Title :

CDK4/CDK6 inhibition as a novel strategy to suppress the growth and survival of BCR-ABL1 T315I+ Clones in TKI-resistant CML

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Presented by:
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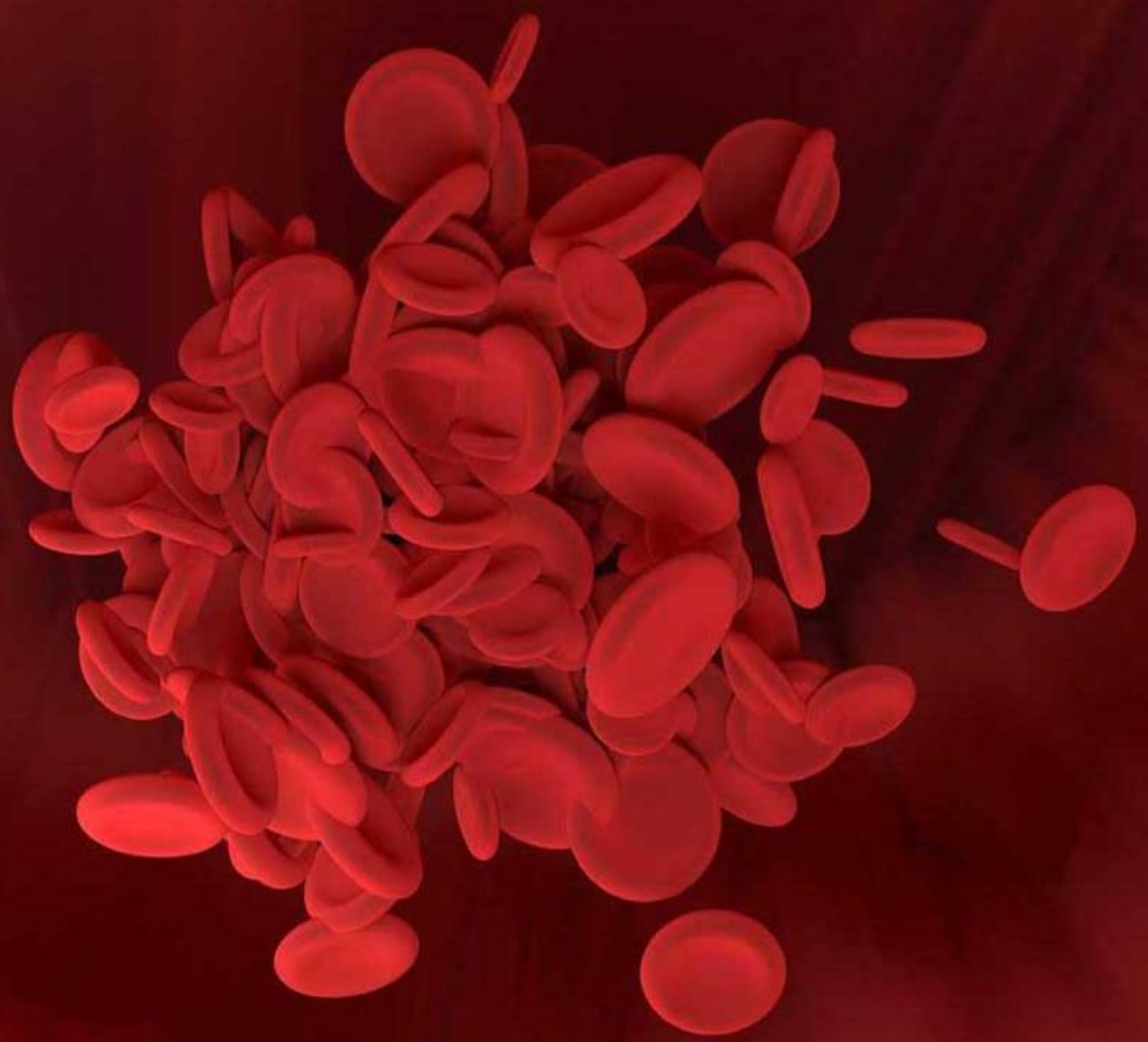
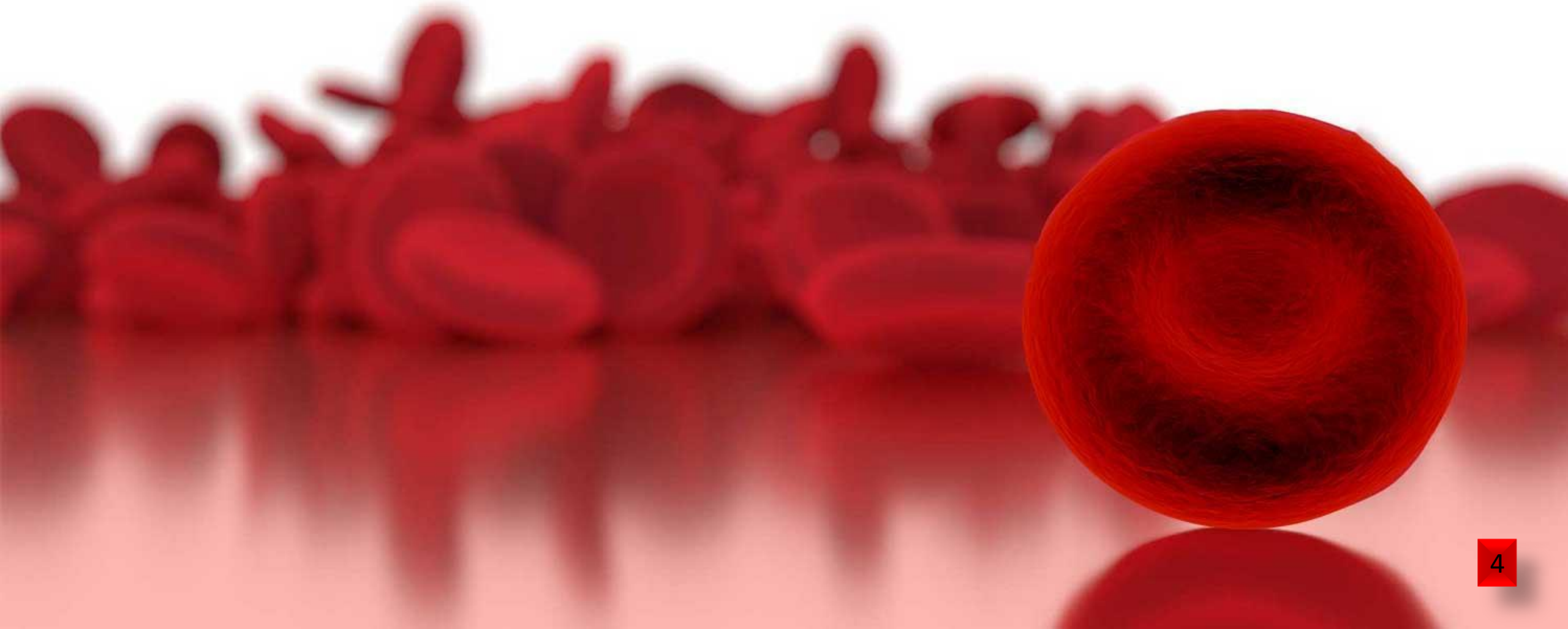
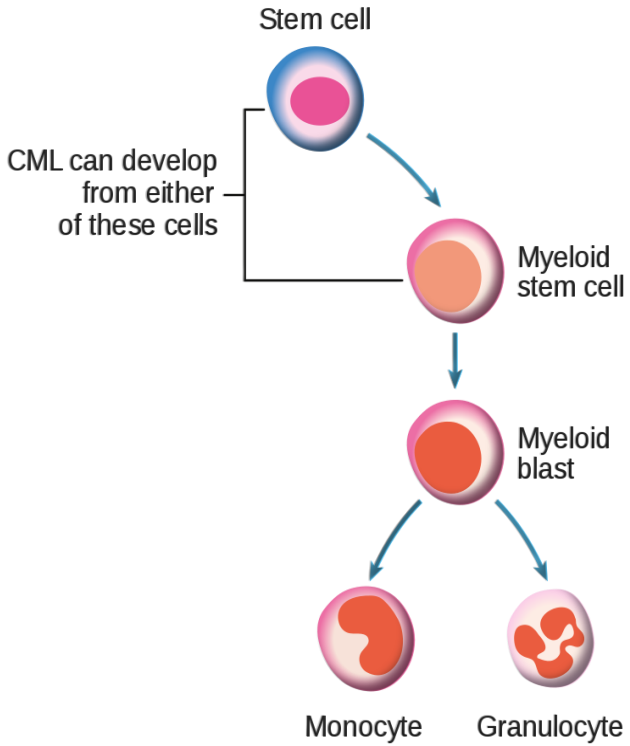
EBioMedicine
IMPACT FACTOR : 8.1

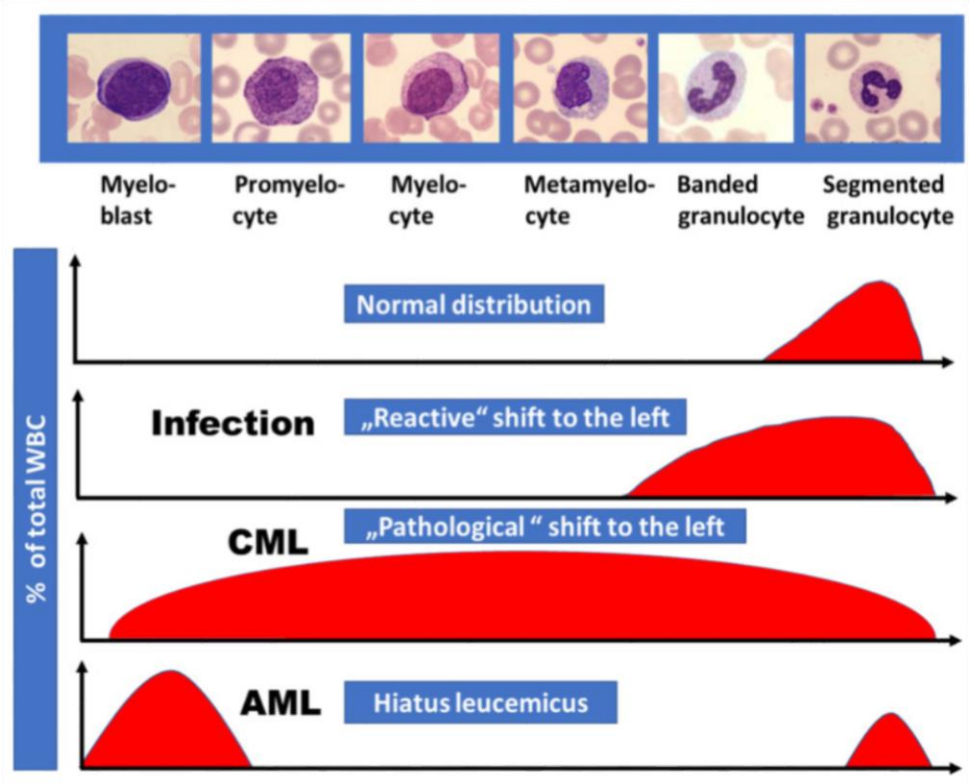


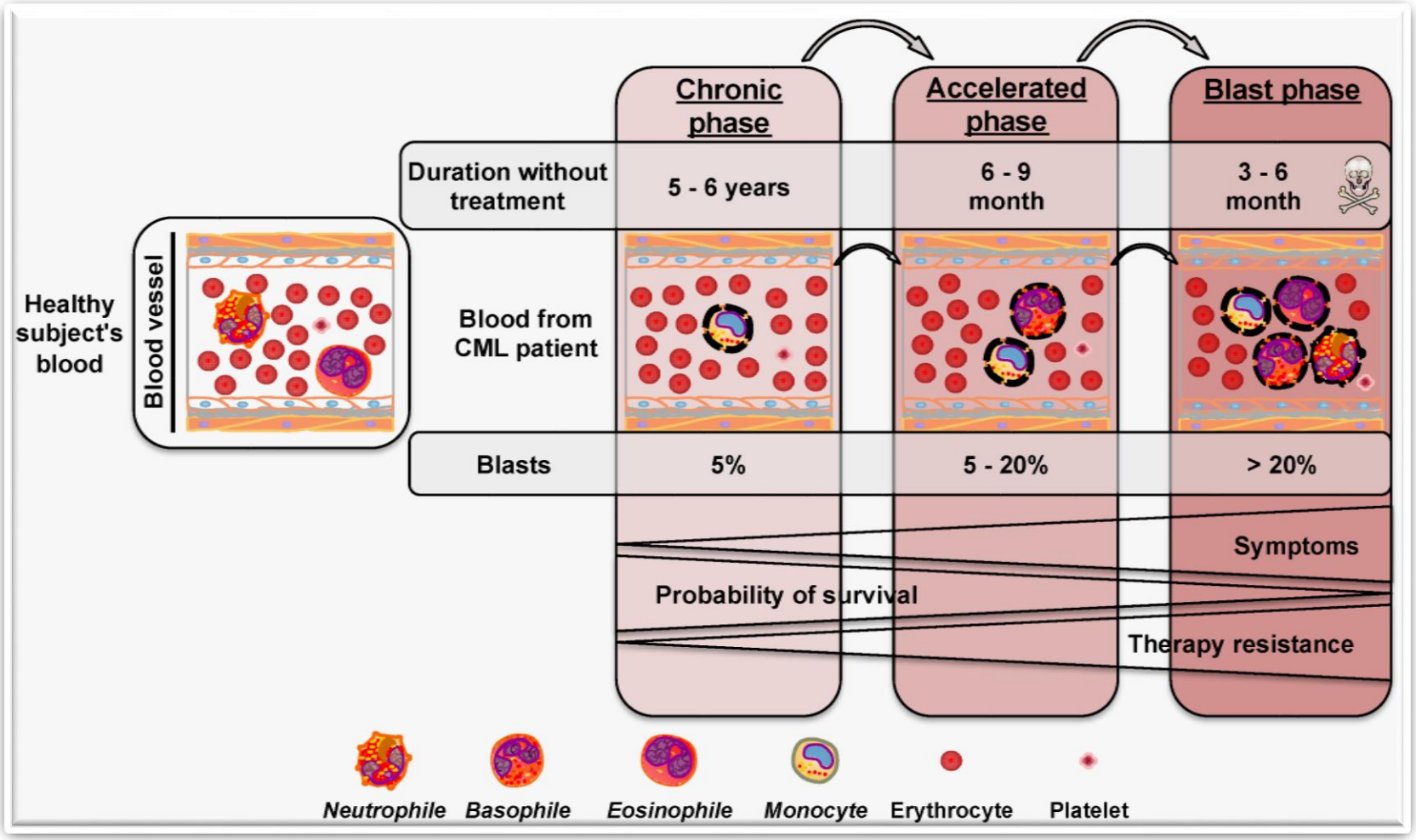
INTRODUCTIN

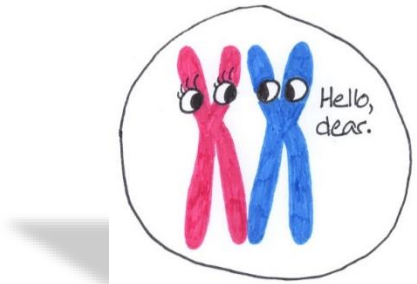


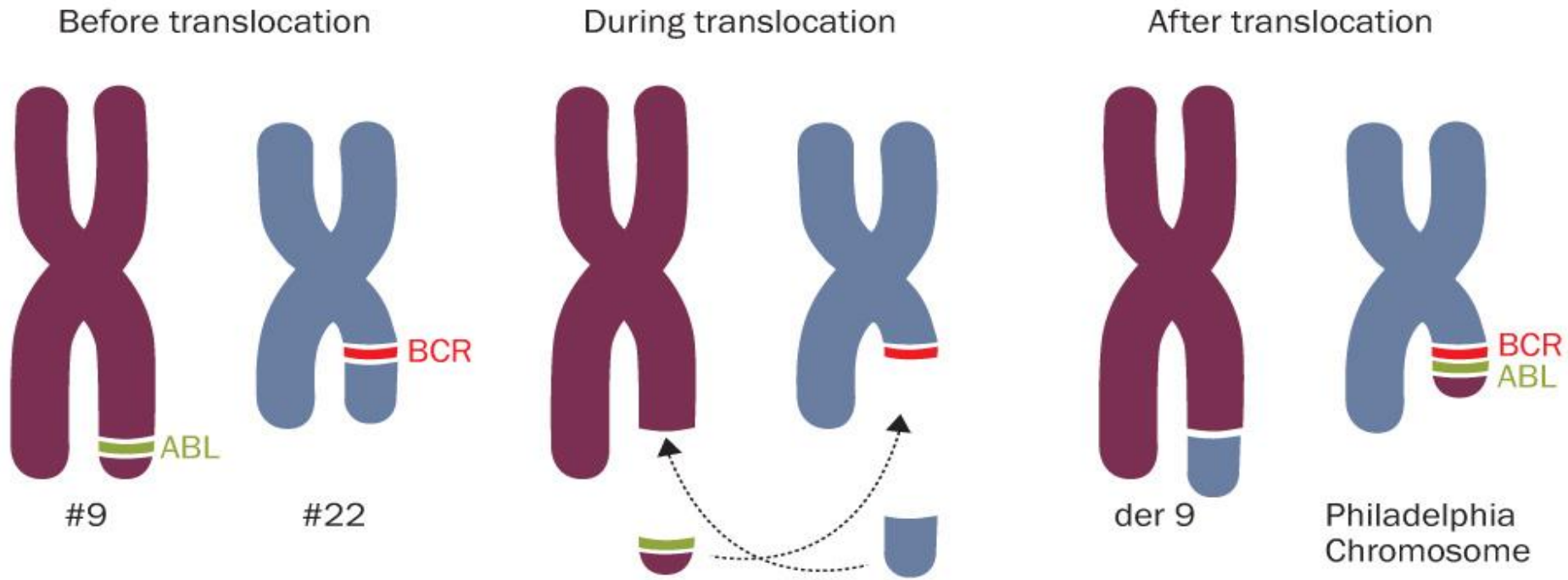
Chronic Myeloid Leukemia :



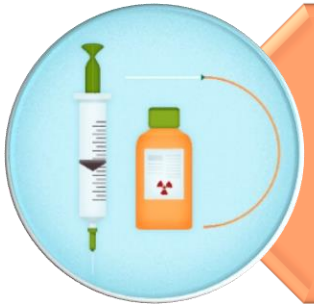



Chronic phase
Accelerated phase
Blast phase
Duration without treatment
5 - 6 years
6 - 9 month
3 - 6 month
Healthy subject's blood
Blood vessel
Blood from CML patient
Blasts
5%
5 - 20%
> 20%
Symptoms
Probability of survival
Therapy resistance
Neutrophile
Basophile
Eosinophile
Monocyte
Erythrocyte
Platelet


Hello, dear.


Before translocation
During translocation
After translocation
ABL
BCR
BCR
ABL
#9
#22
der 9
Philadelphia Chromosome

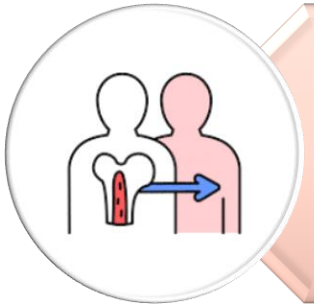
Treatments :



Chemotherapy:

Busulfan

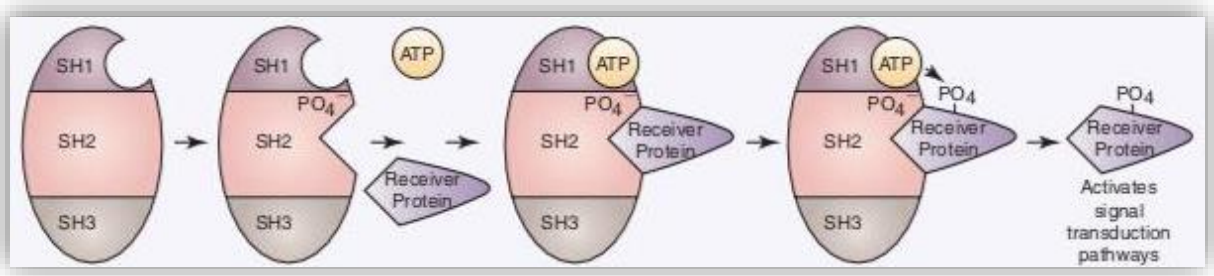
Hydroxyurea

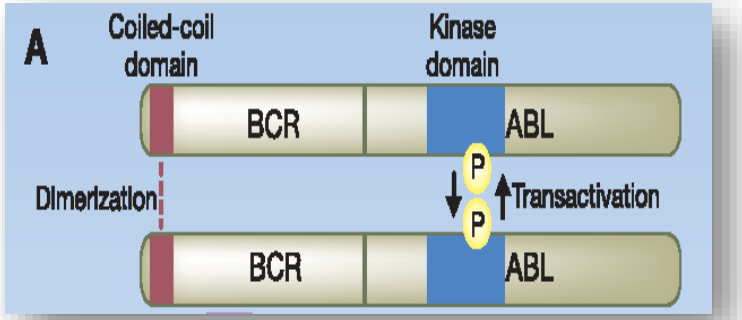


HCST

Tyrosine kinase inhibitors (TKI) :

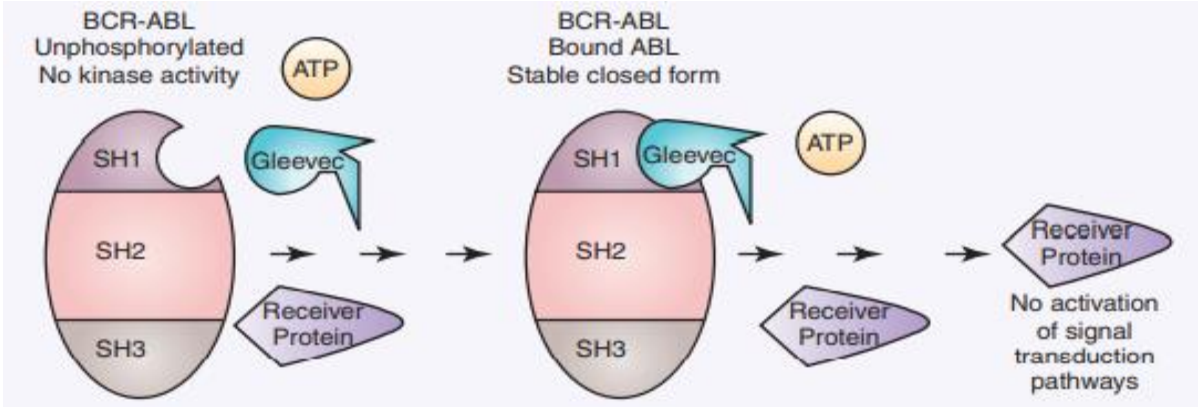
1.





2.

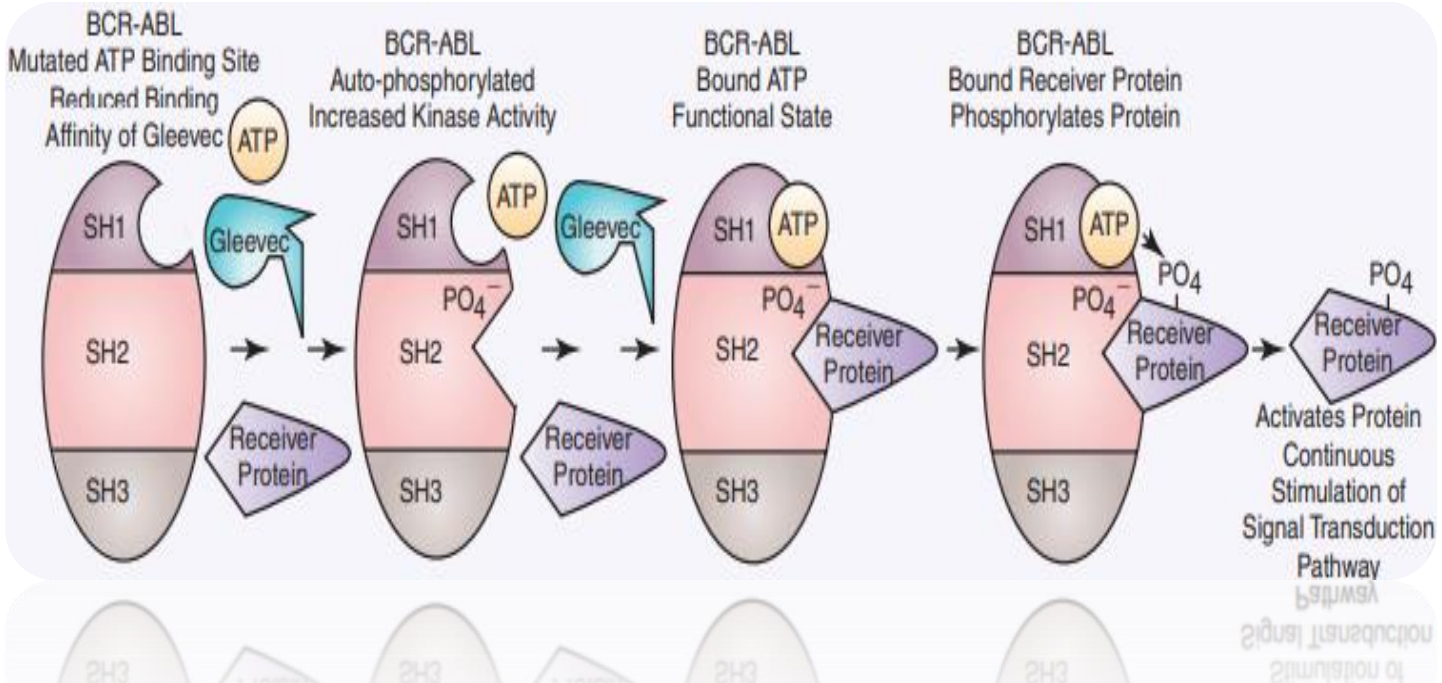
➢ Imatinib :



Imatinib resistance:

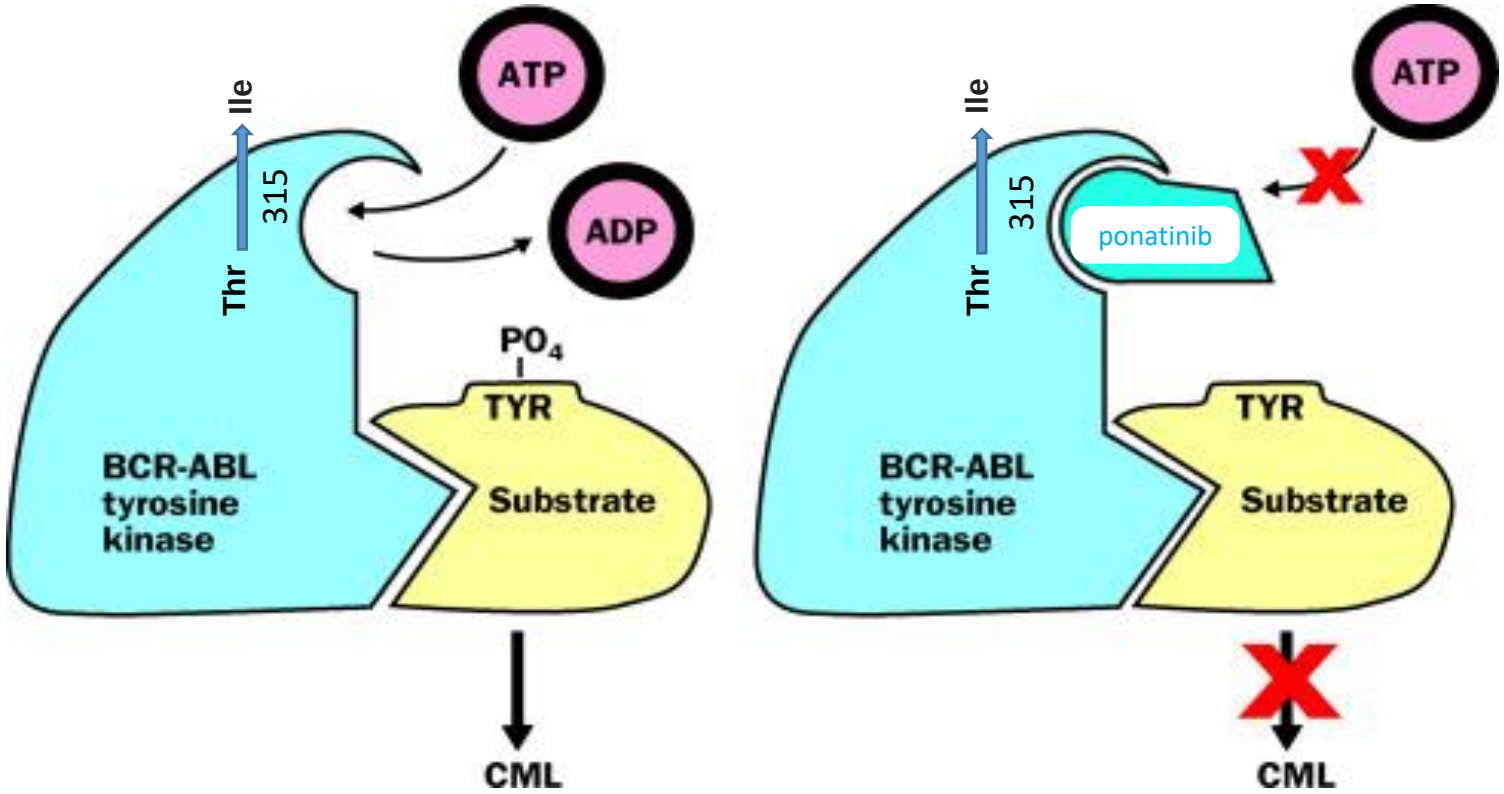
Point mutations in the BCR-ABL kinase domain :

➢**Mutation at the ATP binding site** ⇨ ***T315I***

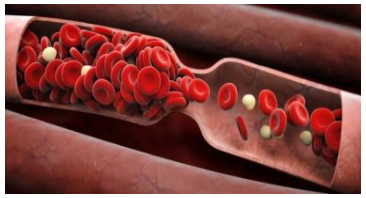


Ponatinib:


45 mg
Ponatinix45
Ponatinib Hydrochloride INN
Composition
Each film coated tablet contains
Ponatinib Hydrochloride INN
equivalent to Ponatinib 45 mg
Manufactured By
BEACON


ATP
ADP
Ile
315
Thr
PO4
TYR
BCR-ABL
tyrosine
kinase
Substrate
CML
ATP
ponatinib
Ile
315
Thr
TYR
BCR-ABL
tyrosine
kinase
Substrate
CML

Side effects of ponatinib :



Vascular occlusion

Tromboemboli

High blood pressure

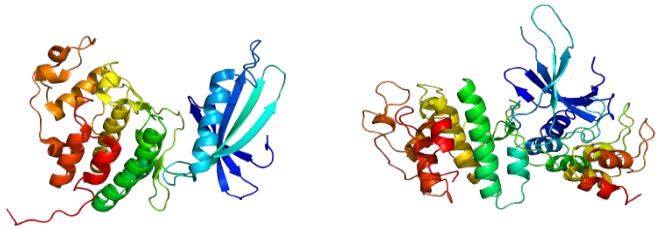
Stomach pain

Nausea

Fever

Nausea

CDK4 , CDK6 :



- Cyclin-dependent kinase 4 , 6
- Transition from phase G1 to S

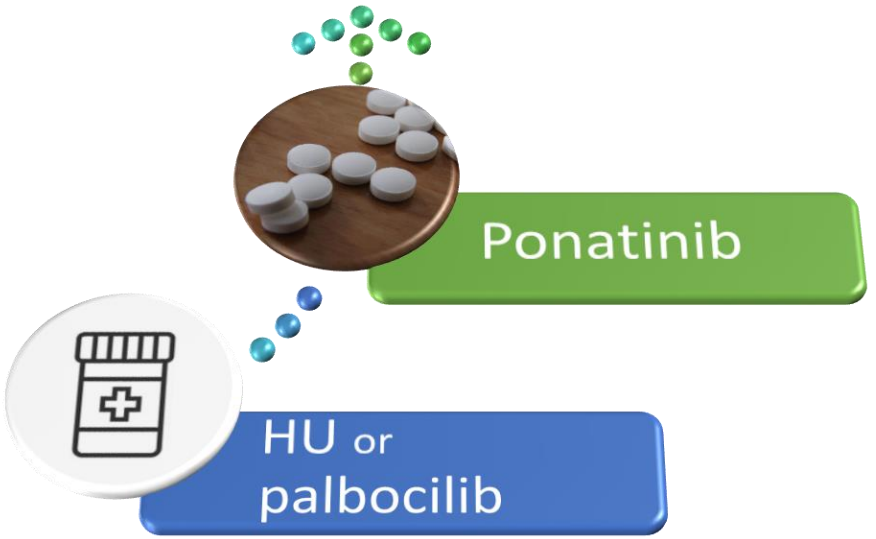
CDK4 , CDK6

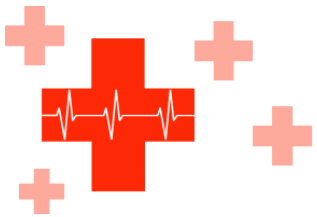
☑ Severe side effects of ponatinib ☑ Use lower doses of

☑ Reduce HU side effects

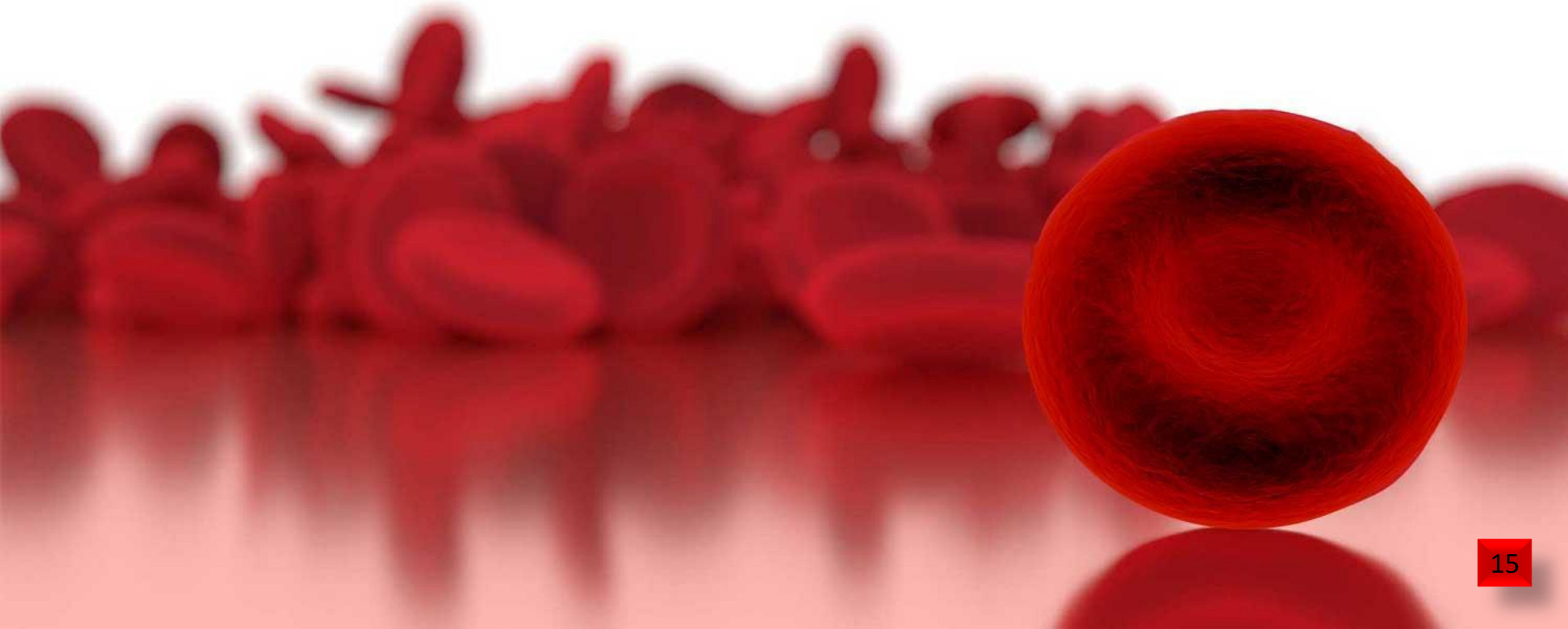
☑ Use of targeted drugs against BCR-ABL mutant forms

1. **Inhibition of CDK4,CDK6**
2. **Combination therapy**





METHODS



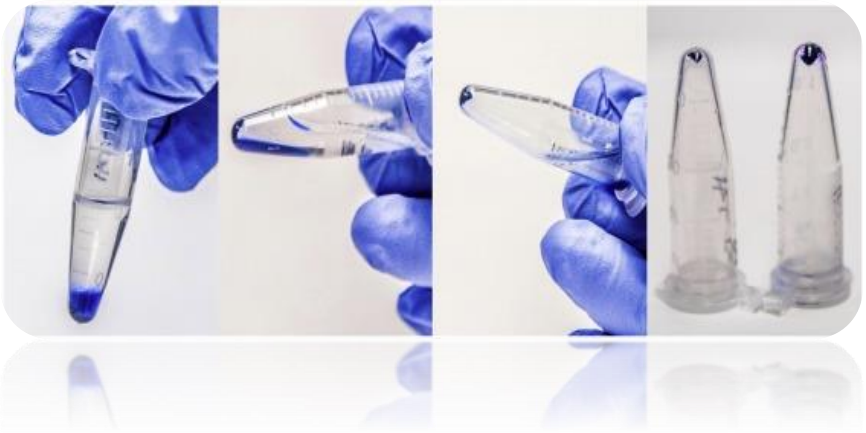
➢ Check in invivo and invitro conditions :

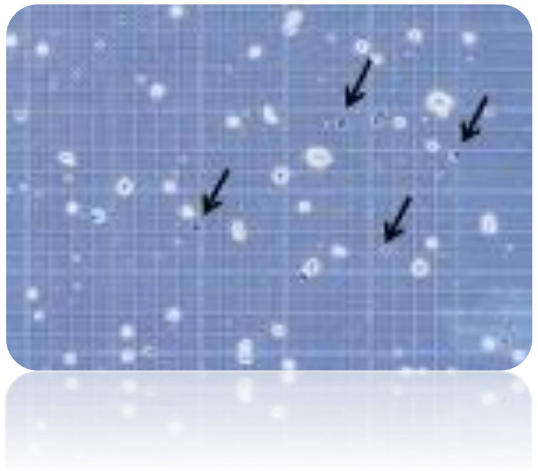
- **invivo**
  - **4 patient**
    - #3 CP
    - #1 BP
- **invitro**
  - **21 sample patient**
    - #16 CP
    - #5 BP
  - **Cell line**
    - K562
    - KU812
    - KCL22
      - ~~T315I~~
      - T315I

RPMI
FCS 10%
ANTIBIOTIC

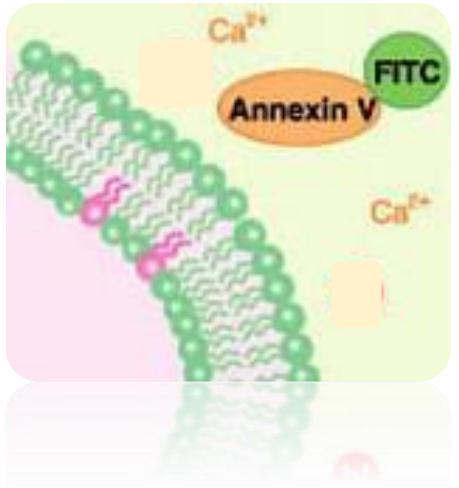
➢ Measurement of apoptosis :

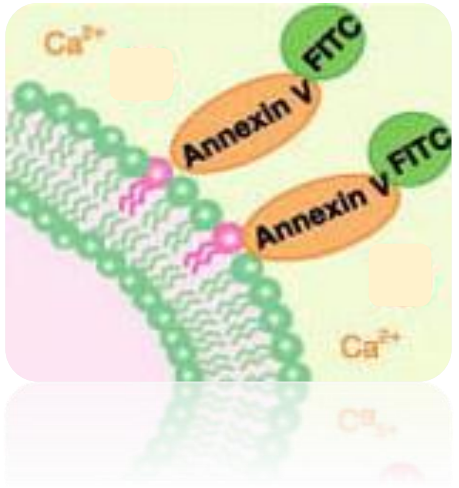
- Trypan blue





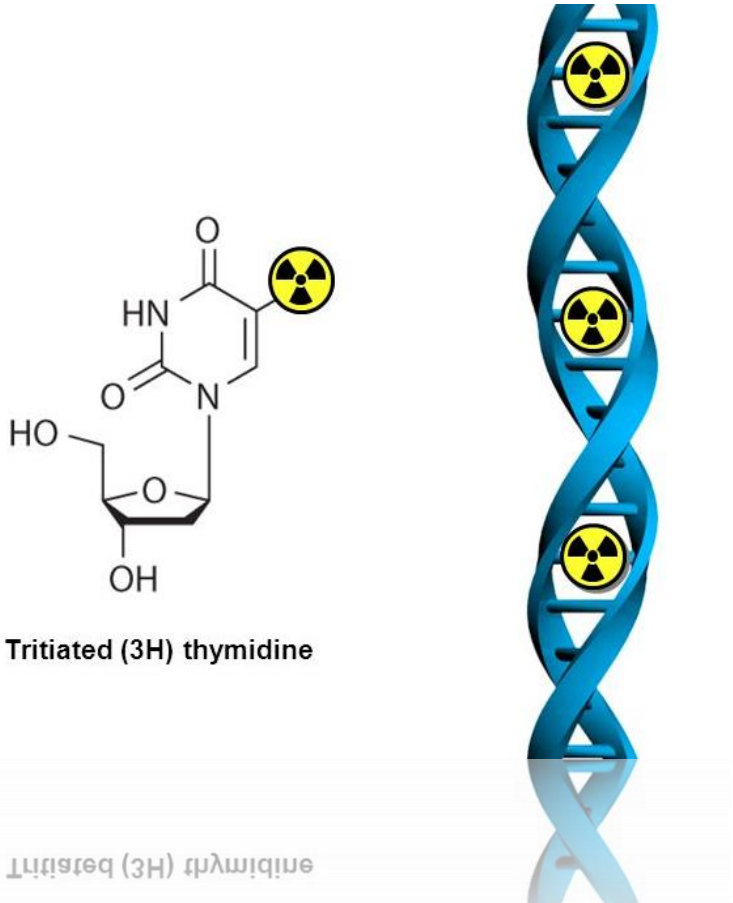
- AnnexinV-FITC

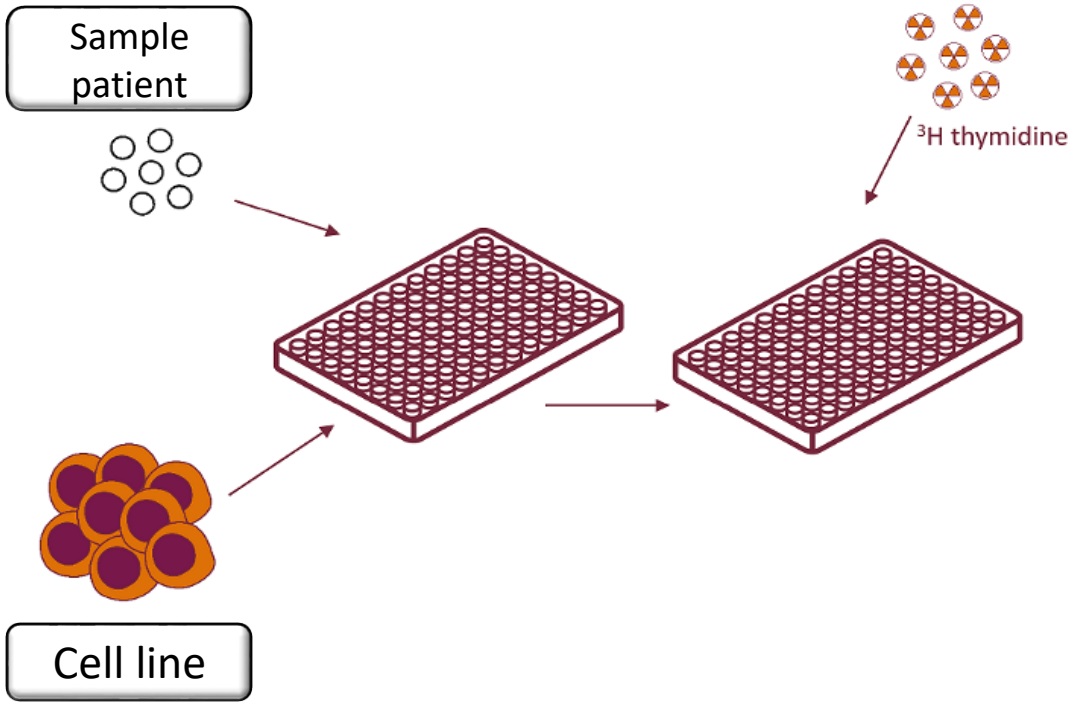




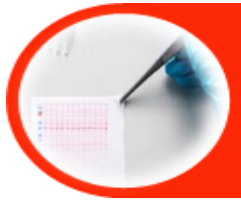
➢ Measurement of proliferation :

- 3H-thymidine-uptake assay



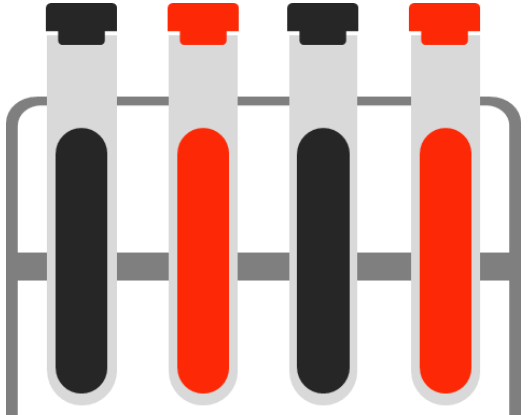


➢ Western blotting and Real-time PCR:

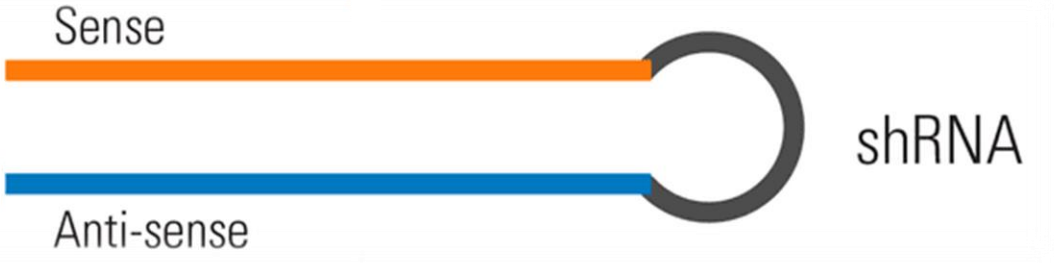


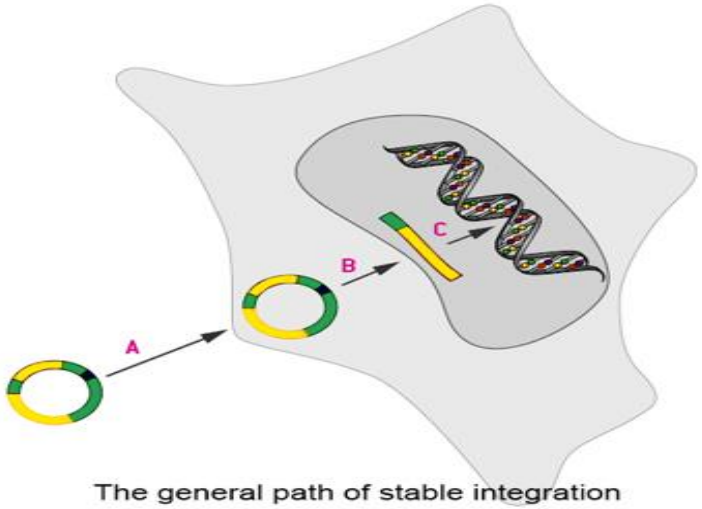
Western blot experiments to study the effects of HU on expression of CDK4/6 were performed Through protein expression on CML cell lines

Real-time PCR is used to determine the level of mRNA expression in CML cell lines



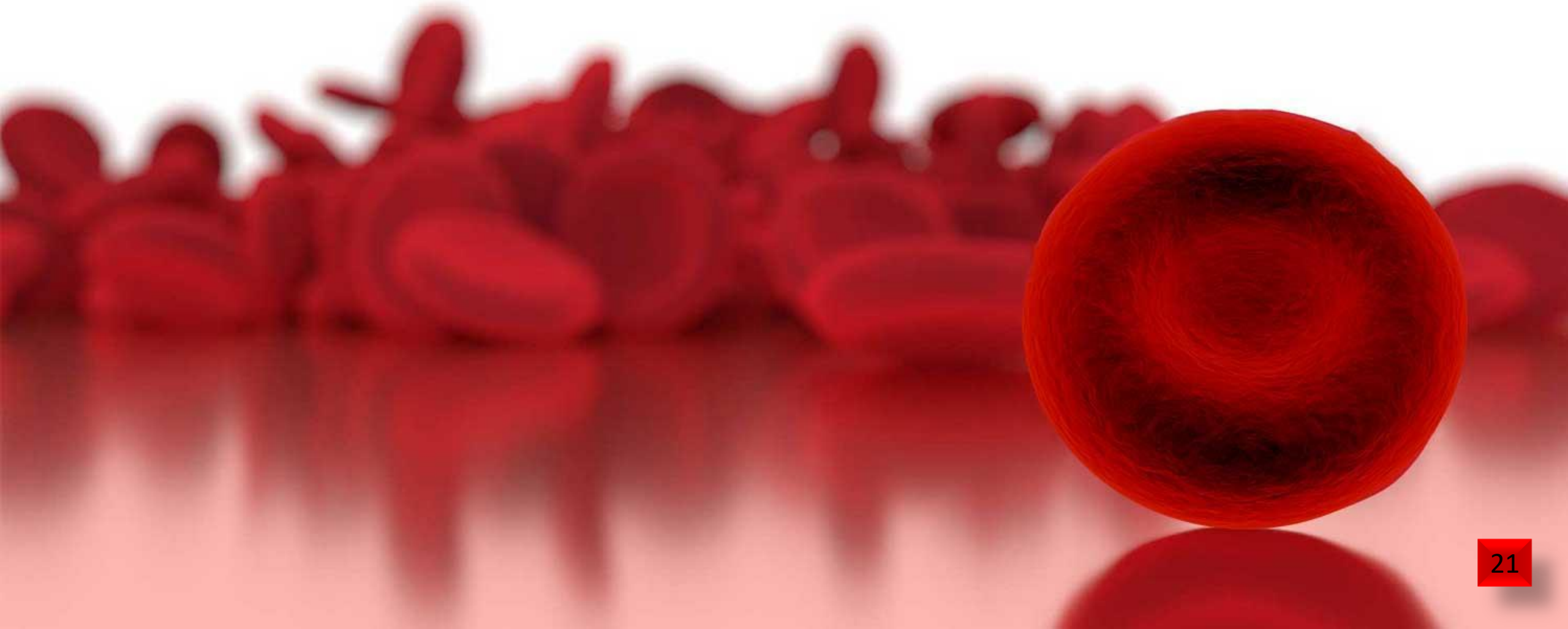
- shRNA induced knockdown of CDK4 and CDK6





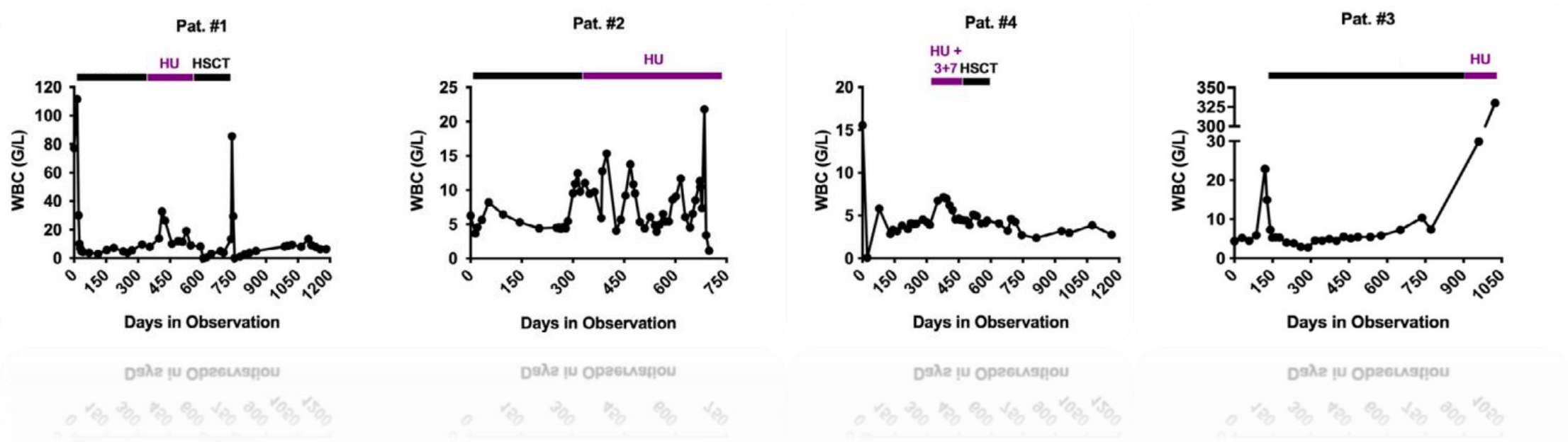
The general path of stable integration

Results



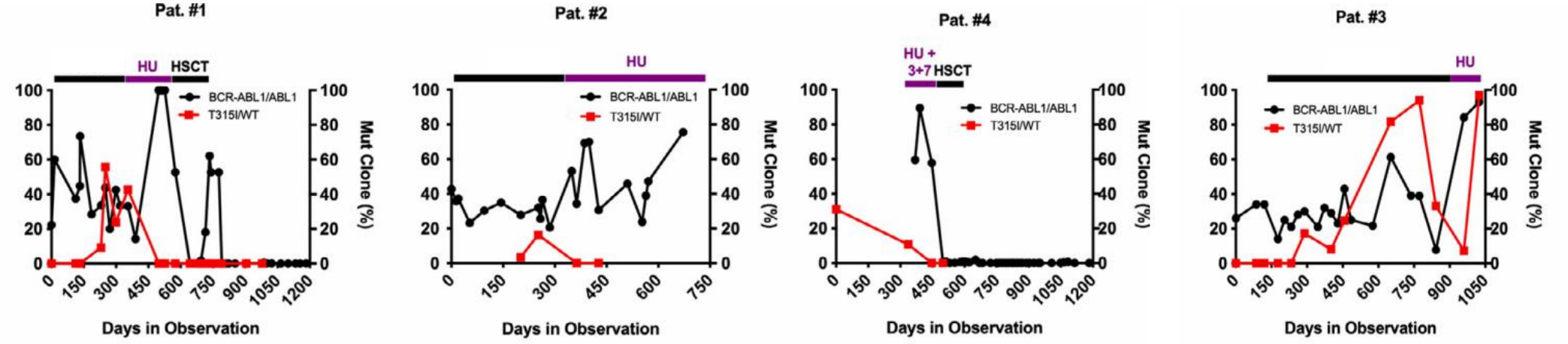
- Response to HU treatment in 4 patients with BCR-ABL1 T315I+ CML *(invivo)* :

1) Stabilization of the leukocyte counts in 3 of 4



2) The percentage of BCR-ABL was significantly decreased in all 4 patients compared to the total ABL

3) In 3 of the 4 patients, the T315I mutant was no longer detectable after therapy



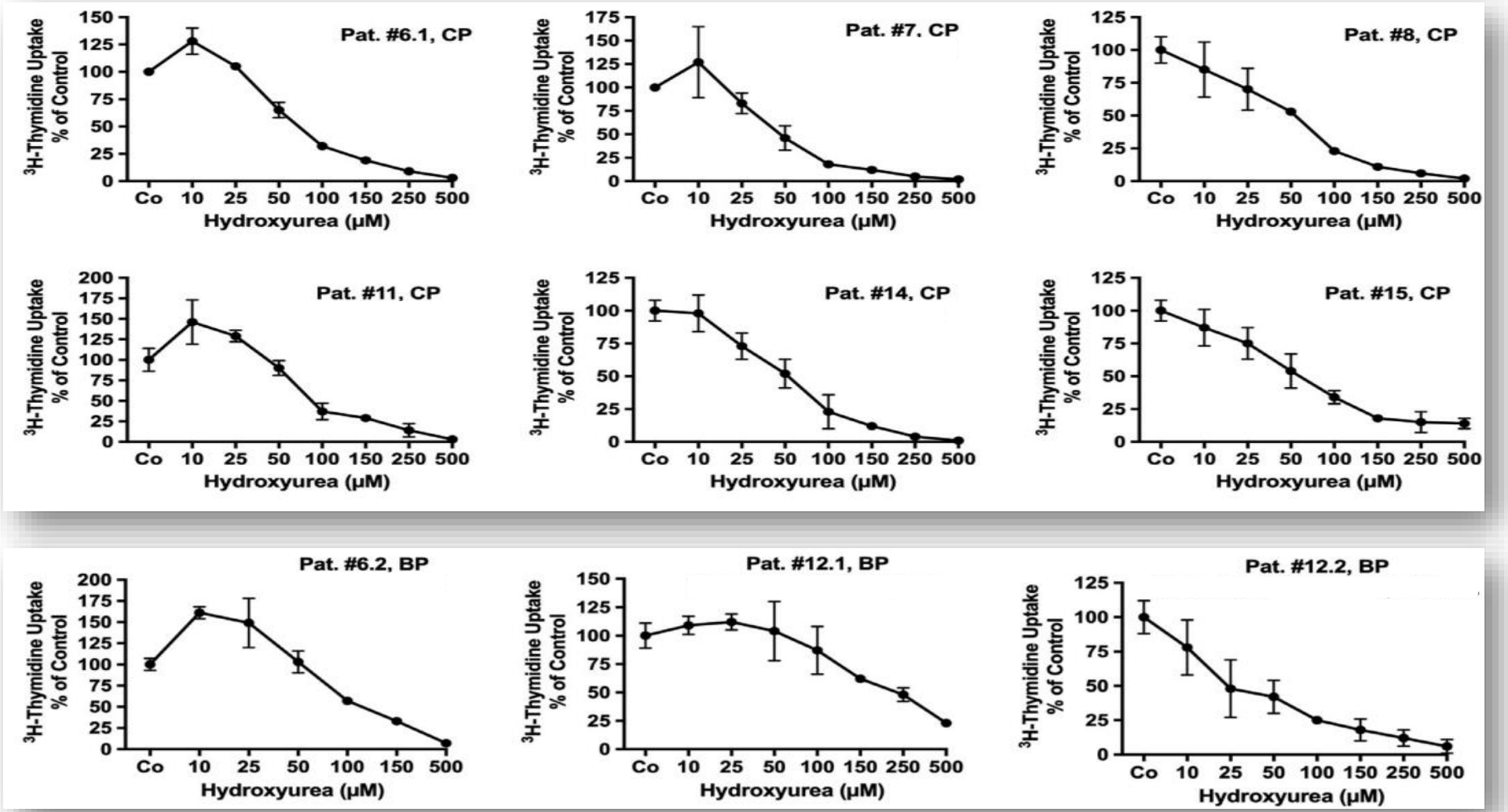
3) These data suggest that HU is able to eliminate BCR-ABL T315I + leukemic cells

4) These observations suggest that HU is able to suppress BCR-ABL T315I+ in patients with CML CP

- Investigation of the effect of HU , ponatinib and Palbociclib on proliferation and survival of BCR-ABL1 + cells sensitive and resistant to TKI *(invitro)* :

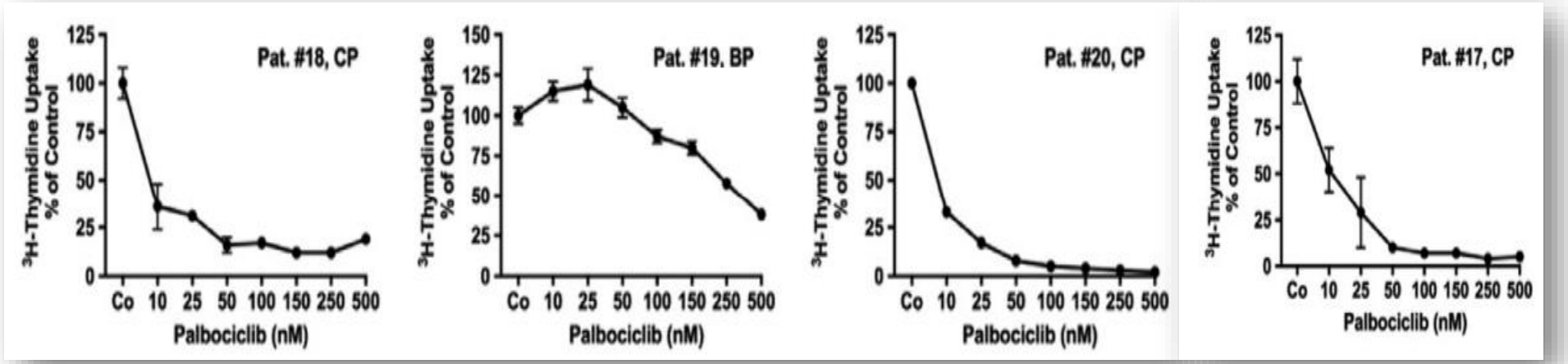
Sample of patients

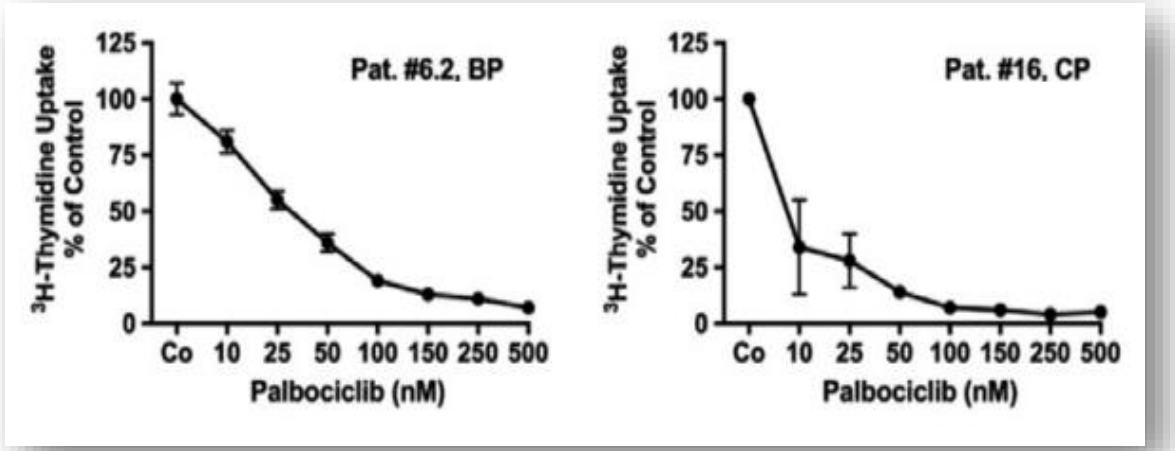
HU



Sample of patients

Palbociclib



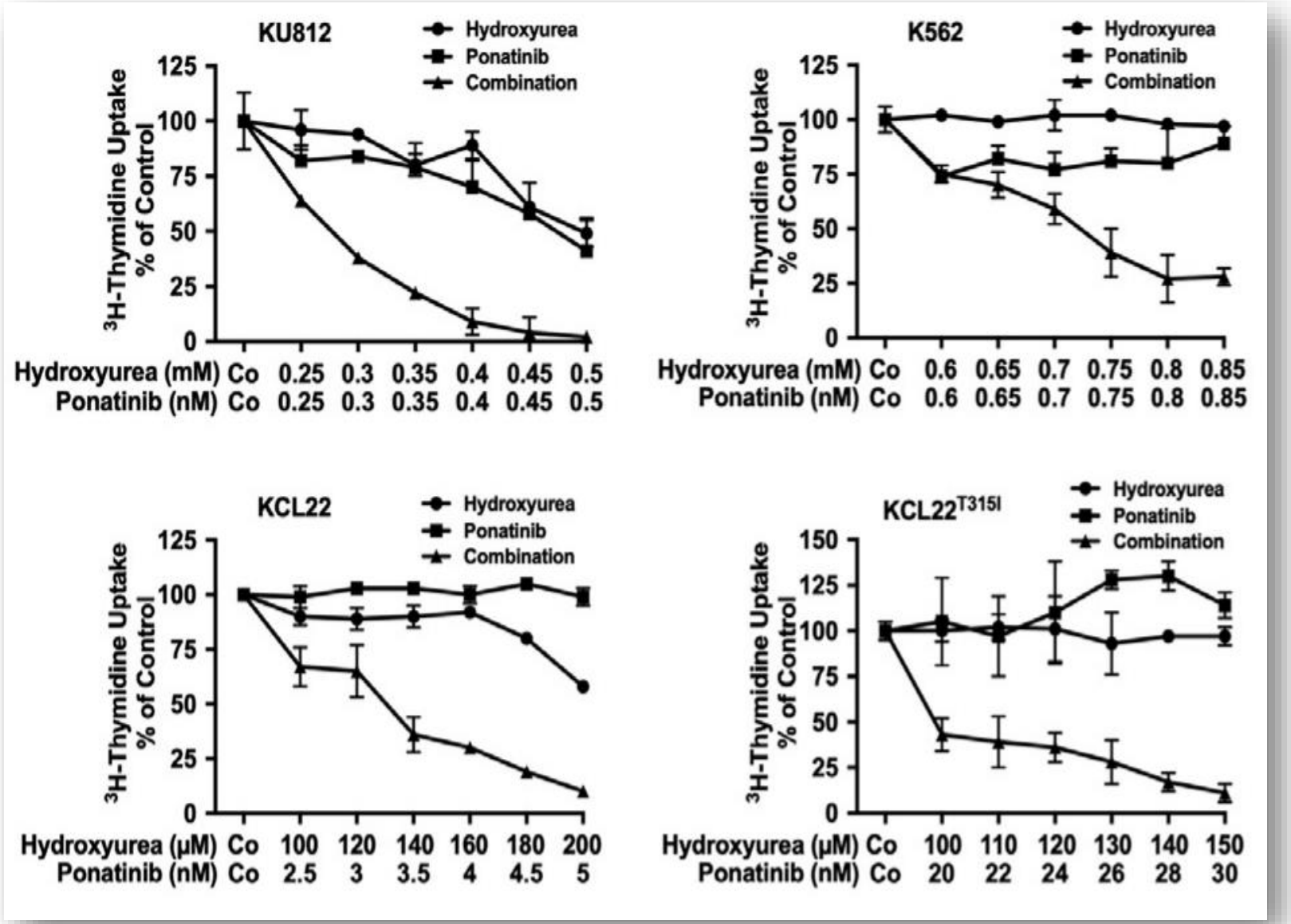


Cell line

HU

and

ponatinib

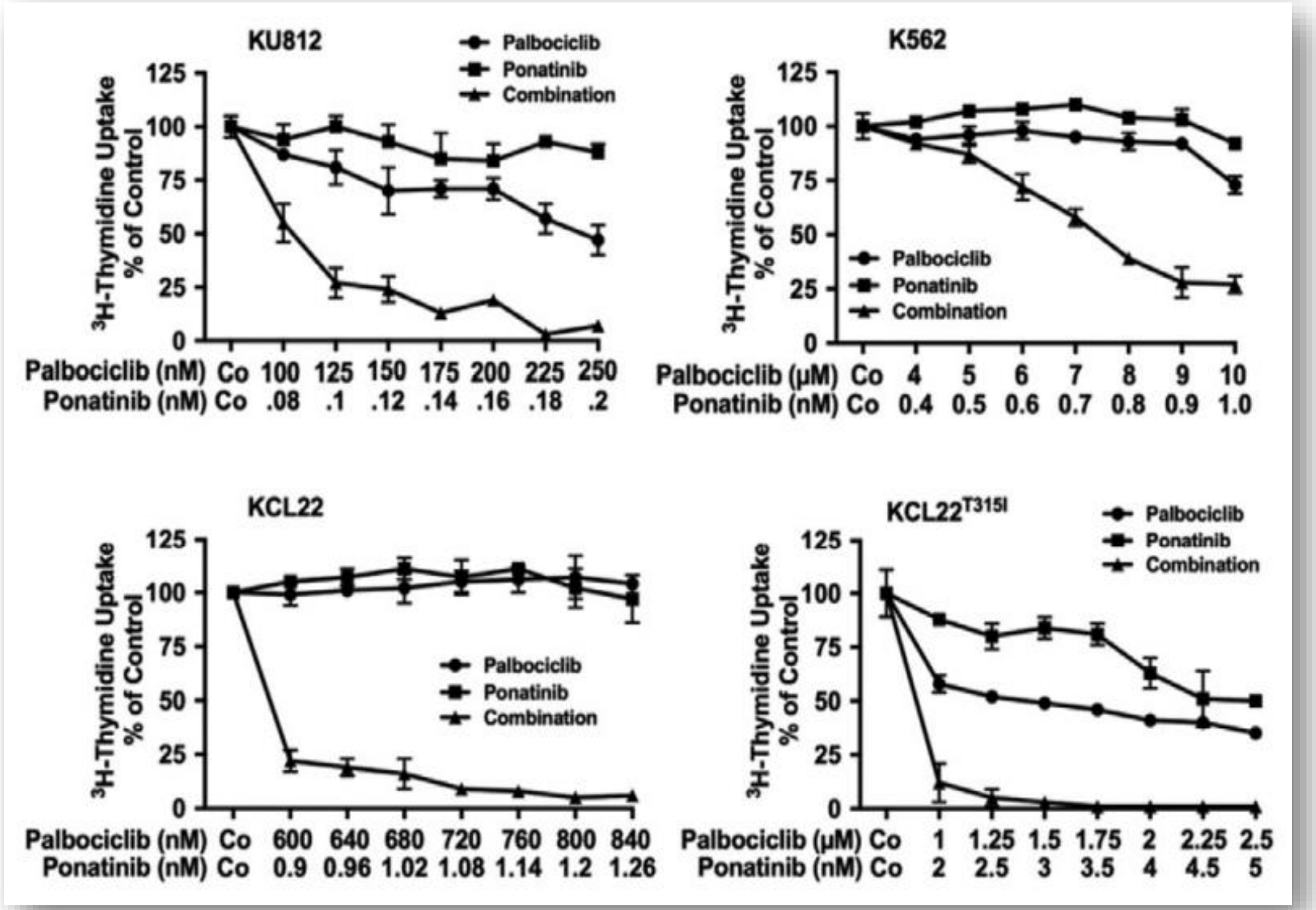


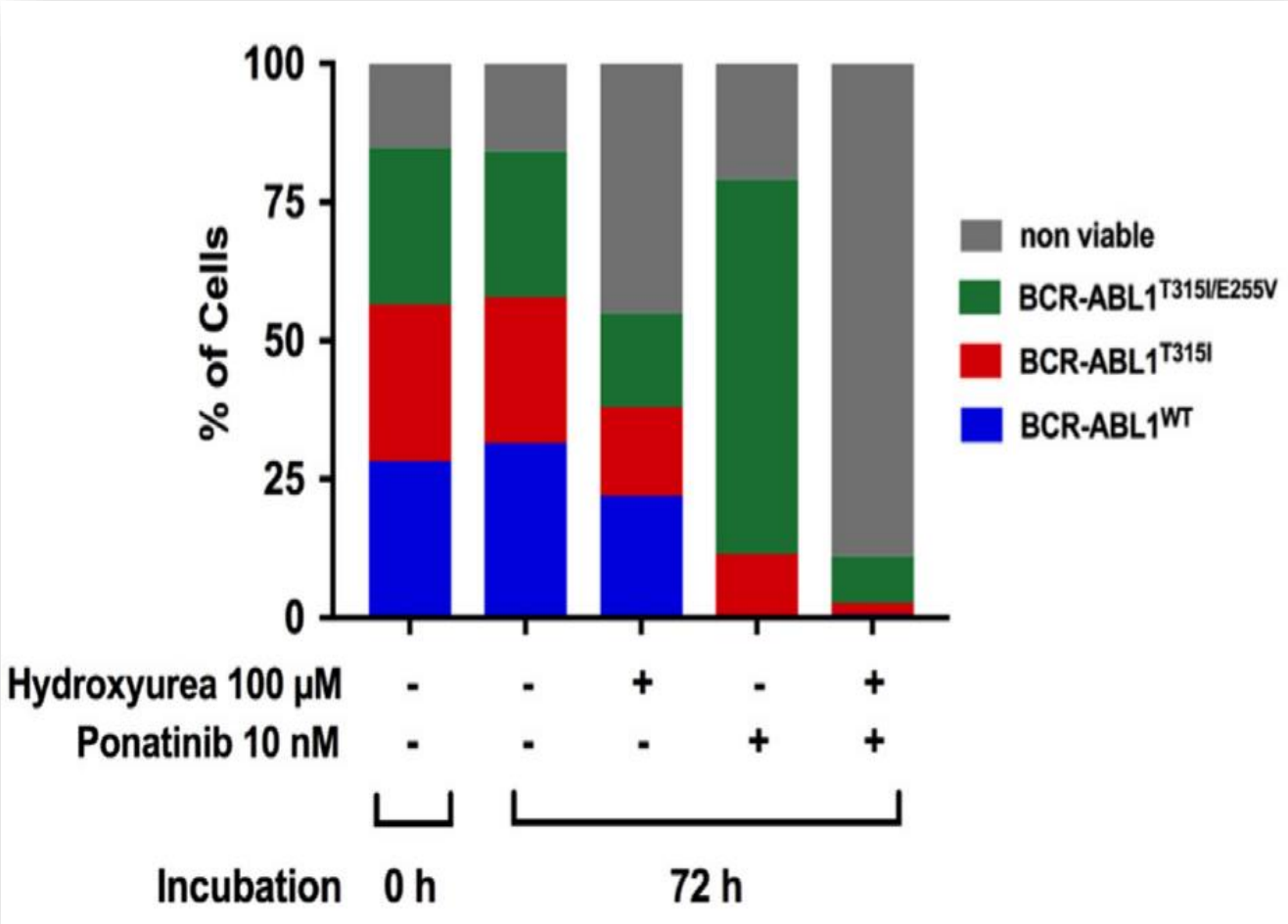
Cell line

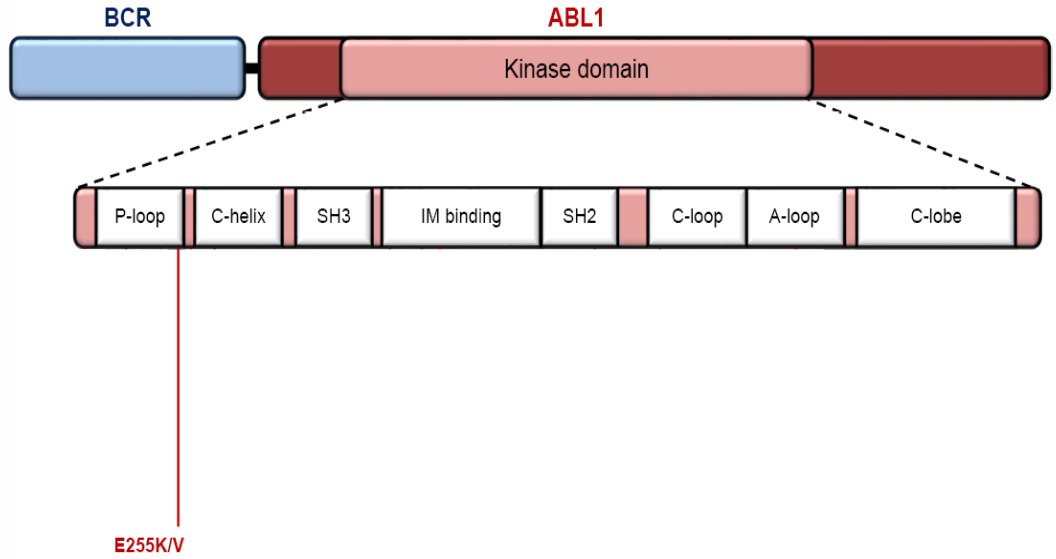
Palbociclib

and

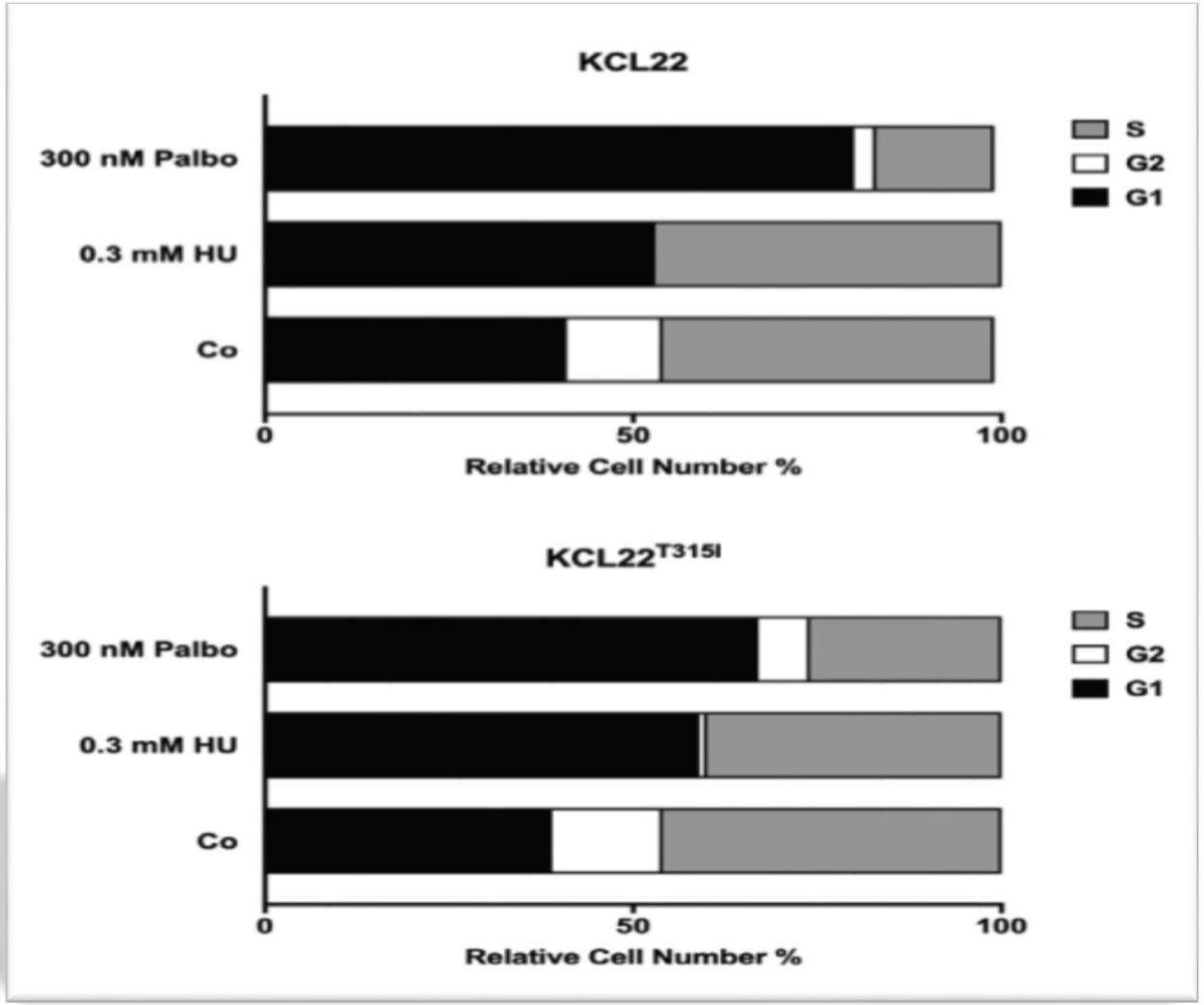
ponatinib



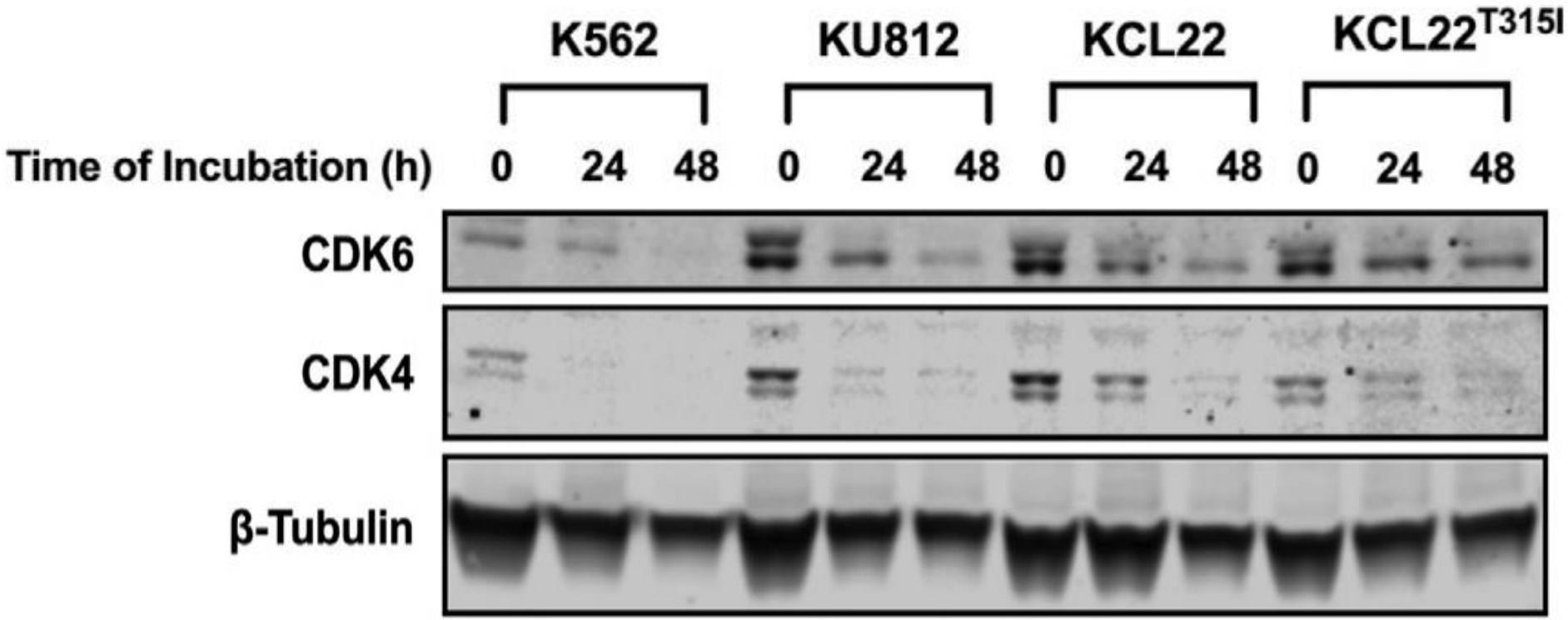

100
75
50
25
0
% of Cells
non viable
BCR-ABL1T315I/E255V
BCR-ABL1T315I
BCR-ABL1WT
Hydroxyurea 100 µM - - + - +
Ponatinib 10 nM - - - + +
Incubation 0 h 72 h


BCR
ABL1
Kinase domain
P-loop
C-helix
SH3
IM binding
SH2
C-loop
A-loop
C-lobe
E255K/V

- Effects of HU and Palbociclib on cell cycle progression :

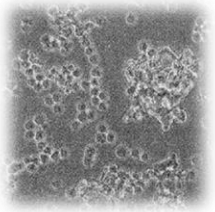


- Effects of HU on the cell cycle regulators CDK4 and CDK6 :



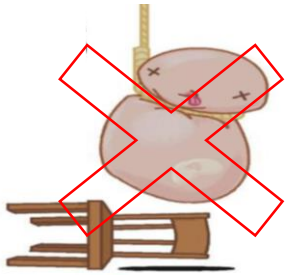
Effects of CDK4/6 knockdown on growth and survival of CML cells :

- K562 , KCL22

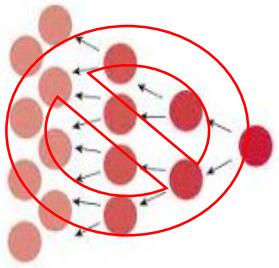


- Growth defect

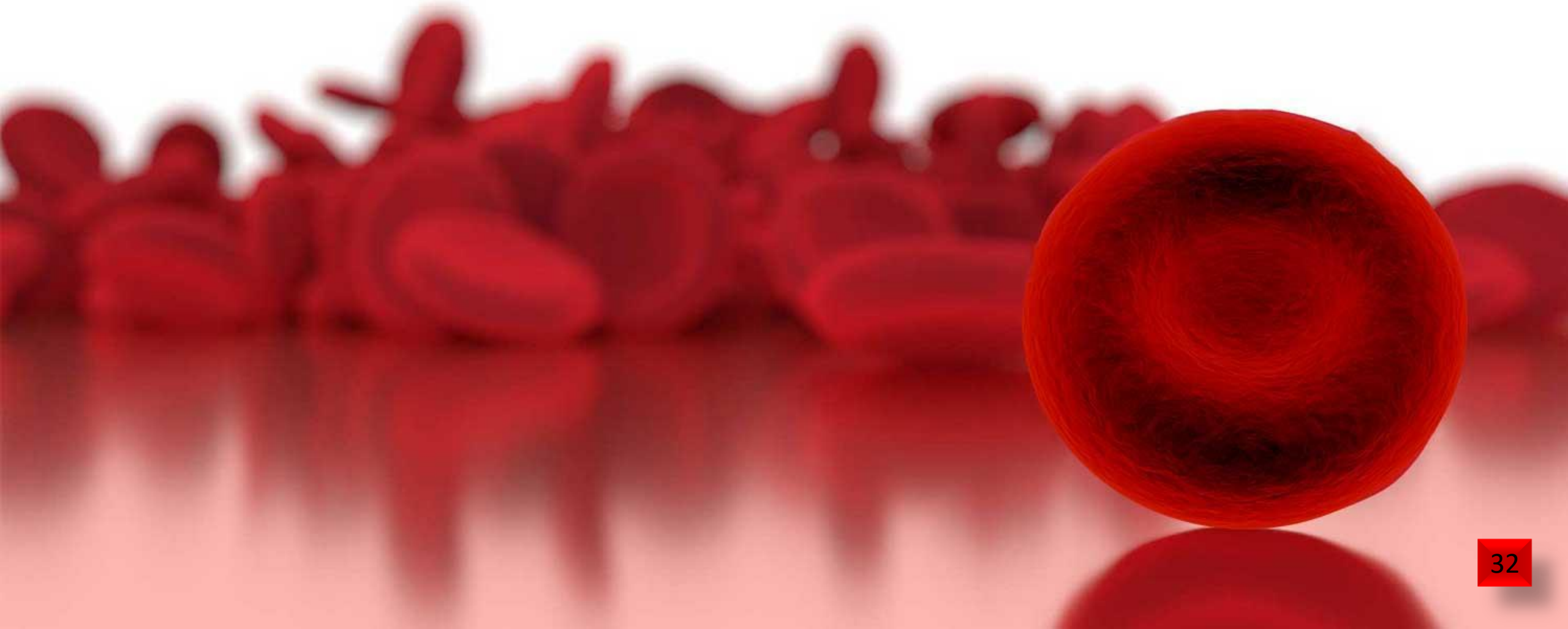
- Deletion of CDK4 and CDK6 did not induce apoptosis



- Inhibition of CDK4 / CDK6 interferes with CML cell proliferation



Discussion



- BCR-ABL1 T315I mutation occurs in 20 to 30% of TKI-resistant in CML patients
- Ponatinib suppresses BCR ABL1T315I but is not an optimal drug for all patients due to its side effects
- HU inhibits the growth of CML cells whose T315I + cells were more sensitive to HU than BCR ABL WT
- HU cooperates with ponatinib in suppressing the growth of leukemic cells expressing BCR ABL1T315I or T315I containing compound mutants
- A direct effect of HU on BCR ABL1 mutants seems unlikely. Indeed, HU is well known to suppress proliferation in neoplastic cells by interfering with cell cycle progression

- Targeting CDK4 / CDK6 may be a strong approach to overcoming resistance to TKIs
- HU reduces the expression of CDK4 and CDK6
- CDK4/6 depletion, or CDK4/6 inhibition, counteracts cell cycle progression and replication in CML
- Palbociclib according to recent data showed high anti-leukemia activity against CML cell lines
- Our data show that clones expressing highly resistant BCR ABL1 hybrid mutations respond to HU and palbociclib


Thank you for your attention