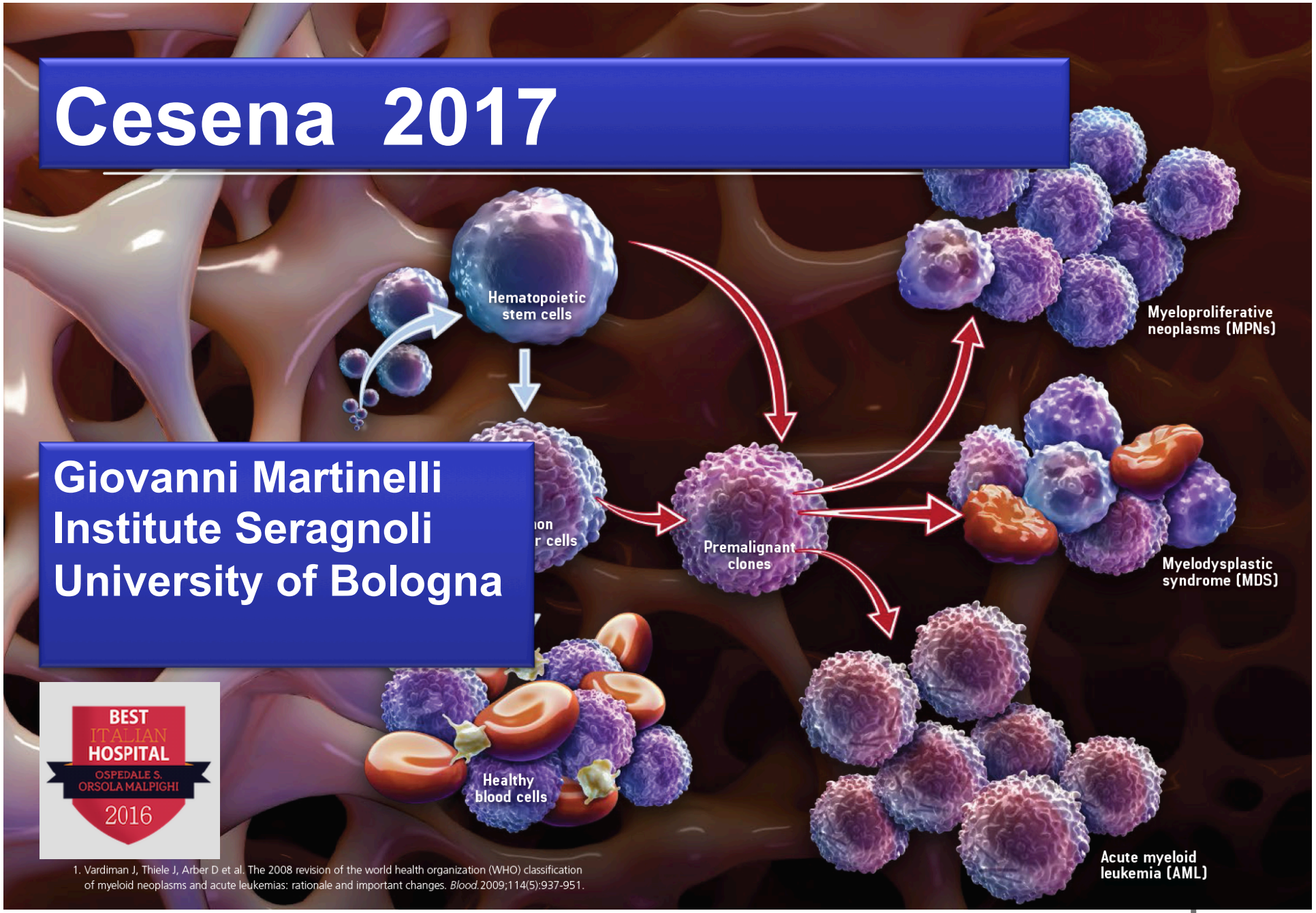
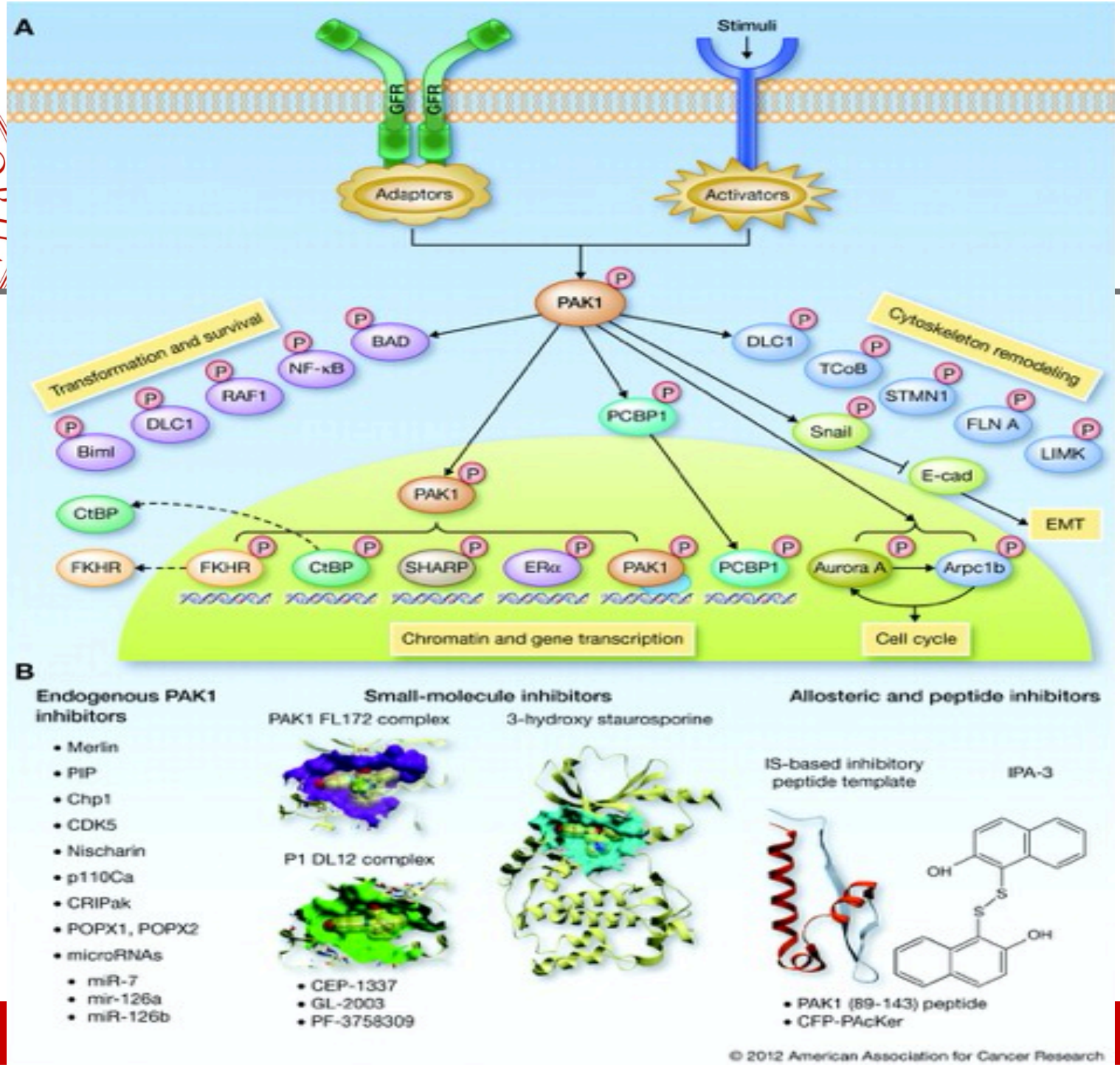
Cesena 2017

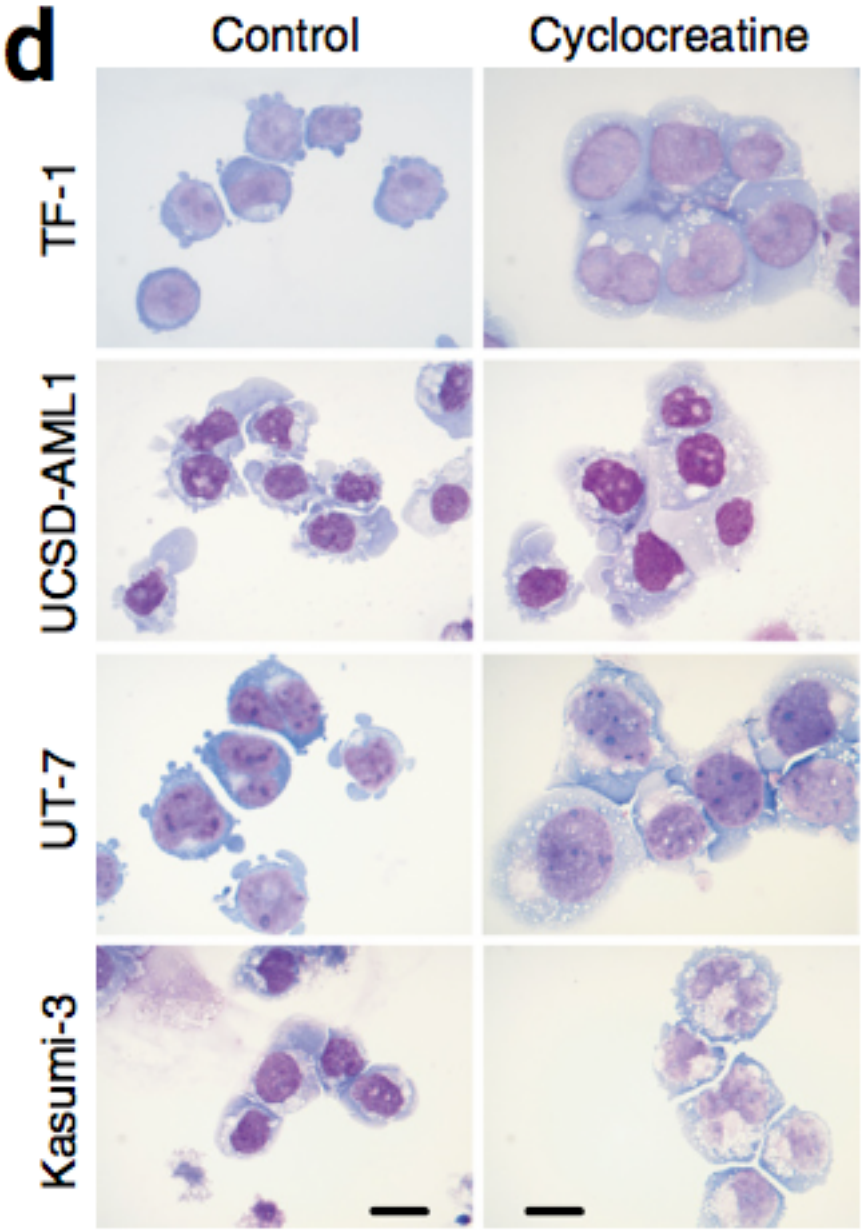


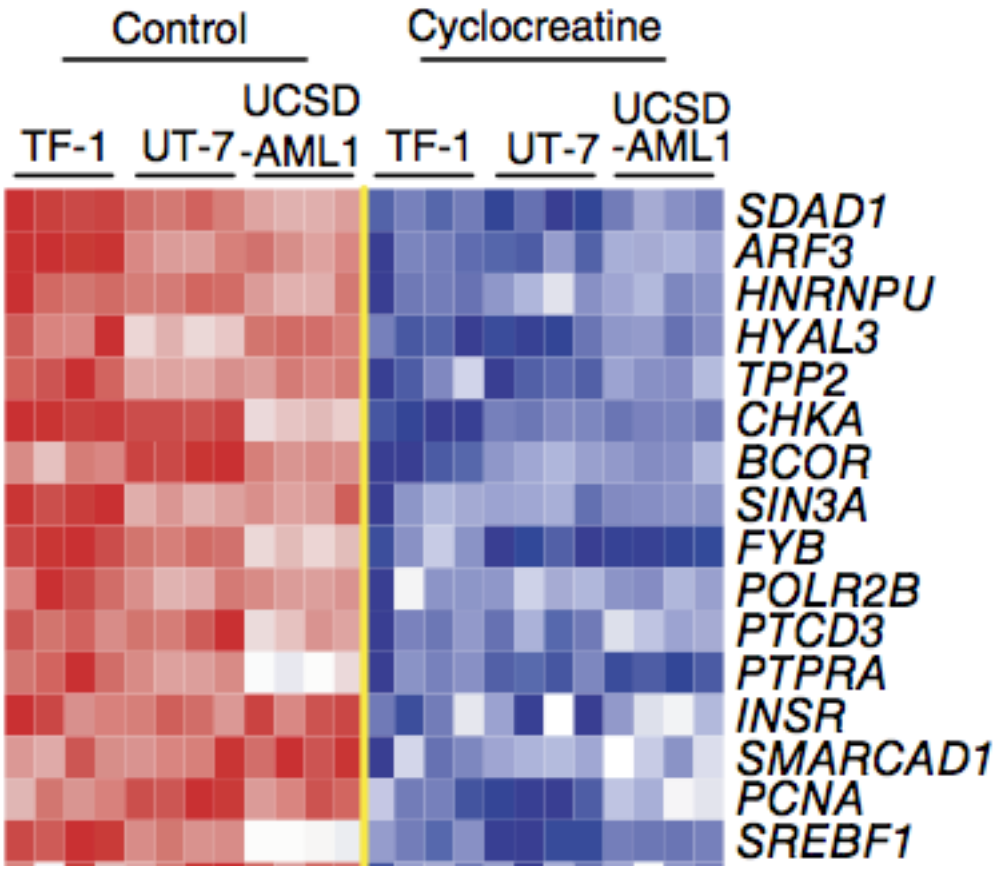
Giovanni Martinelli
Institute Seragnoli
University of Bologna

BEST ITALIAN HOSPITAL
OSPEDALE S. ORSOLA MALPIGHI
2016

1. Vardiman J, Thiele J, Arber D et al. The 2008 revision of the world health organization (WHO) classification of myeloid neoplasms and acute leukemias: rationale and important changes. *Blood*. 2009;114(5):937-951.


A
Stimuli
GFR
GFR
Adaptors
Activators
PAK1
Transformation and survival
BAD
NF-κB
RAF1
DLC1
Bim
PCBP1
DLC1
TCoB
STMN1
FLN A
LIMK
Snail
E-cad
EMT
Cytoskeleton remodeling
PAK1
CtBP
FKHR
FKHR
CtBP
SHARP
ERα
PAK1
PCBP1
Aurora A
Arpc1b
Chromatin and gene transcription
Cell cycle
B
Endogenous PAK1 inhibitors
• Merlin
• PIP
• Chp1
• CDK5
• Nischarin
• p110Ca
• CRIPak
• POPX1, POPX2
• microRNAs
• miR-7
• mir-126a
• miR-126b
Small-molecule inhibitors
PAK1 FL172 complex
3-hydroxy staurosporine
P1 DL12 complex
• CEP-1337
• GL-2003
• PF-3758309
Allosteric and peptide inhibitors
IS-based inhibitory peptide template
IPA-3
OH
S
S
OH
• PAK1 (89-143) peptide
• CFP-PAcKer
© 2012 American Association for Cancer Research



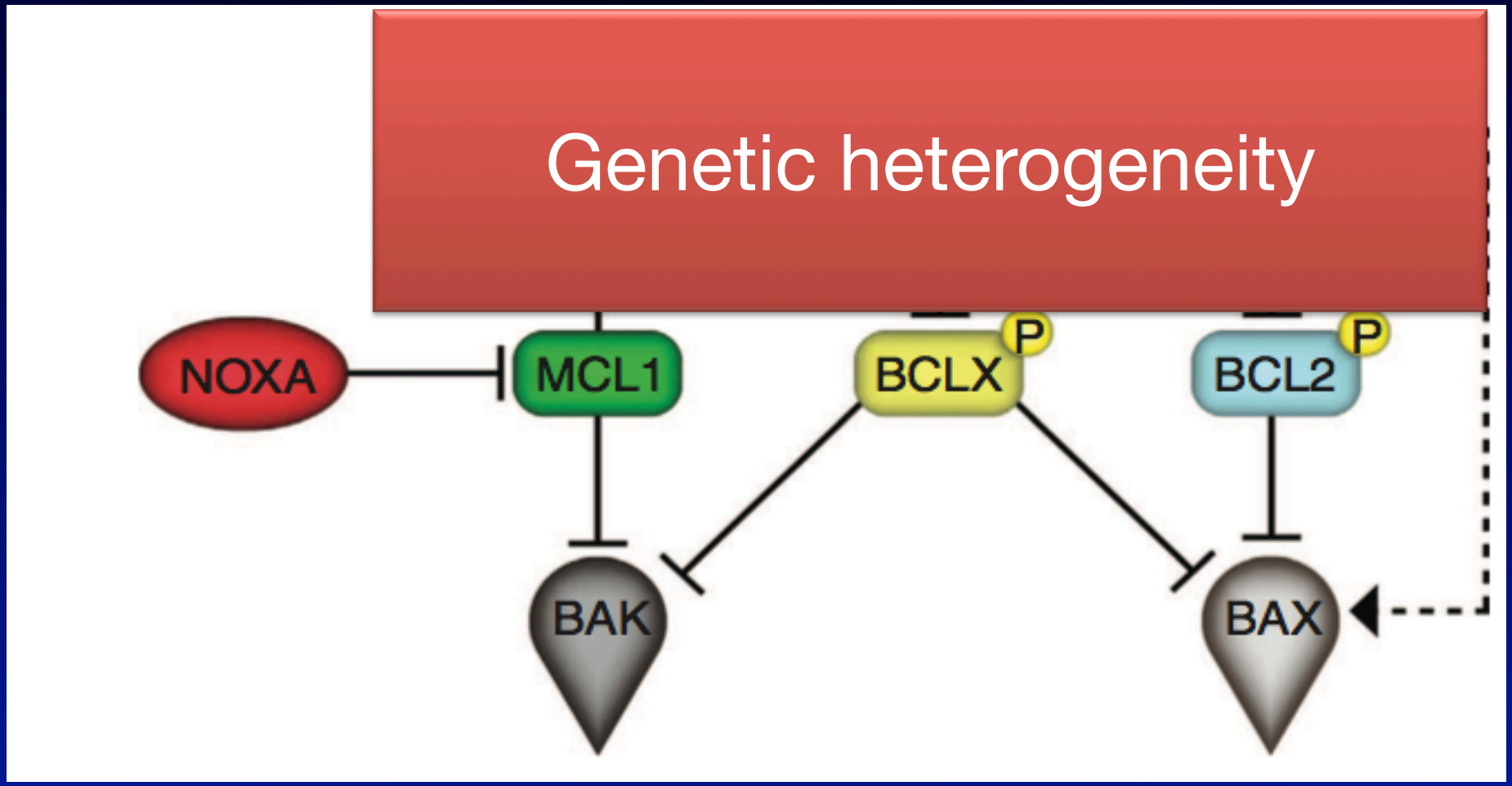


The creatine kinase pathway is a metabolic vulnerability in EVI1-positive acute myeloid leukemia

Nina Fenouille[1,13], Christopher F Bassil[2,13], Issam Ben-Sahra[3], Lina Benajiba[2], Gabriela Alexe[2,4,5], Azucena Ramos[1], Yana Pikman[2], Amy S Conway[2], Michael R Burgess[6], Qing Li[7], Frédéric Luciano[8], Patrick Auberger[8], Ilene Galinsky[9], Daniel J DeAngelo[9], Richard M Stone[9], Yi Zhang[10], Archibald S Perkins[10], Kevin Shannon[11], Michael T Hemann[1,14], Alexandre Puissant[2,12,14] & Kimberly Stegmaier[2,4,14]

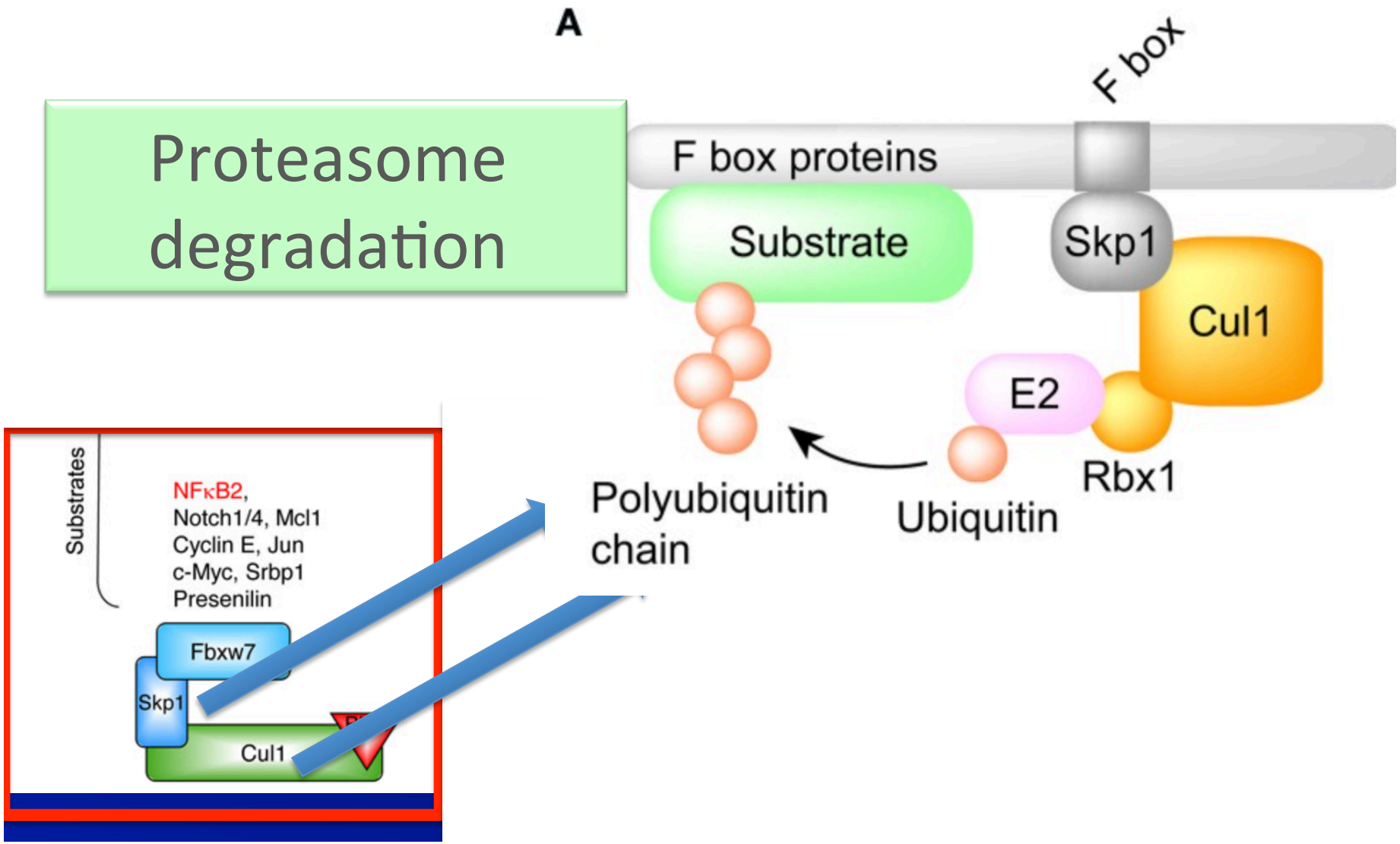
Expression of the *MECOM* (also known as *EVI1*) proto-oncogene is deregulated by chromosomal translocations in some cases of acute myeloid leukemia (AML) and is associated with poor clinical outcome. Here, through transcriptomic and metabolomic profiling of hematopoietic cells, we reveal that EVI1 overexpression alters cellular metabolism. A screen using pooled short hairpin RNAs (shRNAs) identified the ATP-buffering, mitochondrial creatine kinase CKMT1 as necessary for survival of EVI1-expressing cells in subjects with EVI1-positive AML. EVI1 promotes CKMT1 expression by repressing the myeloid differentiation regulator RUNX1. Suppression of arginine–creatine metabolism by *CKMT1*-directed shRNAs or by the small molecule cyclocreatine selectively decreased the viability, promoted the cell cycle arrest and apoptosis of human EVI1-positive cell lines, and prolonged survival in both orthotopic xenograft models and mouse models of primary AML. CKMT1 inhibition altered mitochondrial respiration and ATP production, an effect that was abrogated by phosphocreatine-mediated reactivation of the arginine–creatine pathway. Targeting CKMT1 is thus a promising therapeutic strategy for this EVI1-driven AML subtype that is highly resistant to current treatment regimens.

We need to induce more leukemia cell apoptosis….

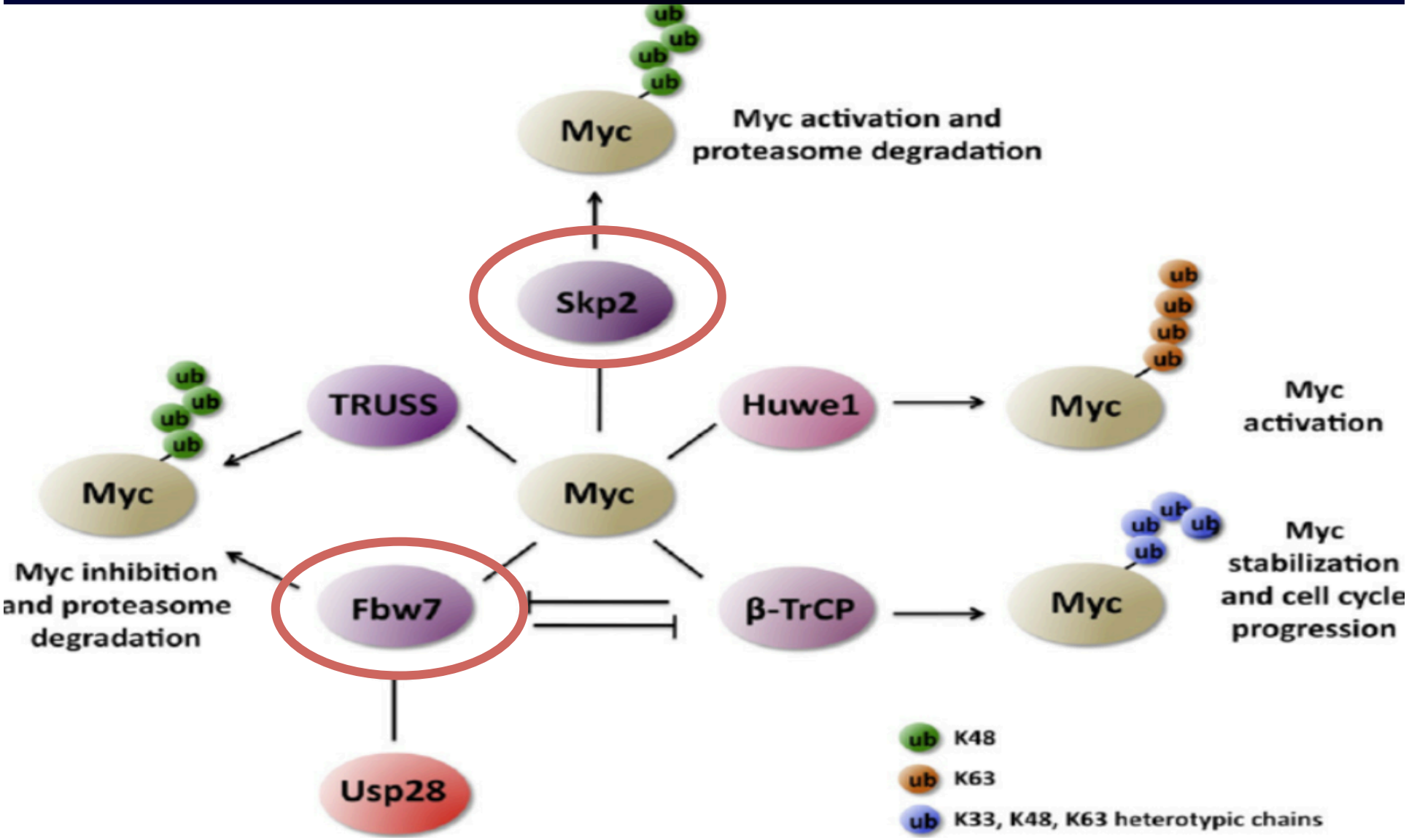


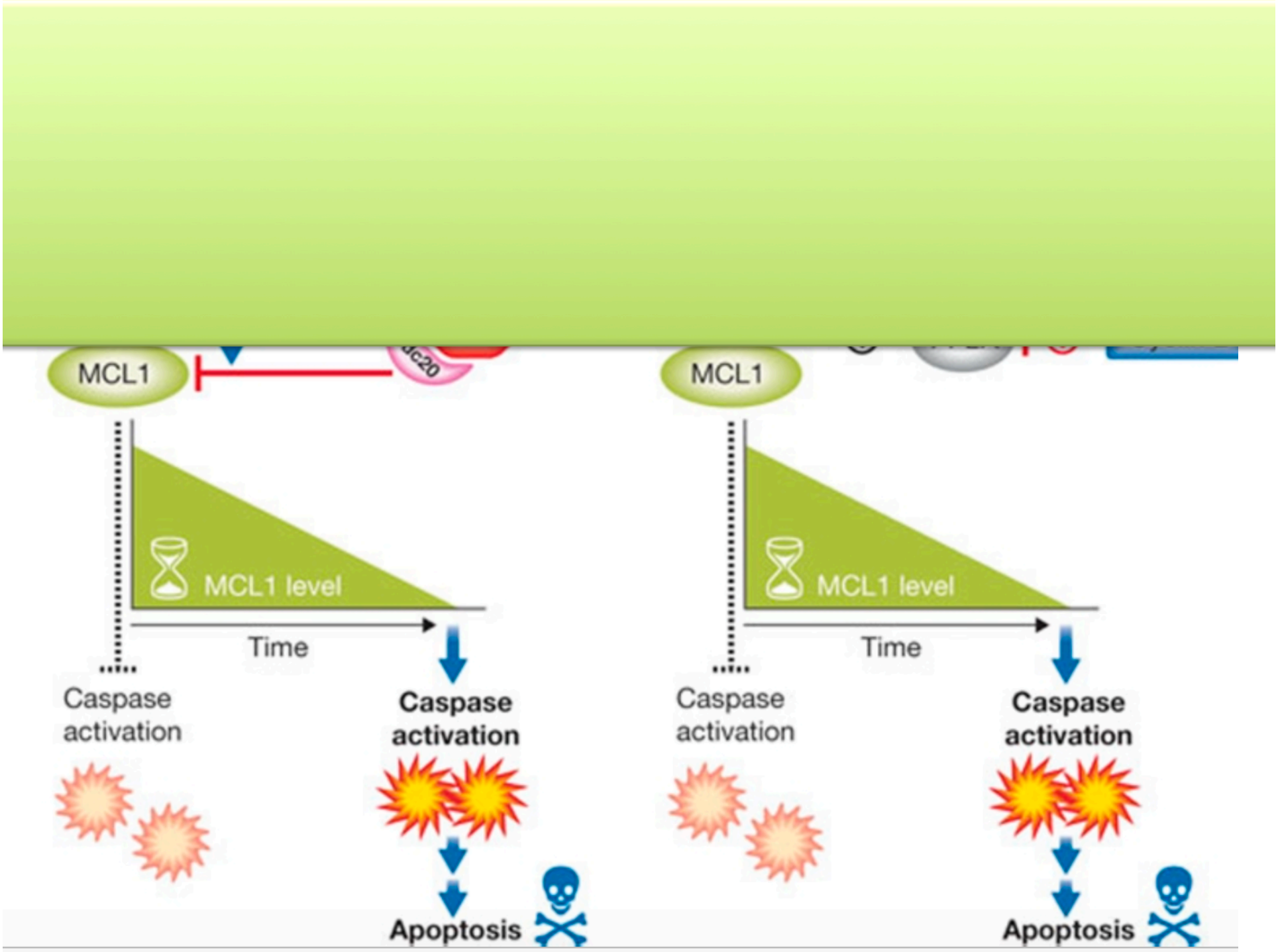
E-Ligase activity in Leukemia

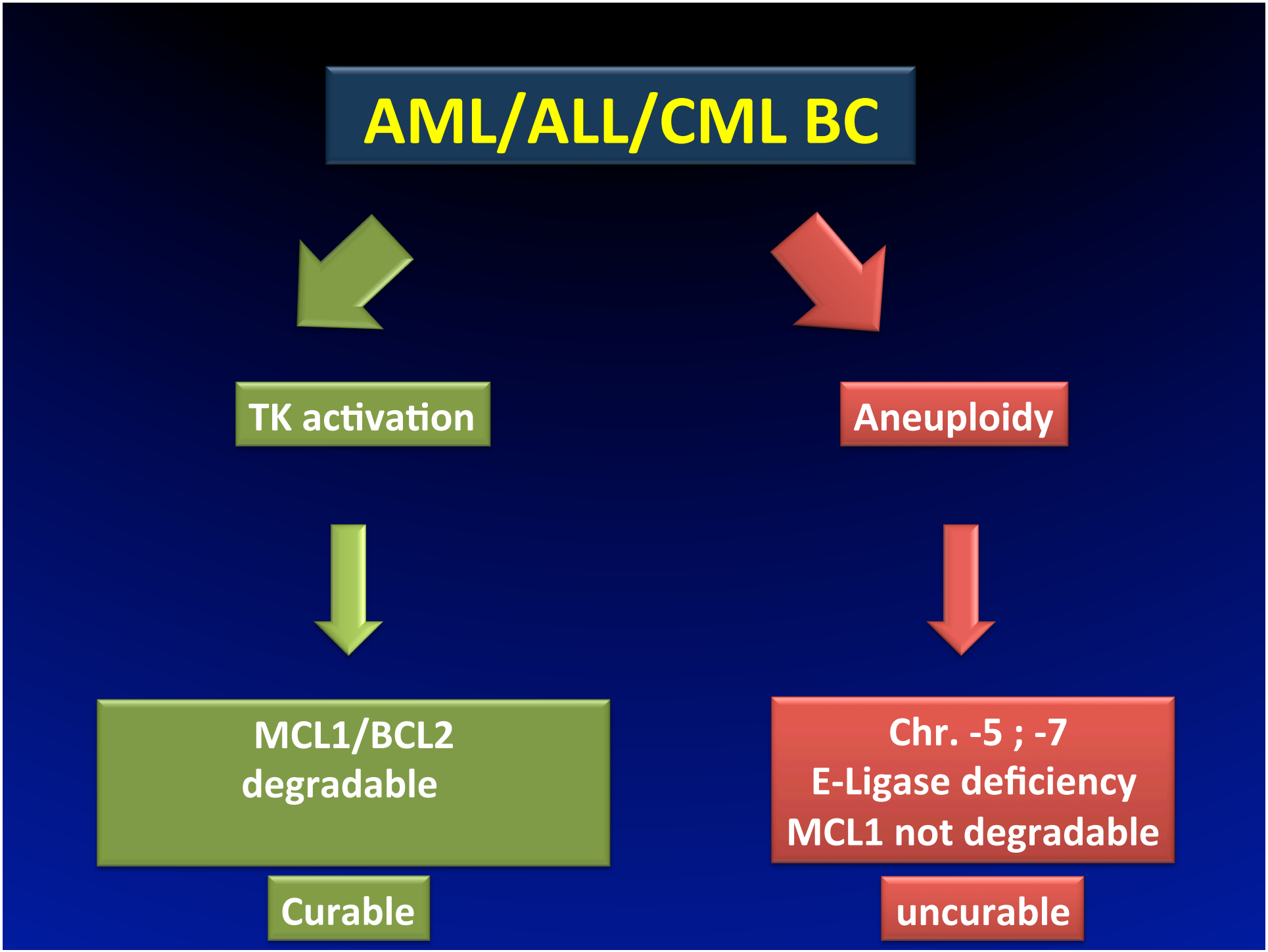
Proteasome degradation



Main route : Chr. 8 trisomy




MCL1
MCL1 level
Time
Caspase activation
Caspase activation
Apoptosis
MCL1
MCL1 level
Time
Caspase activation
Caspase activation
Apoptosis


AML/ALL/CML BC
TK activation
Aneuploidy
MCL1/BCL2
degradable
Curable
Chr. -5 ; -7
E-Ligase deficiency
MCL1 not degradable
uncurable

MYELOID SOLUTION
BY SOPHIA GENETICS

Gene content

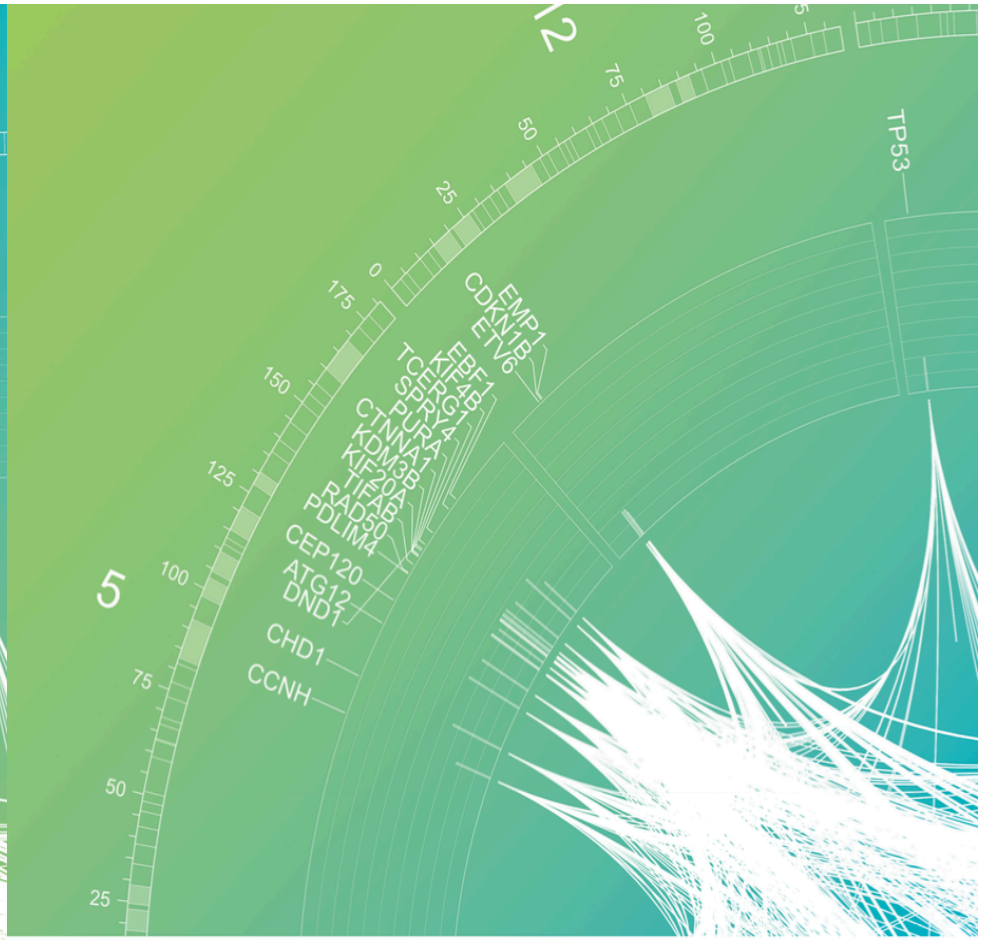
30 genes

Full genes

CEBPA
CSF3R
DNMT3A
ETV6
EZH2
JAK2
RUNX1
TET2
TP53
ZRSR2

Full exons

ABL1 (exons 4-9)
ASLX1 (exons 9, 11-12)
BRAF (exon 15)
CALR (exon 9)
CBL (exons 8-9)
FLT3 (exons 13-15, 20)
HRAS (exons 2-3)
IDH1 (exon 4)
IDH2 (exon 4)
KIT (exons 2, 8-11, 13, 17-18)
KRAS (exons 2-3)
MPL (exon 10)
NPM1 (exons 10-11)
NRAS (exons 2-3)
PTPN11 (exons 3, 7-13)
SETBP1 (exon 4)
SF3B1 (exons 10-16)
SRSF2 (exon 1)
U2AF1 (exons 2, 6)
WT1 (exons 6-10)



UNA COMUNITÀ CLINICA PER NGS
IN ONCO-EMATOLOGIA:
SOPHIA GENETICS E UNIBO

14 giovedì settembre 2017
Aula della Società Medica Chirurgica, Palazzo dell'Archiginnasio, Piazza Galvani 1, 40124
BOLOGNA

NUOVE FRONTIERE DEL
NEXT GENERATION SEQUENCING
NELLA DIAGNOSTICA EMATOLOGICA

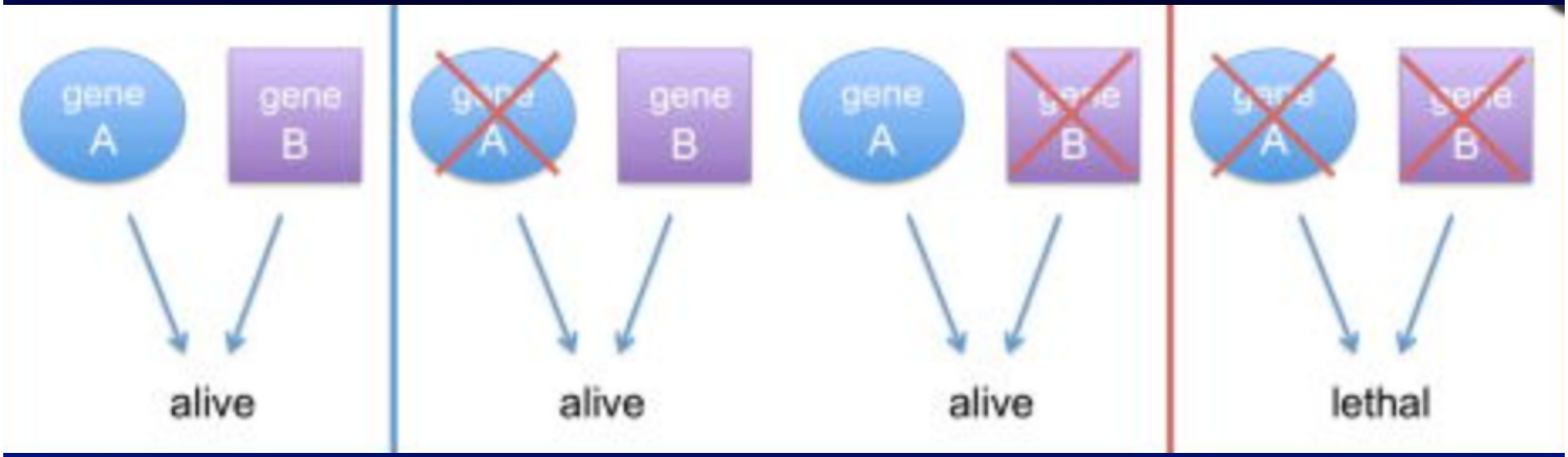
15 venerdì settembre 2017
Aula Chiantore
Istituto L.e A. Serragnoli
Via Massarenti 9 40138
BOLOGNA

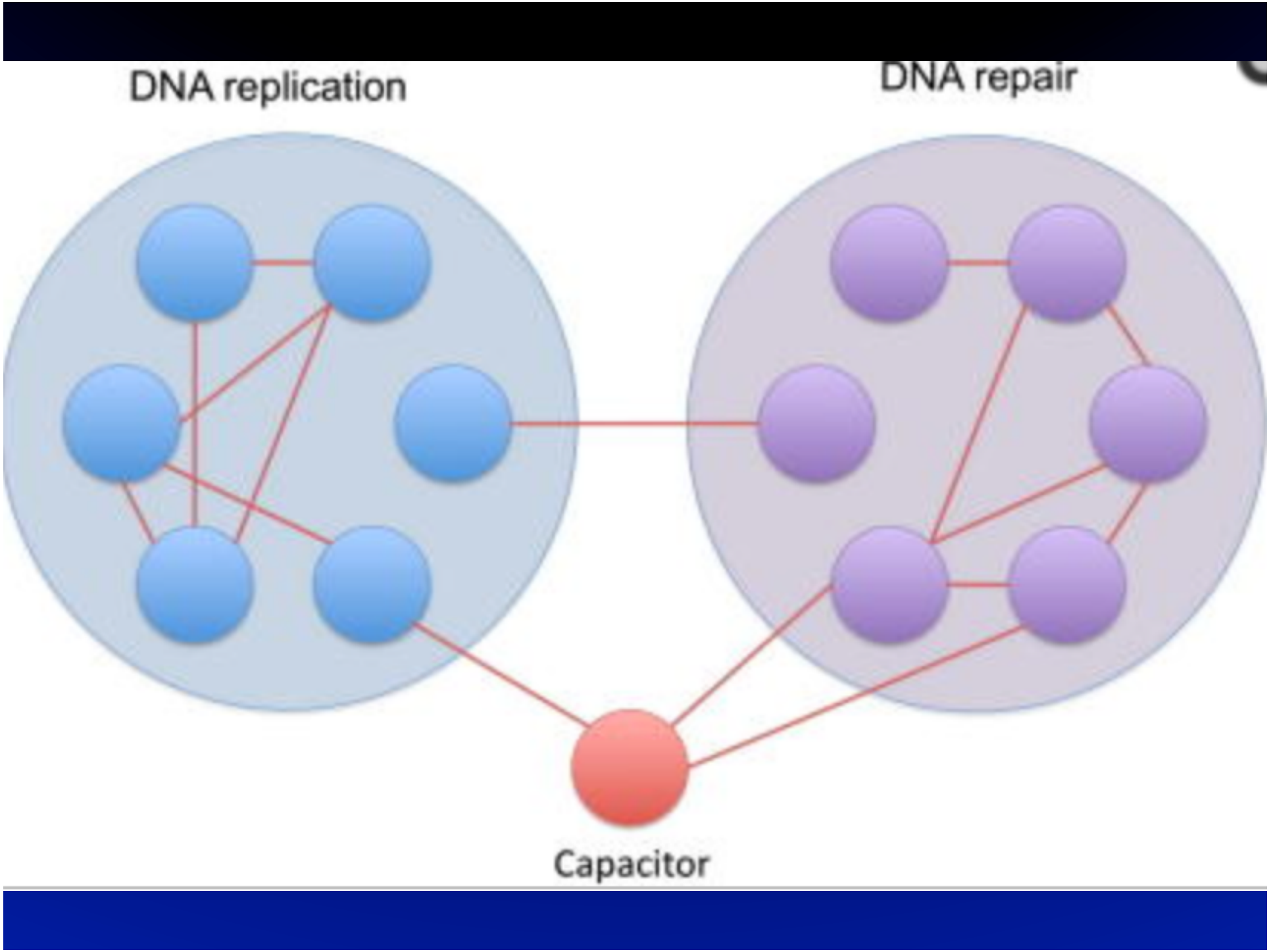
SOPHIA GENETICS · ARROW DIAGNOSTICS · SERVIZIO SANITARIO REGIONALE EMILIA-ROMAGNA Azienda Unità Sanitaria Locale della Romagna · ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA

SOPHIA GENETICS · ARROW DIAGNOSTICS · SERVIZIO SANITARIO REGIONALE EMILIA-ROMAGNA Azienda Unità Sanitaria Locale della Romagna · ALMA MATER STUDIORUM UNIVERSITÀ DI BOLOGNA

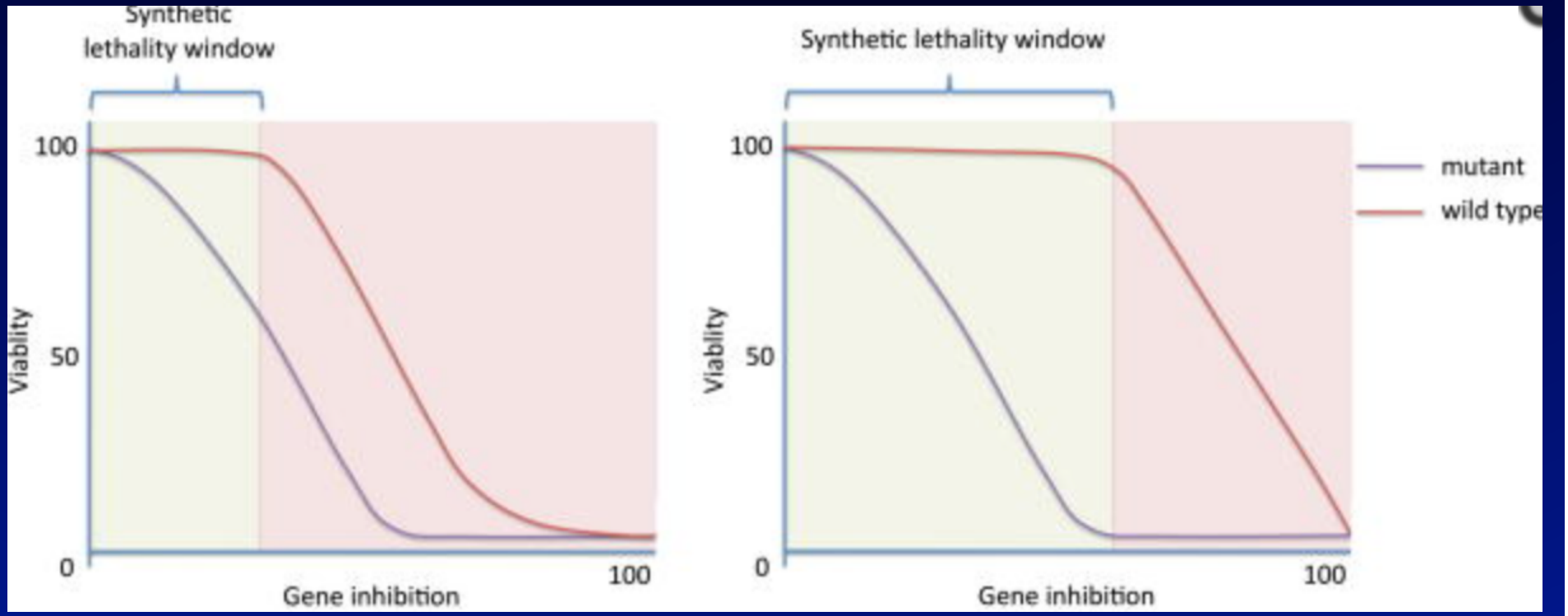
Synthetic Lethality in Leukemias

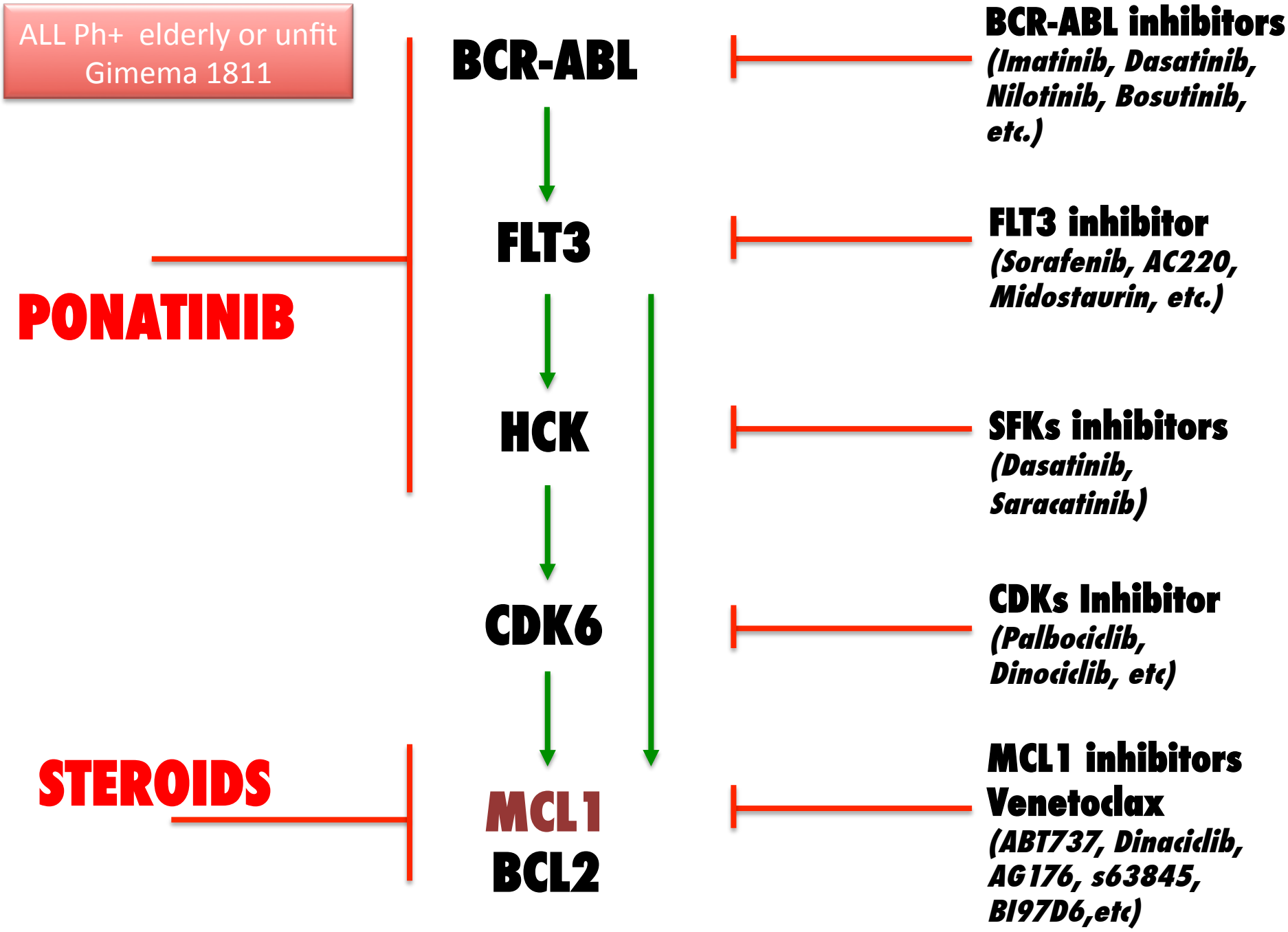
Synthetic Lethality




DNA replication
DNA repair
Capacitor

"Synthetic Lethality"
Therapy Increases The Therapeutic Window




ALL Ph+ elderly or unfit
Gimema 1811
PONATINIB
STEROIDS
BCR-ABL
FLT3
HCK
CDK6
MCL1
BCL2
BCR-ABL inhibitors
(Imatinib, Dasatinib, Nilotinib, Bosutinib, etc.)
FLT3 inhibitor
(Sorafenib, AC220, Midostaurin, etc.)
SFKs inhibitors
(Dasatinib, Saracatinib)
CDKs Inhibitor
(Palbociclib, Dinociclib, etc)
MCL1 inhibitors
Venetoclax
(ABT737, Dinaciclib, AG176, s63845, BI97D6,etc)

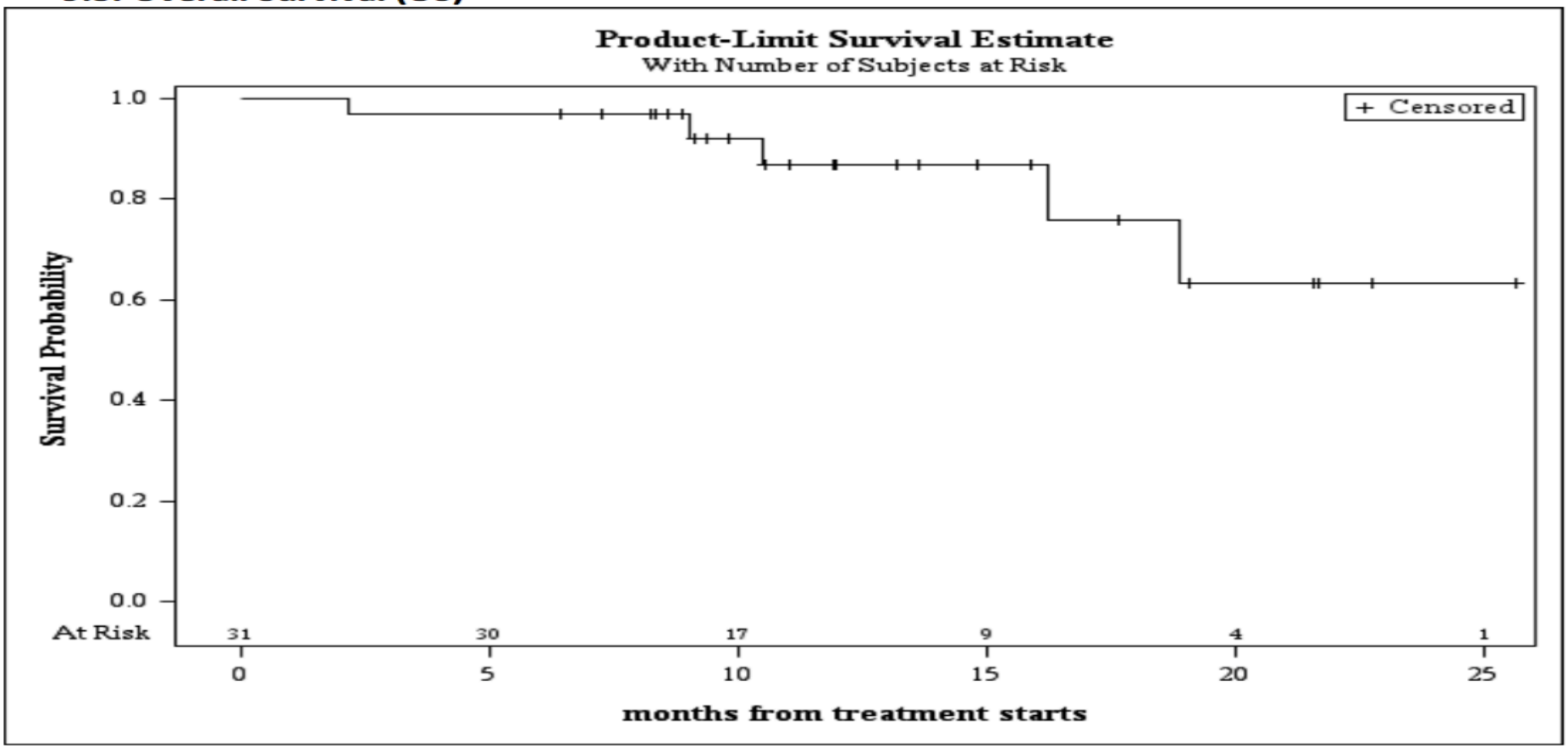
Steroid + Ponatinib monotherapy in Ph-Positive ALL. Overall Results at w24 (6 mths)

Parameter	N (%)
CHR*	34/38** (90)
CCyR	34/38** (90)
Deep CMR*** continuously (undetectable)	11/24 (45)
Deep CMR*** at least one time (undetectable)	23/28 (82)
Early death	0 (0)

- ** (primary endpoint): 4 pt are in HCR, CCyR and CMR at less than 6 months FU;
- * one pts died in CHR; 2 pts in CR of which: 1 pt to undergo HSCT; 1 pt for investigator choice; 1 for Molecular Relapse

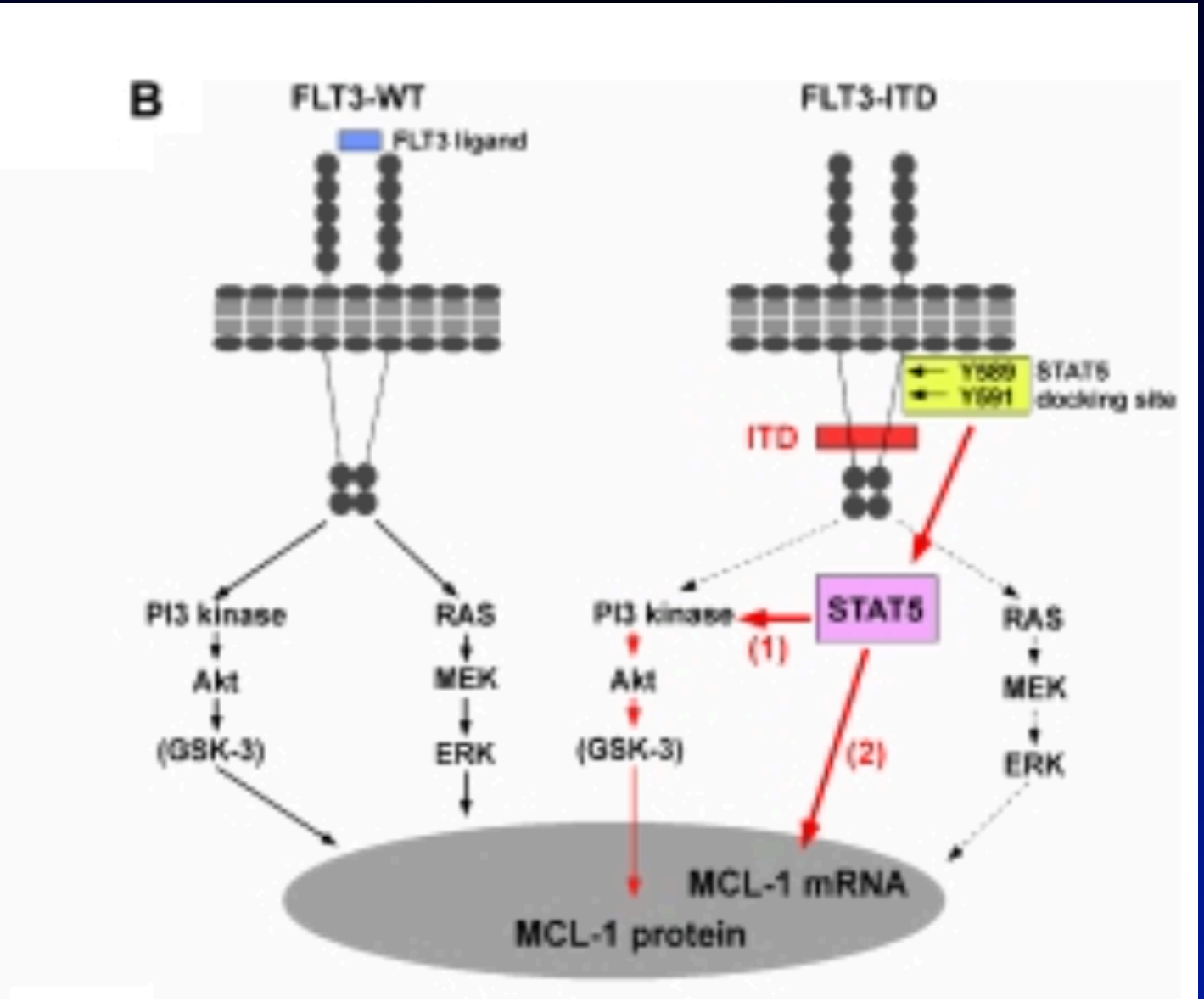
Gimema 1811
Survival at 30 / March 2017

9.3. Overall Survival (OS)

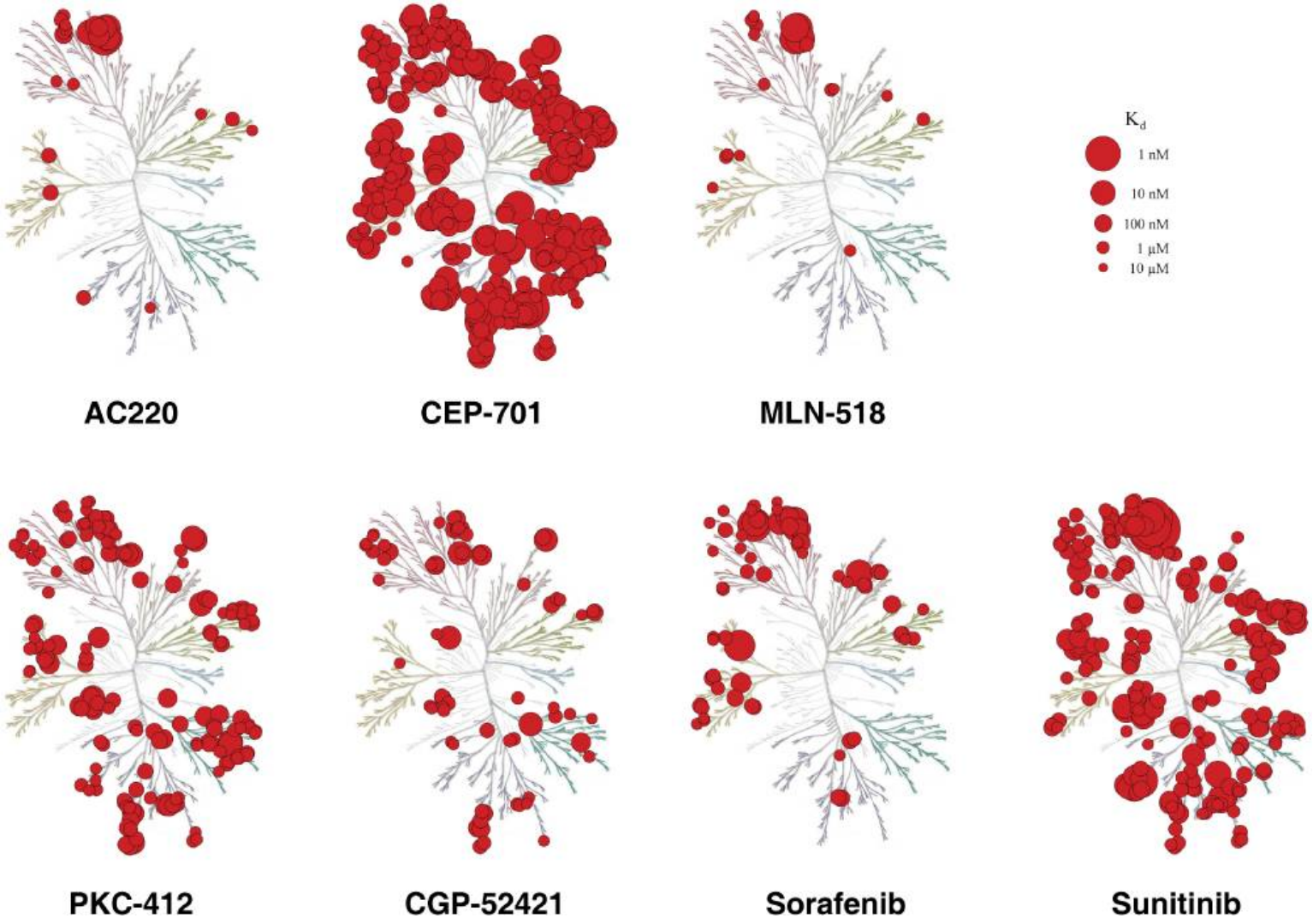


months	Survival Distribution Function Estimate	SDF Lower 95.00% Confidence Limit	SDF Upper 95.00% Confidence Limit
6	96.8	90.8	100
12	86.7	73.5	100

MCL1 as THE real, final, target?



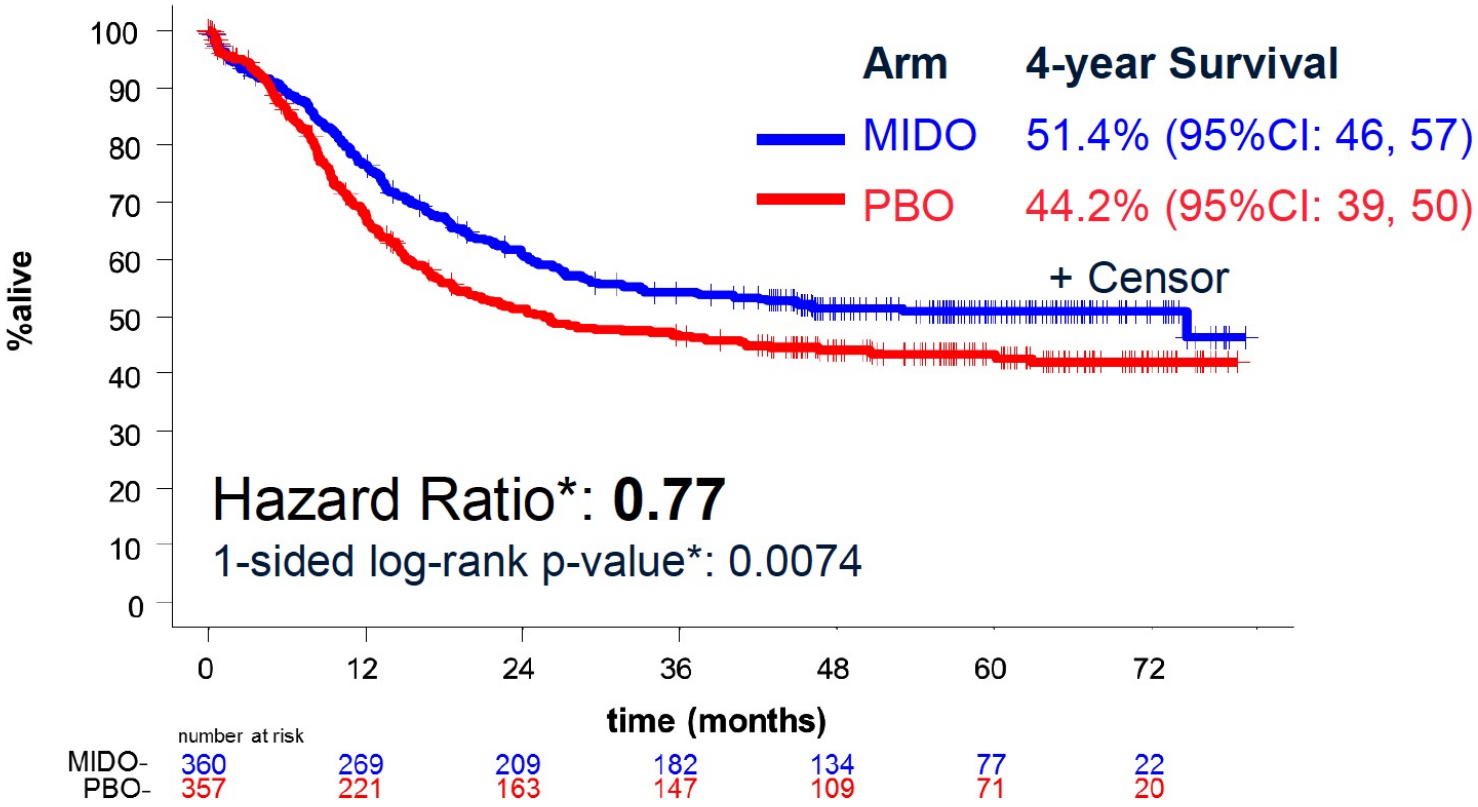
Small Molecule Kinase Interaction Maps for FLT3 Inhibitors



Zarrinkar *Blood* 2009

New Drugs in AML– RATIFY Trial

OS: 23% reduction in risk of death in midostaurin arm

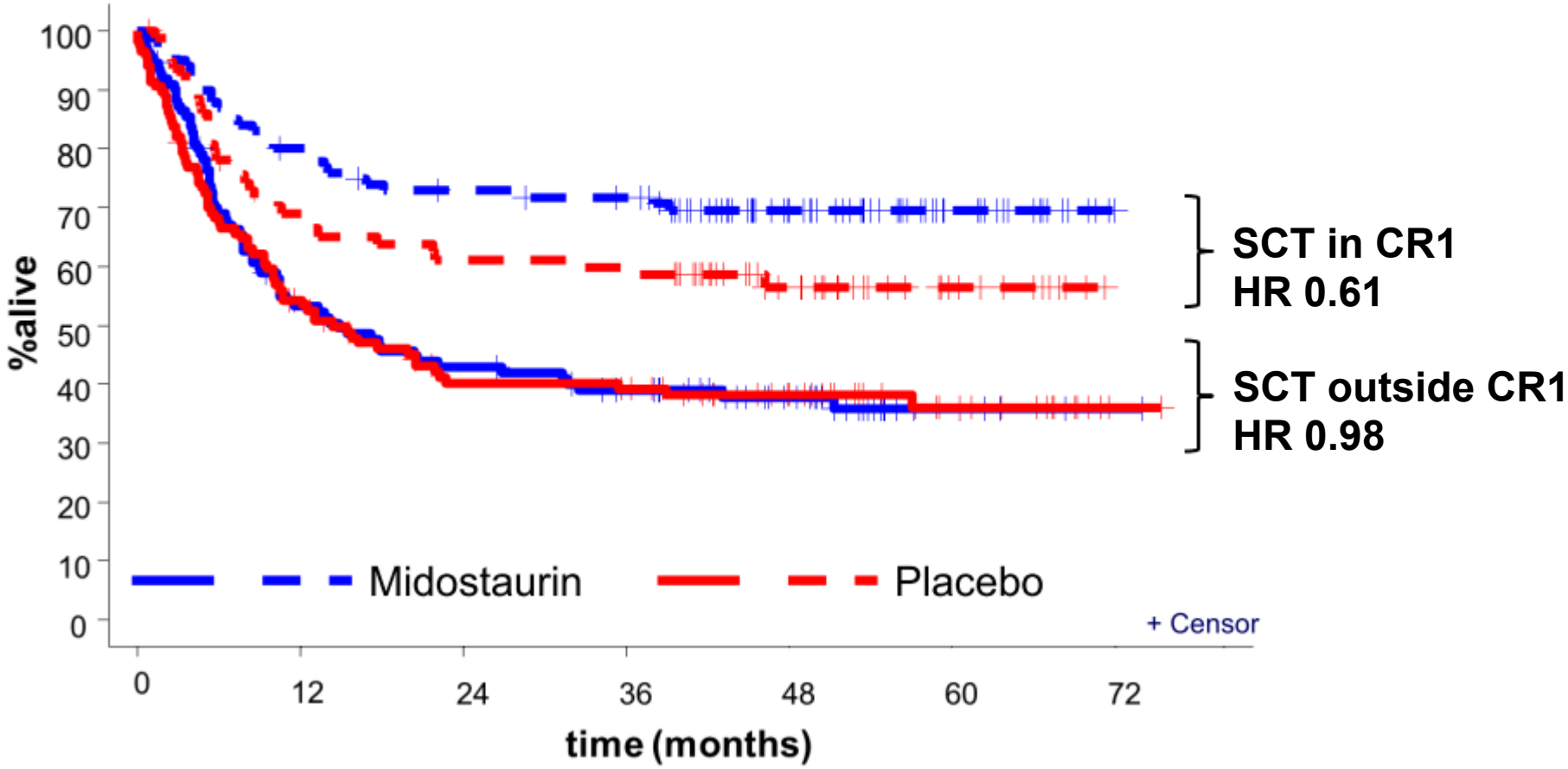


Median OS: midostaurin 74.7 months (31.7–NE); placebo 25.6 months (18.6–42.9)

Stone et al. ASH 2015. Abstract 6.

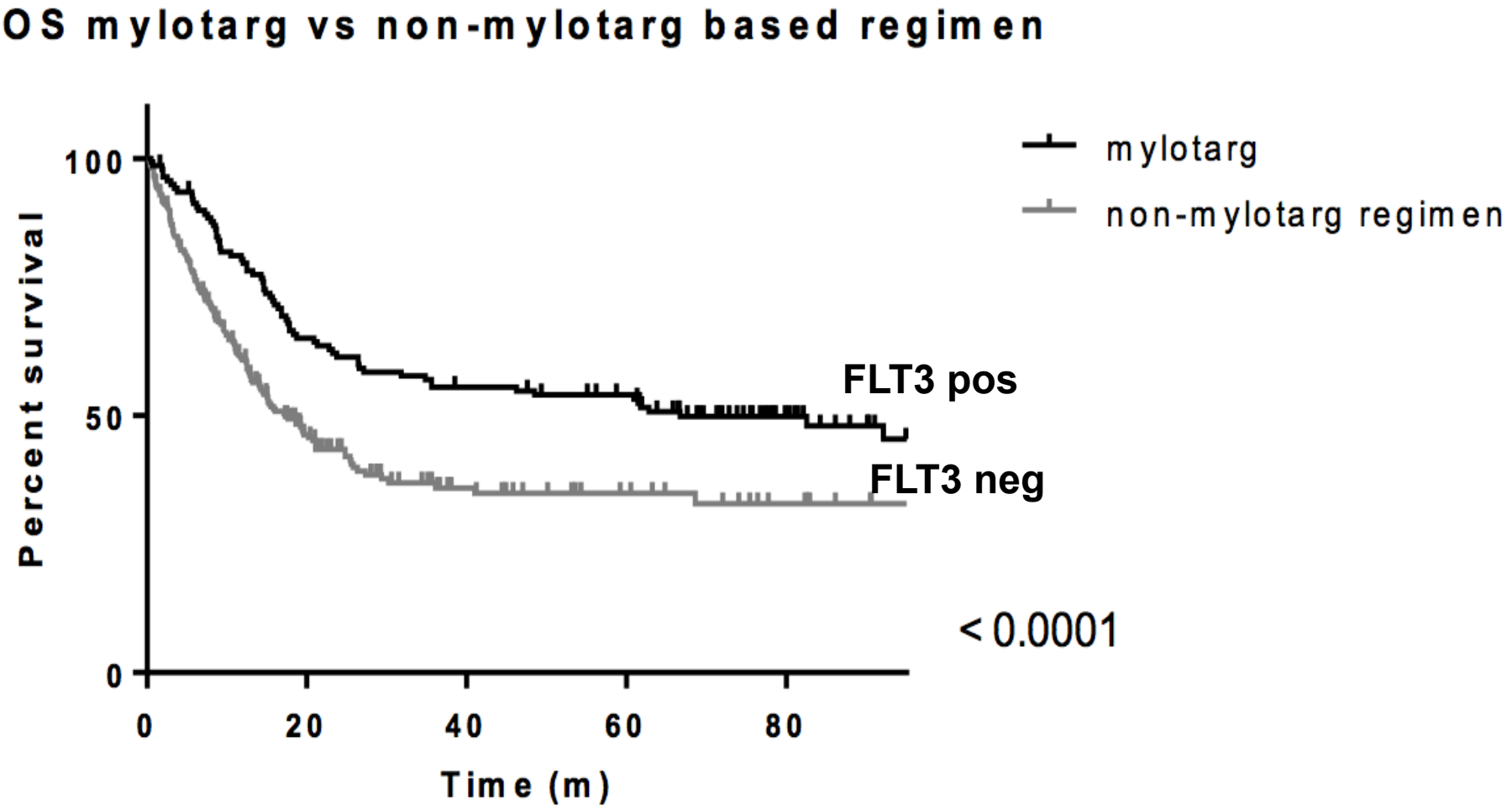
New Drugs in AML– RATIFY Trial

OS Post-transplant

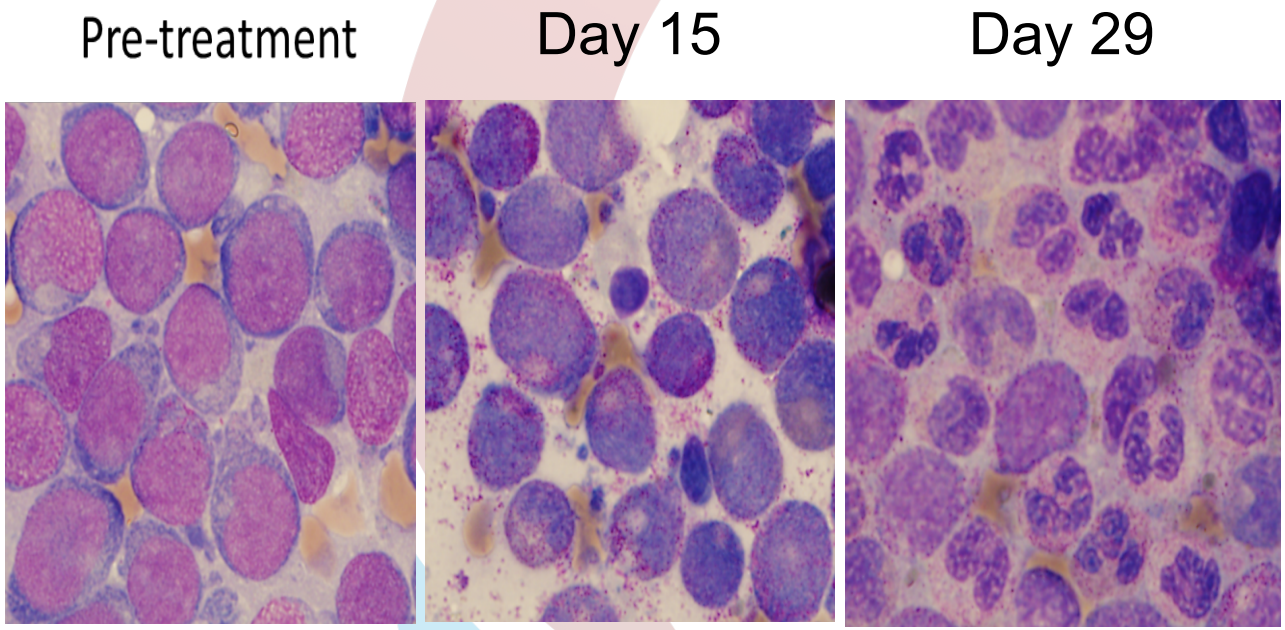


Stone et al. ASH 2015. Abstract 6.

My –FLAI
(Italian Experience)



Quizartinib Induces Blasts in the Bone Marrow to Undergo Terminal Myeloid Differentiation



No significant change in overall cellularity between Day 1 and Day 29

Sexauer *Blood* 2012

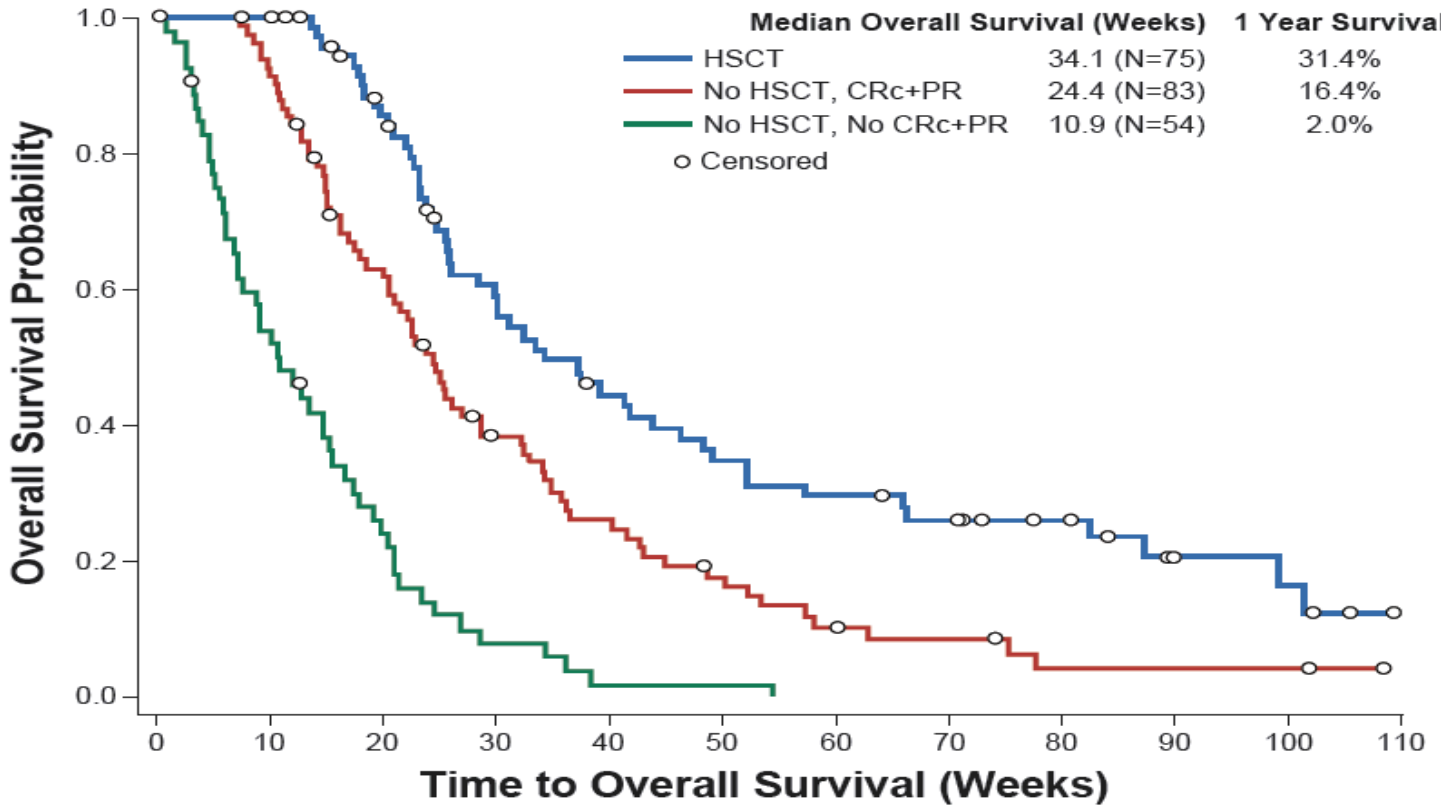
QuANTUM-FIRST STUDY

Daiichi-Sankyo

Overall Survival of Quizartinib (Phase 2)

Figure 1. AC220-002 and 2689-CL-2004 (N=212)

Survival of FLT3-ITD(+) Patients by Response to Quizartinib and Subsequent HSCT or No HSCT

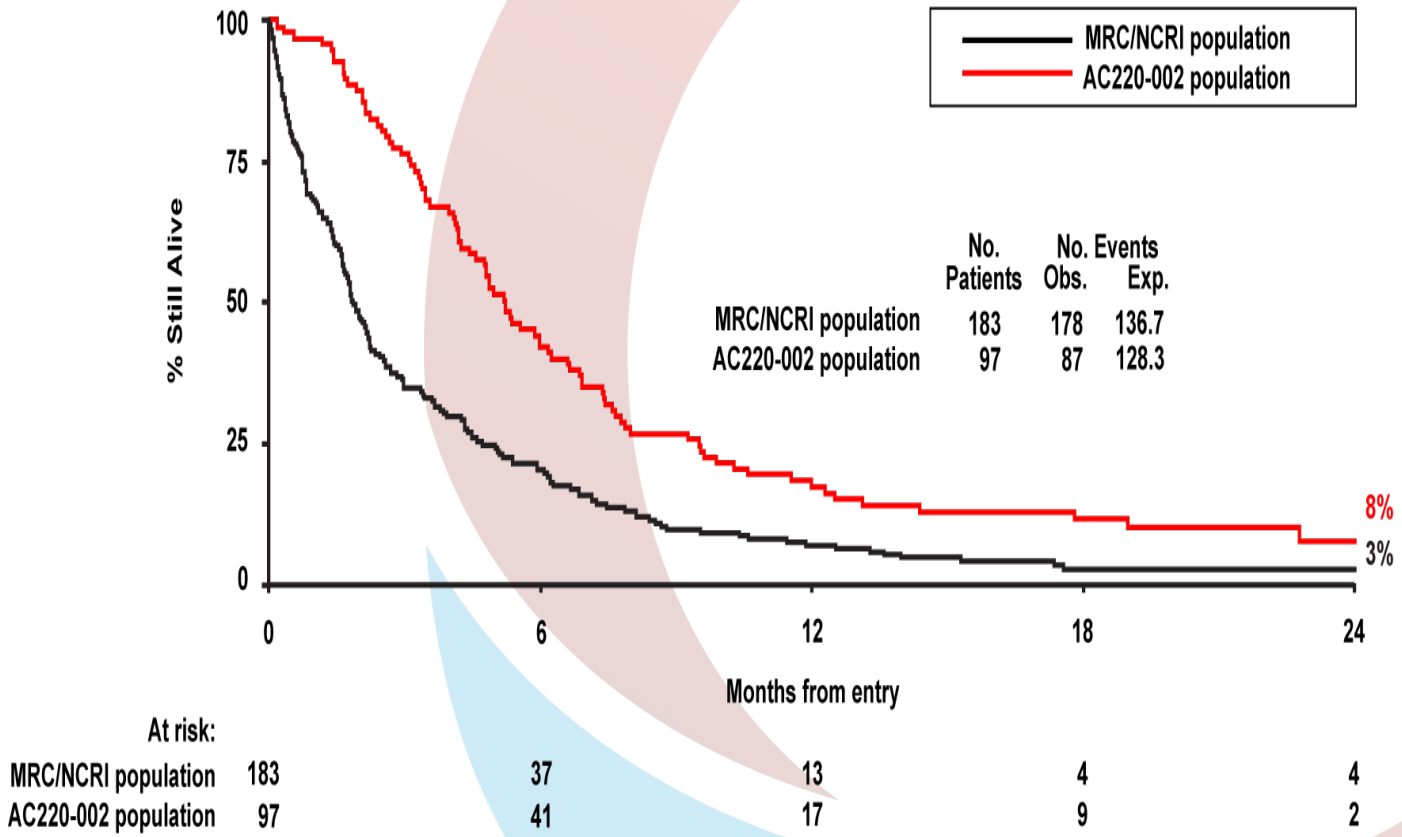


QuANTUM-FIRST STUDY

Levis ASCO 2014

Daiichi-Sankyo

Overall Survival of AC220-002 Cohort 2 and a Matched Group from UK NCRI Studies

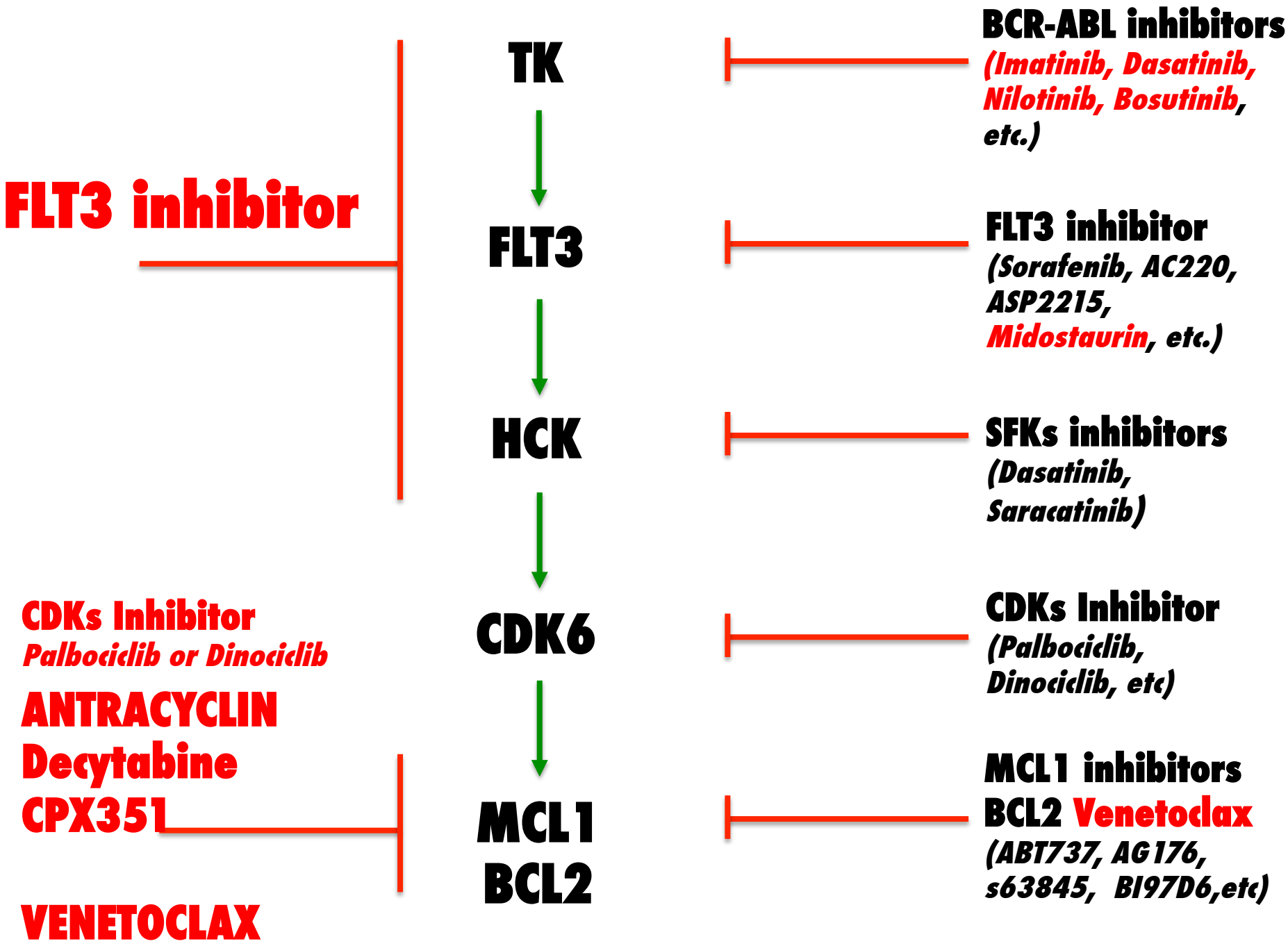


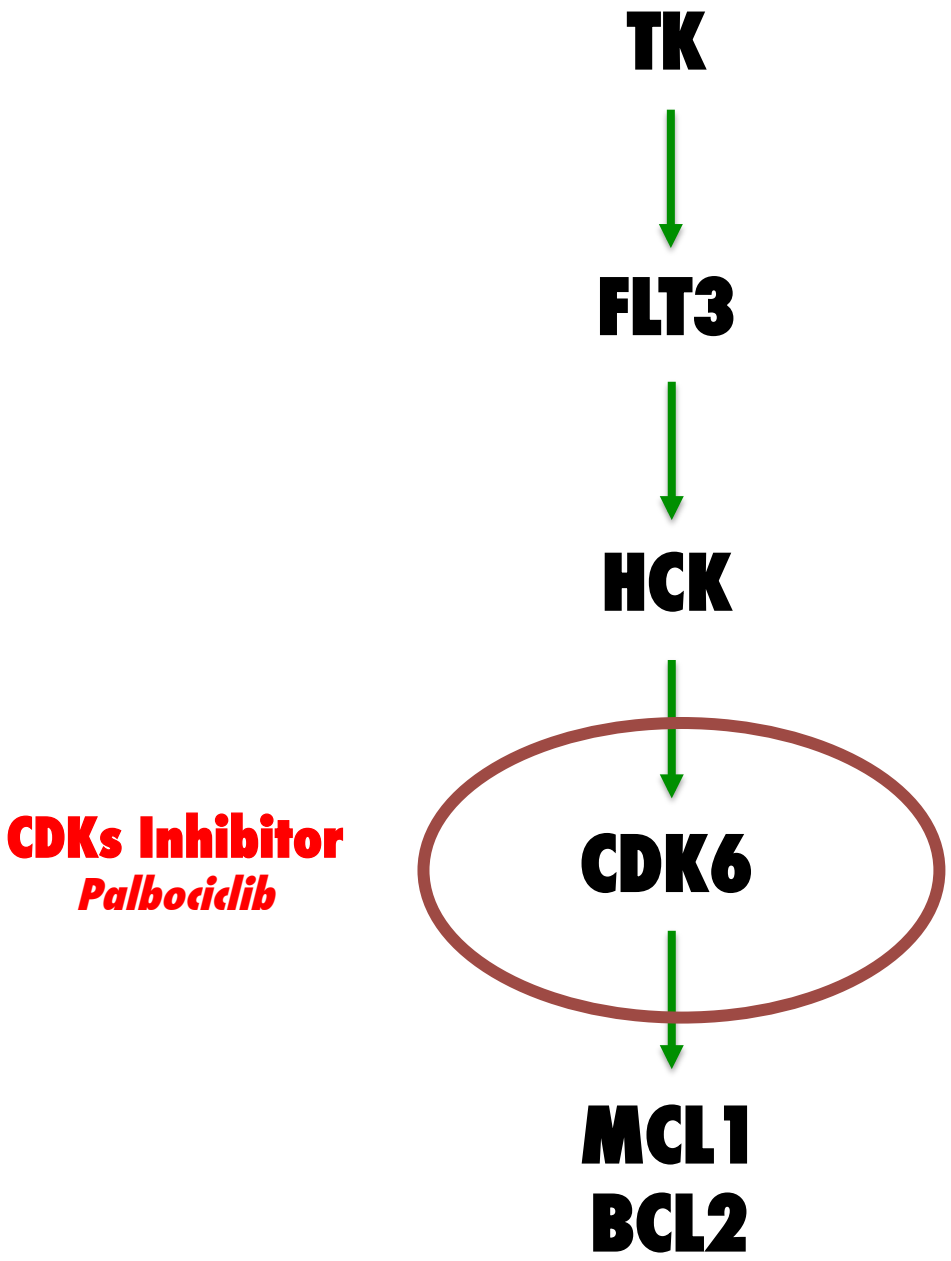
Cox regression model HR = 0.53 (0.41, 0.68) p = < 0.00001

Hills, ASH 2015

QuANTUM-FIRST STUDY

Daiichi-Sankyo


FLT3 inhibitor
CDKs Inhibitor
Palbociclib or Dinociclib
ANTRACYCLIN
Decytabine
CPX351
VENETOCLAX
TK
FLT3
HCK
CDK6
MCL1
BCL2
BCR-ABL inhibitors
(Imatinib, Dasatinib, Nilotinib, Bosutinib, etc.)
FLT3 inhibitor
(Sorafenib, AC220, ASP2215, Midostaurin, etc.)
SFKs inhibitors
(Dasatinib, Saracatinib)
CDKs Inhibitor
(Palbociclib, Dinociclib, etc)
MCL1 inhibitors
BCL2 Venetoclax
(ABT737, AG176, s63845, BI97D6,etc)


TK
FLT3
HCK
CDK6
MCL1
BCL2
CDKs Inhibitor
Palbociclib

CDK6 is a target in LSC

blood
Leading the world in reporting basic and applied hematology research
JOURNAL OF THE AMERICAN SOCIETY OF HEMATOLOGY
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Blood. 2015 Jan 1; 125(1): 90–101.
Prepublished online 2014 Oct 23. doi: 10.1182/blood-2014-06-584417
PMCID: PMC4281832

CDK6 as a key regulator of hematopoietic and leukemic stem cell activation

Ruth Scheicher,[1] Andrea Hoelbl-Kovacic,[1] Florian Bellutti,[1] Anca-Sarmiza Tigan,[1] Michaela Prchal-Murphy,[1] Gerwin Heller,[2] Christine Schneckenleithner,[1] María Salazar-Roa,[3] Sabine Zöchbauer-Müller,[2] Johannes Zuber,[4] Marcos Malumbres,[3] Karoline Kollmann,[1] and Veronika Sexl[✉1]

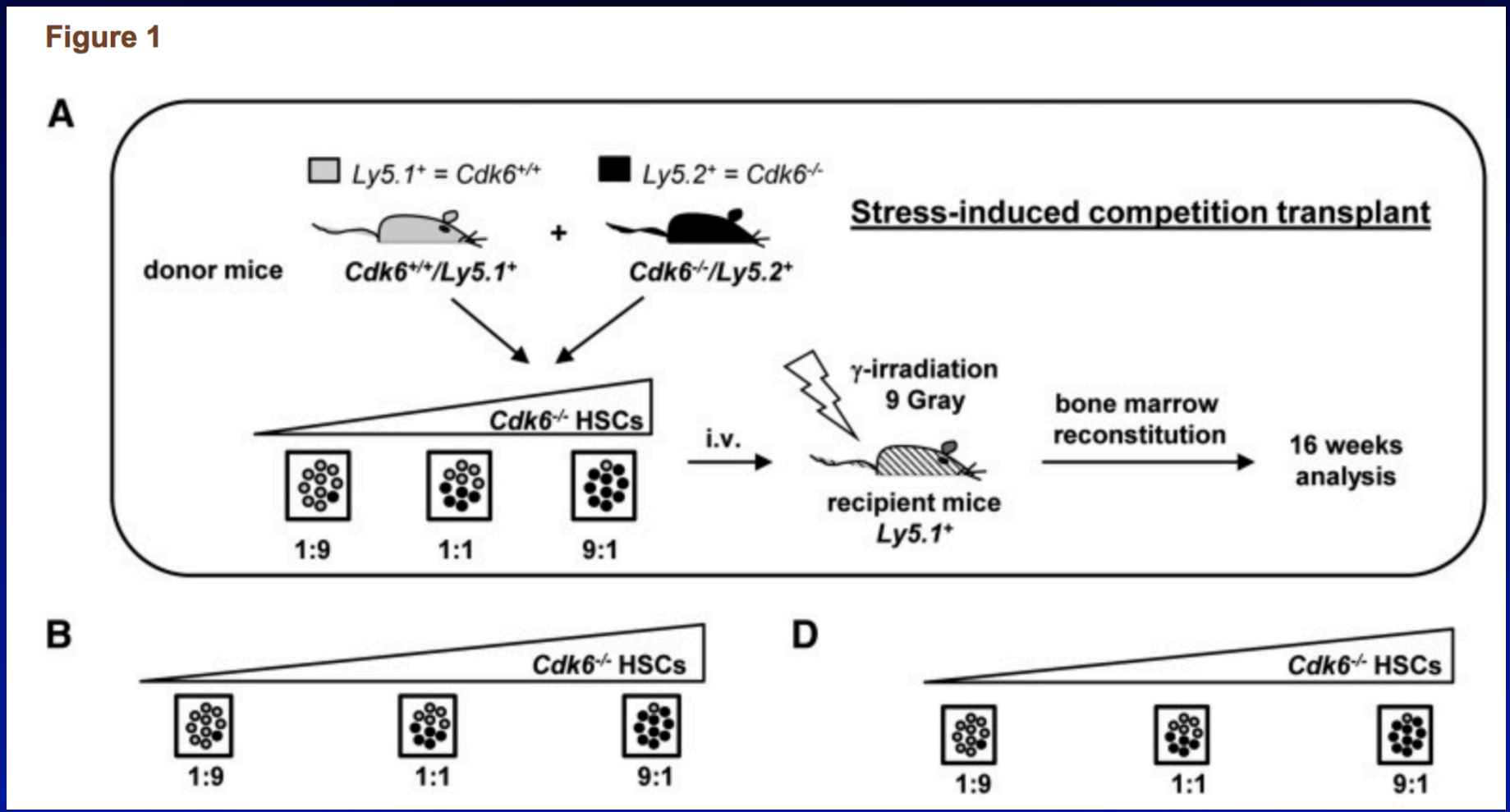
[1]Institute of Pharmacology and Toxicology, University of Veterinary Medicine, Vienna, Austria;
[2]Clinical Division of Oncology, Department of Medicine I, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria;
[3]Cell Division and Cancer Group, Molecular Oncology Programme, Centro Nacional de Investigaciones Oncológicas, Madrid, Spain; and
[4]Research Institute of Molecular Pathology, Vienna, Austria
[✉]Corresponding author.

Received 2014 Jun 24; Accepted 2014 Oct 17.

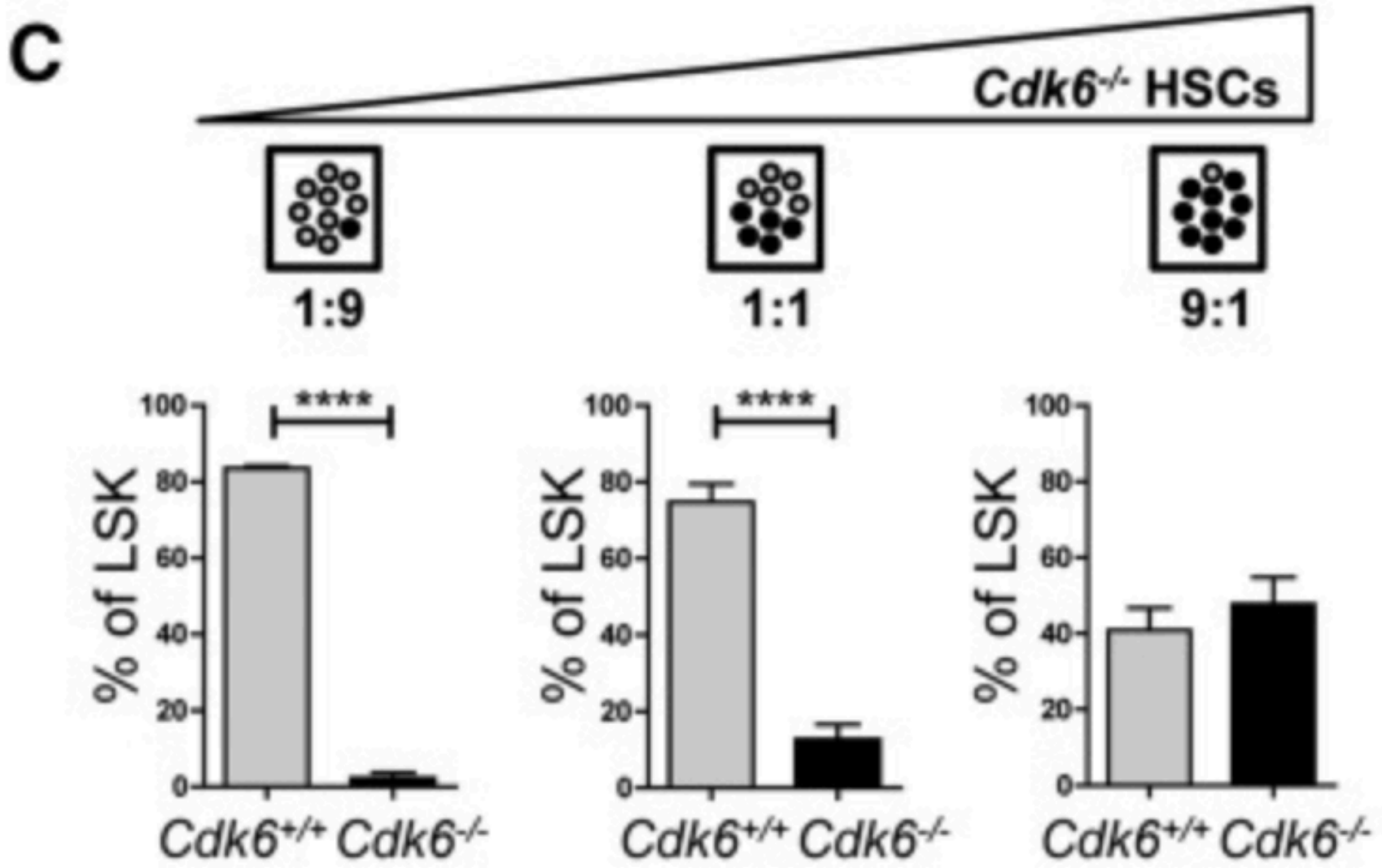
Key Points

- CDK6 acts as a transcriptional regulator to suppress *Egr1* in HSCs and LSCs, allowing their activation.
- *Cdk6*$^{-/-}$ HSCs fail to contribute to repopulation in competitive transplants, and BCR-ABL^{p210+} *Cdk6*$^{-/-}$ LSCs fail to inflict disease.

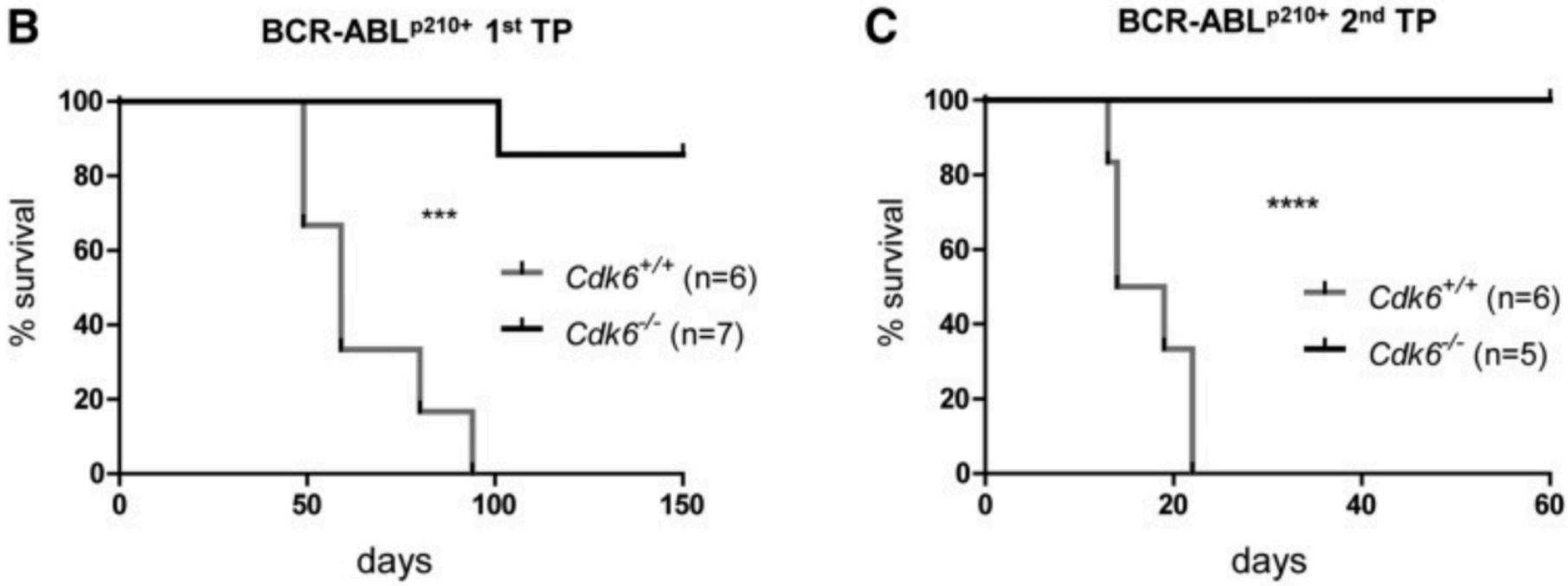
Without CDK6: no Ph+ LSC

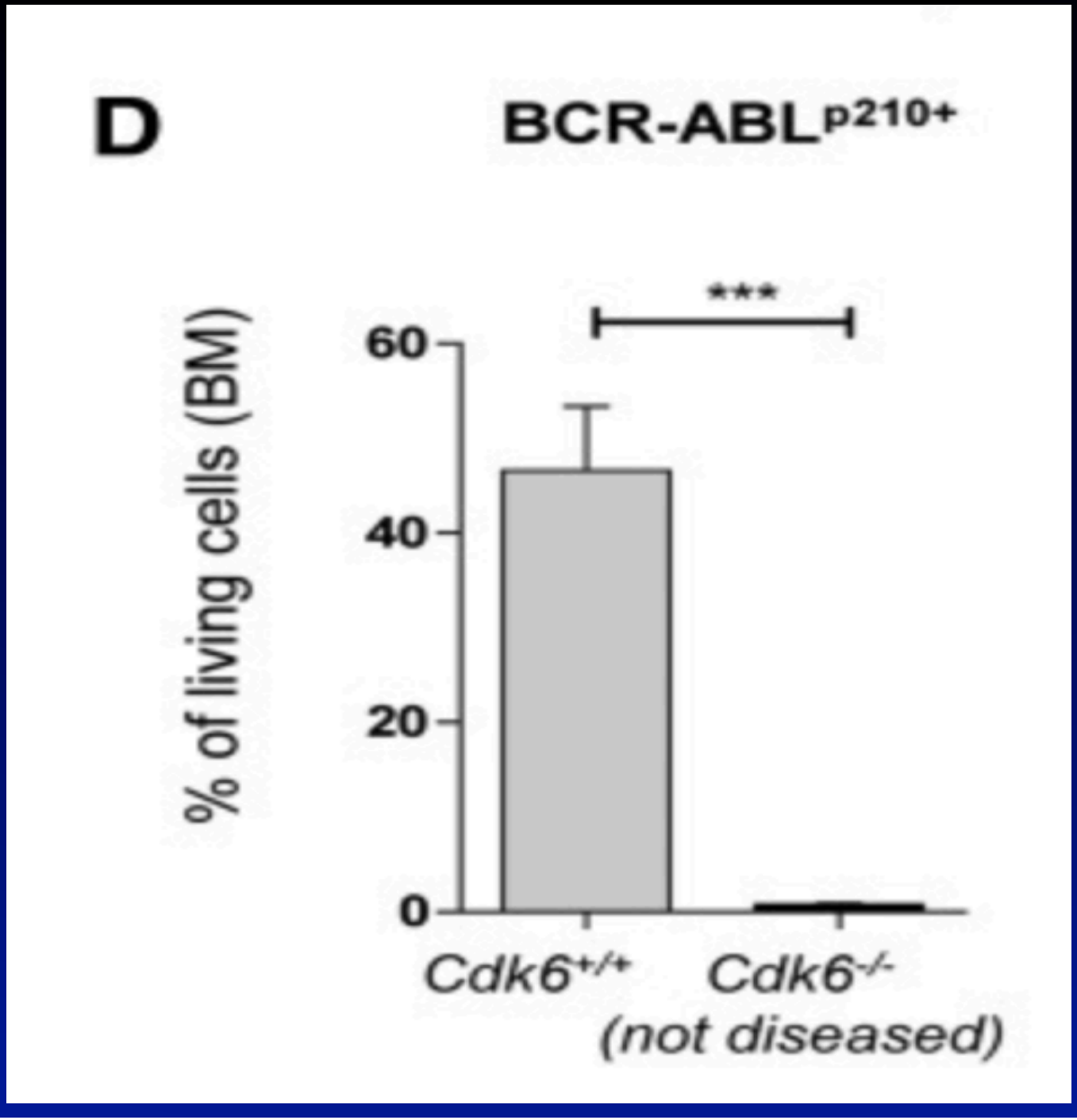


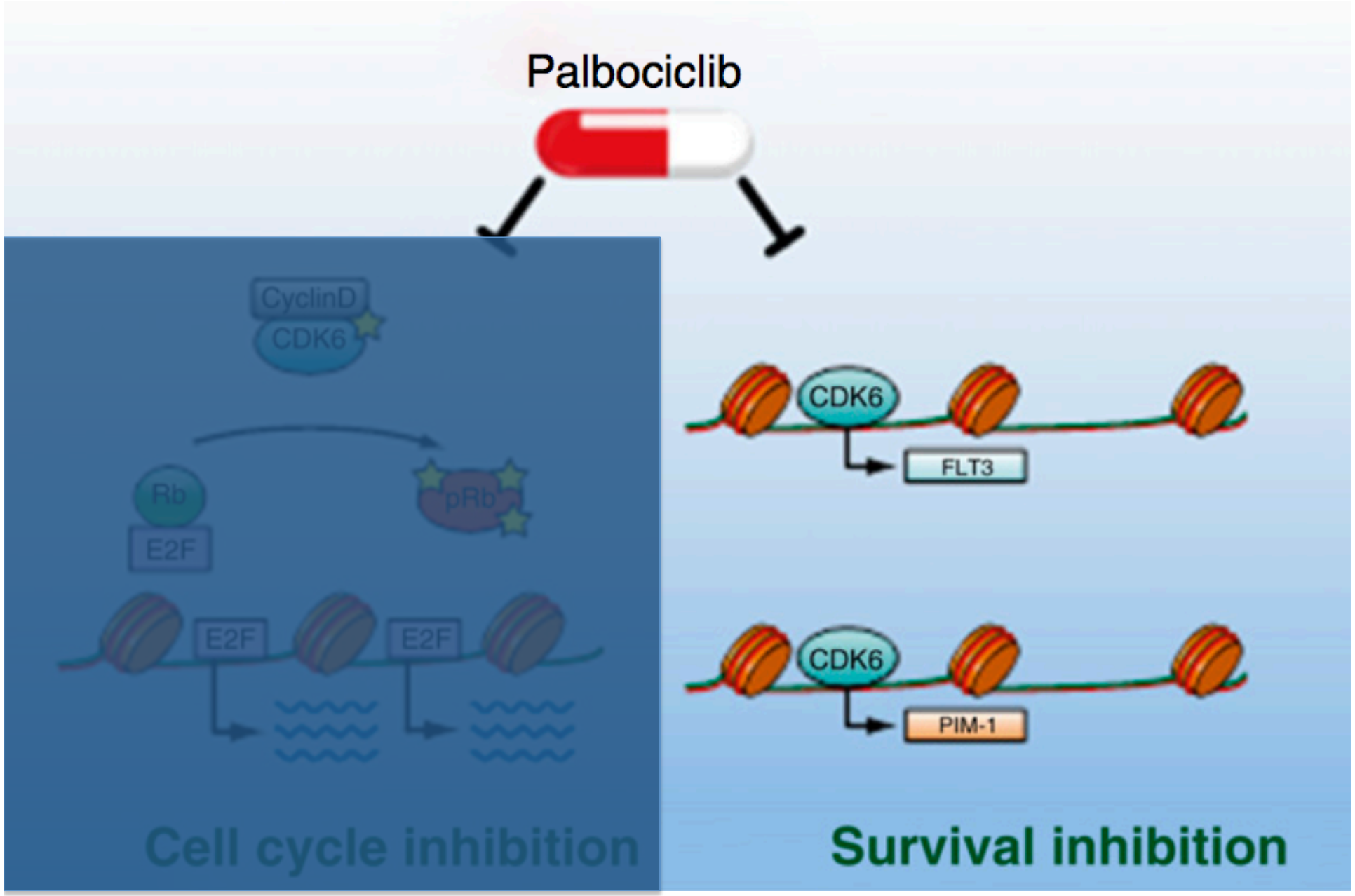
CDK6 is required for CML leukemia formation in vivo

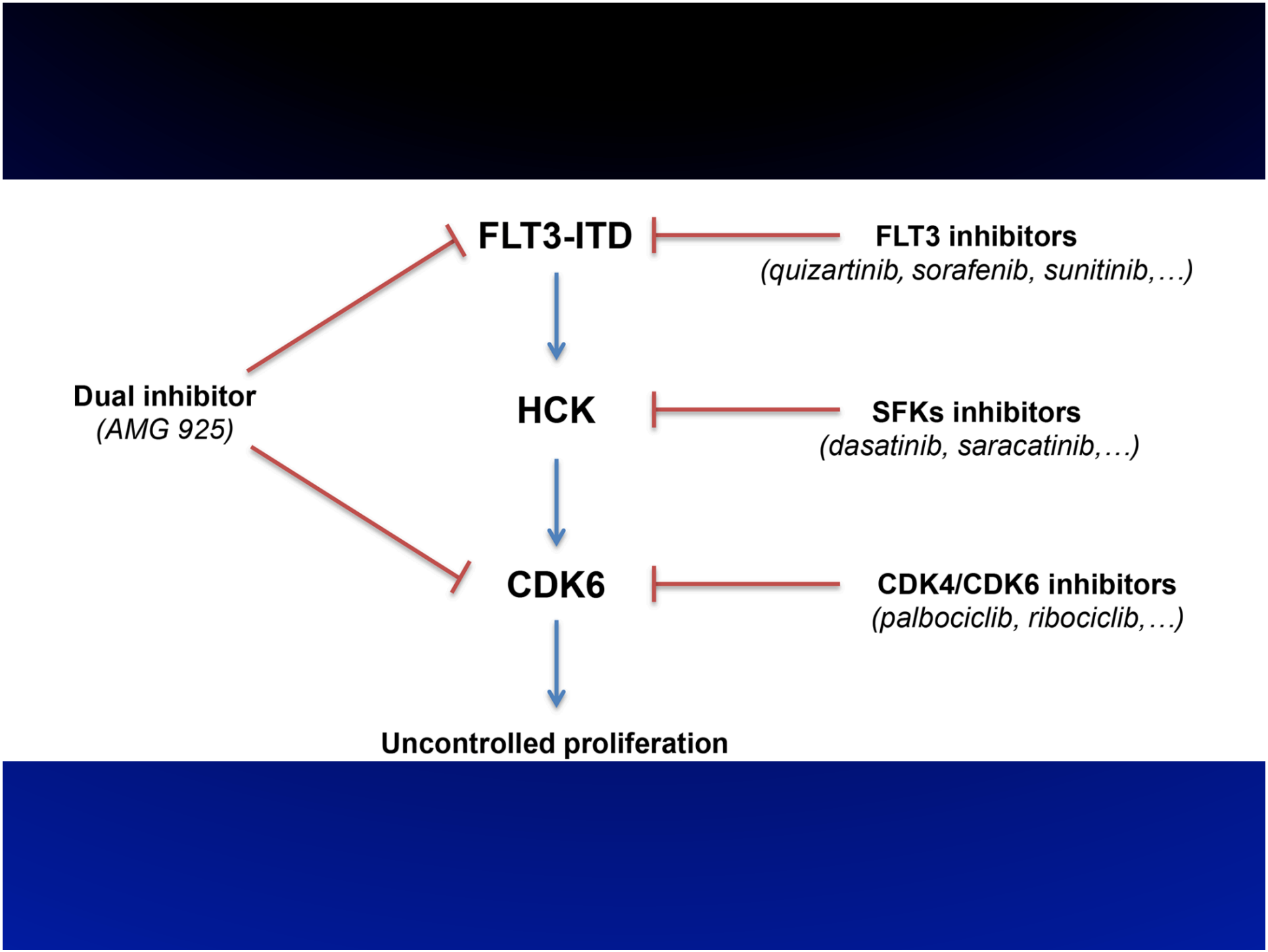


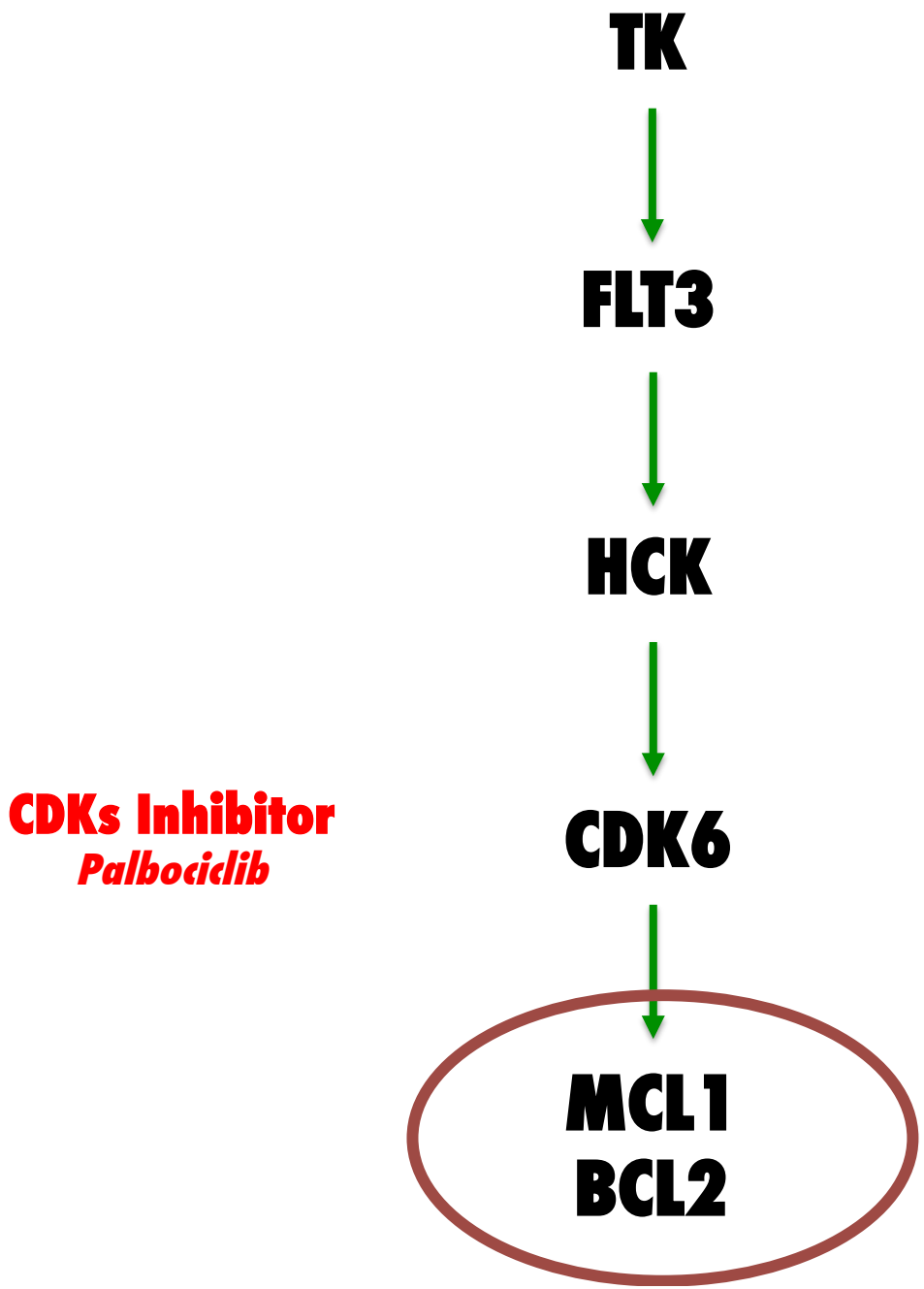
CDK6 is required for CML leukemia formation in vivo




D
BCR-ABL p210+
***
60
40
20
0
% of living cells (BM)
Cdk6+/+
Cdk6-/-
(not diseased)

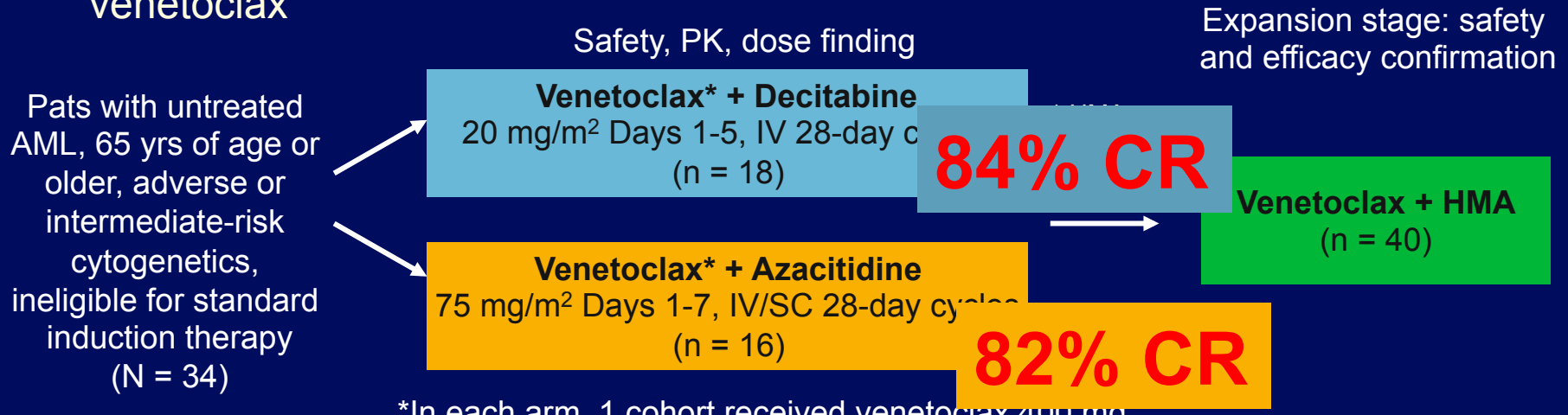

Palbociclib
CyclinD
CDK6
Rb
E2F
pRb
E2F
E2F
Cell cycle inhibition
CDK6
FLT3
CDK6
PIM-1
Survival inhibition


FLT3-ITD
FLT3 inhibitors
(quizartinib, sorafenib, sunitinib,…)
Dual inhibitor
(AMG 925)
HCK
SFKs inhibitors
(dasatinib, saracatinib,…)
CDK6
CDK4/CDK6 inhibitors
(palbociclib, ribociclib,…)
Uncontrolled proliferation


TK
FLT3
HCK
CDK6
MCL1
BCL2
CDKs Inhibitor
Palbociclib

Frontline Venetoclax + HMAs in Elderly AML Pts

- Open-label, nonrandomized, 2-arm, 2-stage, phase Ib study of BCL-2 inhibitor venetoclax



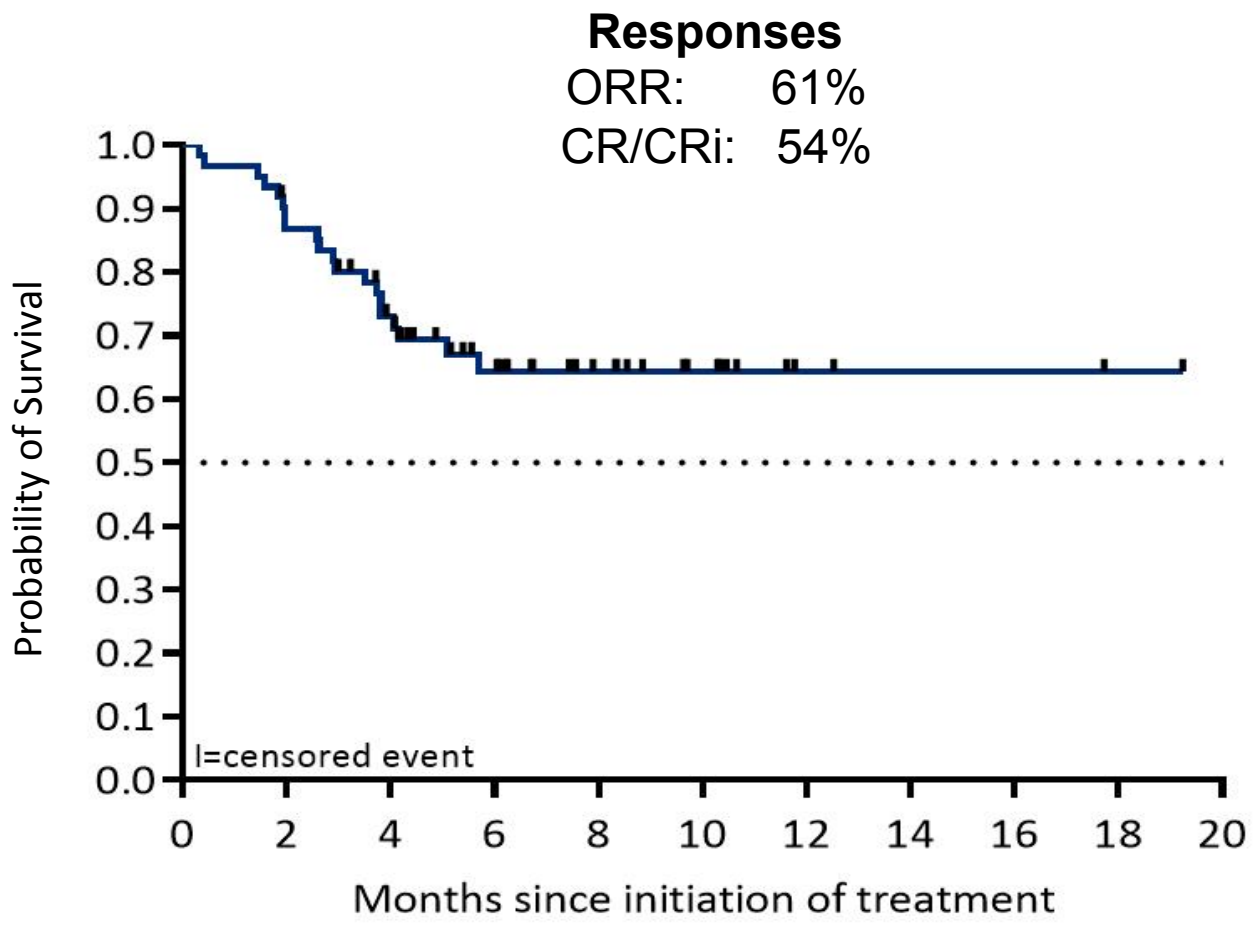
*In each arm, 1 cohort received venetoclax 400 mg PO and 2 cohorts received 800 mg PO d 2-28 (cycle 1) and d 1-28 (following cycles) of 28-day cycle.

- Endpoints
  - Safety: MTD, DLTs, RP2D, AEs, early deaths, PK
  - Efficacy: ORR per IWG AML criteria, response duration, TTP, PFS, OS, MRD (assessed after cycles 1 and 4, then every 12 weeks)
  - Exploratory: mutational profiling and BCL-2 characterization, molecular markers, ex vivo testing of pt samples

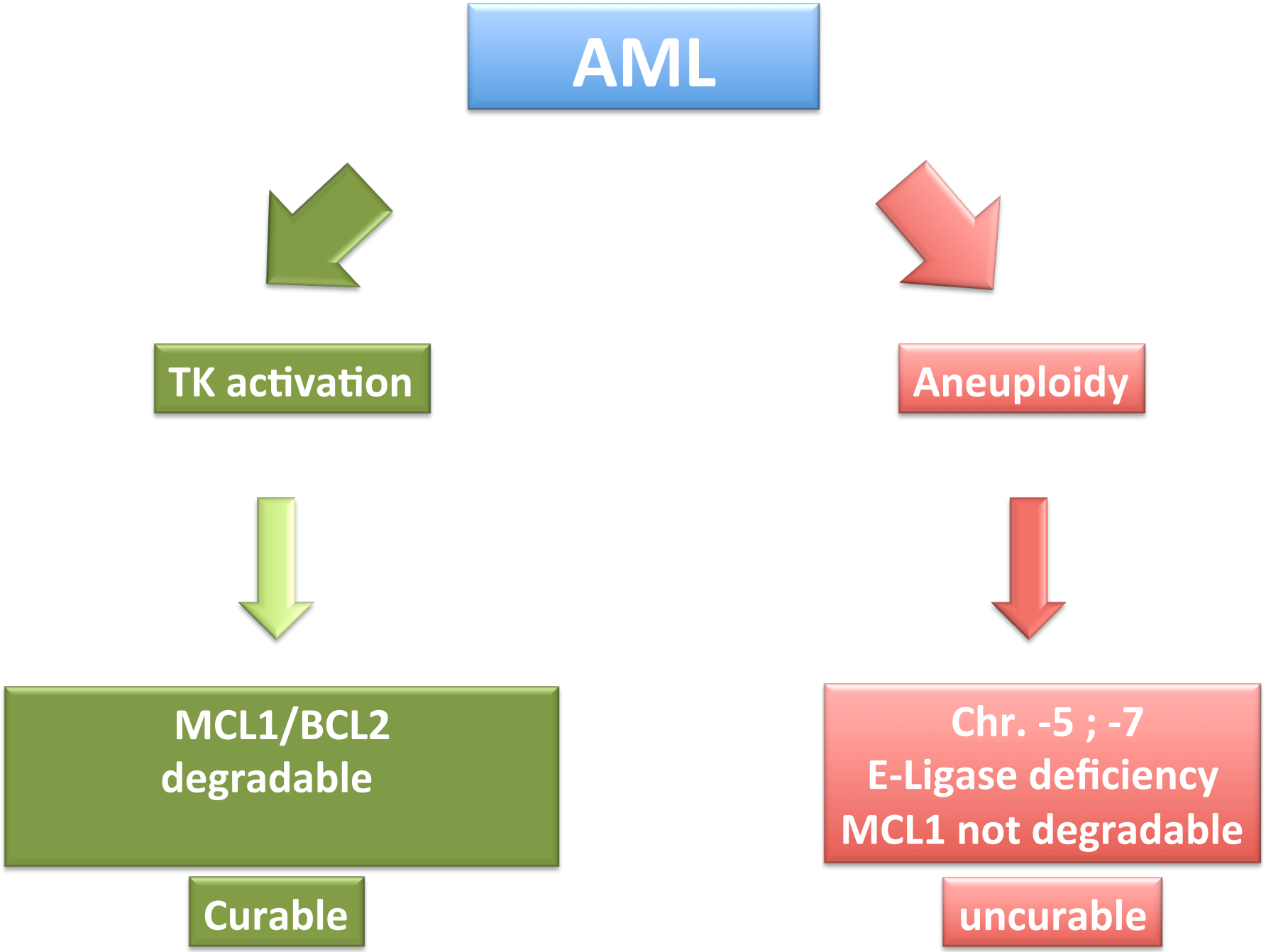
DiNardo C, et al. ASH 2015. Abstract 327.

ASH 2015

Outcome with Venetoclax and LDAC (n=61)



Wei et al., ASH 2016, abstract #102


AML
TK activation
Aneuploidy
MCL1/BCL2
degradable
Curable
Chr. -5 ; -7
E-Ligase deficiency
MCL1 not degradable
uncurable

Acknowledgments

Supported by: FP7, European LeukemiaNet, AIL, AIRC, FIRB 2006, Fondazione del Monte di Bologna e Ravenna

Different fuel for Leukemias?

Rationale:

- 6-19% of AML patients carry mutations of *IDH1* and *IDH2* genes.
- *IDH2* mutations are frequently found in cytogenetically normal (CN)-AML.
- *IDH2* mutations in AML are stable disease markers and evidence suggests they may confer favorable prognosis, especially when associated to NPM1 mutations.
- Targeted inhibition of mutant *IDH1/2* through small molecules represents a promising therapeutic strategy

2 ottobre 2017

Italian Network for IDH1/2 mutational analysis in Acute Myeloid Leukemia

≈30 Italian Hematological Institutions

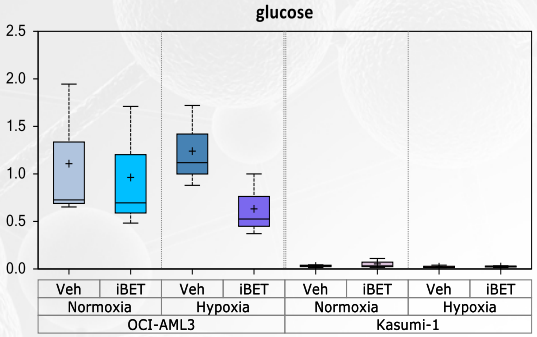
Coordinated by Gimema WP and

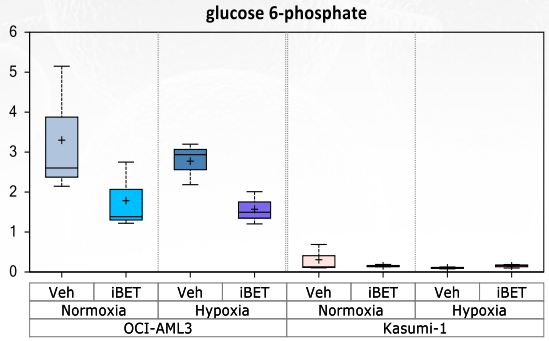
University of Bologna

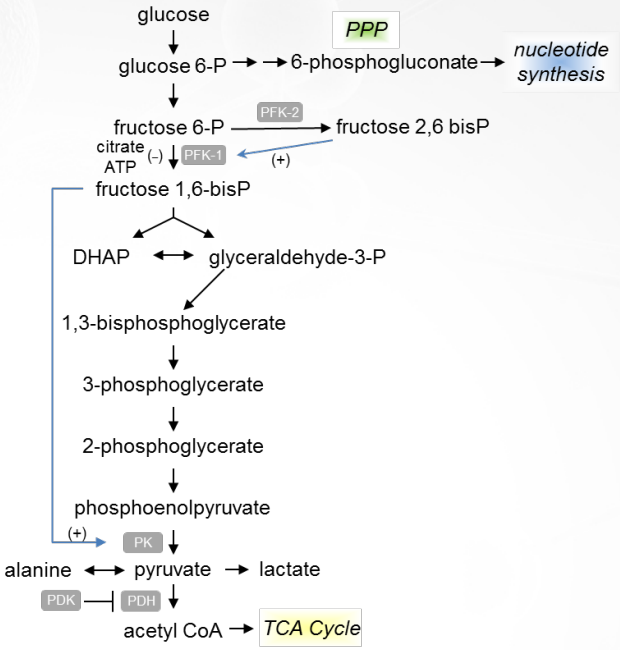
Acute myeloid leukemia cell lines and primary cells; metabolic alterations consequent to hypoxia and BET inhibitor treatment

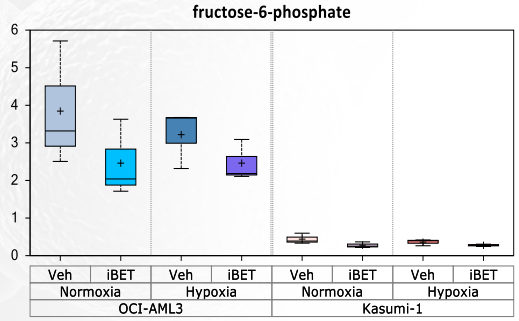
Università di Bologna
Giorgia Simonetti, PhD
UNBO-01-14VW
January 19, 2016
Author: Steven Stirdivant, PhD

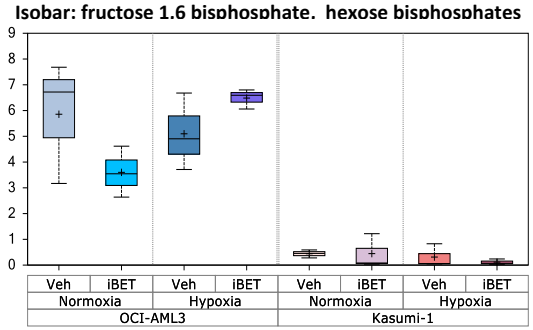
Glycolysis in OCI-AML3 cells was impacted by iBET treatment

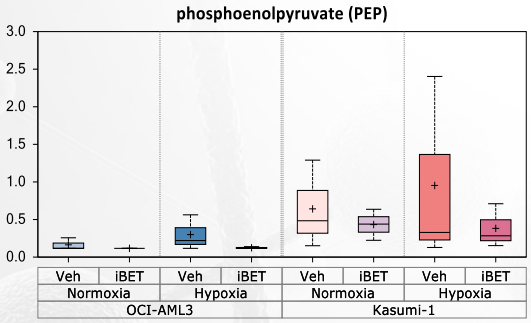


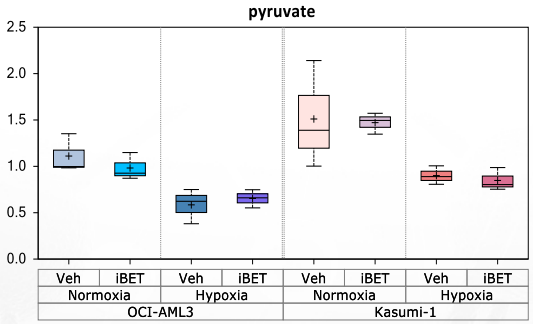


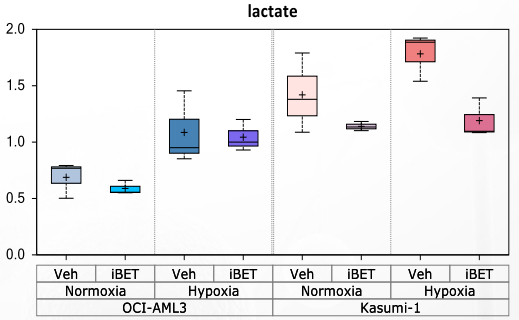




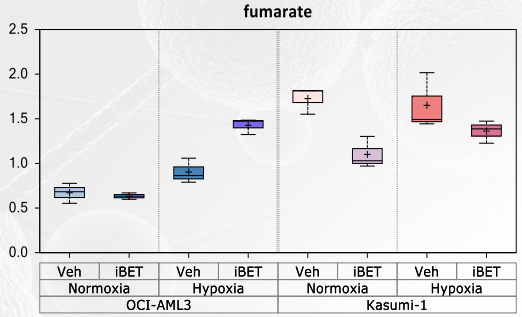


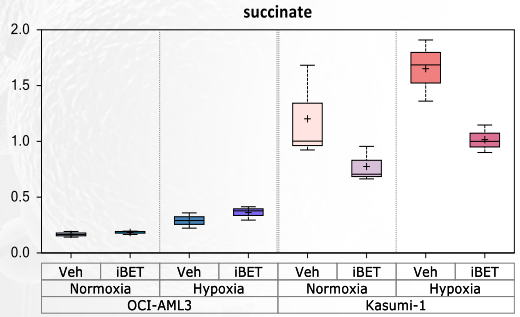


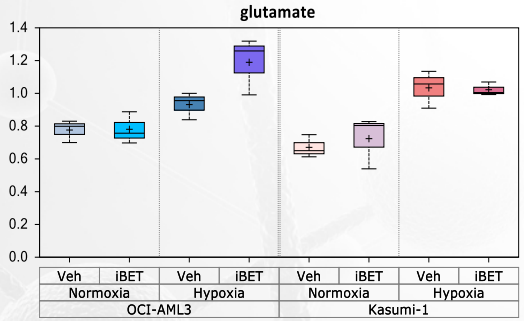


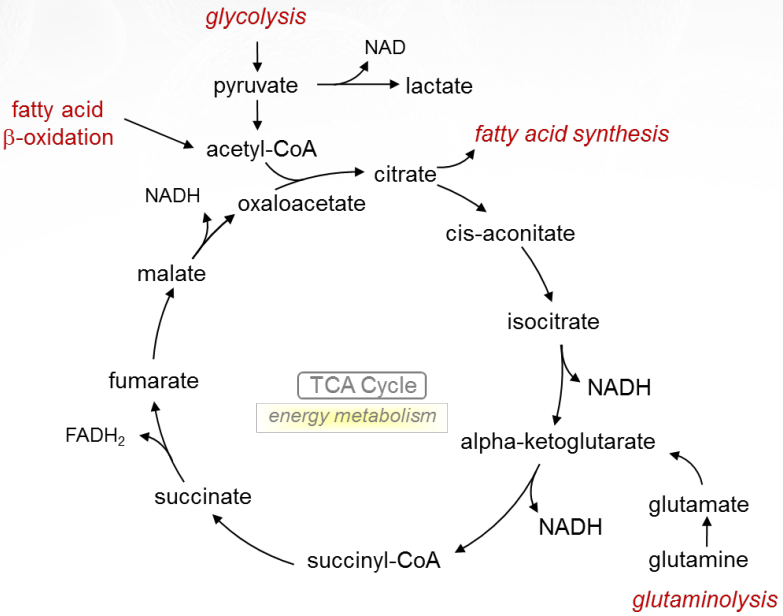


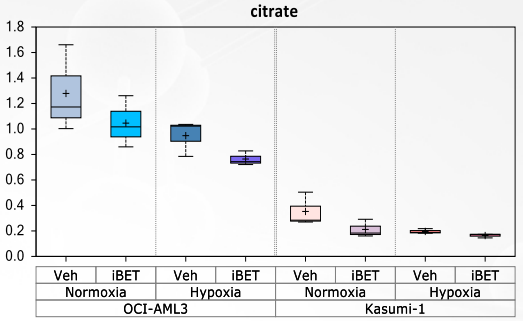
Cell line specific effects of iBET on TCA cycle metabolites

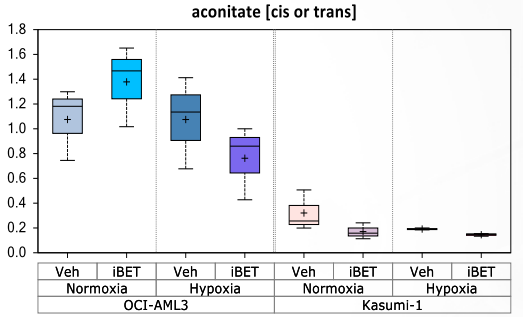


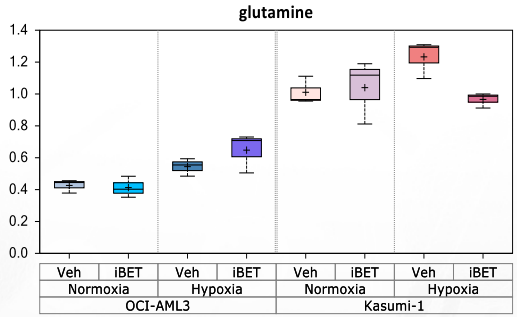


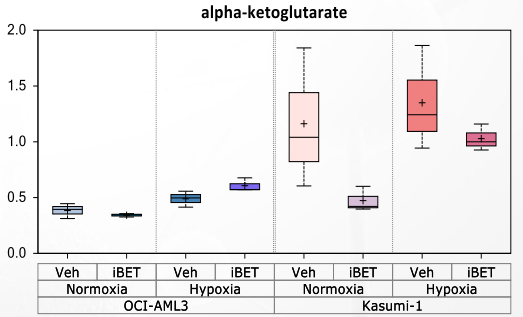




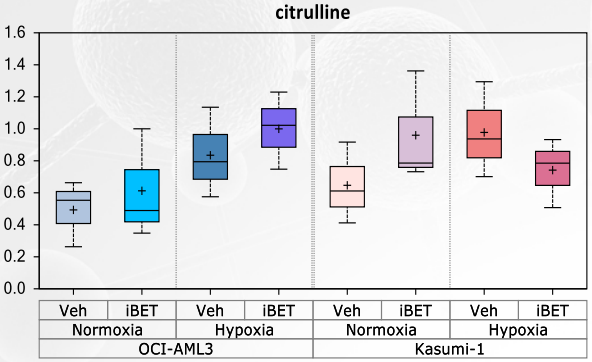


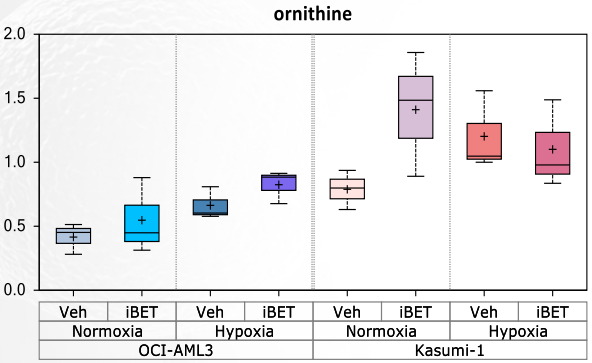


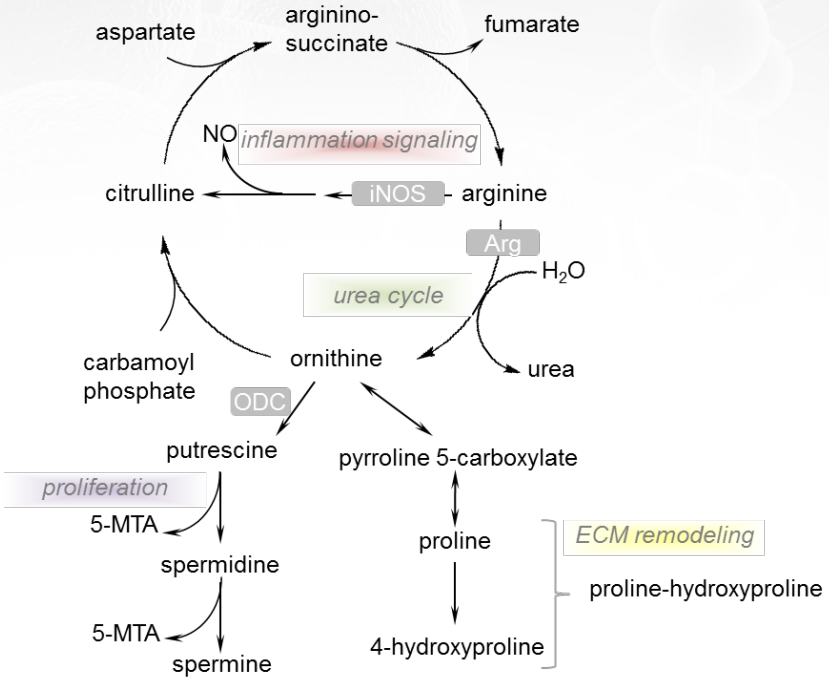


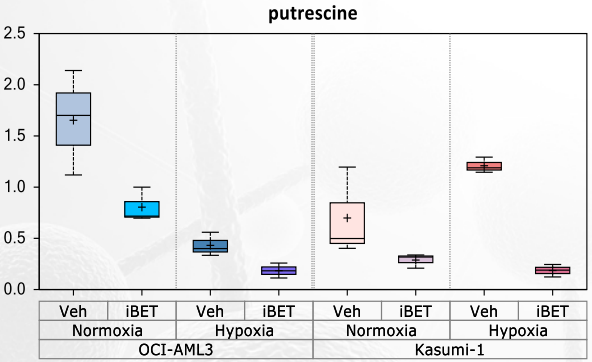


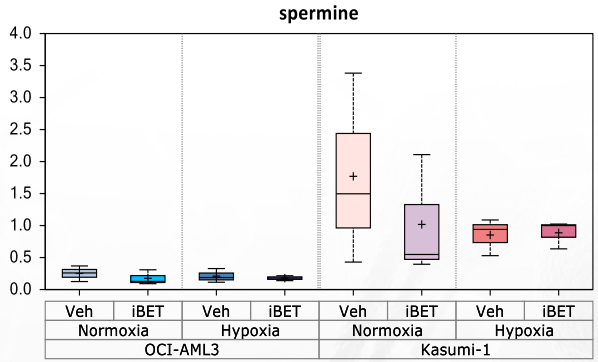
Putrescine levels were reduced by iBET treatment



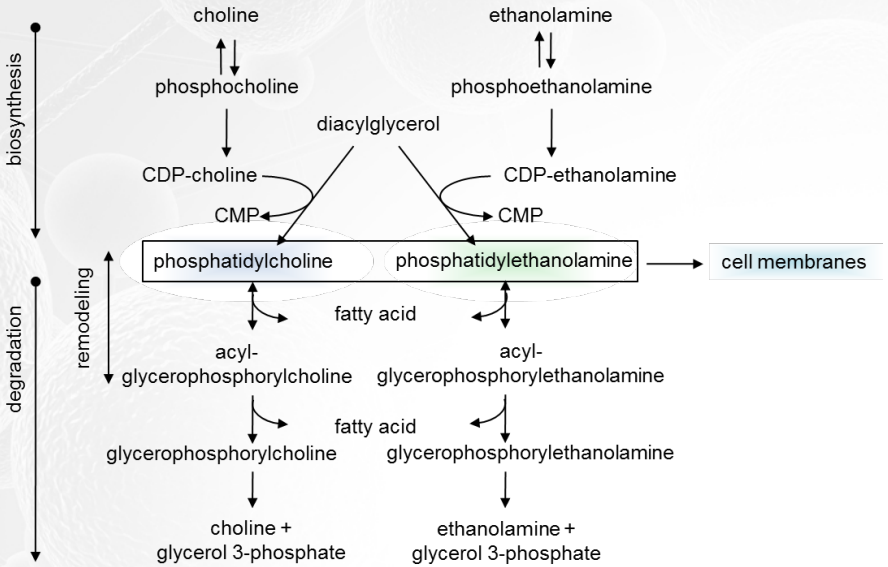




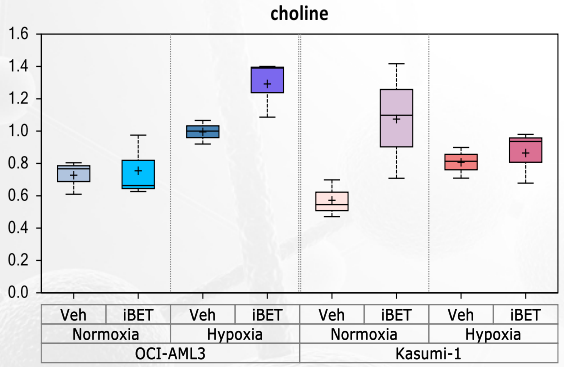


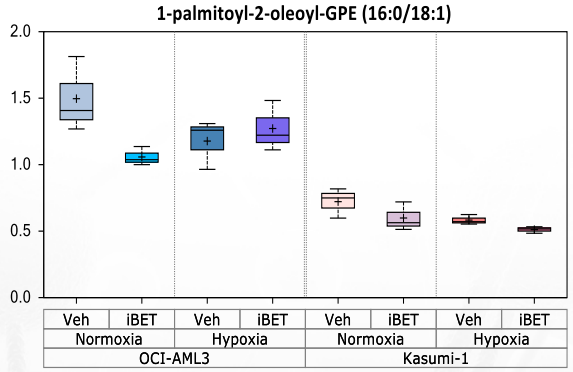


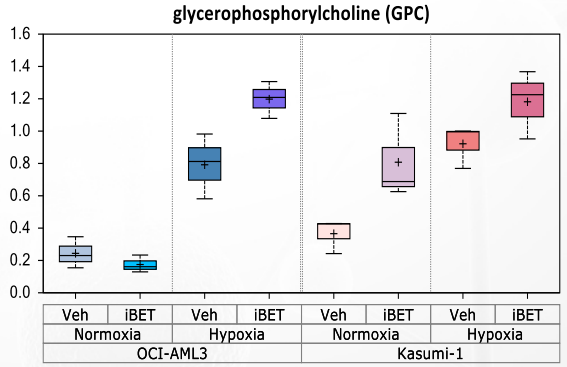
Phospholipid levels reduced in iBET treated normoxic OCI-AML3 cells



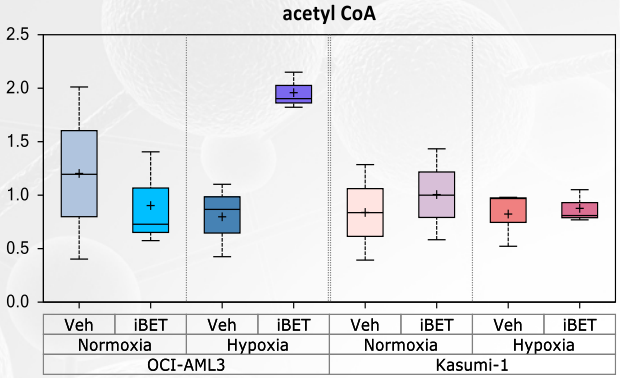
Sub Pathway	Biochemical Name	Two-Way ANOVA: Culture and Treatment Main	Two-Way ANOVA: Cell Line Main Effect	Two-Way ANOVA: Main Effects Interaction	Fold of Change, ANOVA Contrasts: iBET / Vehicle: OCI Norm	OCI Hyp	Kas Norm	Kas Hyp
Phospholipid Metabolism	choline	■		■	1.04	1.30	1.88	1.07
	choline phosphate		■		0.81	1.25	1.10	0.95
	cytidine 5'-diphosphocholine	■	■		0.89	1.54	1.32	0.94
	glycerophosphorylcholine (GPC)	■	■	■	0.72	1.51	2.21	1.28
	phosphoethanolamine	■	■		0.97	1.45	2.24	2.52
	cytidine-5'-diphosphoethanolamine		■		0.91	1.02	1.63	0.96
	glycerophosphoethanolamine	■	■	■	0.83	1.44	1.82	1.34
	1-stearoyl-2-oleoyl-GPC (18:0/18:1)	■	■	■	1.05	1.24	1.02	1.02
	1-stearoyl-2-oleoyl-GPI (18:0/18:1)*	■	■		0.62	1.13	0.58	0.85
	1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	■	■	■	0.76	0.96	0.88	0.86
	1-palmitoyl-2-oleoyl-GPG (16:0/18:1)	■			0.51	0.88	0.81	0.95
	1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	■	■		0.71	1.08	0.83	0.88
	1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)				0.83	1.06	1.18	0.92
	1-stearoyl-2-oleoyl-GPE (18:0/18:1)		■	■	0.76	1.07	0.94	0.87
	1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*				0.75	1.03	1.17	0.89
	1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)		■		0.80	1.00	0.96	0.89
	1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	■	■		0.64	1.14	0.71	0.93
	1,2-dioleoyl-GPG (18:1/18:1)		■		0.96	1.18	1.18	1.17
	1,2-dioleoyl-GPI (18:1/18:1)	■	■		0.70	1.11	0.74	0.88
	1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	■	■		1.09	0.97	1.05	0.83
	1,2-dioleoyl-GPE (18:1/18:1)		■		0.79	1.07	0.93	0.96
	1-palmitoyl-2-oleoyl-GPI (16:0/18:1)*	■	■		0.61	1.09	0.75	1.02
	1-palmitoyl-2-oleoyl-GPS (16:0/18:1)	■	■		0.68	1.08	0.87	0.90

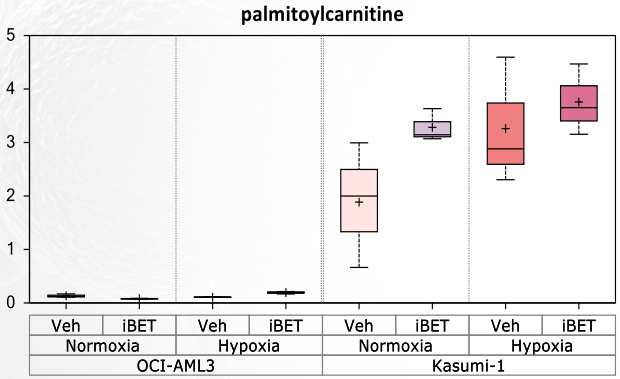


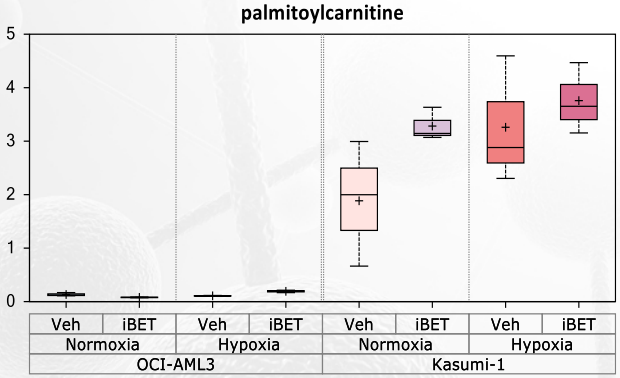




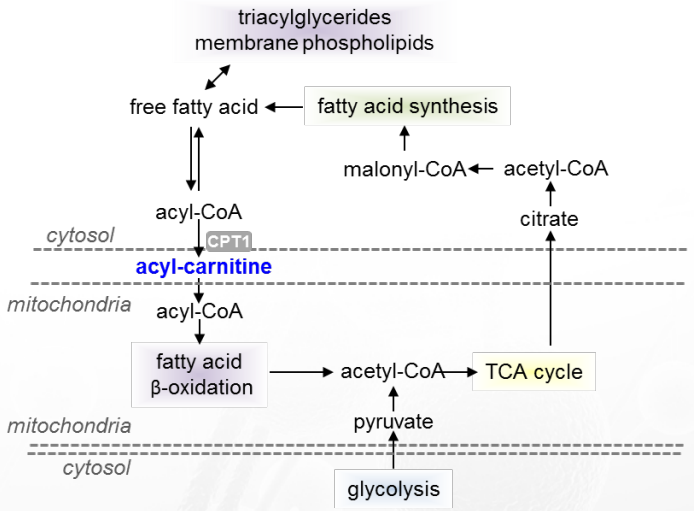
iBET raises acylcarnitine levels in normoxic Kasumi-1 cells



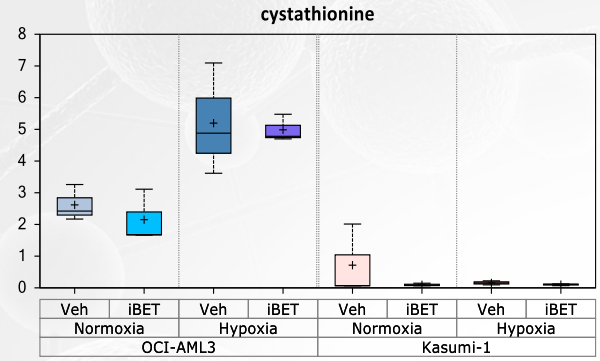


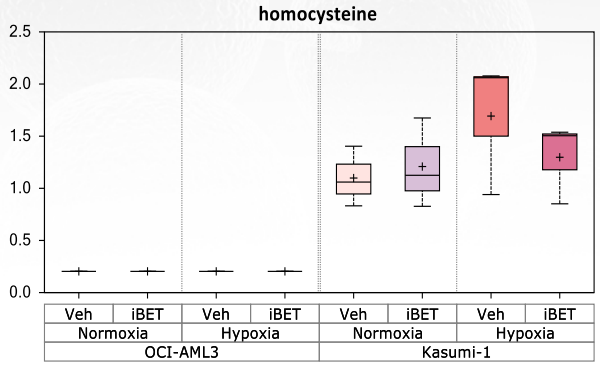


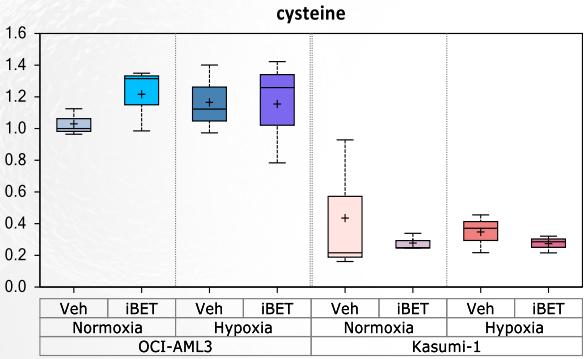
Sub Pathway	Biochemical Name	Two-Way ANOVA: Culture and Treatment Main	Two-Way ANOVA: Cell Line Main Effect	Two-Way ANOVA: Main Effects Interaction	Fold of Change, ANOVA Contrasts: iBET / Vehicle: OCI Norm	OCI Hyp	Kas Norm	Kas Hyp
Fatty Acid Metabolism	acetyl CoA				0.75	2.45	1.20	1.06
Fatty Acid Metabolism(Acyl Carnitine)	acetylcarnitine				1.72	3.54	1.69	1.13
	3-hydroxybutyrylcarnitine (1)				1.10	1.17	1.73	1.04
	3-hydroxybutyrylcarnitine (2)				0.77	1.06	1.41	0.98
	valerylcarnitine				1.00	1.00	1.12	0.88
	hexanoylcarnitine				0.54	1.55	1.26	0.92
	octanoylcarnitine				1.00	1.00	1.61	1.33
	laurylcarnitine				0.80	0.96	1.56	1.40
	myristoylcarnitine				0.64	1.47	1.57	1.13
	palmitoylcarnitine				0.59	1.78	1.74	1.15
	palmitoleoylcarnitine*				0.82	1.51	1.71	1.09
	stearoylcarnitine				0.72	1.77	1.66	1.38
	linoleoylcarnitine*				0.86	1.28	1.83	1.14
	oleoylcarnitine				0.88	1.29	1.76	1.16
	myristoleoylcarnitine*				0.80	1.93	1.84	1.04

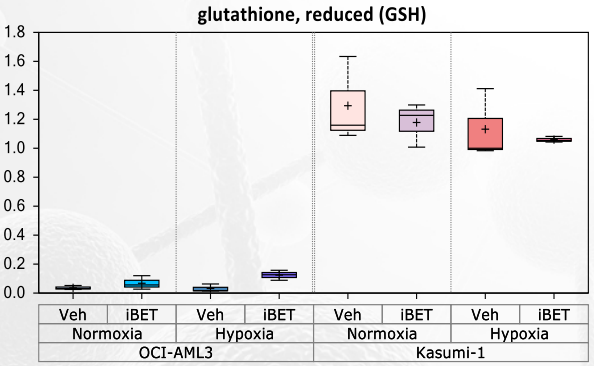


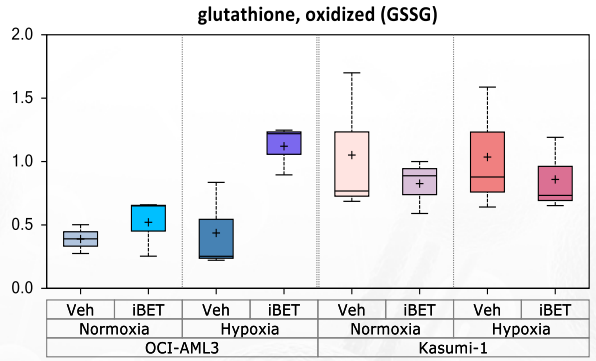
Glutathione levels increased in iBET treated hypoxic OCI-AML3 cells







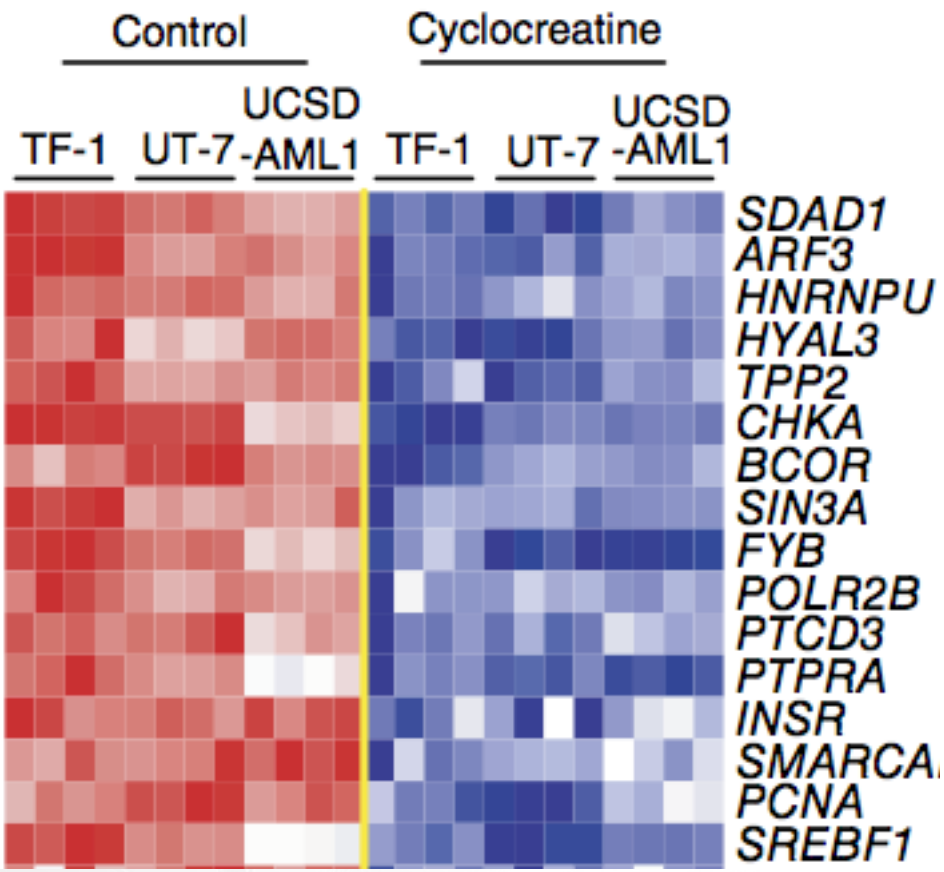




The creatine kinase pathway is a metabolic vulnerability in EVI1-positive acute myeloid leukemia

Nina Fenouille[1,13], Christopher F Bassil[2,13], Issam Ben-Sahra[3], Lina Benajiba[2], Gabriela Alexe[2,4,5], Azucena Ramos[1], Yana Pikman[2], Amy S Conway[2], Michael R Burgess[6], Qing Li[7], Frédéric Luciano[8], Patrick Auberger[8], Ilene Galinsky[9], Daniel J DeAngelo[9], Richard M Stone[9], Yi Zhang[10], Archibald S Perkins[10], Kevin Shannon[11], Michael T Hemann[1,14], Alexandre Puissant[2,12,14] & Kimberly Stegmaier[2,4,14]

Expression of the *MECOM* (also known as *EVI1*) proto-oncogene is deregulated by chromosomal translocations in some cases of acute myeloid leukemia (AML) and is associated with poor clinical outcome. Here, through transcriptomic and metabolomic profiling of hematopoietic cells, we reveal that EVI1 overexpression alters cellular metabolism. A screen using pooled short hairpin RNAs (shRNAs) identified the ATP-buffering, mitochondrial creatine kinase CKMT1 as necessary for survival of EVI1-expressing cells in subjects with EVI1-positive AML. EVI1 promotes CKMT1 expression by repressing the myeloid differentiation regulator RUNX1. Suppression of arginine–creatine metabolism by *CKMT1*-directed shRNAs or by the small molecule cyclocreatine selectively decreased the viability, promoted the cell cycle arrest and apoptosis of human EVI1-positive cell lines, and prolonged survival in both orthotopic xenograft models and mouse models of primary AML. CKMT1 inhibition altered mitochondrial respiration and ATP production, an effect that was abrogated by phosphocreatine-mediated reactivation of the arginine–creatine pathway. Targeting CKMT1 is thus a promising therapeutic strategy for this EVI1-driven AML subtype that is highly resistant to current treatment regimens.


a
Control
Cyclocreatine
TF-1
UT-7
UCSD -AML1
TF-1
UT-7
UCSD -AML1
SDAD1
ARF3
HNRNPU
HYAL3
TPP2
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PTCD3
PTPRA
INSR
SMARCA
PCNA
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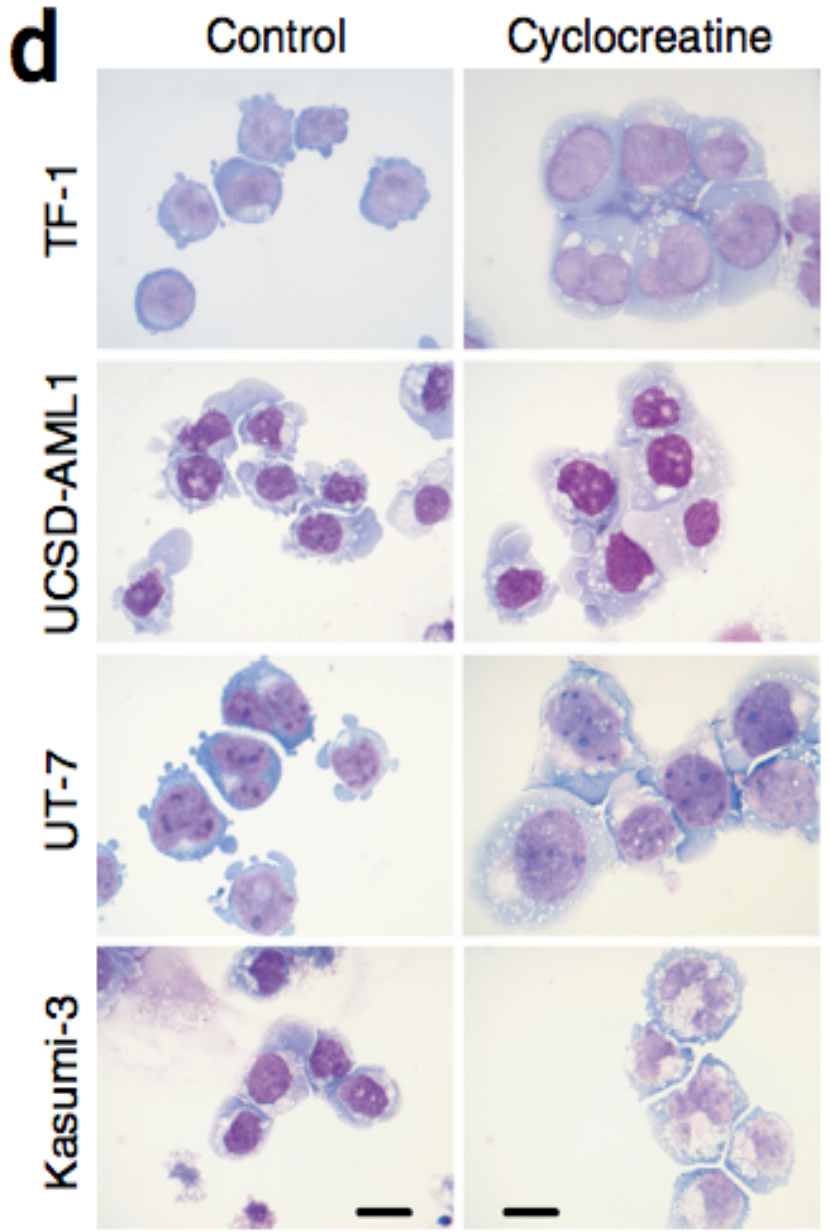

d
Control
Cyclocreatine
TF-1
UCSD-AML1
UT-7
Kasumi-3

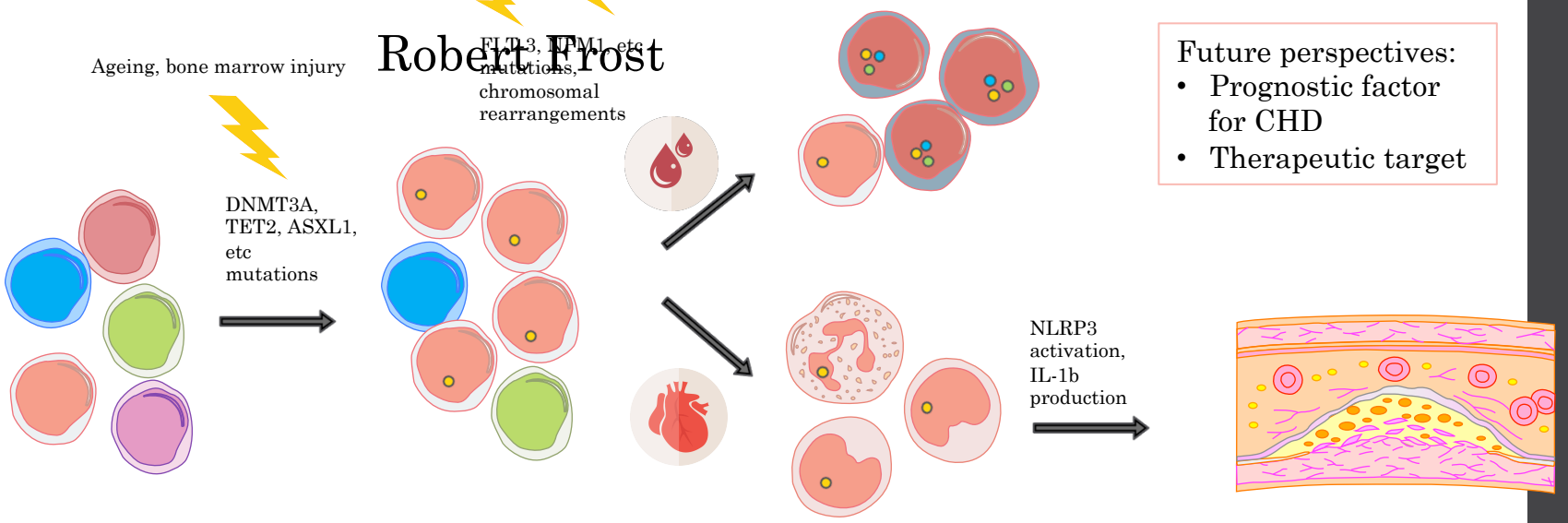
Acknowledgments

Supported by: FP7, European LeukemiaNet, AIL, AIRC, FIRB 2006, Fondazione del Monte di Bologna e Ravenna

Two roads diverge in a ”clonal wood”

“Two roads diverged in a wood, and I
I took the one less travelled by,
And it made all the difference.”

Robert Frost



Future perspectives:
- Prognostic factor for CHD
- Therapeutic target

Could it make all the difference?