Article

Cell

# Patterns of Early p21 Dynamics Determine Proliferation-Senescence Cell Fate after Chemotherapy

Authors

Chien-Hsiang Hsu, Steven J. Altschuler, Lani F. Wu

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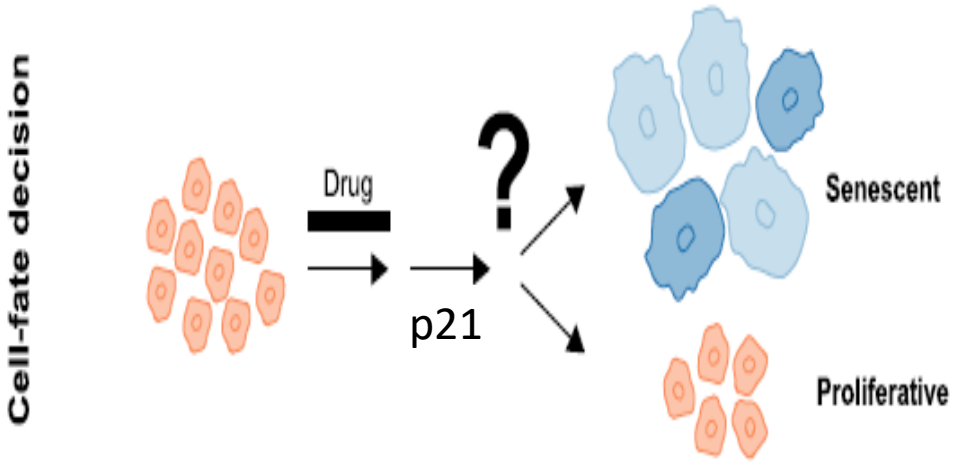
Dimitrios Veroutis, Master Student

# Introduction

- At non lethal doses of chemotherapy cancer cells can choose to remain proliferative or become senescent
- Key molecular mediator of therapy-induced senescence is the cyclin-dependent kinase inhibitor p21 (CDKN1A)
- p21 has the ability to promote both proliferation and senescence cell- fate outcomes
- p21 induction at 5 h after DNA-damaging treatment is predictive of a loss of proliferative potential at 24h
  **(promotion of senescence cell- fate)**
- Radiation- induced p53- independent up- regulation of p21 in stem cells limited damage accumulation and promoted the expansion of a stem cell pool
  **(promotion of proliferative cell-fate)**




How do early signaling events connect to final proliferation- senescence cell fate?

# 1-Day Pulsed Drug Treatment Leads to Mixed Proliferation and Senescence Fates

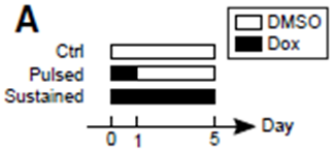
A549 cells

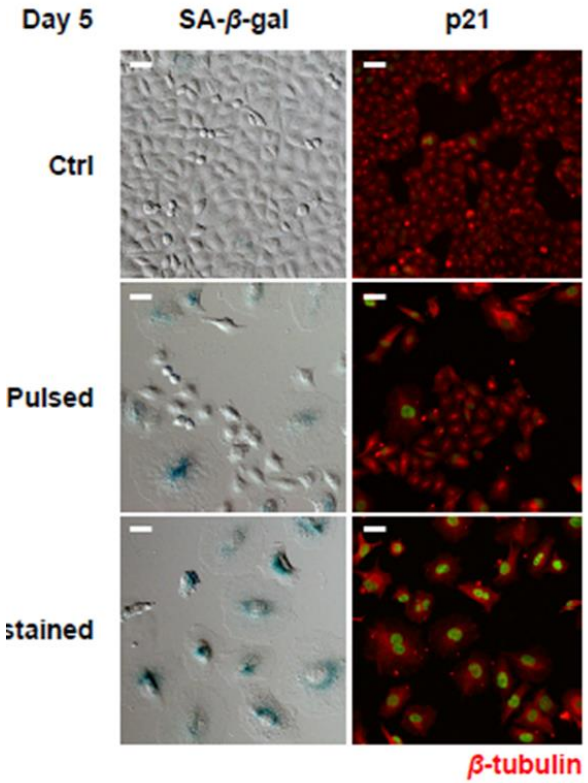
Dox/ 24h

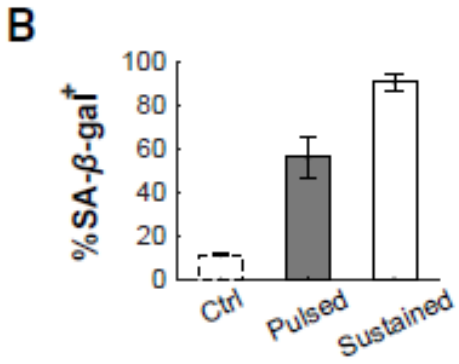
Dox/ 24h

Proliferation → p21, Ki-67

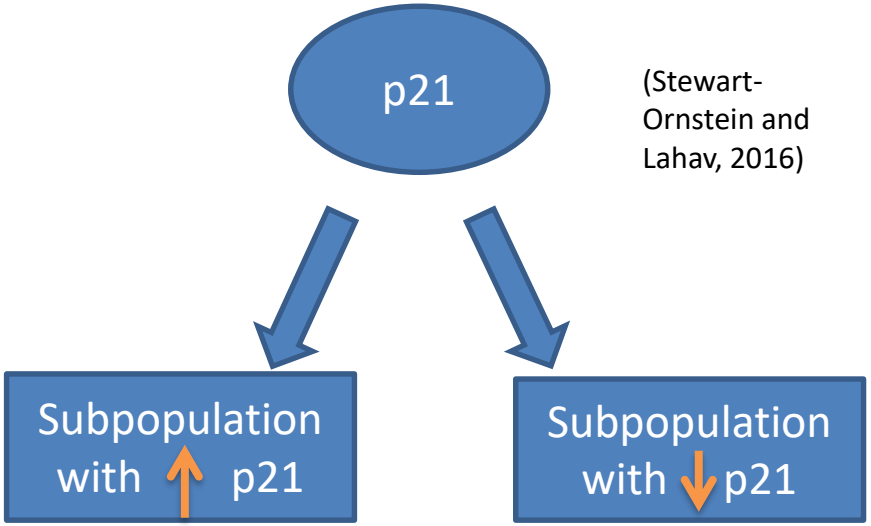
Senescence → SA-βgal, p21



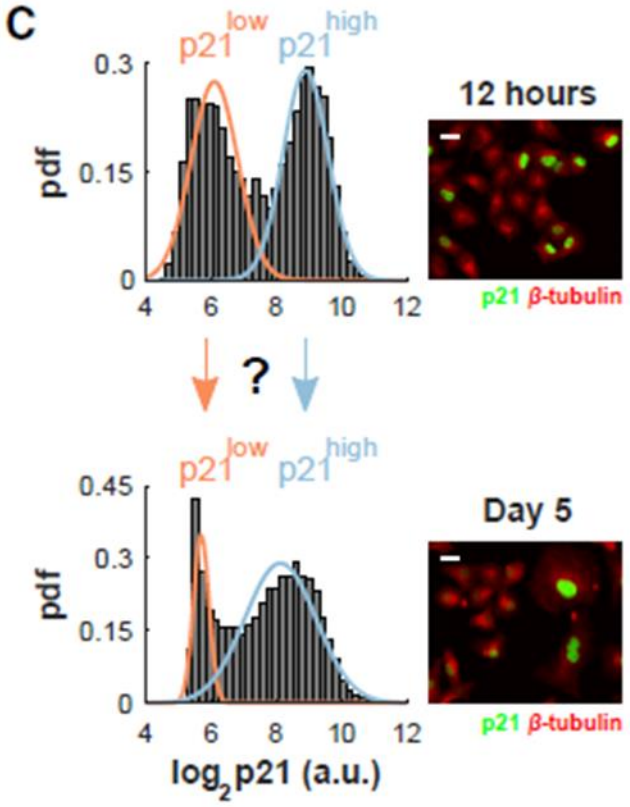



# What early signaling event govern cell- fate decision ?




(Stewart-Ornstein and Lahav, 2016)

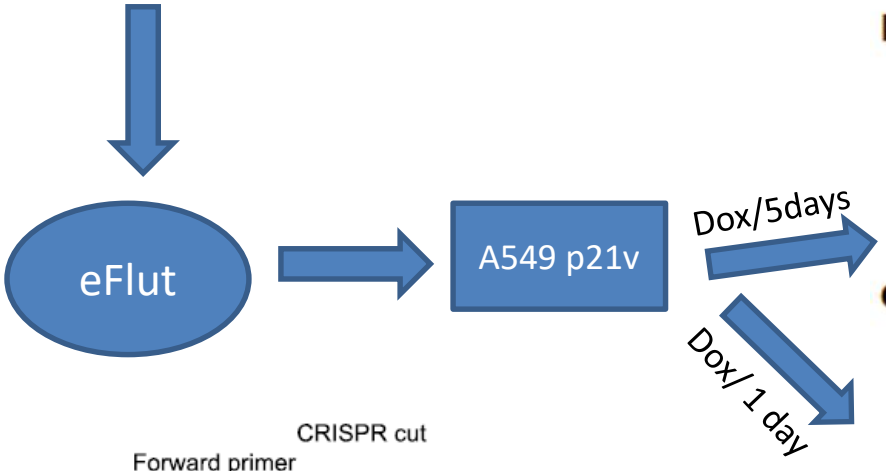


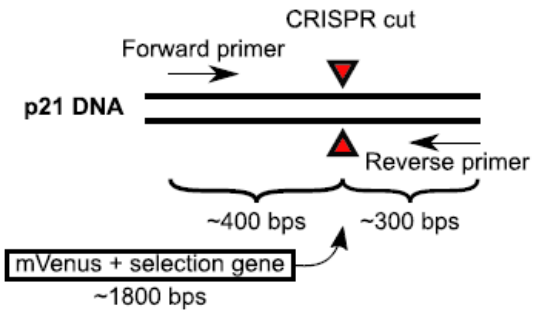

Hypothesis: early low p21 levels stay low and lead to a final proliferation state and early high p21 levels stay high and lead to a final senescence fate

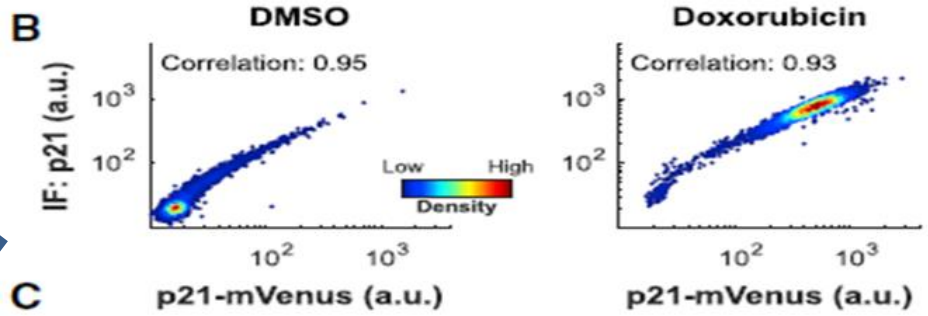
# How p21 expression during drug pulse determines the final cell fate ?

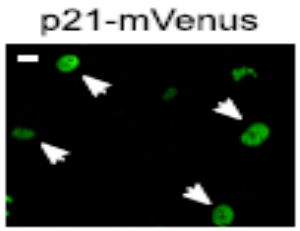
A Live- Cell Reporter System Links Early p21 Expression to Final Cell Fate

1. monitor p21 levels at a single- cell resolution
2. link early p21 expression levels to final cell fates



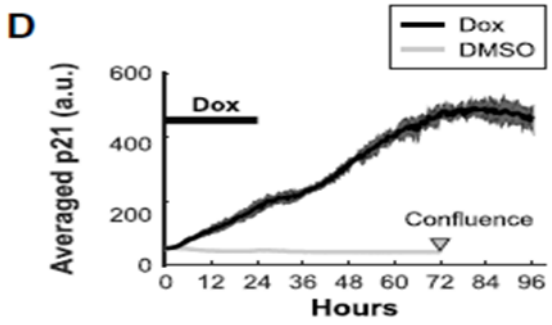



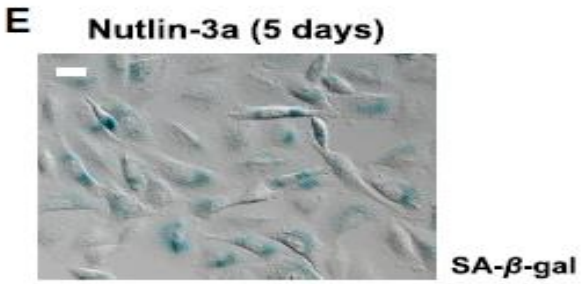

- Bimodal distribution of p21-mVenus was observed

✓ A549p21V

# How p21 expression during drug pulse determines the final cell- fate ?

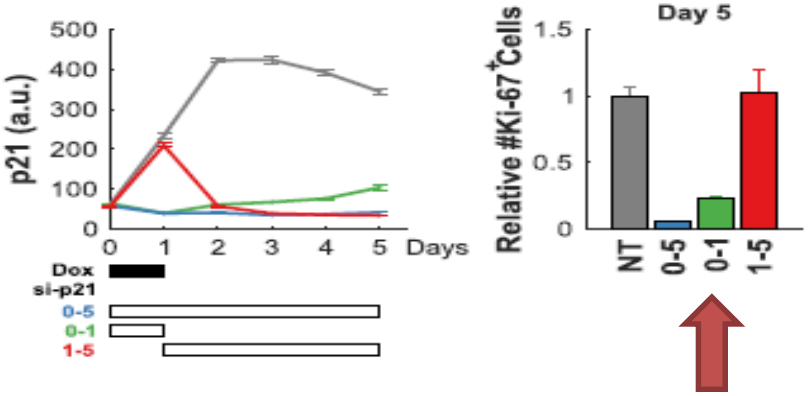



Nutil-3a: sustaind induction of p21

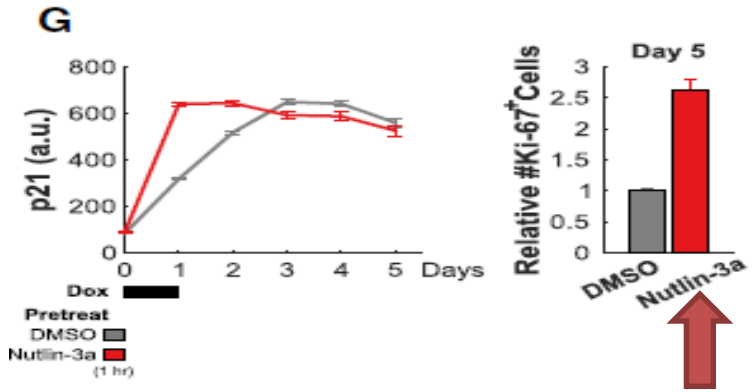



Hypothesis: Inhibiting or enhancing p21 expression during drug pulse should increase or decrease the fraction of cells with proliferation fates after drug removal ?

## Inhibition of p21

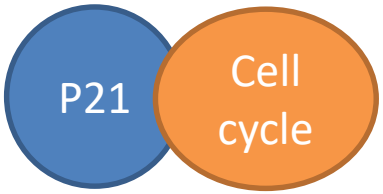



## Enhanced of p21 expression




p21 induction before or during- but not after- drug pulse is essential for promoting proliferation fate choice

# In Silico Cell-Cycle Detection Links p21 and Cell-Cycle Dynamics to Cell Fate



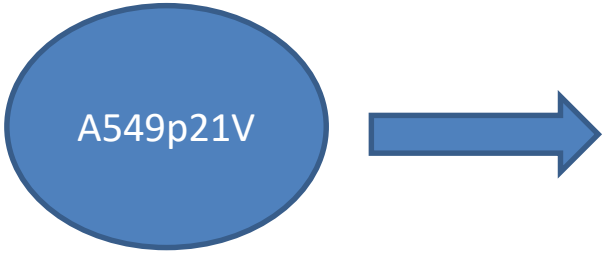

In silico cell- cycle detector consist of three steps

1. Cell division events (M)
2. Period when p21 is undetectable levels (S)
3. Remaining time points ( G1 ή G2)

Main assumption

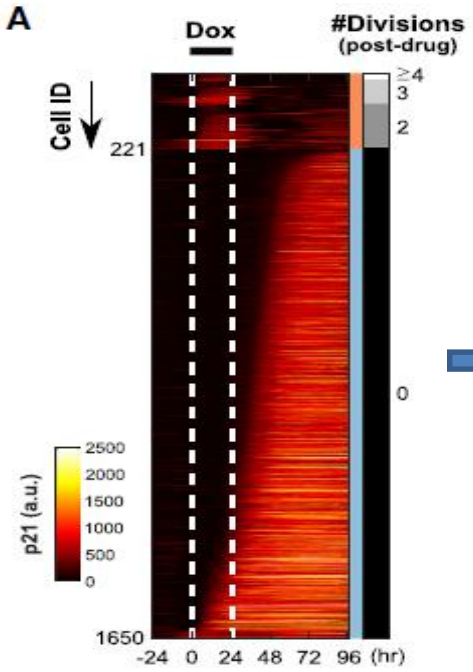
p21 undetectable - S phase

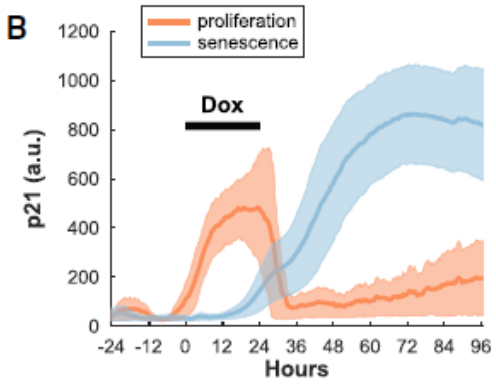
How early p21 expression during drug pulse determines final cell fate?



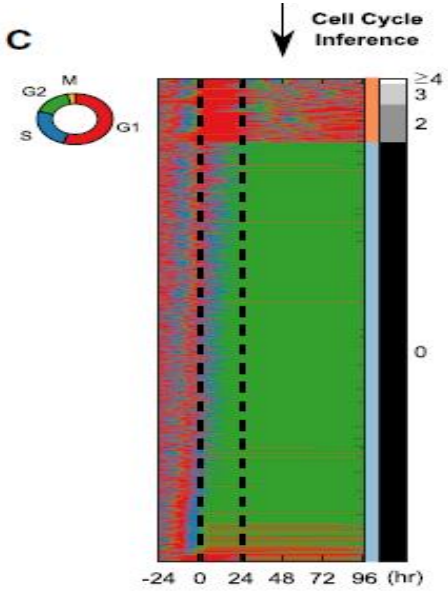

Imaging every 20 min, starting from 24h before the pulsed doxorubicin treatment

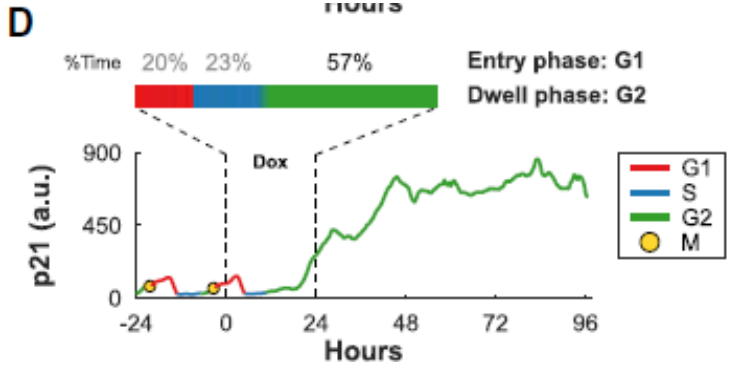
# Different Cell-Fate Outcomes Have Different Patterns of p21 Dynamics



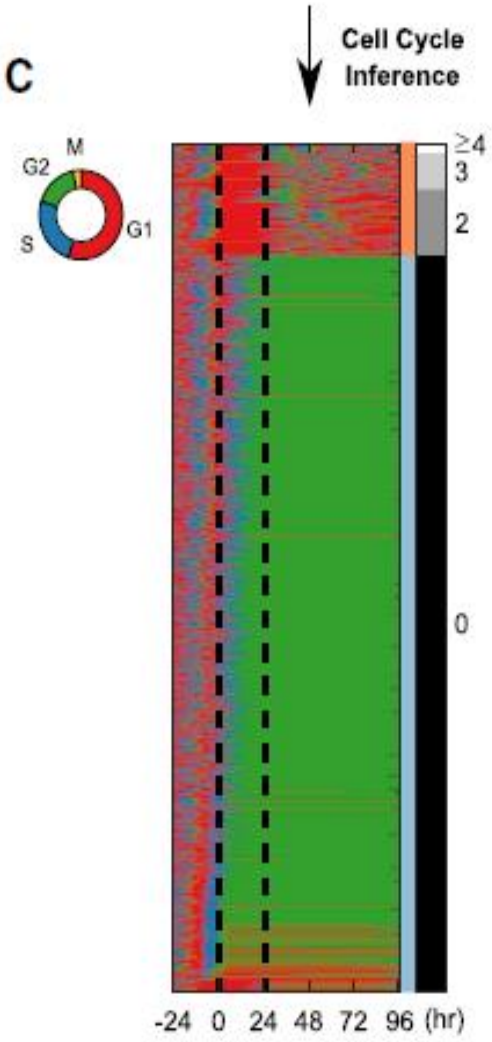

Pulsed p21 dynamics during drug treatment has a causal role in determining final cell fates

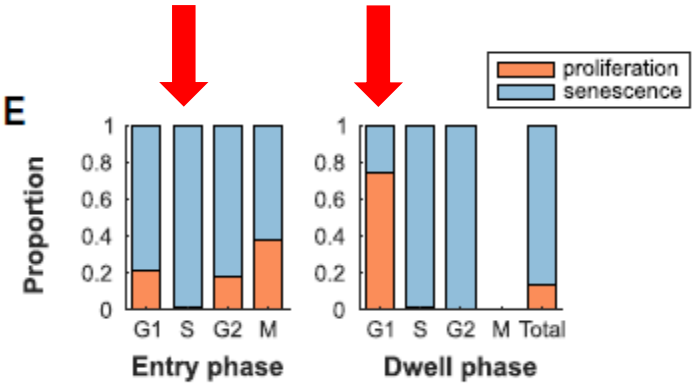


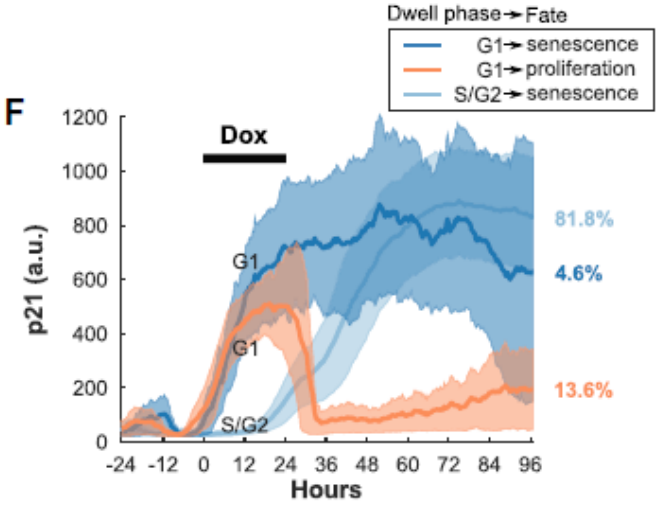

**entry phase**: cell cycle phase at treatment onset (o h)
**dwell phase**: the longest cell- cycle phase a cell stayed in during drug pulse

# How entry and dwell phases related to cell fate?



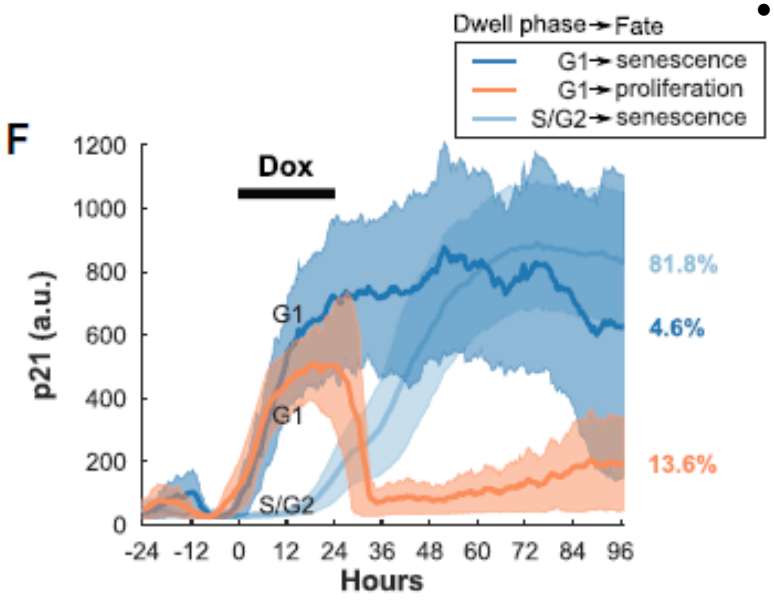

- S phase good indicator of senescence- fated cells A
- Proliferation- fated cells almost entirely emerge from G1 dwell phase




| Patterns of p21 dynamics | |
|---|---|
| Senescence fate | Proliferation fate |
| Delayed- low p21 response | Acute-intermediate response |
| Acute- high response | |

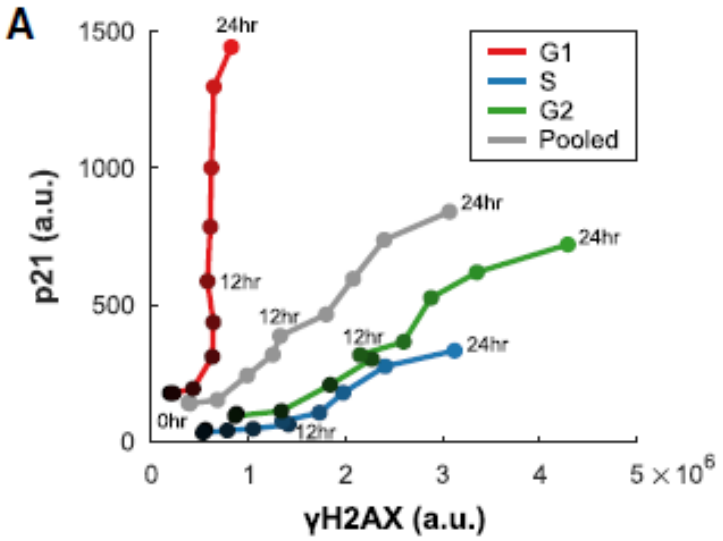
p21 can promote opposing proliferation and senescence fates depending on the patterns of its dynamics

# Why cells in G1 dwell phase have higher p21 expression than cells in S/G2 dwell phases?



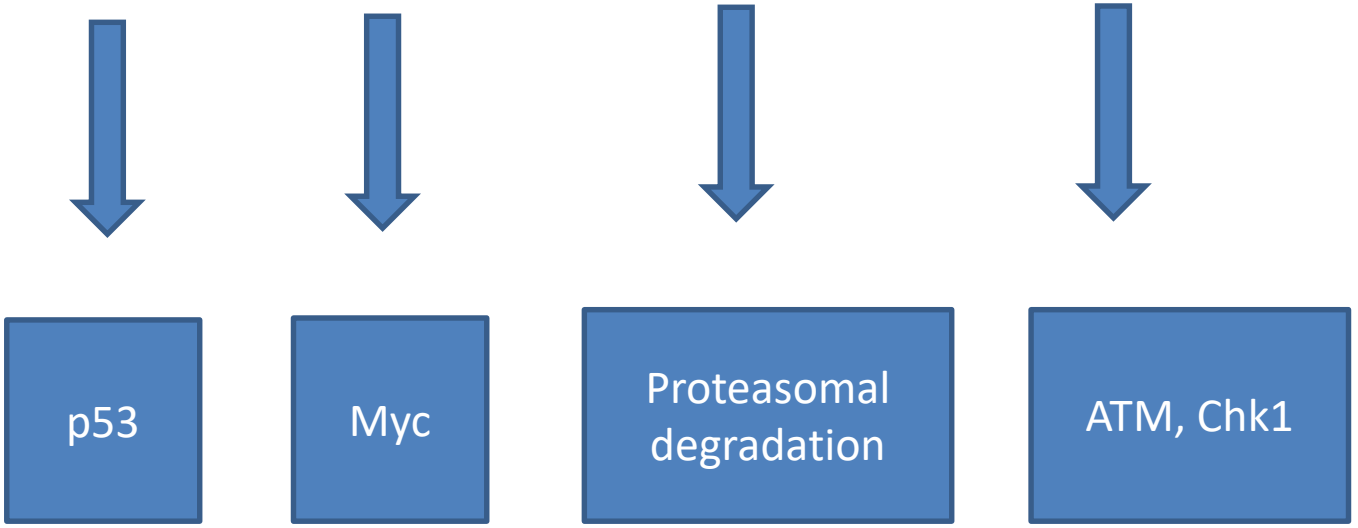

- Dox. is most damaging to cells in S/G2 phases

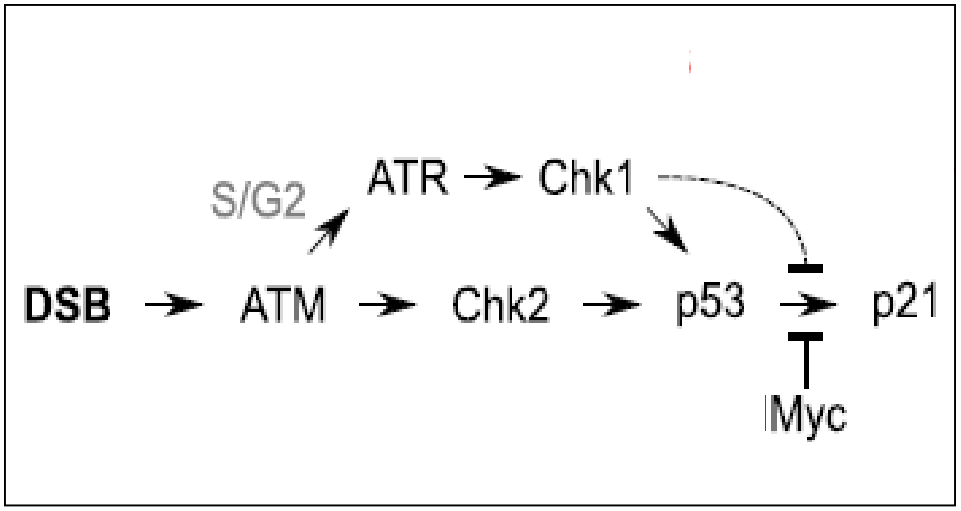
DNA damage



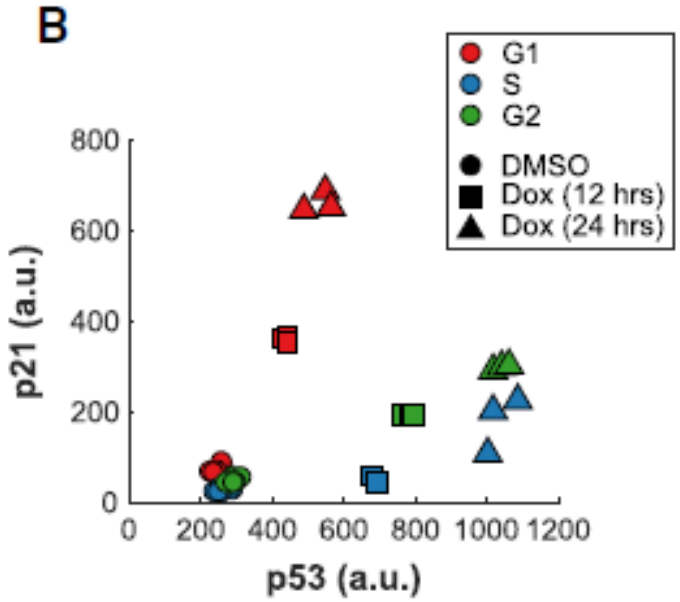

- Single cell analysis revealed p21- DNA damage relation in different cell- cycle phases after Dox. treatment
- G1 had the lowest level of damage and expressed highest level of p21 during treatment than S and G2 cells

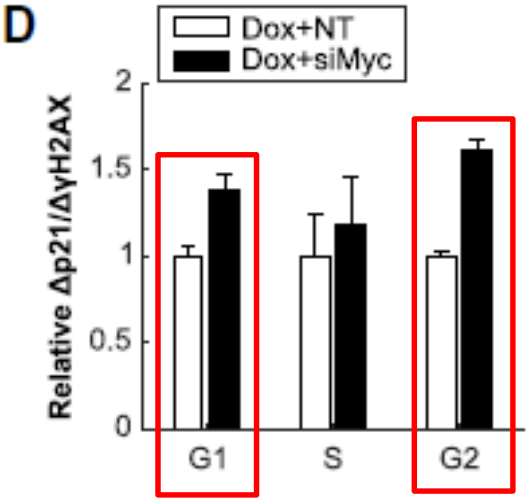
What mechanism prevented higher DNA damage from having higher p21 expression in S/G2 cells?

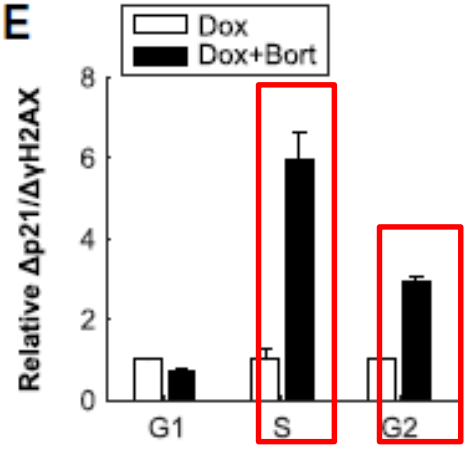


# Drug-Induced p21 Expression Is Repressed by Chk1 and Proteasomal Degradation in S/G2

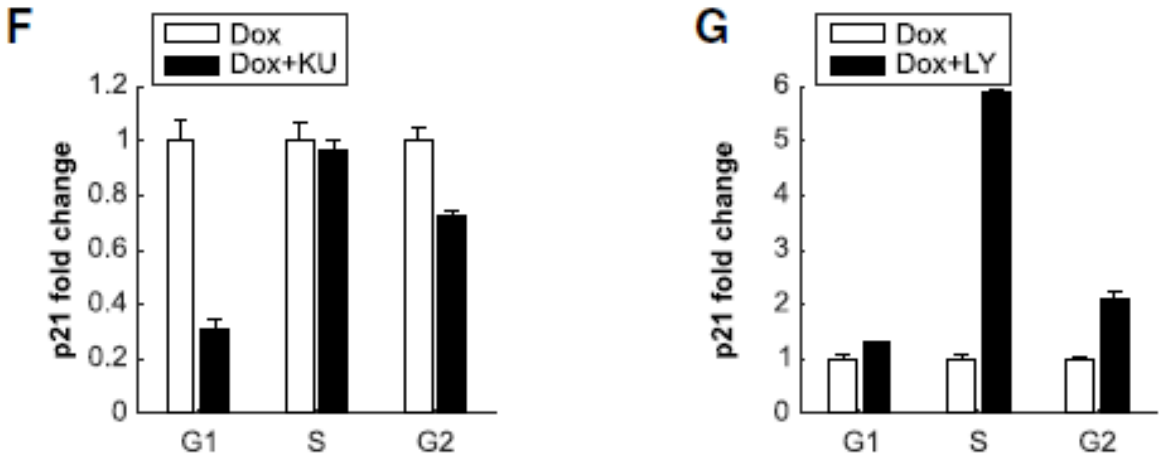


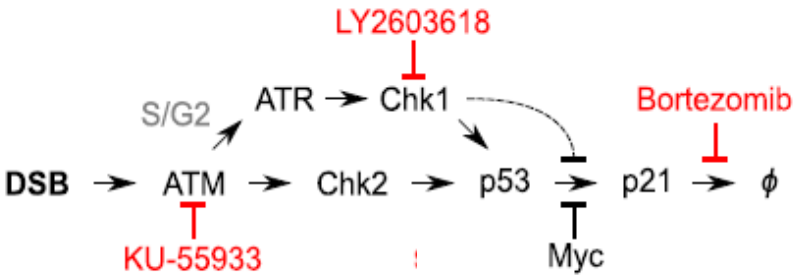




Proteasomal degradation of p21 plays role in differential activation of p21 at the different phases of the cell cycle

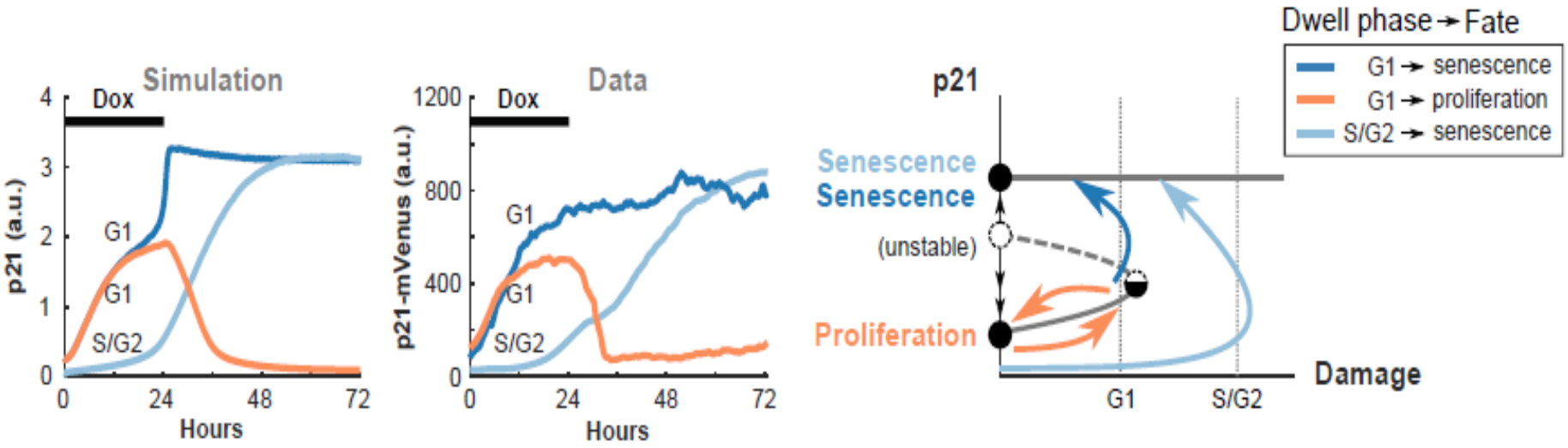
# Drug-Induced p21 Expression Is Repressed by Chk1 and Proteasomal Degradation in S/G2




ATM signaling is required for high levels of p21 expression in G1, while the lower level of p21 expression in S/G2 is due to repression by Chk1 signaling and proteasomal degradation



# A Mathematical Framework for Understanding the Emergence of Different Patterns of p21 Dynamics

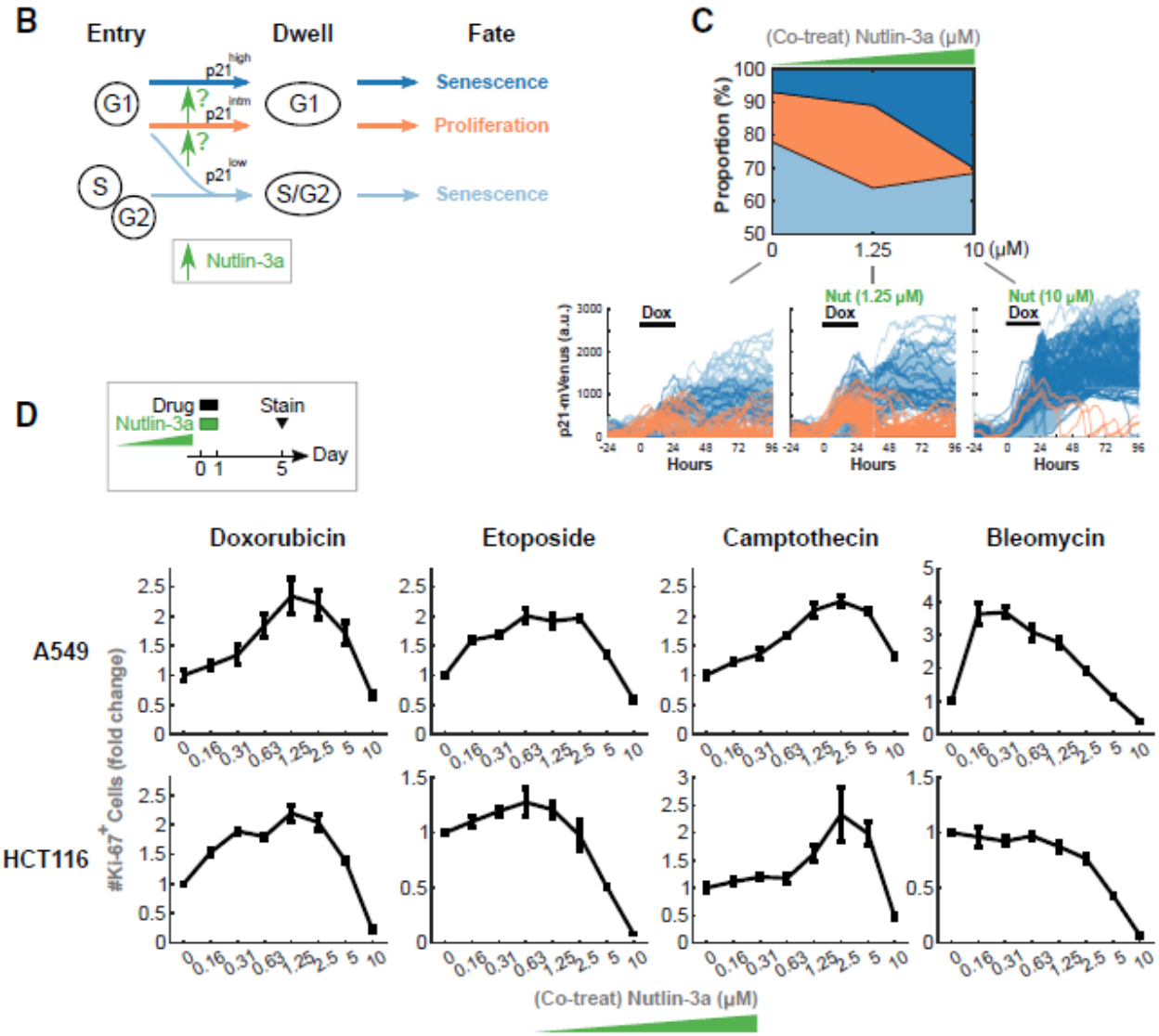


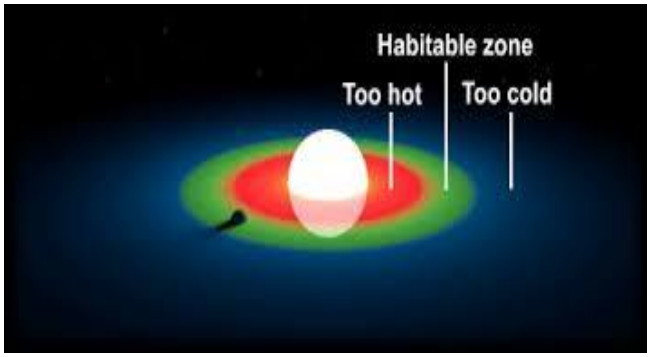

Model incorporated three processes

1. cell- cycle- dependent DNA damage
2. feedback of p21
3. cell- cycle- dependent degradation

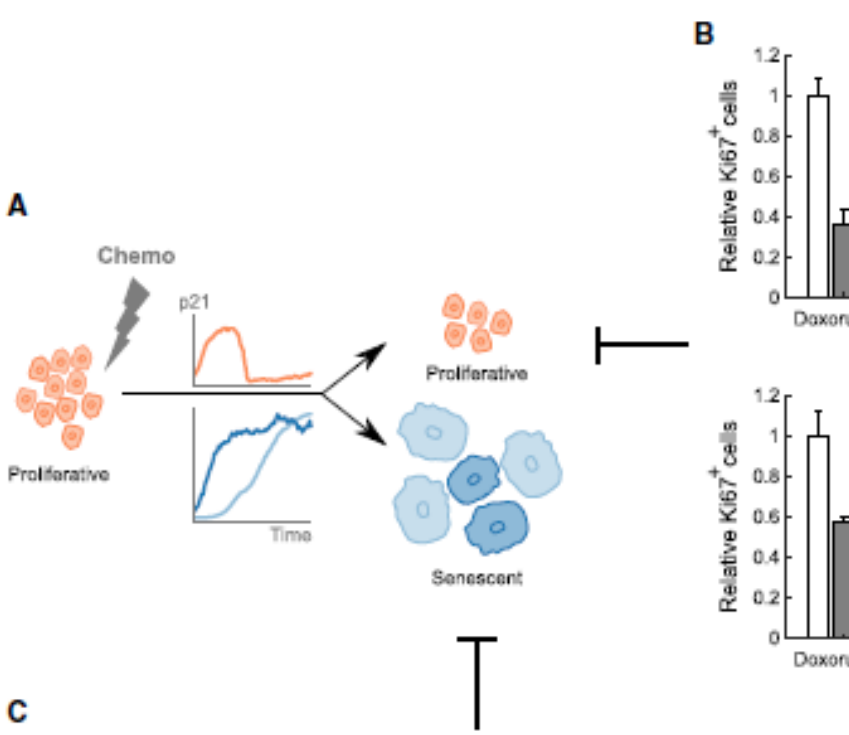
Model captured the observed p21 dynamics and suggested possible mechanisms underlying the three observed patterns of p21 dynamics with the two cell- fate outcomes

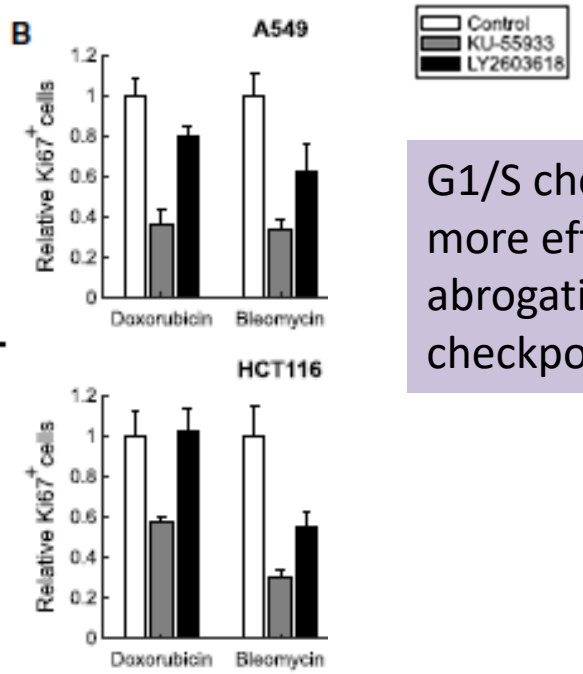
# A p21 "Goldilocks Zone" Promotes the Proliferative Cell Fate after Chemotherapy



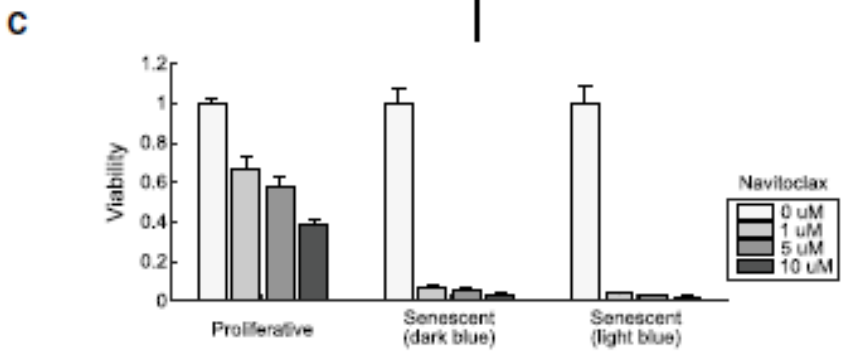



# Strategies for Reducing the Proliferative or Senescent



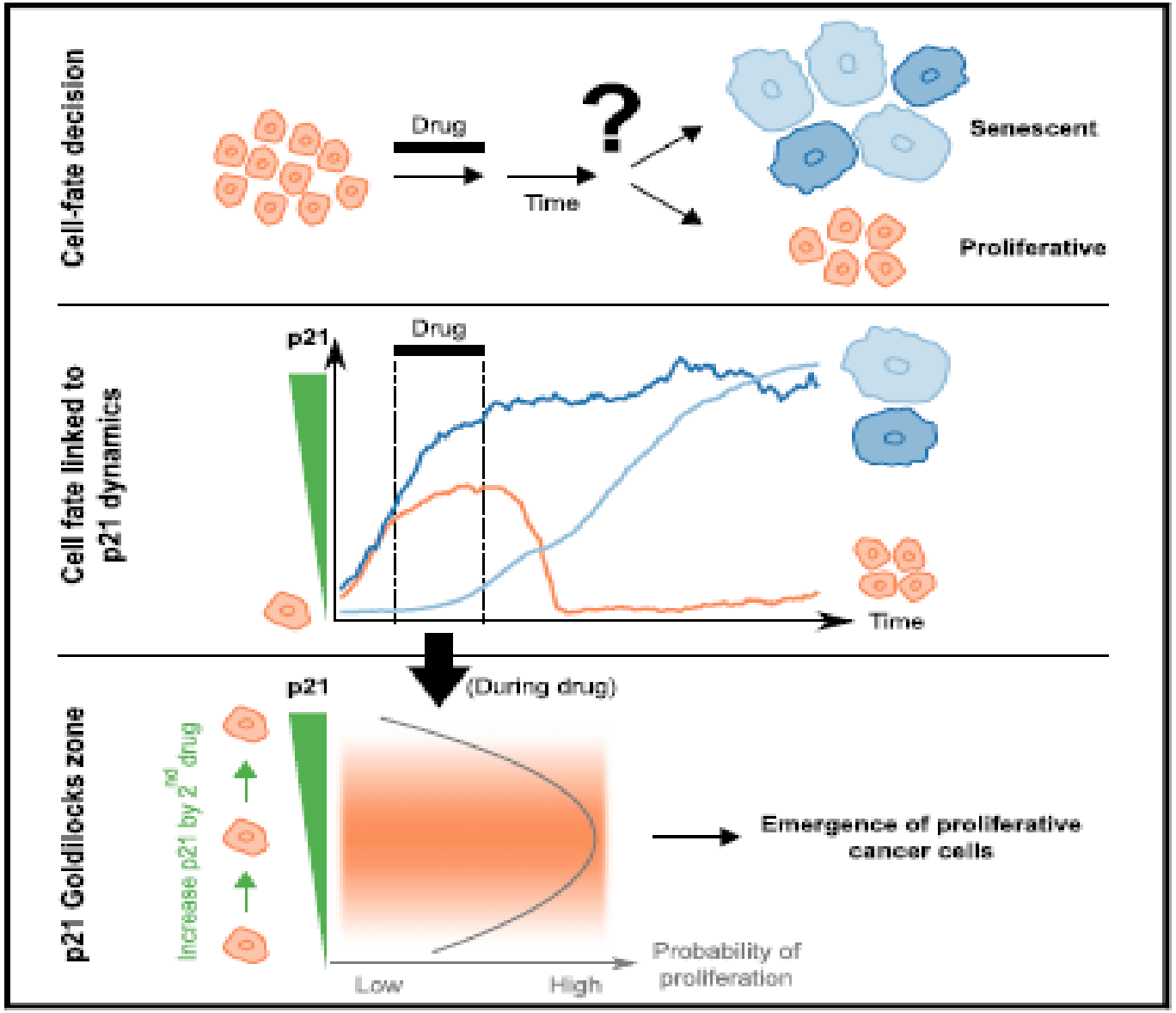




G1/S checkpoint was more effective than abrogating the G2/M checkpoint




Abrogating G1/S checkpoint during treatment could reduce the final proliferative subpopulation and that application of senolytic drugs after treatment could reduce the remaining senescent subpopulation

# Conclusion


Cell-fate decision
Drug
Time
?
Senescent
Proliferative
Cell fate linked to p21 dynamics
p21
Drug
Time
p21 Goldilocks zone
p21
(During drug)
Increase p21 by 2nd drug
Low
High
Probability of proliferation
Emergence of proliferative cancer cells