27th *The Japanese Society for the Study of Xenobiotics* Annual Meeting in Chiba on 11/21/2012

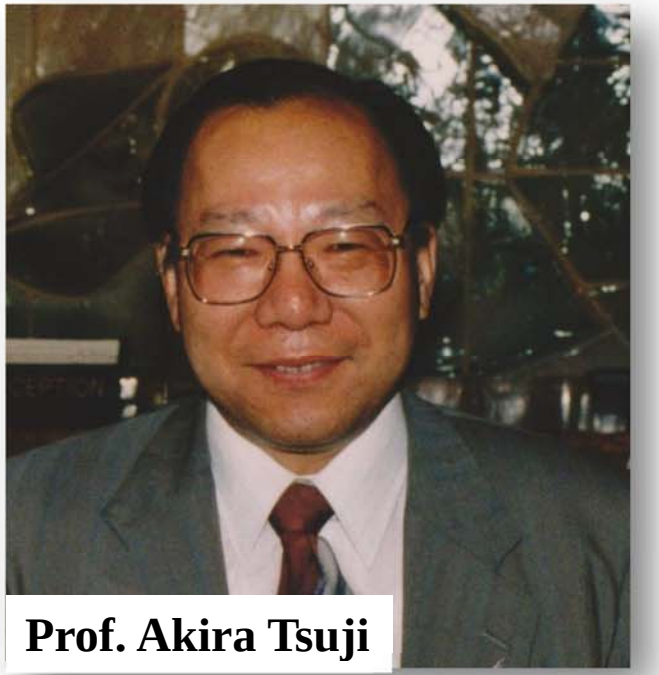
Transporter as an Emerging Target for Cancer Therapy

Takeo Nakanishi, PhD

Department of Membrane Transport and Biopharmaceutics,
School of Pharmaceutical Sciences, Kanazawa University

1. ***Oligopeptide transporter in cancer cells***
2. ***Functional characterization of amino acid transporters***
3. ***Role of BCRP in multi-drug resistance (MDR) of cancer***
4. ***Role of SLC transporter in cancer (OATPs, Amino Acid TP & PGT)***
5. ***Transporter-targeted drug delivery (OATPs, OCT & Urate TP)***
6. ***Development of quantitative analysis for DDIs on transporters (OATPs, MRP2 & BSEP)***

The Three Who Taught Me Transporters



Prof. Akira Tsuji

A/Prof. Ikumi Tamai

As/Prof. Yoshimichi Sai

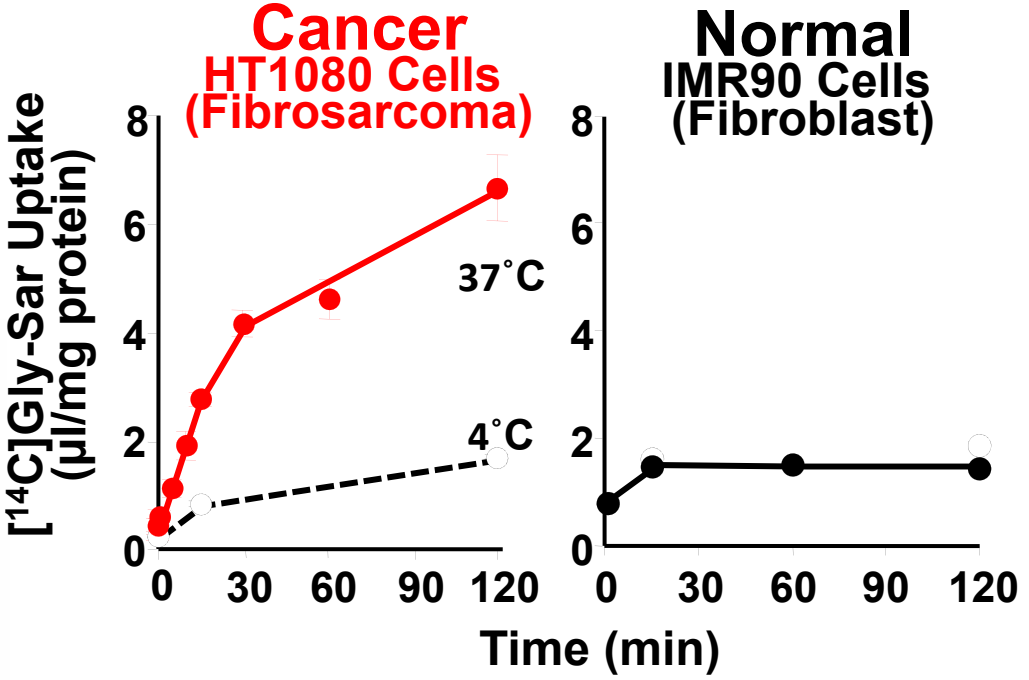
1996 - 1999

[CANCER RESEARCH 57, 4118–4122, September 15, 1997]

Carrier-mediated Transport of Oligopeptides in the Human Fibrosarcoma Cell Line HT1080[1]

Takeo Nakanishi, Ikumi Tamai, Yoshimichi Sai, Takuma Sasaki, and Akira Tsuji[2]

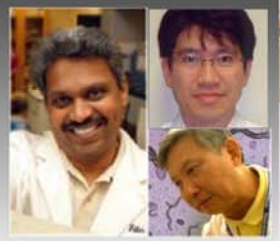
Department of Pharmacobio-dynamics, Faculty of Pharmaceutical Sciences [T. N., I. T., Y. S., A. T.], and Cancer Research Institute [T. S.], Kanazawa University, Kanazawa 920, Japan



1 *Oligopeptide transporter in cancer cells*

MCG
GEORGIA'S HEALTH SCIENCES UNIVERSITY

1999 - 2001

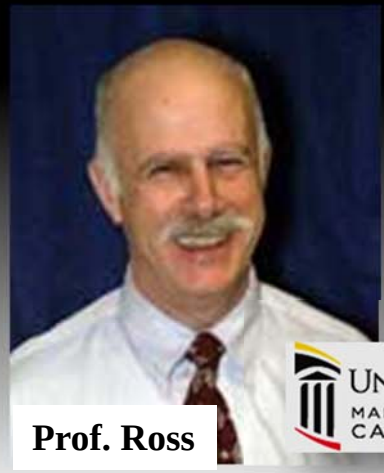


Prof. Ganapathy

St. Jude Children's Research Hospital

Collaborate from 2003

2001 - 2008



Prof. Ross

UNIVERSITY of MARYLAND
MARLENE AND STEWART GREENEBAUM CANCER CENTER

2 *Functional characterization of amino acid transporters*

3 *Role of BCRP in multi-drug resistance in cancer*

4 *Role of SLC transporter in cancer (OATPs, Amino Acid TP & PGT)*

5 *Transporter-targeted drug delivery (OATPs, OCT & Urate TP)*

6 *Development of quantitative analysis for DDIs on transporters (OATPs, MRP2 & BSEP)*

金沢大学

2009 - present

MICHIGAN

Collaborate from 2012

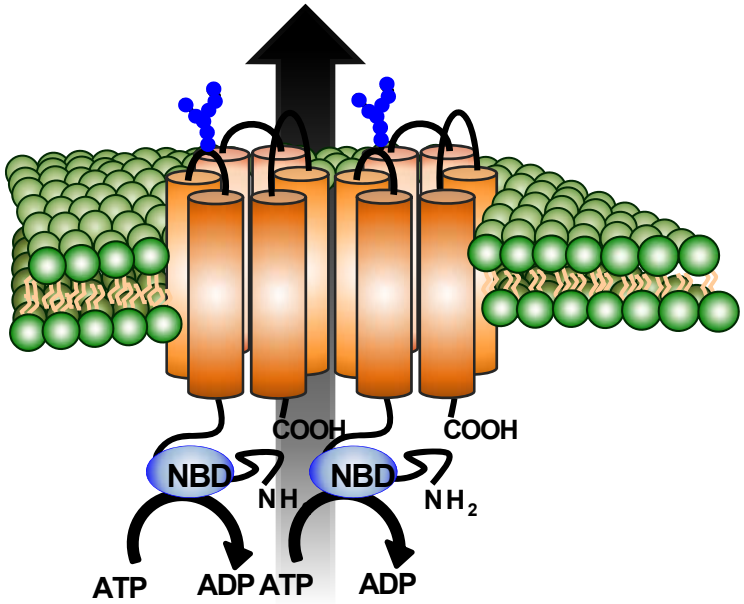
THE UNIVERSITY OF AUCKLAND
NEW ZEALAND
Te Whare Wānanga o Tāmaki Makaurau

Collaborate from 2010

Breast Cancer Resistance Protein BCRP/ABCG2

Specific Inhibitors

- **FTC, Ko143, Elacridar**



Substrates

- **Natural Substrates**
- **Xenobiotics**
- **Chemotherapeutics**

In Normal Cells

- **Liver and gut; Limits absorption and enhances elimination of drugs**
- **BBB and Testis; Limits penetration of drug into brain or testicular tumors**
- **Normal stem cells; Protect stem cells from damages by drugs**

Pharmacokinetics

Poor Cancer Treatment Outcomes

Pharmacology

In Cancer Cells

- **Limits attainment of therapeutic intracellular concentration of drugs (Multi-Drug Resistance)**
- **Effluxes drugs from cancer stem cells**

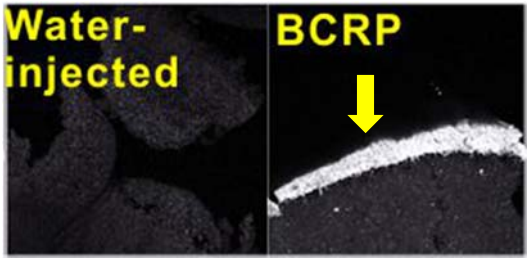
Reviewed in *Chin J Cancer*, 2012 and Natarajan et al *Biochemical Pharmacol, 2012*

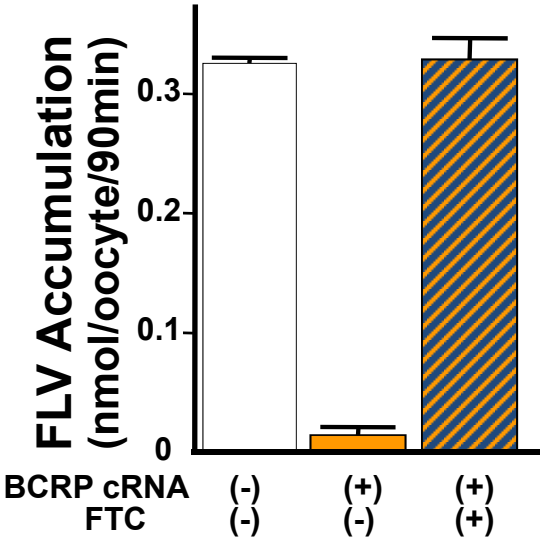
BCRP Causes Resistance to Small Molecule Kinase Inhibitors Used in Targeted Therapy

CDK Inhibitor for CLL

Alvocidib (Flavopiridol)

Xenopus L. Oocytes



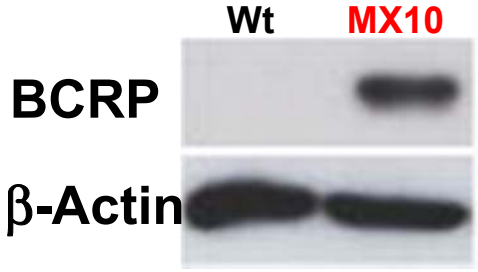


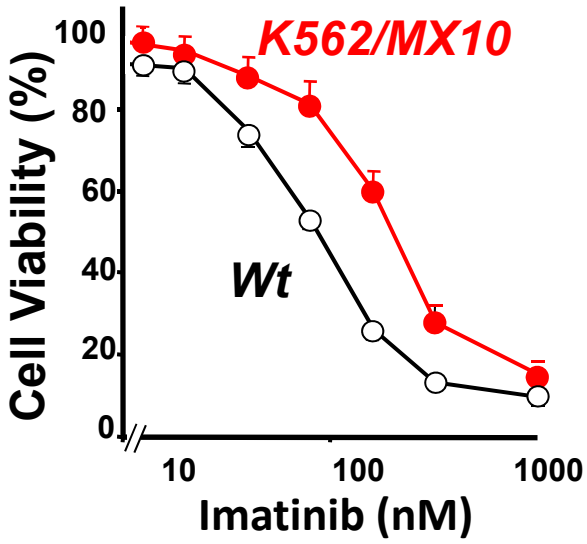
Mol Pharmacol, 2003
Clin Cancer Res, 2003

BCR-ABL Inhibitor for CML

Imatinib

BCR-ABL +ive K562 cells



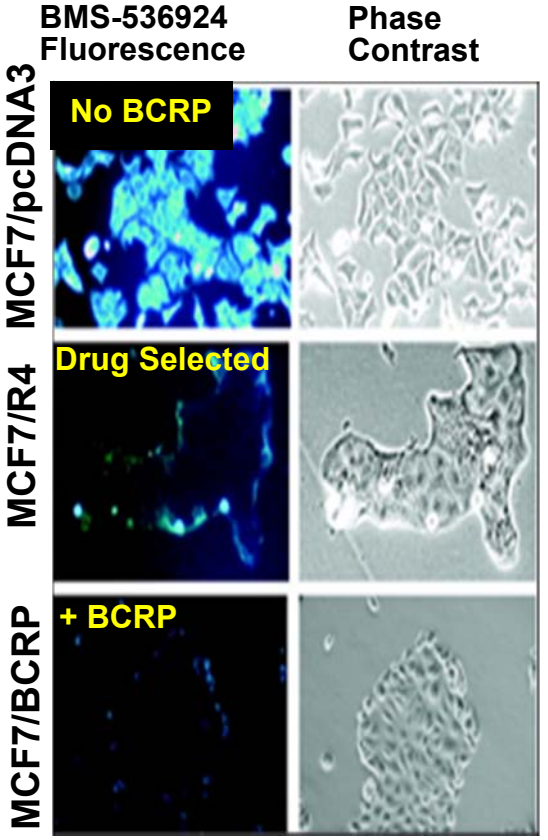


Blood, 2006

IGFR1 Inhibitor

BMS-536924

Drug Resistant MCF7 cells

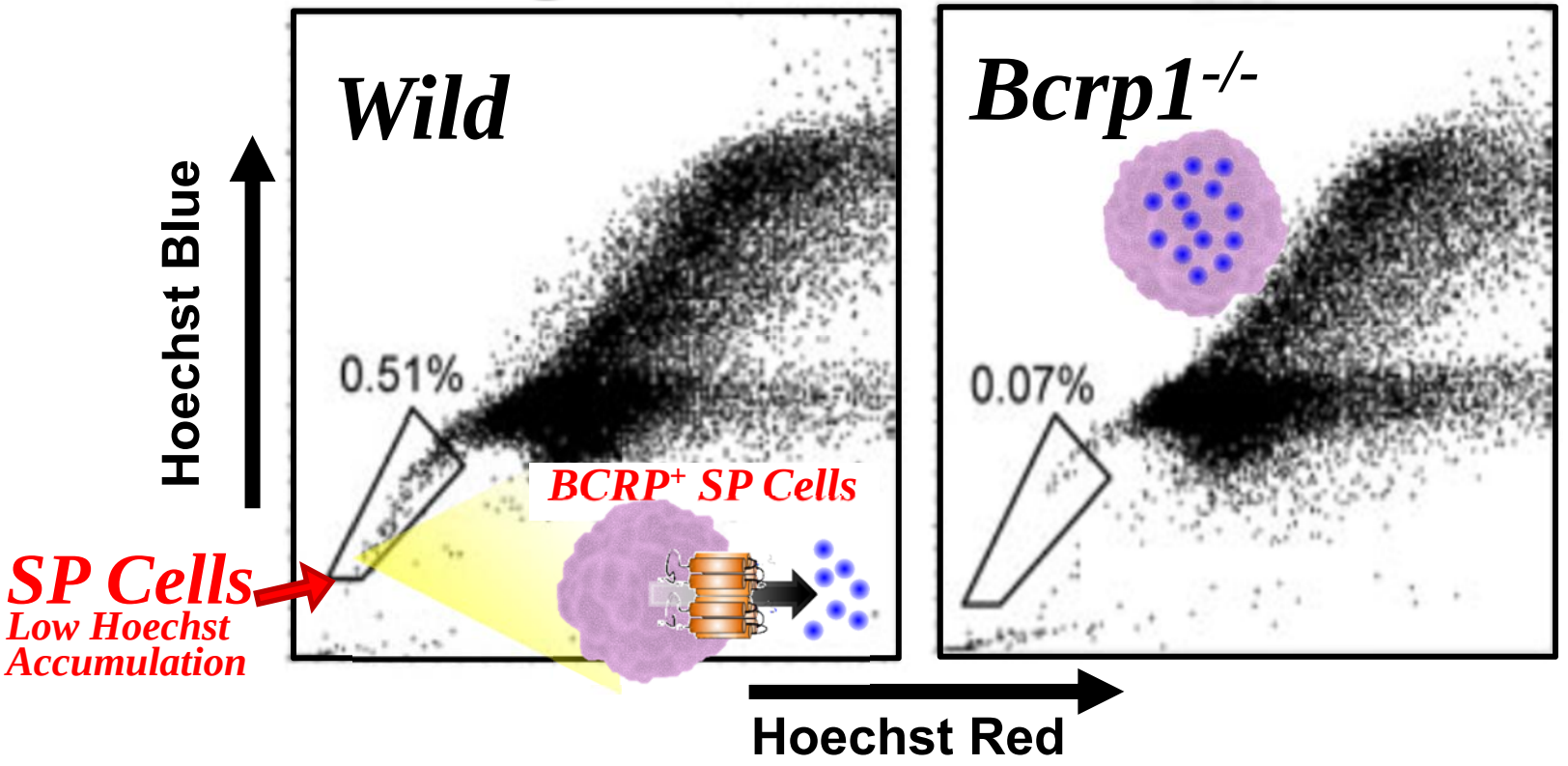


With Mayo Clinic
Mol Cancer Ther, 2011

TKI; Tyrosine kinase inhibitors, CDK; Cyclin dependent kinase, FLV; Flavopiridol, CLL; Chronic lymphocytic leukemia, CML; Chronic myelogenous leukemia, IGFR; Insulin-like growth factor 1 (IGF-1) receptor.

BCRP is a Universal Marker for Side Population (SP) Stem Cells in Normal Tissues and Malignant Tumors

FACS Analysis of Mouse Bone Marrow Cells

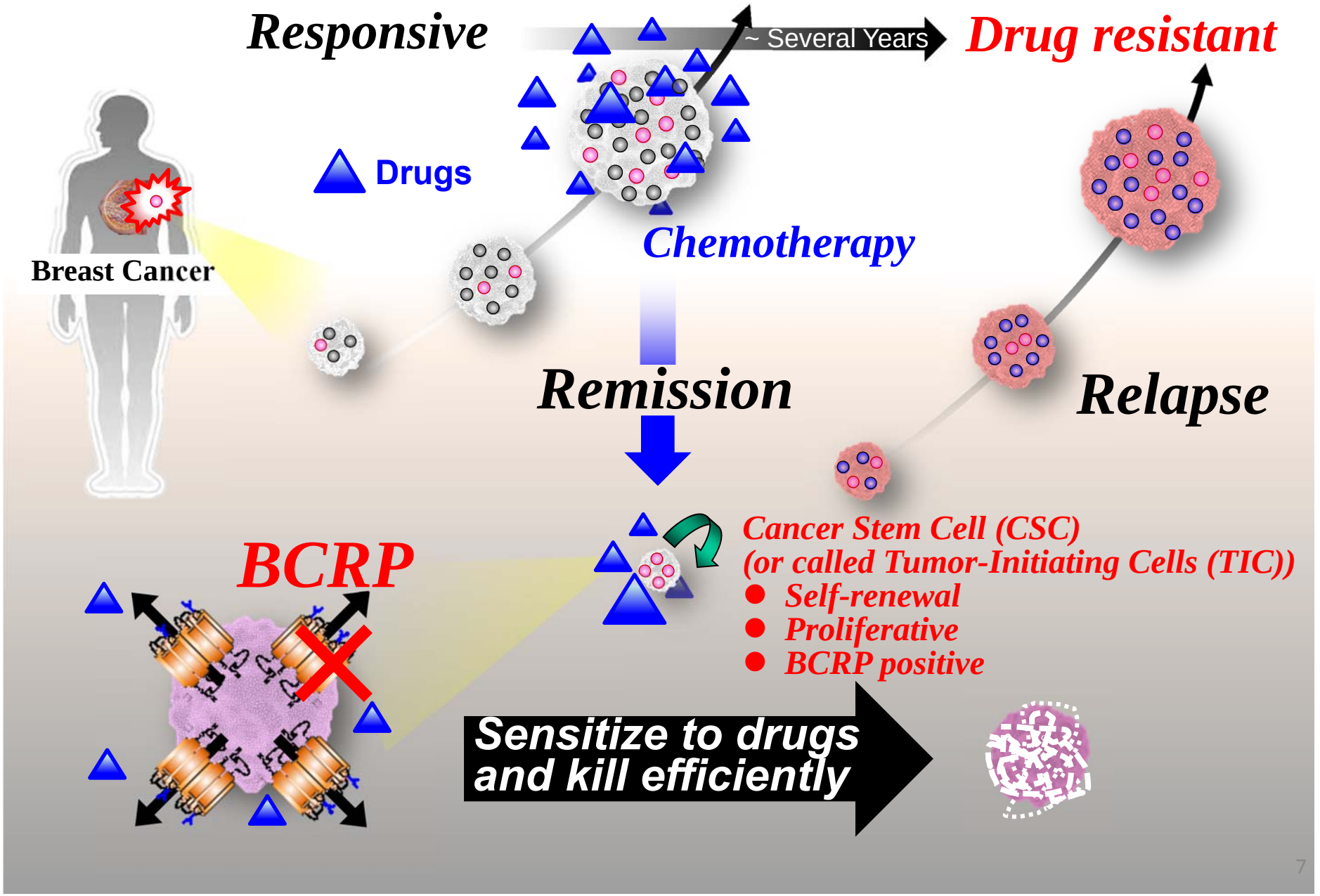


- *Shields stem cells from the lethal effect of PPIX generated in hypoxia*
- *Protects cancer stem cells from damages by chemotherapeutic drugs*

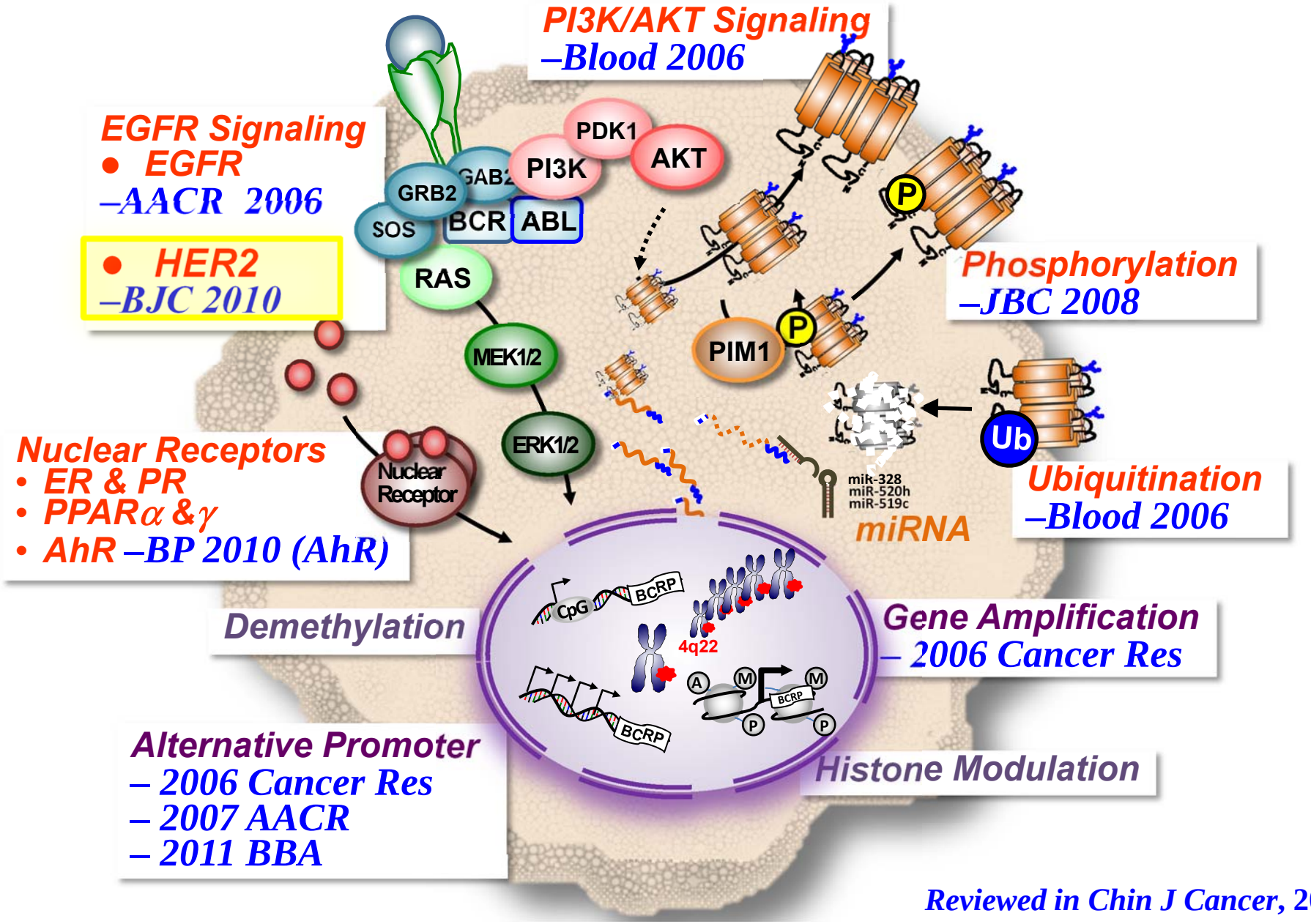
FACS; Florescence activation cell sorting, Hoechst 33342; DNA-binding dye and substrate of BCRP

With Dr. Schuetz (St Jude Children's Research Hospital) in *J Biol Chem*, 2003

BCRP May Protect Cancer Stem Cells



Known Mechanisms of Regulation of *BCRP* Gene Expression



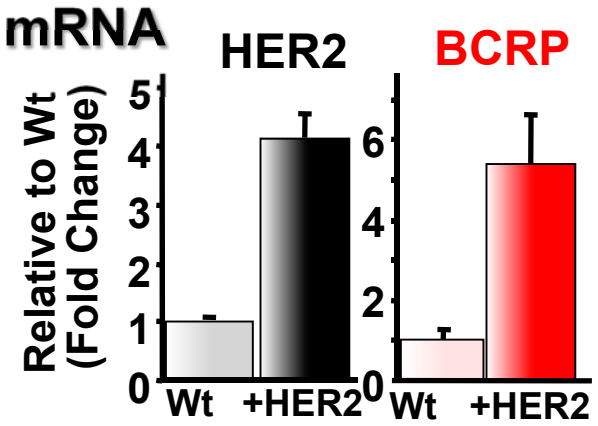
Reviewed in Chin J Cancer, 2012

HER2 Enhances BCRP in Breast Cancer (BC)

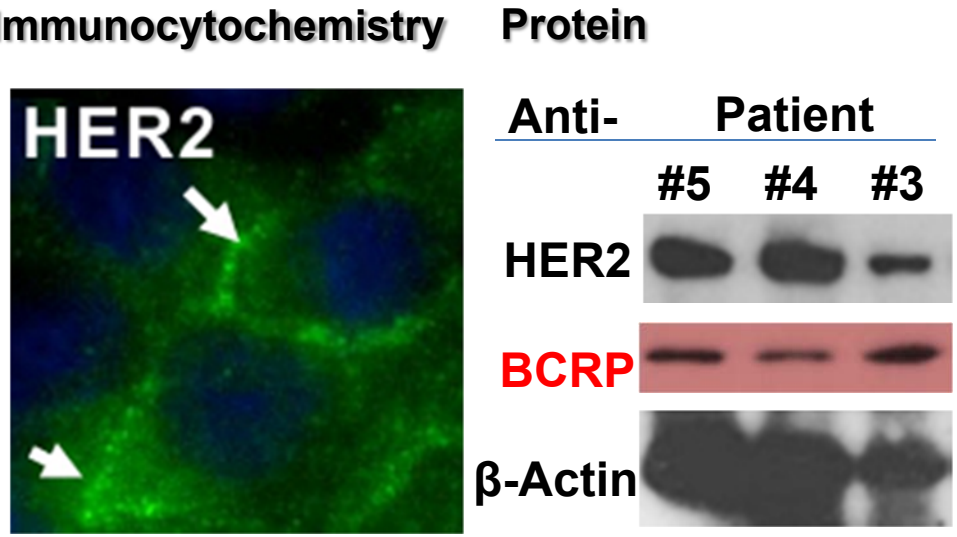
HER2 is

- An oncogenic RTK
- Overexpressed in 30% of BC patients
- Associated with
  - high proliferation,
  - high rate of metastasis (brain, lung)
  - resistance to chemotherapy

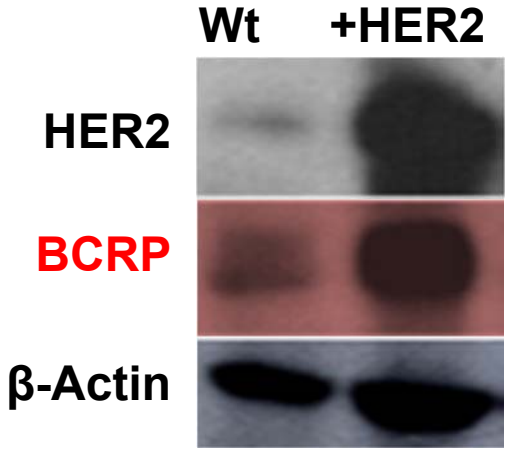
MCF-7 Cells



Patient-derived Breast Cancer



Protein

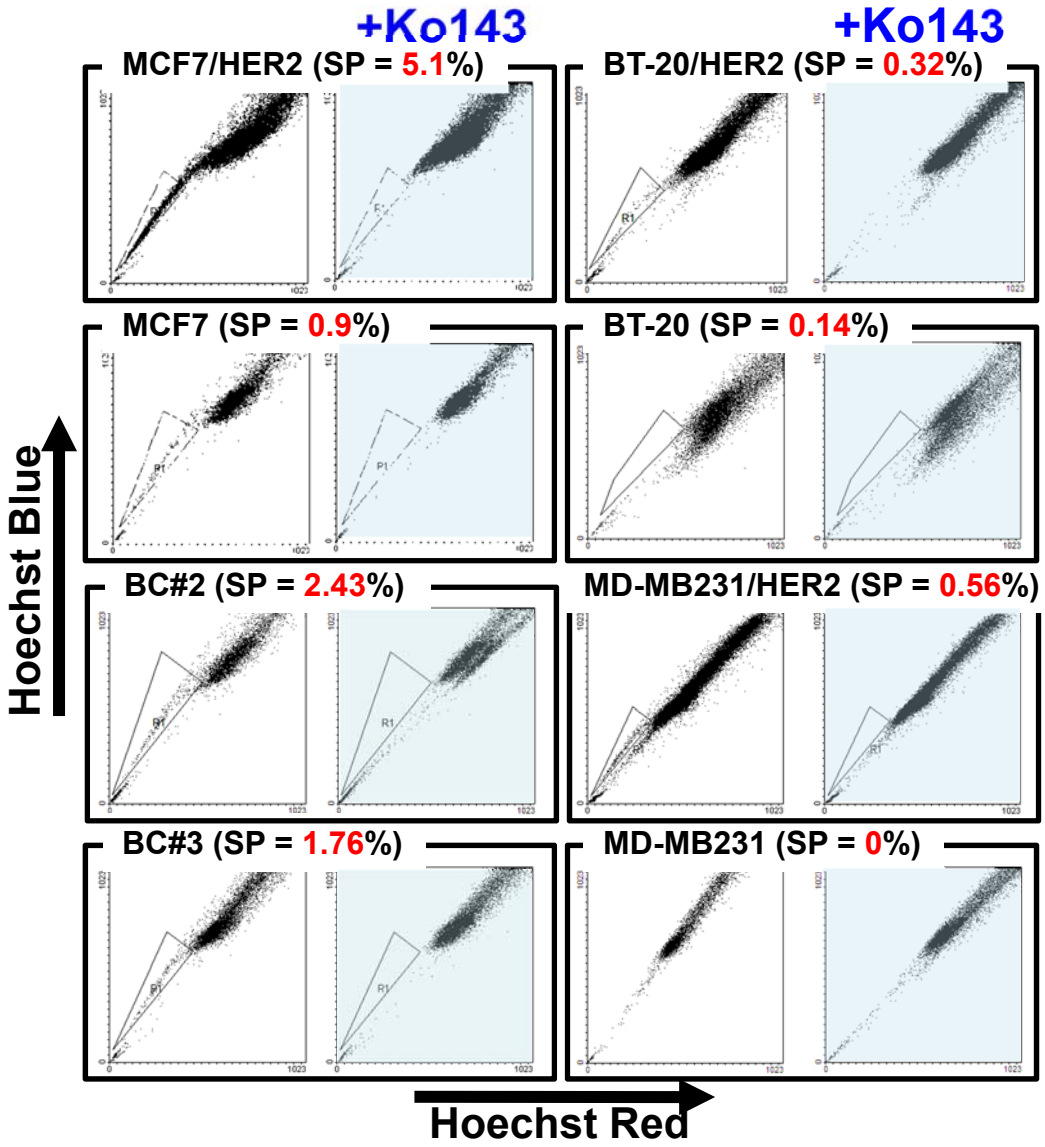


RTK; Receptor tyrosine kinase

Br J Cancer, 2010

Correlation of HER2 Expression and SP Cells in Human Breast Cancer

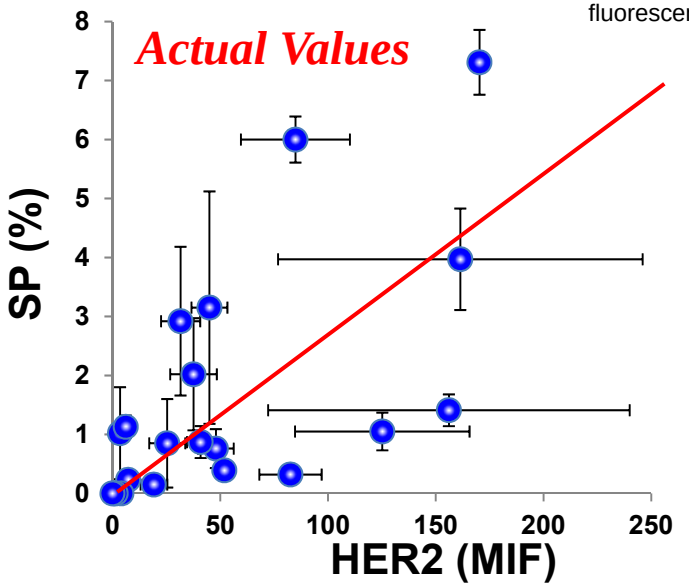
SP Cells in Breast Cancer

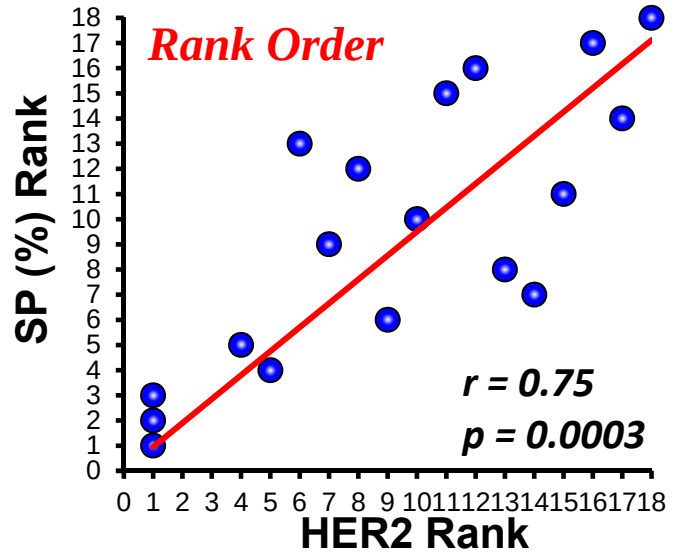


Hoechst33342; 5 μg/ml, Ko143 (specific inhibitor of BCRP); 1 μM

HER2 and SP Correlation

MIF:
Mean intensity of fluorescence

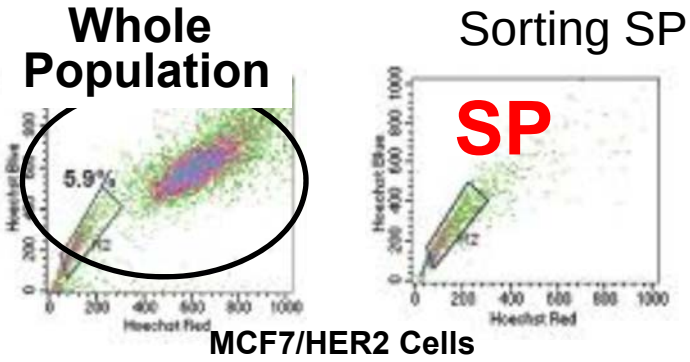


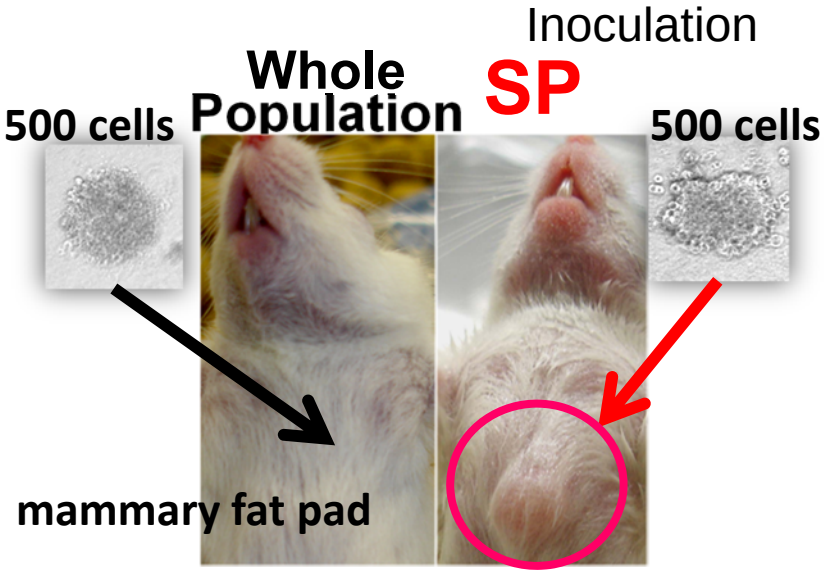


Br J Cancer, 2010

Inhibiting HER2 Diminishes Tumorigenicity of SP Cells in NOD/SCID Mice

Inoculation of SP





Repopulation of SP in NOD/SCID Mice

SP Cell Type	SP Cells Injected	Control	+AG825	+Herceptin
		Tumors/Injections		
MCF7/HER2	100	11/14	0/7	0/5
	500	10/13	0/9	0/8
	1000	8/8	0/2	2/3
BC (Patient #4)	100	4/4	1/4	Not tested
	500	4/4	0/4	Not tested
Total		37/43	1/26	2/16

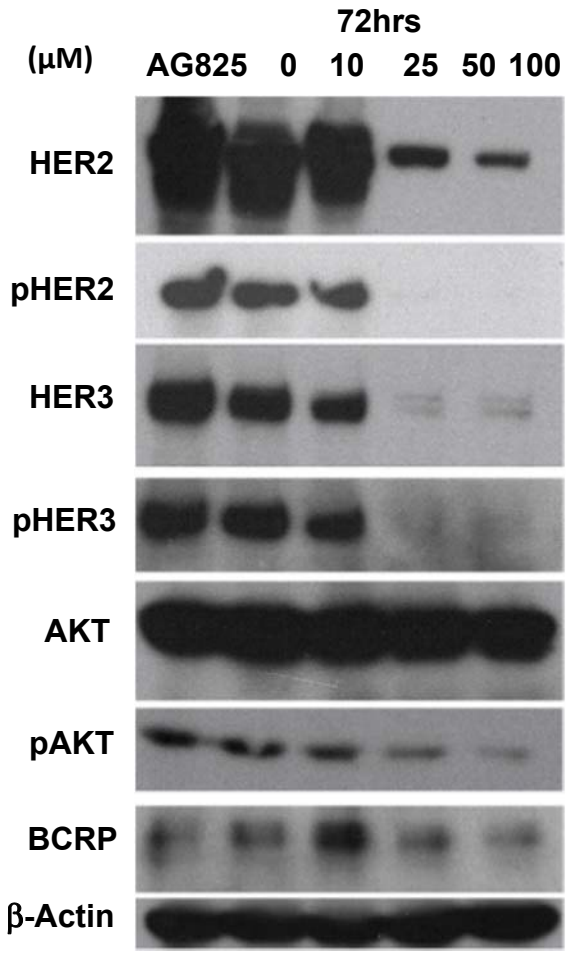
AG825; HER2-specific inhibitor

Sorted cells (100-1000) were Suspended with BD Matrigel™, incubated with either AG825 (100μM) or Trastuzumab (160μg/mL) for 2 hours before injection. Trastuzumab (160ug/mL) was injected into mice every other day during experiments. Tumors were observed eight to ten weeks.

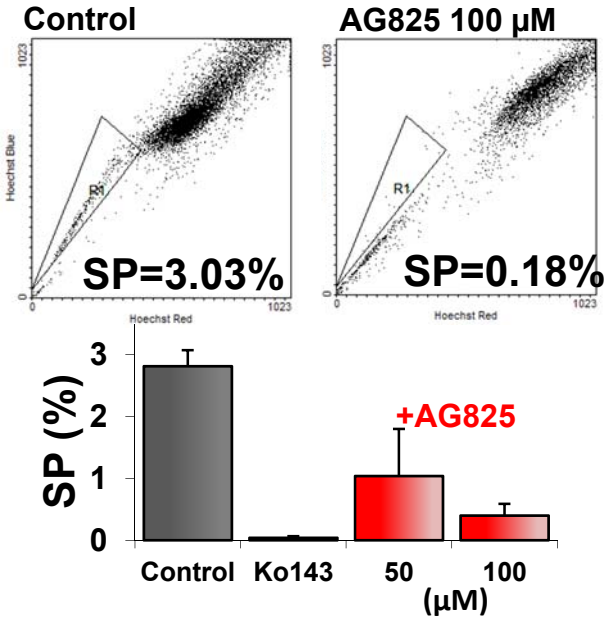
Br J Cancer, 2010

HER2/HER3 Signaling Plays a Role In Expansion of SP Cells in Breast Cancer Cells

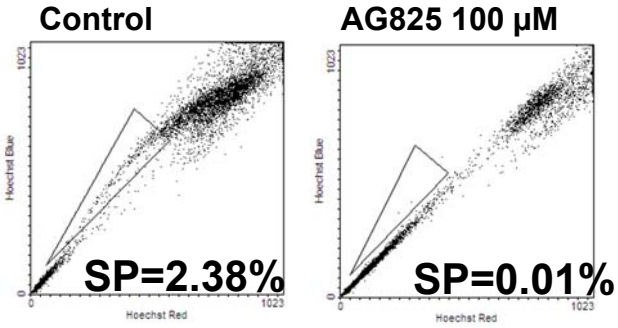
Western Blot – MCF7/HER2 Cells

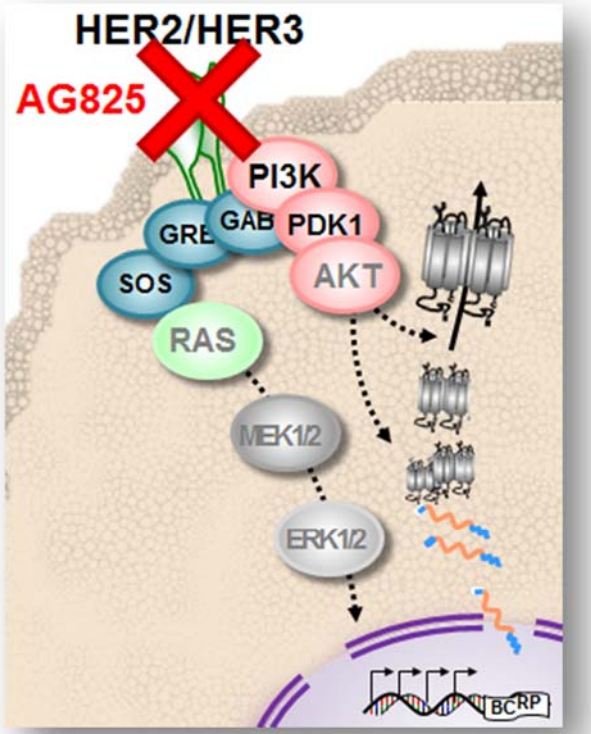


MCF7/HER2 Cells (72 hr)



Patient BC#4 Cells (72 hr)



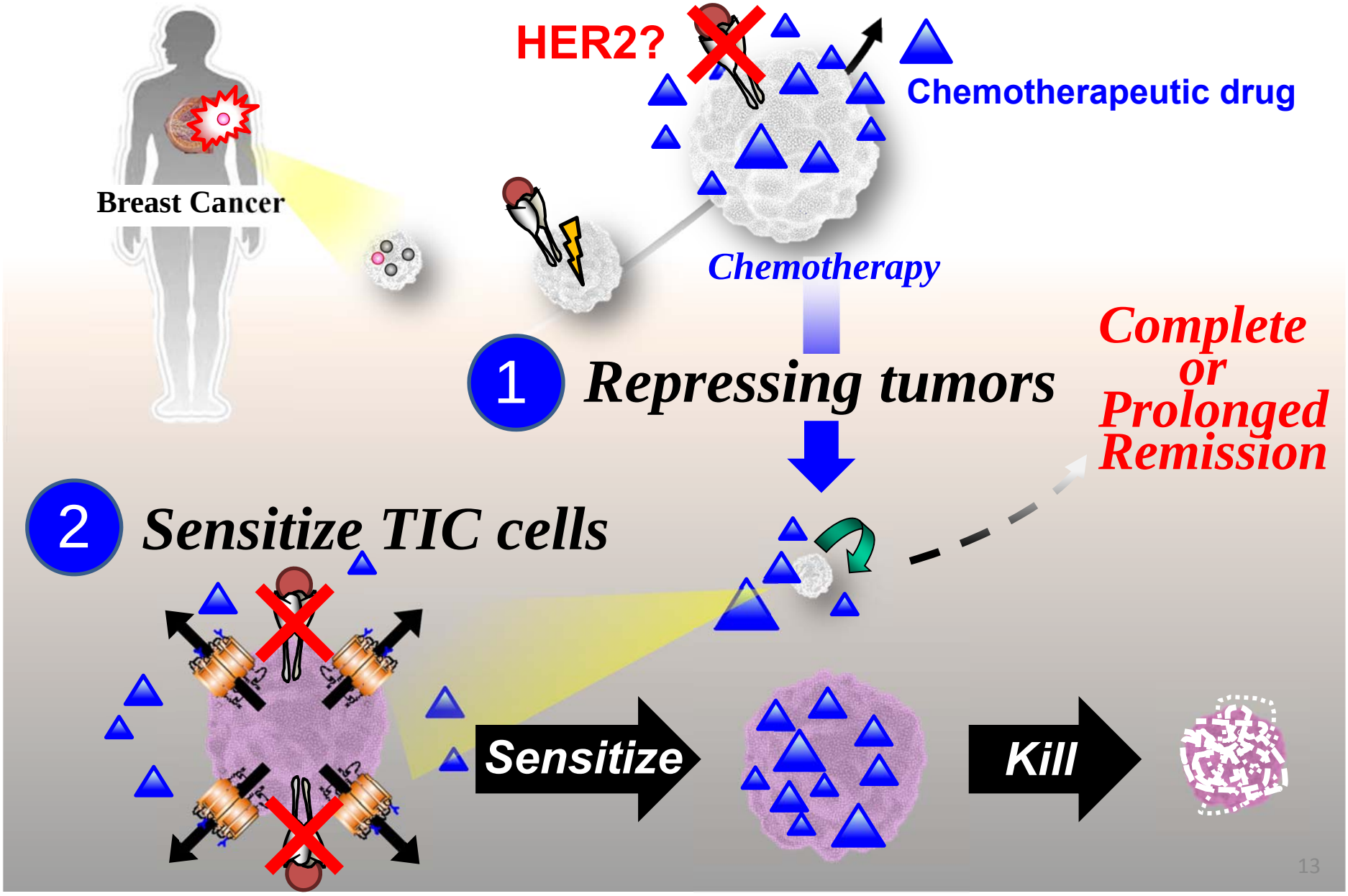


Shutting off HER2 oncogenic network is also effective to remove BCRP expression from TIC cells.

AG825; HER2-specific inhibitor

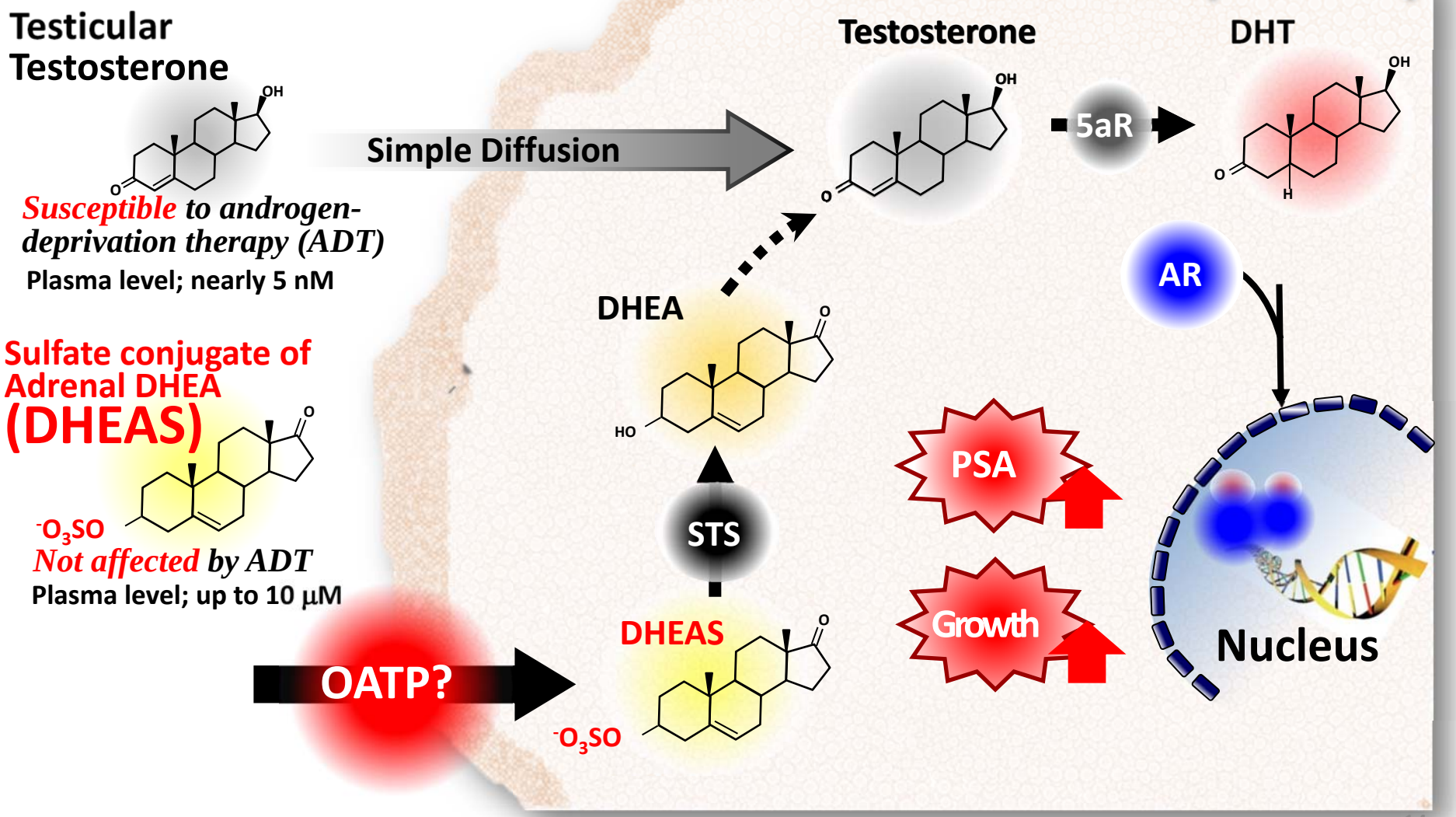
Br J Cancer, 2010

Blocking HER2 Is an Ideal Strategy to Kill Two Birds with One Stone



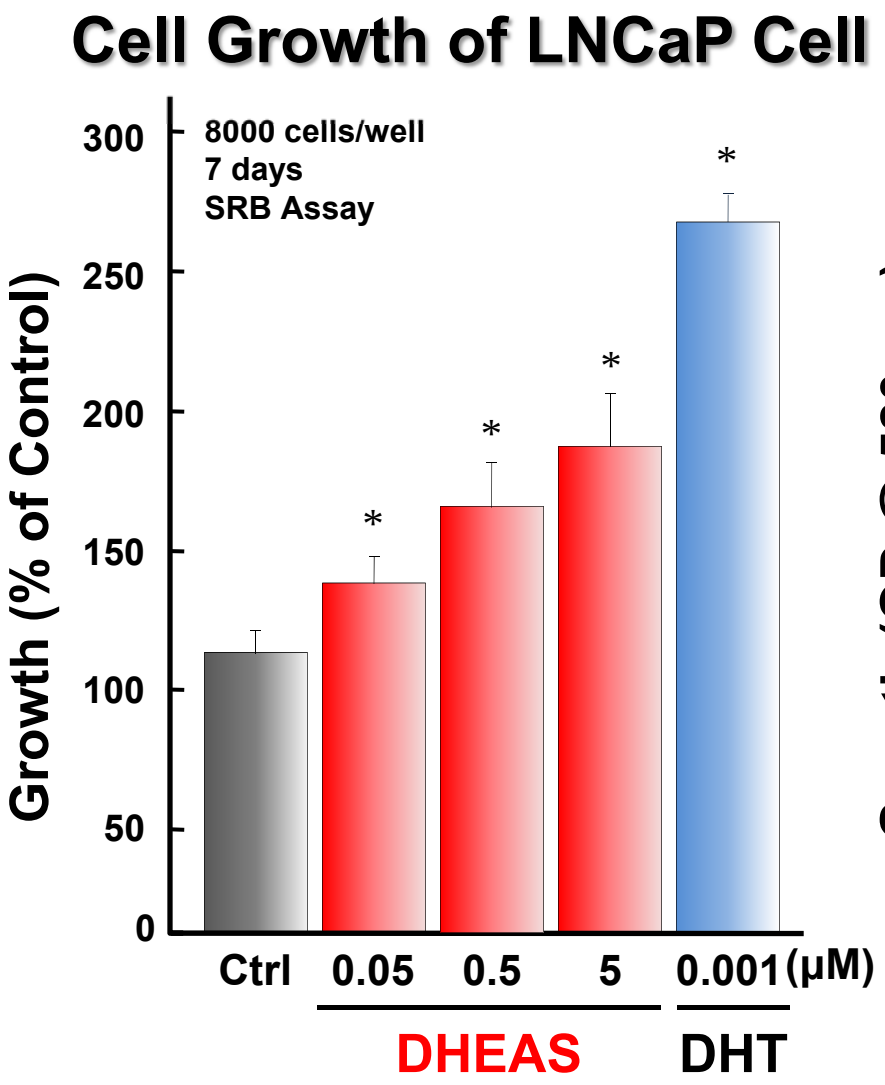
A Hypothesized Role of OATPs in Survival of Prostate Cancer Cells through Androgen-deprivation Therapy (ADT)

Castration Resistant Prostate Cancer (CRPC)

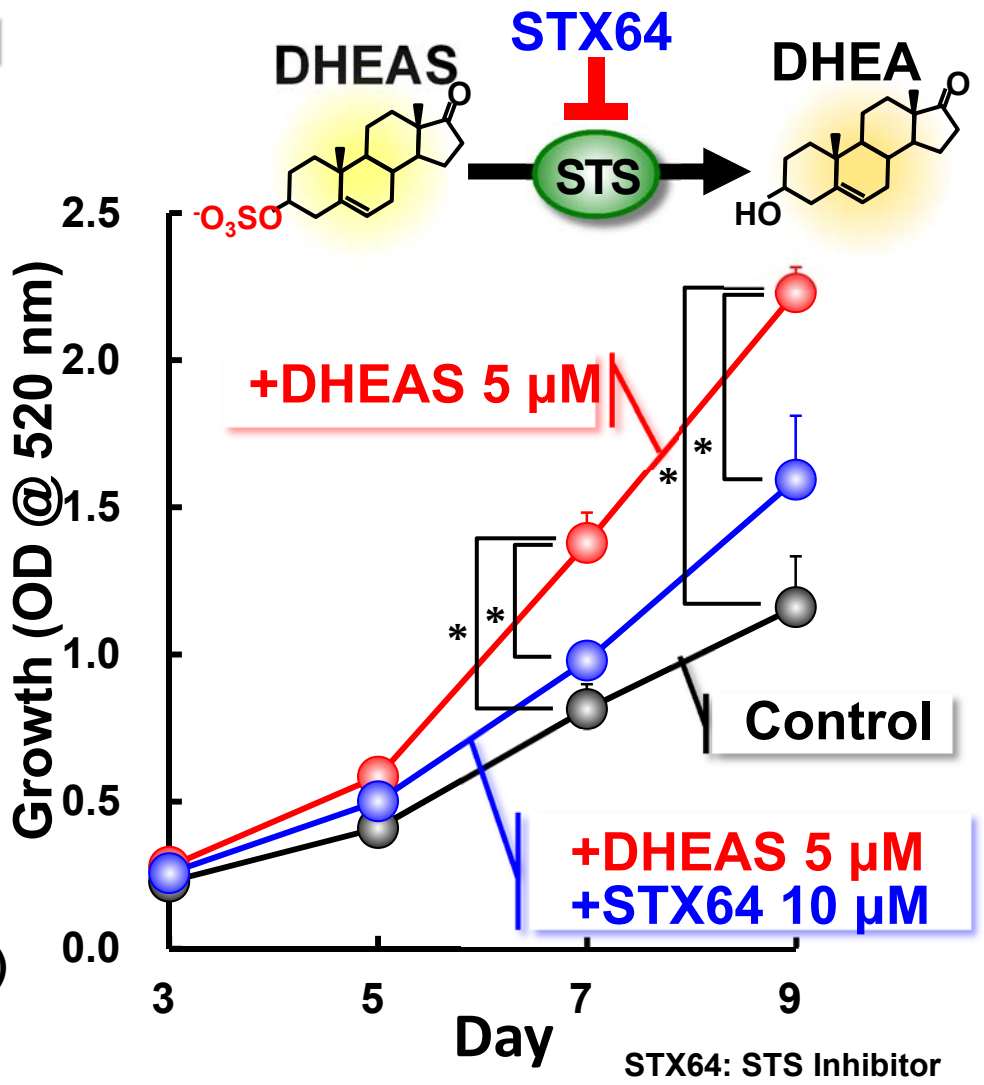


DHEA; Dehydroepiandrosterone, OATP; Organic anion transporting polypeptide,
AR; Androgen receptor, STS: Steroid sulfatase, 5αR: 5α-Reductase, PSA; Prostate specific antigen.

Stimulation Effect of DHEAS on Cell Growth of AR-positive Prostate Cancer LNCaP Cells



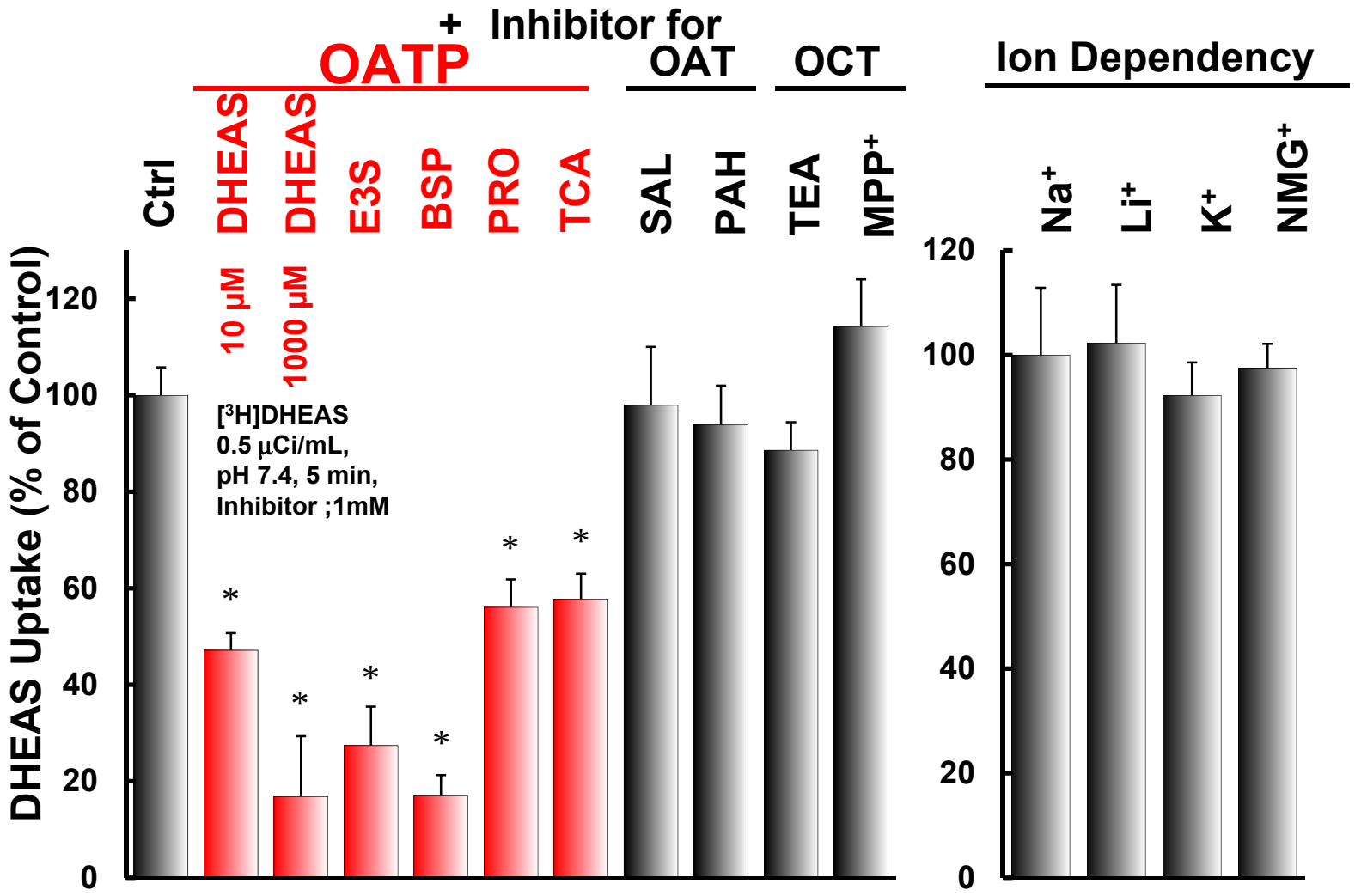
Mean ± S.E.M. (n = 6), * : *p<0.05* (vs. Ctrl)



Mean ± S.E.M. (n = 6), * : *p<0.05*

Biochem Pharmacol, 2012

Characterization of DHEAS Transport into LNCaP Cells



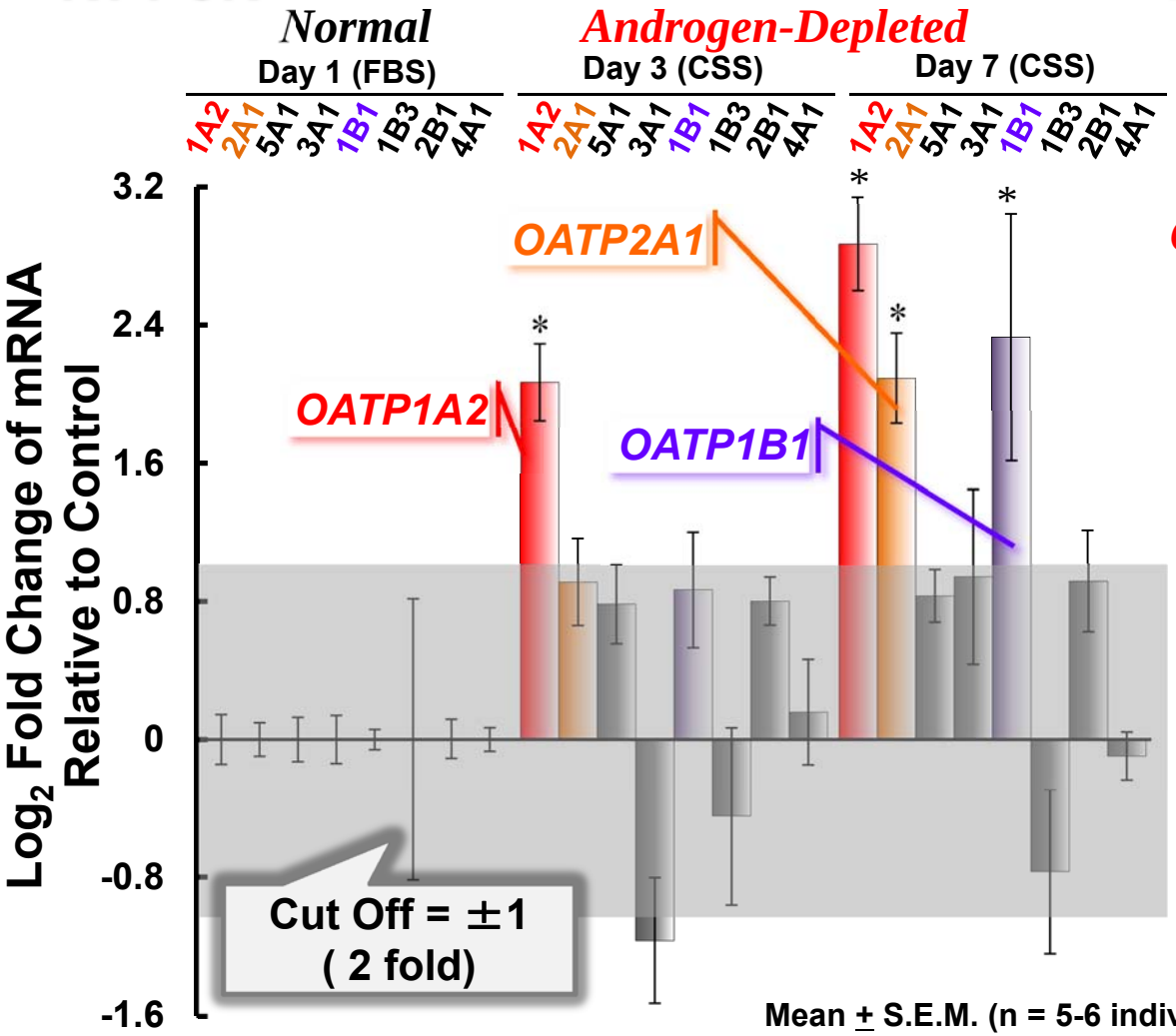
E3S; Estrone-3-sulfate, TCA; Taurocholate, PRO; Probenecid, SAL; Salicylate, PAH; *p*-Amminohippuric acid, TEA; Tetraethylammonium, MPP^+; 1-methyl-4-phenylpyridinium

Mean ± S.E.M. (n = 3), * : *p<0.05 (vs. Ctrl)*

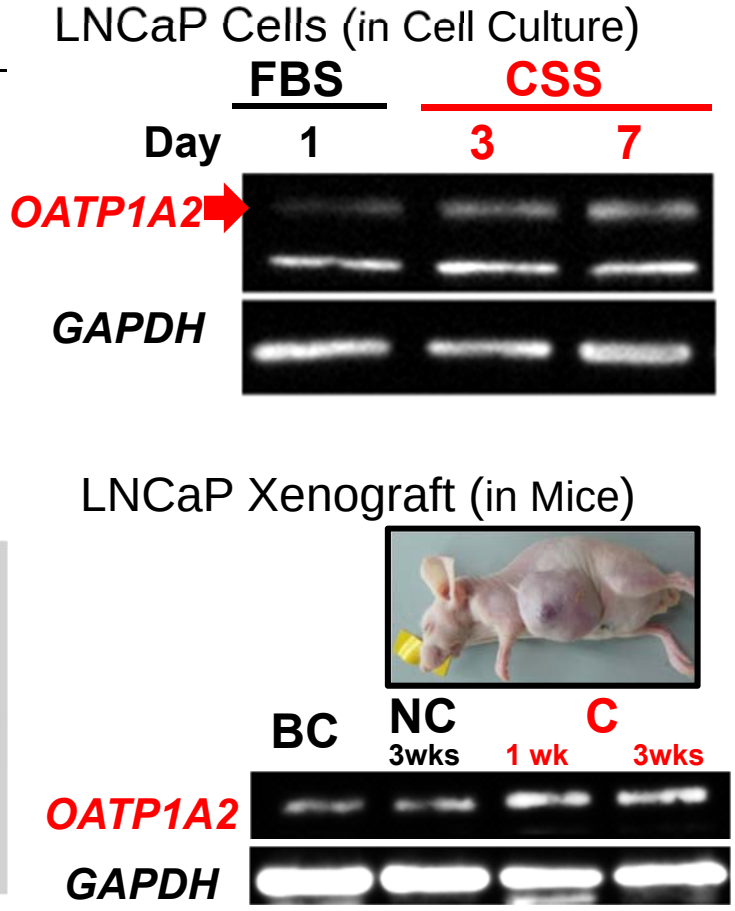
Biochem Pharmacol, 2012

Enhanced Expression of OATPs in LNCaP Cells under Androgen-Depletion

RT-PCR



Western Blotting

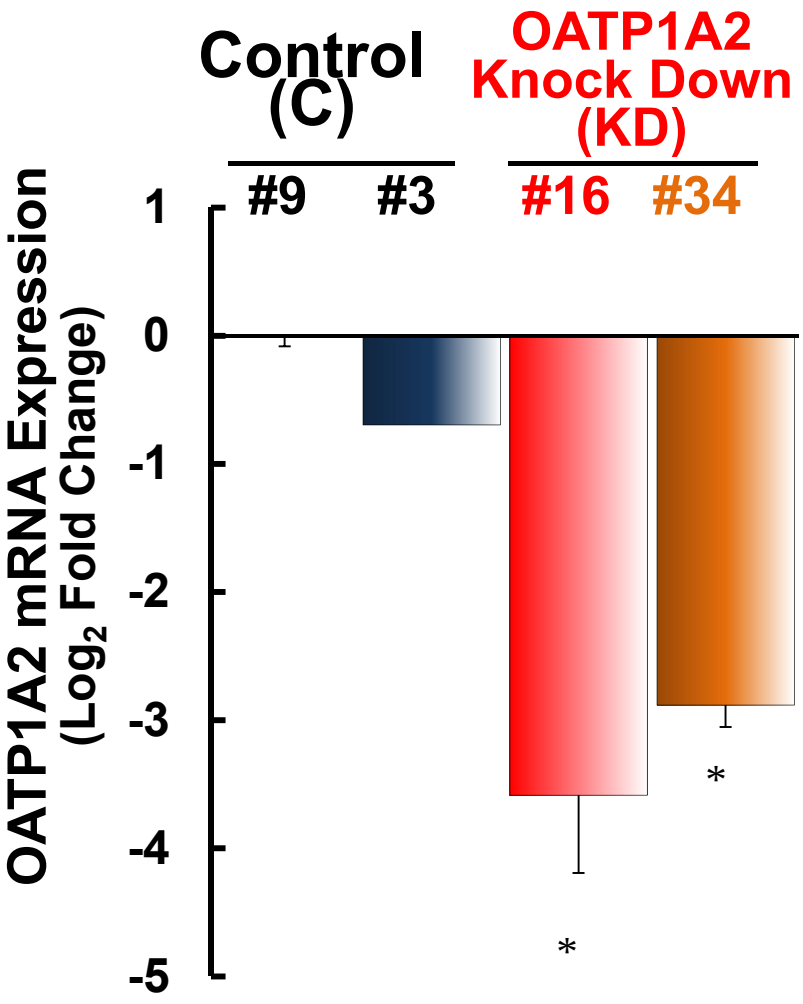


Mean ± S.E.M. (n = 5-6 individual PCR results), * : *p<0.05* (vs. Day1)
BC; Before castration, NC; Non-castration, C; Castration
CSS; Charcoal-stripped serum.

Biochem Pharmacol, 2012

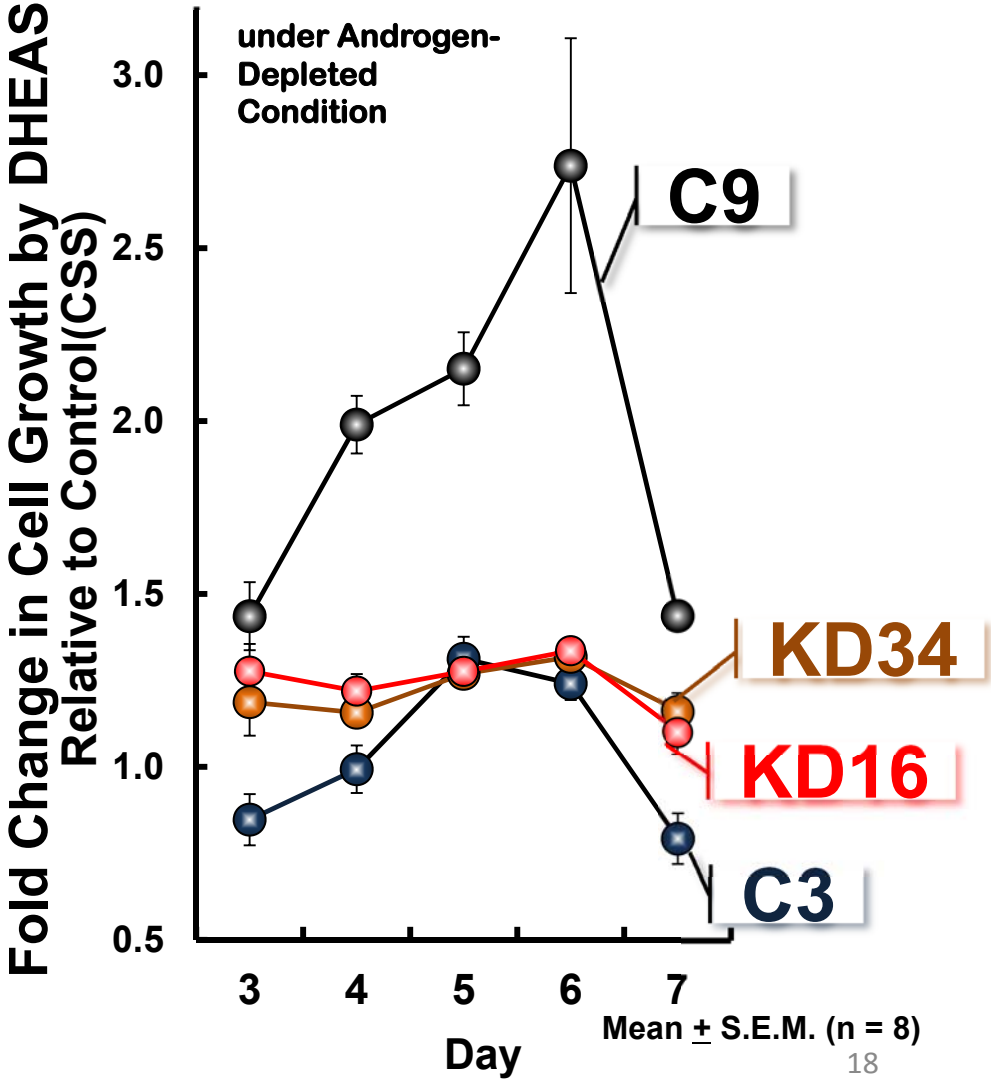
Knocking-down of OATP1A2 Diminishes Stimulation Effect of DHEAS in LNCaP Cells

RT-PCR



Mean ± S.E.M. (n = 3) * : *p<0.05*

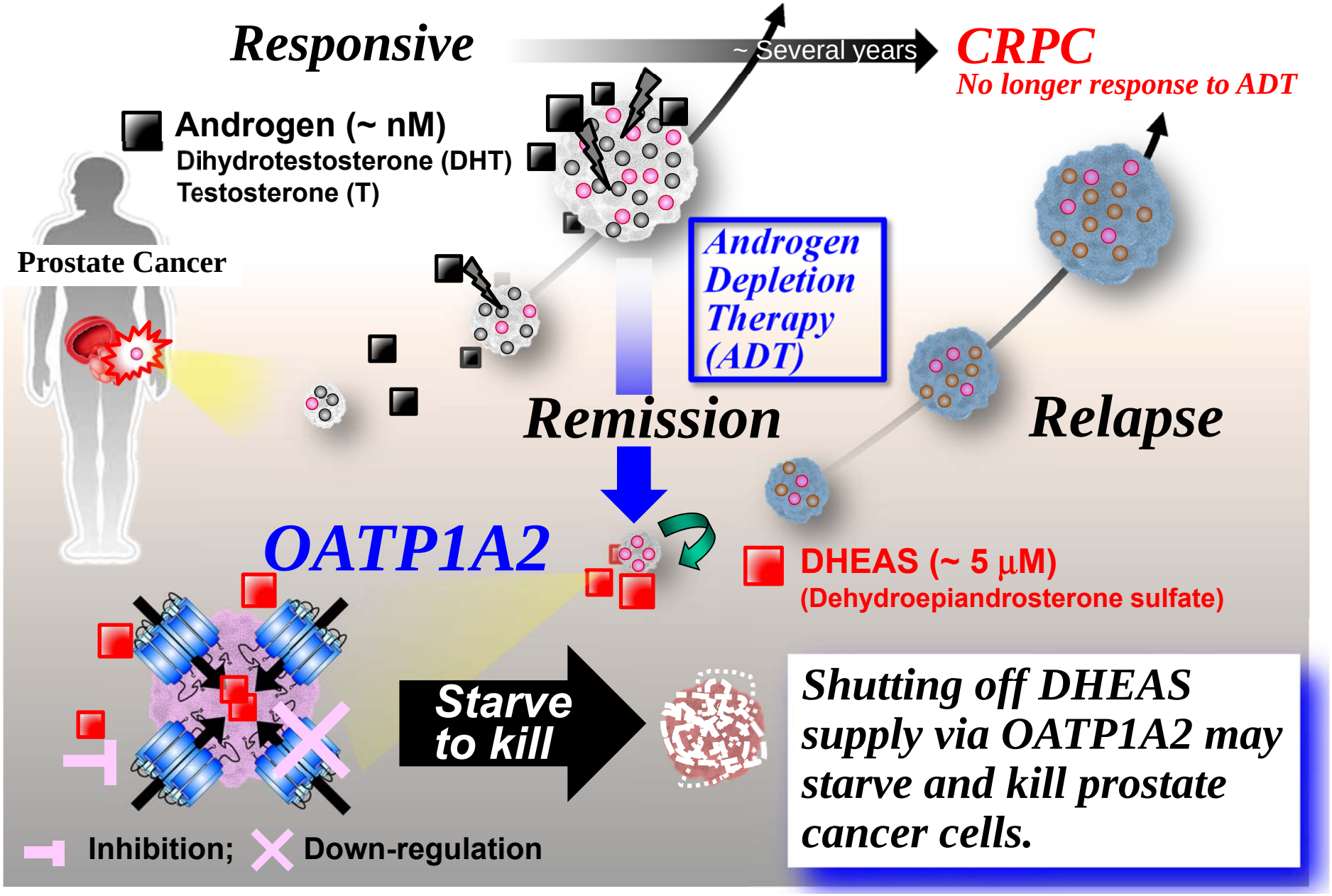
Stimulation Effect of DHEAS



Mean ± S.E.M. (n = 8)

Biochem Pharmacol, 2012

DHEAS Transporter OATPs May Play an Essential Role in Progression of CRPC



To Make Transporter-targeted Cancer Therapy Successful…..

From BCRP Study in Breast Cancer

- **Strategy to "kill two birds with one stone"**

  Find an oncogenic growth signal which regulates gene expression of MDR transporter as well, it may serve as a target to overcome a transporter-based MDR.

From OATP Study in Prostate Cancer

- **Strategy to "shut off supply for survival of damaged cells by chemotherapy"**

  Identify a specific transporter that tumor relies on for substance/nutrients to escape and survive chemotherapy, and it may serve as a target to starve and kill cancer cells.

Again, THANK YOU