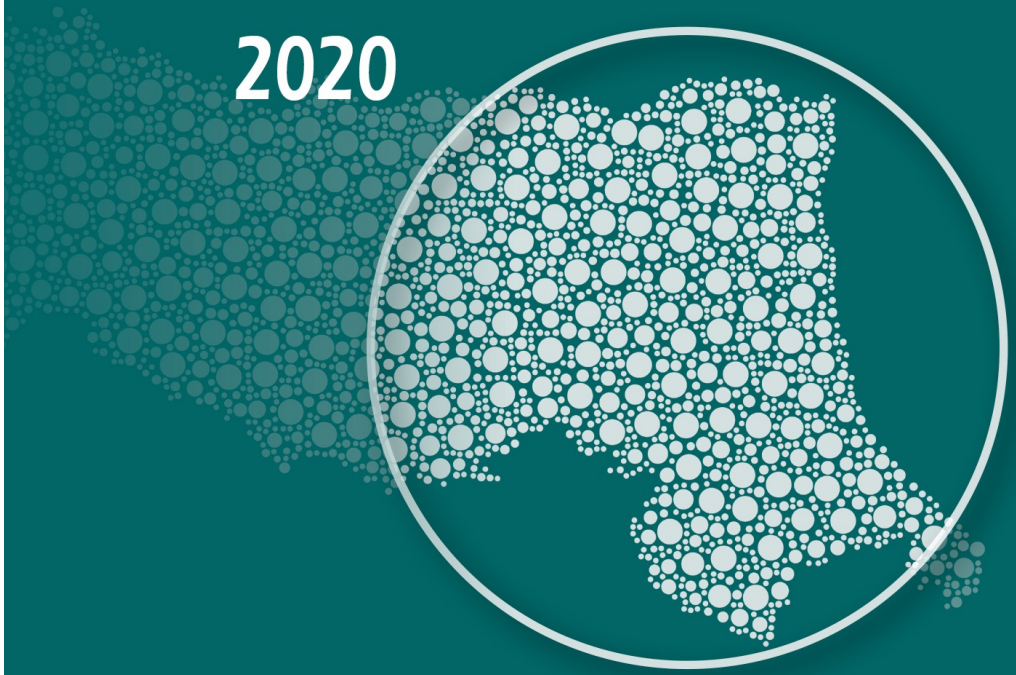



Progetto
Ematologia
Romagna

# DALLA BIOLOGIA ALLA TERAPIA: UNA STORIA A LIETO FINE PER LA LEUCEMIA MIELOIDE CRONICA E UNA STORIA TUTTA DA SCRIVERE PER LA MASTOCITOSI SISTEMICA

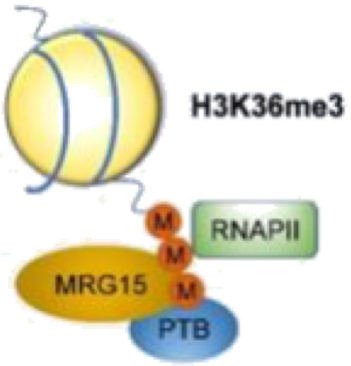
**MANUELA MANCINI**
*ISTITUTO DI EMATOLOGIA LORENZO E ARIOSTO SERÀGNOLI*

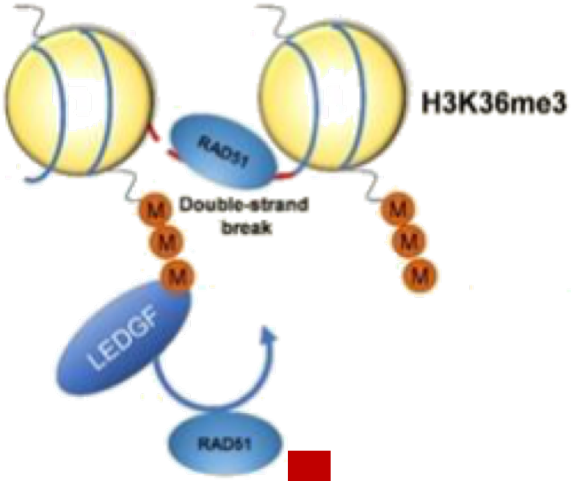
# Disclosures

I have nothing to disclose

# SETD2 functions

## TRIMETHYLATES H3 ON K36 AND CONTROLS



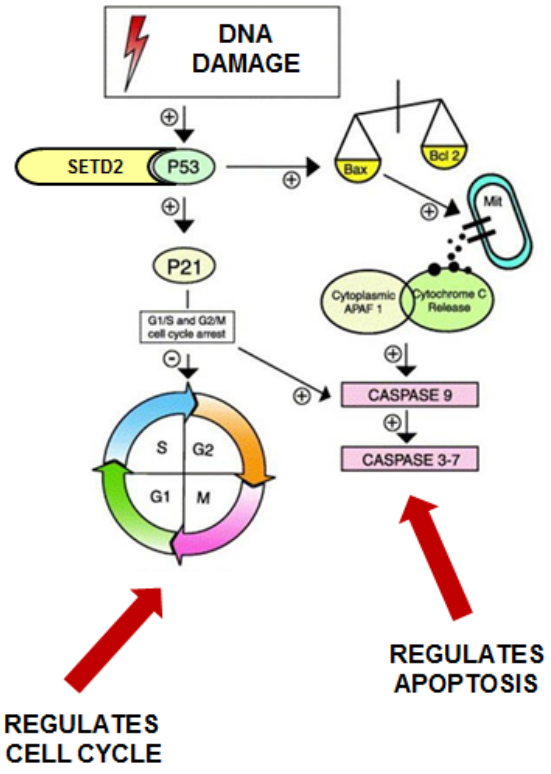




**TRANSCRIPTIONAL REGULATION**

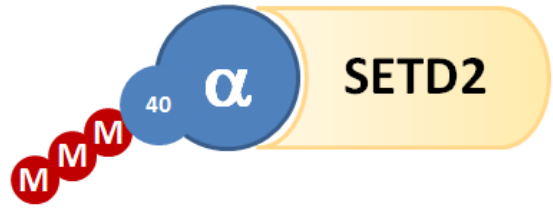
**DNA DAMAGE REPAIR**

# SETD2 functions

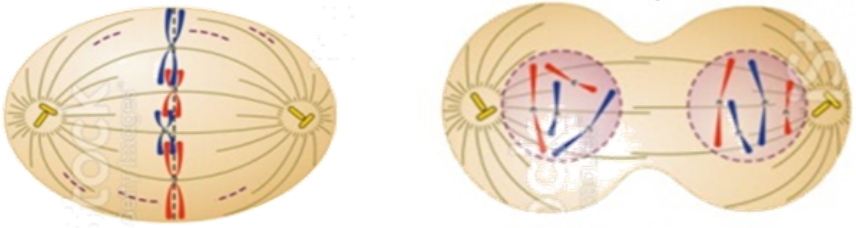
## INTERACTS WITH P53




## METHYLATES α–Tubulin

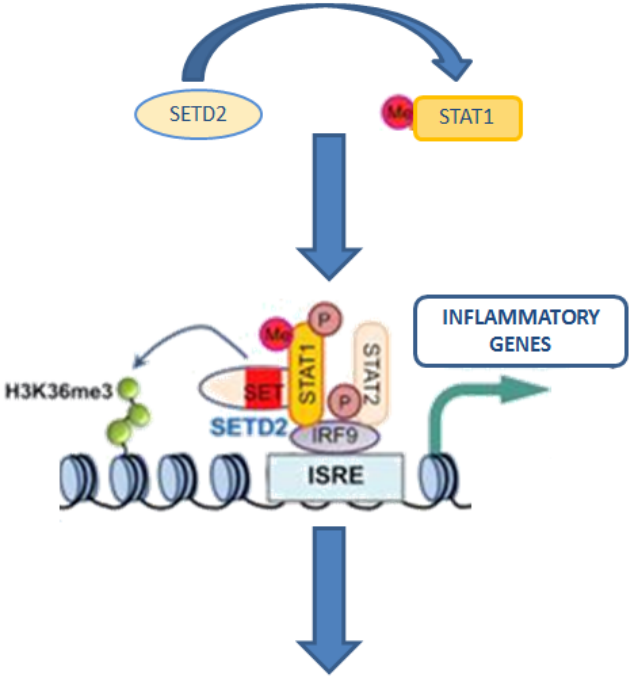



METHYLATION OF MITOTIC MICROTUBULES



REGULATION OF MITHOSIS AND CYTOKINESIS

## METHYLATES STAT1



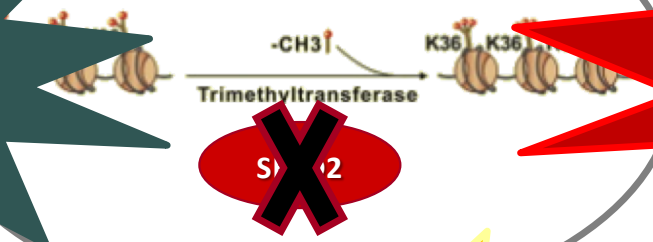

MEDIATES INTERFERON ANTIVIRAL ACTIVITY

# Effects of SETD2 loss of function

TRANSCRIPTION AL REGULATION

ALTERNATIVE SPLICING

GENOME INSTABILITY




APOPTOSIS INHIBITION

CROSS-TALK OF HISTONE MODIFICATIONS

LOSS OF CELL CYCLE CONTROL

DNA DAMAGE REPAIR

# Background

SETD2 loss of function has been reported in many solid tumors and in a variety of hematologic myeloid and lymphoid malignancies, including:

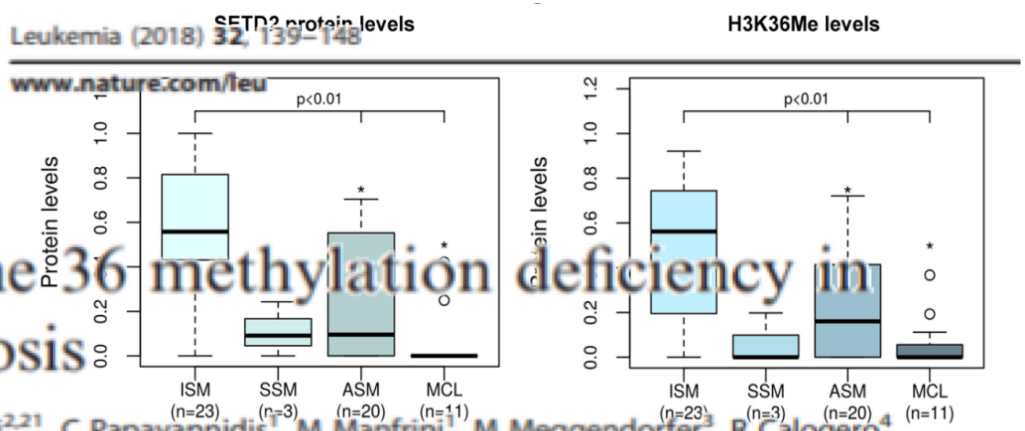
- acute leukemia
- chronic lymphocytic leukemia
- T-cell lymphoma
- systemic mastocytosis
- chronic myeloid leukemia

# SETD2 loss of function was found in advSM

## Validation cohort

**57 SM pts:**

- **23 pts with ISM [3 with AHN]**
- **3 pts with SSM**
- **20 advanced SM [3 with AHN]**
- **11 with MCL [2 with AHN]**




OPEN

Leukemia (2018) 32, 139–148

www.nature.com/leu

ORIGINAL ARTICLE

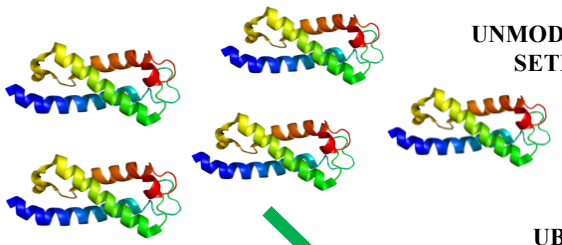
SETD2 and histone H3 lysine 36 methylation deficiency in advanced systemic mastocytosis

G Martinelli[1,20], M Mancini[1,20], C De Benedittis[1,21], M Rondoni[2,21], C Papayannidis[1], M Manfrini[1], M Meggendorfer[3], R Calogero[4], V Guadagnuolo[1], MC Fontana[1], L Bavaro[1], A Padella[1], E Zago[5,6], L Pagano[7], R Zanotti[8,9], L Scaffidi[8,9], G Specchia[10], F Albano[10], S Merante[11], C Elena[11], P Savini[12], D Gangemi[13], P Tosi[14], F Ciceri[15,16], G Poletti[17], L Riccioni[18], F Morigi[18], M Delledonne[5,6], T Haferlach[3], M Cavo[1], P Valent[19] and S Soverini[1]

✓ Proteasomal inhibition was found to rescue SETD2 expression and H3K36me3 and to result in accumulation of hyper-ubiquitinated SETD2 protein (*Manuela Mancini et al., Blood 2018 132:1726; Manuela Mancini et al., Blood 2018 132:1779*)

# SETD2 loss of function is due to proteasomal degradation

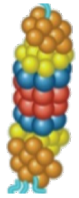
## HEALTHY CELL



UNMODIFIED SETD2

SETD2 IS NOT UBIQUITINATED BY MDM2 AND DEGRADATED BY PROTEASOME

MDM2

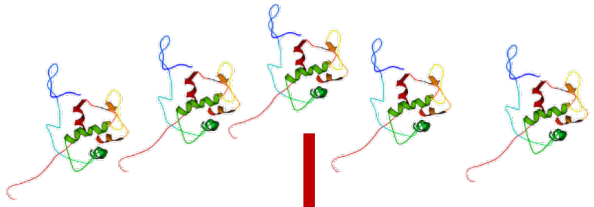


ACTIVE SETD2:

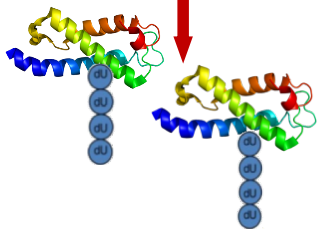
- TRIMETHYLATES H3K36
- ACTIVATES HR REPAIR
- ACTIVATES MISMATCH REPAIR
- REGULATES MITOSIS AND CYTOKINESIS

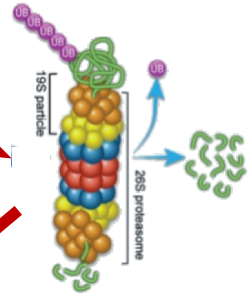
## advSM CELL

MDM2



UBIQUITINATION





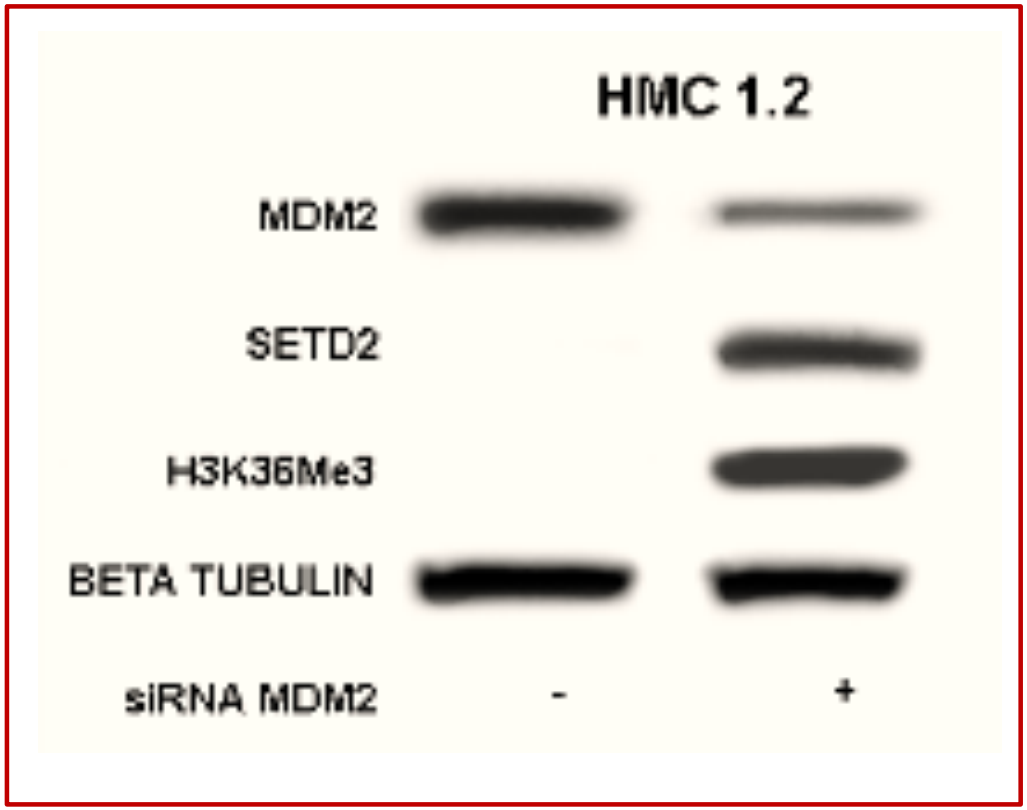

SETD2 PROTEASOMAL DEGRADATION:

- H3K36ME3 DEMETHYLATION
- INACTIVATION OF HR REPAIR
- INACTIVATION O MISMATCH REPAIR
- DEREGULATION OF MITOSIS AND CYTOKINESIS

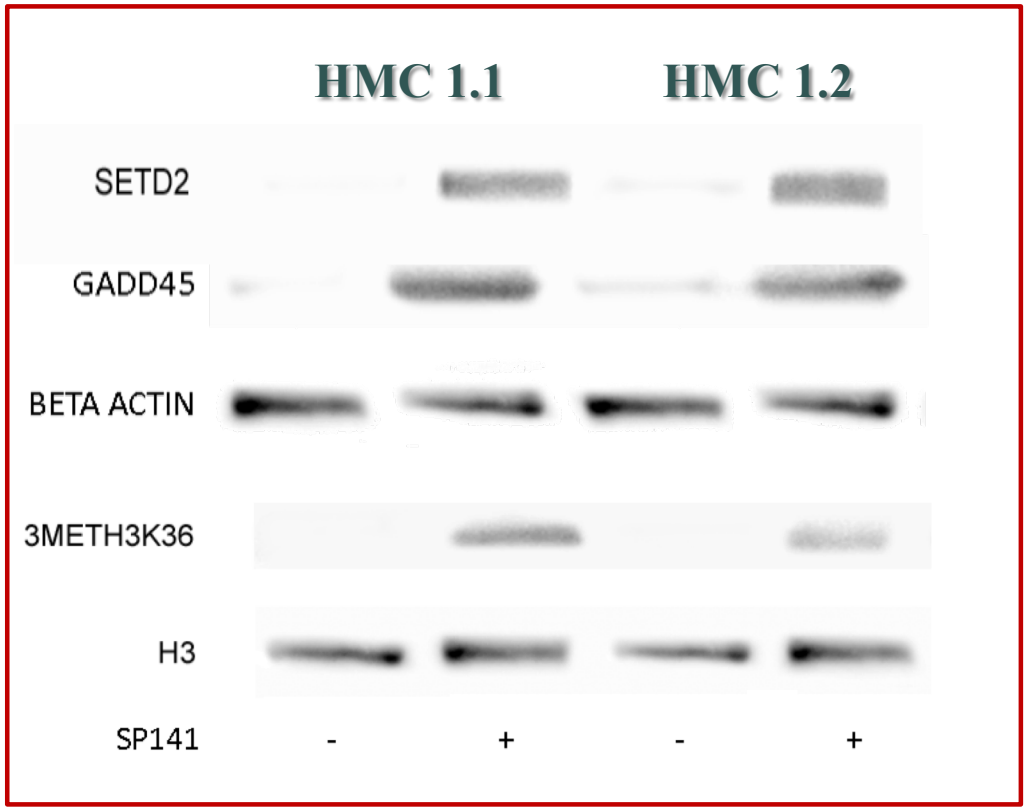
**GENETIC AND GENOMIC INSTABILITY**

# MDM2 inhibition rescues SETD2 expression and function

**siRNA-mediated knock-down of MDM2 rescues SETD2 expression and activity**

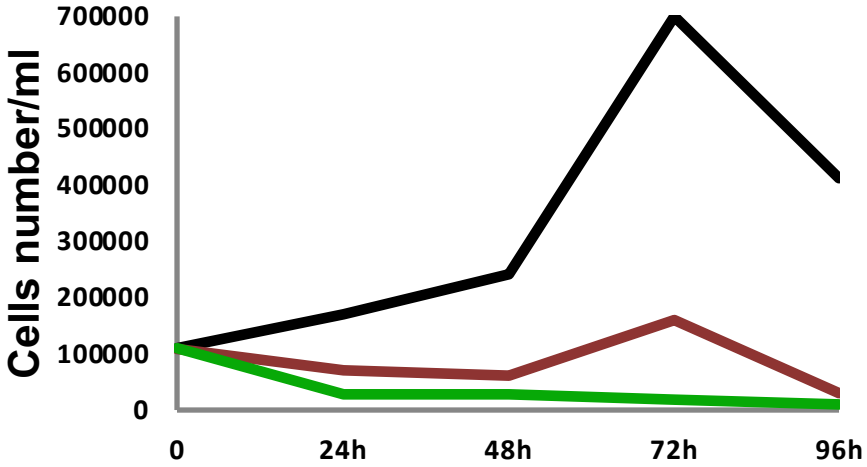



**Pharmacological inhibition of MDM2 by SP-141 rescues SETD2 expression and H3K36Me3**

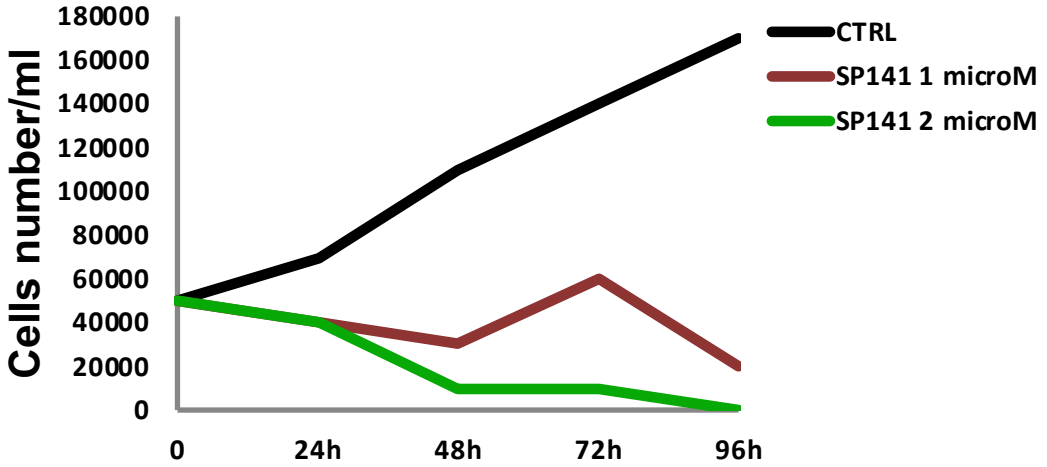


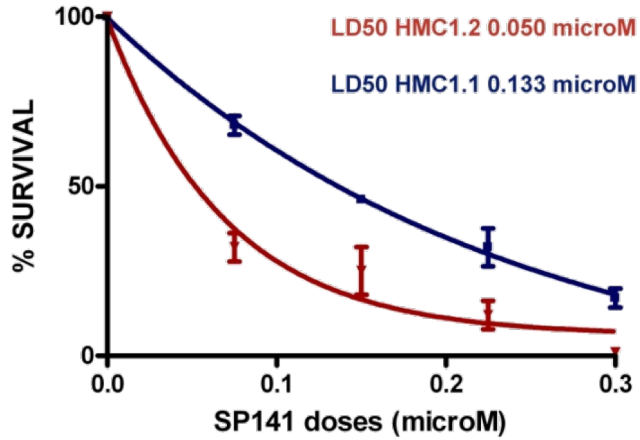
# SP-141 treatment induces cytostatic effects in HMC-1

## HMC 1.1

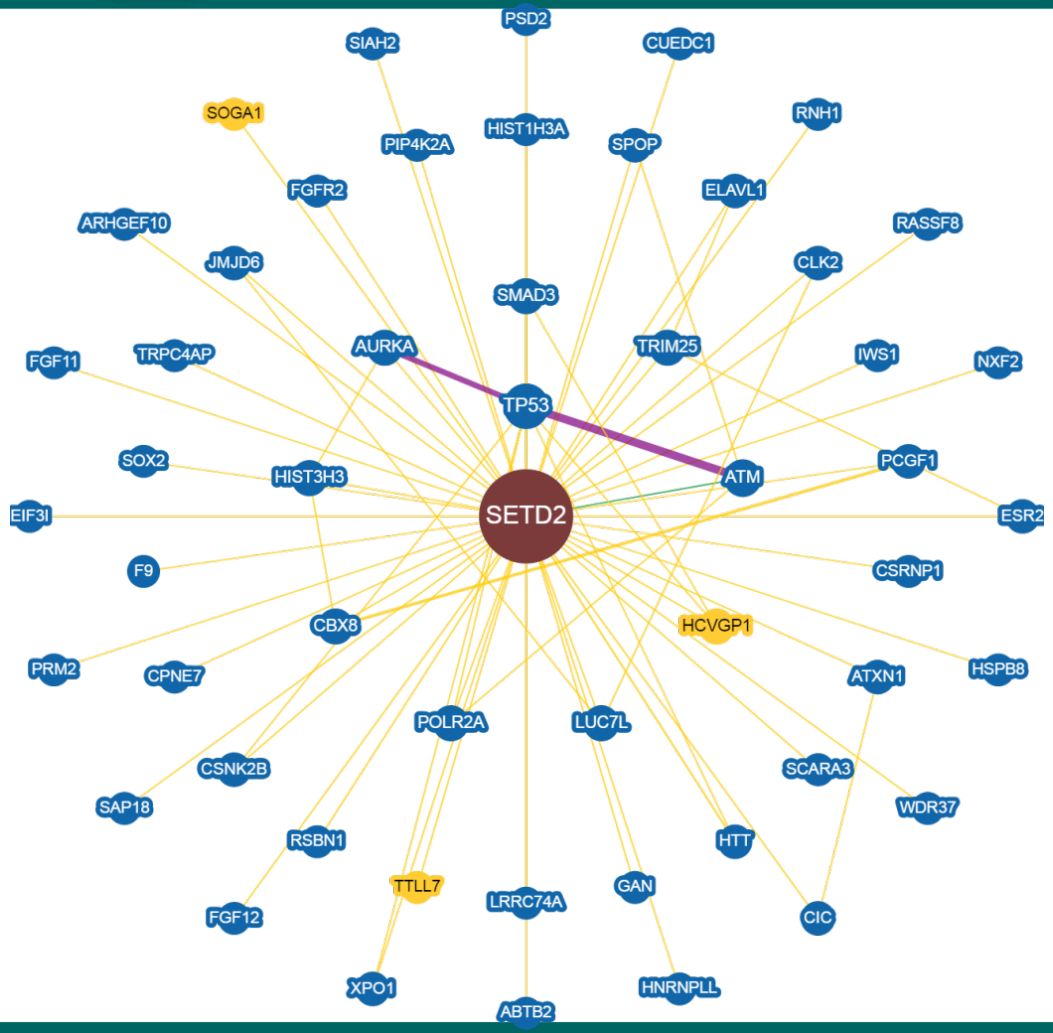



## HMC 1.2

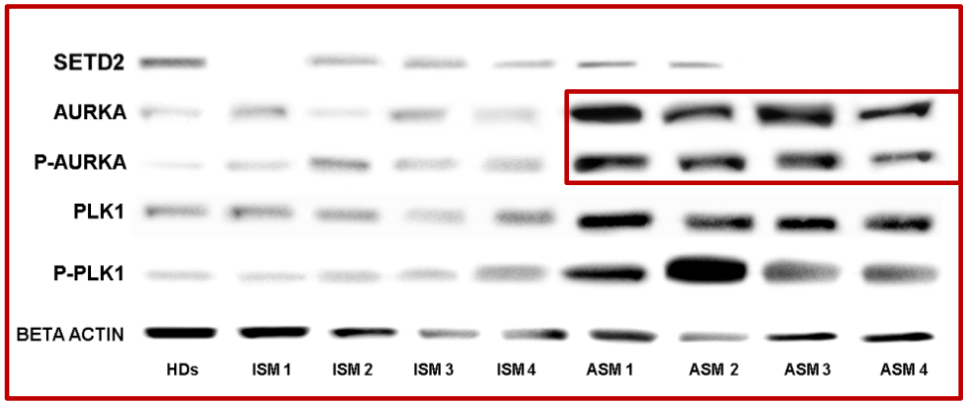


# SETD2 interacts with Aurora Kinase A (AKA)

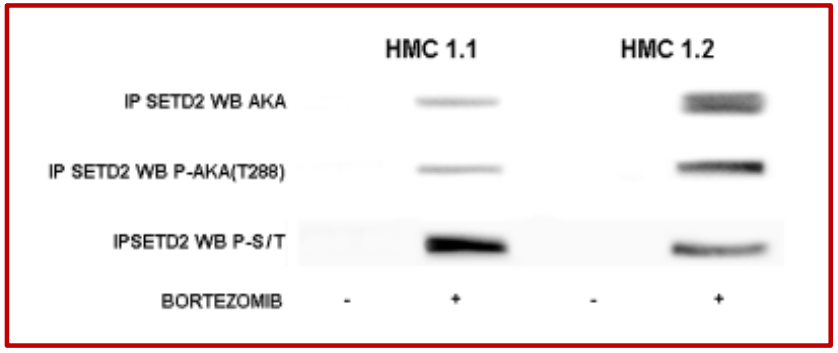



## AKA IS OVEREXPRESSED AND HYPER-ACTIVATED IN ADVSM

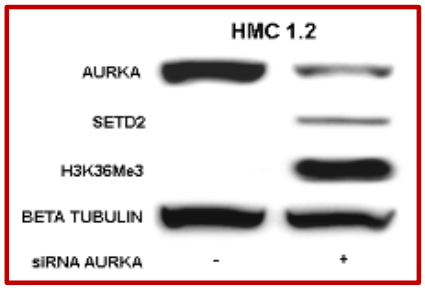


# AKA is involved in SETD2 loss of function

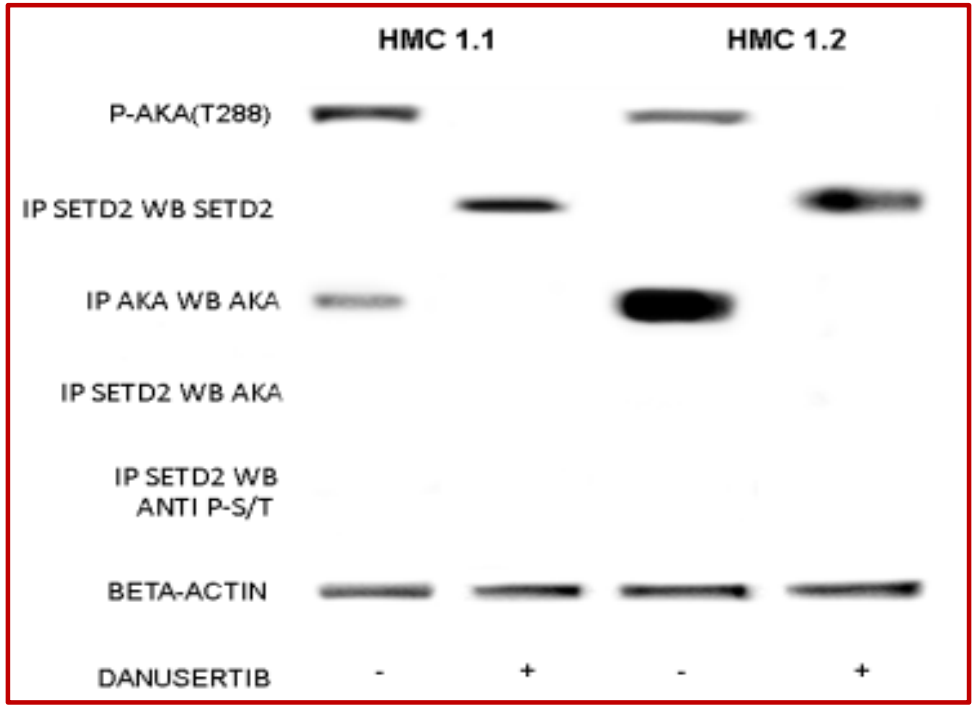
**AKA phosphorylates SETD2 and may be involved in SETD2 proteasomal degradation**




**siRNA-mediated knock-down of AKA rescues SETD2 expression and activity**



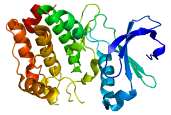

**AKA Inhibition by Danusertib rescues SETD2 expression and activity**



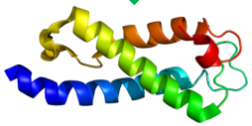
# Mdm 2 and Aurora Kinase A reduces SETD2 stability

## HEALTHY CELL

UNPHOSPHORYLATED AURORA KINASE A



UNPHOSPHORYLATED SETD2



UNPHOSPHORYLATED SETD2 IS NOT UBIQUITINATED BY MDM2 AND DEGRADATED BY PROTEASOME

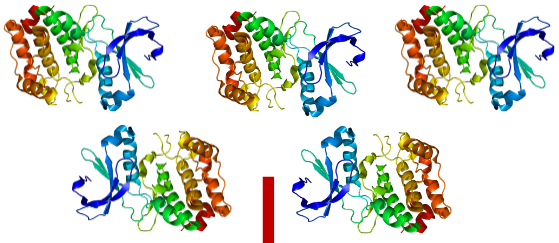
MDM2

ACTIVE SETD2:

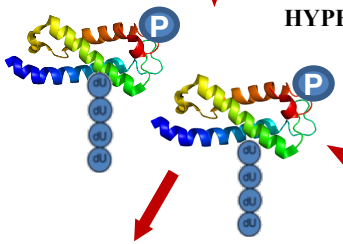
- TRIMETHYLATES H3K36
- ACTIVATES HR REPAIR
- ACTIVATES MISMATCH REPAIR
- REGULATES MITOSIS AND CYTOKINESIS

## advSM CELL

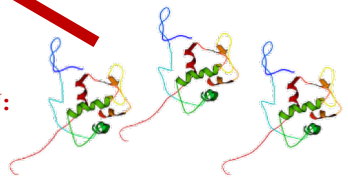
HYPER-PHOSPHORYLATED AND OVER-EXPRESSED AURORA KINASE A



HYPER-PHOSPHORYLATION OF SETD2




UBIQUITINATION



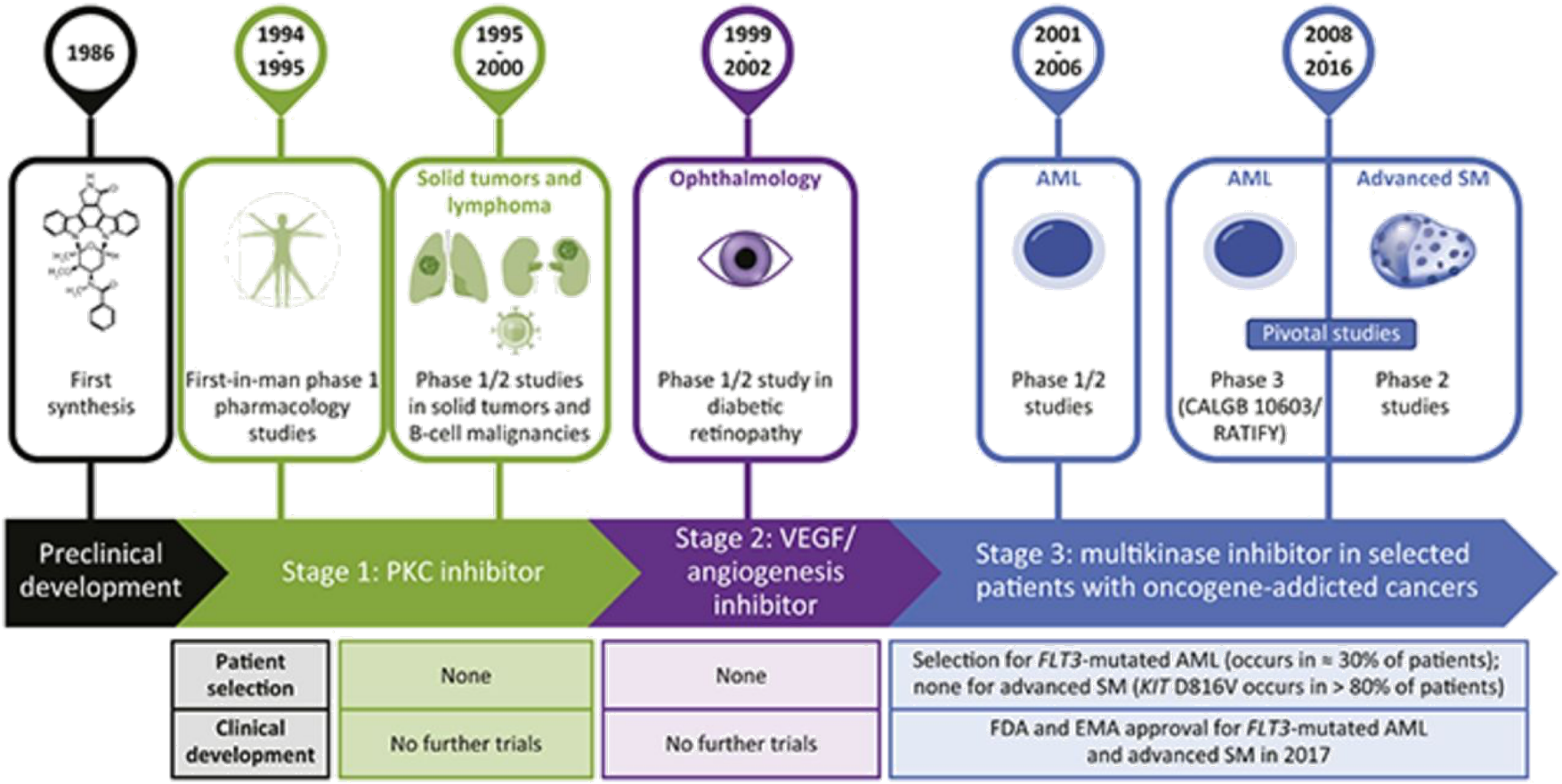
MDM2

SETD2 PROTEASOMAL DEGRADATION:

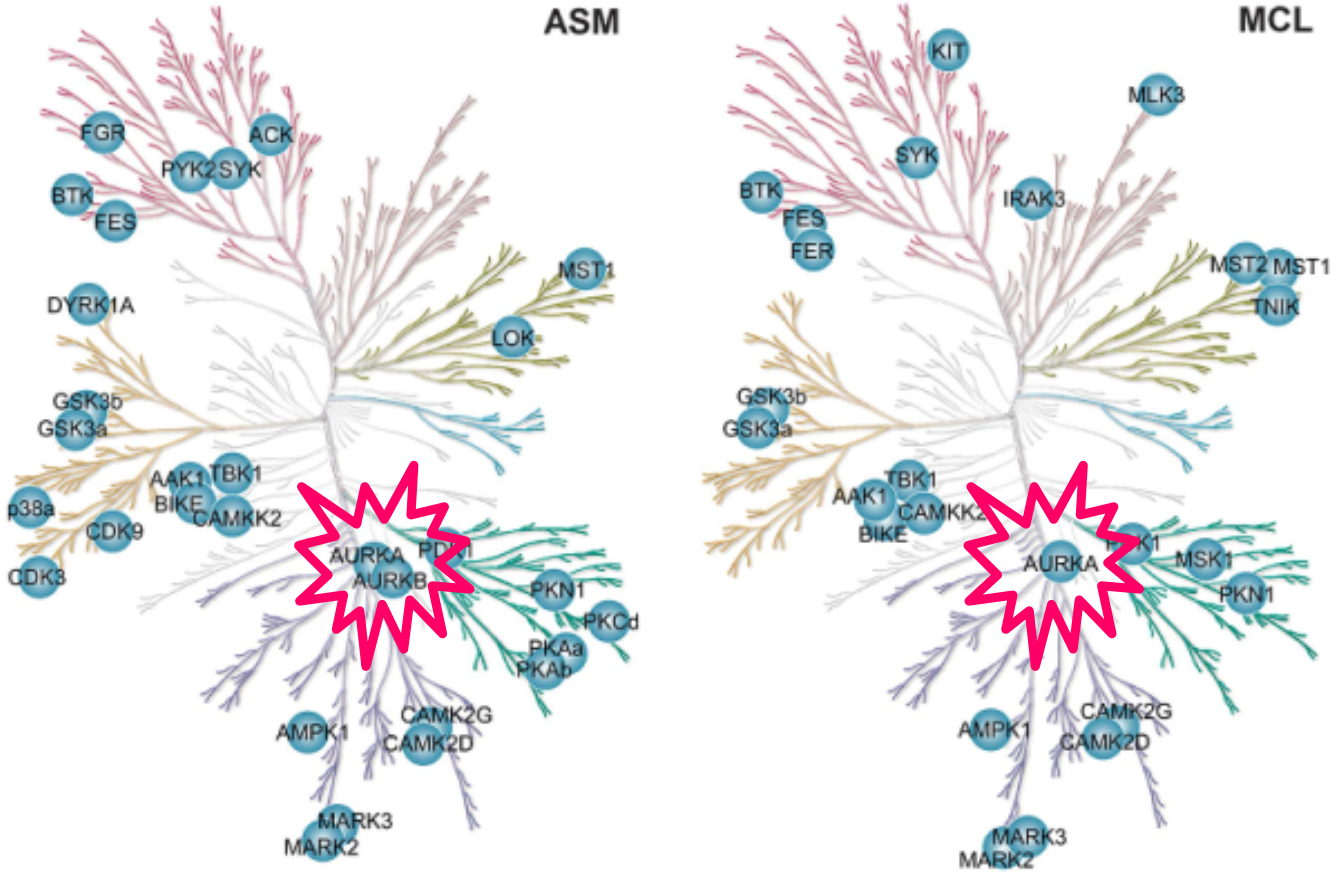
- H3K36ME3 DEMETHYLATION
- INACTIVATION OF HR REPAIR
- INACTIVATION O MISMATCH REPAIR
- DEREGULATION OF MITOSIS AND CYTOKINESIS

**GENETIC AND GENOMIC INSTABILITY**

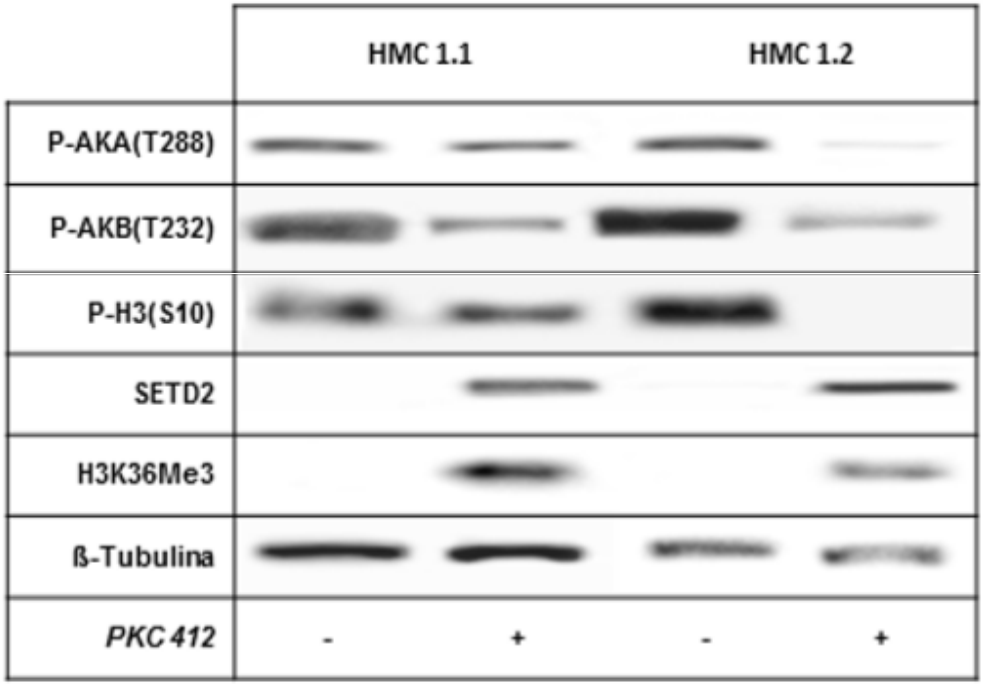
# Midostaurin: its odyssey from discovery to approval for treating advanced systemic mastocytosis



# Midostaurin: a multi-kinase inhibitor



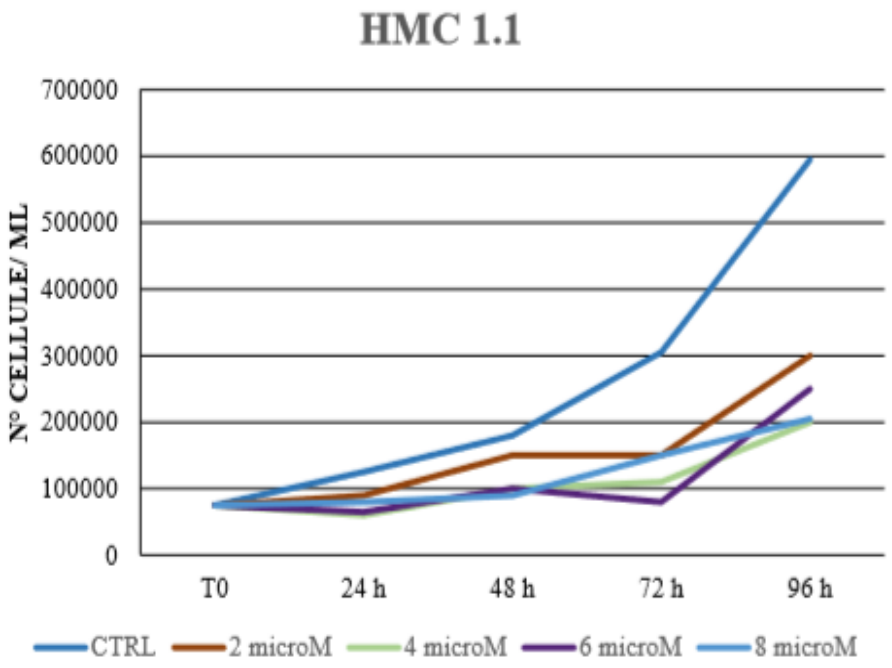
# Midostaurin treatment partially inhibits AKA and AKB

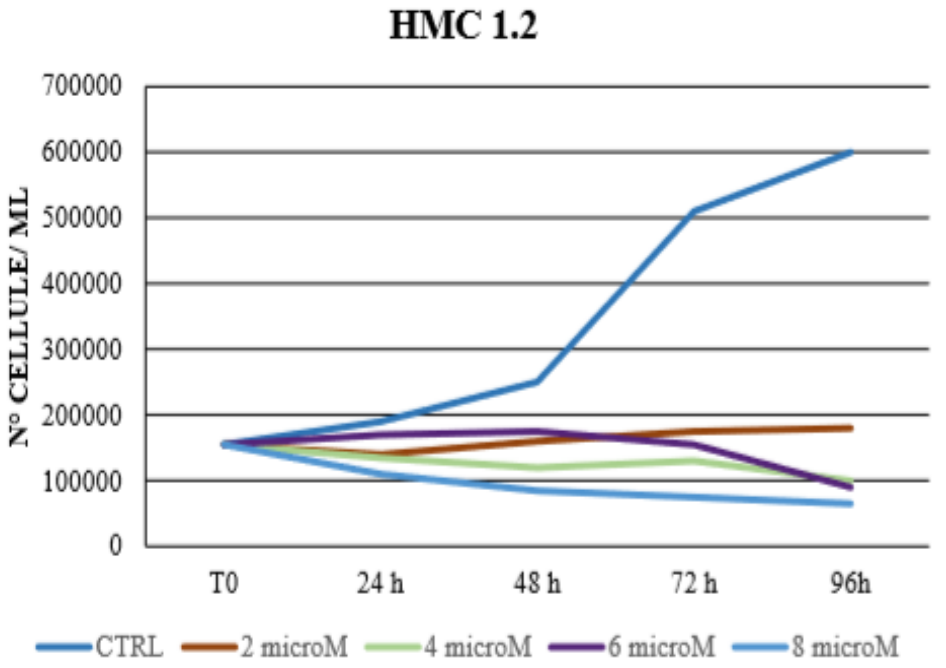



**Midostaurin treatment rescues Setd2 expression and function in SM cell lines**

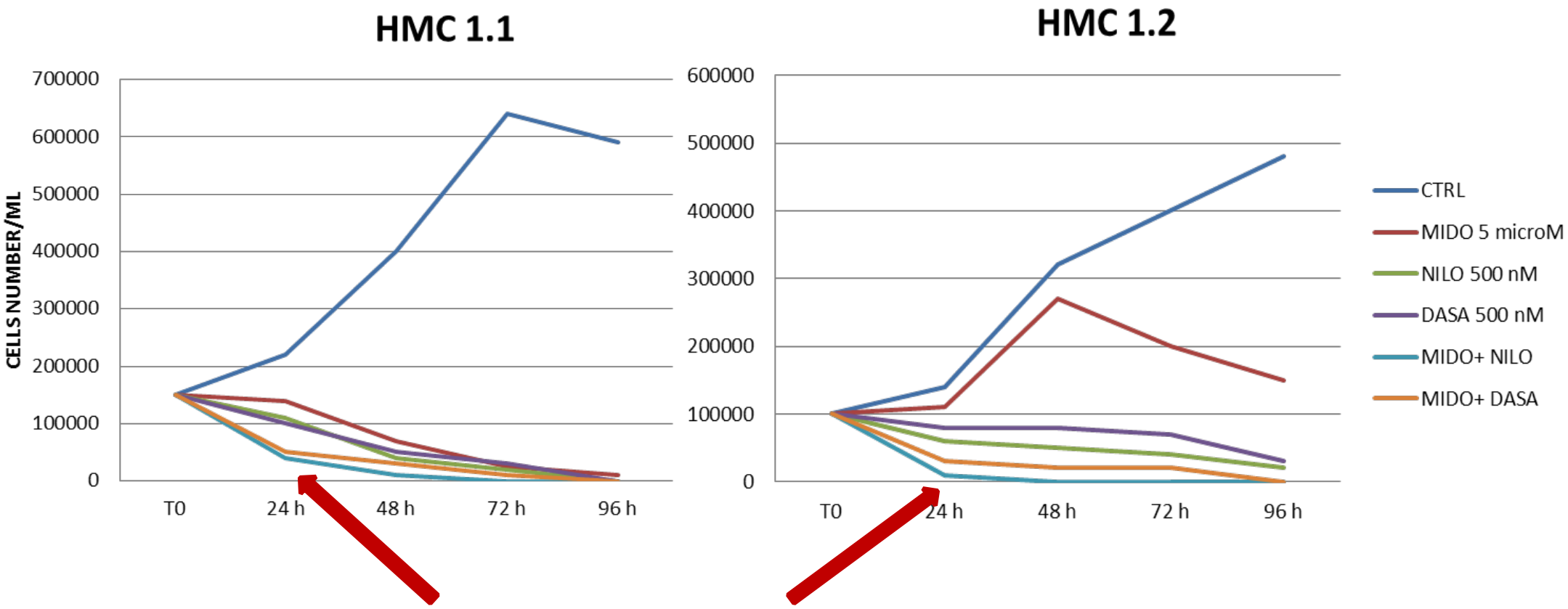
# Midostaurin treatment shows cytostatic effects in SM cell lines

## TIME-COURSE AND DOSE-ESCALATION EXPERIMENTS PERFORMED IN LIQUID MEDIUM



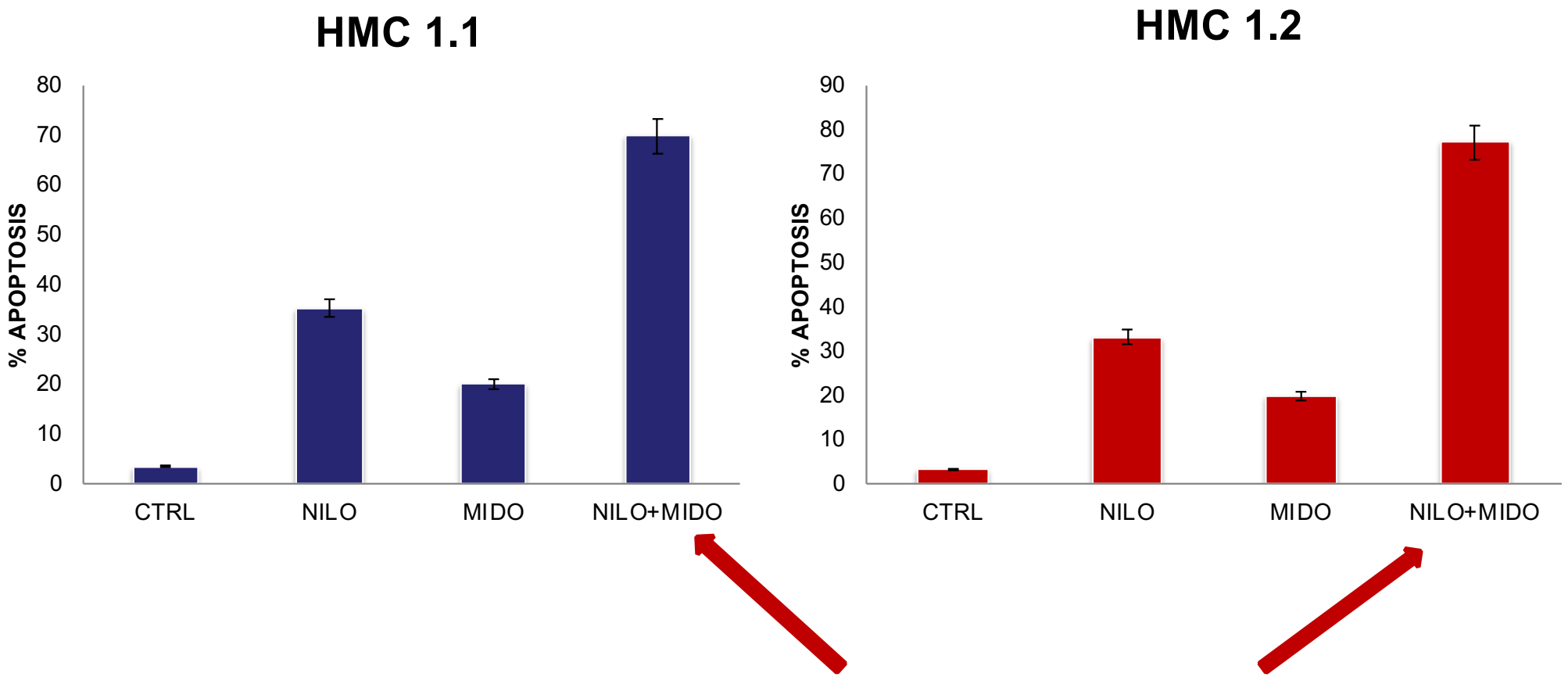



# Effects of Midostaurin combination with second generation TKIs



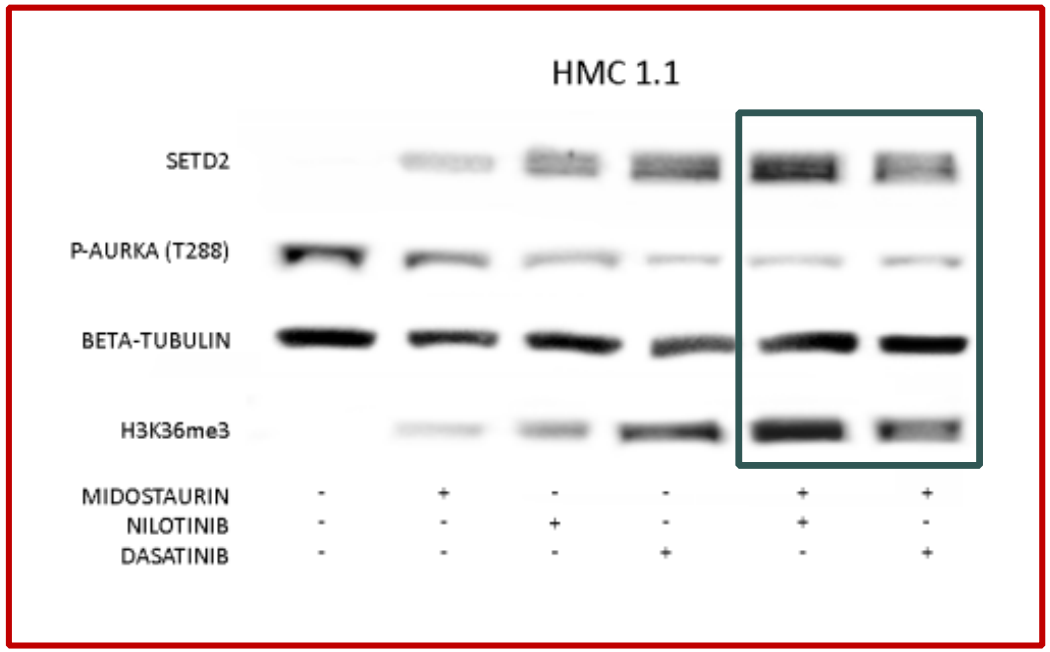

**SYNERGISTIC EFFECTS**

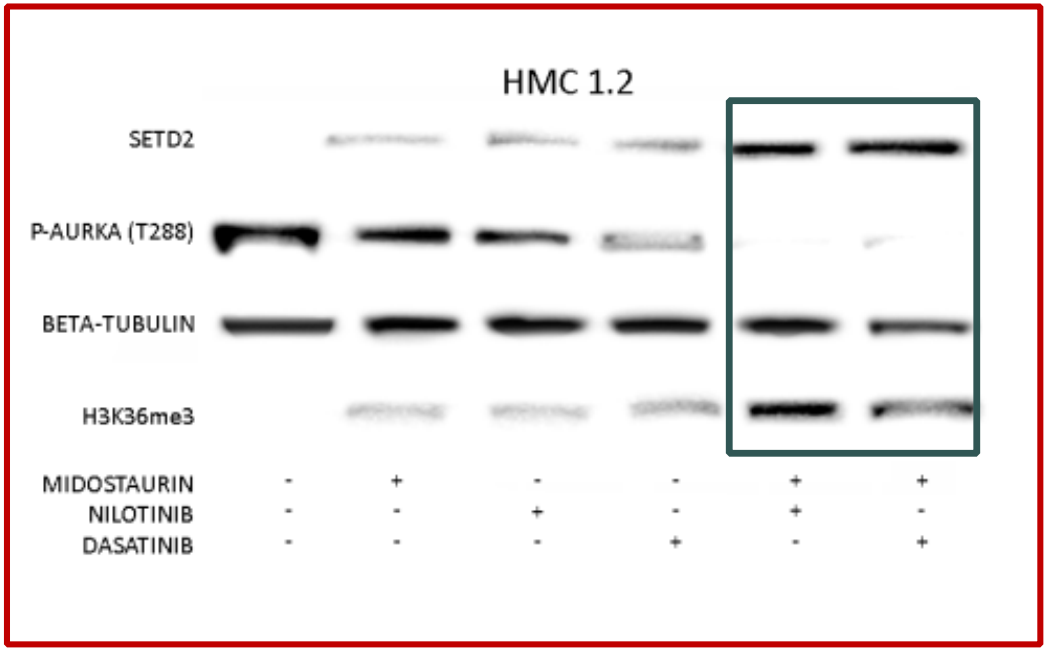
# Effects of Midostaurin combination with Nilotinib




**SYNERGISTIC EFFECTS**

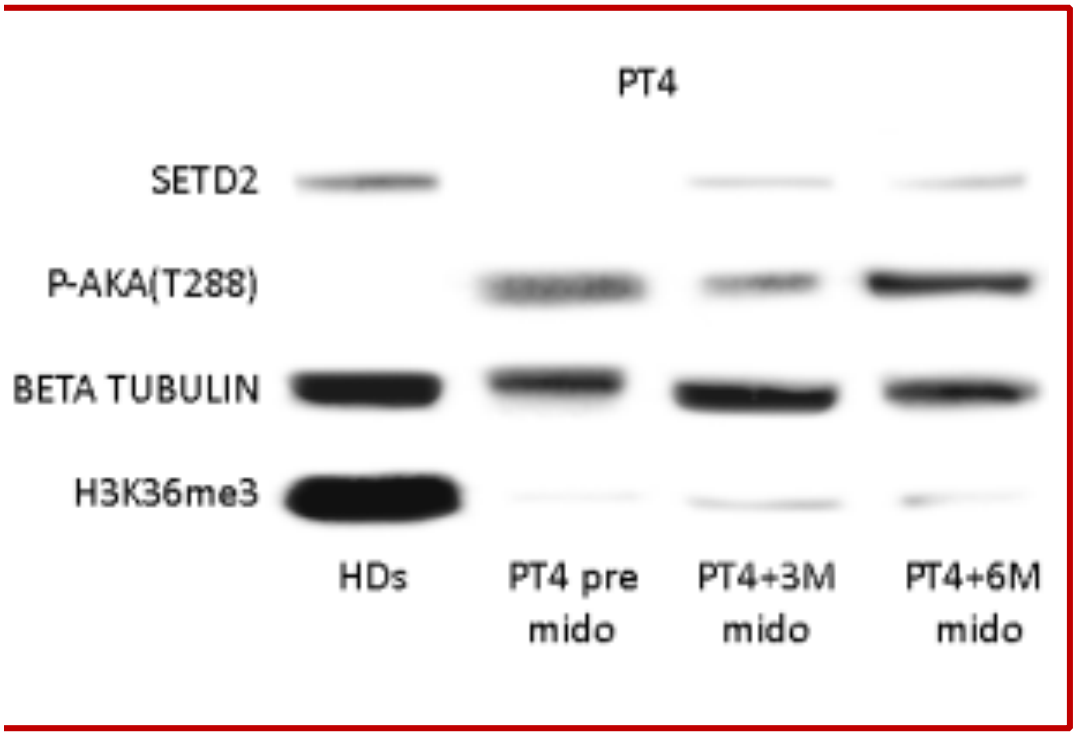
# Midostaurin combination with second generation TKIs rescue SETD2 expression in SM cell lines

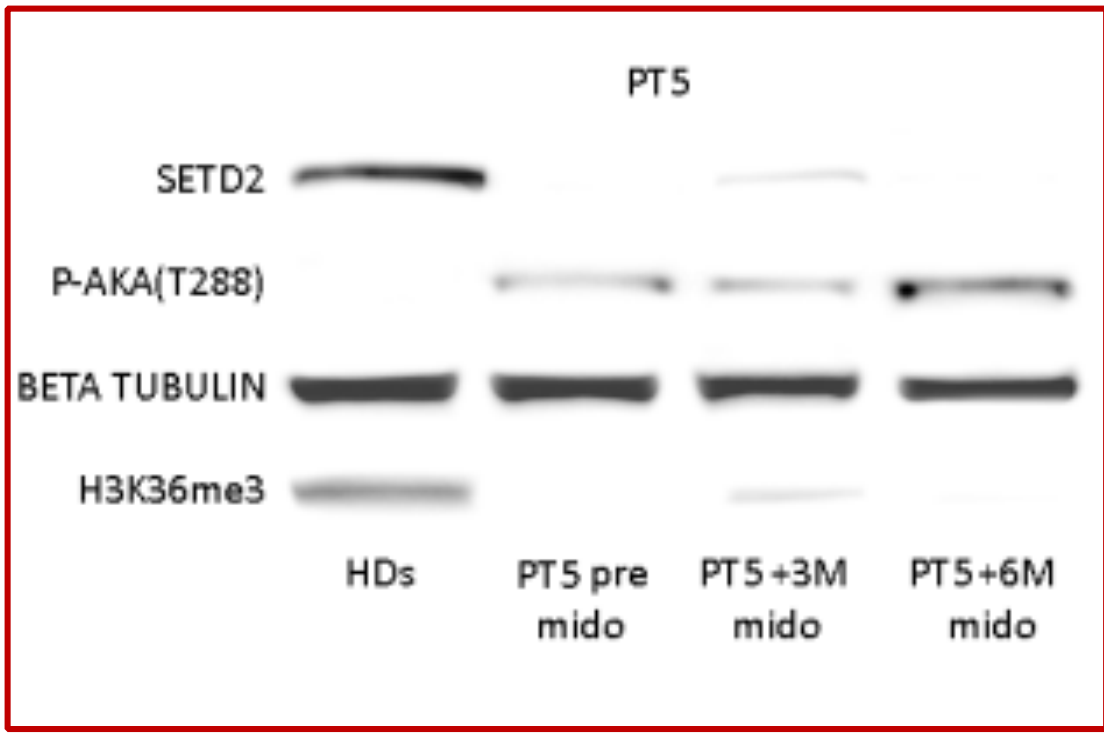


# Midostaurin rescues Setd2 expression and activity *in vivo*

# Midostaurin only partially inhibits AKA *in vivo*




| | | | | | | |
|---|---|---|---|---|---|---|
| **C-KIT D816V** | **26,32%** | **23,39%** | **15,60%** | | **25,62 %** | **18,36%** | **15,31%** |

# Conclusions

- ✓ Impaired function or loss of function of SETD2 is a general phenomenon in SM
- ✓ MDM2 and AKA-mediated post-translational modifications contribute to SETD2 non-genomic loss of function in ASM and MCL
- ✓ Inhibition of AKA and c-Kit activity by midostaurin, associated with a second generation TKI, is a promising therapeutic strategy in patients with low SETD2 expression levels

# SETD2 in Chronic Myeloid Leukemia

blood

ISSUES FIRST EDITION ABSTRACTS COLLECTIONS

631. CHRONIC MYELOID LEUKEMIA: BIOLOGY AND PATHOPHYSIOLOGY, EXCLUDING THERAPY: NOVEL TARGETS AND CML STEM CELL BIOLOGY | DECEMBER 7, 2017

## SETD2 Loss of Function Is a Recurrent Event in Advanced-Phase Chronic Myeloid Leukemia

Manuela Mancini, PhD, Sara De Santis, Cecilia Monaldi, Luana Bavaro, Margherita Martelli, Fausto Castagnetti, MD PhD, Gabriele Gugliotta, MD PhD, Gianantonio Rosti, MD, Alessandra Iurlo, MD PhD, Elisabetta Abruzzese, MD, Marzia Salvucci, MD, Patrizia Pregno, MD, Antonella Gozzini, MD, Monica Crugnola, MD, Francesco Albano, MD, Massimiliano Bonifacio, MD, Elisabetta Calistri, MD, Mario Tiribelli, MD, Gianni Binotto, MD, Annalisa Imovilli, MD, Elena Trabacchi, MD, Sara Galimberti, PhD MD, Claudia Baratè, MD PhD, Elena Tenti, PhD, Michele Baccarani, MD, Michele Cavo, MD, Giovanni Martinelli, MD, Simona Soverini, PhD



*Blood* (2017) 130 (Supplement 1): 43.

https://doi.org/10.1182/blood.V130.Suppl_1.43.43

## Aurora Kinase a/MDM2–Mediated SETD2 Loss of Function in Chronic Myeloid Leukemia Patients in Blast Crisis Induces Genetic Instability and Can be Therapeutically Targeted

Manuela Mancini, Sara De Santis, Cecilia Monaldi, Luana Bavaro, Margherita Martelli, Fausto Castagnetti, Gabriele Gugliotta, Gianantonio Rosti, Maria Chiara Fontana, Elisa Dan, Barbara Sinigaglia, Alessandra Iurlo, Nicola Orofino, Elisabetta Abruzzese, Marzia Salvucci, Patrizia Pregno, Antonella Gozzini, Monica Crugnola, Francesco Albano, Massimiliano Bonifacio, Elisabetta Calistri, Mario Tiribelli, Gianni Binotto, Annalisa Imovilli, Elena Trabacchi, Sara Galimberti, Claudia Baratè, Elena Tenti, Michele Baccarani, Giovanni Martinelli, Michele Cavo, and Simona Soverini

Blood 2018 132:1726; doi: https://doi.org/10.1182/blood-2018-99-112908

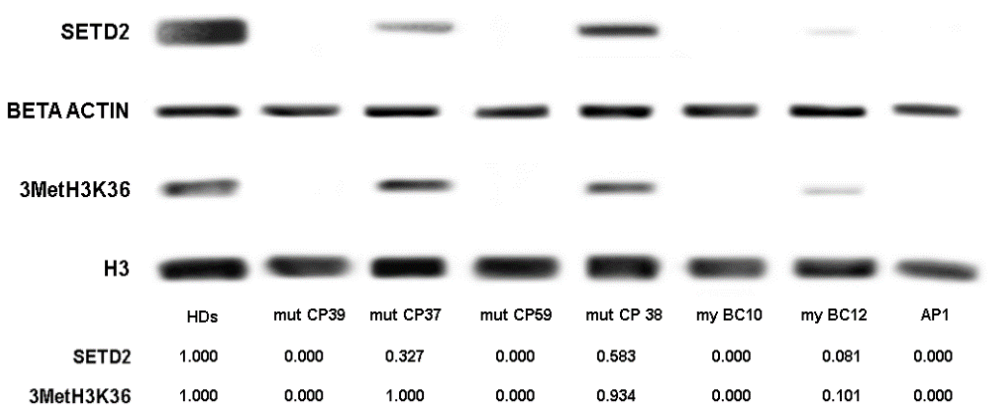
# Cohort description

96 CML patients:

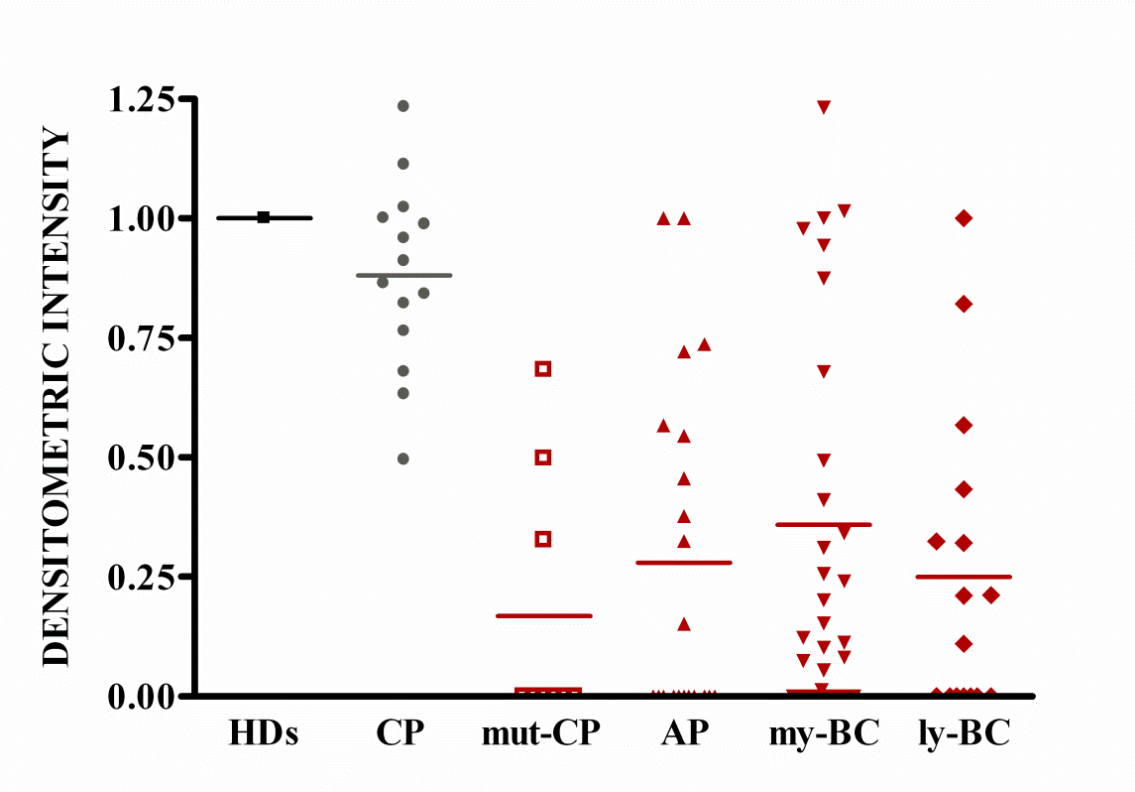
- accelerated phase (AP; n=21),
- myeloid blast crisis (my-BC; n=35),
- lymphoid blast crisis (ly-BC; n=17)
- tyrosine kinase inhibitor (TKI)-resistant chronic phase harbouring 2 or more BCR-ABL kinase domain mutations (mut-CP; n=9)

Samples collected at diagnosis from chronic phase patients (n=14) were studied for comparison

# SETD2 is down-modulated in advanced phases of CML




| | HDs | mut CP39 | mut CP37 | mut CP59 | mut CP 38 | my BC10 | my BC12 | AP1 |
|---|---|---|---|---|---|---|---|---|
| SETD2 | 1.000 | 0.000 | 0.327 | 0.000 | 0.583 | 0.000 | 0.081 | 0.000 |
| 3MetH3K36 | 1.000 | 0.000 | 1.000 | 0.000 | 0.934 | 0.000 | 0.101 | 0.000 |



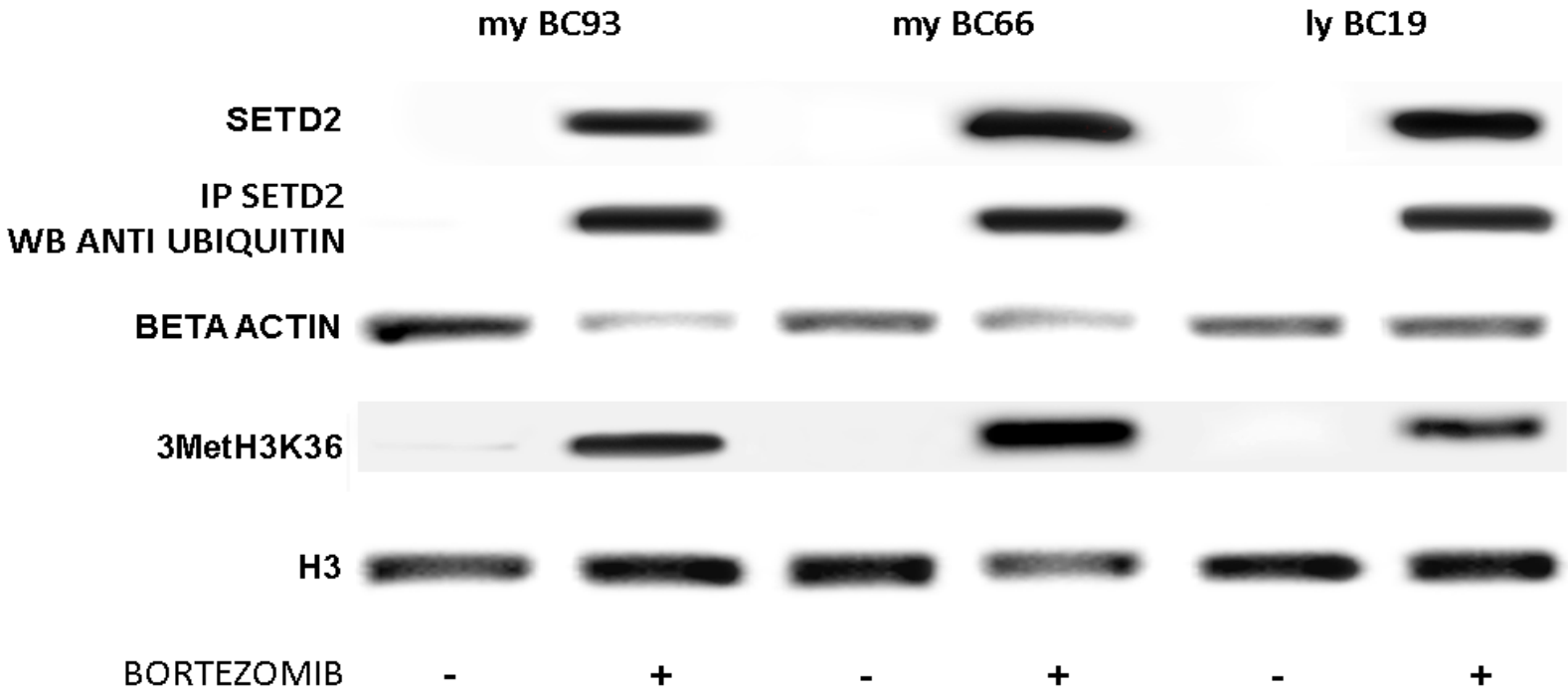
# 2020 Neither mutations nor transcriptional down modulation can be observed in SETD2 deficient patients

- ✓ Only 1 missense mutation at a splicing site in a CML patient, no truncated forms were identified by WB

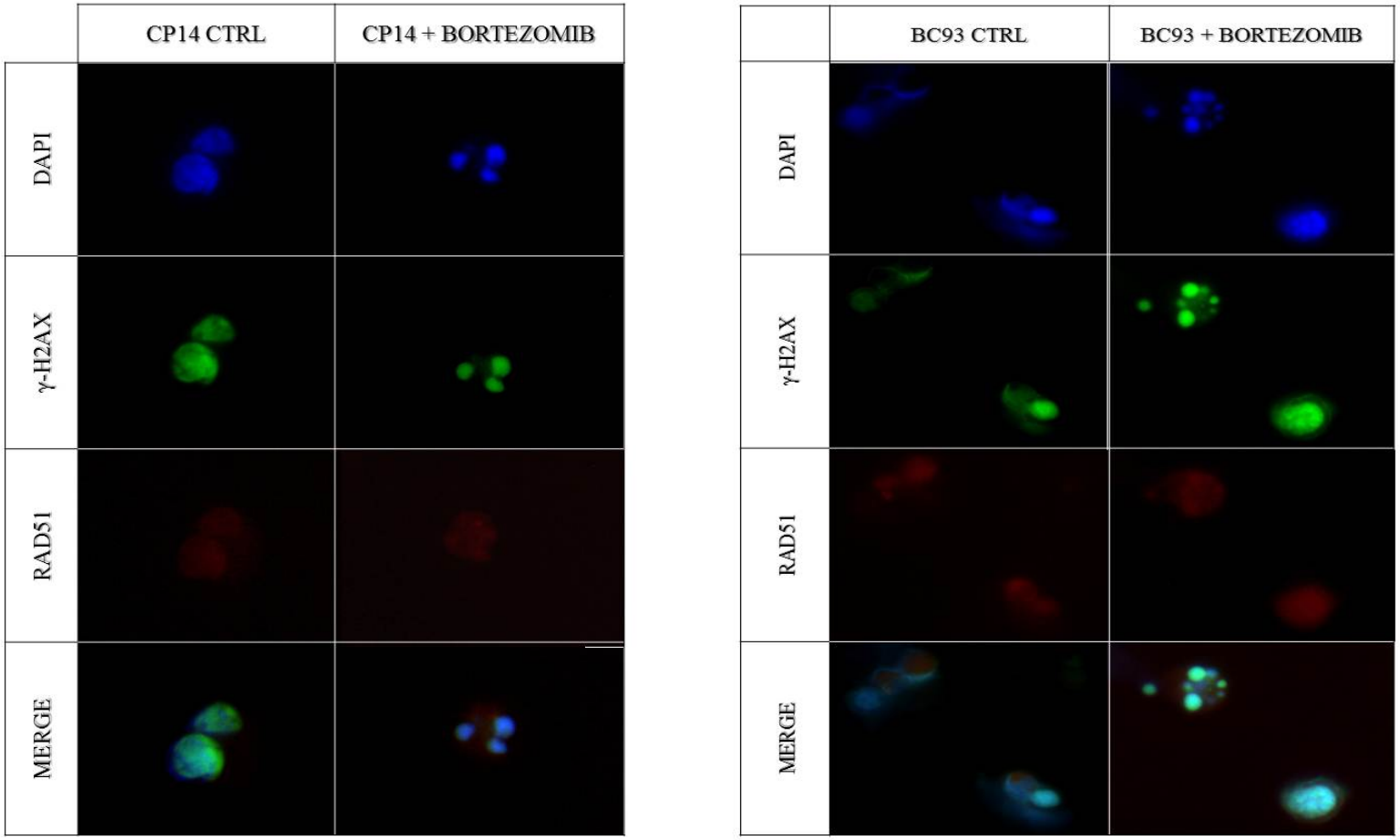
- ✓ SETD2 transcript levels not significantly lower in patients with no or low SETD2 expression as compared to healthy donors

# Proteasomal inhibition induces SETD2 up-modulation

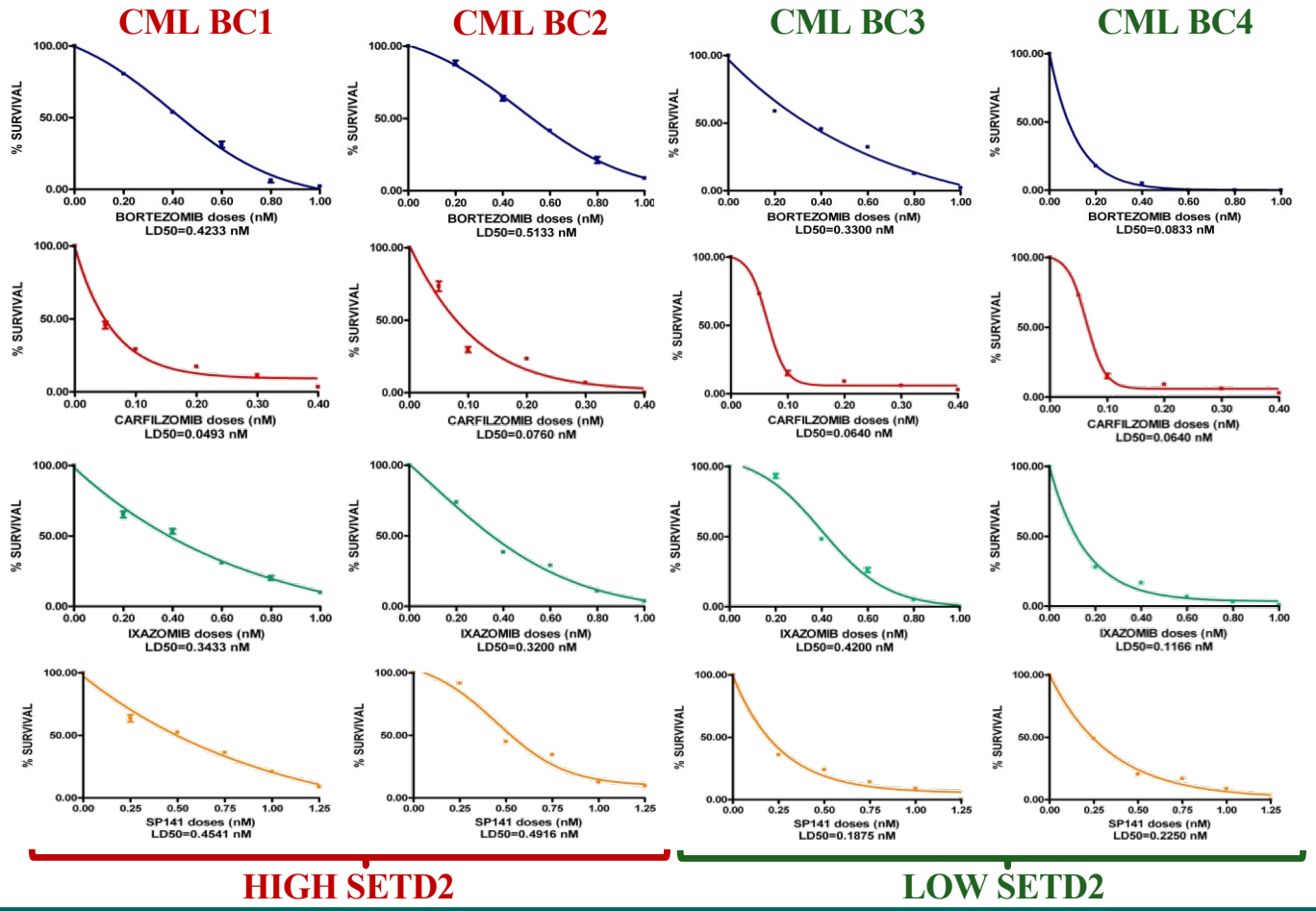
Proteasomal inhibition was found to rescue SETD2 expression and H3K36Me3 and to result in accumulation of hyper-ubiquitinated SETD2 protein



# Bortezomib induces apoptosis in Setd2 negative patients



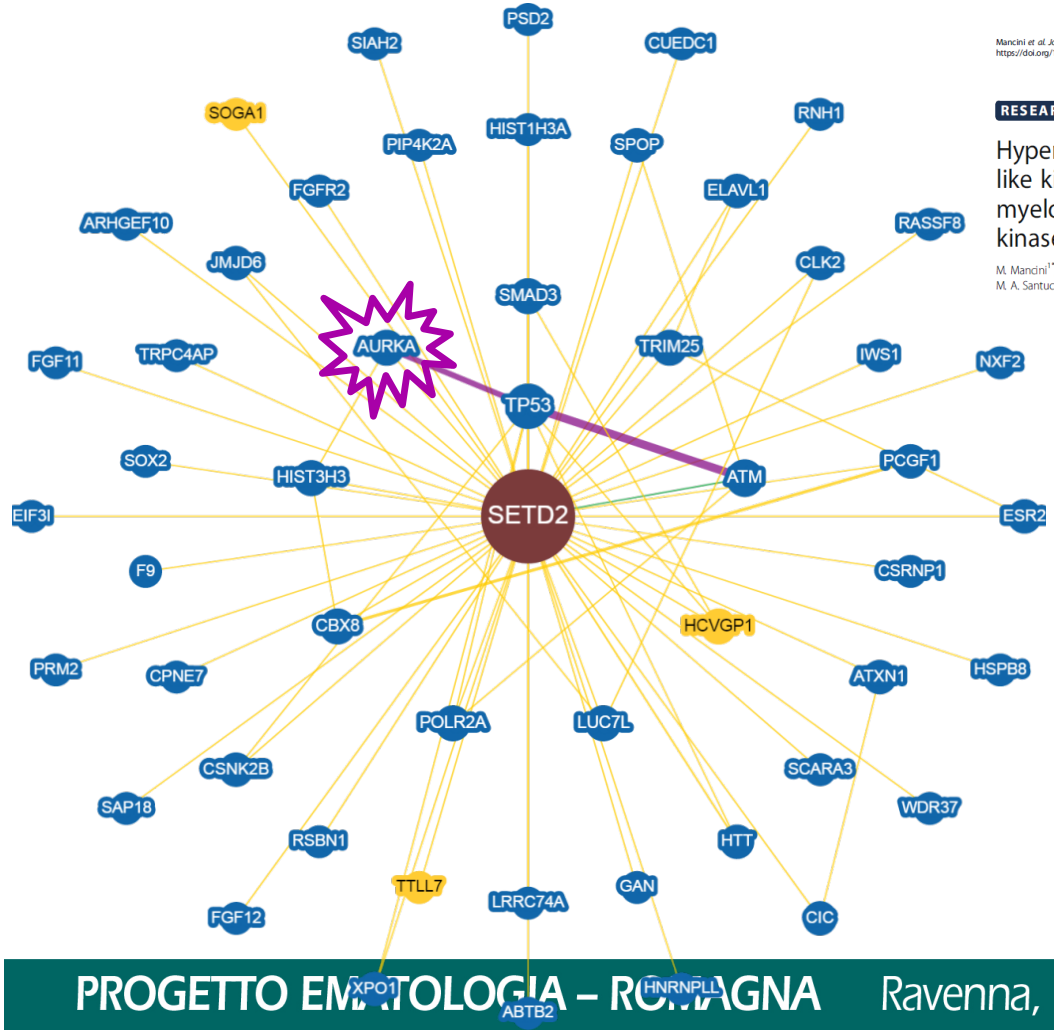
# Reduction of clonogenic growth after proteasomal inhibition is indeed SETD2-dependent




| | CML BC1 | CML BC2 | CML BC3 | CML BC4 |
|---|---|---|---|---|
| $LD_{50}$ BORTEZOMIB | 0,42 nM | 0,51 nM | 0,33nM | 0,08nM |
| $LD_{50}$ CARFILZOMIB | 0, 05 nM | 0, 08 nM | 0, 06 nM | 0,02 nM |
| $LD_{50}$ IXAZOMIB | 0,34 nM | 0, 32 nM | 0,42 nM | 0,12 nM |
| $LD_{50}$ SP141 | 0,45 nM | 0,49 nM | 0,19 nM | 0,22 nM |

| | CML BC1 | CML BC2 | CML BC3 | CML BC4 |
|---|---|---|---|---|
| SETD2 | 0.545 | 0.679 | 0 | 0 |
| H3K36Me3 | 0.657 | 0.722 | 0 | 0 |

# SETD2 interacts with Aurora Kinase A




Mancini et al. Journal of Experimental & Clinical Cancer Research (2019) 38:216
https://doi.org/10.1186/s13046-019-1197-9

Journal of Experimental & Clinical Cancer Research

RESEARCH Open Access

## Hyper-activation of Aurora kinase a-polo-like kinase 1-FOXM1 axis promotes chronic myeloid leukemia resistance to tyrosine kinase inhibitors

M. Mancini[1*], S. De Santis[1], C. Monaldi[1], L. Bavaro[1], M. Martelli[1], F. Castagnetti[1], G. Gugliotta[1], G. Rosti[1], M. A. Santucci[1], G. Martinelli[2], M. Ca

Medical Oncology (2018) 35:26
https://doi.org/10.1007/s12032-018-1079-6

ORIGINAL PAPER

## Expression of Hippo signaling pathway and Aurora kinase genes in chronic myeloid leukemia

Ana Paula Zambuzi Cardoso Marsola[1] · Belinda Pinto Simões[2] · Leonardo Carvalho Palma[2] · Maria Gabriela Berzoti-Coelho[1] · Sandra Mara Burin[1] · Fabíola Attié de Castro[1]

www.oncotarget.com Oncotarget, 2018, Vol. 9, (No. 65), pp: 32496-32506

Research Paper

## Therapeutic targeting of Aurora A kinase in Philadelphia chromosome-positive ABL tyrosine kinase inhibitor-resistant cells

Seiichi Okabe[1], Tetsuzo Tauchi[1], Yuko Tanaka[1] and Kazuma Ohyashiki[1]
[1]Department of Hematology, Tokyo Medical University, Tokyo, Japan

INTERNATIONAL JOURNAL OF ONCOLOGY 46: 2488-2496, 2015

## A novel compound against oncogenic Aurora kinase A overcomes imatinib resistance in chronic myeloid leukemia cells

ZI-JIE LONG[1*], LE-XUN WANG[1*], FEI-MENG ZHENG[2,3], JIA-JIE CHEN[1], YU LUO[4], XI-XIANG TU[3], DONG-JUN LIN[1], GUI LU[4] and QUENTIN LIU[1-3]

SCIENTIFIC REPORTS

OPEN

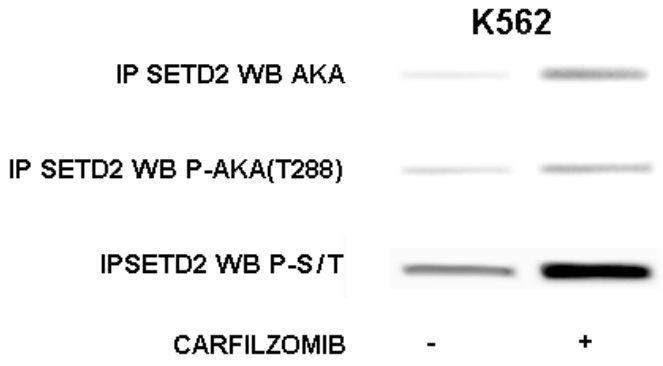
## Aurora A Kinase Inhibitor AKI603 Induces Cellular Senescence in Chronic Myeloid Leukemia Cells Harboring T315I Mutation

Received: 14 April 2016
Accepted: 29 September 2016
Published: 08 November 2016

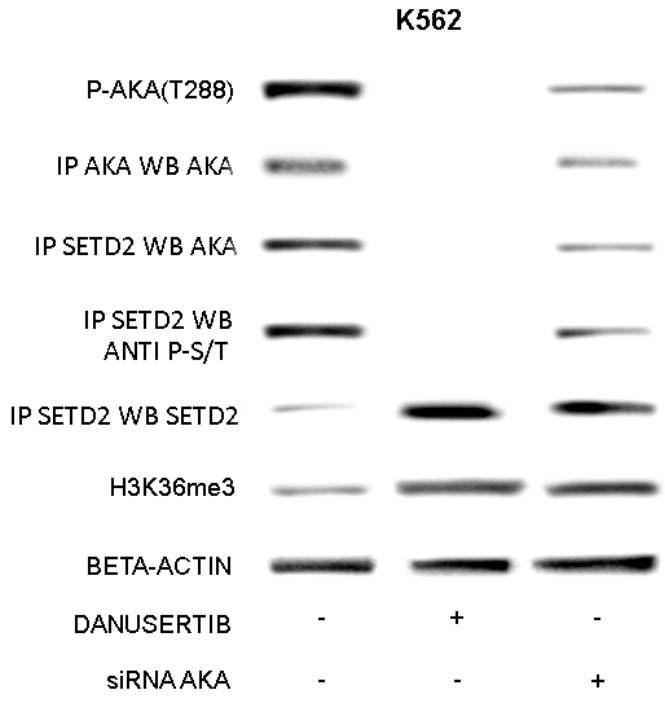
Le-Xun Wang[1,2,*], Jun-Dan Wang[1,*], Jia-Jie Chen[1,*], Bing Long[1], Ling-Ling Liu[1], Xi-Xiang Tu[3], Yu Luo[4], Yuan Hu[1], Dong-Jun Lin[1], Gui Lu[4], Zi-Jie Long[1] & Quentin Liu[1,2,3]

*https://thebiogrid.org/118845/summary/homo-sapiens/setd2.html*

# SETD2 co-immunoprecipitates with Aurora kinase A




% APOPTOSIS 5,5 67,8

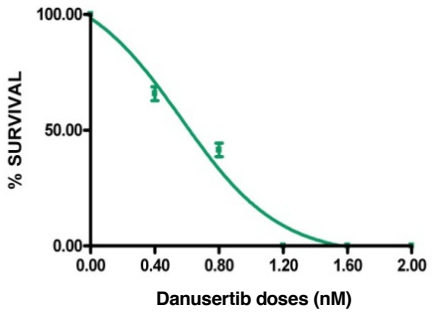



% APOPTOSIS 5,5 70,8 58,4

SETD2 hyper-phosphorylation by Aurora kinase A induces its proteasomal mediated degradation

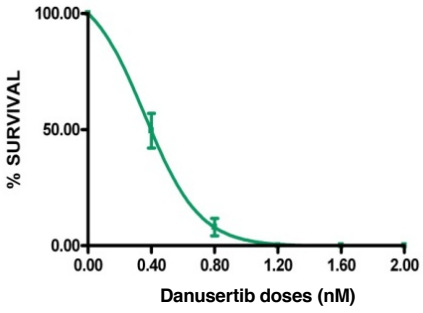
# AKA inhibition induces reduction of clonogenic growth

**LAMA 84**




**HIGH SETD2**

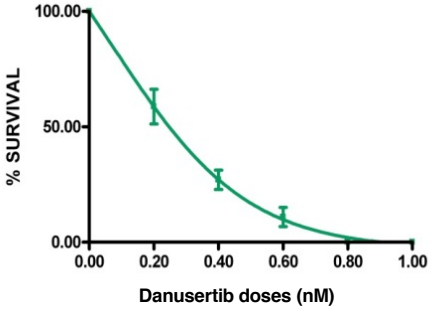
**LAMA 84 KD$^{SEDT2}$**




**LOW SETD2**

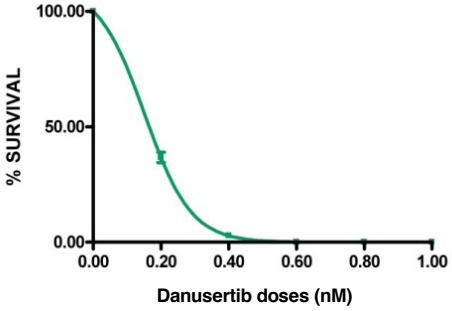
| | LAMA 84 | LAMA 84 siRNA |
|---|---|---|
| $LD_{50}$ Danusertib | 0,7200 nM | 0,3800 nM |

**K562**




**LOW SETD2**

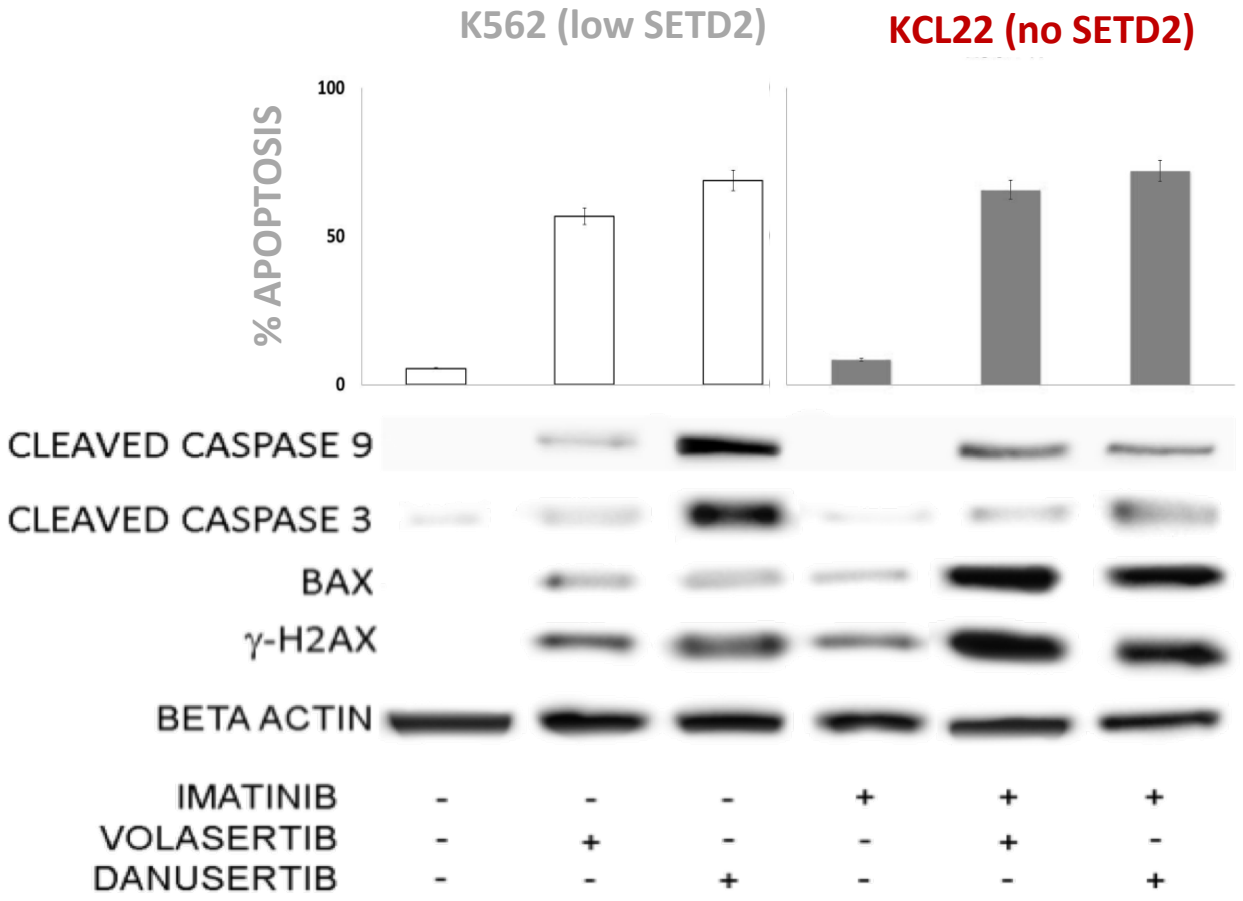
**KCL22**



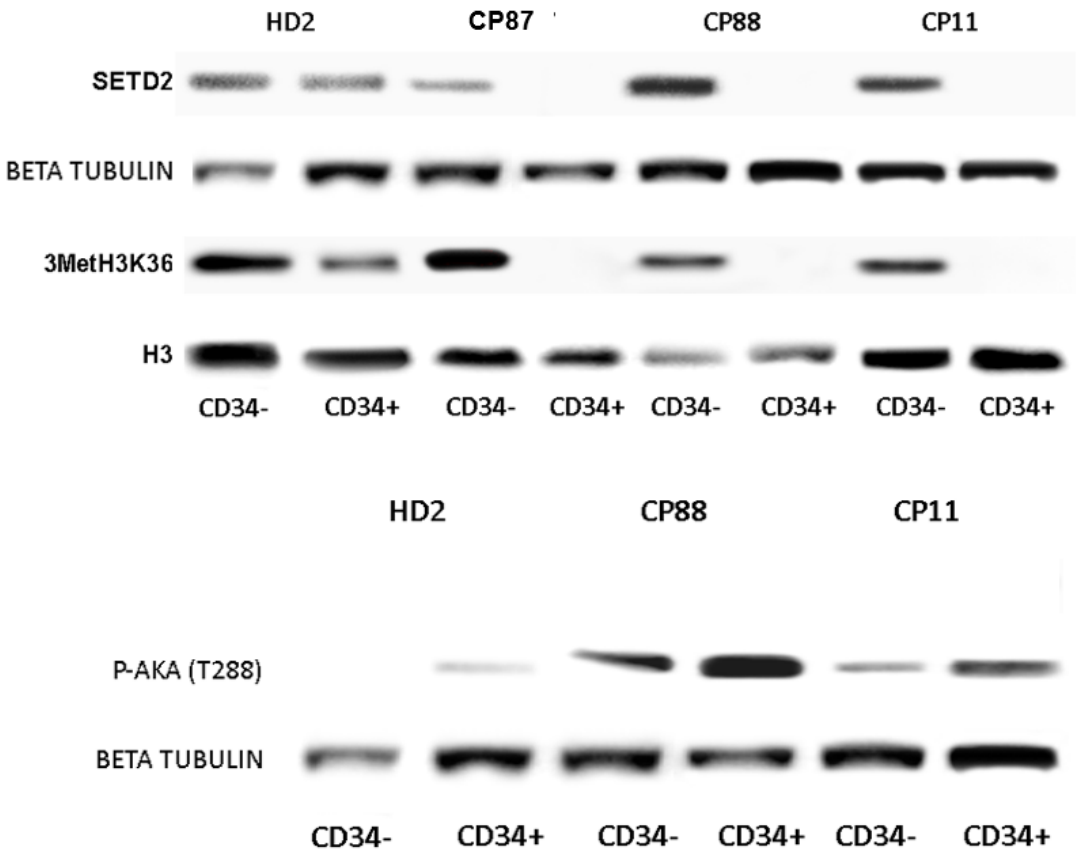

**NO SETD2**

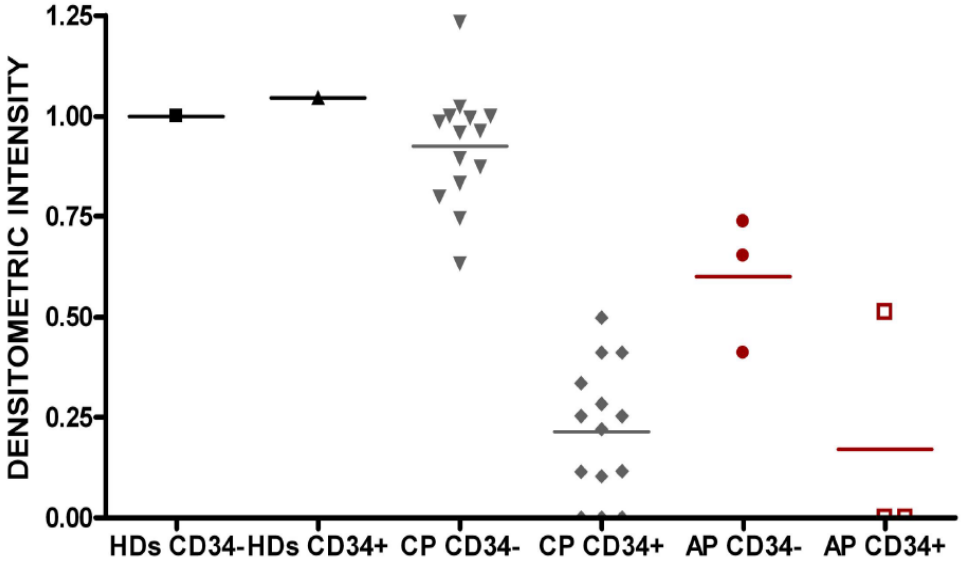
| | K562 | KCL22 |
|---|---|---|
| $LD_{50}$ Danusertib | 0,2367 nM | 0,1633 nM |

# AKA inhibition induces p53 dependent apoptosis

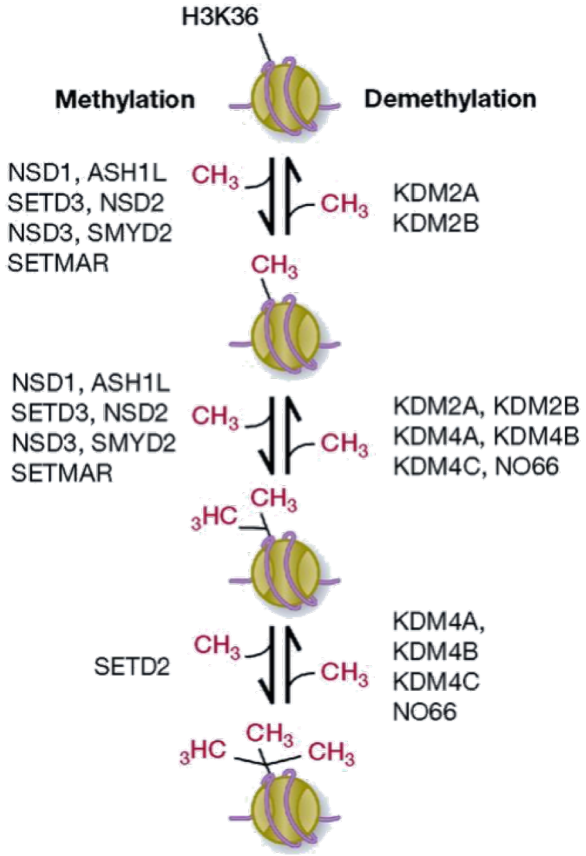


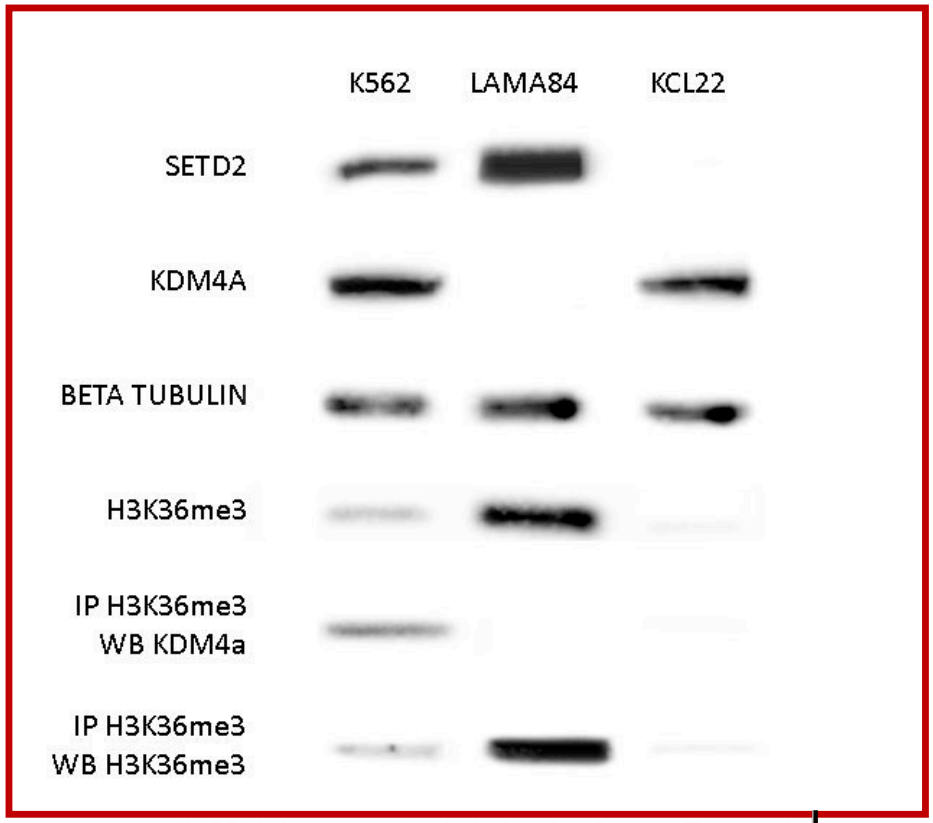
# SETD2 is down-modulated in CD34+ cells of CP-patients



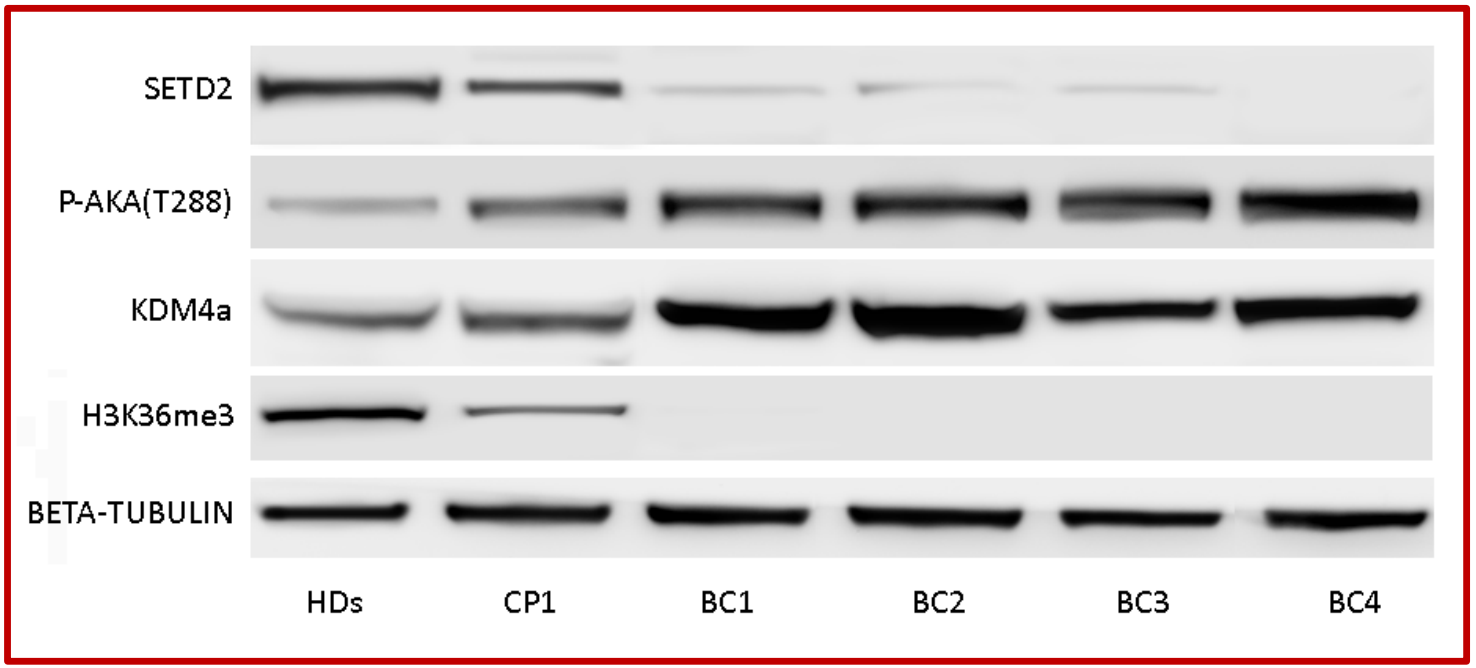



# KDM4A is overexpressed and de-methylates H3K36me3 in K562

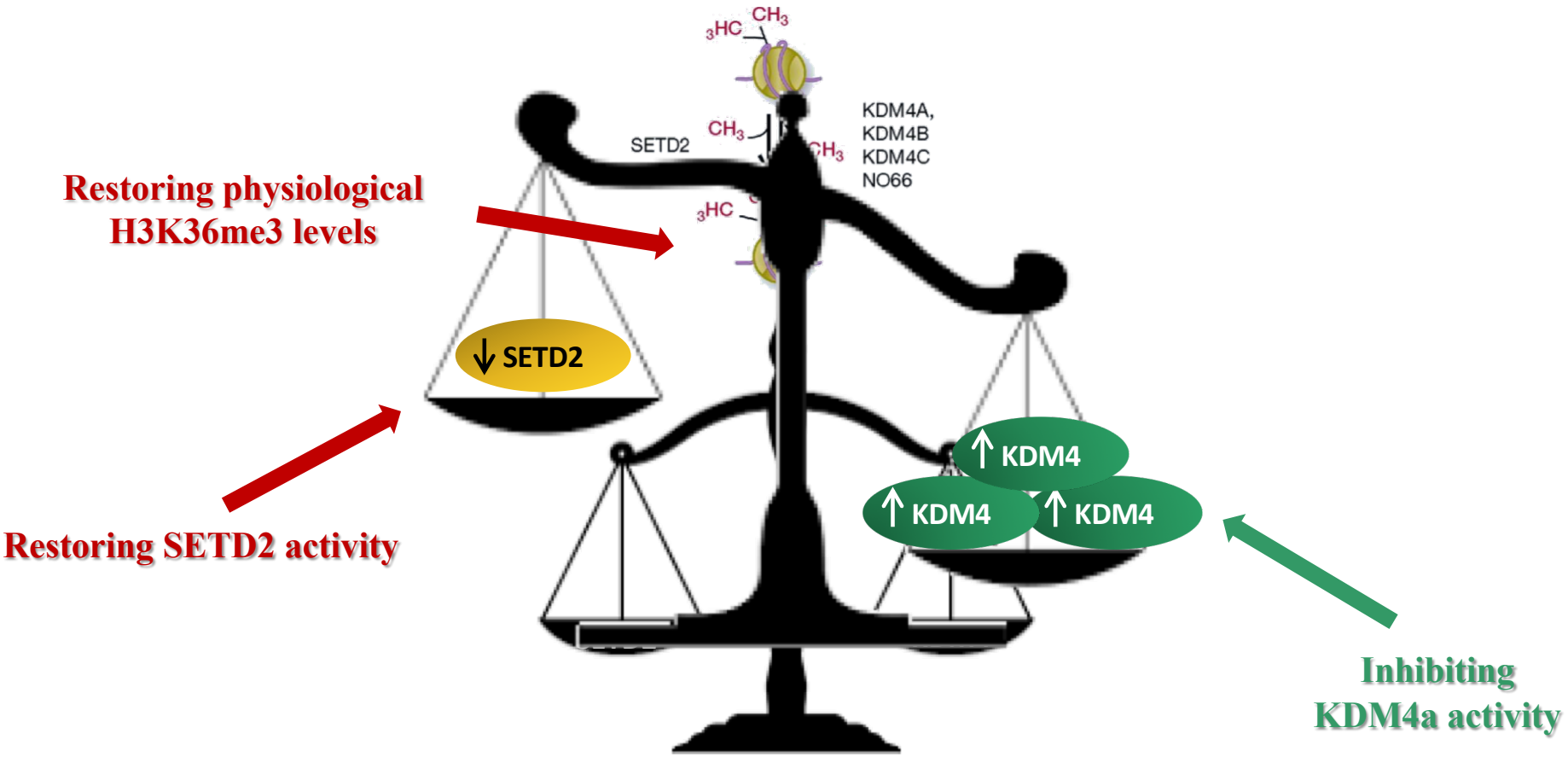


# 2020 KDM4A is overexpressed and de-methylates H3K36me3 in BC-CML patients



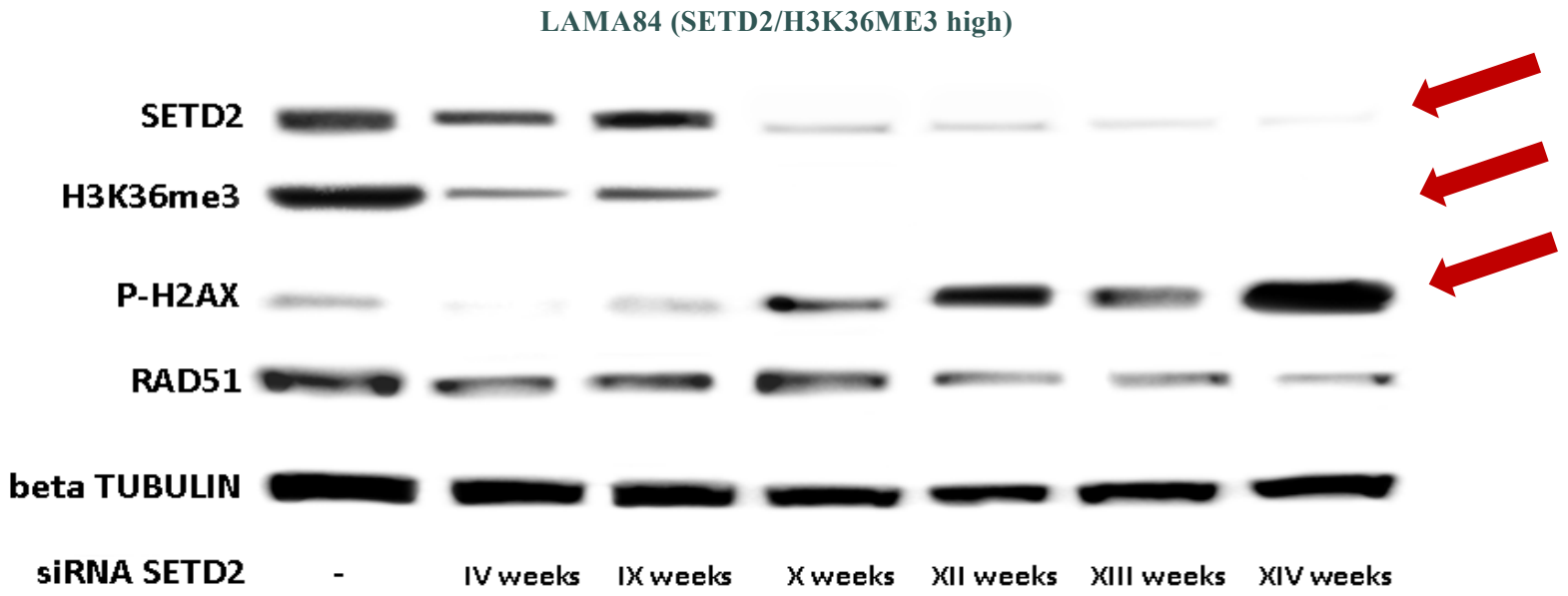
# CONCLUSIONS




**MAY IMPROVE THE OUTCOME OF ADVANCED SM AND CML PATIENTS**

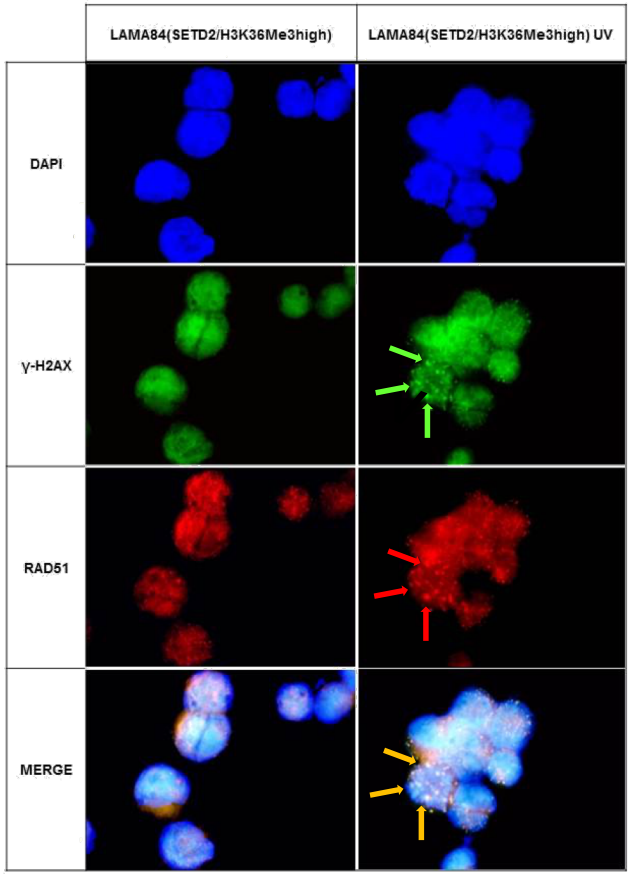
# SETD2/H3K36me3 loss contribute to genetic instability

LAMA 84 (SETD2/H3K36me3high) cells were studied by western blotting to assess phosphorylated histone 2A.X (γH2AX) and Rad51 in steady state conditions after silencing SETD2 by siRNA for 14 weeks

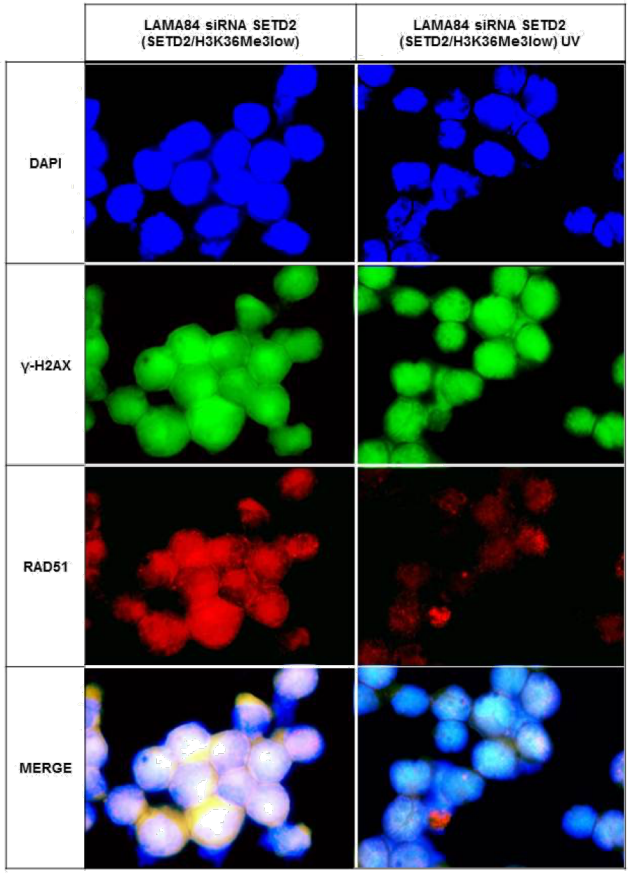


# SETD2 loss affects cell ability to repair to DNA damage

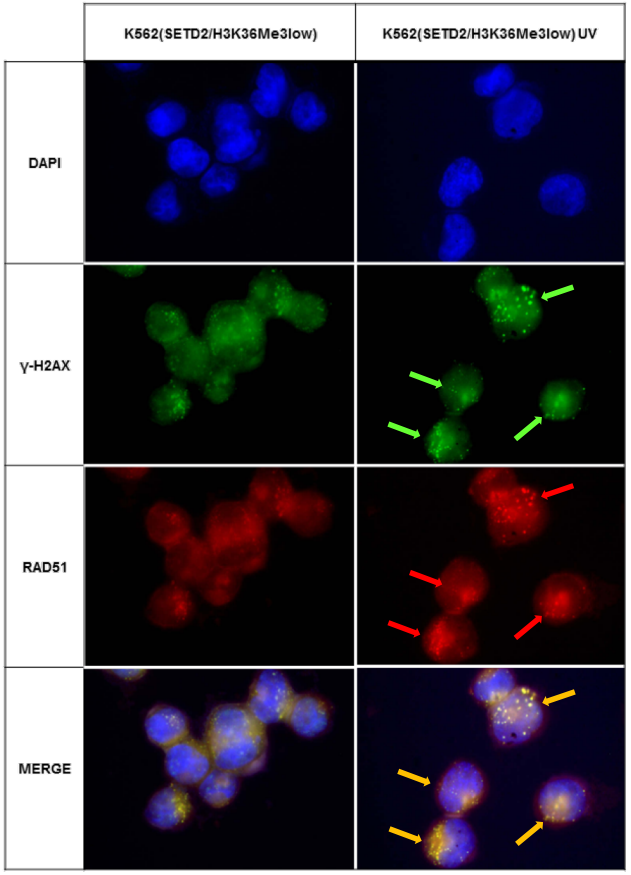
**HIGH SETD2**




**NO SETD2**




**LOW SETD2**



# Take home messages

- ✓ SETD2 down-modulation and H3K36Me3 deficiency are frequently associated with advanced forms of SM and CML
- ✓ SETD2 loss of function is due to post-translational modification rather than being the results of genetic/genomic hits or transcriptional repression
- ✓ Proteasomal inhibition rescues SETD2 expression

# Take home messages

✓AKA and MDM2 induce druggable post-translational modifications

✓CD34+ cells from CP-CML patients display reduced SETD2 and H3K36Me3 levels

✓SETD2 and H3K36Me3 loss might contribute to the genetic instability that is the hallmark of advanced-phase CML

# ACKNOWLEDGEMENTS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PROF. MICHELE CAVO

Ministero della Salute

*Project GR-2016-02364880*

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GIMEMA CML WP

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