Pharmacological Quiescence

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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) → CDK4/6 → RB → CDK2 → p21, p27 → RB → E2F’s

Cyclin E1, E2 and A
CDK4/6 CDK2 RB E2F's

D-Type Cyclins (x3) Cyclin E1, E2 and A

p16\textsuperscript{INK4a} p18\textsuperscript{INK4c} p21 p27

Mitogens

Mitogens

INK4’s

p16\textsuperscript{INK4a} p21 p27

Mitogens

Cyclin E1, E2 and A

2002 2005

E2F’s

Mitogens

Mitogens

UNC Comprehensive Cancer Center
For $G_1 \rightarrow S$, two things have to happen:

- D-Type Cyclins
  - CDK4/6
- E,A-Type Cyclins
  - CDK2 (CDK1 / cdc2)

Also, lots of other things:
- Histones
- DNA repair proteins
- Other kinases
- Etc.
Pharmacological Quiescence (PQ)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Islet</th>
<th>Pituitary</th>
<th>Lymph node</th>
<th>Small bowel</th>
</tr>
</thead>
<tbody>
<tr>
<td>No treatment</td>
<td><img src="image1" alt="Islet" /></td>
<td><img src="image2" alt="Pituitary" /></td>
<td><img src="image3" alt="Lymph node" /></td>
<td><img src="image4" alt="Small bowel" /></td>
</tr>
<tr>
<td>Treatment w/ PD 0332991</td>
<td><img src="image5" alt="Islet" /></td>
<td><img src="image6" alt="Pituitary" /></td>
<td><img src="image7" alt="Lymph node" /></td>
<td><img src="image8" alt="Small bowel" /></td>
</tr>
</tbody>
</table>
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

- EPO®
  - 4.6B
- Unmet*
- Neupogen®
  - 4.3B
- Unmet

*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

**WM2664** (wt Rb)

**A2058** (Rb null)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>WM2664</th>
<th>A2058</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cdk4/6 Inhibitor</td>
<td></td>
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</table>

**Graphs**

- **WM2664**
  - Log nM PD 0332991 vs. Ki67+
  - Log nM PD 0332991 vs. S

- **A2058**
  - Log nM PD 0332991 vs. G1
  - Log nM PD 0332991 vs. G2
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

DMSO  
0 Gy IR

CDKi – PD0332991  
6 Gy IR

γH2AX  
Phalloidin

Mean Nuclear Intensity

DMSO No IR  
PD No IR

DMSO 6 Gy  
PD 6 Gy

DMSO 6 Gy  
PD 6 Gy
Cdk4/6 inhibition protects from doxorubicin cytotoxicity
Cdk4/6 inhibition protects from ionizing radiation

<table>
<thead>
<tr>
<th>Time after IR</th>
<th>n/a</th>
<th>3 hr</th>
<th>6 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD0332991</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>p-P53</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>ACTIN</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Normalized pp53 Intensity

Growth Area PD0332991:DMSO

DMSO
PD0332991

No IR
6Gy, 3hr
6Gy, 6hr

Gy XRays

0
2
4
6
8
10

Growth Area PD0332991:DMSO

tHDF
A2058
Step 3: Does cdk4/6 inhibition affect hematopoietic proliferation \textit{in vivo}?
Bone marrow proliferation assay procedure

I.P. BrdU
1 mg / 6 hrs

8AM – Euthanize, Harvest
Bone Marrow, FACS

Cdk4/6 Inhibitor Vs. vehicle

8AM
Hematopoiesis

Lin⁻ cKit⁺ Sca1⁺ SLAM⁺ CD48⁻

Lin⁻ cKit⁺ Sca1⁺ SLAM⁻ CD48⁻

Lin⁻ cKit⁺ Sca1⁻
MEP: CD34<sup>lo</sup> CD16/32<sup>lo</sup>
CMP: CD34<sup>hi</sup> CD16/32<sup>lo</sup>
GMP: CD34<sup>hi</sup> CD16/32<sup>hi</sup>

Bryder, Rossi, Weissman 2006 American J Pathology
Adult HSPC need CDK4/6 too:

HSCs

MPPs

Lin- cKit+ Sca1-

Untreated

CDK-I

BrdU

Ki67

BrdU+

Ki67+

HSCs

MPPs

Lin- cKit+ Sca1-

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

Radiation Exposure

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

Percent Survival

p<0.001

Days after 7.5 Gy TBI

- Minus 4 hrs (N=22)
- Untreated (N=28)
In Vivo Radioprotection

Nadir Counts 21 days post-TBI

- Hematocrit
- Platelets
- Lymphocytes
- Granulocytes

- 7.5Gy TBI, untreated (N=5)
- 7.5Gy TBI, treated (N=5)
- 0Gy TBI (N=7)
Protection AFTER Radiation: Mitigation

Radiation Exposure

PQ Drug

12-24 hours duration of PQ effect

Percent Survival

Days after 7.5 Gy TBI

p<0.01

Plus 20 hrs (N=10)

Untreated (N=28)
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*<sup>CIP</sup> 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.)
Possible radioprotection mechanisms

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INK4's

p16\textsuperscript{INK4a}

p21

CDK4/6

CDK2

D-Type Cyclins (x3)

Cyclin E1, E2 and A

Mitogens

DNA Damage

p53

Fbxo31

RB’s (x3)
Role of p21<sup>Clp</sup> 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
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.)*

See also Lobrich and Jeggo, (Deckbar, Canc Res. 2010)
Step 6: Beyond radioprotection:
Chemoprotection

- Chemo
- PQ Drug
- 1-2 Days
- 21 Days

- Chemo
- PQ Drug
- 1-2 Days
- 21 Days

- Chemo
- PQ Drug
- 1-2 Days
- 21 Days
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

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