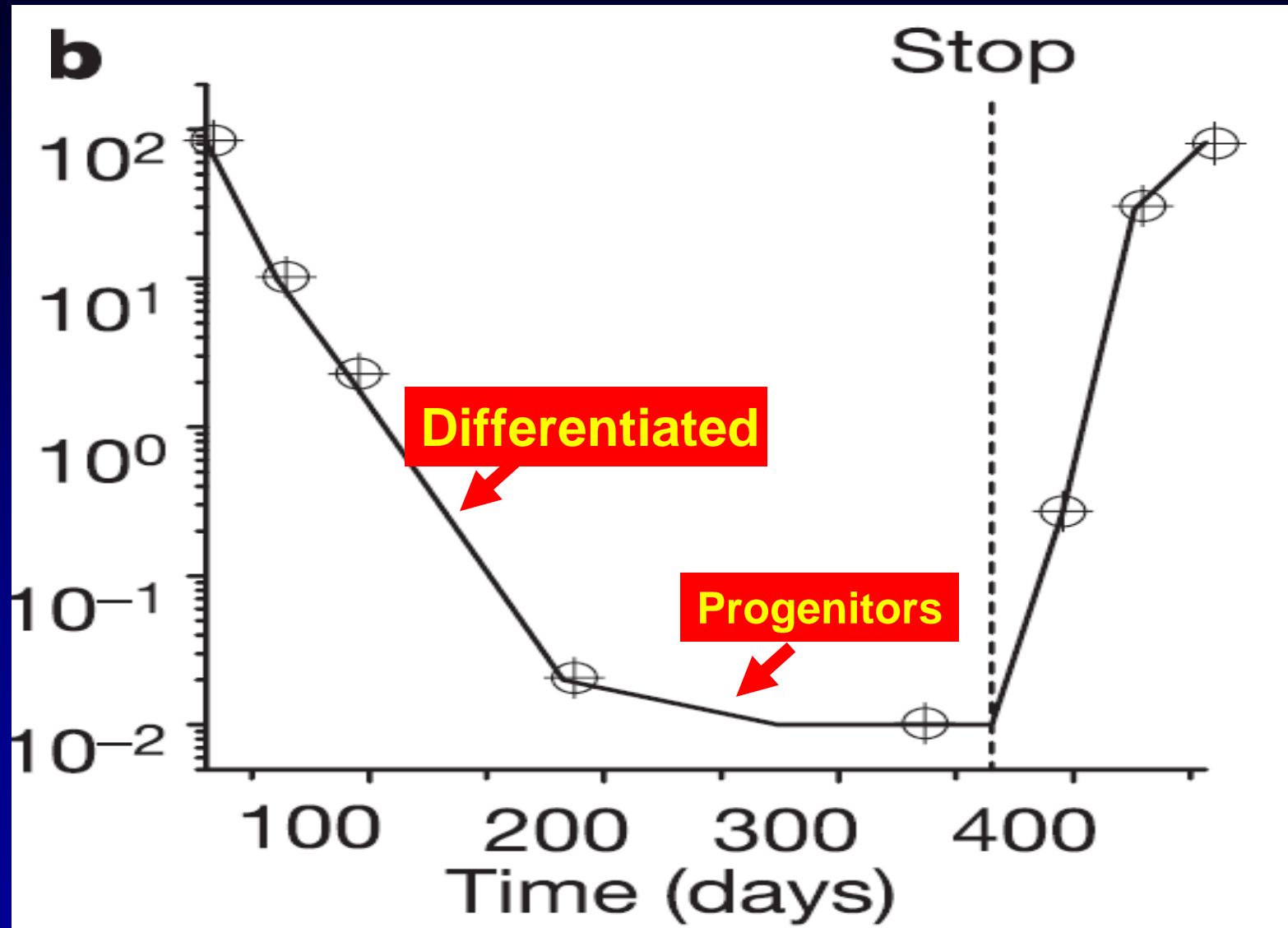


# Minimal residual disease in responding patients: therapeutic strategies

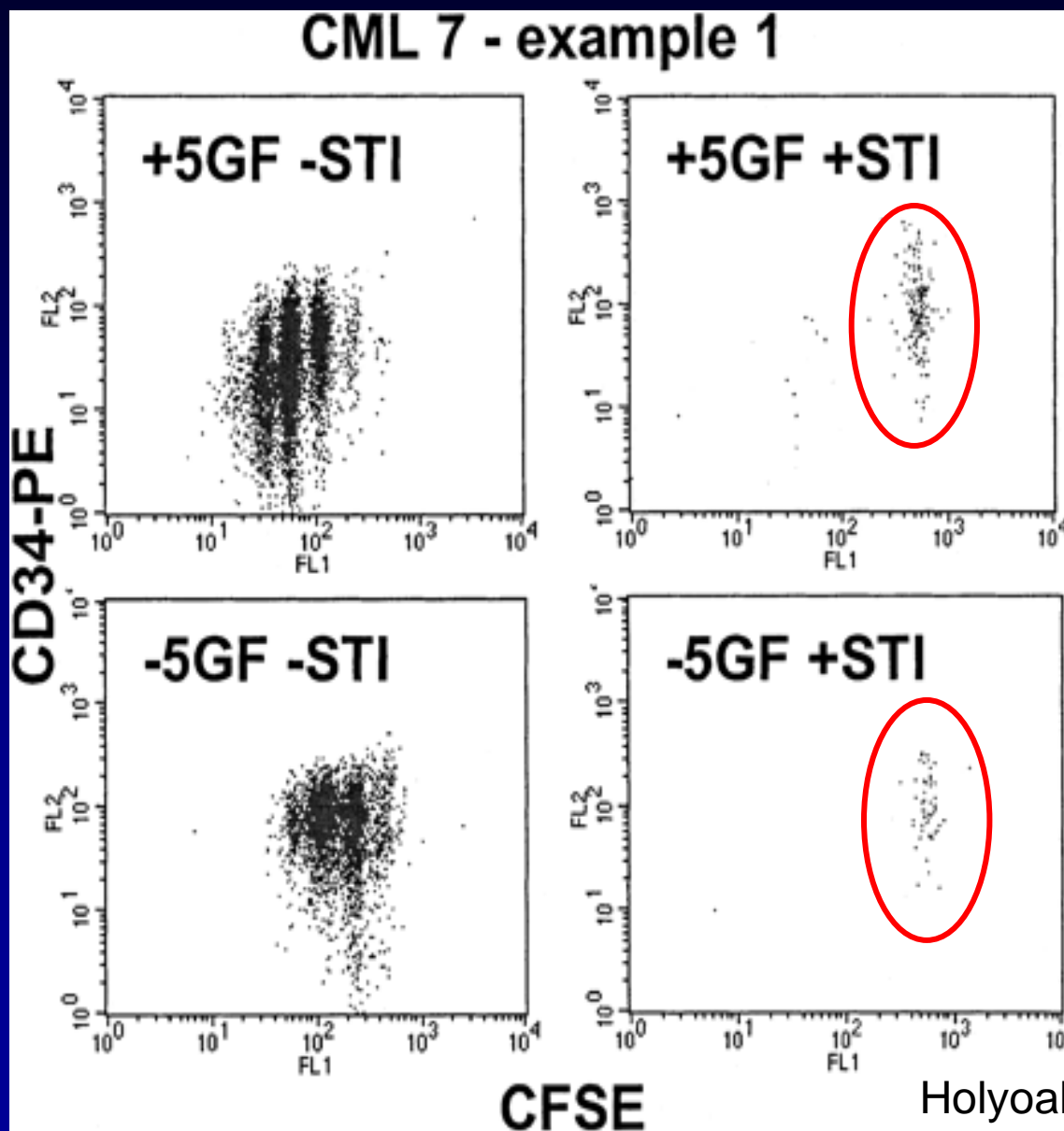
Giuseppe Saglio  
University of Turin

# Dynamics of chronic myeloid leukaemia



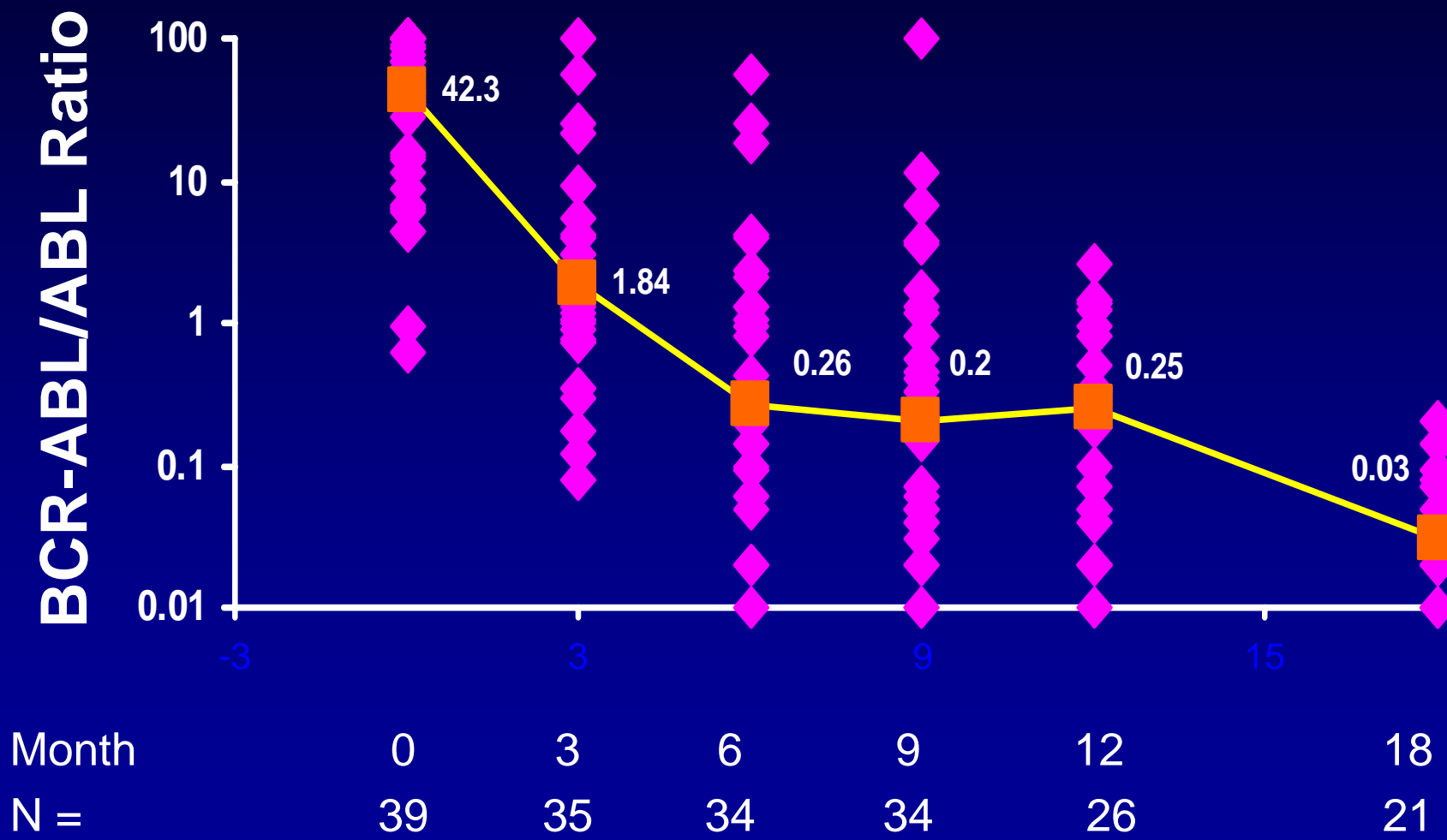
Michor et al., Nature 2005

# Primitive quiescent BCR-ABL+ leukemic stem cells are less sensitive to imatinib



# Dasatinib in Early CP CML

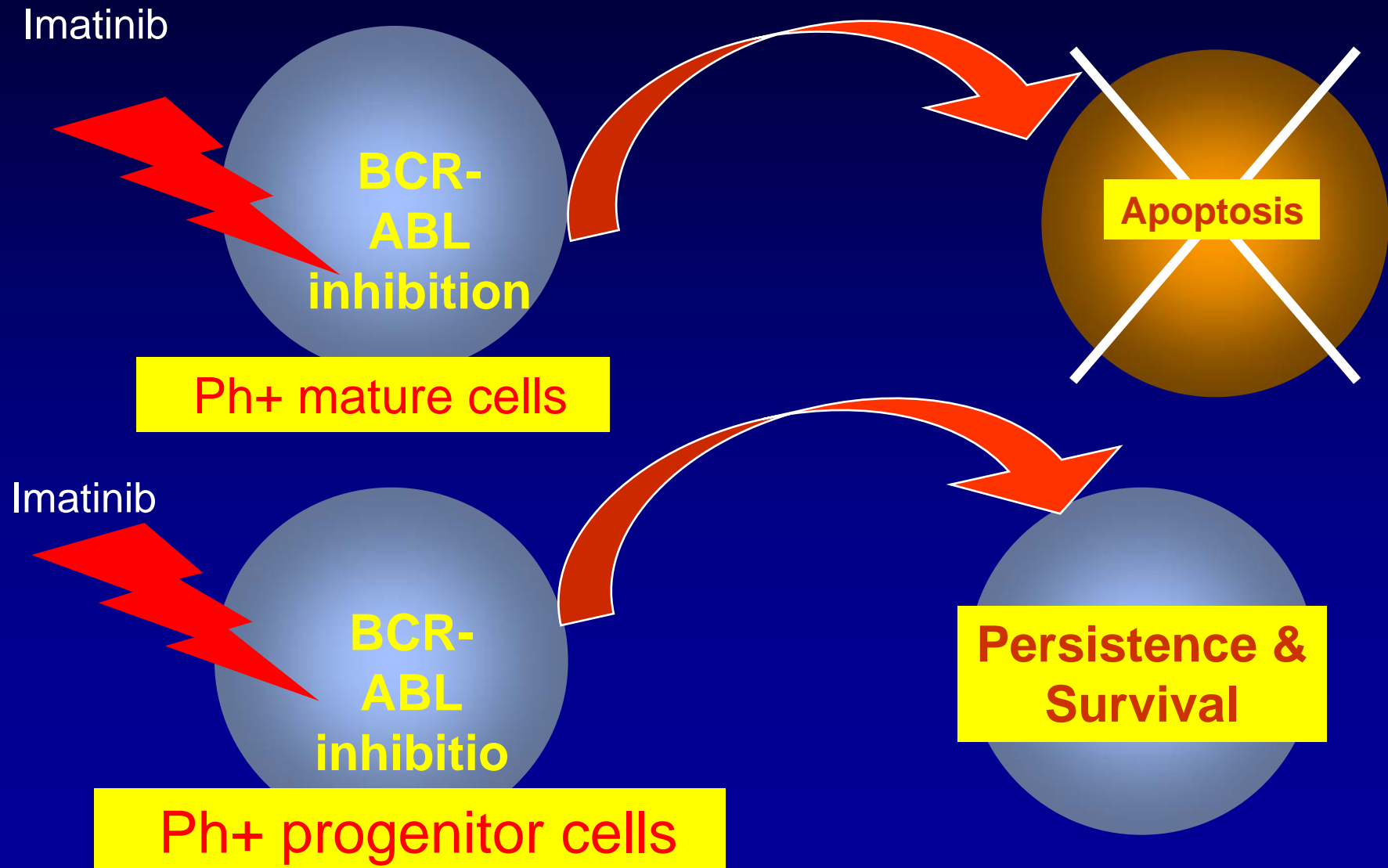
## Molecular Responses



# Mechanisms of resistance of Ph+ progenitors to TKI

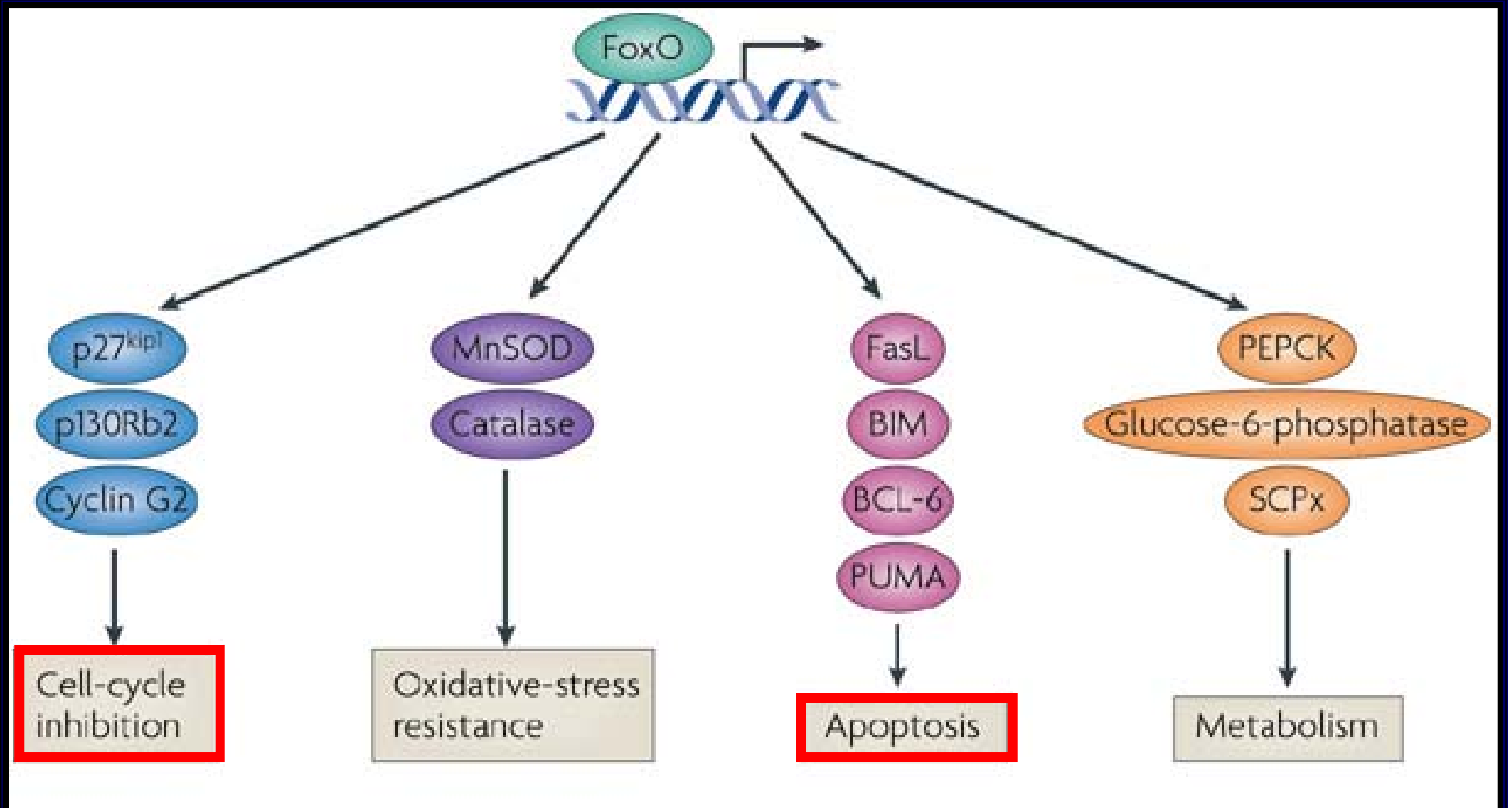
- Intrinsic mechanisms
  - Higher expression of BCR-ABL
  - Lower concentration of intracellular drugs
- Natural phenomenon:
  - the stem cells behave as stem cells
  - BCR-ABL TK activity for them is “redundant”
  - the BCR-ABL TK activity has different effect at different levels of myeloid differentiation

In the progenitor cell population of CML, the BCR-ABL TK activity may be suppressed without any damage

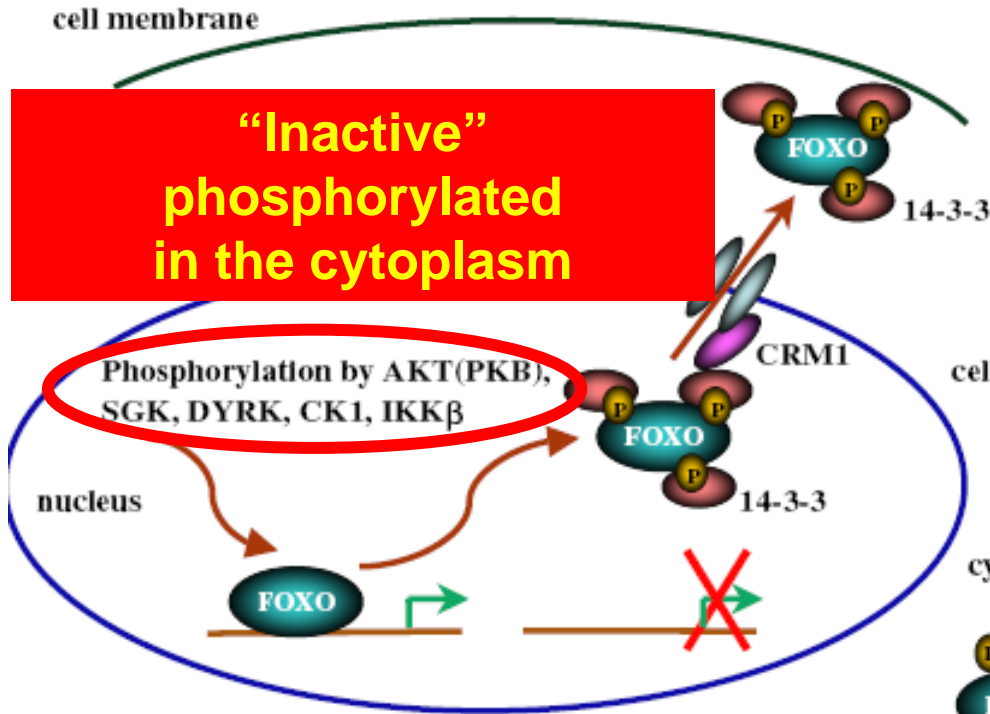


# Example: FoxO transcription factors

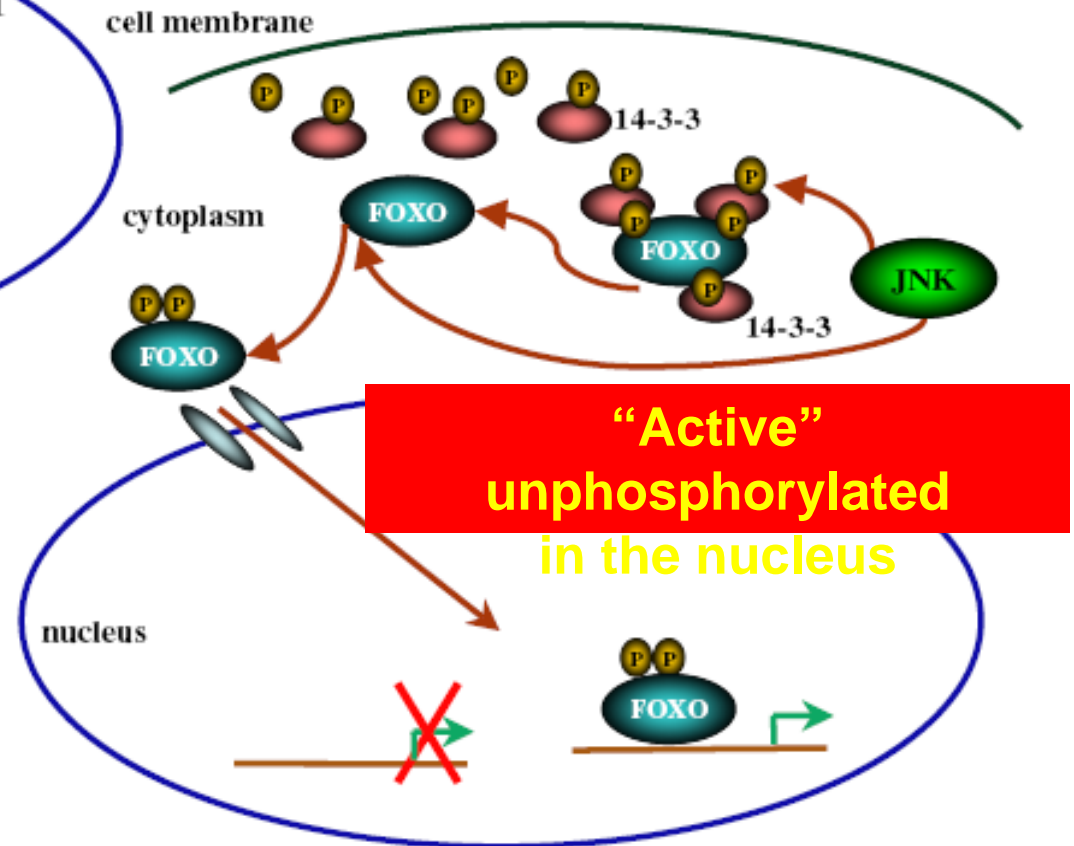
- FoxOs code for transcription factors belonging to the Forkhead family of transcription factors
- Part of PI3K/AKT pathway
- FoxO transcription factors mediate apoptosis and regulate cell cycle



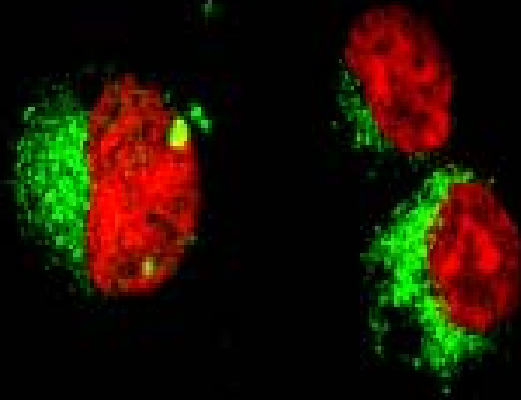
A In the presence of growth and/or survival signals



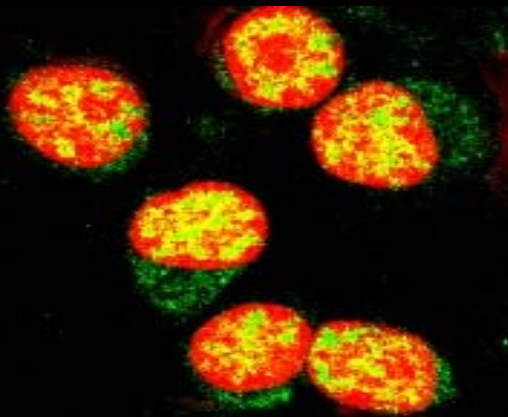
B In the presence of stress stimuli



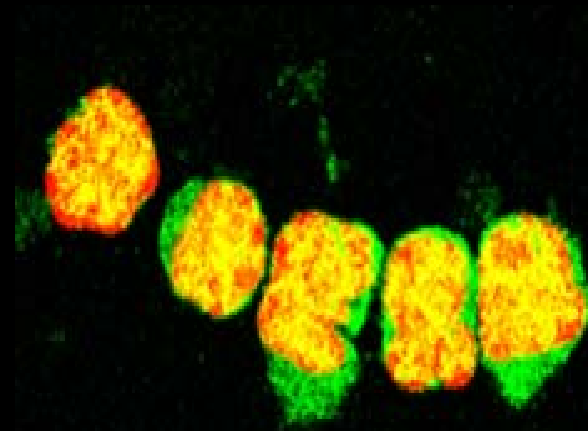
# Immunofluorescence for detection of FOXO in CML and in controls



CML

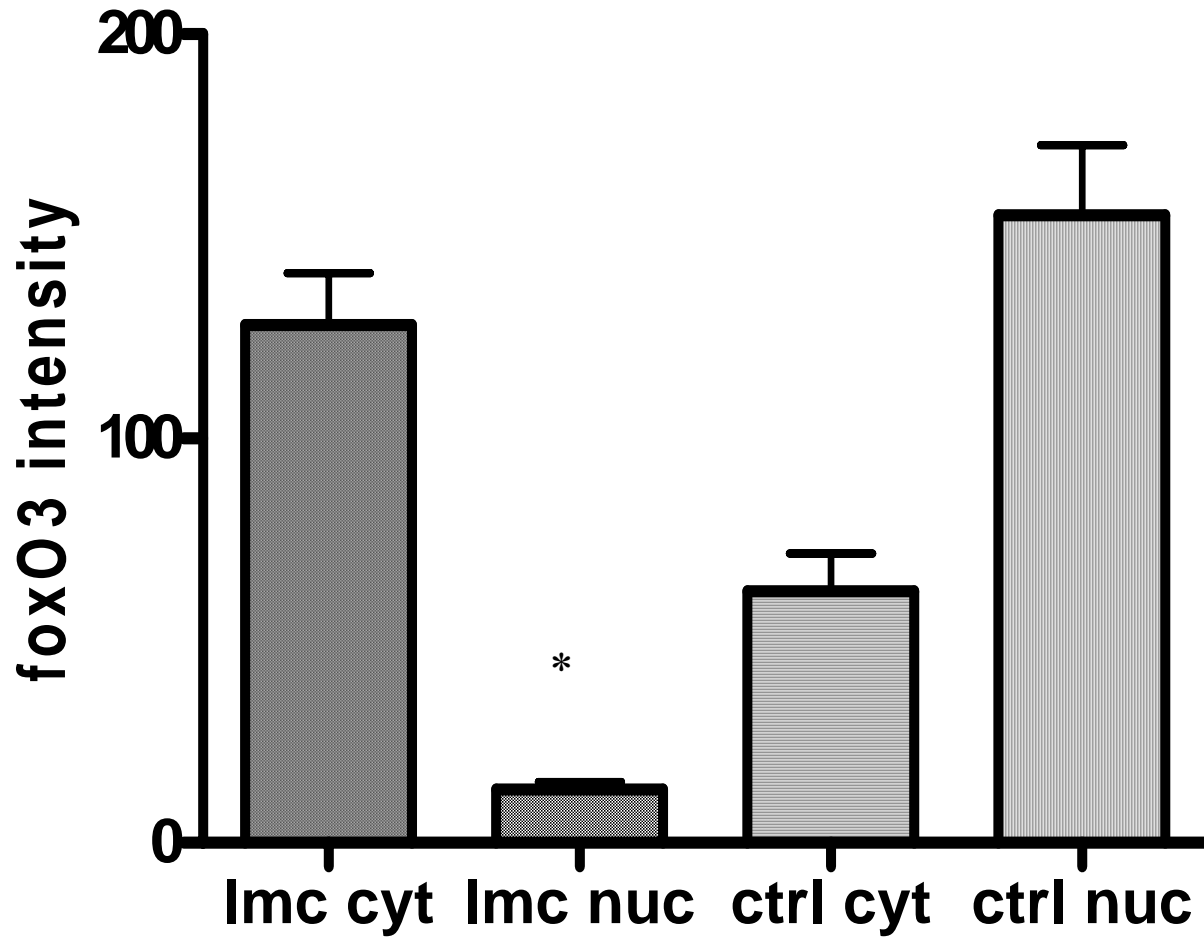


Control

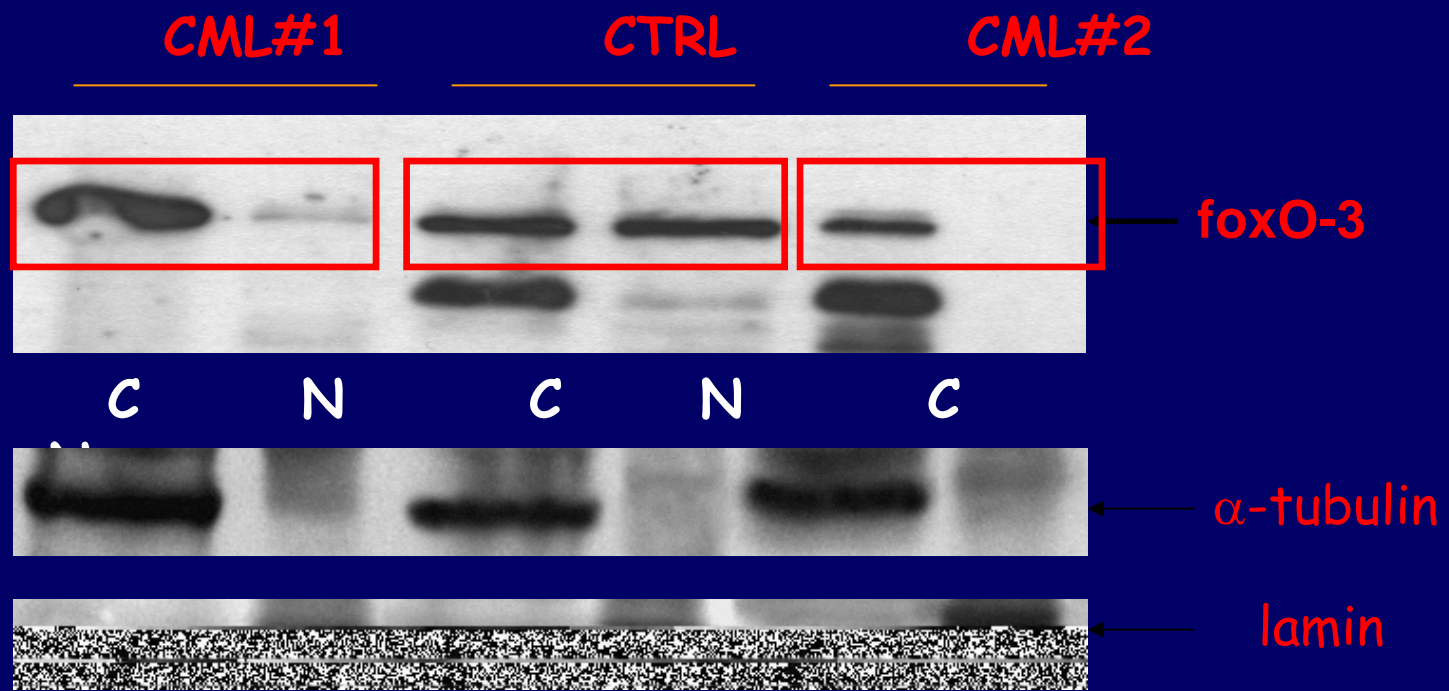


CML in CCyR

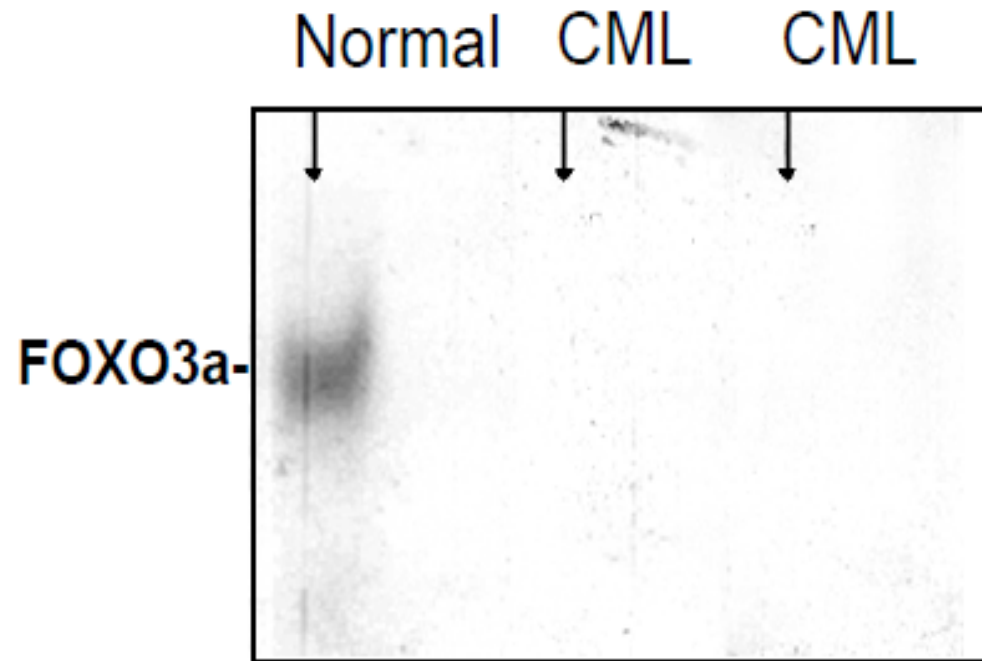
# Quantification of FoxO signal



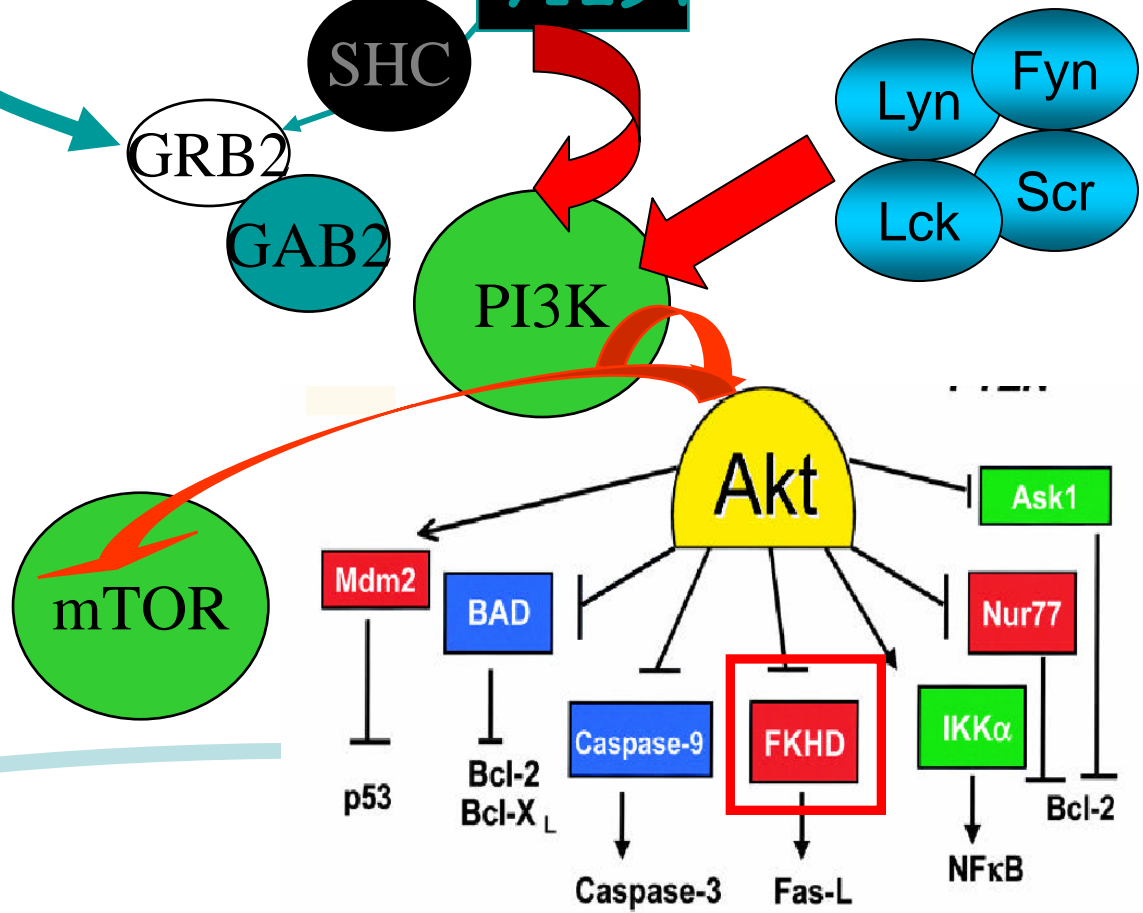
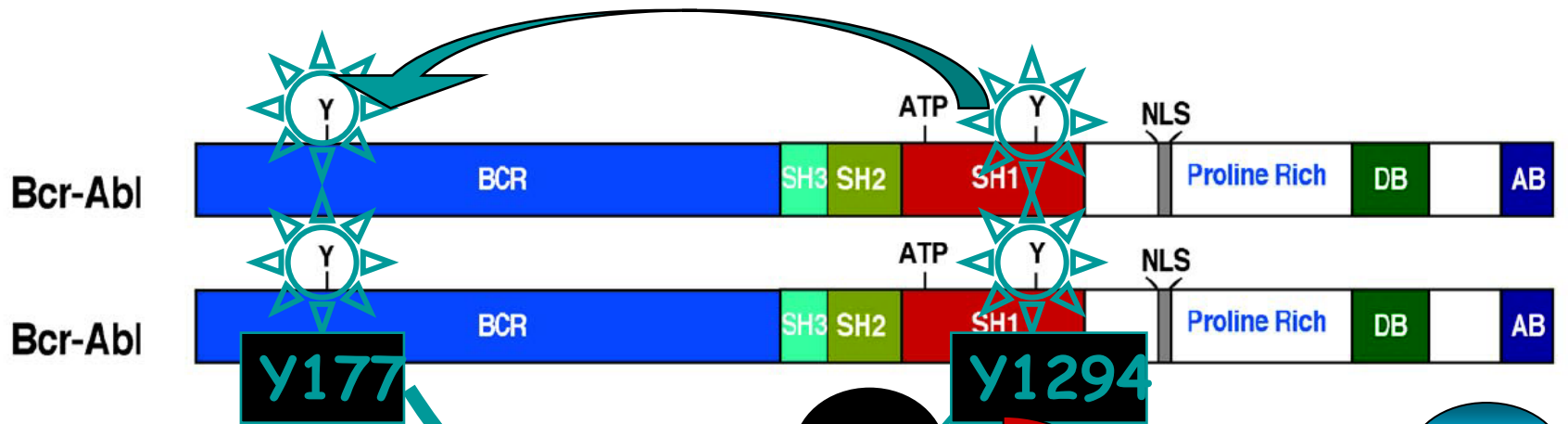
# FoxO 3 protein in CML



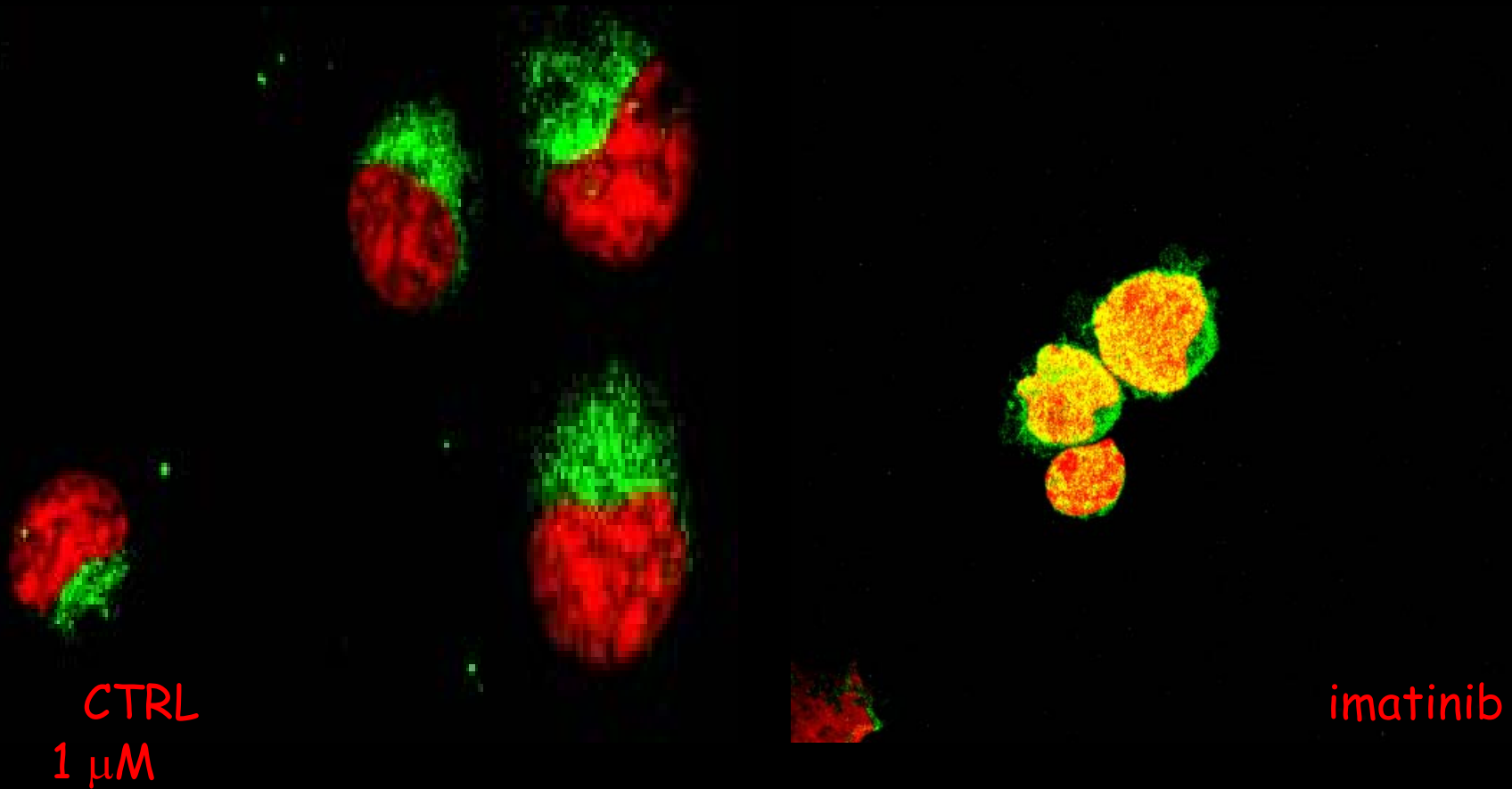
# EMSA for the evaluation of FoxO DNA binding activity



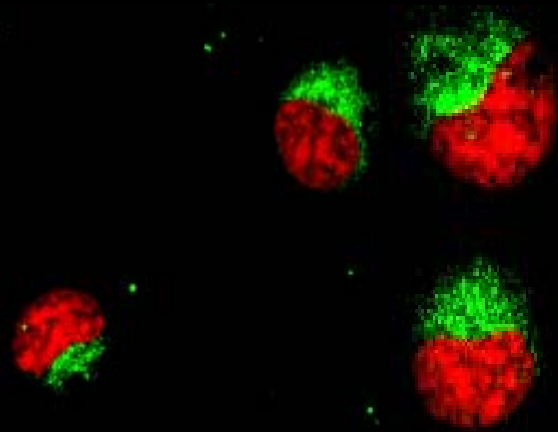
Consensus sequence foxO3: TACTGTTTTGACG



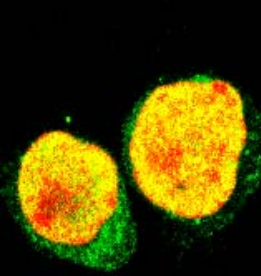
# FoxO re-localization in CML following imatinib treatment



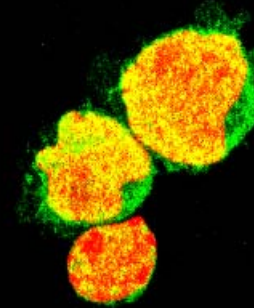
# IKK Inhibition also induces FoxO re-localization



CTRL

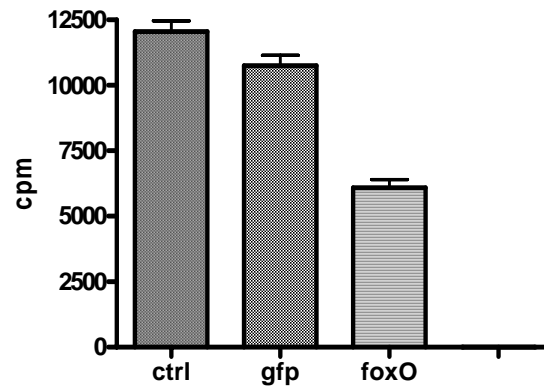


PS1145

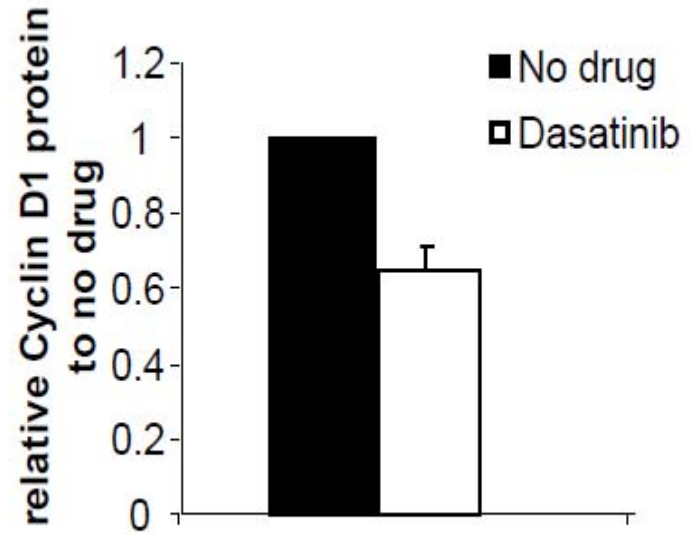
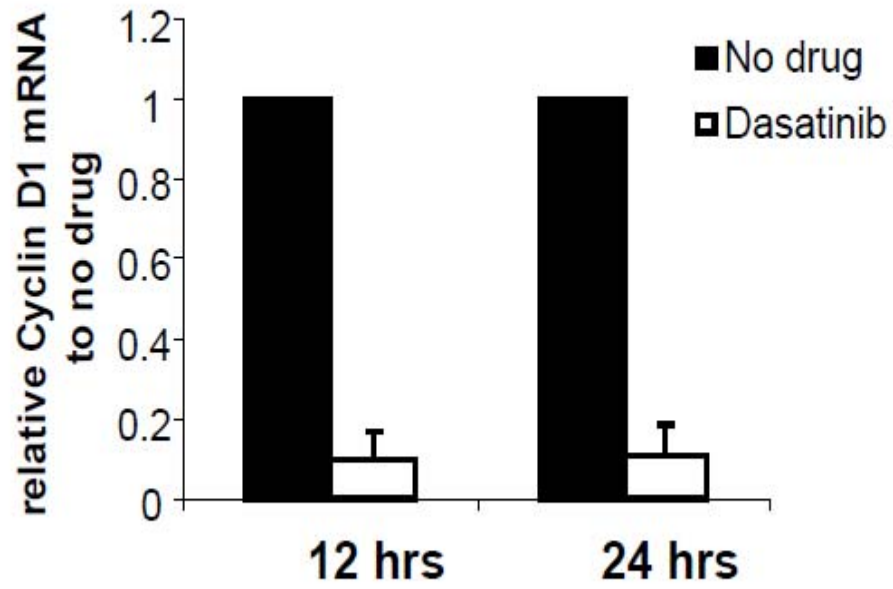


PS1145+LY294002

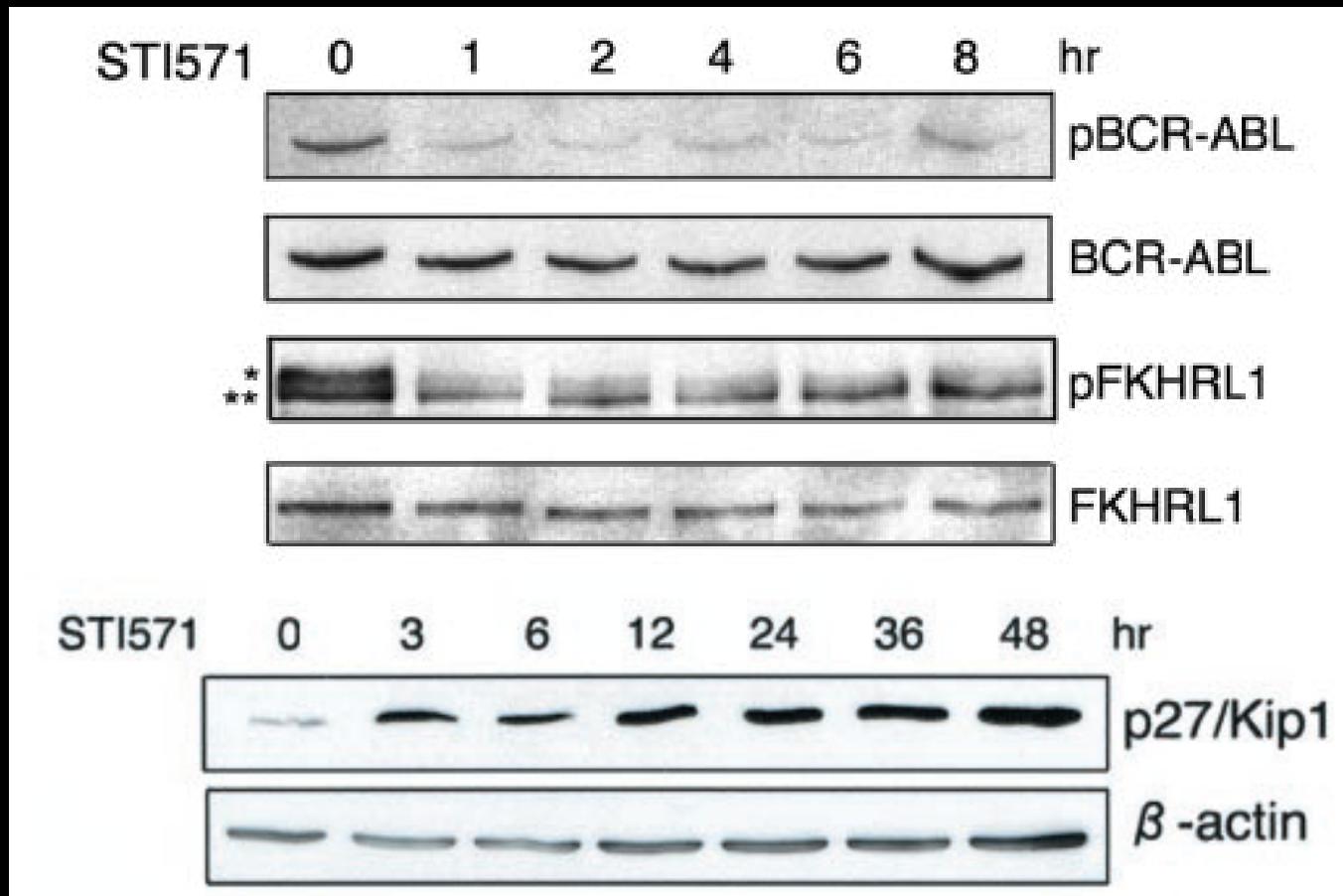
# Proliferation of K562 after transfection with FoxO mutant form that cannot be phosphorylated (constitutively activated)



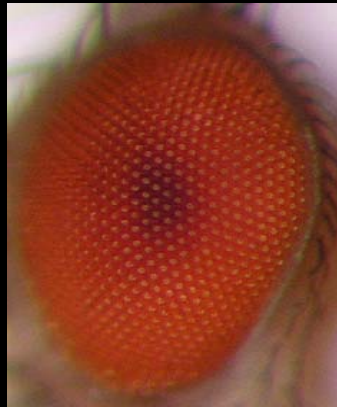
**A**



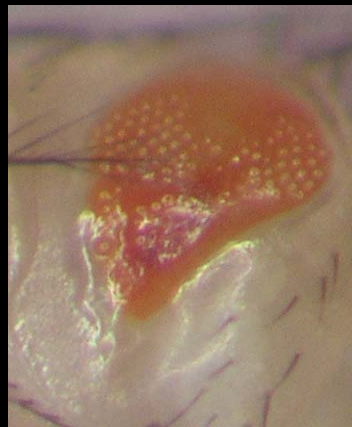
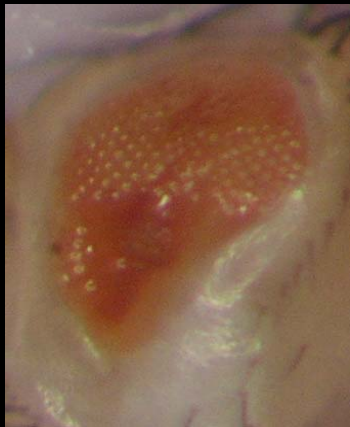
# A Member of Forkhead Transcription Factor FKHRL1 Is a Downstream Effector of STI571-induced Cell Cycle Arrest in BCR-ABL-expressing Cells\*



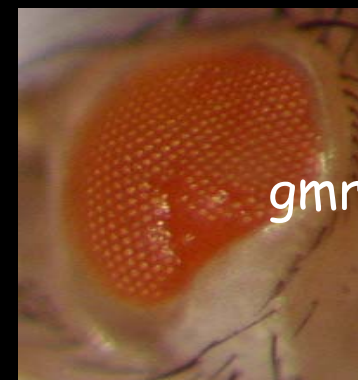
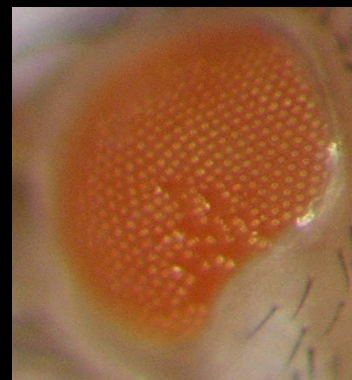
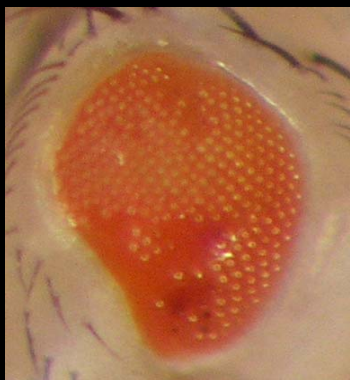
*Komatsu N et al. The Journal of Biological Chemistry, 2003; 6411*



gmrG4/ uas **bcr-abl** 1M



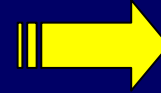
gmrG4, uas **dfoxO**/uas  
lacZ



gmrG4, uas **dfoxO**/uas **bcr-abl**1M

**FoxO is directly inactivated by BCR-ABL**

BCR-ABL inhibition by imatinib  
inhibition



PI3K/Akt



FoxO re-activation



Stem and Progenitor cells quiescence

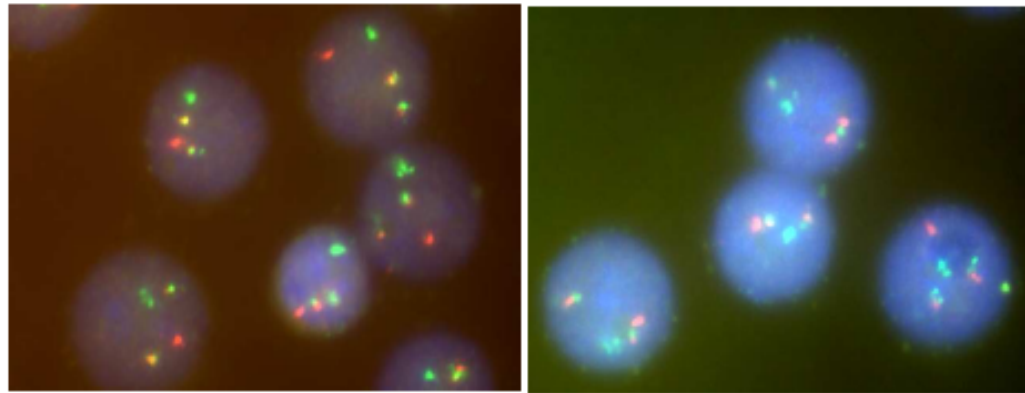


Resistance to TKIs

Is imatinib resistance of CML progenitor cells mainly due to re-activation of FoxO?

Imatinib therapy may help cells to  
restore  
a number of “normal functions” and this  
may lead to prolong survival of Ph+  
early progenitor cells

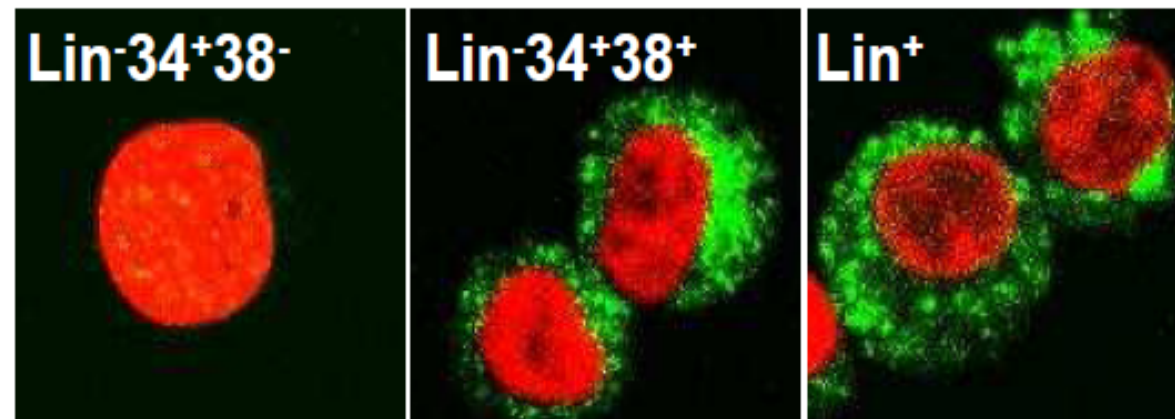
**B**



**CD34<sup>+</sup>38<sup>+</sup>**

**CD34<sup>+</sup>38<sup>-</sup>90<sup>+</sup>**

**C**



**Lin<sup>-</sup>34<sup>+</sup>38<sup>-</sup>**

**Lin<sup>-</sup>34<sup>+</sup>38<sup>+</sup>**

**Lin<sup>+</sup>**

# Conclusions

- TKI-induced G1 arrest is mediated by re-activation of FOXOs, whilst maintenance and quiescence of CML stem/progenitor cells is regulated by on-going FOXO activity despite high BCR-ABL expression.
- Need of new strategies to target CML stem/progenitor cells by preventing or reversing “TKI-induced quiescence” and forcing these cells into cycle and apoptosis.

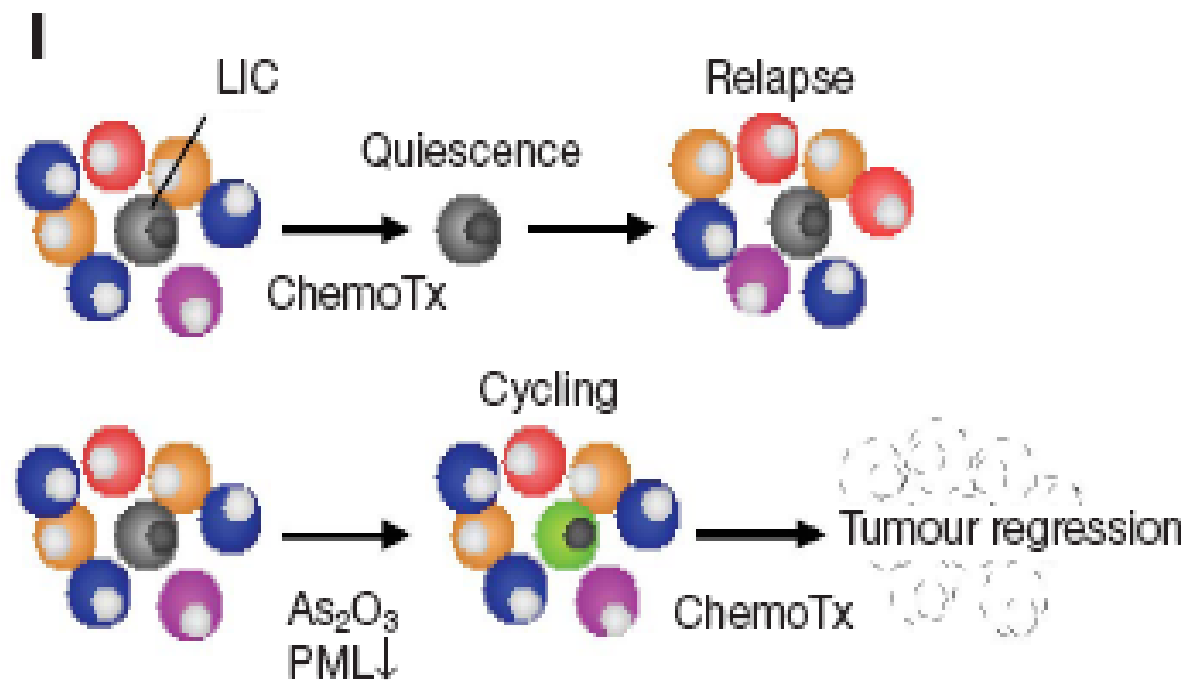
# Strategies to try to eradicate Ph+ stem cells

- It may occur spontaneously after a while (FX Mahon docet) without recurrence of the disease in 50% of the cases
- It may be “stimulated” by immunomodulatory agents like IFN (A. Hochhaus docet)
- We may try to force it
  - Through vaccination processes (Monica Bocchia)
  - Drugs affecting stem cell activated pathways (G. Martinelli approach)

# 1 PML targeting eradicates quiescent leukaemia-initiating cells

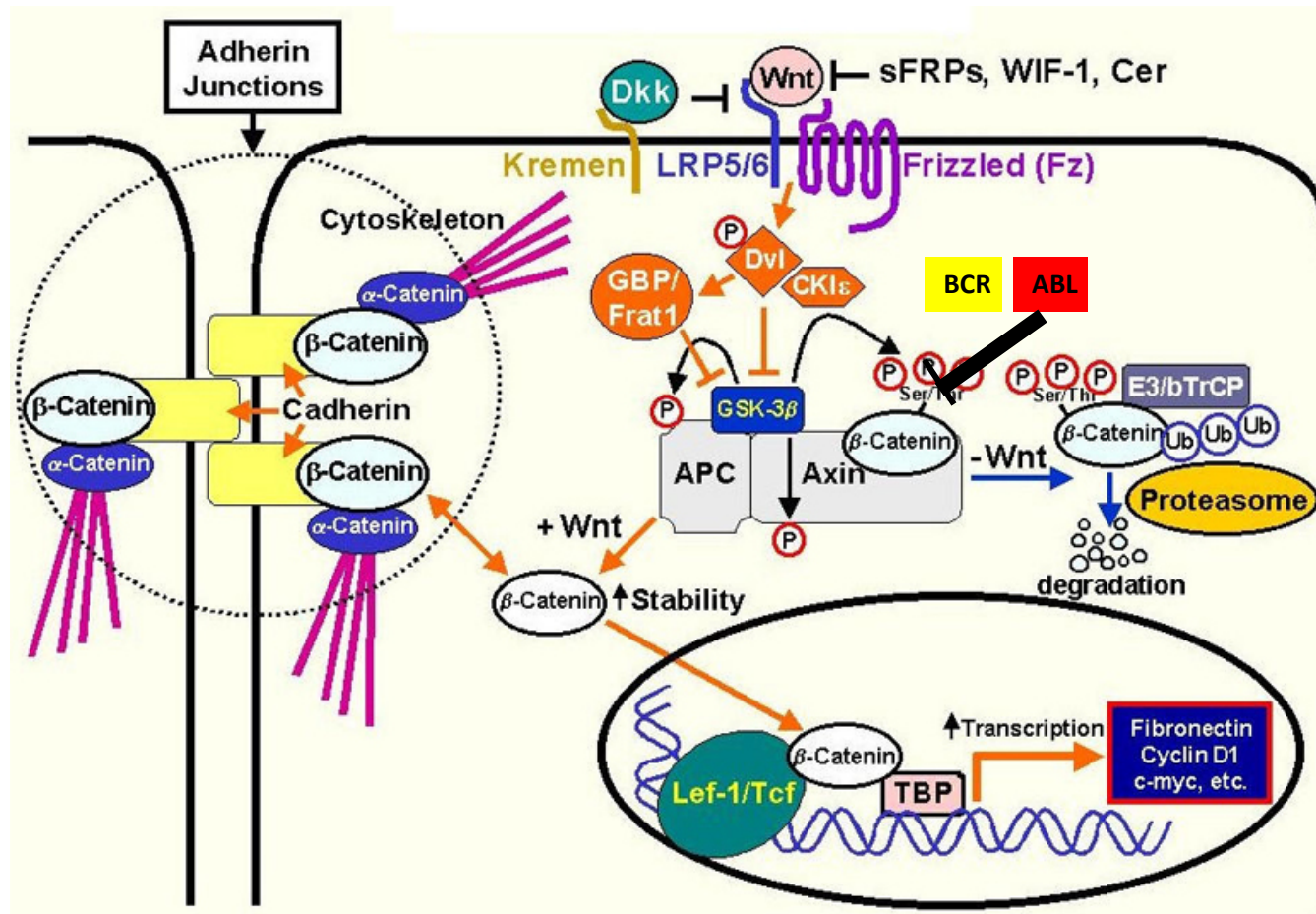
Keisuke Ito<sup>1,3,4</sup>, Rosa Bernardi<sup>1,3,4</sup>, Alessandro Morotti<sup>1,3,4</sup>, Sahoko Matsuoka<sup>5</sup>, Giuseppe Saglio<sup>6</sup>, Yasuo Ikeda<sup>5</sup>, Jacalyn Rosenblatt<sup>2</sup>, David E. Avigan<sup>2</sup>, Julie Teruya-Feldstein<sup>4</sup> & Pier Paolo Pandolfi<sup>1,3,4</sup>

2008



# self renewal targeting

## CML: Wnt1



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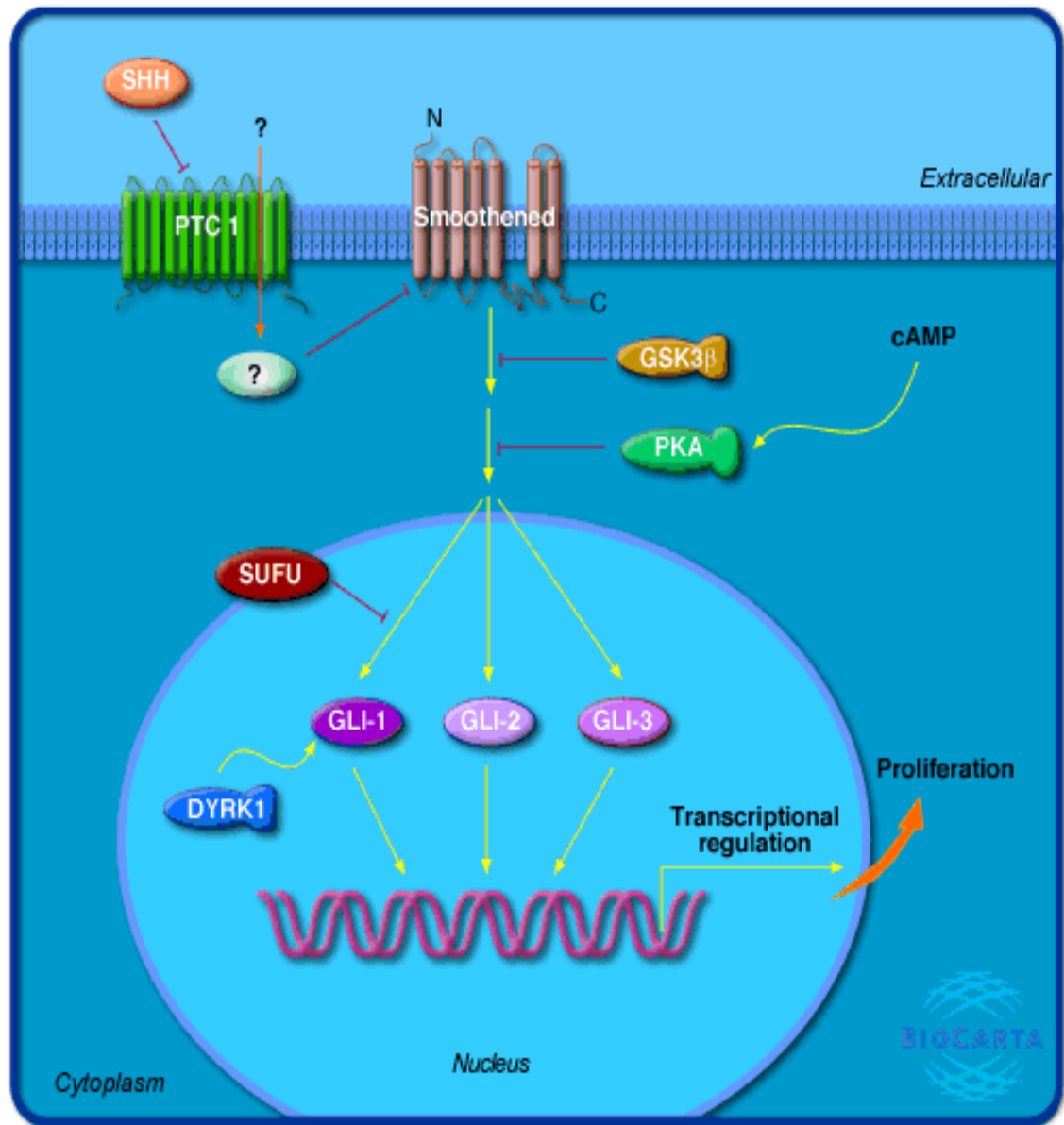
# Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia

Chen Zhao<sup>1\*</sup>, Alan Chen<sup>1\*</sup>, Catriona H. Jamieson<sup>3</sup>, Mark Fereshteh<sup>1</sup>, Annelie Abrahamsson<sup>3</sup>, Jordan Blum<sup>1</sup>, Hyog Young Kwon<sup>1</sup>, Jynho Kim<sup>4</sup>, John P. Chute<sup>2</sup>, David Rizzieri<sup>2</sup>, Michael Munchhof<sup>5</sup>, Todd VanArsdale<sup>6</sup>, Philip A. Beachy<sup>4</sup> & Tannishtha Reya<sup>1</sup>

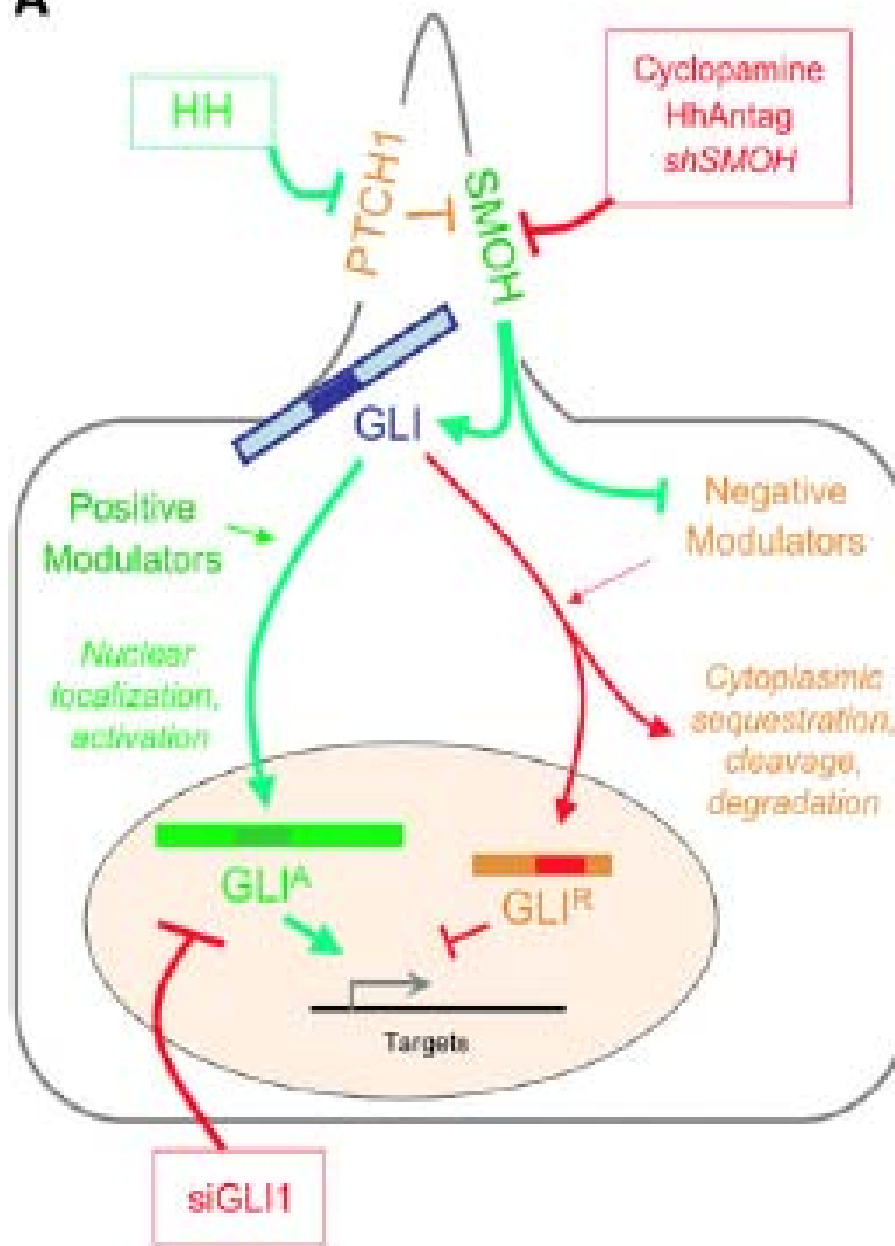
Nature 2009

# Hh signaling and CML self renewal

- Hedgehog and Gli signaling (Hh-GLI) remains one of the most critical pathways in animal patterning during embryogenesis, and may play a key role in human malignancies when aberrantly activated.
- Post birth, the Hh pathway is normally repressed.
- Hh signaling is initiated when Hh binds to Patched (PTCH) and deactivates its function, which in the absence of Hh, negatively regulates signaling.



A



# SMO inhibitor (Hh inhibitors)

- LDE225 (Novartis) (BCC, pancreas, CML?)
- ICG001 (Genetech- Roche)
- BMS114 +/- dasatinib (CML)
- PF0441933 (hematological diseases)
- Cyclophamine (Infinity Pharmaceutical) (BCC, SNC c. pancreas)

- **University of Turin**
- Daniela Cilloni
- Francesca Messa
- Cristina Panuzzo
- Francesca Arruga
  
- **University of Bologna**
- Giovanni Martinelli
- Ilaria iacobucci
  
- **University of Glasgow**
- Francesca Pellicano
- Tessa Holyoake

