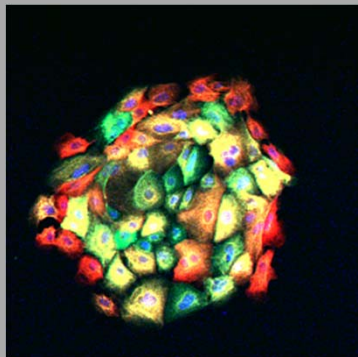


NCAB

Discussion of Cancer Stem Cell Theory  
September 15, 2009

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Kathleen Kelly, Ph.D.  
Cell and Cancer Biology Branch, CCR, NCI



# Cancer Stem Cell Theory

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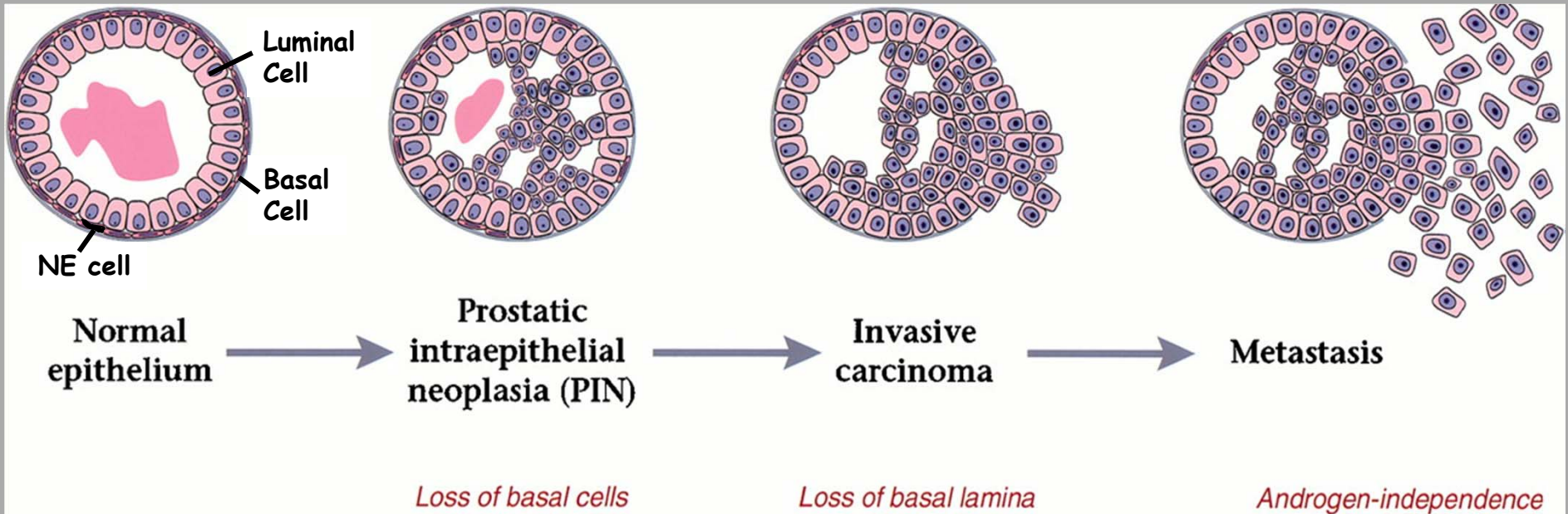
- Attributes the heterogeneity of cellular populations within tumors to a differentiation hierarchy
  - Tumor initiation
  - Self-renewal
  - Marker expression
- Tumor initiating populations
  - Express markers of progenitors
  - Recreate the differentiation hierarchy in transplanted tumors

# The Value of Investigating Tumor Heterogeneity

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- Defining the cell of origin may lead to better early detection markers
- Treatments must target all populations
- Cancer stem cells and metastasis initiating cells share several properties

# Prostate Cancer Progression



**Luminal Cells:** CK8+

**Basal Cells:** CK5+, p63

**Neuroendocrine Cells:** synaptophysin+,  $\beta$ -3 tubulin

# Properties of PC Metastasis

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- Poorly differentiated CK8+ carcinomas
- Metastases can demonstrate mixed lineage markers, especially luminal and neuroendocrine
- A large percentage of castrate-resistant prostate cancers express mutated AR, suggesting evolution from an AR+ cell

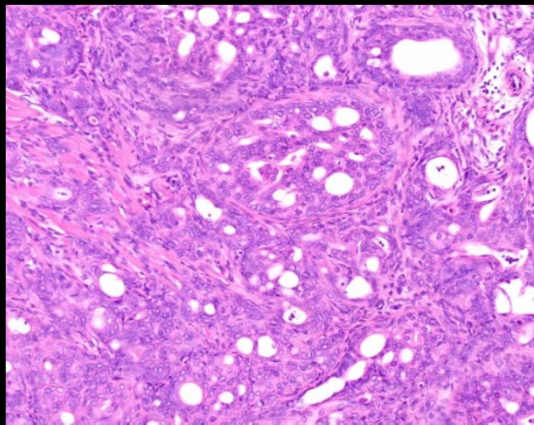
# Questions Being Addressed

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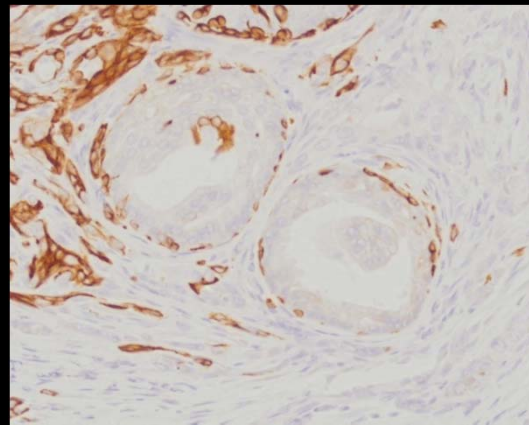
- Mechanistic effect of specific common gene mutations on prostate progenitor populations
- Cellular origins of castrate-resistant PC and physiological role of AR

## Modeling PC in the mouse: $(PbCre^+) PTEN^{fl/fl}, P53^{fl/fl}, Luc^+$

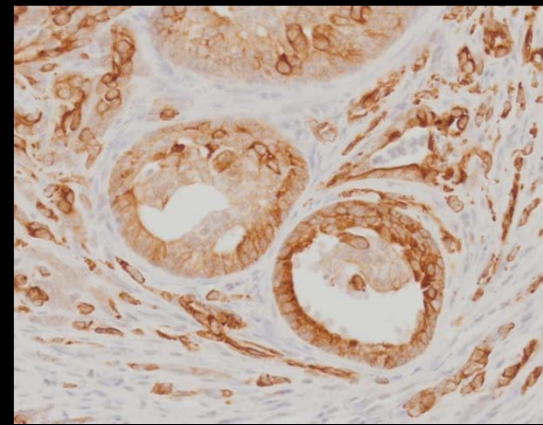
- The PTEN pathway is frequently altered in human PC
- Development of invasive and disseminated adenocarcinoma, but not clinically-apparent metastatic tumors
- Death from urinary outflow obstruction at ~ 6 mos.
- Proliferation of cells with intermediate ( $CK5^+/CK8^+$ ) and luminal phenotypes



H&E



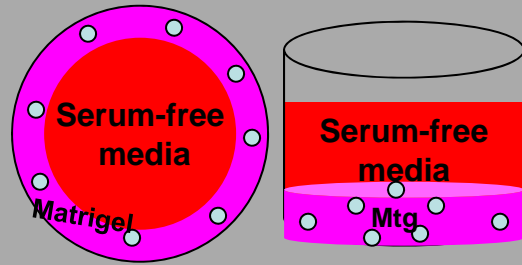
CK5



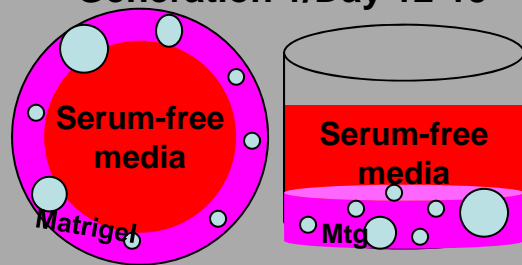
CK8

# Protosphere-forming assay (3-D)

# Colony-forming assay (2-D)



Generation 1/Day 0



Generation 1/Day 12-15

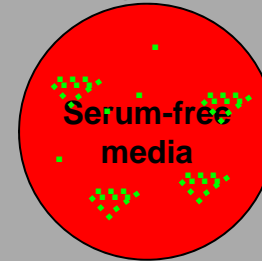
- Single cell
- Sphere



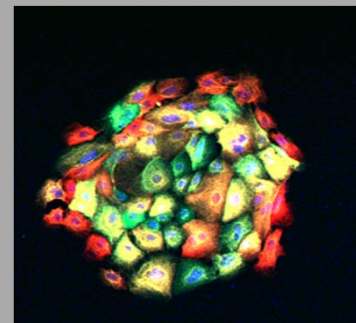
Passage 0/Day 0



Passage 0/Day 5-8



- Single cell
- Colony

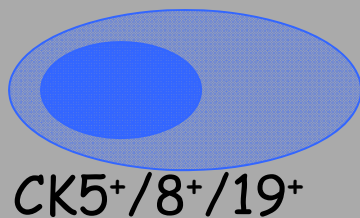




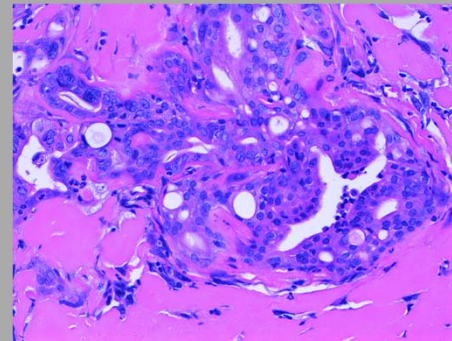
# Prostate progenitors are tumor initiating cells

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1. Cell clones established from tumors and expressing markers of progenitor cells give rise to adenocarcinoma



Orthotopic  
injection →



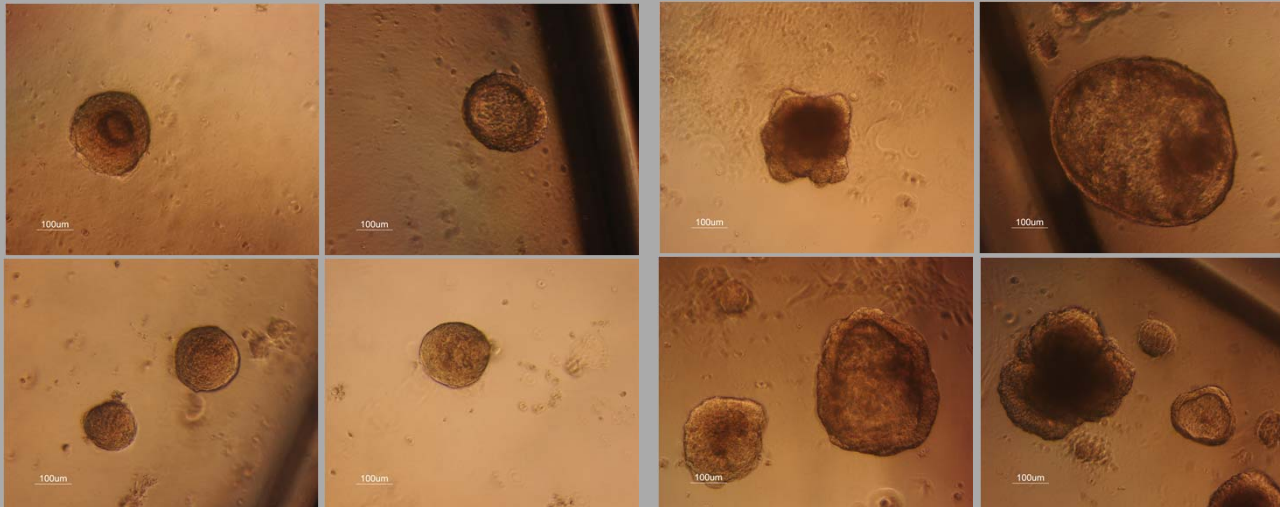
1. Single cell suspensions made from protospheres give rise to prostate carcinoma

# Protosphere Morphologies

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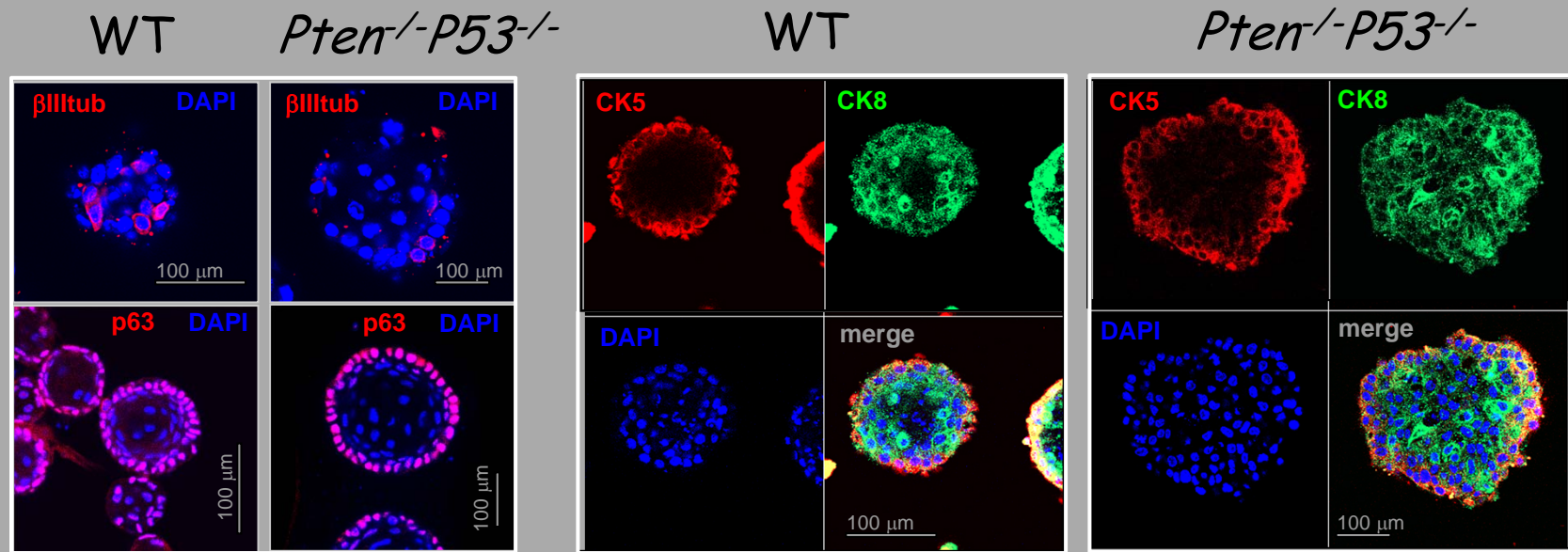
WT G1/D12

*Pten<sup>-/-</sup>P53<sup>-/-</sup>* G1/D12



*Pten<sup>-/-</sup>P53<sup>-/-</sup>* protospheres relative to wt are  
3X larger in diameter  
Contain 50% more cells

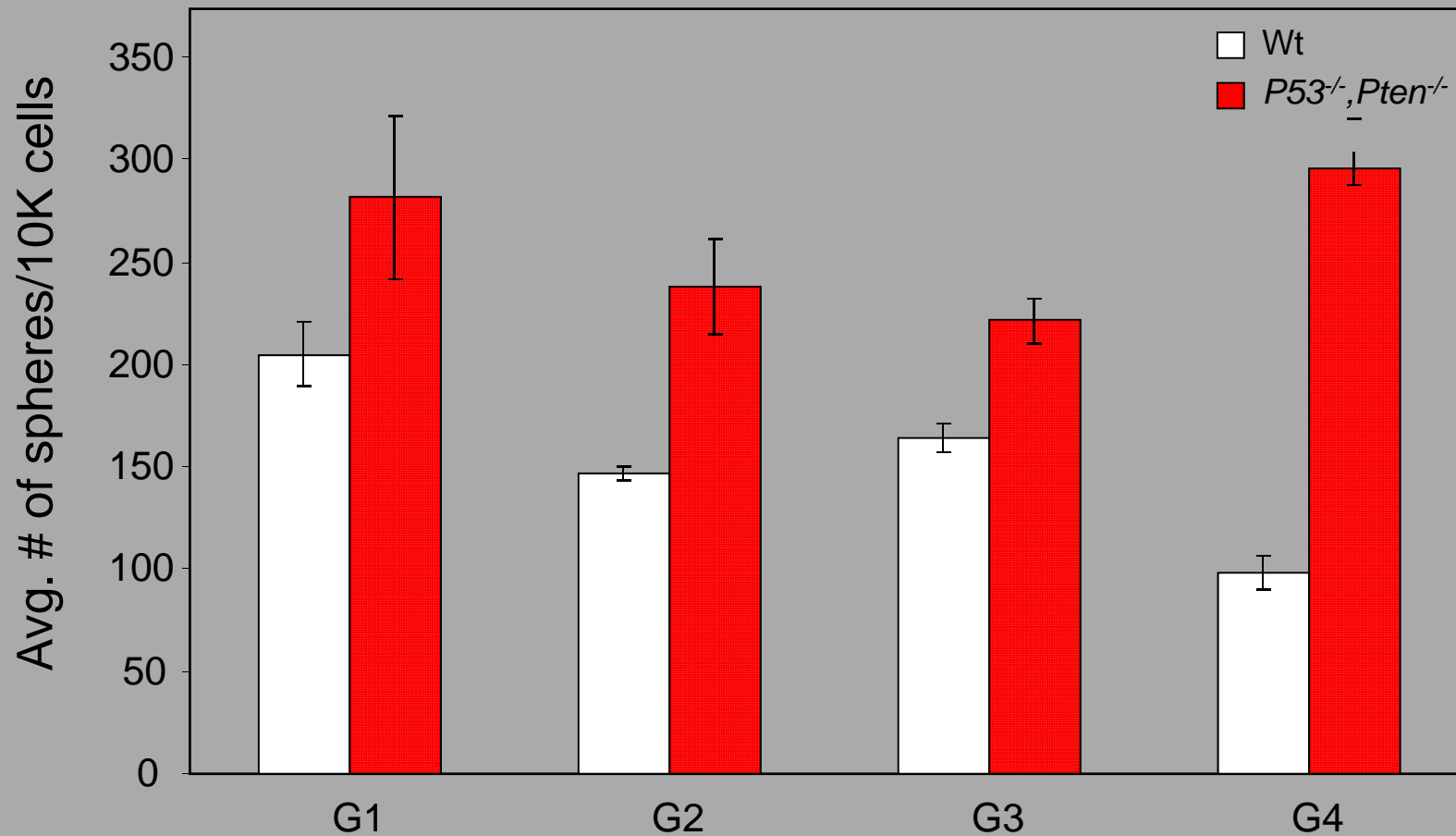
# Differentiation Potential in Transformed spheres



- Sphere-forming cells are rare (~1%)
- Spheres have a defined architecture
- Basal cells form the outermost layer
- Spheres contain multipotent progenitors that produce basal, intermediate (CK5<sup>+</sup>/CK8<sup>+</sup>), and neuroendocrine cells
- *Pten*<sup>-/-</sup>, *P53*<sup>-/-</sup> progenitors produce more CK8<sup>+</sup> cells

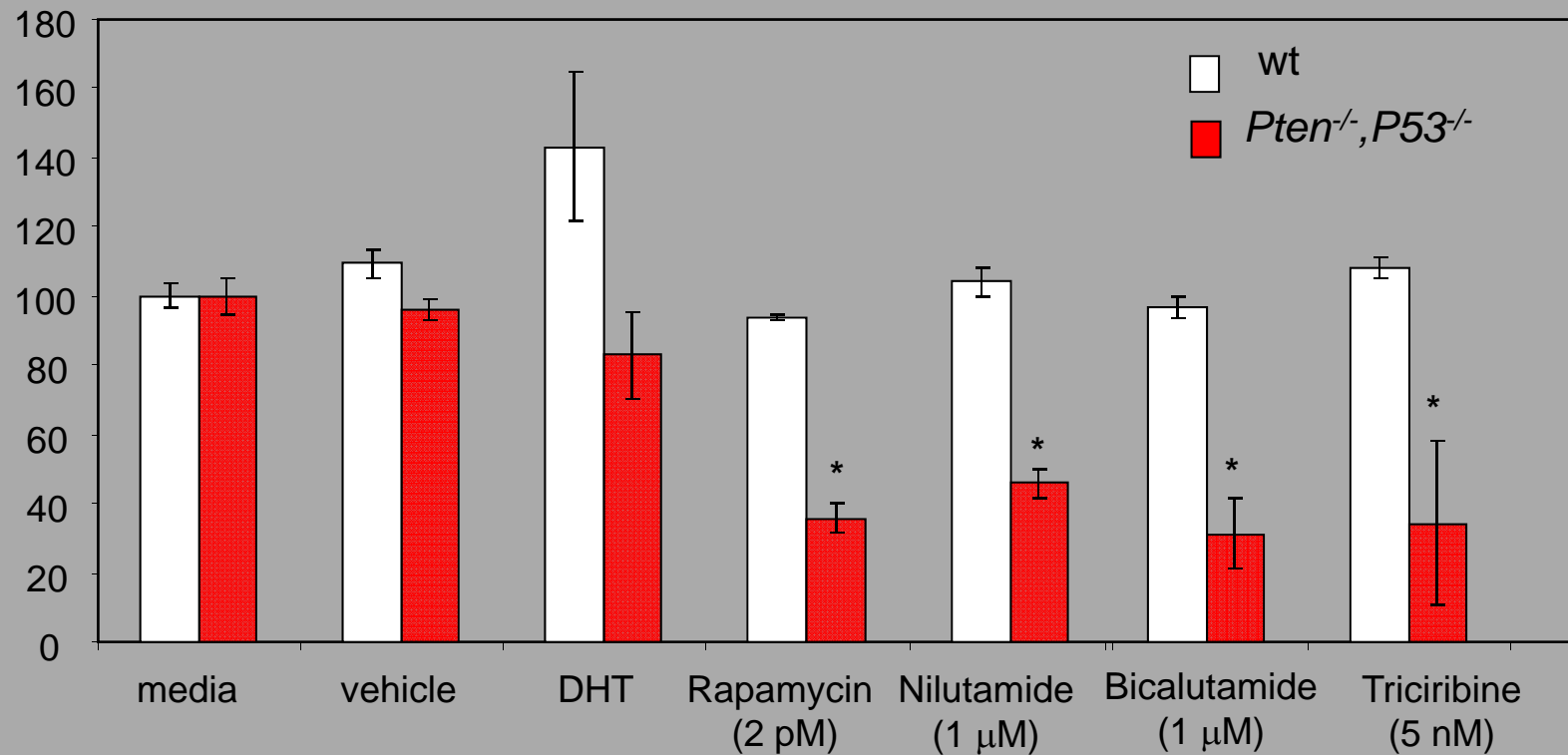
# Transformed Progenitors Show Increased Self-Renewal

Sphere formation assay



# Transformed Progenitors Are Differentially Inhibited by Drugs

Colony formation (% of mock treated)



# Conclusions

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- PTEN<sup>-/-</sup>;P53<sup>-/-</sup> prostate progenitors demonstrate perturbations of self renewal and differentiation
- These progenitors express altered drug sensitivity- i.e. AKT "addiction" and acquired AR dependence

# Implications

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- Establishing the relationship of specific gene mutations to *CSC* function is important for improved mechanistic understanding of cancer progression and treatment
- Therapeutic screening methodologies that target unique *CSC* signaling properties should be developed



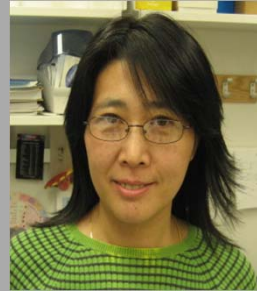
Philip Martin  
Rachel Pierce



Wassim Abou-Kheir



Paul Hynes



Ivy Yin



Orla Casey



Luhua Zhang



Yvona Ward



Ross Lake



# Questions

- What criterion should be applied to the development of CSC lines used for therapeutic screening purposes?
- What is the best approach to analyze the diagnostic and/or prognostic value of CSC markers in human cancer?

# Properties of Normal Prostate Stem Cells

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- Resistant to castration
- Give rise to luminal, basal, and NE cells
- Cofractionate with basal cells- AR status unknown

# Clarifications re CSC Theory

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- Does not make assumptions about the frequency of tumor initiating cells
- Does not make assumptions about the cell of origin
- Does not discount the possibility of plasticity: non-CSC may convert to CSC's