Pharmacological Quiescence

Norman E. Sharpless, MD
Associate Professor of Medicine and Genetics
The Lineberger Comprehensive Cancer Center
The University of North Carolina

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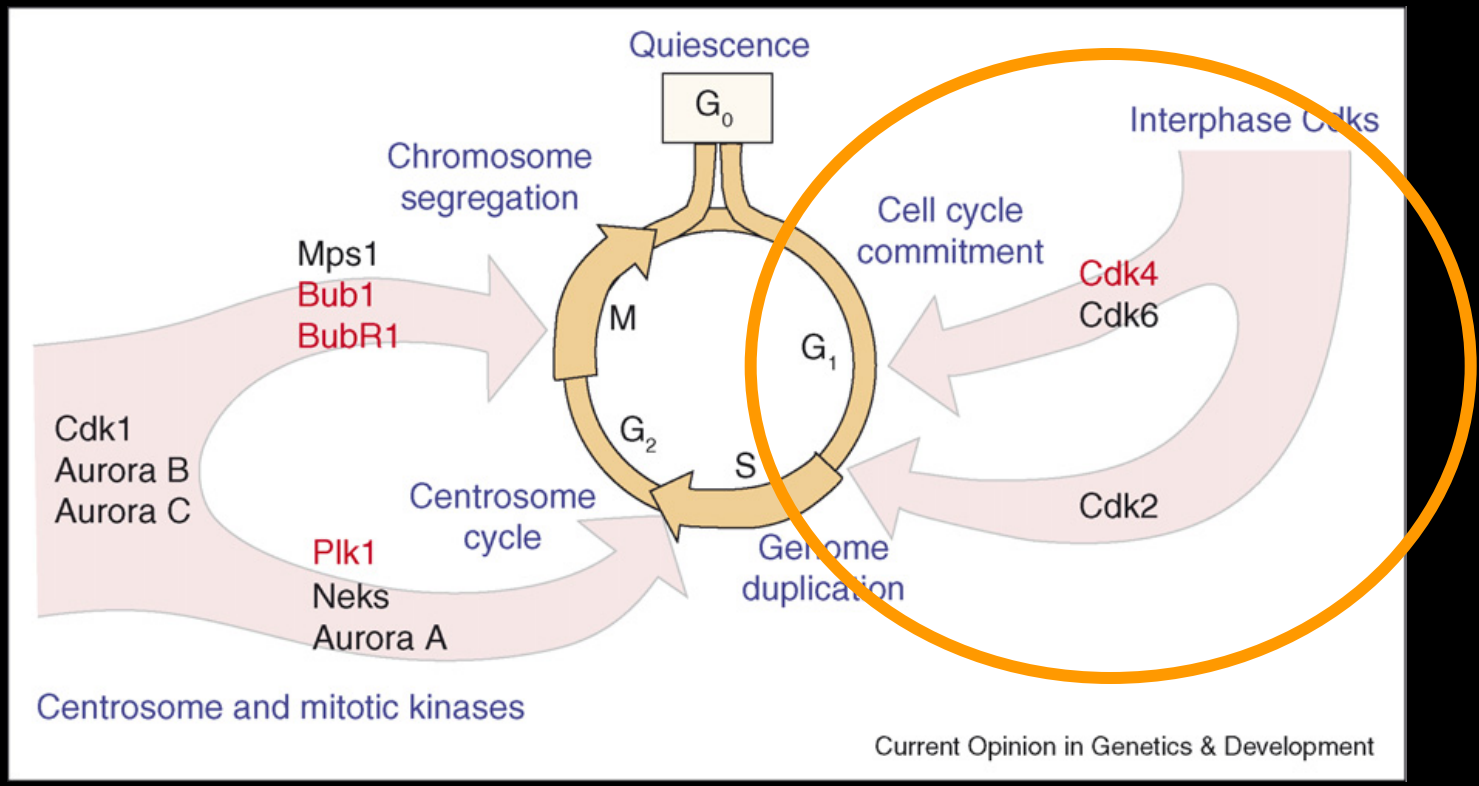
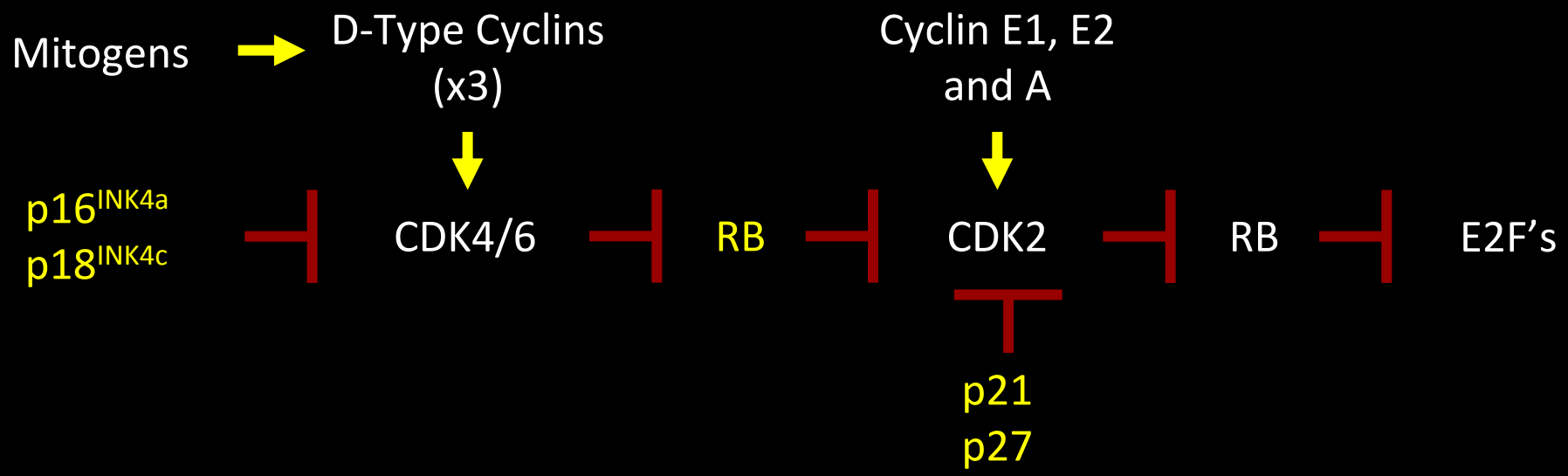
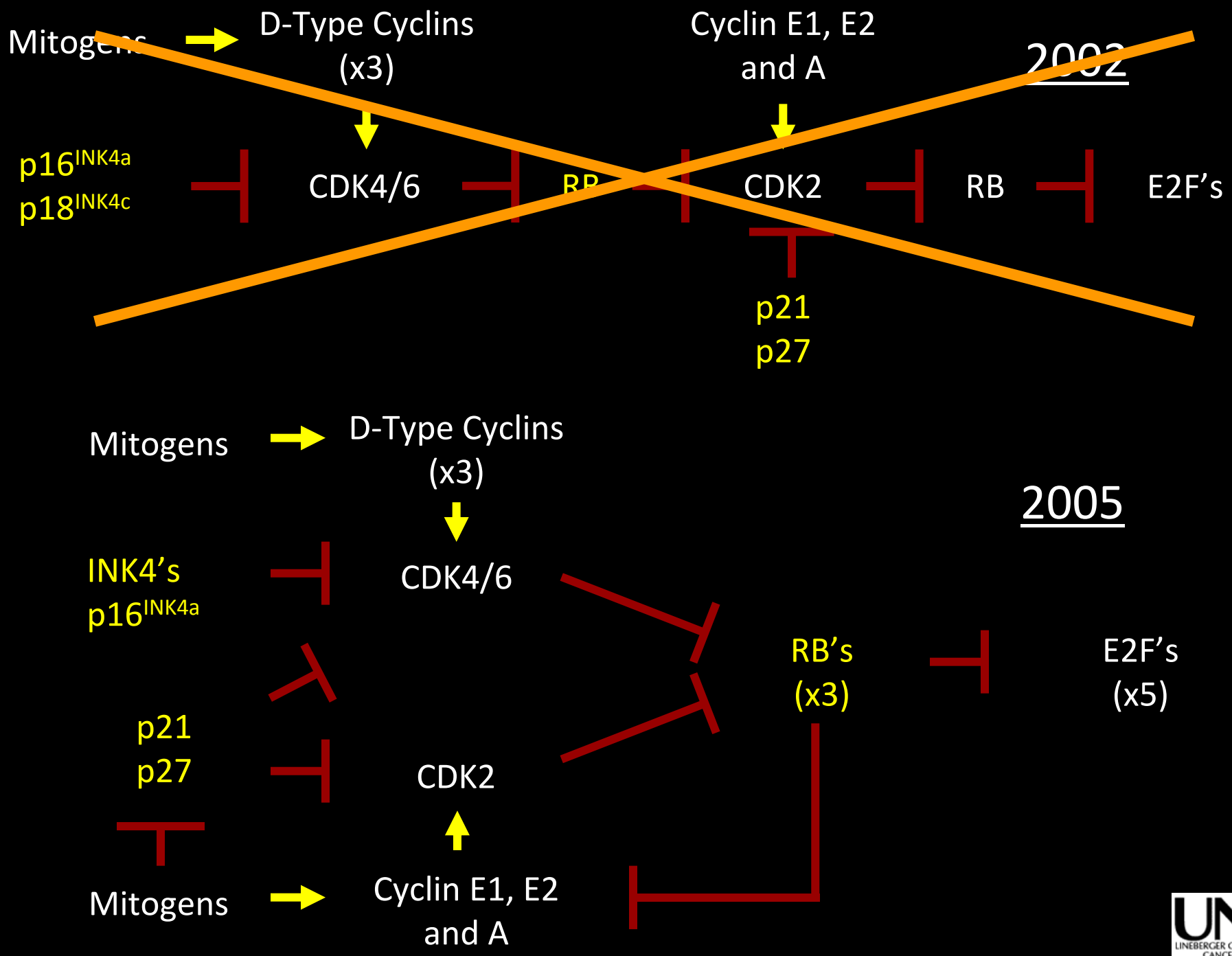


- We all know about the boring, old cell cycle.
- Nobel given in 2001 to Hartwell, Hunt and Nurse for understanding yeast cell cycle.
- Cell cycle not nearly as cool as telomeres or angiogenesis or PARP inhibitors or RNAi or Stem Cells…..

G1→S in 2002:


Mitogens
D-Type Cyclins (x3)
Cyclin E1, E2 and A
p16INK4a
p18INK4c
CDK4/6
RB
CDK2
RB
E2F's
p21
p27


Mitogens
D-Type Cyclins
(x3)
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2002
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2005
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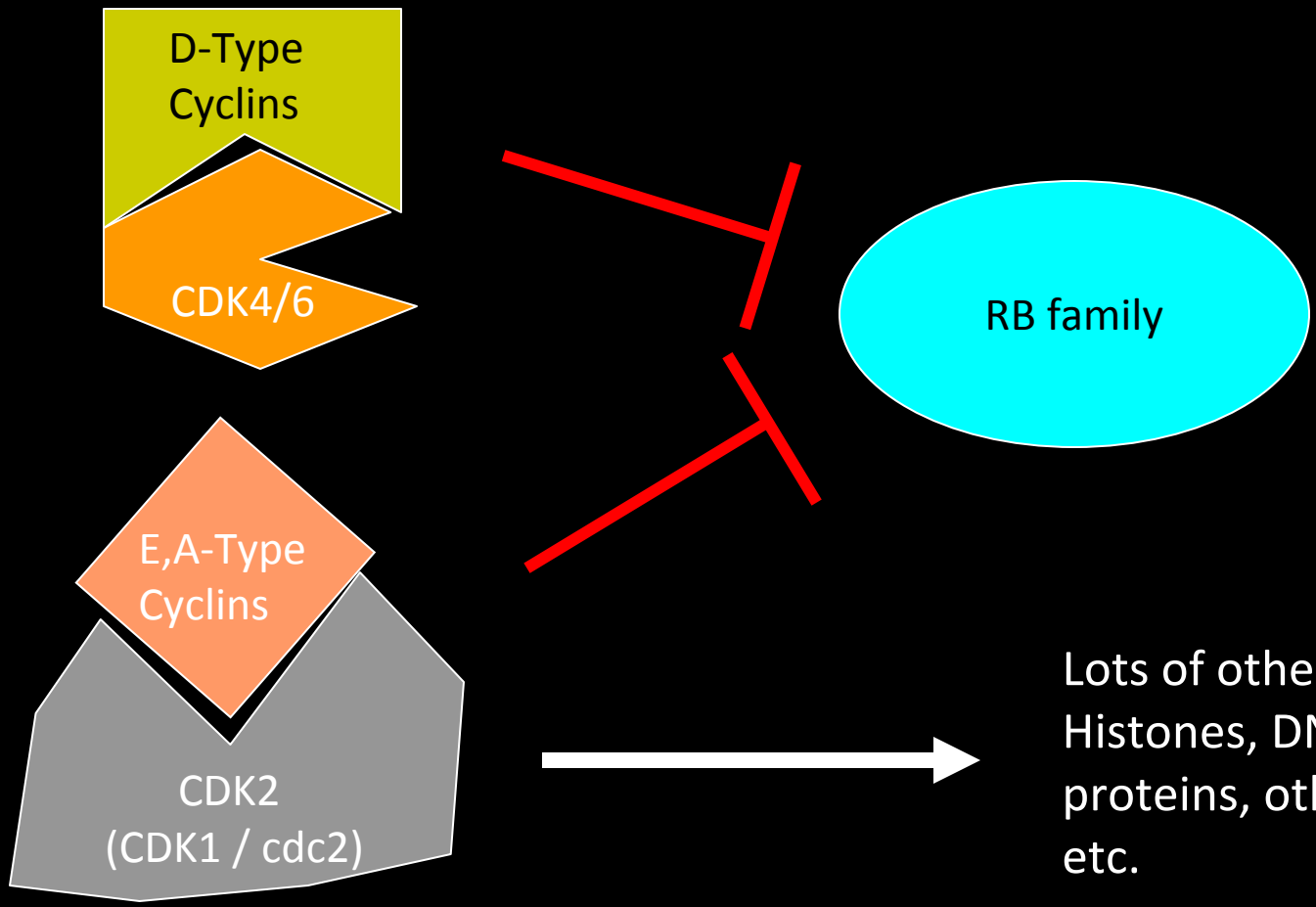
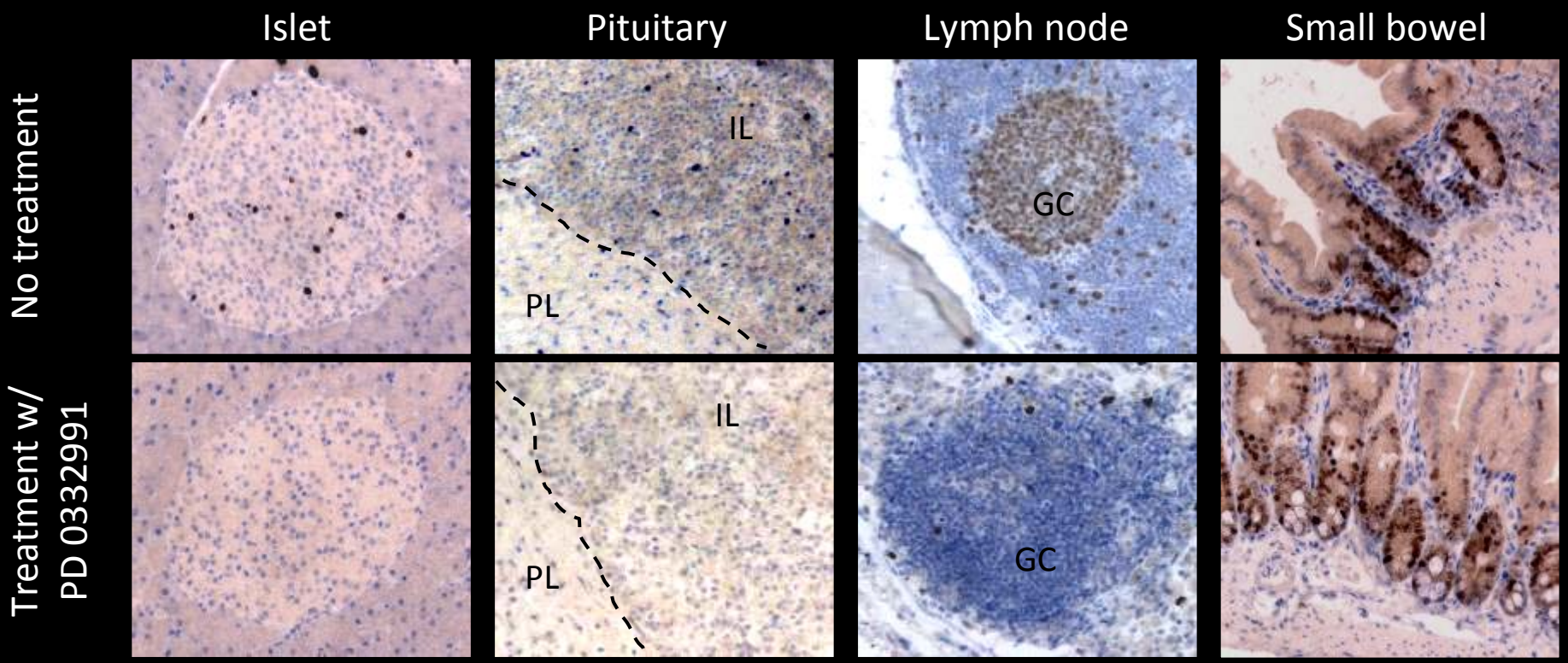
CDK4/6
RB's
(x3)
E2F's
(x5)
p21
p27
CDK2
Mitogens
Cyclin E1, E2
and A

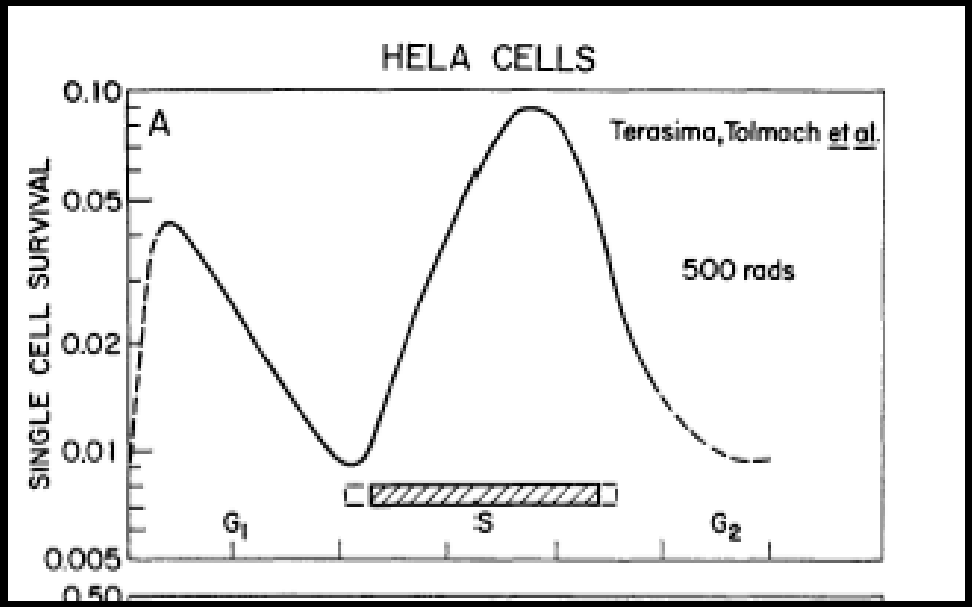
For G1→S, two things have to happen:



Pharmacological Quiescence (PQ)



Can in vivo modulation of cell cycle alter toxicity of DNA damaging agents?

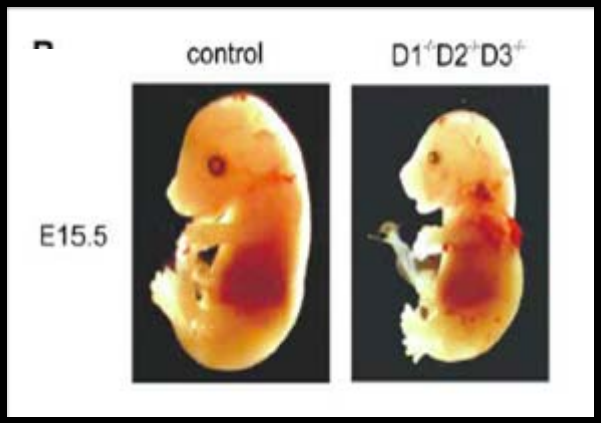


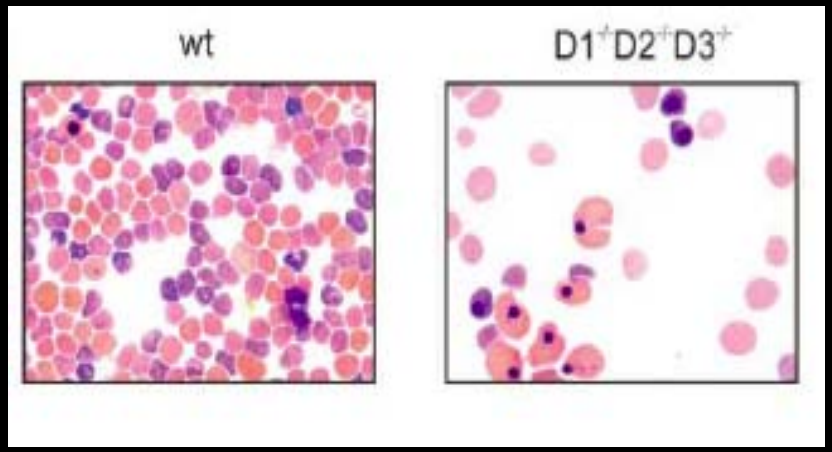
Terasima, Tolmach et al, 1963

Hematopoietic cell cycle regulation

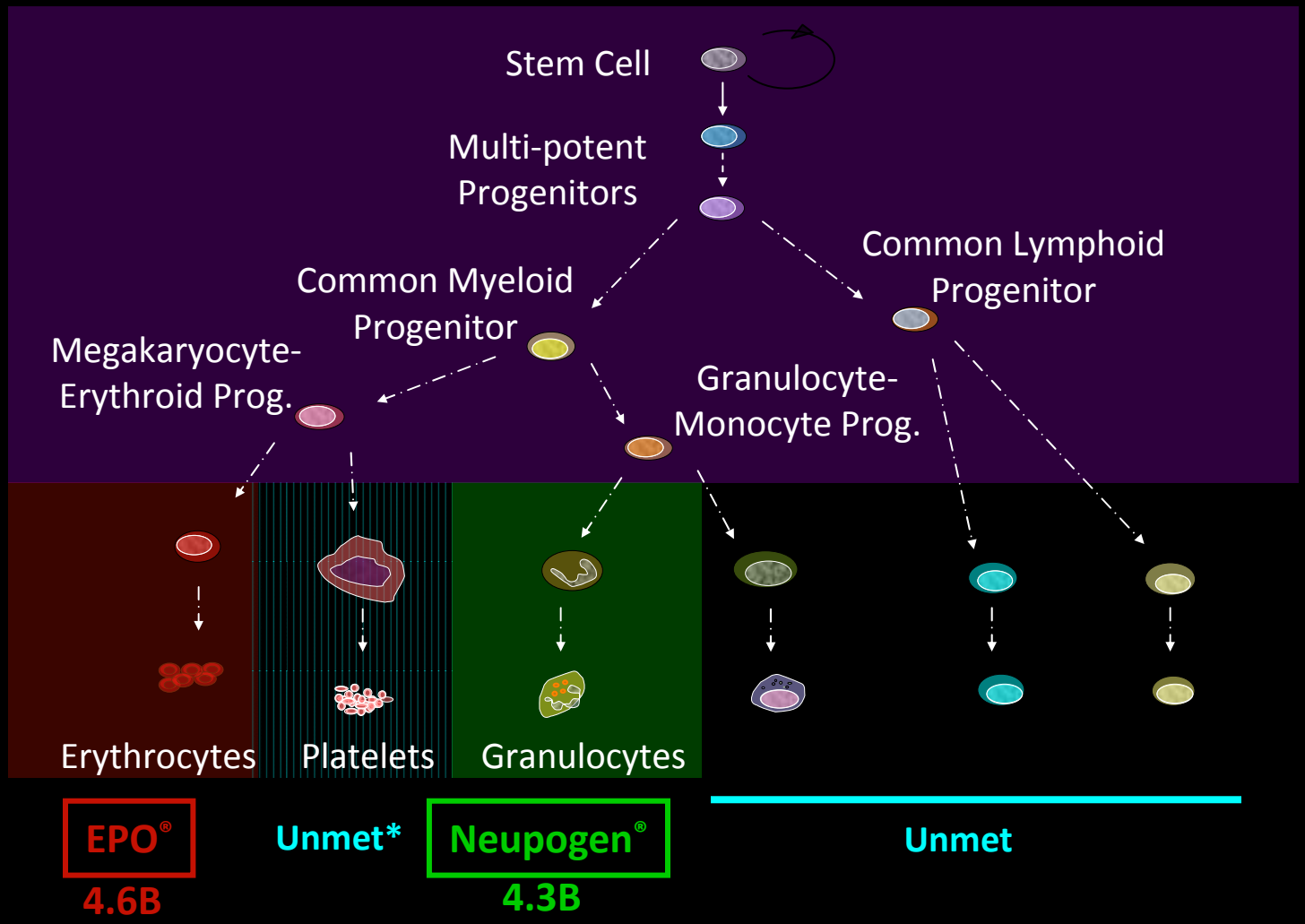
Cyclin D triple KO mice have massive HSC defect

Kozar et al. 2004 *Cell*





Treating Bone Marrow Suppression

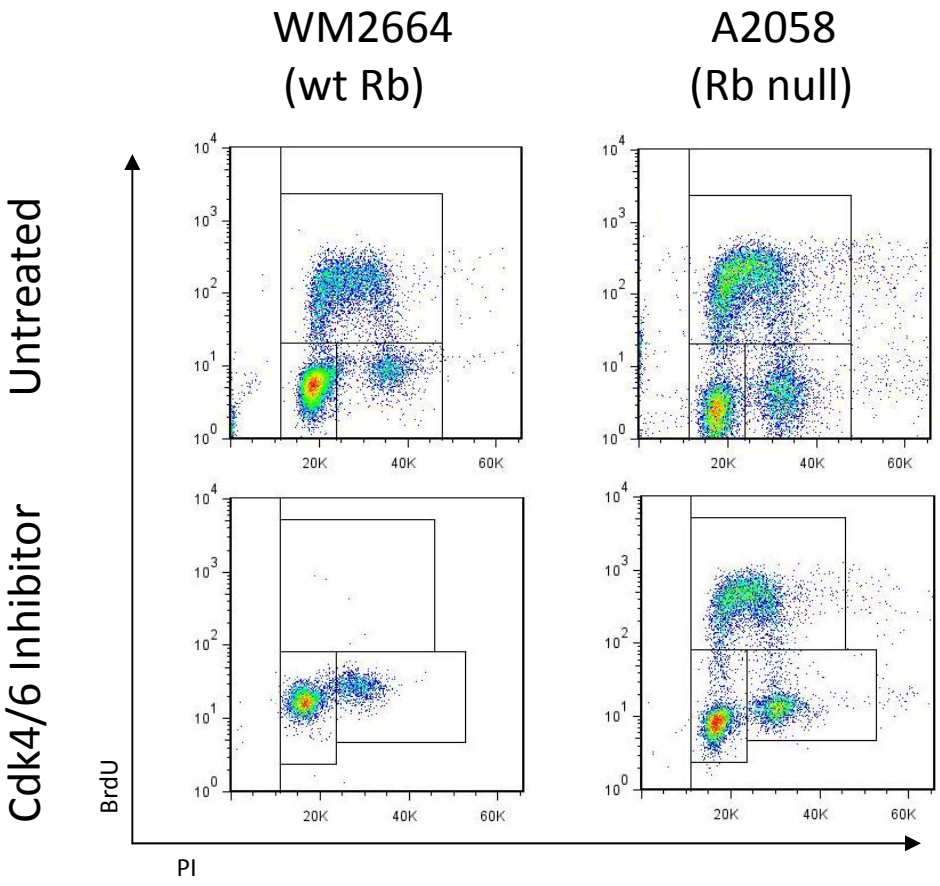


*Neumega ®—minor product

HOW TO MODULATE THE CELL CYCLE AND PROTECT FROM DNA DAMAGE IN VIVO?

Step 1: Screen for highly selective CDK4/6 inhibitors

CDK4/6 Dependent cells arrest in G1 with cdk4/6 inhibitor



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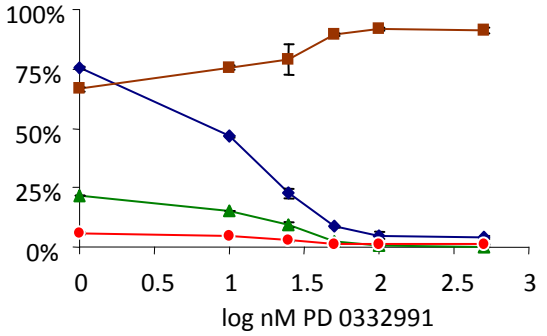

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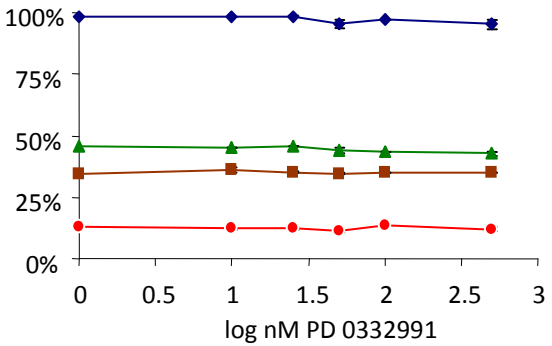

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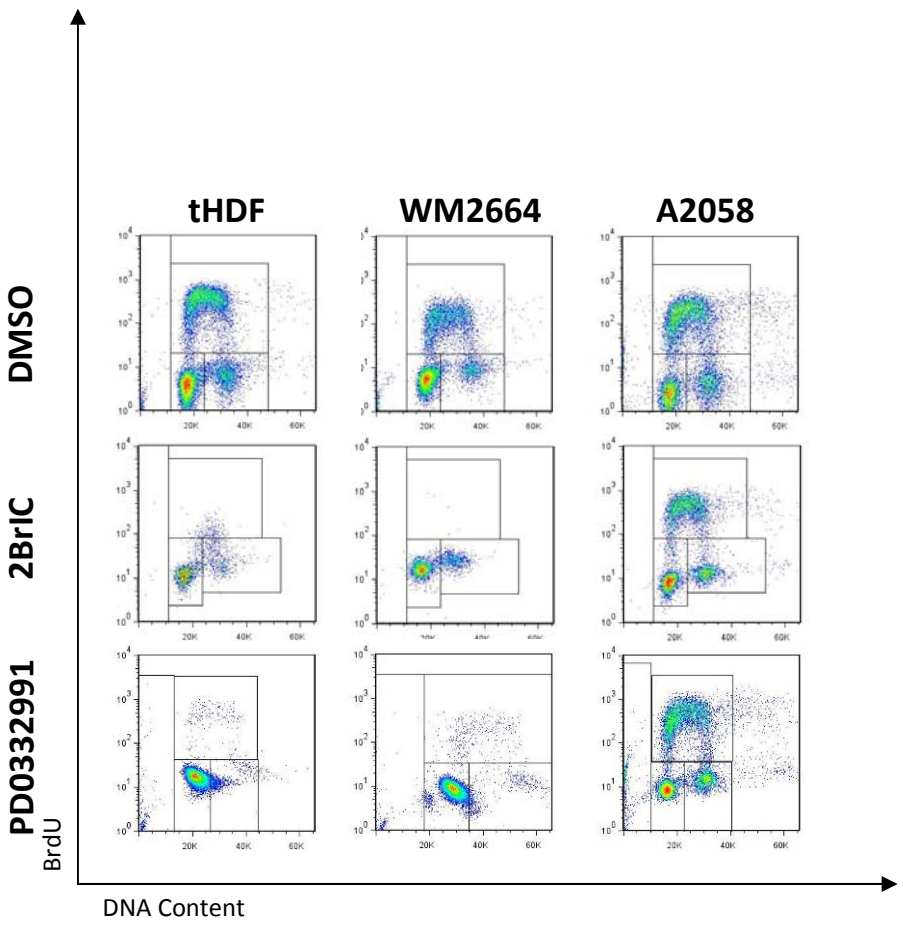
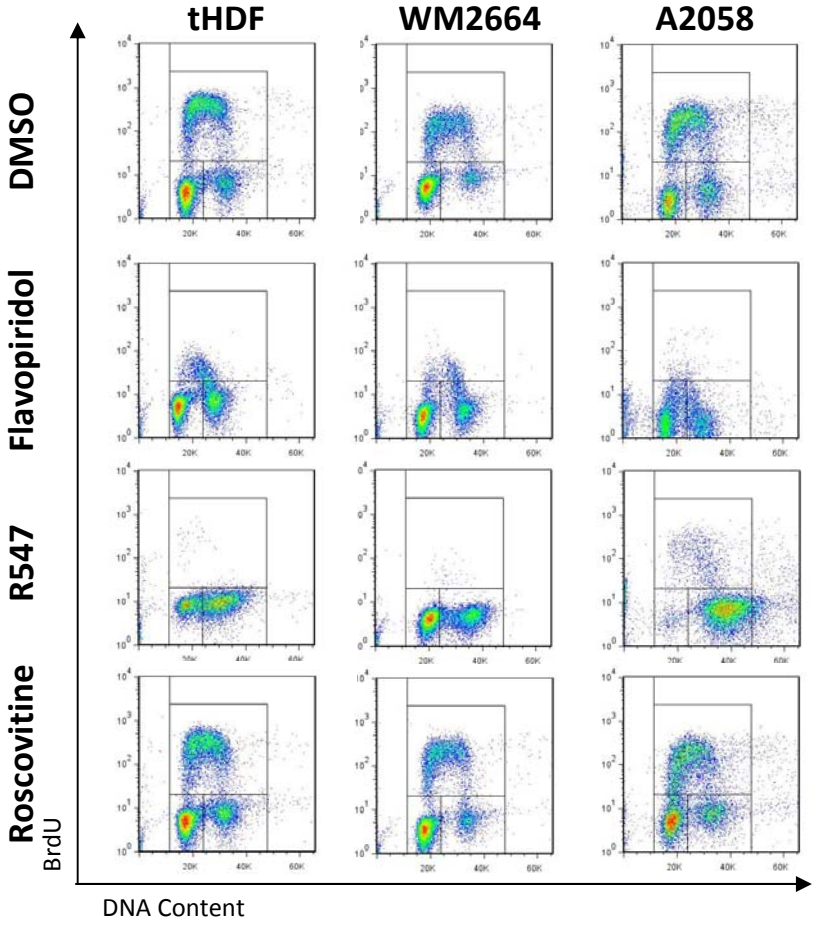
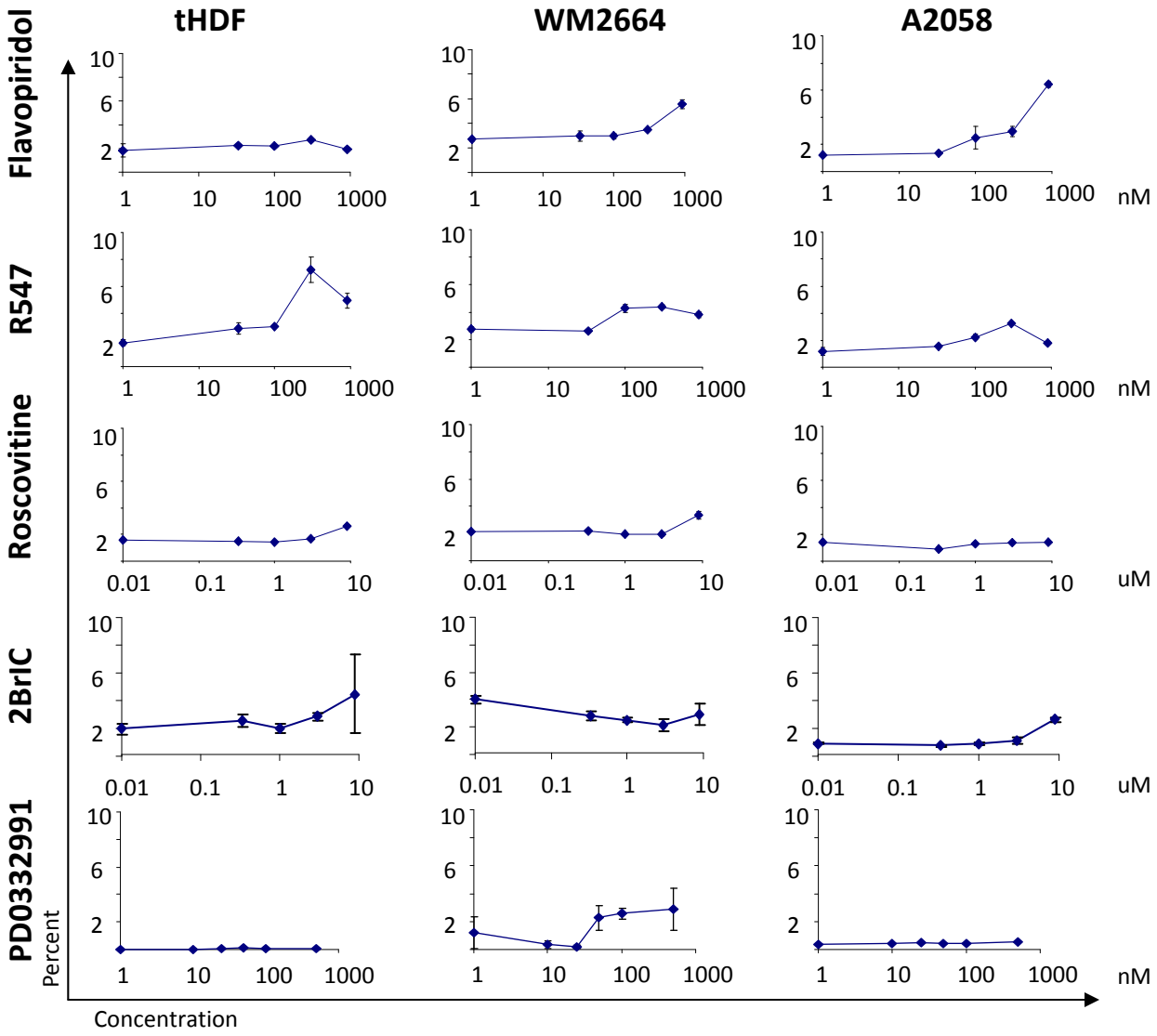
Non-selective cdk4/6 inhibitors do not cause “clean” G1-arrest



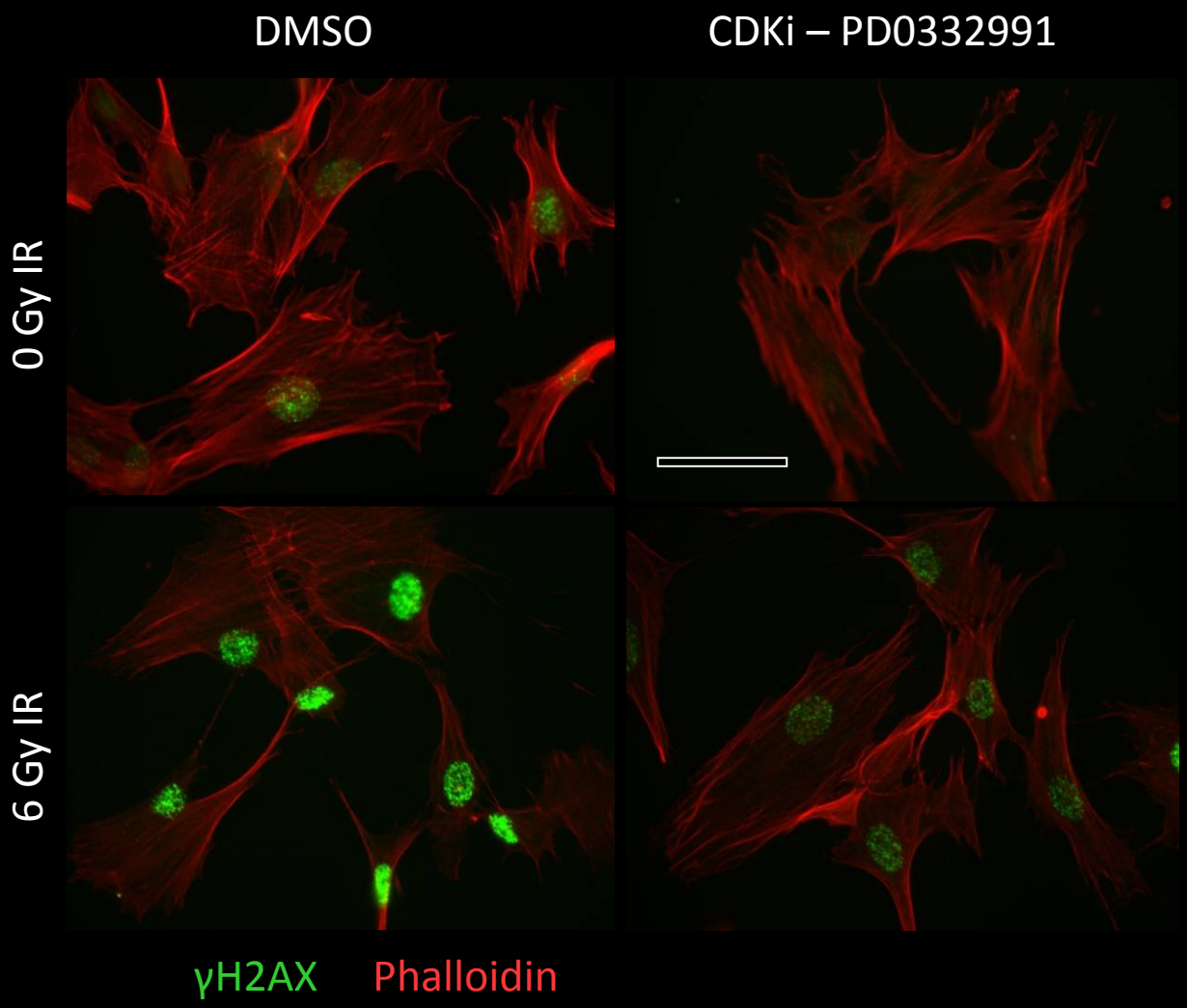


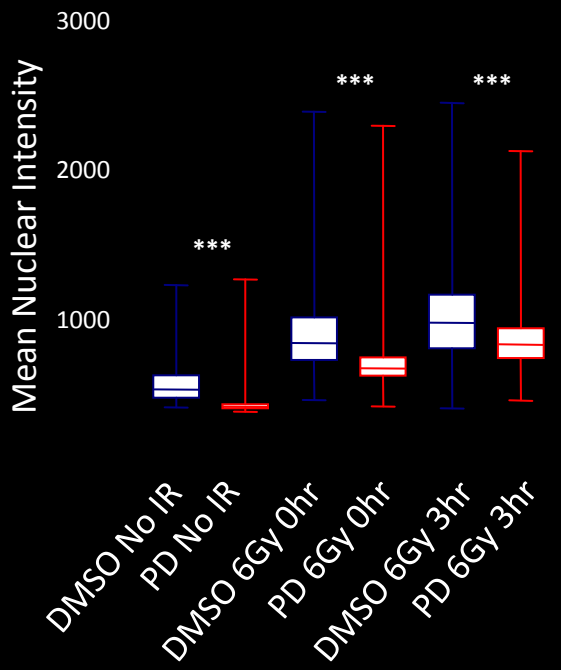
Non-specific CDK inhibitors are toxic



Step 2: Does CDK4/6 inhibition protect cells from radiation *in vitro*?

Cdk4/6 inhibition protects from radiation damage





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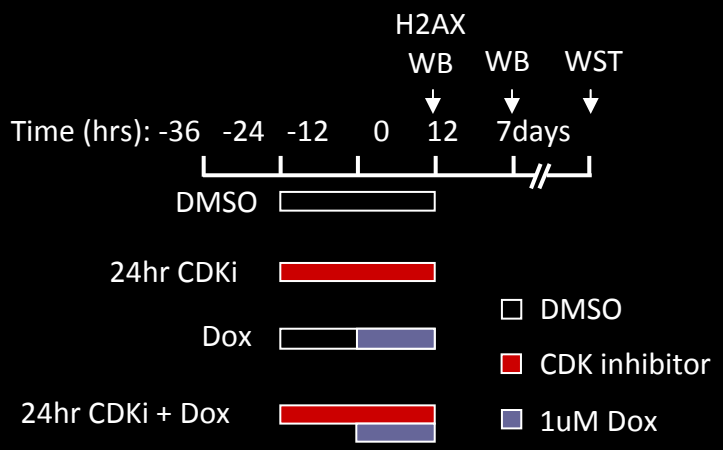
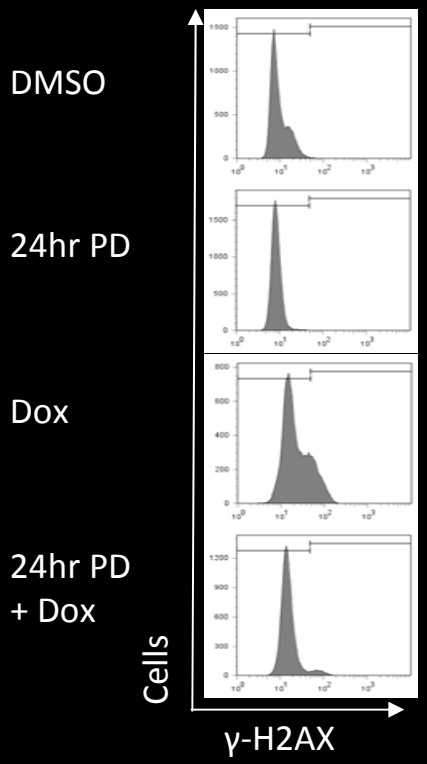
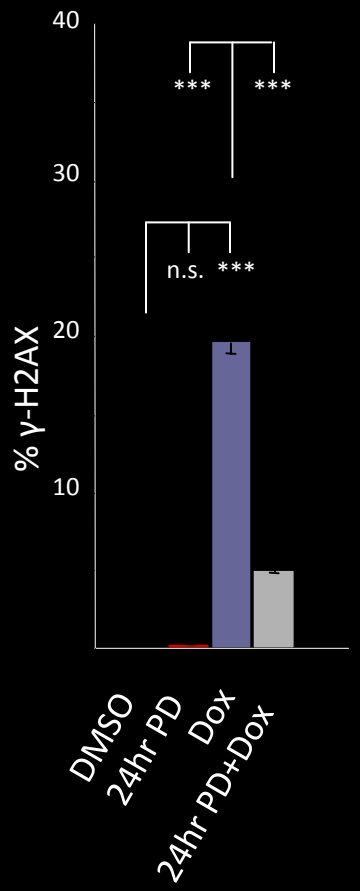
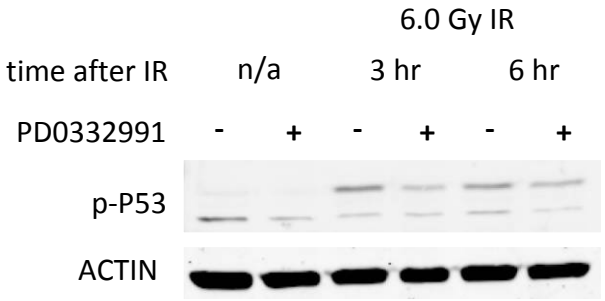
Cdk4/6 inhibition protects from doxorubicin cytotoxicity

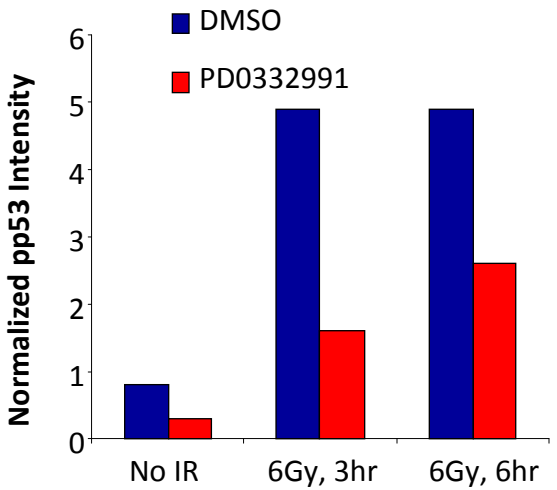


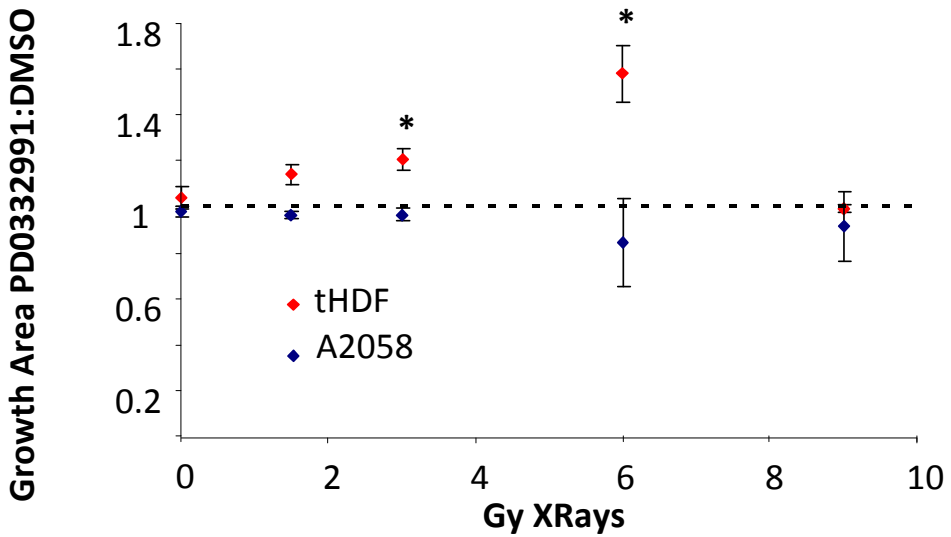




Cdk4/6 inhibition protects from ionizing radiation







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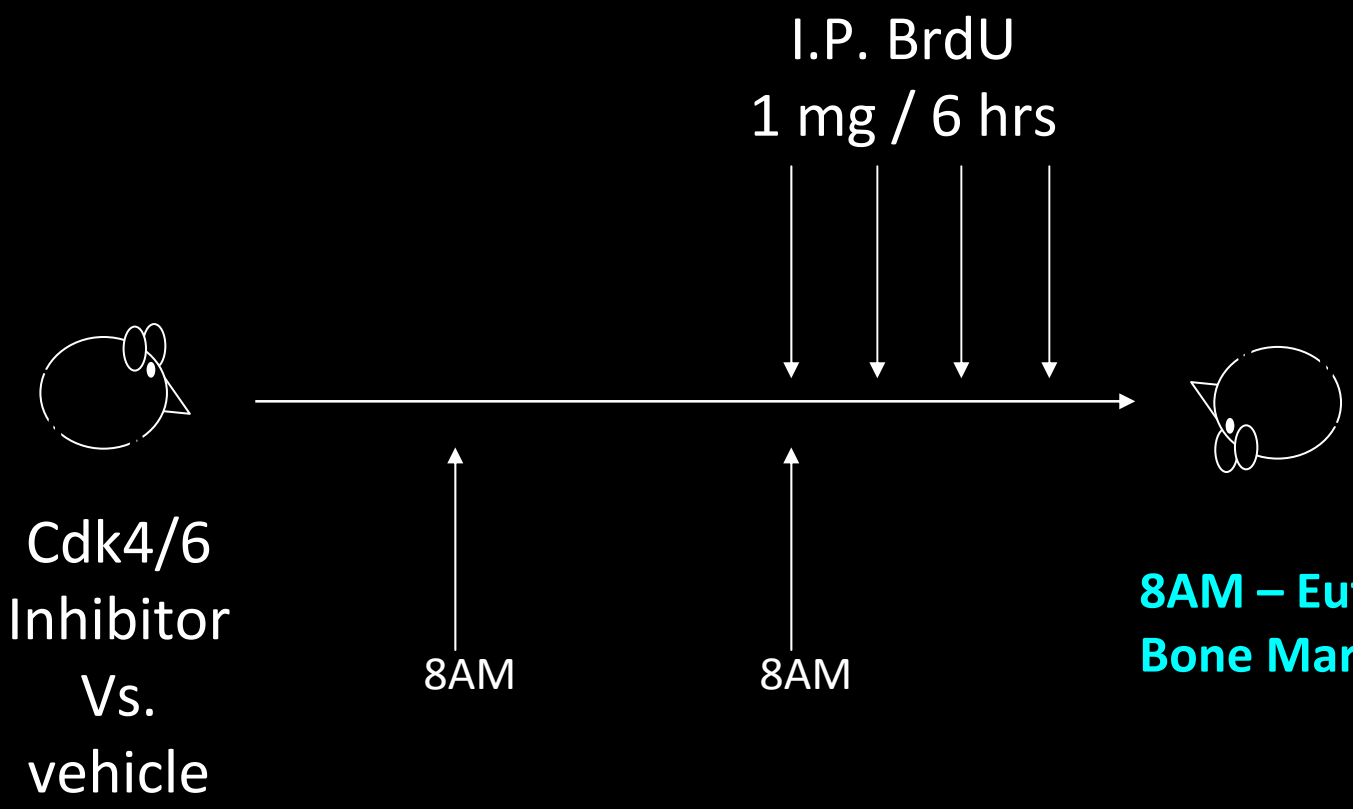
Step 3: Does cdk4/6 inhibition affect hematopoietic proliferation *in vivo*?

Bone marrow proliferation assay procedure

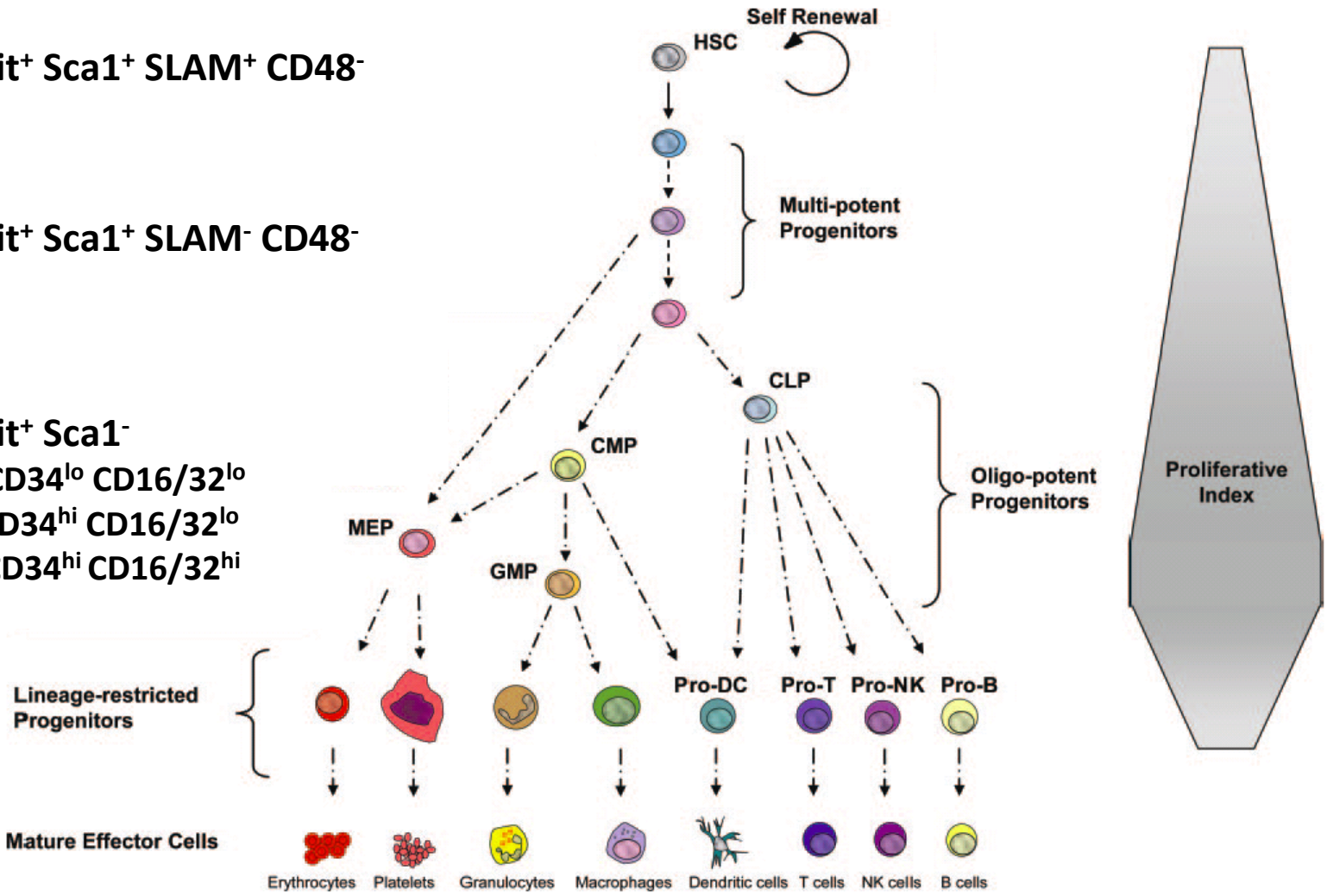


Hematopoiesis

Lin^{-} cKit^{+} Sca1^{+} SLAM^{+} CD48^{-}

Lin^{-} cKit^{+} Sca1^{+} SLAM^{-} CD48^{-}

Lin^{-} cKit^{+} Sca1^{-}
MEP: CD34^{lo} CD16/32^{lo}
CMP: CD34^{hi} CD16/32^{lo}
GMP: CD34^{hi} CD16/32^{hi}



Bryder, Rossi, Weissman 2006 *American J Pathology*

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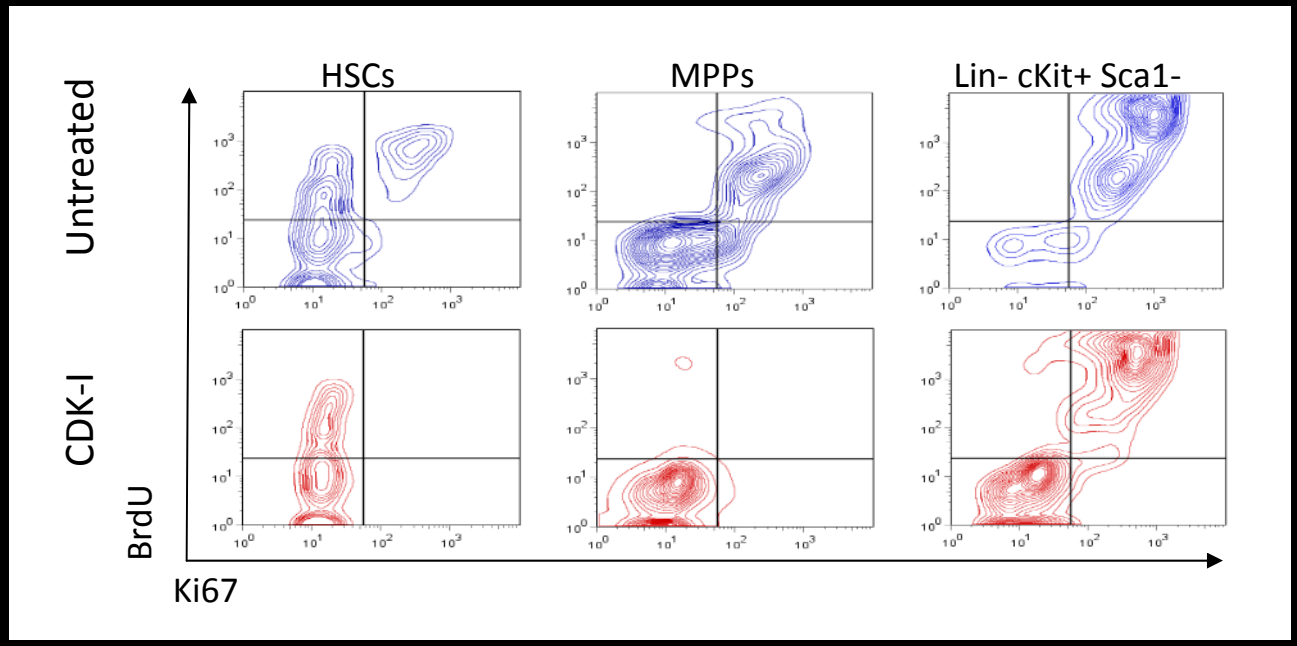
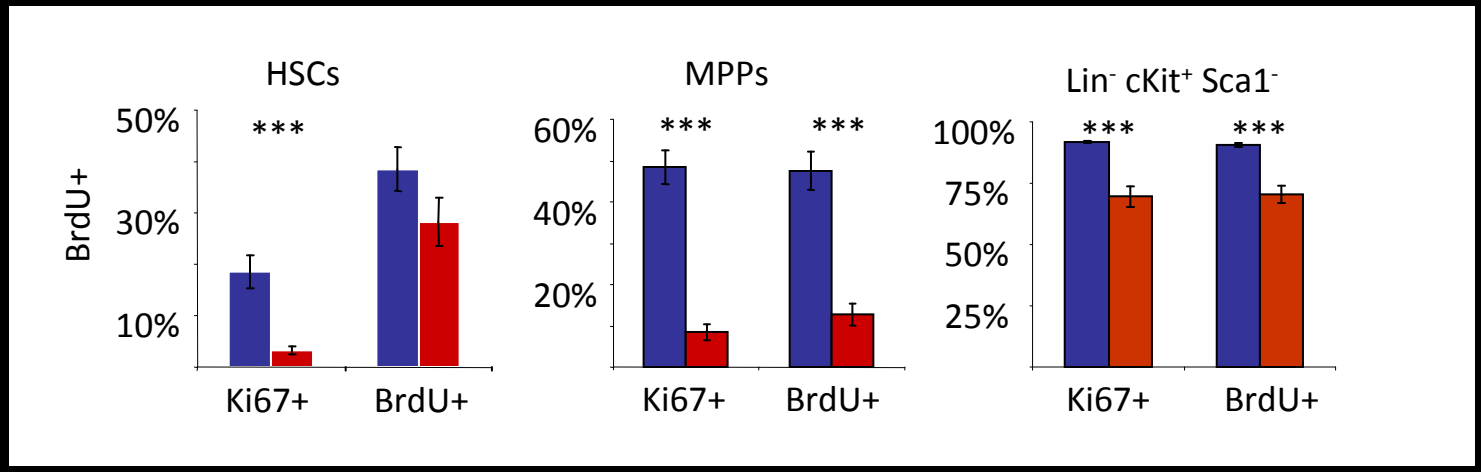
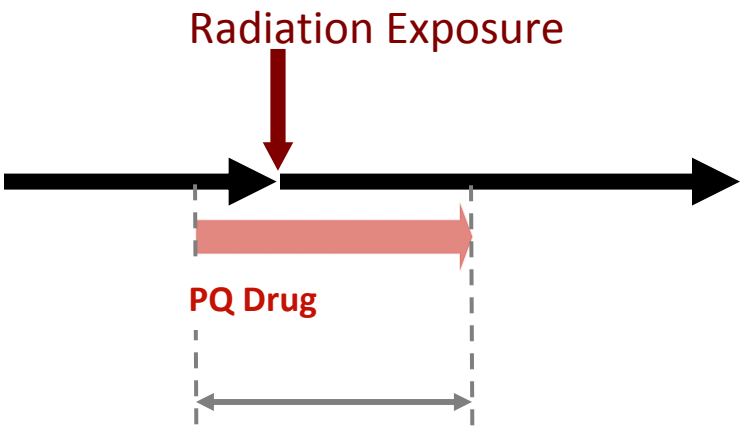
Adult HSPC need CDK4/6 too:



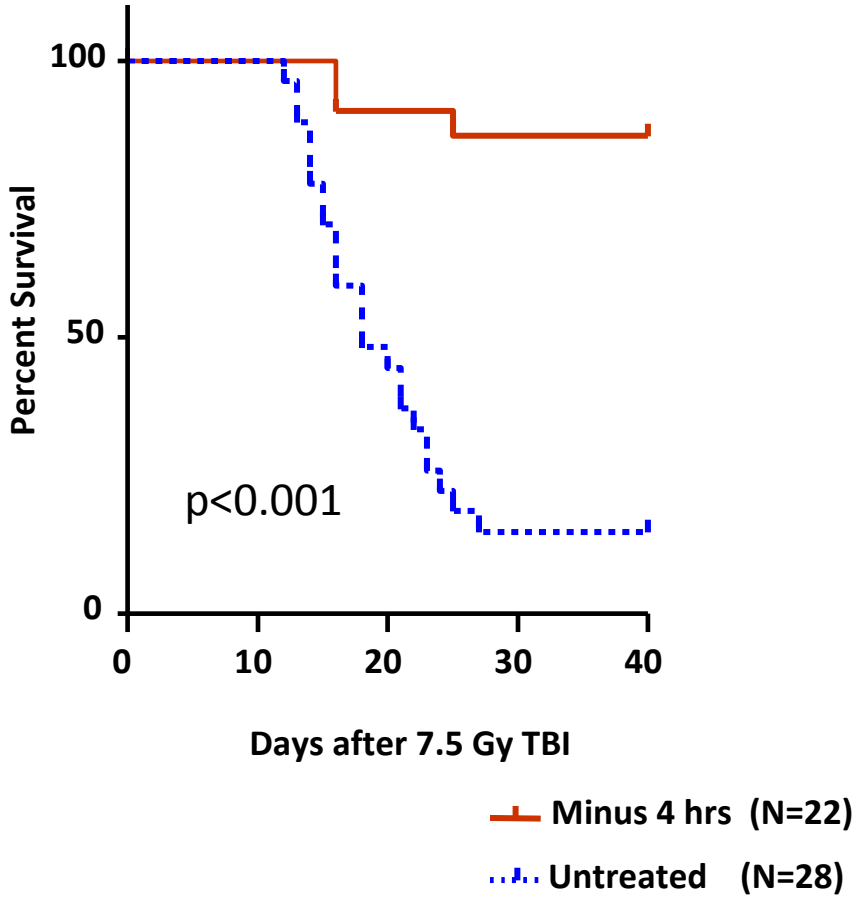


Step 4: Does cdk4/6 inhibition protect mice from radiation toxicity?

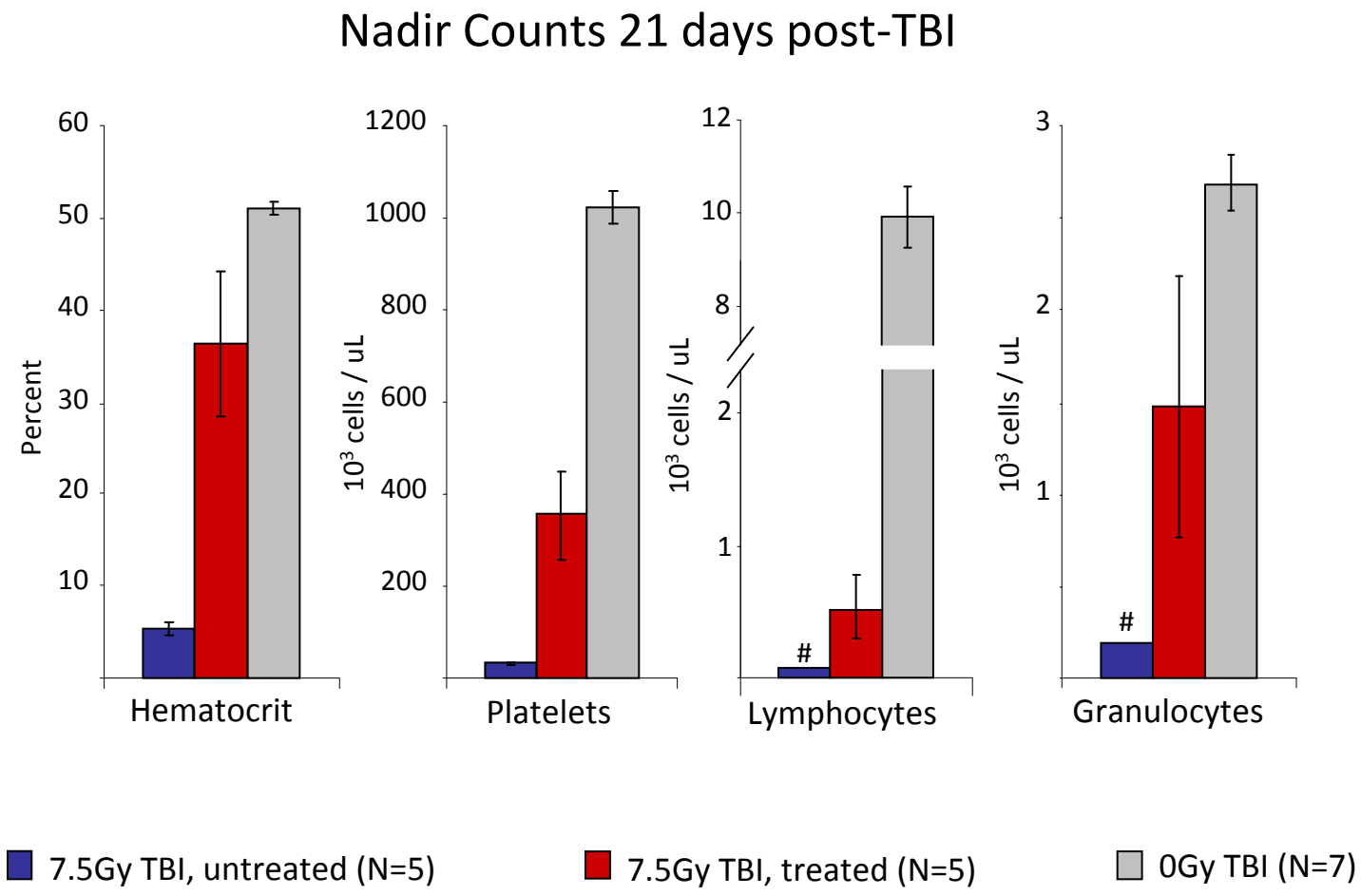
In Vivo Radioprotection



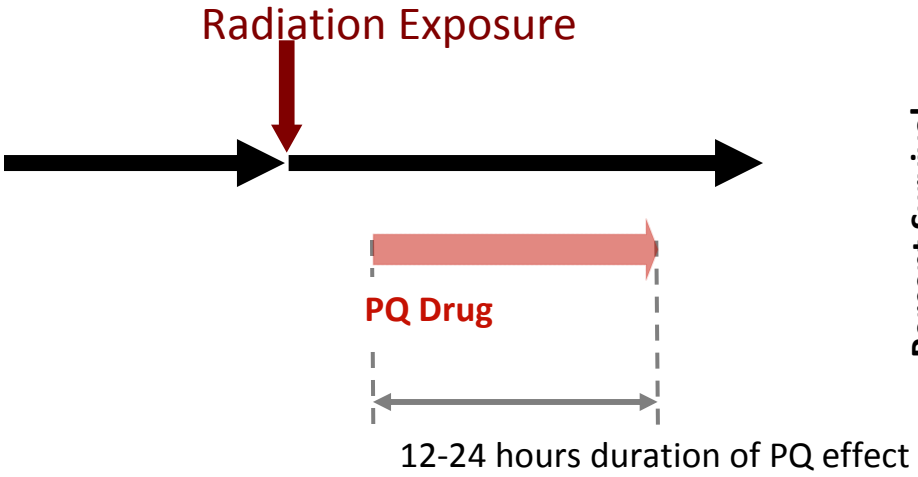
24+ hours duration of PQ effect
starting 0-12 hours prior to radiation

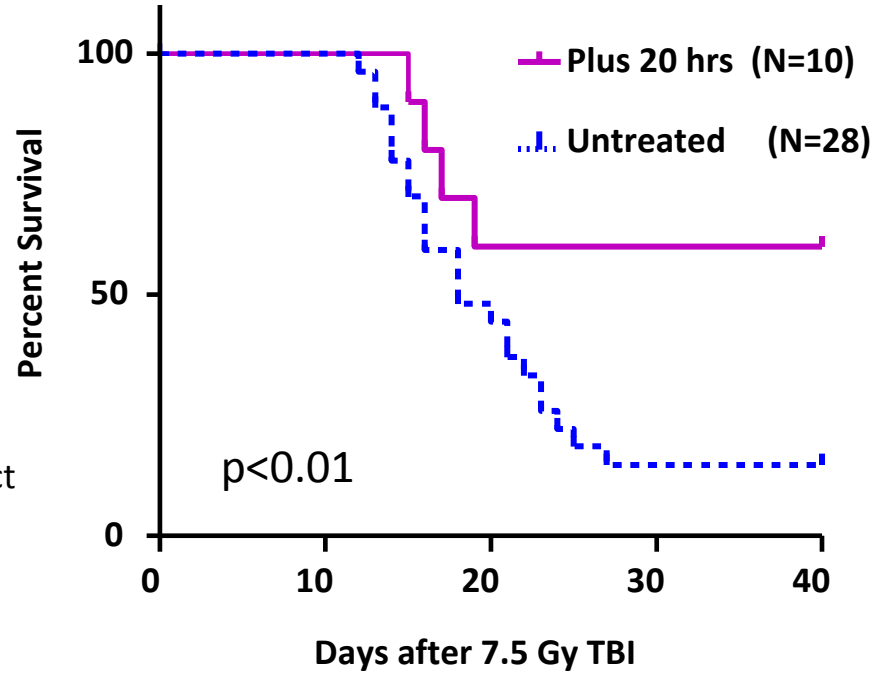


In Vivo Radioprotection



Protection AFTER Radiation: Mitigation





PQ is Robust (*in vivo*):

- Benefits:
  - C3H mice, FVB/n, C57Bl/6
  - Old mice, young mice, male mice, female mice (n>100)
  - Tumor-bearing mice (melanoma, breast cancer)
  - Wild-type, *Ink4a/Arf* KO and *p21^{CIP}* KO mice
  - Multiple doses of X-rays (XRAD) and Gamma rays (Cs)
  - As *protectant* (before) or as *mitigant* (up to 20 hours after) of TBI
  - Protects all 4 hematopoietic lineages from a wide variety of cytotoxic chemotherapy drugs.

Step 5: What are the mechanisms of radioprotection through PQ?

Possible radioprotection mechanisms

A. Prevention

B. Repair

C. Damage conversion

Possible radioprotection mechanisms

A. Prevention -

Radiation causes less damage when cells are in G1

B. Repair

C. Damage conversion

Possible radioprotection mechanisms

A. Prevention

B. Repair -

Increased rates and capacity for repair during G1

C. Damage conversion

Possible radioprotection mechanisms

A. Prevention

B. Repair

C. S-phase entry effects -

Predilection to apoptosis in early S-phase

Non-lethal DNA lesions are converted to lethal damage during DNA replication (DSBs, stalled replication forks, etc.)

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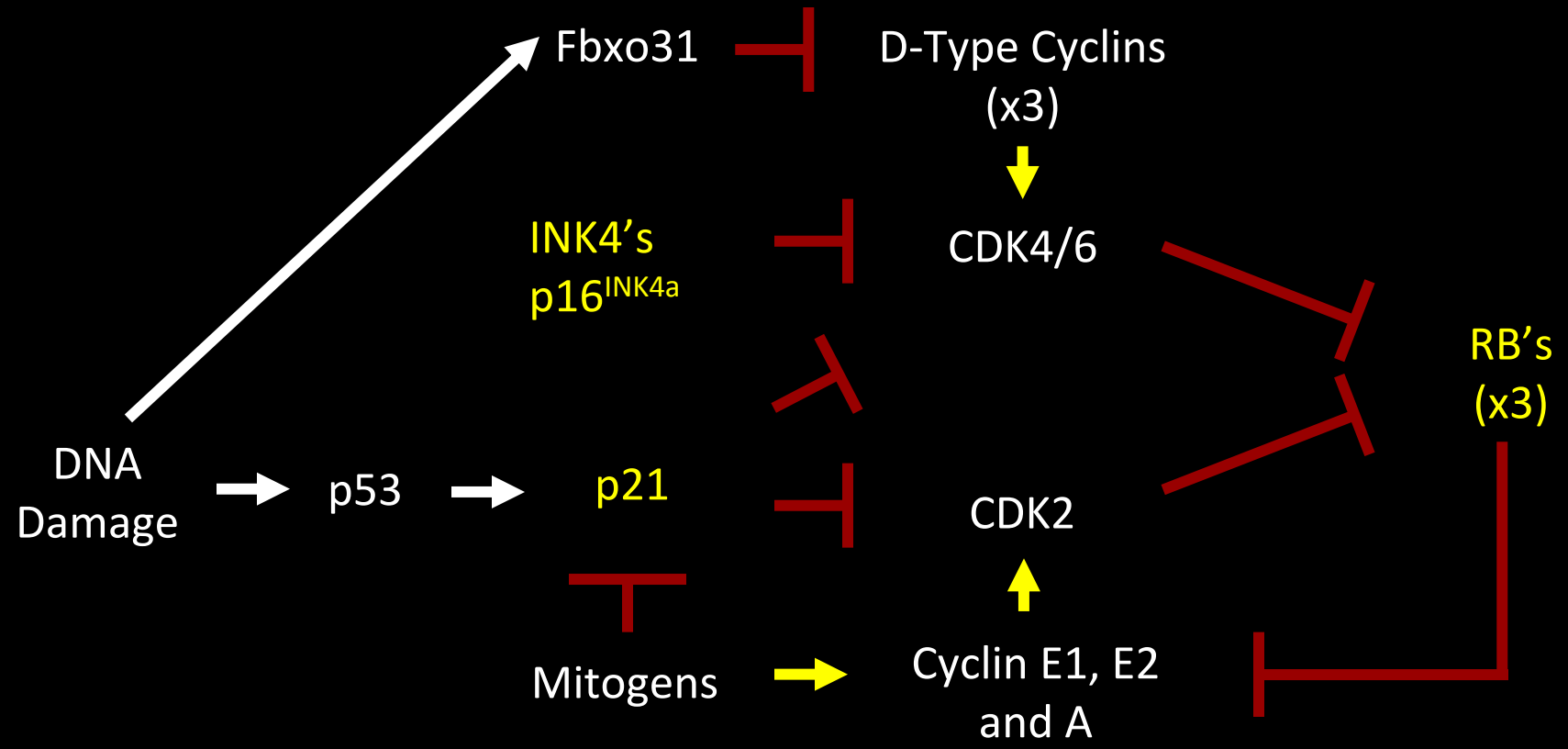
Fbxo31
D-Type Cyclins
(x3)
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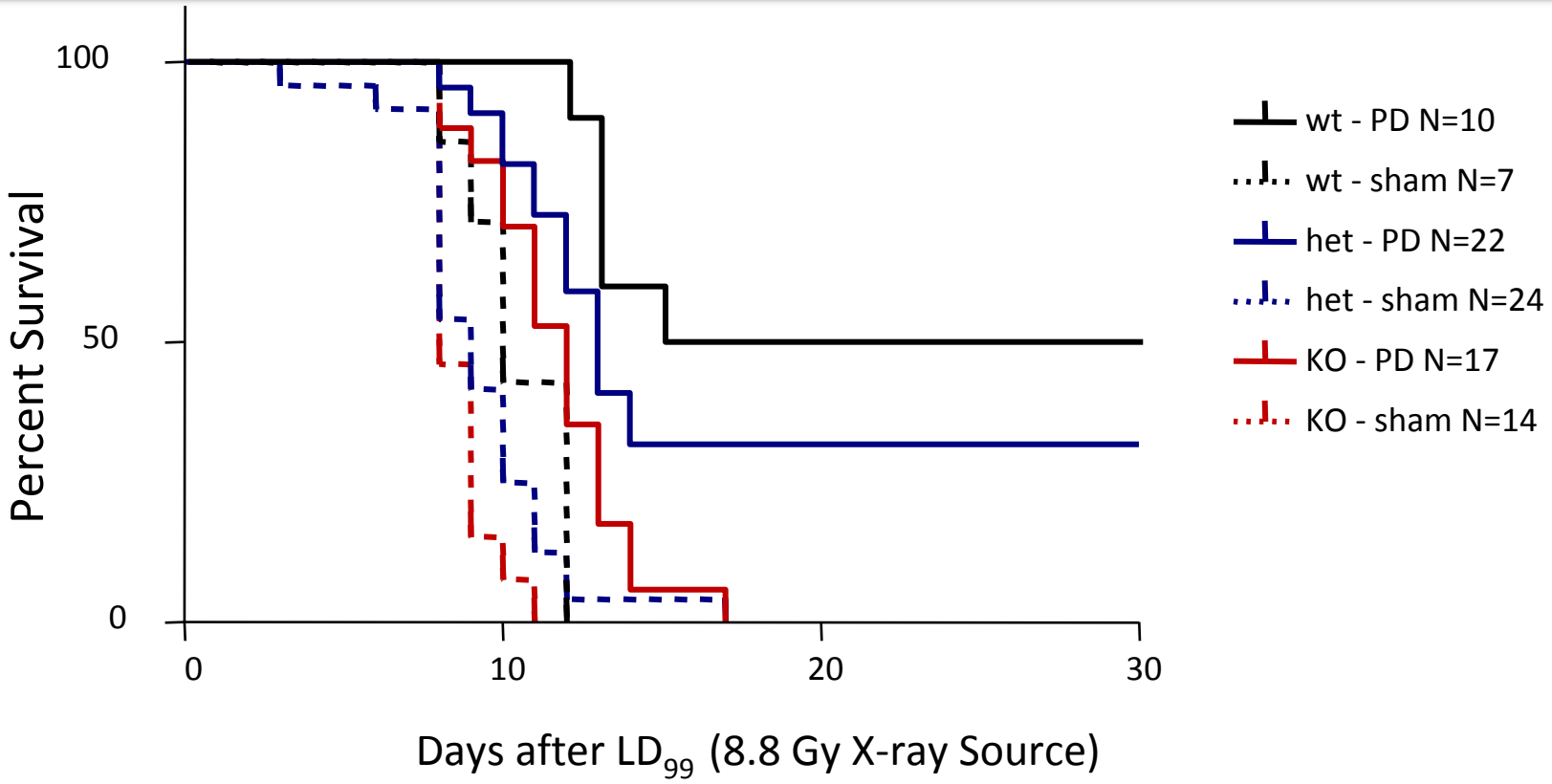
RB's
(x3)
DNA
Damage
p53
p21
CDK2
Mitogens
Cyclin E1, E2
and A

Role of $p21^{CIP}$ in radiation survival



Log Rank Test:

Wt PD vs wt sham	<0.0001
Het PD vs het sham	<0.0001
KO PD vs KO sham	<0.0001

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Possible radioprotection mechanisms

A. Prevention

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C. S-phase entry effects -

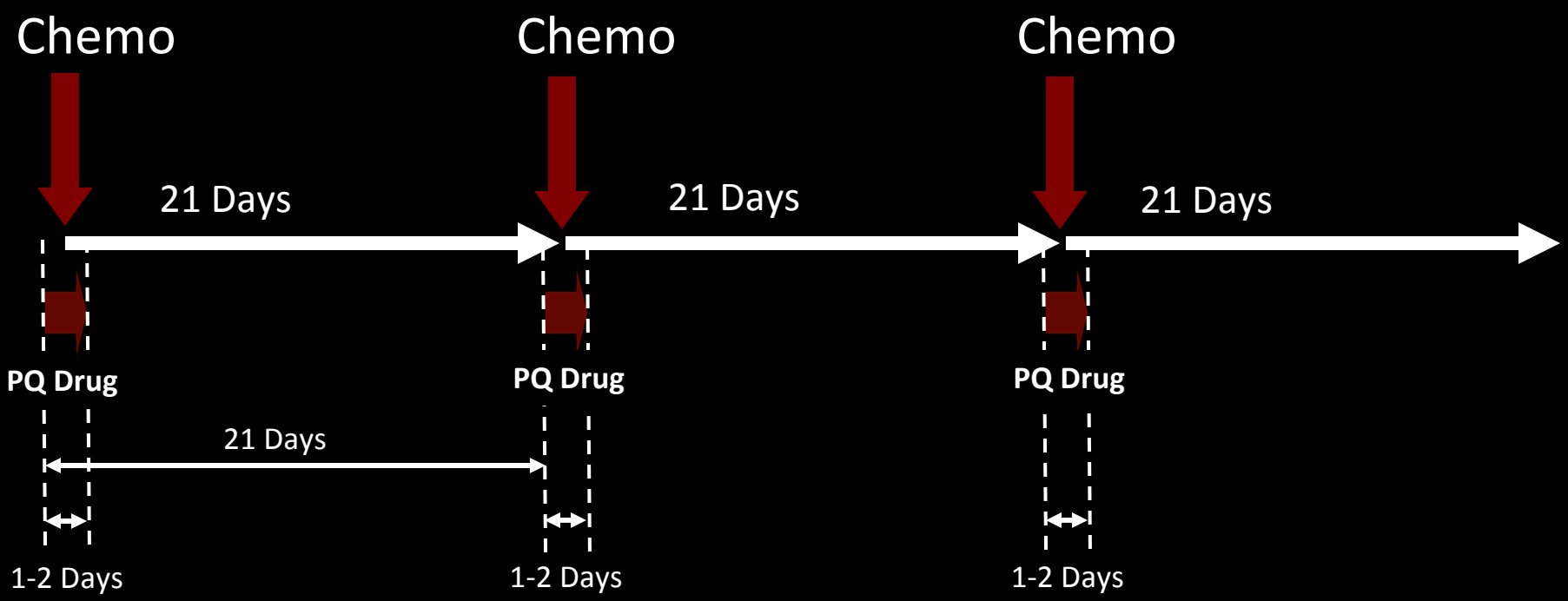
Predilection to apoptosis in early S-phase

Non-lethal DNA lesions are converted to lethal damage during DNA replication (DSBs, stalled replication forks, etc.)

See also Lobrich and Jeggo, (Deckbar, Canc Res. 2010)

Step 6: Beyond radioprotection:

Chemoprotection



Protecting the Tumor?

- Although PQ protects the bone marrow, if it also causes tumor to stop dividing, the tumor would become resistant to DNA damage.
- Most tumors are not addicted to CDK4/6.
- RB null tumors almost always overexpress p16 (CDK4/6 inhibitor) and do not require CDK4/6 activity for proliferation.
- Per COSMIC, 11% of human cancer is RB-null.

PQ vs. Existing Therapies

Existing Treatments (Epo, GCSF)	Pharmacological Quiescence
❑ Biologics by injection	❑ Orally available small molecule
❑ Mortality liability (EPO in oncology patients)	❑ Well-tolerated (animals, human Ph1/2)
❑ Therapies boost blood cell production but do not prevent BMS	❑ Protects bone marrow
❑ Two therapeutics to treat RBC, WBC; no extant therapy for platelets or lymphocytes	❑ One agent treats all lineages

SUMMARY

CDK4/6 activity is required for proliferation in highly, specific cellular compartments, regulating self-renewal and transformation.

Selective pharmacologic modulation of CDK4/6 (PQ) in vivo is well-tolerated, causing transient cell cycle arrest in HSPC.

PQ affords marked resistance to the hematopoietic toxicities of DNA damaging agents in vivo.

PQ provides radiomitigation: protecting from lethal myelosuppression even when initiated AFTER the radiation exposure.

Acknowledgements

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Yong Wang

Pfizer

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Gerit Los

Harvard

Kwok-Kin Wong
Ben Humphreys
Derek DiRocco

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