



Targeting Prostate Cancer Stem Cells

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Outline

- Historical Research, Identification of Cancer Stem Cells in Leukemia
- Techniques to Identify Cancer Stem Cells
- Prostate (Cancer) Stem Cells
 - Identification
 - Therapy

Definition of (Cancer) Stem Cells

Benign and Cancer Stem Cells:

Self-renewal - the ability to go through numerous cycles of cell division while maintaining the undifferentiated state

Potency - the capacity to differentiate into specialized cell types. In the strictest sense, this requires stem cells to be either totipotent or pluripotent - to be able to give rise to any mature cell type.

Cancer Stem Cells:

Generate all heterogeneous lineages of cells within a tumor

Suggests a hierarchy of tumor initiating capabilities

Cancer Stem Cell Hypothesis is NOT New

1950-1970's

G. Barry Pierce: proposed organ cell hierarchy of organogenesis of tumorigenesis

1990's

John Dick: evidence for the existence of cancer stem cells in Acute Myeloid Leukemia

Cancer Stem Cells in the Headlines

BBC August 1, 2012

Cancer stem cell discovery could signal 'paradigm shift'

By Pallab Ghosh

Fox News September 27, 2012

Common cancer treatments may create dangerous cancer stem cells

By Charles Q. Choi

Forbes October 09, 2012

Cancer Stem Cell Therapy: Real Or Just Hype?

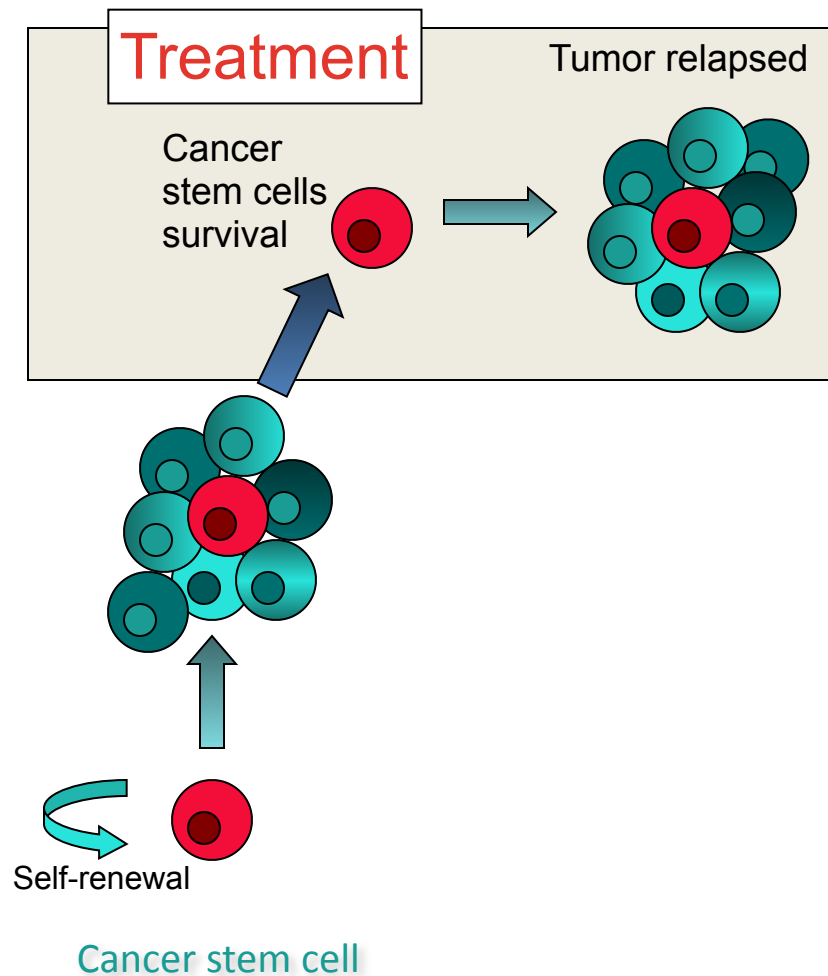
By Nathan Sadeghi-Nejad

The New Yorker September 7, 2014

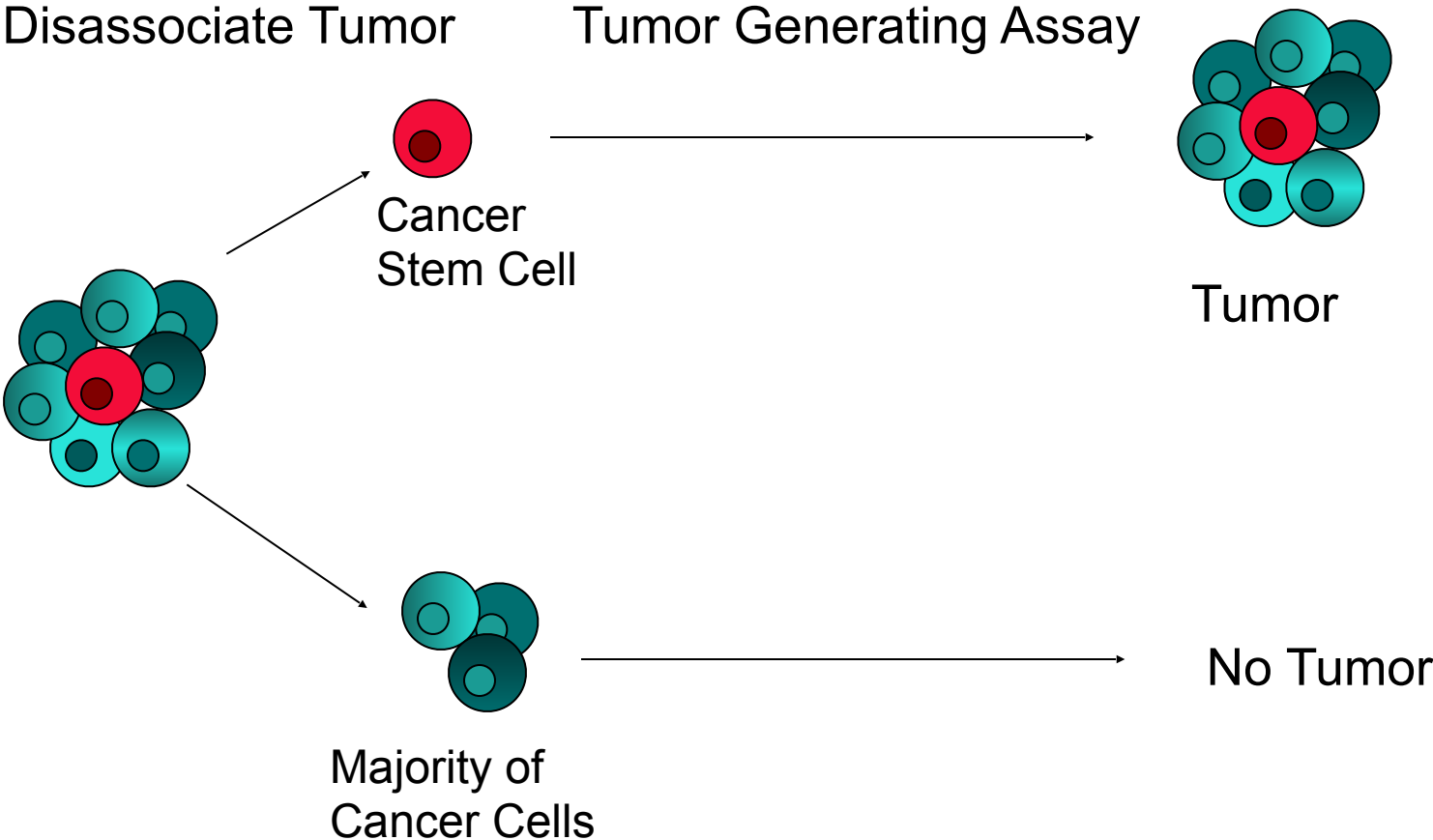
The Transformation

By Jerome Groopman

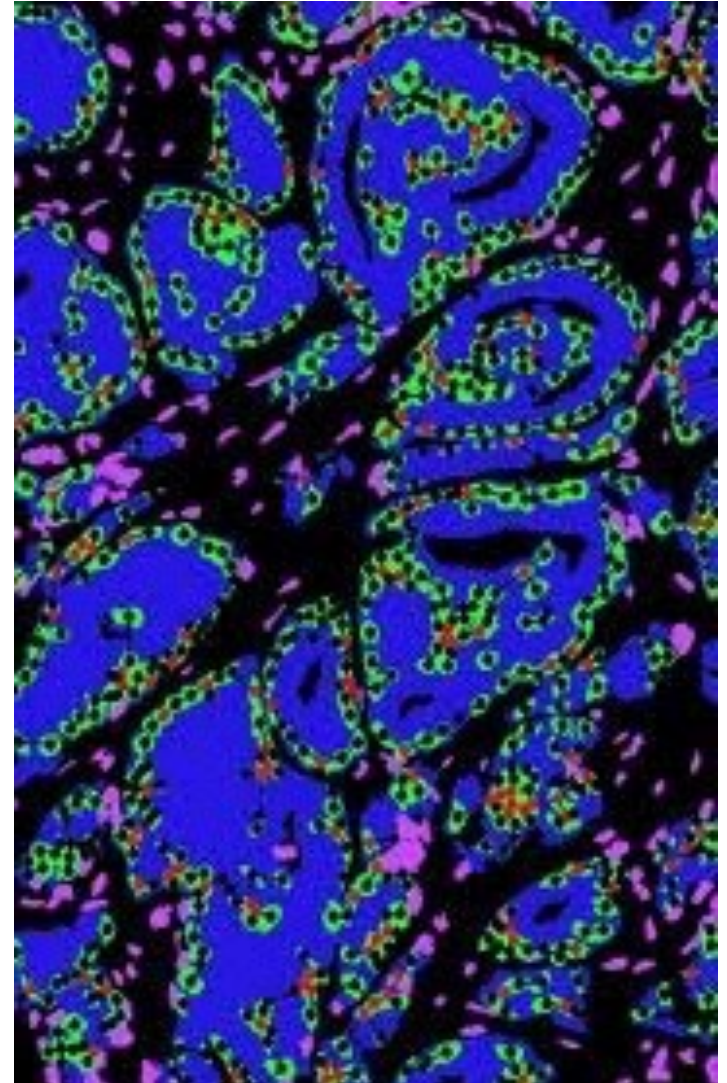
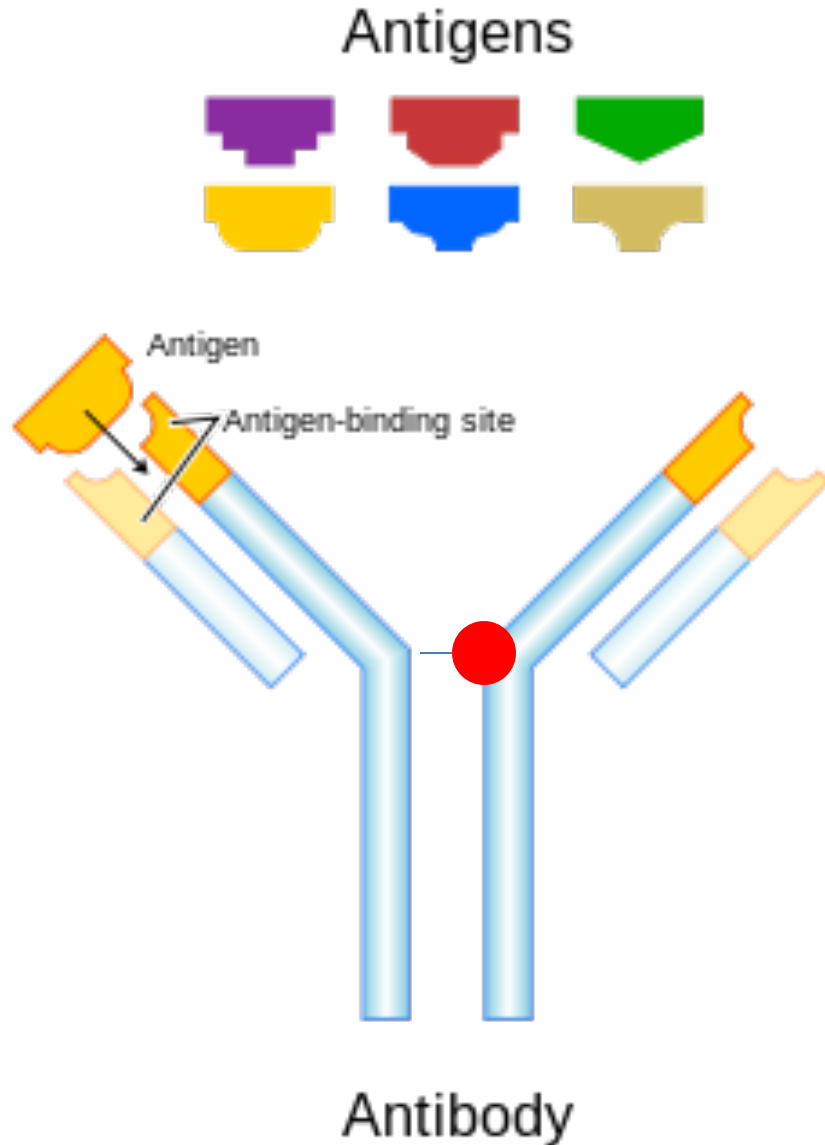
Cancer Stem Cell Hypothesis



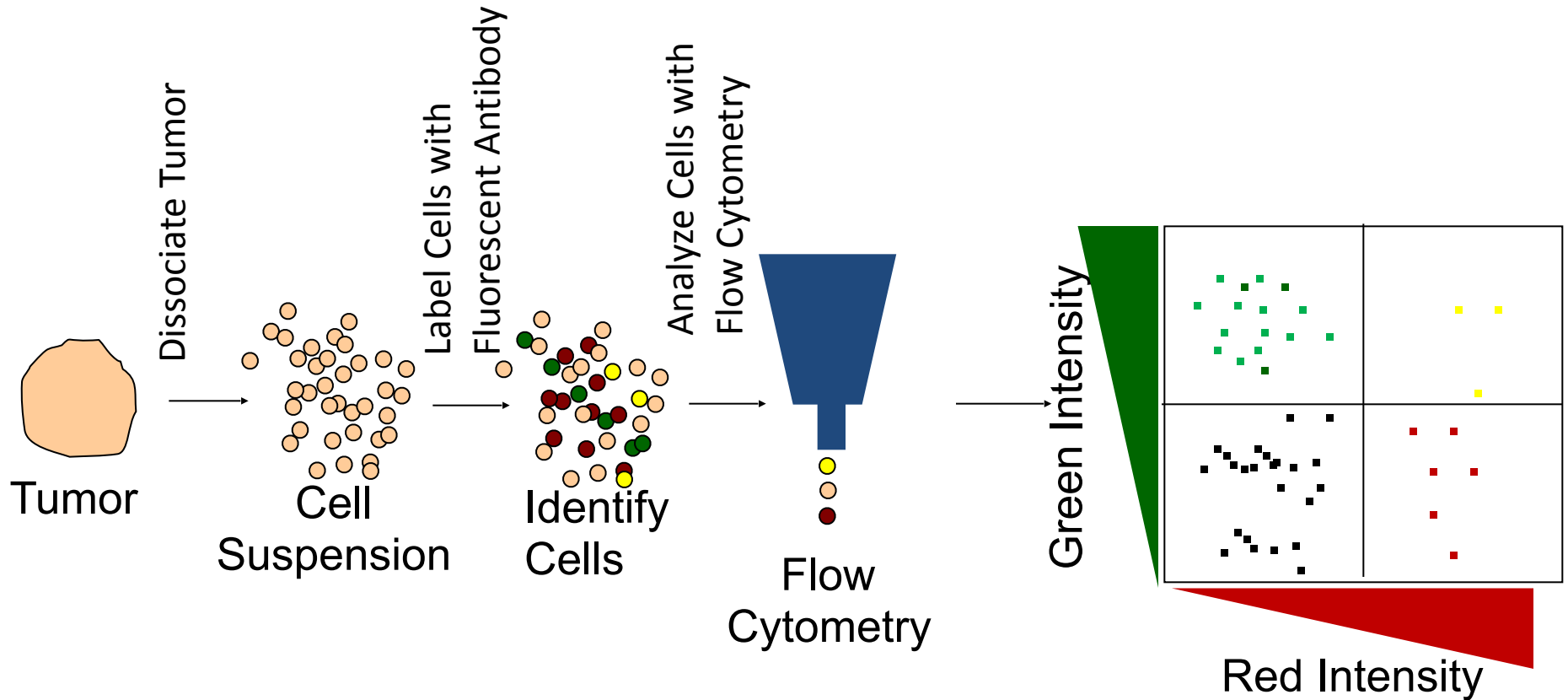
Cancer Stem Cell Identification



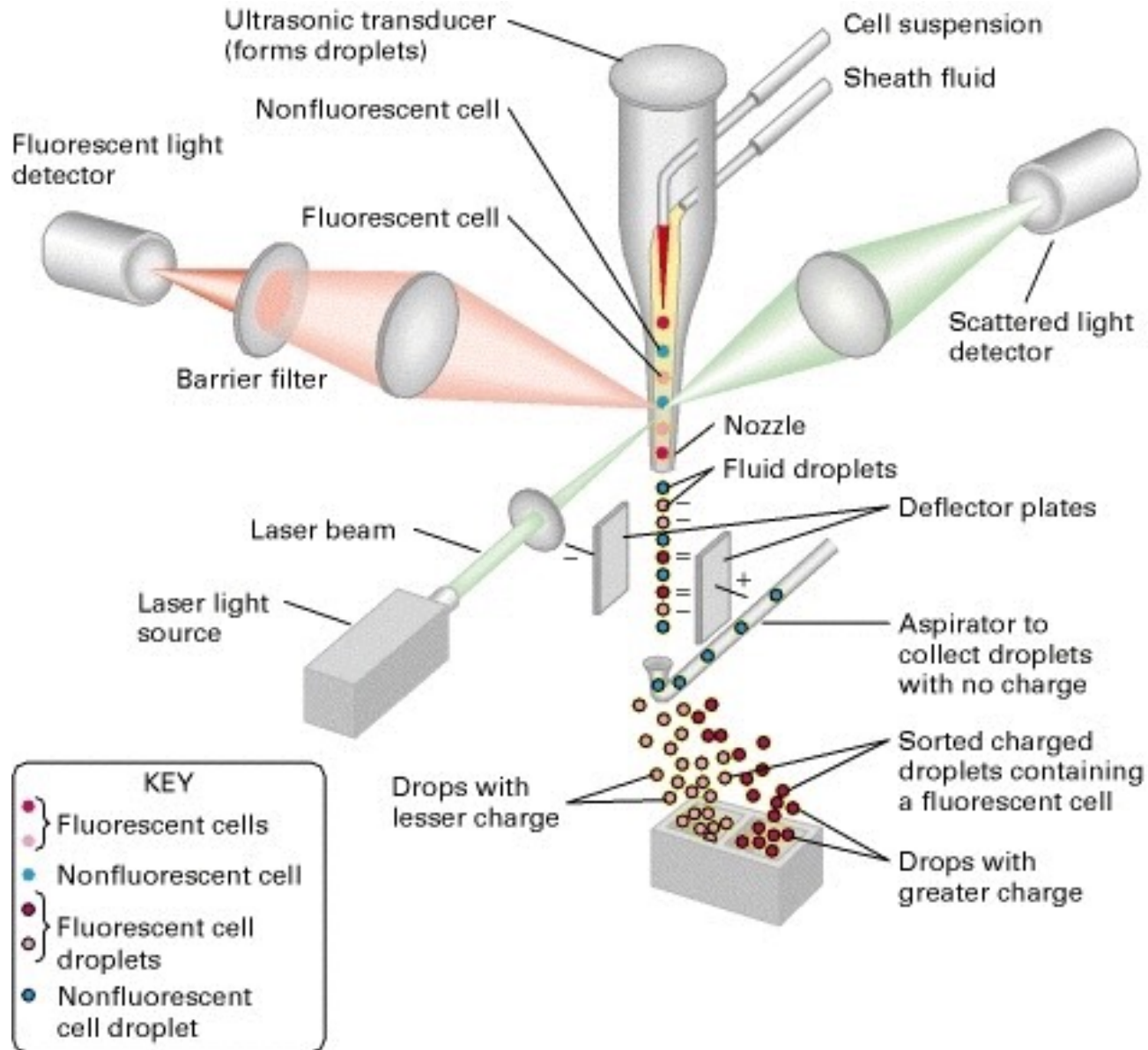
Cell Identification with Antibodies



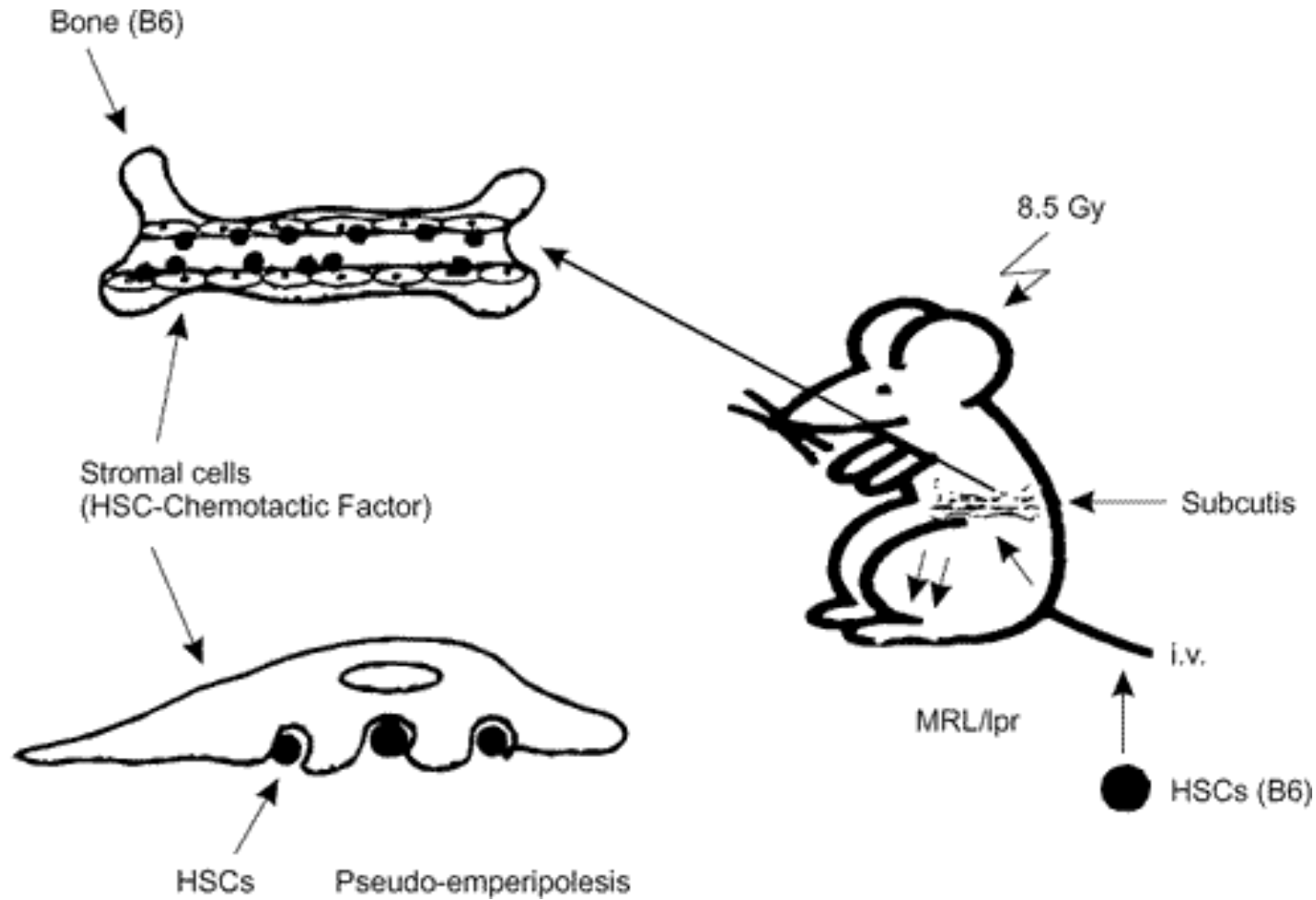
Flow Cytometry



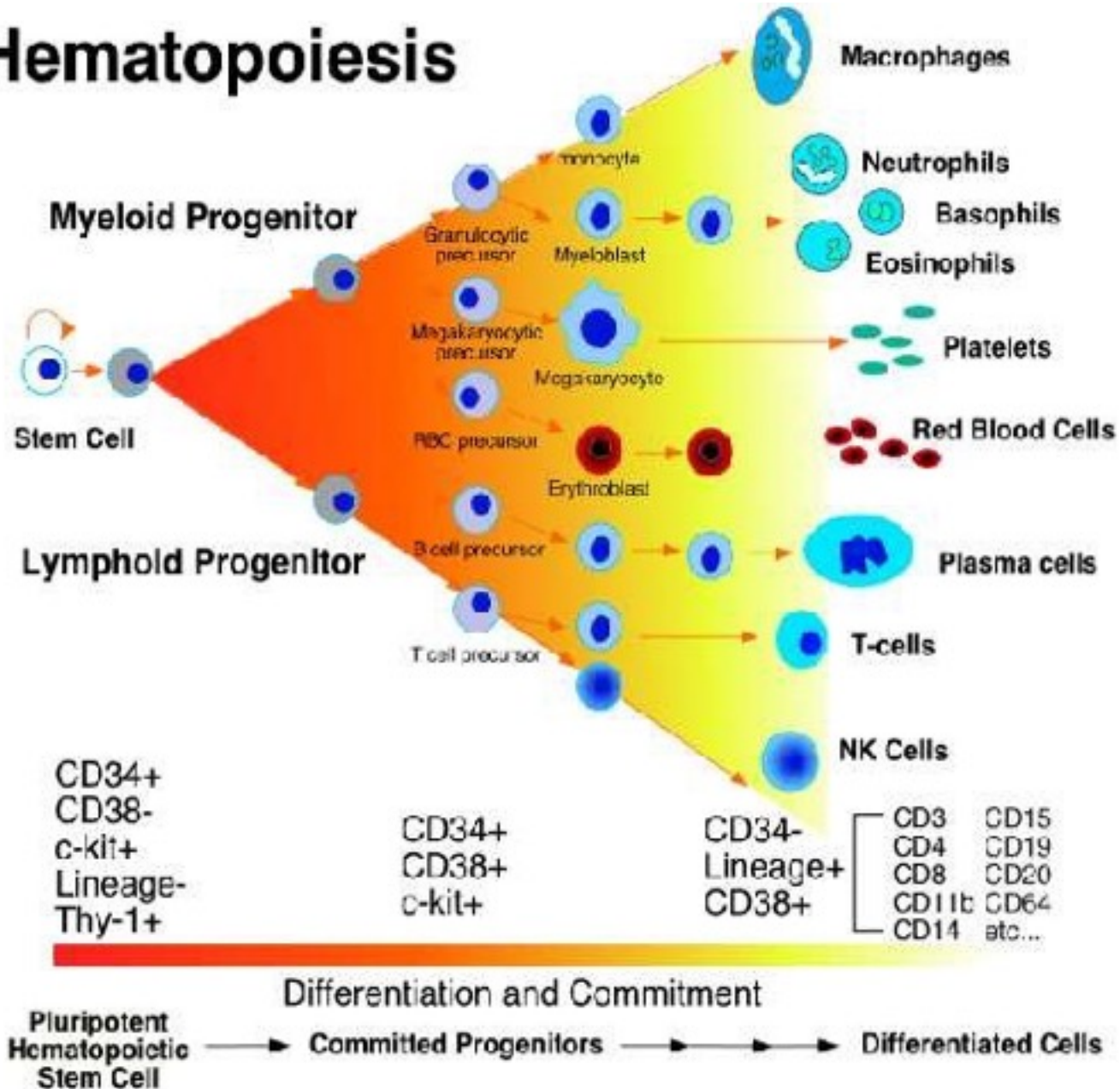
Flow Activated Cell Sorting



Bone Marrow Transplants in Mice



Hematopoiesis



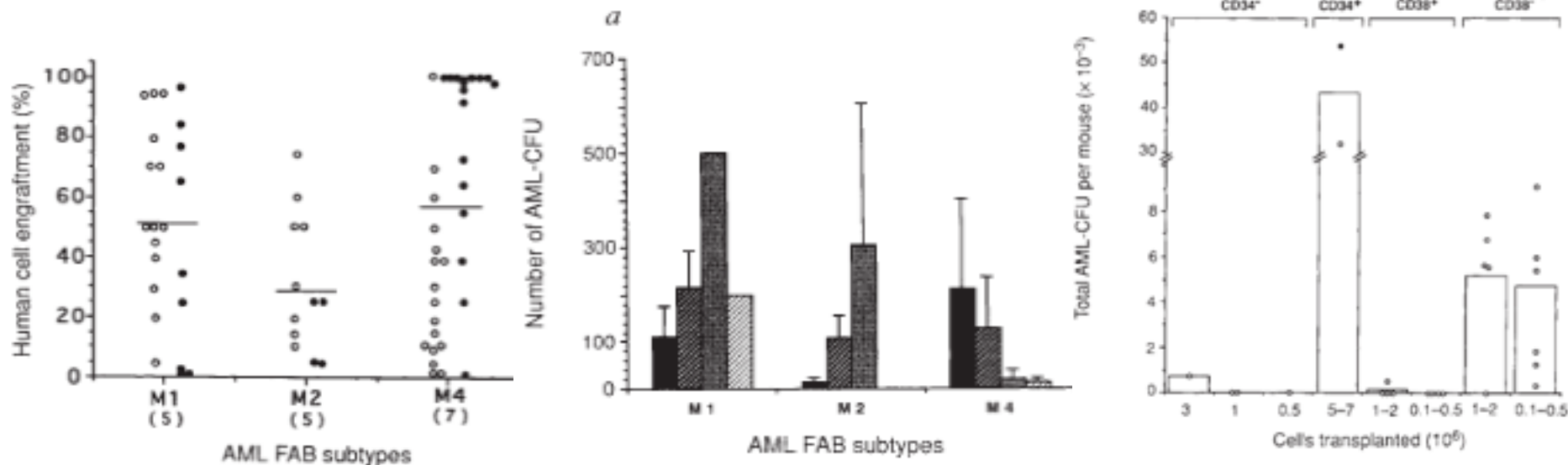
First Demonstration of Cancer Stem Cells

In vivo assay for human leukemia – Demonstrated different stages of AML

could engraft in irradiated mice

had colony formation

CD34+CD38-, but not CD34+CD38+ cells could recapitulate human AML in mice



Stem Cell Protection Mechanisms

Common protective mechanisms between benign and cancer stem cells assays for discrimination and isolation

Multidrug resistance pumps

Hoechst Efflux Side Population

Vybrant[®] DyeCycle[™] Violet Side Population

High Aldehyde Dehydrogenase Activity

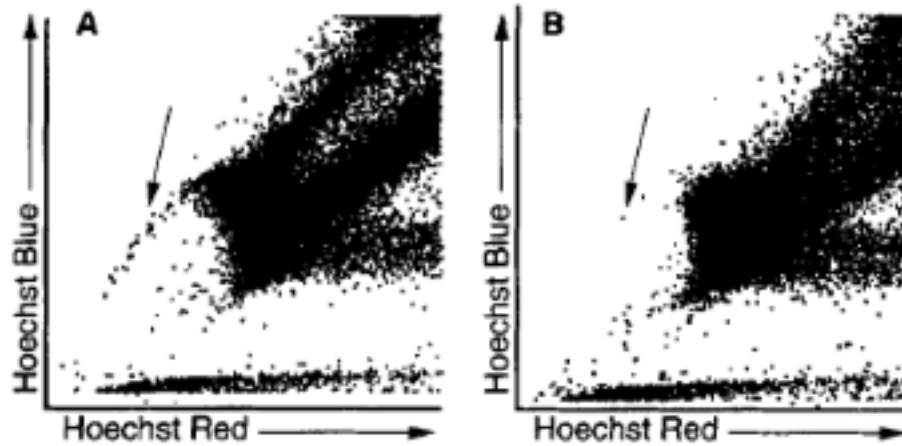
ALDEFLOUR[®]

Radiation Protection: ↑Chk1 & Chk2 mediated DNA repair capacity

Telomerase Activity

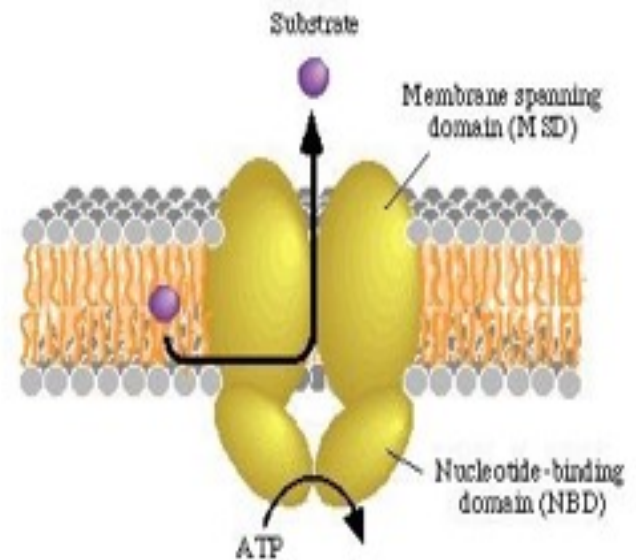
Hematopoietic Side Population Phenotype

+ verapamil



ABC Transporters

- ATP binding cassette transporters- the largest family of drug transporters
- Evolutionarily conserved
- Present in plasma membrane and membranes of intracellular compartments
- Use of cellular ATP to drive transport
- 7 subfamilies and 50 ABC transporters

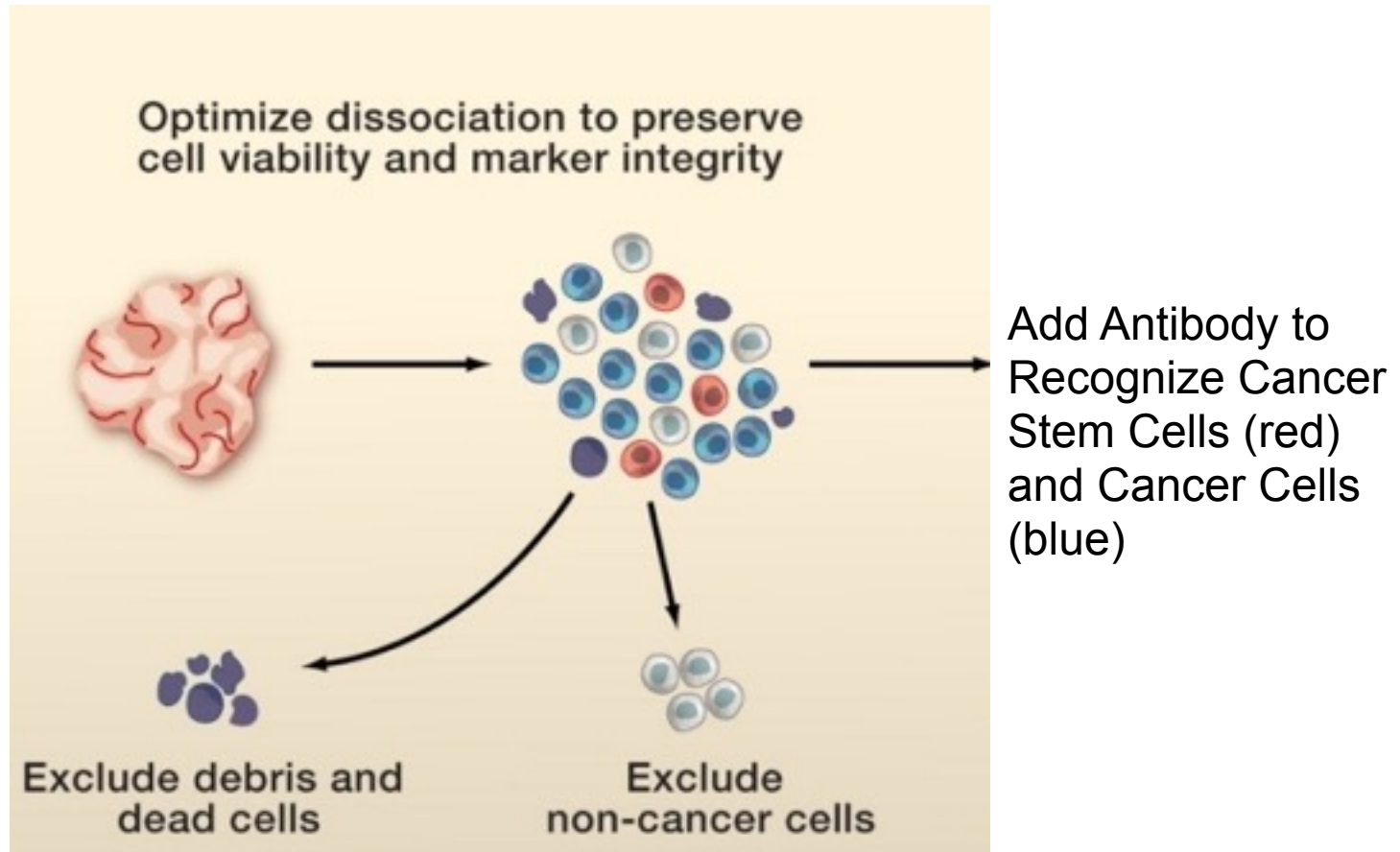


Dean, M. and R. Allikmets (2001). J Bioenerg Biomembr **33**(6): 475-479.

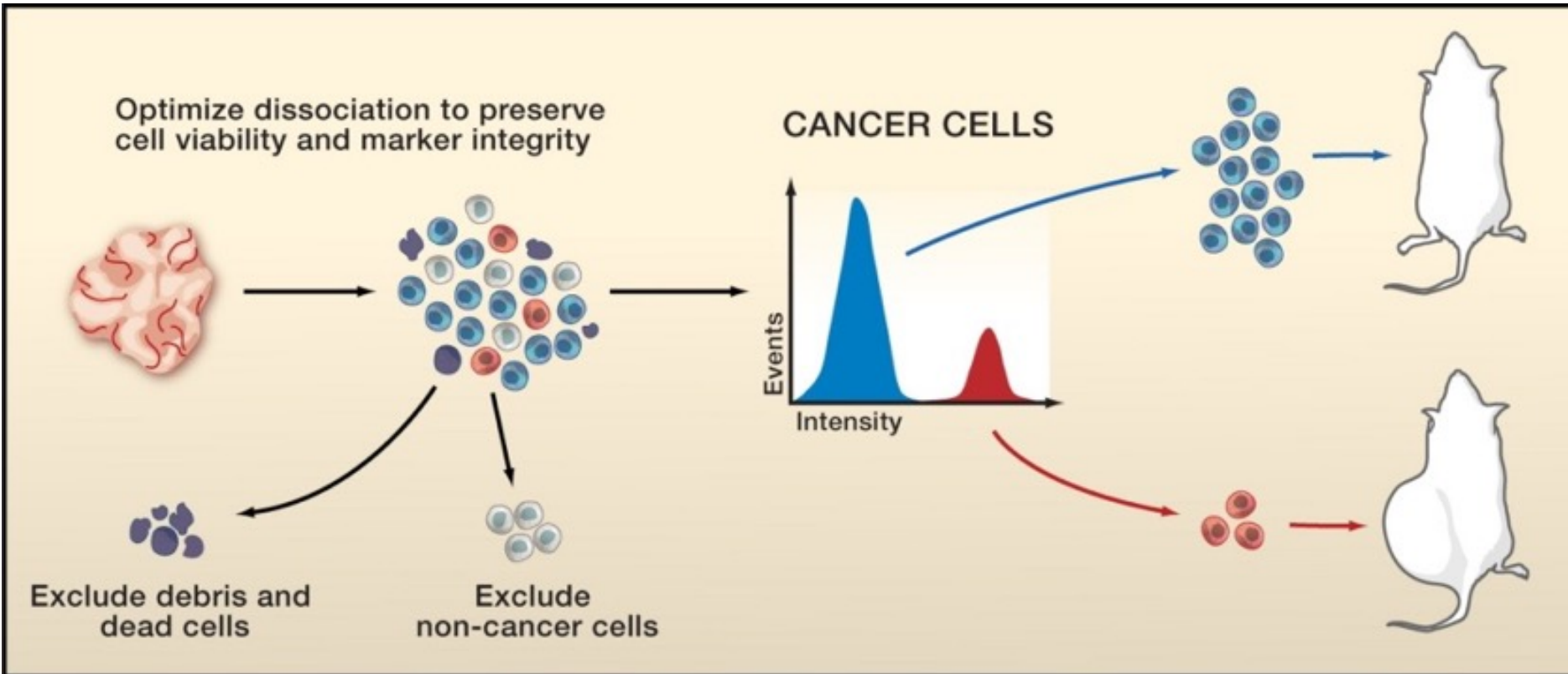
ABC Transporter Super Family

Subfamilies	Most studied member	Role of the most studied member
ABCA1-12	ABCA1 and 2	Cholesterol efflux (A1), Drug resistance (A2)
ABCB1-11	ABCB1,6,11	Multi drug resistance, Hoechst (B1) Iron transport (B6) Bile salt transport (B11)
ABCC1-13	ABCC1-5	Drug resistance (C1,3) Nucleotide transport (C4,5) Chloride channels (C7)
ABCD1-4	-	-
ABCF1-3	-	-
ABCE1	-	-
ABCG1-8	ABCG1, 2, 5, 8	Cholesterol efflux (G1) Toxins, drugs, dyes e.g. Hoechst , rhodamine, DCV, steroids (G2)

Identification of Cancer Stem Cells in Solid Tumors

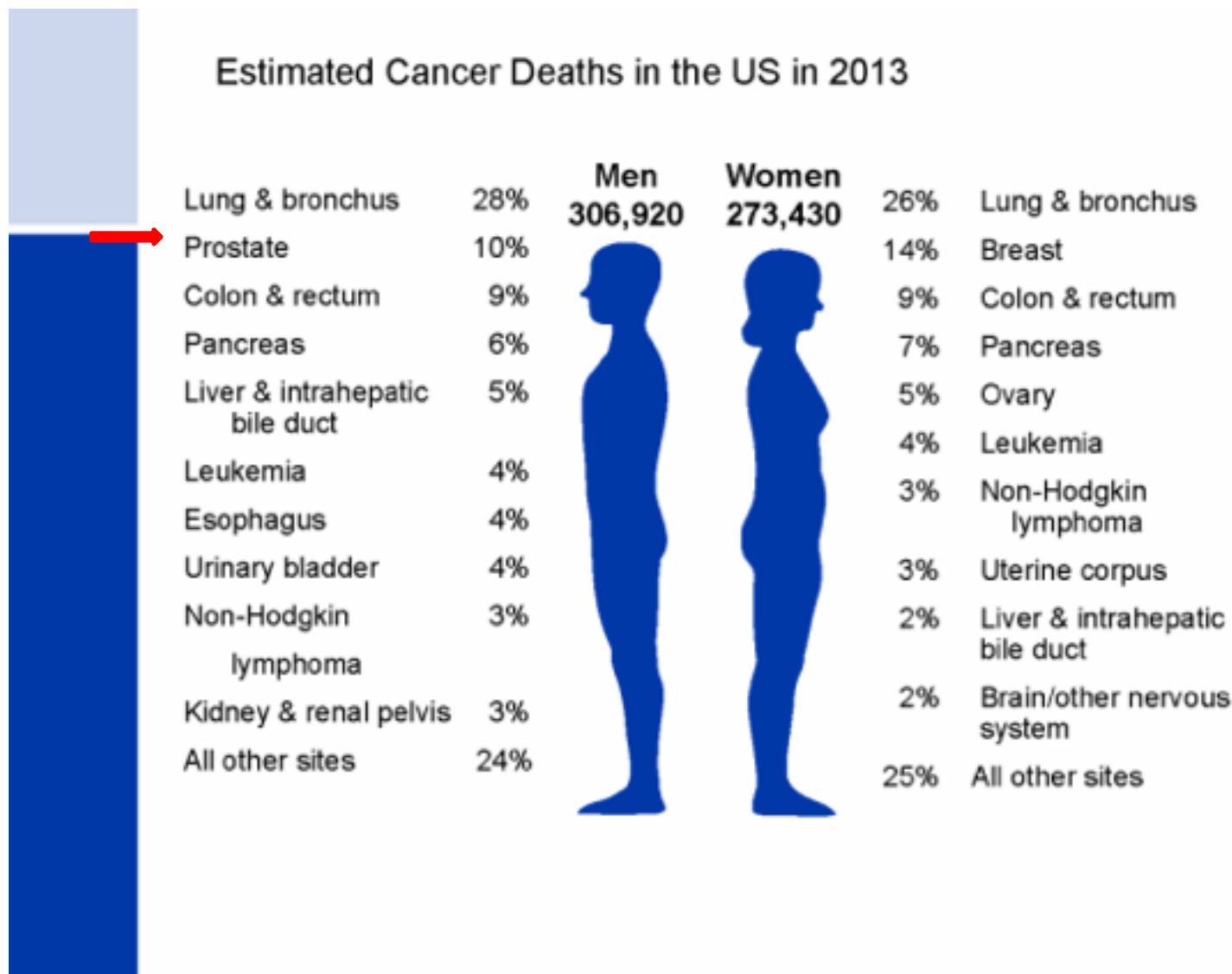


Identification of Cancer Stem Cells in Solid Tumors

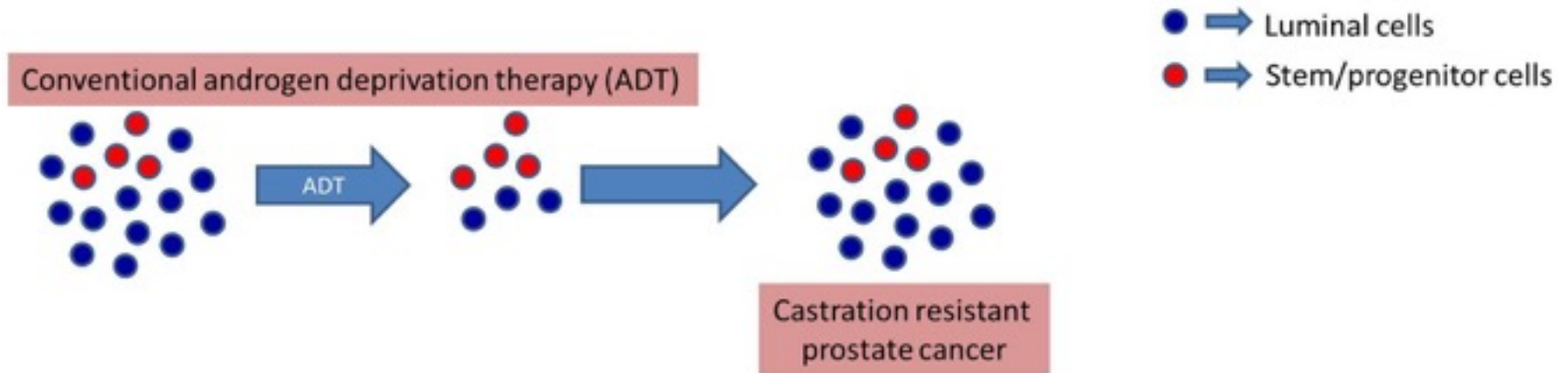


Prostate Cancer is Second Cause of Cancer Related Death in American Men

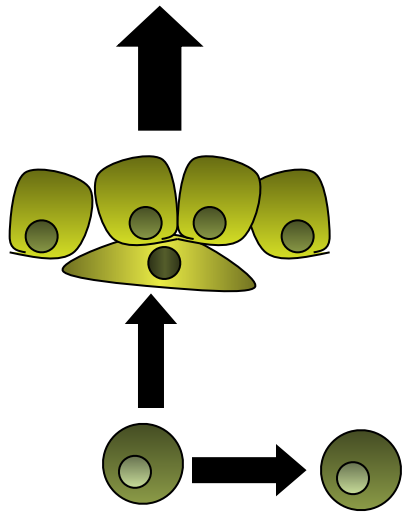
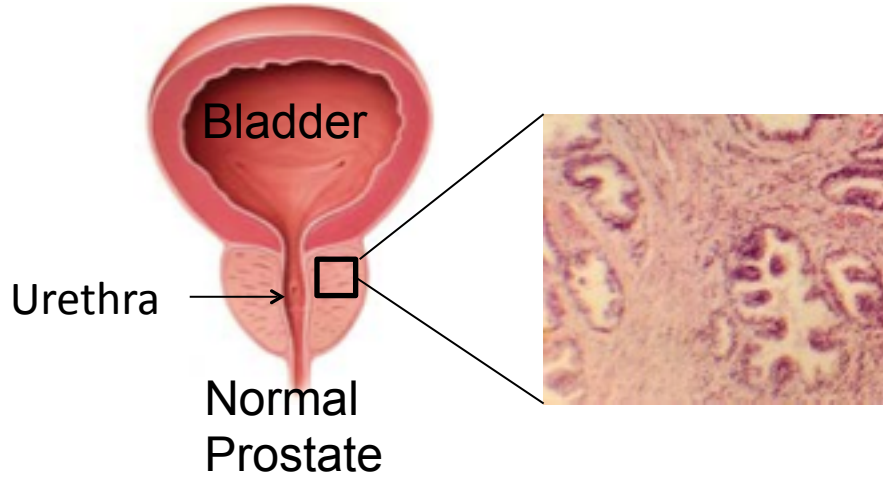
Estimated Cancer Deaths in the US in 2013



Clinical Significance: Castration Resistant Prostate Cancer

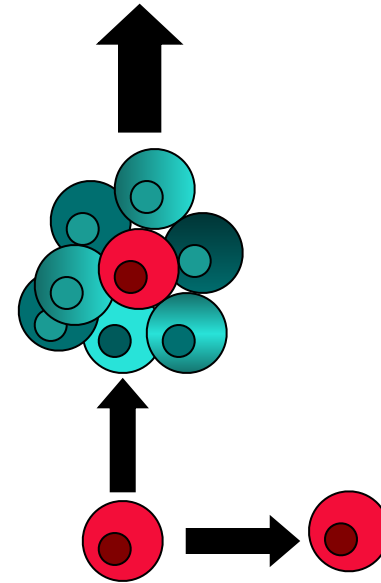
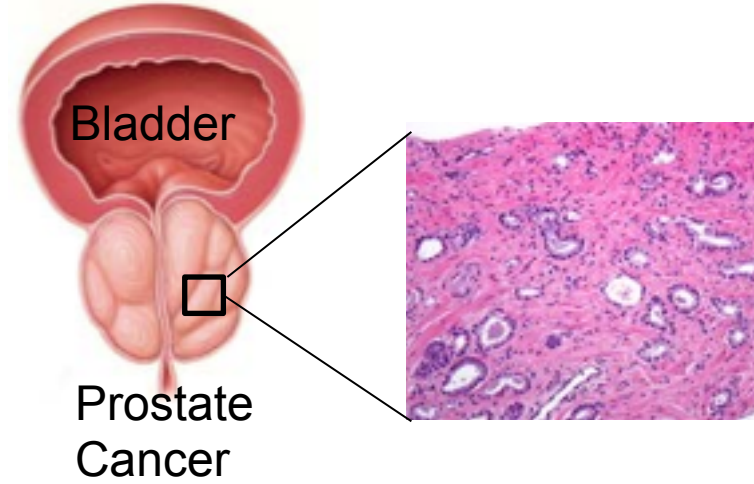


Organ Stem Cells



Normal adult stem cell

Cancer Stem Cells



Cancer stem cell

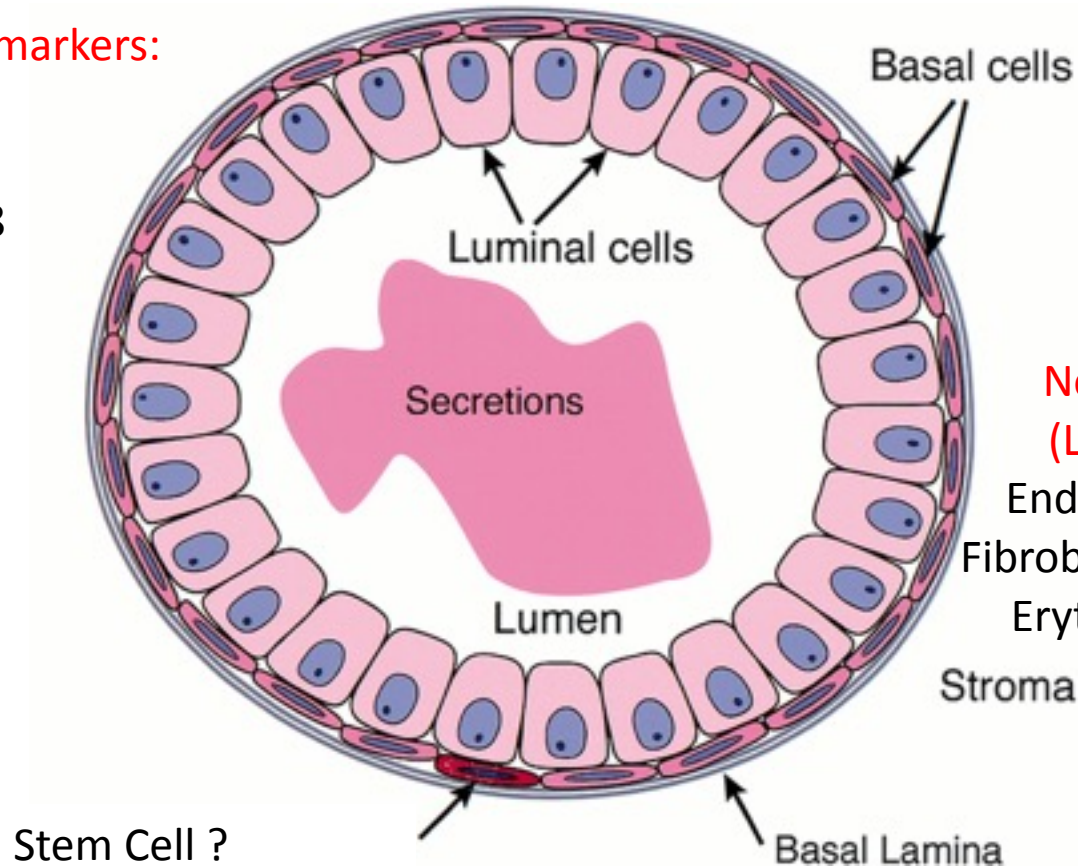
Schematic Depiction of the Prostatic Duct

Luminal cell markers:

AR,
CK8,
CK18

Basal cell markers:

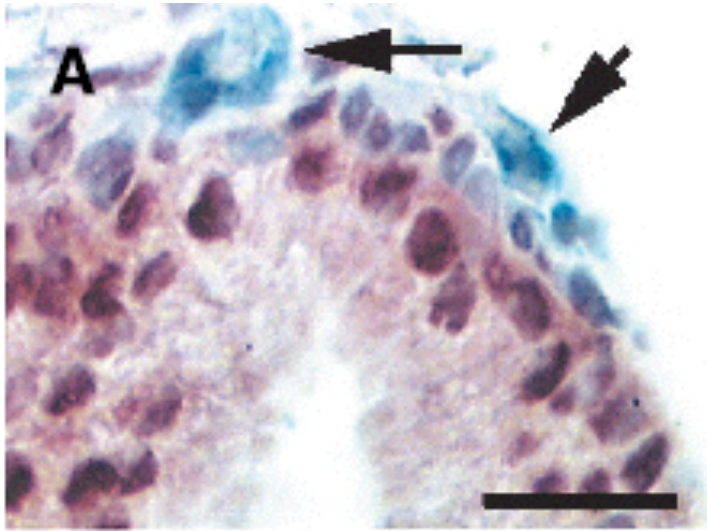
p63,
CK5,
CK14



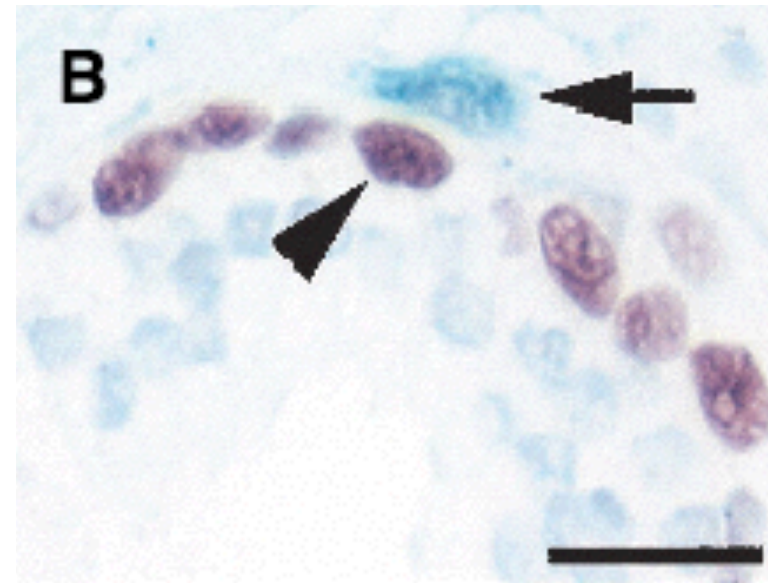
Non-epithelial cells (Lineage markers):

Endothelial cells: CD31
Fibroblasts: CD45 and CD34
Erythroid cells: Ter119

Abcg2+ Cells are Located in Stem Cell Compartment

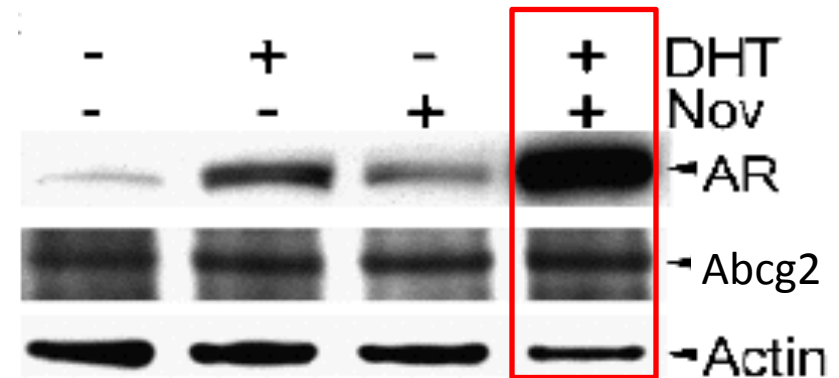
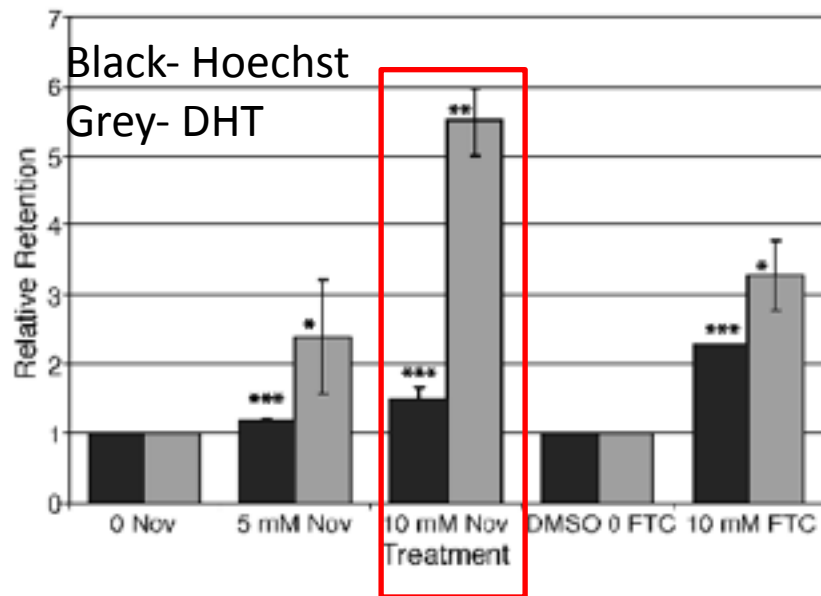


ABCG2- Blue
AR- Red

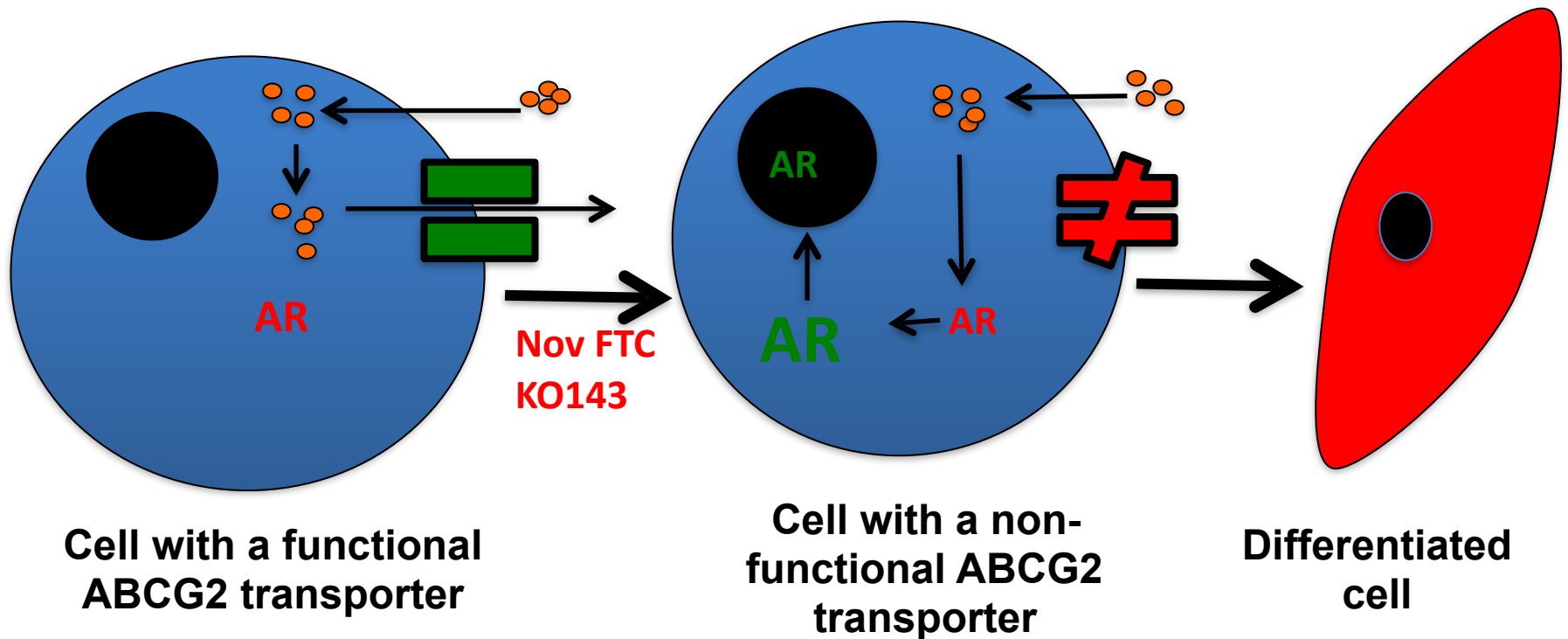


ABCG2- Blue
p63- Red

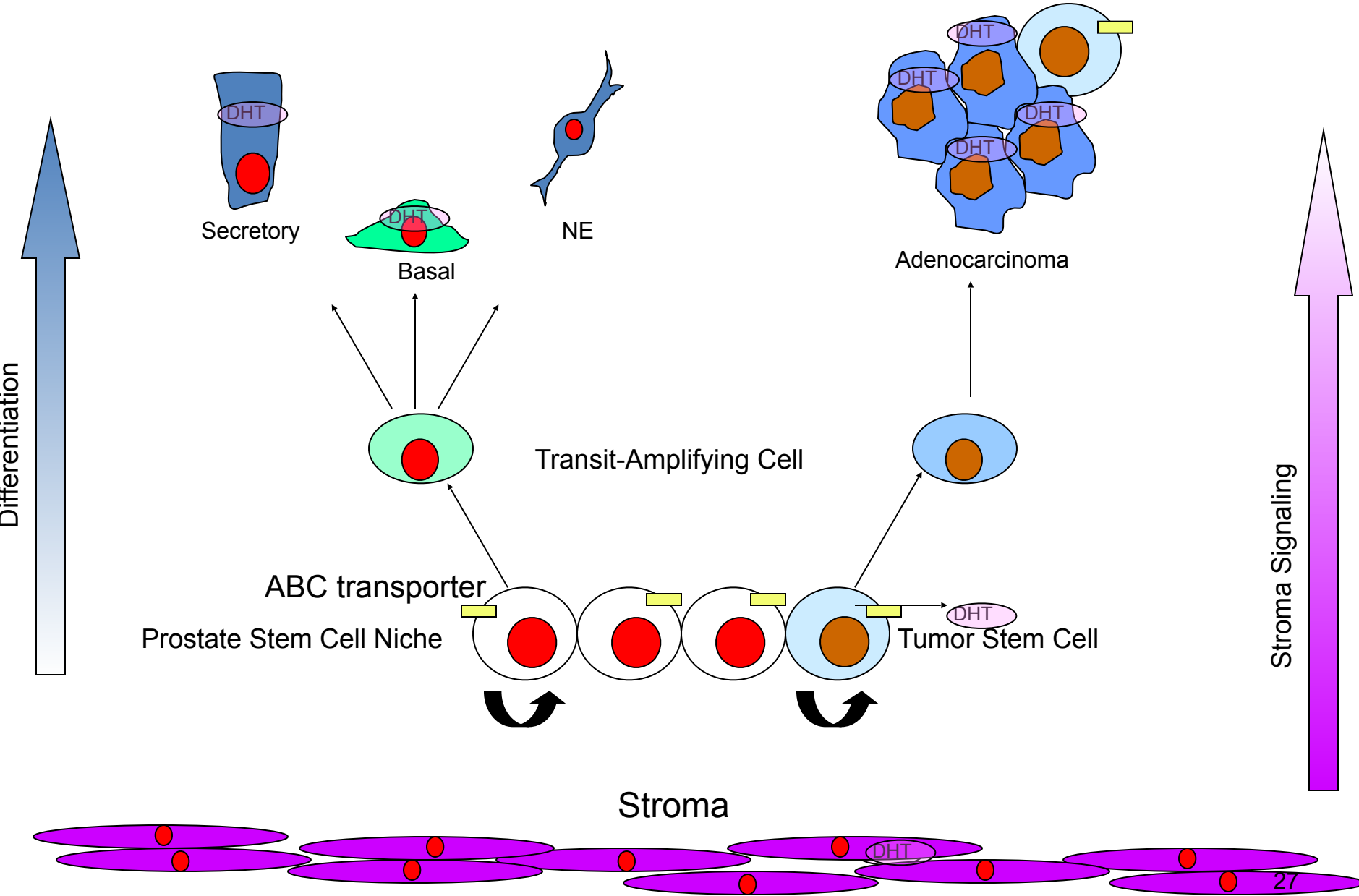
ABCG2 Inhibition Increases Intracellular Androgen and Androgen Receptor



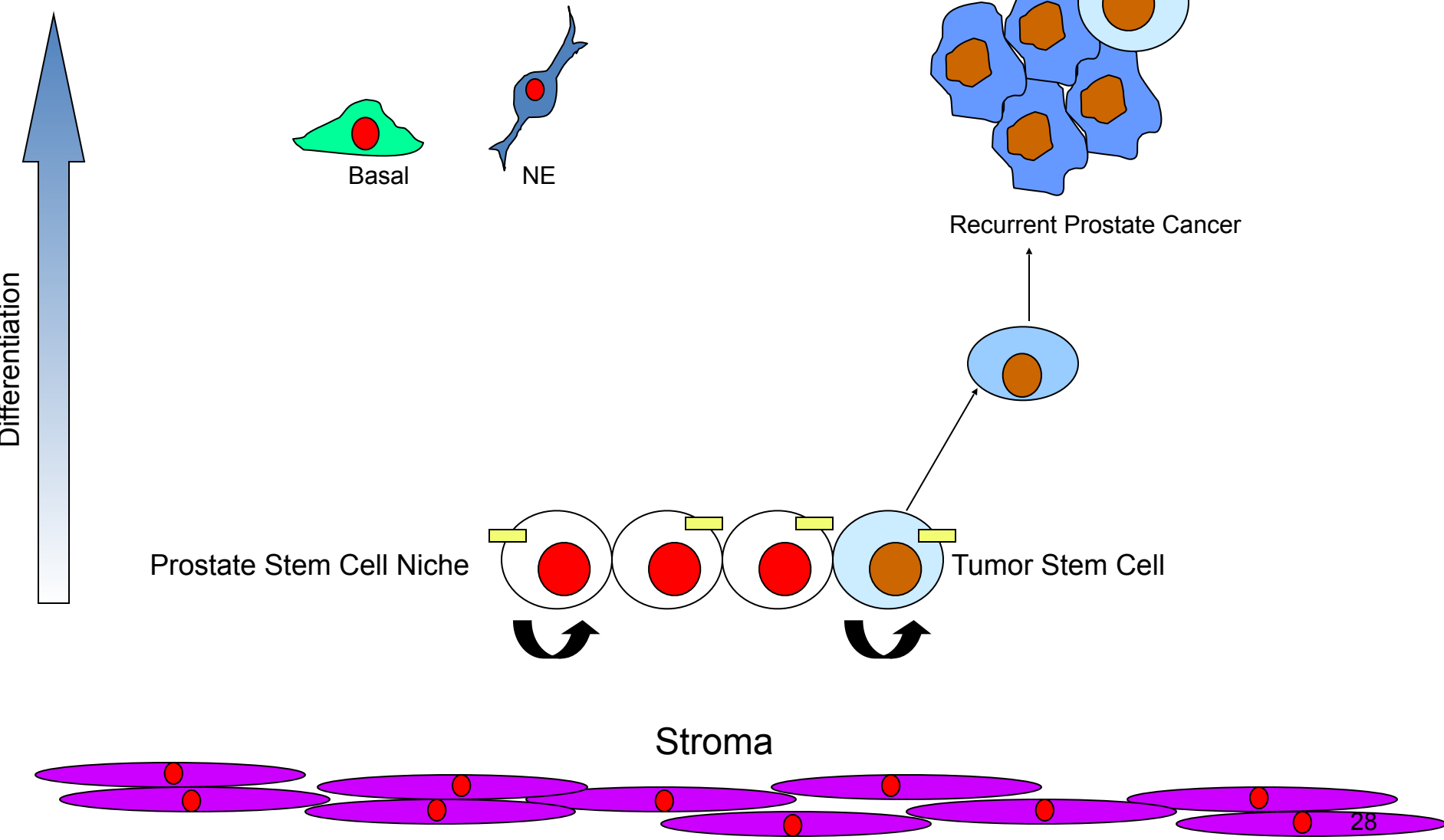
Androgen Receptor (AR) Regulation by Androgens



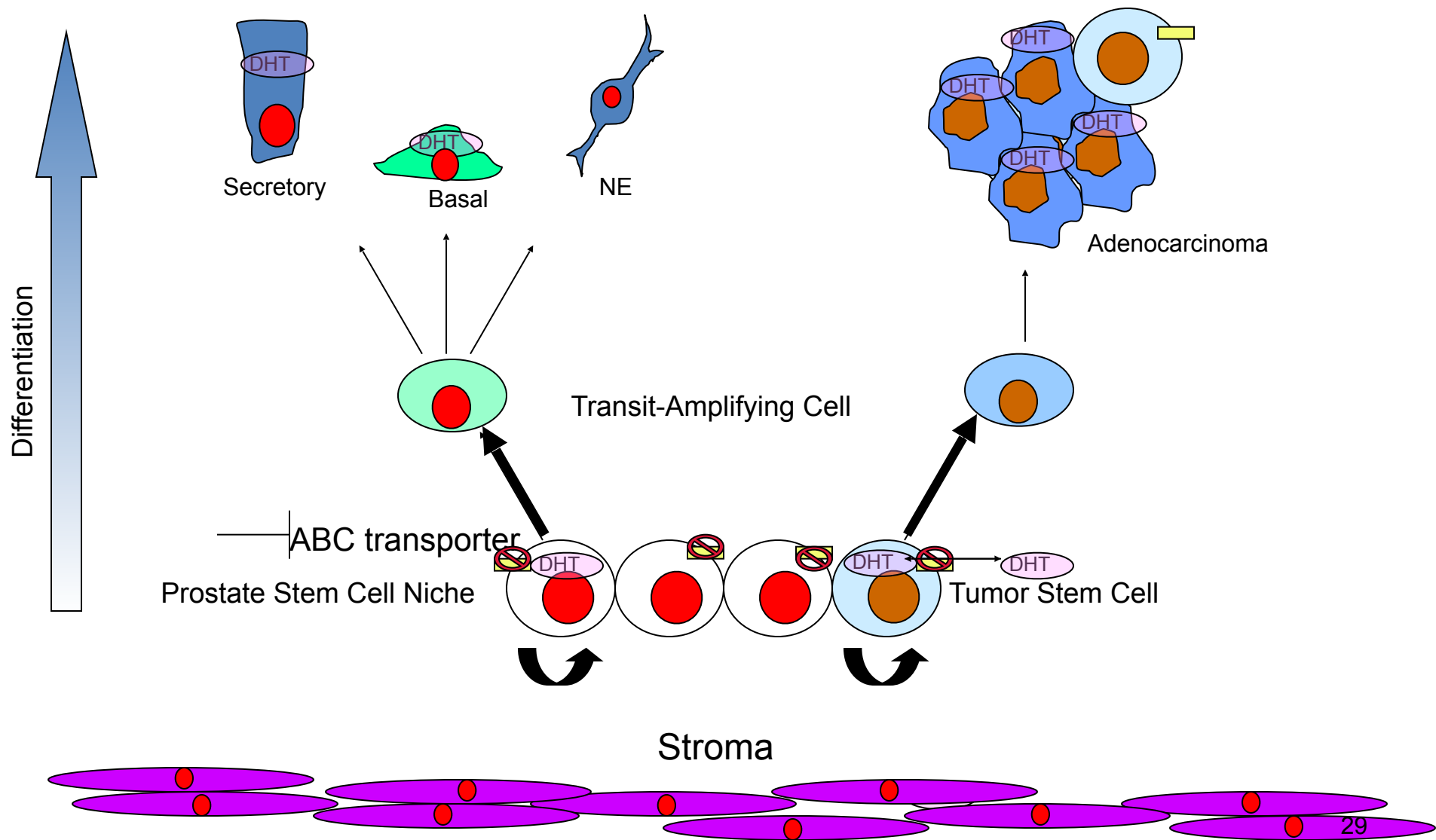
Prostate Benign and Cancer Stem Cells



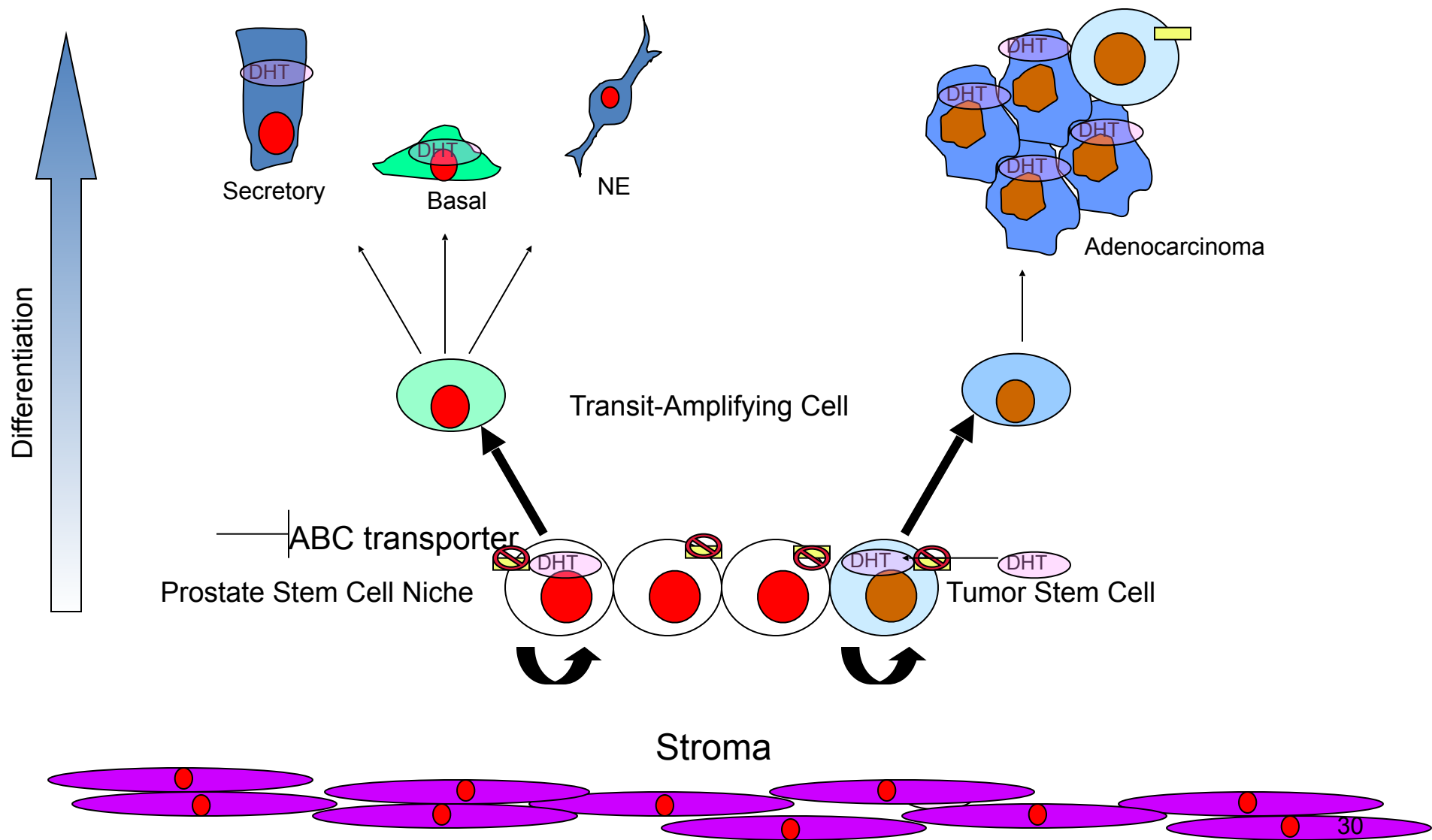
Androgen Deprivation Therapy Recurrent Prostate Cancer



Inhibition of ABC Transporters Before Androgen Deprivation to Target Cancer Stem Cells



Inhibition of ABC Transporters Before Androgen Deprivation to Target Cancer Stem Cells



Hypothesis: ABCG2 efflux of androgen inhibits prostate stem cell differentiation to maintain stem cell properties

Specific aims:

1. *Determine the mechanism of androgen efflux to maintain stem cell properties.*

ABCG2 mediated androgen efflux inhibits AR induced prostate stem cell differentiation.

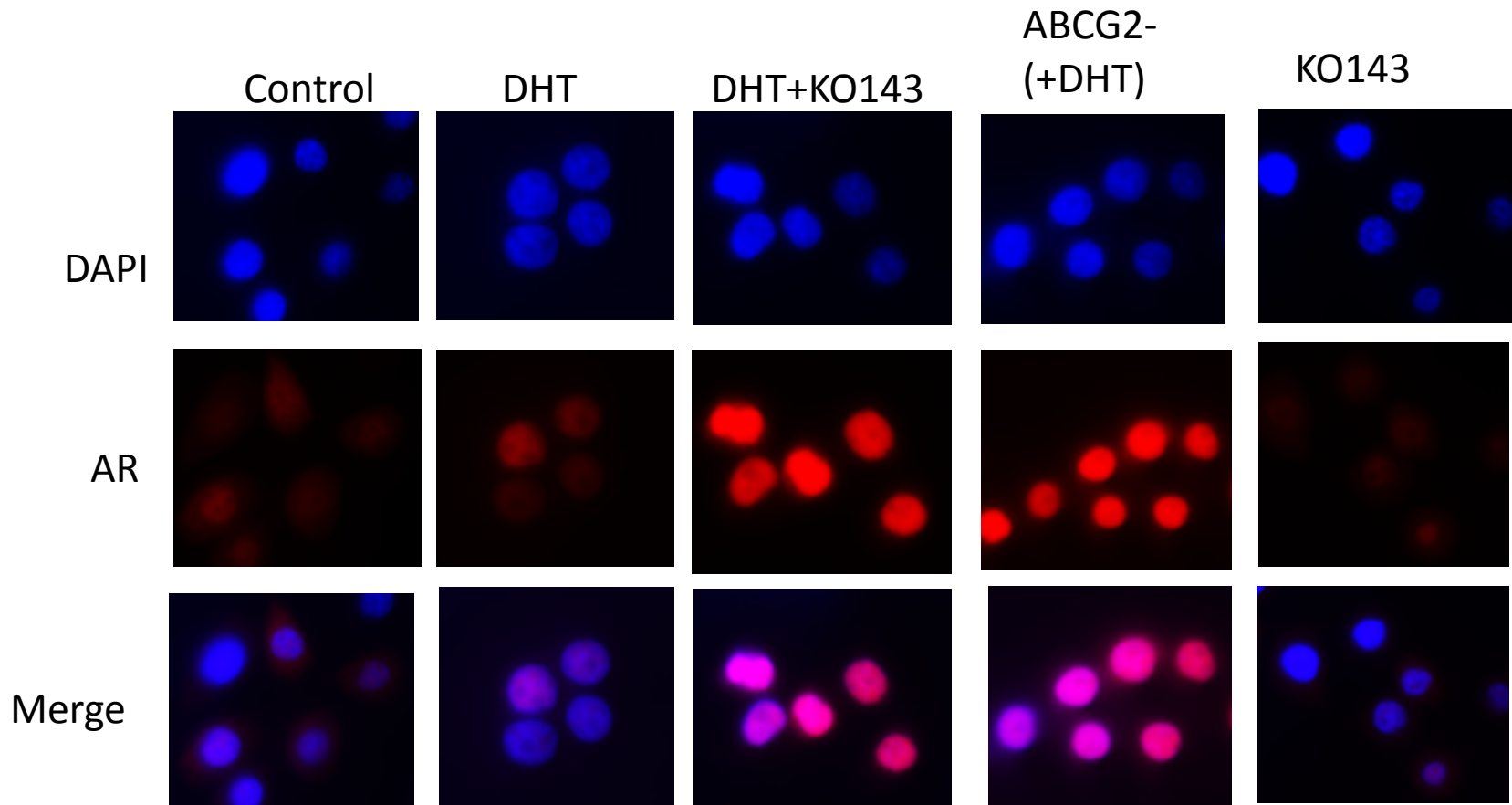
2. *Identify regulators of the side population phenotype that contribute to maintaining prostate stem cell properties.*

Prostate stem cells within the side population require ABCG2 expression.

3. *Determine the effect of abrogated ABCG2 function on the prostate stem cell niche.*

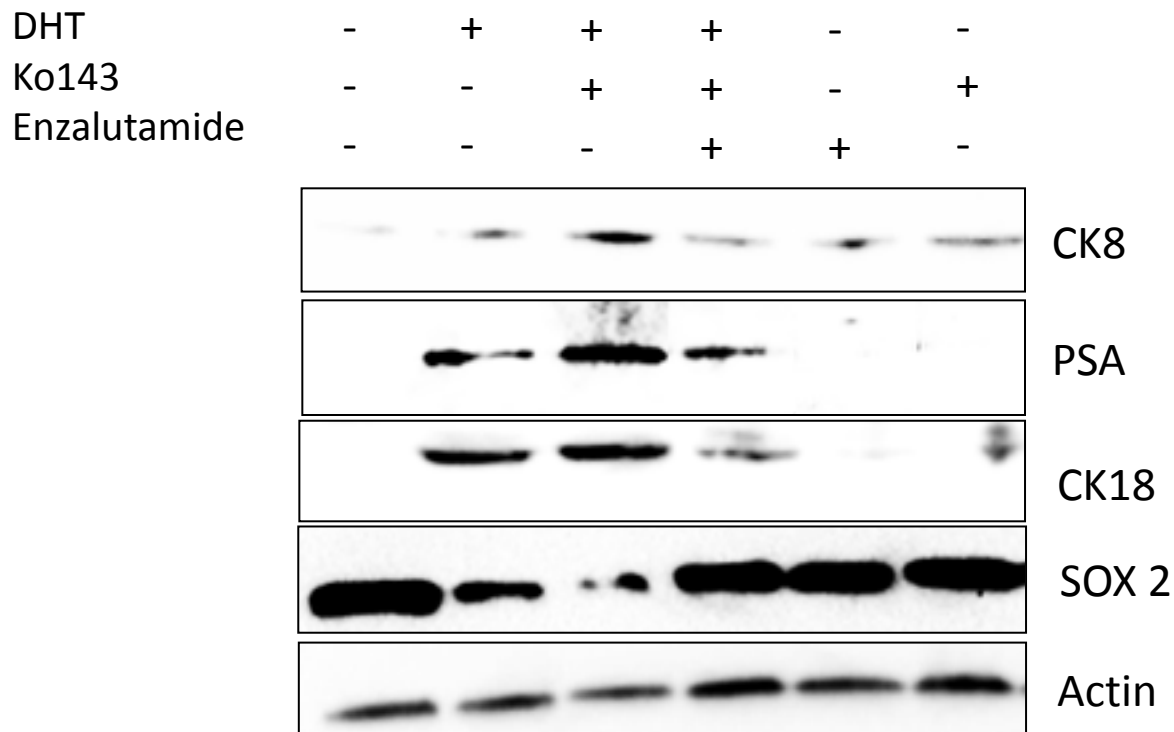
ABCG2 inhibition depletes stem cell compartment and the prostate is unable to serially regenerate.

Aim 1: Inhibiting ABCG2 Mediated Androgen Efflux Increases AR Nuclear Translocation



ABCG2 expressing HPr-1-AR cells (Similar Results in CWR-R1)

Aim 1: Inhibiting ABCG2 Mediated Androgen Efflux Increases Expression of Luminal Differentiation Markers



ABCG2-expressing HPr-1-AR cells
(Similar Results in CWR-R1)

Summary Aim 1 and Future Directions

ABCG2 inhibition increases nuclear AR &
expression of AR regulated differentiation markers

Determine if differentiation is regulated by AR activation
inhibit AR and determine differentiation capabilities

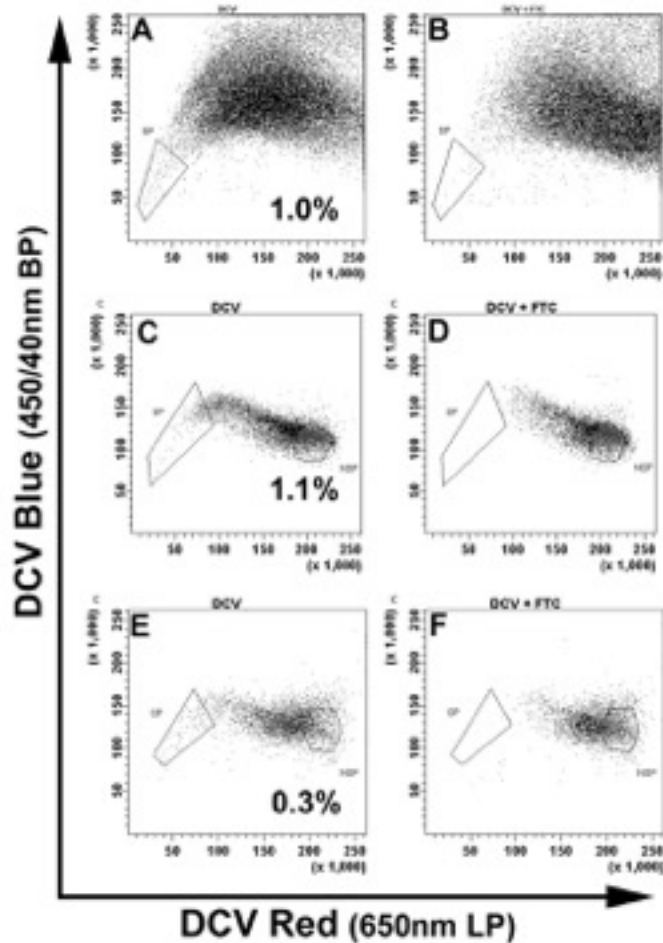
Hypothesis-

Inhibition of ABCG2-Mediated Androgen Efflux Eliminates the Prostate Cancer Stem Cell Compartment

Aims

- Determine AR Function with ABCG2 Inhibition
- Determine Stem Cell Properties of ABCG2 Expressing Cells
- Determine the Role of ABCG2 in Stem Cell Maintenance

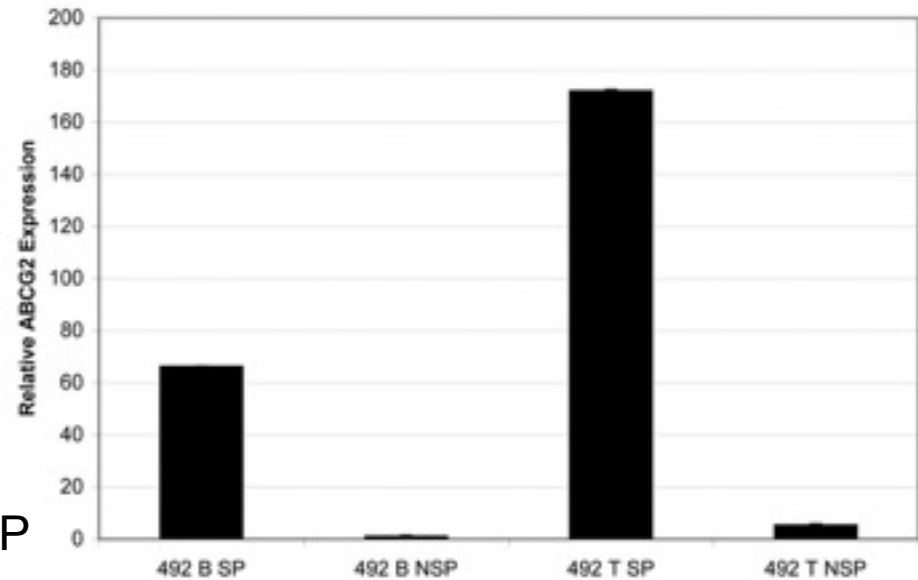
ABCG2 Expression in Side Population from Human Prostate Specimens



Non-Tumor

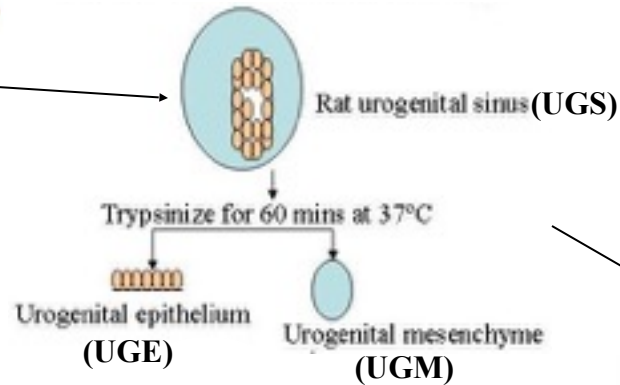
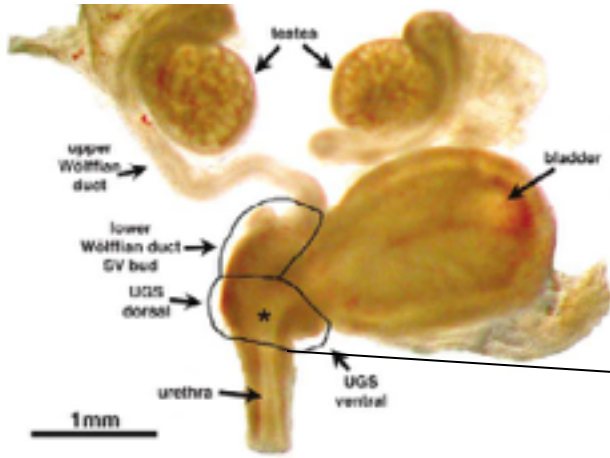
Tumor

TRAMP



Testing Prostate Stem Cell Properties with Tissue Recombination

Embryonic Rodent Urogenital Tract

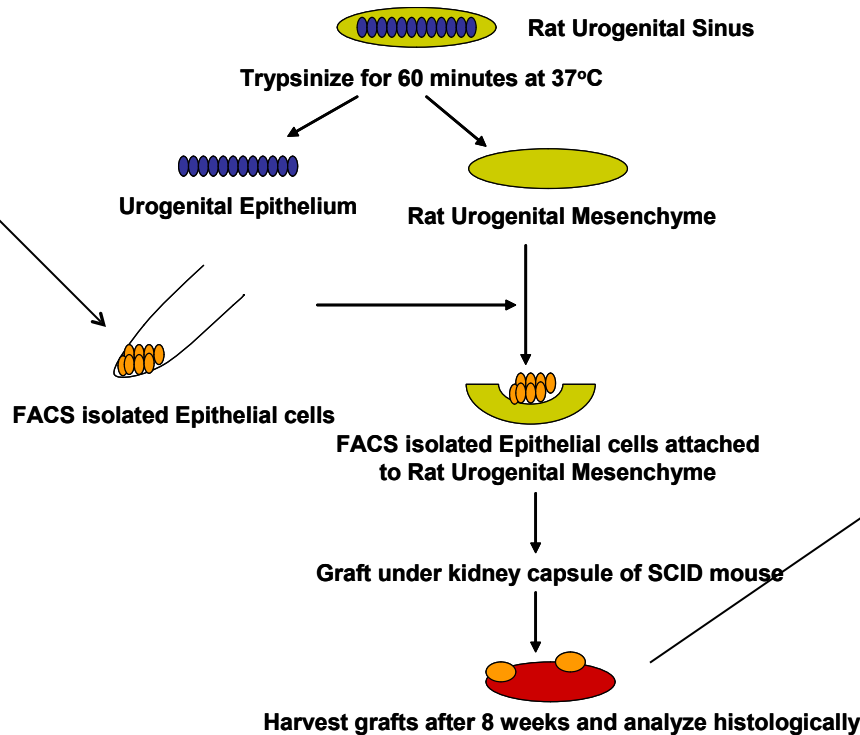
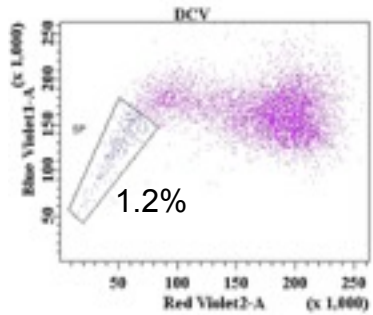


Graft *in vivo* under renal capsule

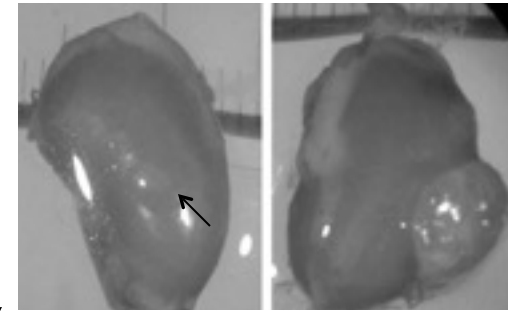


Tissue Recombination of rUGM and Putative Stem Cells

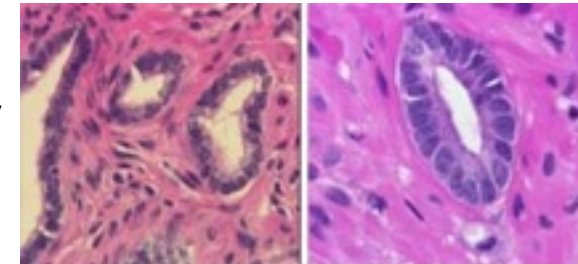
Side Population Assay



Side population cells isolated from radical prostatectomy specimens



Cells + collagen = no growth
Cells + UGM = growth

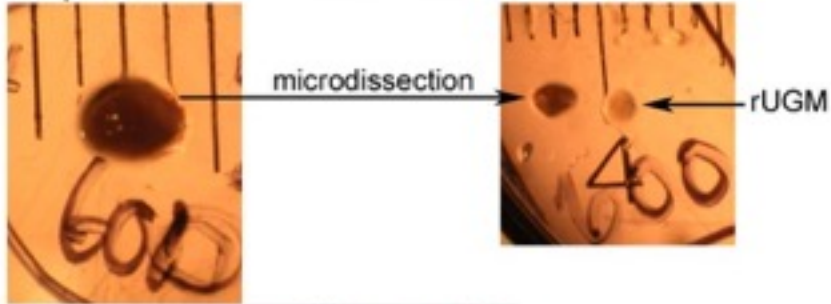


H&E analysis of side population cells + UGM

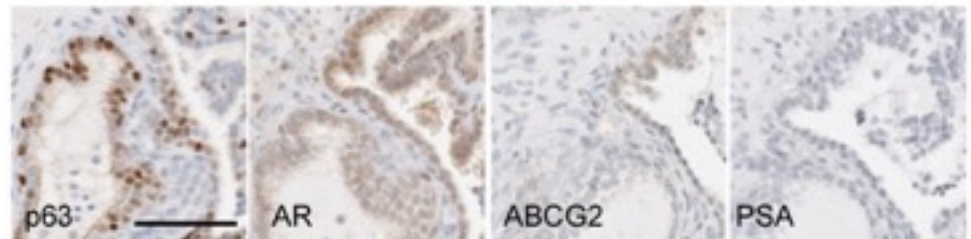
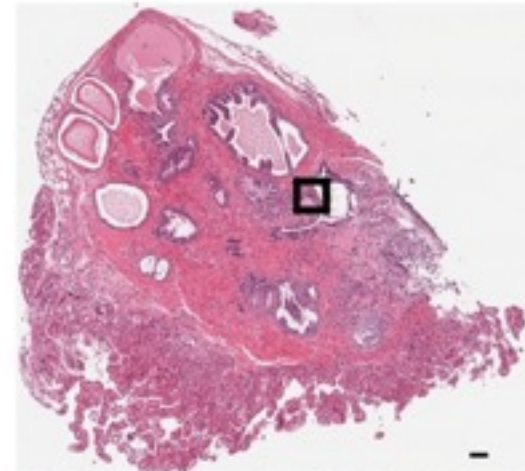
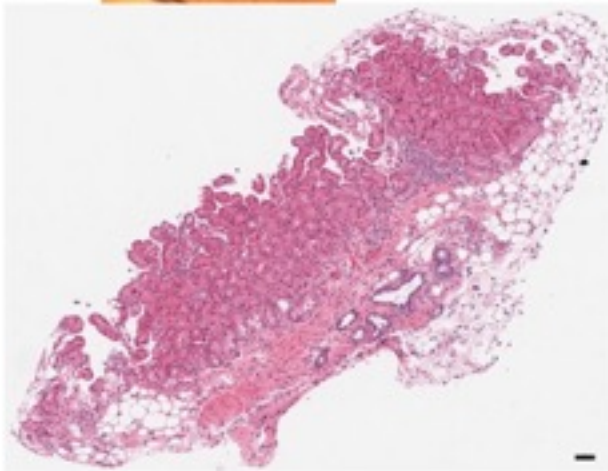
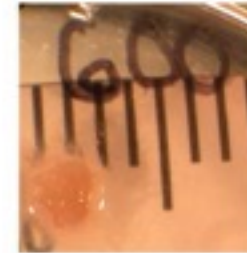
FISH analysis for epithelial species identification, IHC analysis for 38 differentiation markers

Serial Tissue Recombinations

A Side Population 1st Generation



2nd Generation



Summary Aim 2 and Future Directions

Side Population Assay enriches for Prostate Stem Cells

Next-Determine if Side Population Assay enriches for Prostate Cancer Stem Cells

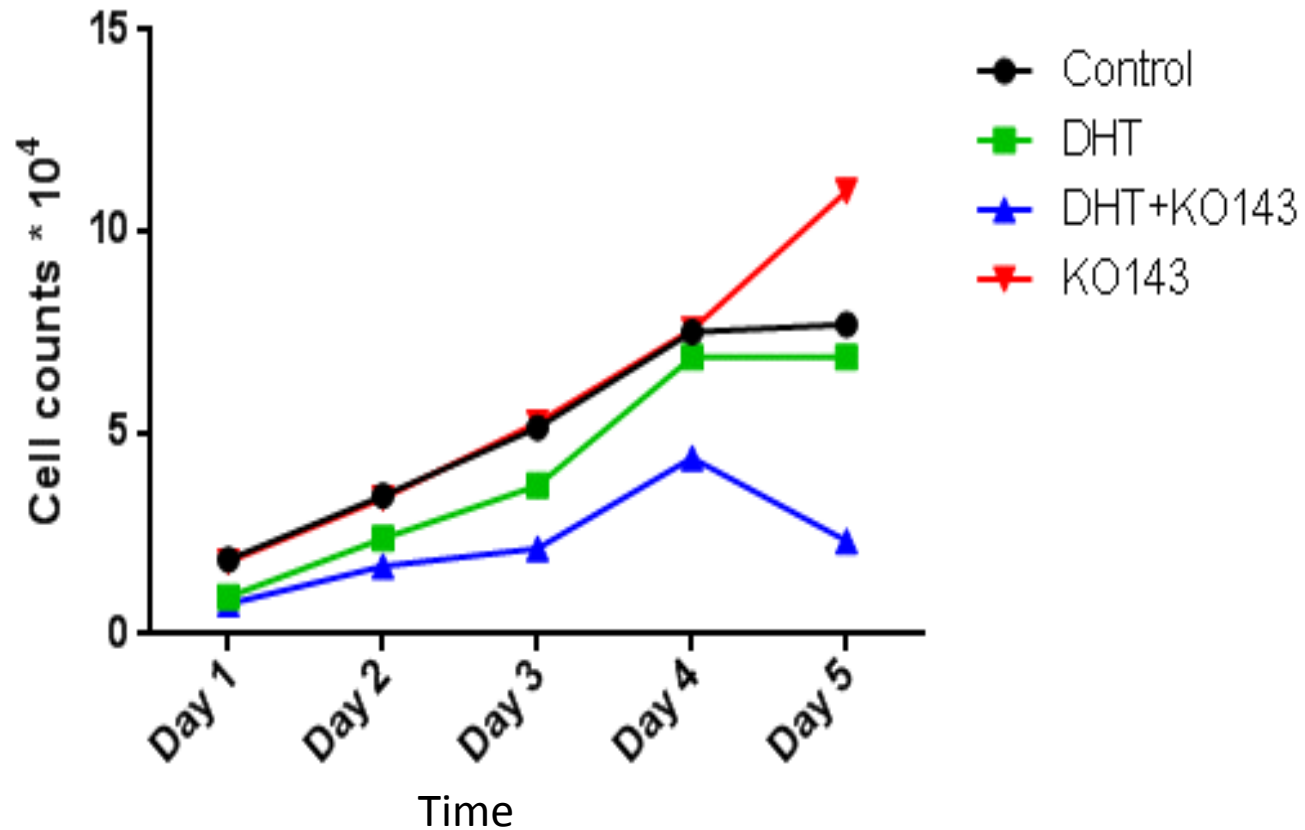
Hypothesis-

Inhibition of ABCG2-Mediated Androgen Efflux Eliminates the Prostate Cancer Stem Cell Compartment

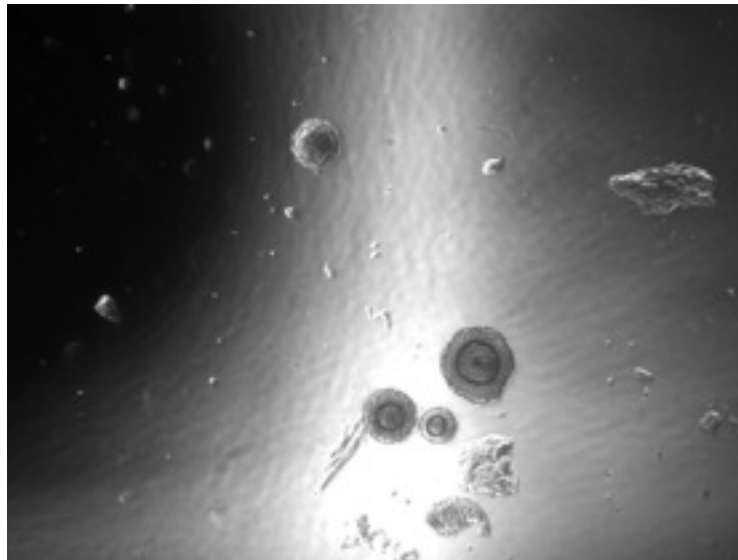
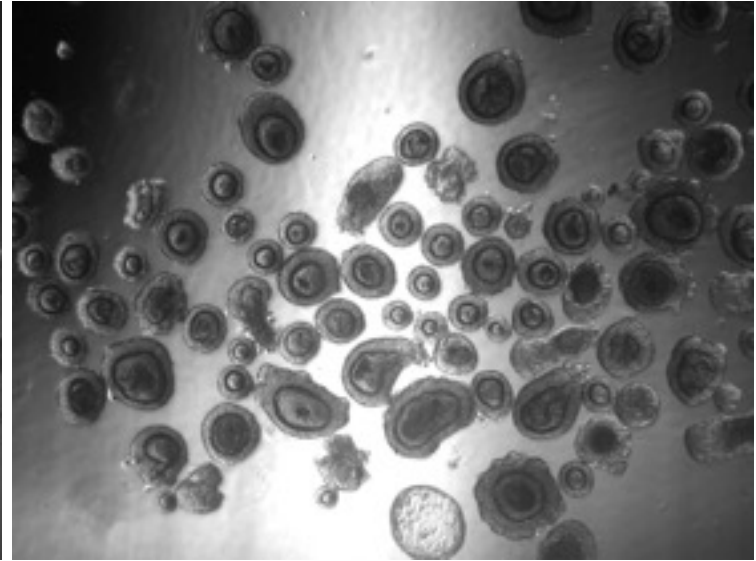
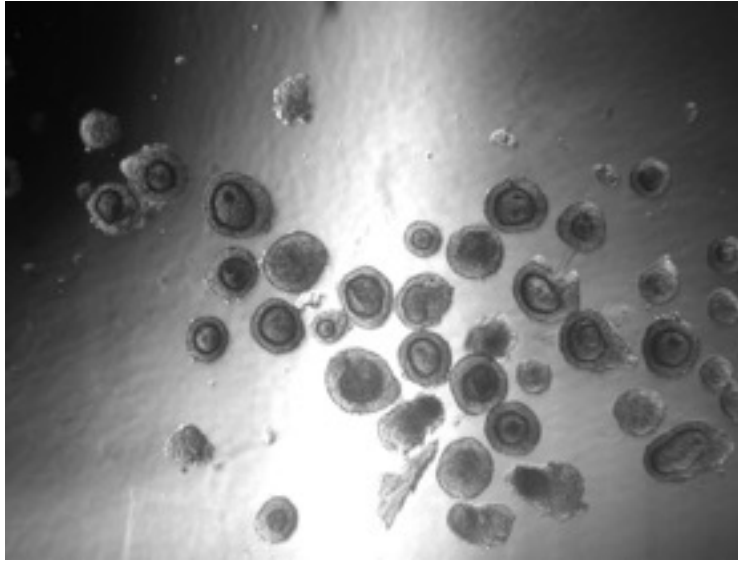
Aims

- Determine AR Function with ABCG2 Inhibition
- Determine Stem Cell Properties of ABCG2 Expressing Cells
- Determine the Role of ABCG2 in Stem Cell Maintenance

ABCG2 Inhibition and Androgen Retention Leads to Delayed Growth Response

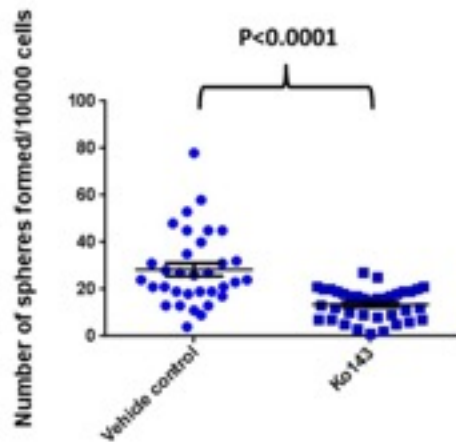


Sphere Formation Assay



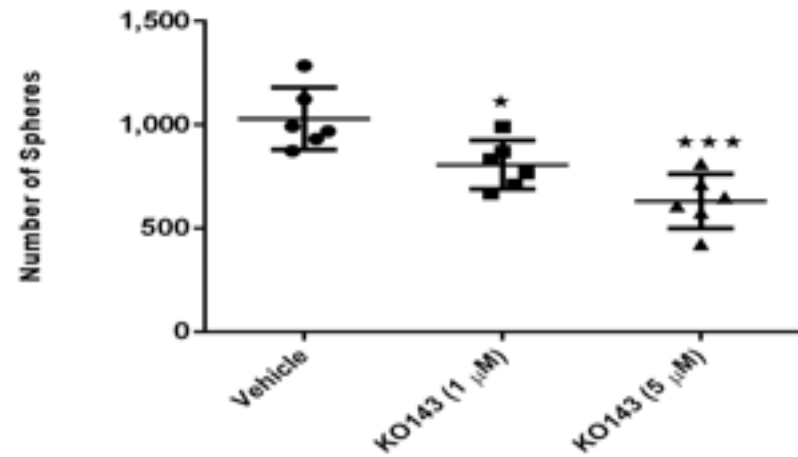
Inhibiting ABCG2 Function Reduces Sphere Forming Capabilities

Primary mouse ventral prostate cells



Samant MD, Jackson CM, Felix CL, Jones AJ, Goodrich DW, Foster BA, and Huss WJ. *Stem Cells and Development* 2015, 24(10): 1236-1251.

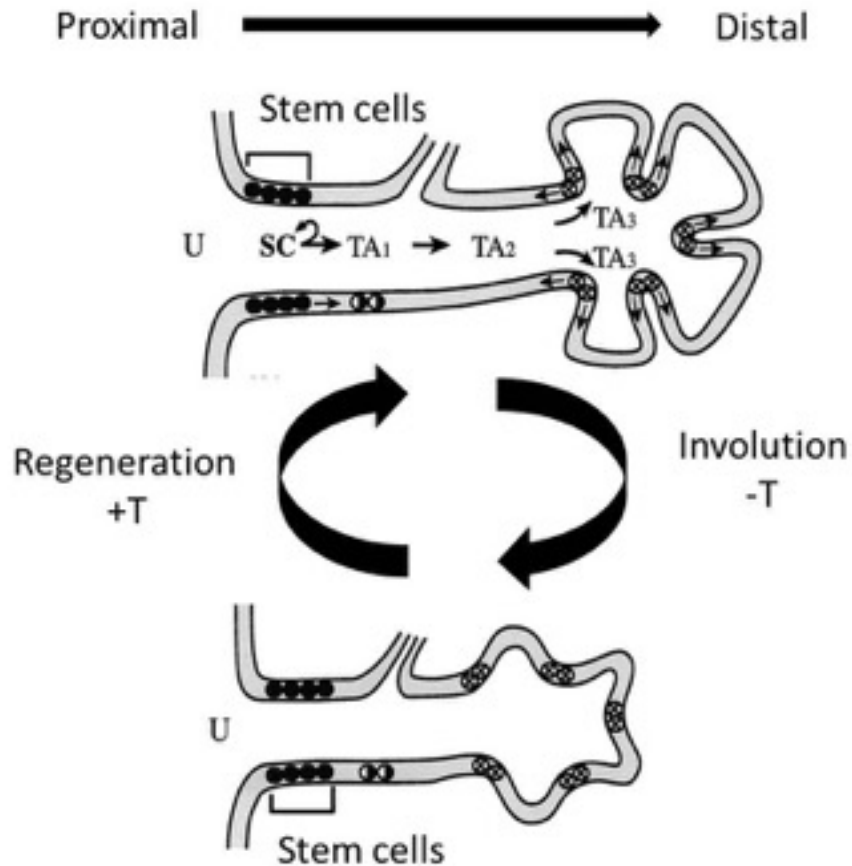
CWR-R1 cells



Gangavarapu KJ, Azabdaftari G, Morrison CD, Miller A, Foster BA, Huss WJ. *Stem Cell Research and Therapy* 2013; 4:132.

Ko143 inhibits ABCG2>ABCB1>ABCC1

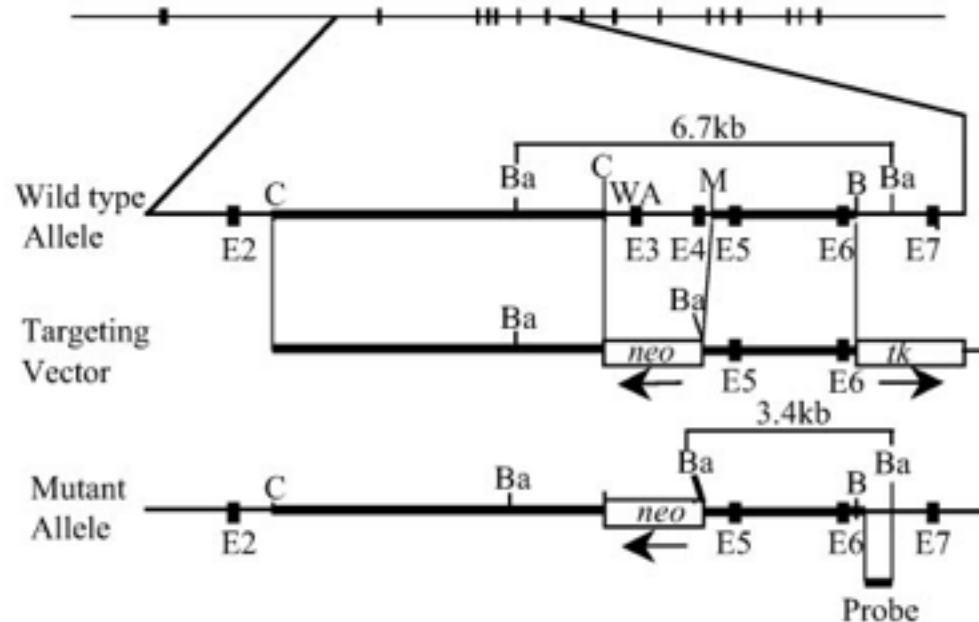
The Role of Androgens in Prostate



Apoptosis in the luminal cell compartment

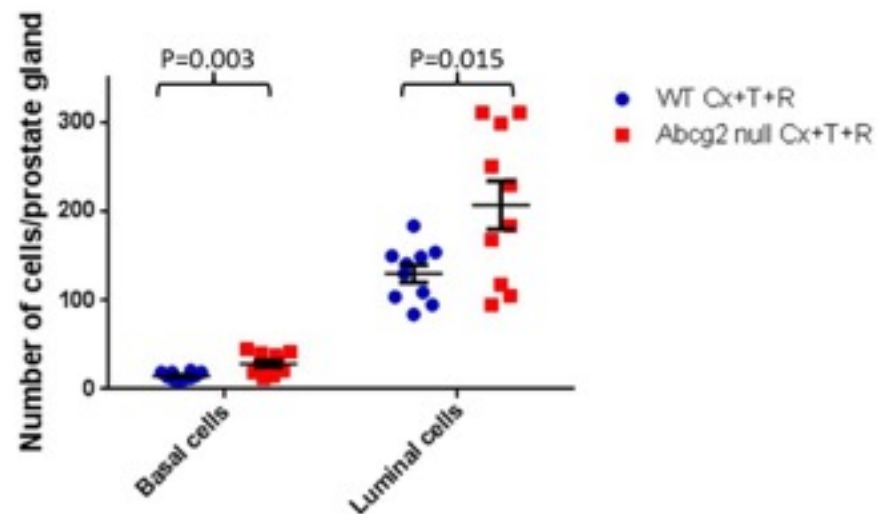
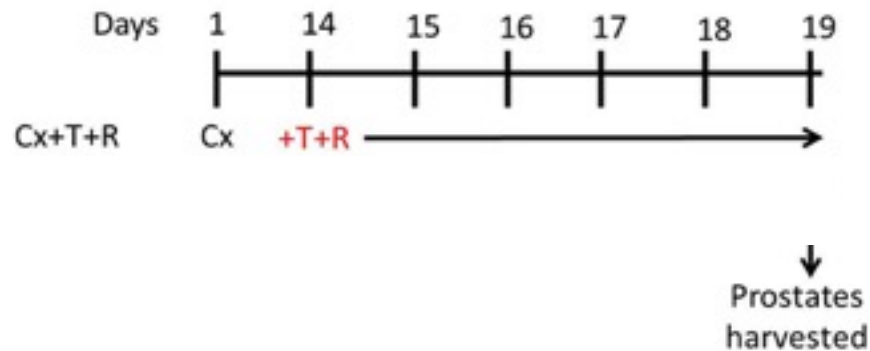
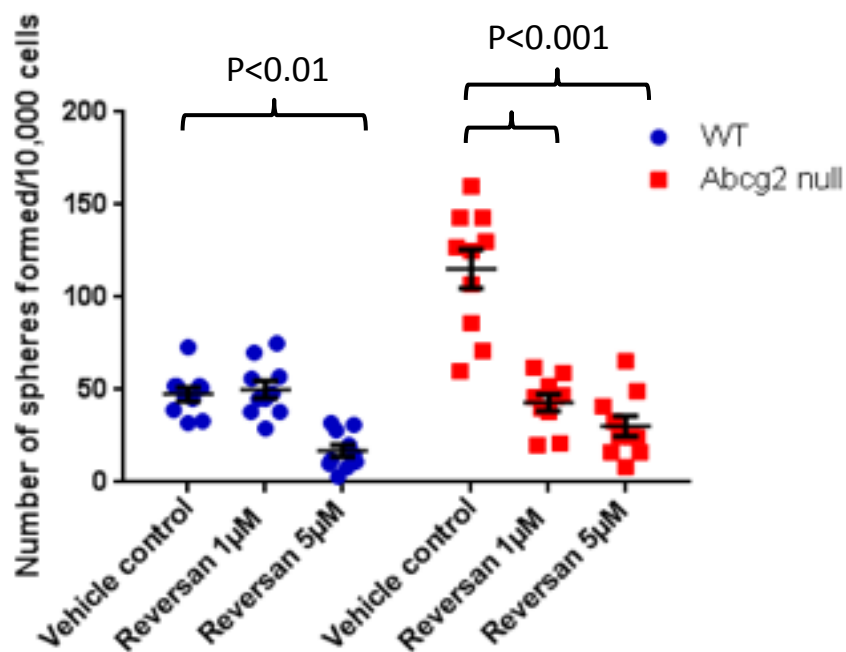
ABCG2 null mouse model

- *Abcg2* is deleted embryonically.
- Systemic deletion.
- By replacing exons 3 and 4 with a neomycin cassette via homologous recombination.



Zhou, S., et al. (2002). "Bcrp1 gene expression is required for normal numbers of side population stem cells in mice, and confers relative protection to mitoxantrone in hematopoietic cells in vivo." *Proc Natl Acad Sci U S A* **99**(19): 12339-12344.

Aim 3: Abcg2 null prostate cells were more sensitized to reversan treatment than WT controls



Reversan inhibits ABCC1>ABCB1>ABCG2

Summary

ABCG2 is a marker of prostate (cancer) stem cells

Inhibition of ABCG2 forces prostate (cancer) stem cell differentiation

- AR nuclear translocation

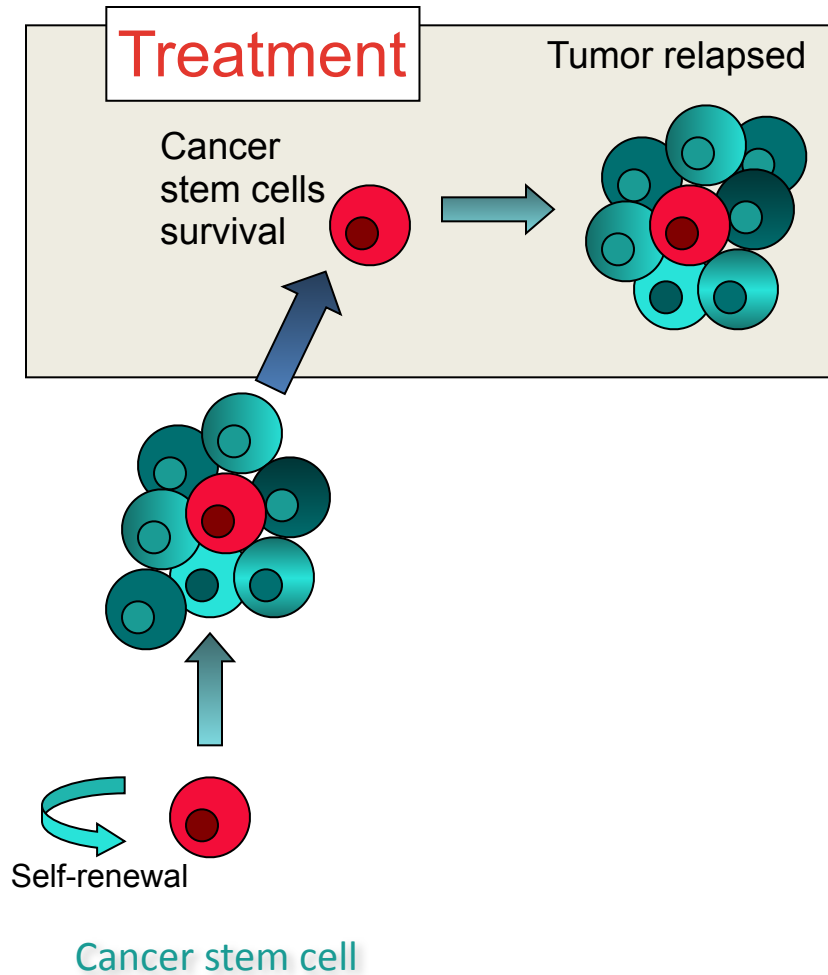
- Elevated differentiation markers

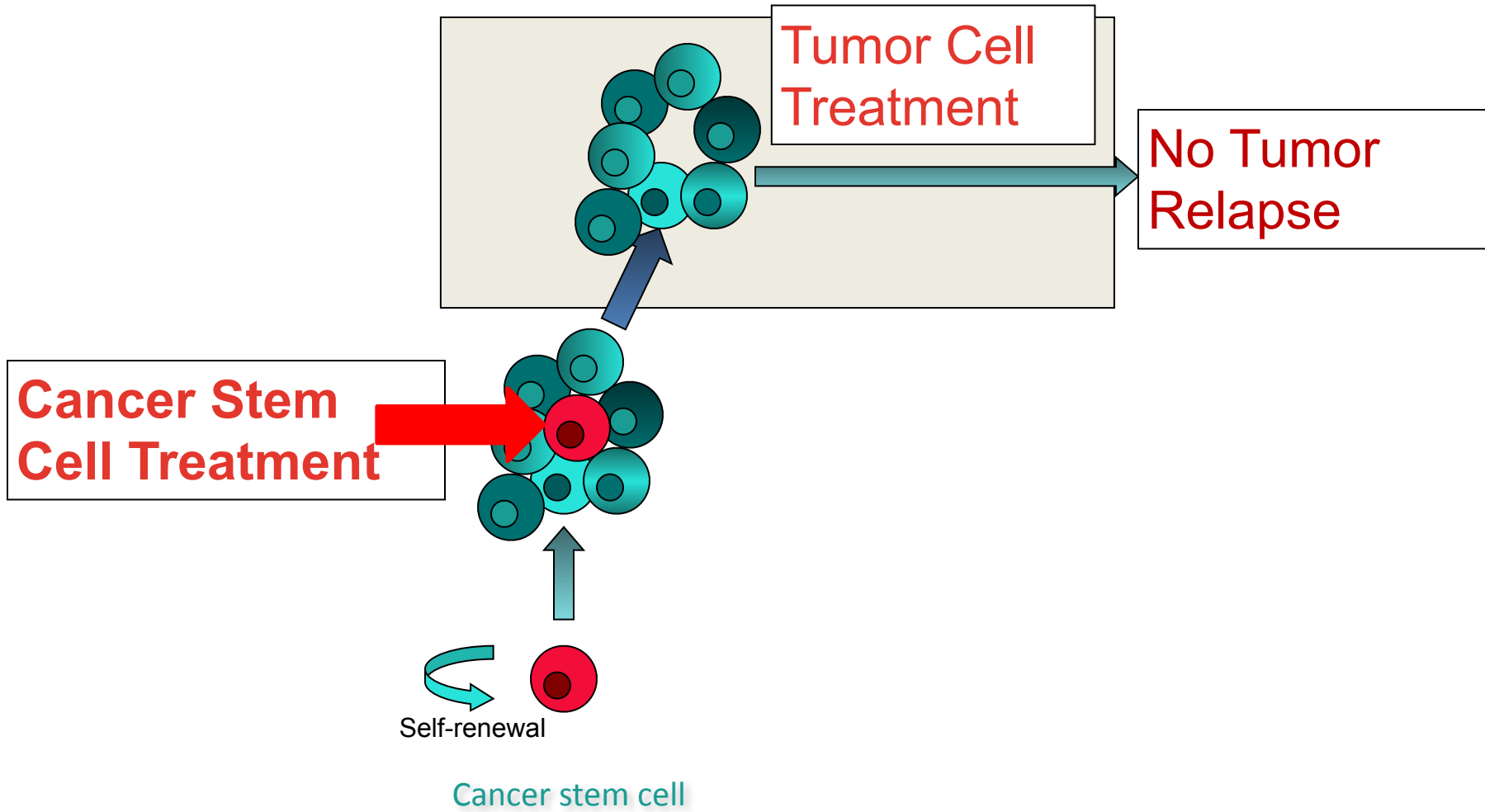
- Decreased cell growth

- Decreased sphere formation

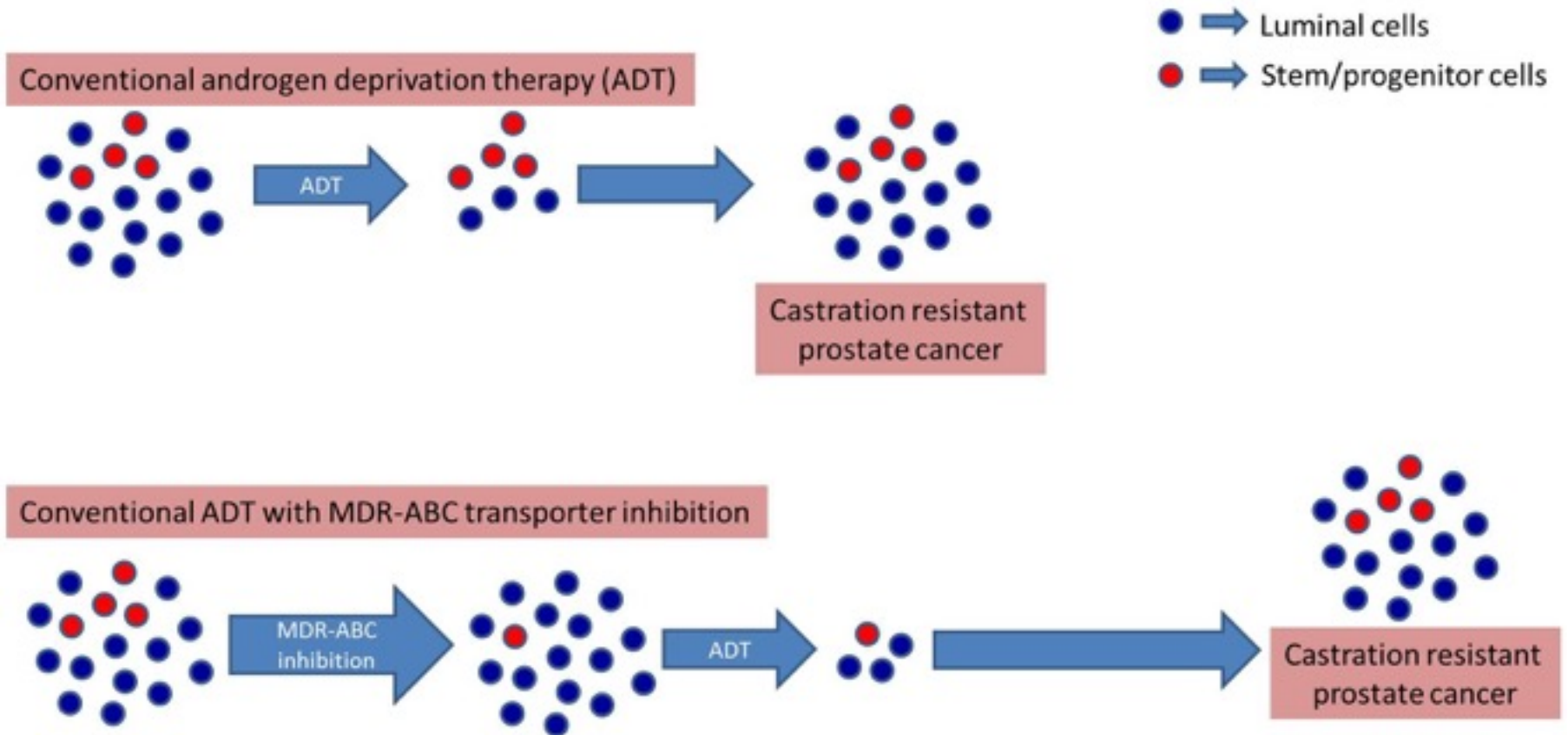
Future Directions

Determine if decreased cancer stem cells reduces tumor recurrence





Clinical significance: Differentiation therapy for prostate cancer



Acknowledgments

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Gissou Azabdaftari, MD

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