Global Breast Cancer Conference 2011

Individualization for Therapeutic Management of Luminal Subtype Breast Cancer

Shin-ichi Hayashi

Tohoku University
Graduate School of Medicine

TOHOKU
UNIVERSITY

Individualization of Breast Cancer and Transition of Predictive and Prognostic Factors

Traditional risk estimation of relapse by clinicopathological factors

Biological characteristics of tumors

ER, PgR, Her2, Ki67 (Intrinsic subtype)
Oncotype DX, Mammaprint (IVDMIA)

In Vitro Diagnostic Multivariate Index Assay

Judgements must be made in the care of individual patients of whether to use or withhold each treatment modality. (St. Gallen 2009)

Understanding the molecular biological background of breast cancer

Present Classification for Eligibility of Therapy in Breast Cancer

- HER2-positive (HER2 type)

  Anti-HER2 + Chemotherapy

- Triple negative (Basal-like) (Sub-classification should be needed)

  Chemotherapy

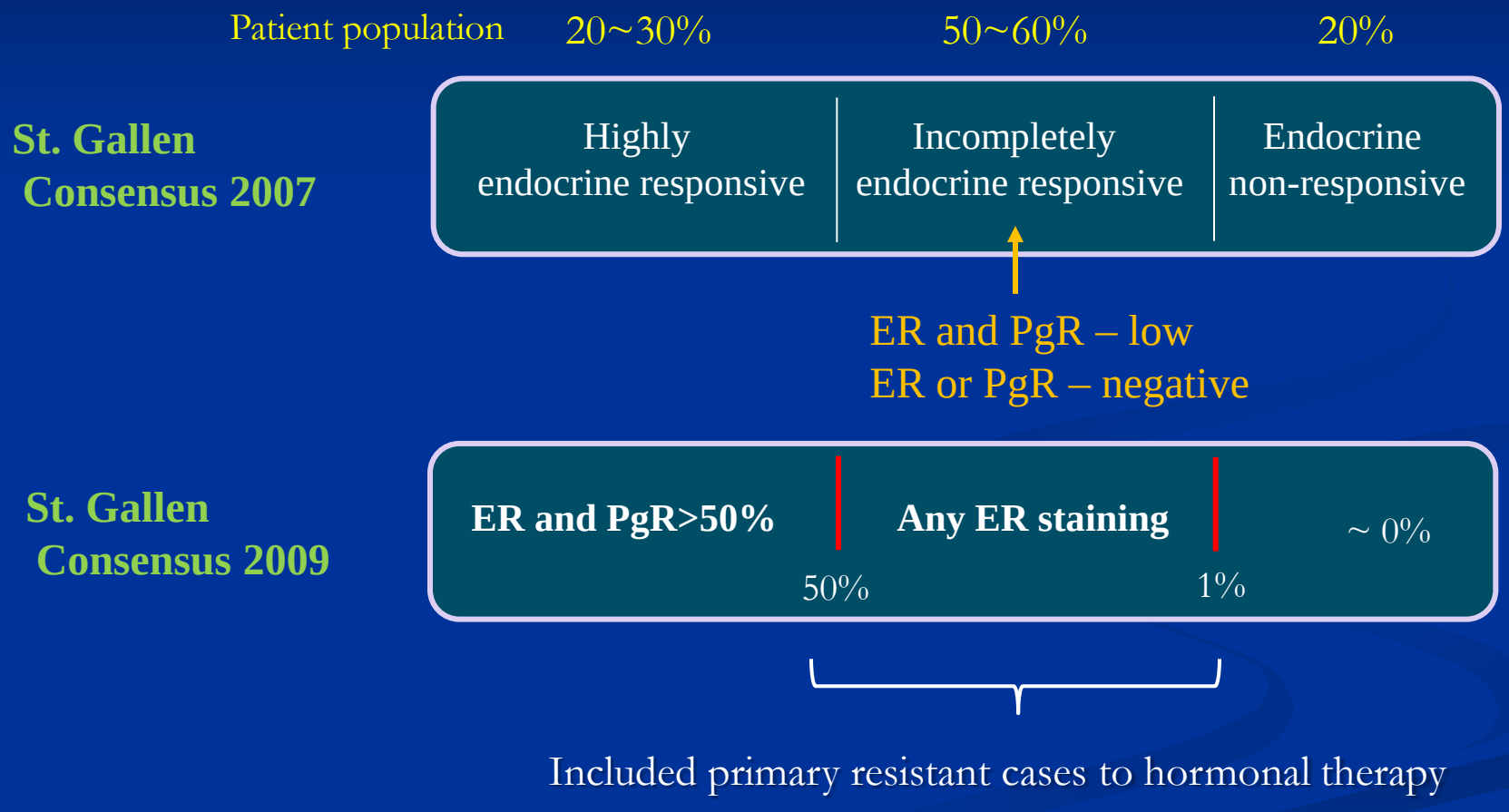
- HR-positive (Luminal type)

  Hormonal therapy alone or Chemothrapy + Hormonal therapy

  (Luminal A) (Luminal B)

  Classified by the expression of ER, PgR, Her2, Ki-67, clinicopathological factors, Oncotype DX

Classification of Hormonal Response



Estrogen Responsive DNA Microarray

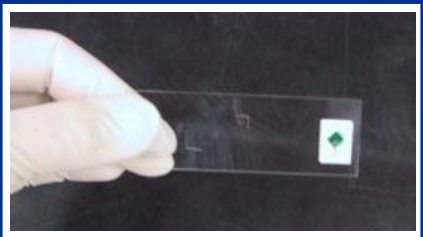
Comprehensive DNA microarray analysis of 10000 genes

↓

Identified 200 estrogen regulated genes

J Mol Endocrinol 29: 175 (2002)

Custom-made estrogen responsive cDNA microarray



Basic studies using this microarray

Oncogene 22: 5011 (2003)
Cancer Sci 95: 496 (2004)
J Mol Endocrinol 32: 649 (2004)
Biomed Pharmacotherapy 58: 1 (2004)

↓

Clinical application for diagnosis of hormone-dependent cancer

Screening of novel prognostic factors

Clin Cancer Res 10: 1962 (2004)
Oncogene 24: 4531 (2005)
Endocrine-Related Cancer, 14: 279 (2007)

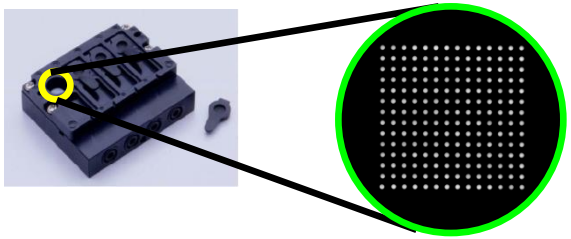
HDAC6、EGR3、IGFBP4

New focused array

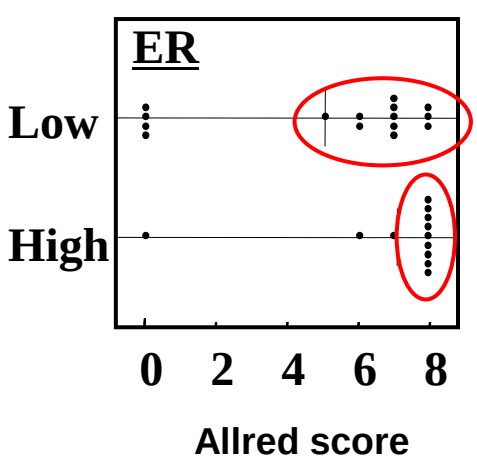
3D-microarray

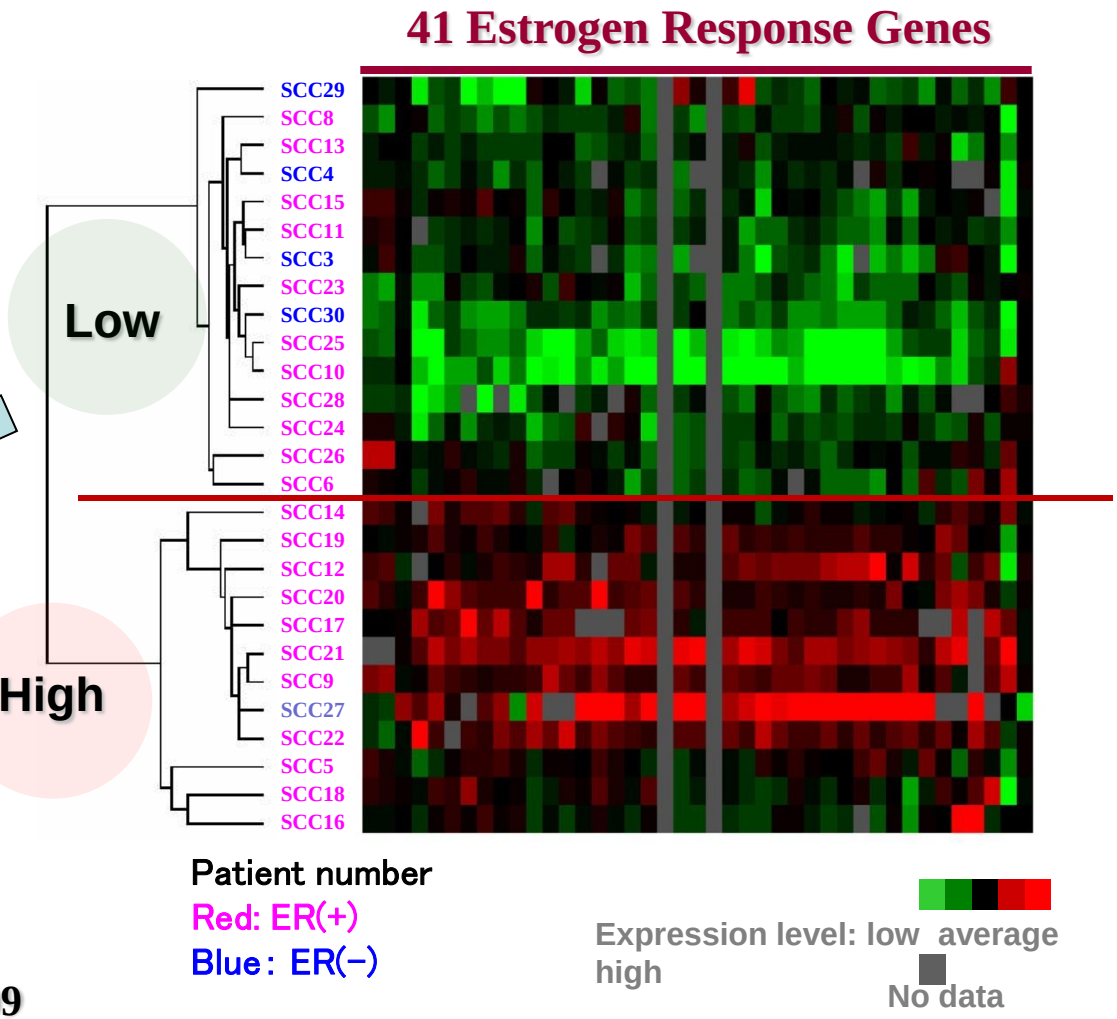
- Prediction of hormone therapy
- Classification of patients for individualized therapy
- Monitoring of hormone therapy

Expression Profile of Estrogen Responsive Genes in Primary Breast Cancer Analyzed by Focused DNA Microarray



Analyzed by estrogen-response 3D-microarray



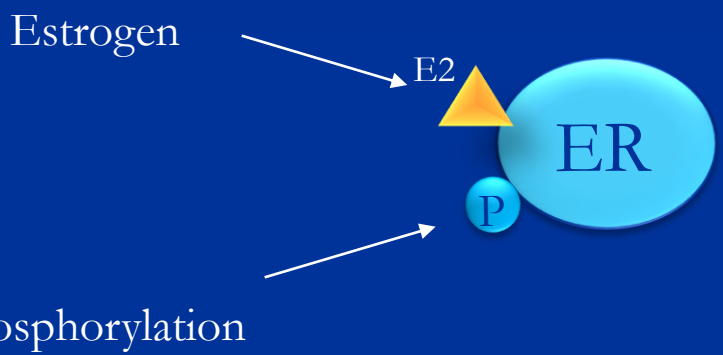


Anticancer Res., 29: 3971-3976, 2009

Relationship among ER protein, Transcription Activity, and Target Genes

Present predictive marker

Expression status of ER



Transcription

Expression profile of target genes

Target genes (PgR)

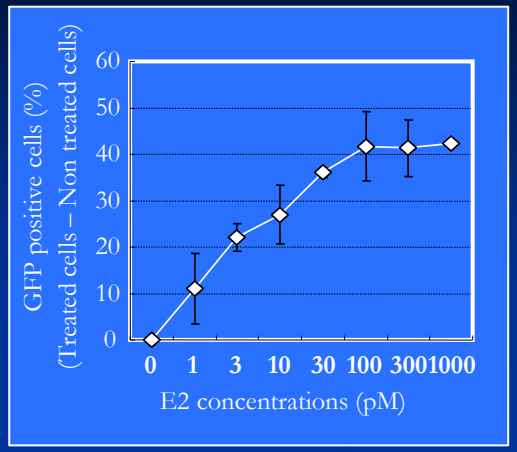
DNA microarray

Transcription activity of ER

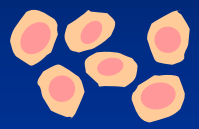
Adenovirus ERE-GFP assay

For analyzing ER transcription activity in small amount of tissue specimens, such as needle biopsy.

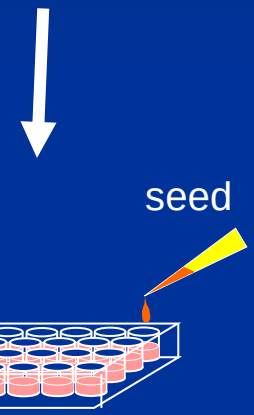
ERE-GFP Assay by Adenovirus Vector



Breast cancer tissue



collagenase treatment
(37ºC, 1-3 h)



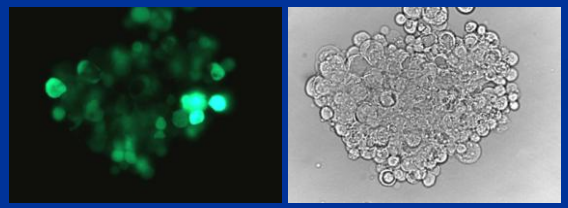
seed

Adenovirus

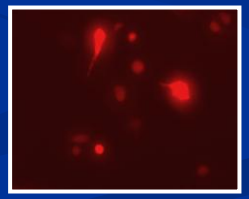
AdV-ERE-GFP

After 3 days

Observation and measurement of GFP luminous cells by fluorescence microscopy



Ad-CMV-DsRed
for control



Endocrine-Related Cancer 15: 451-463, 2008

Relation between Clinical Characterization and ER Activity in 62 Patients with Breast Cancer

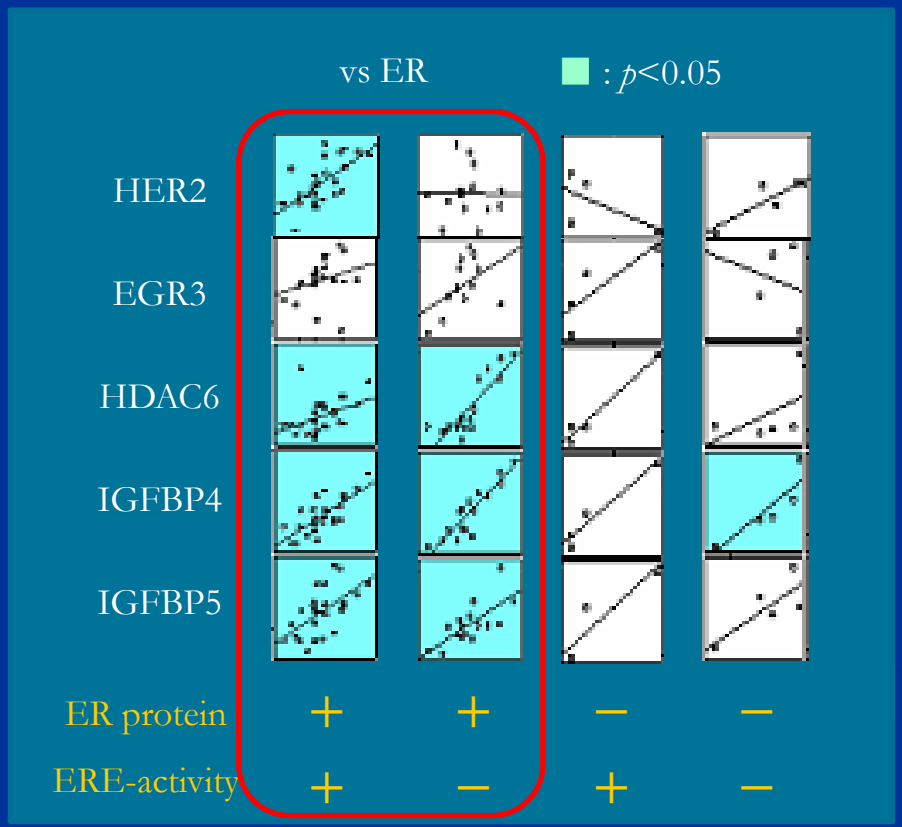
	n	*P* Value		n	*P* Value		n	*P* Value
Total	62		**ER**			**Grade (NA)**		
Age			negative	13	0.1427*	2	30	0.2952*
< 50	27	0.1366*	positive	46		3	19	
≥ 50	35		unknown	3		unknown	11	
Menopausal			**PgR**			**Grade (NM)**		
pre	28	**0.0188***	negative	17	**0.0403***	1	9	0.1862**
post	33		positive	46		2	9	
No (men)	1		unknown	3		3	32	
						unknown	12	
Size			**Her2**					
< 2 cm	27	0.6129*	negative	31	0.1115*	**Grade (NG)**		
≥ 2 cm	30		positive	23		1	7	0.5350**
unknown	5		unknown	8		2	9	
						3	33	
Stage			**V**			unknown	13	
0	3		-	53	0.9626*			
1	13	0.7199**	+	4		**Ly**		
2	33		unknown	5		0	30	0.6525**
3	5					1	21	
unknown	8					2	3	
						3	3	
						unknown	5	

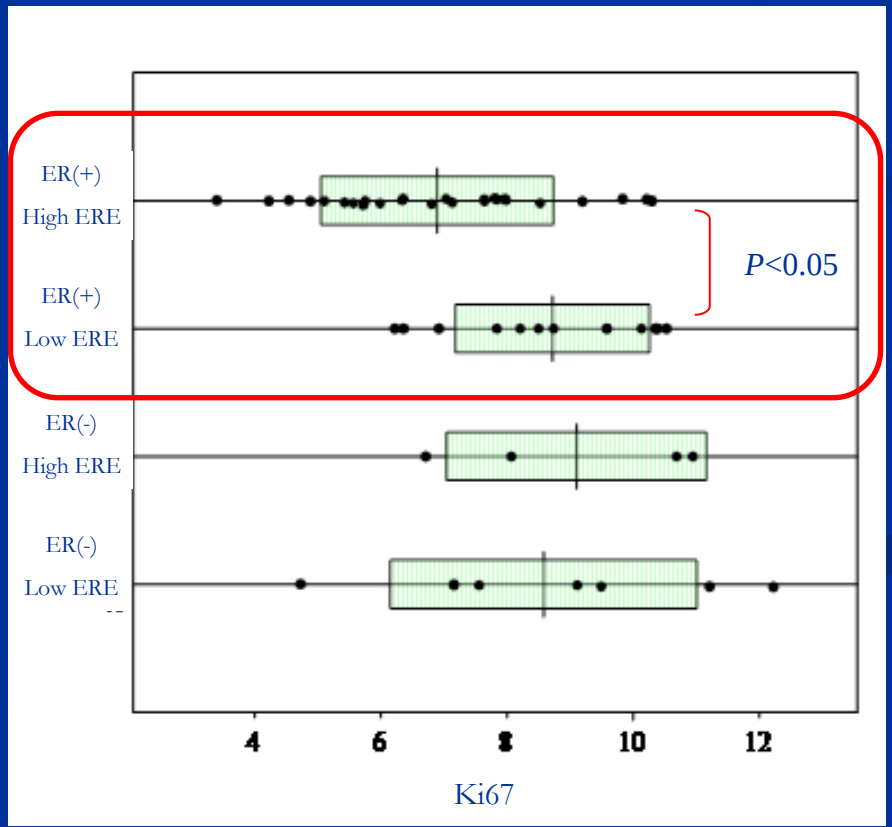
*Mann-Whitney test, **Kruskal-wallis test

Gohono et al. in preparation.

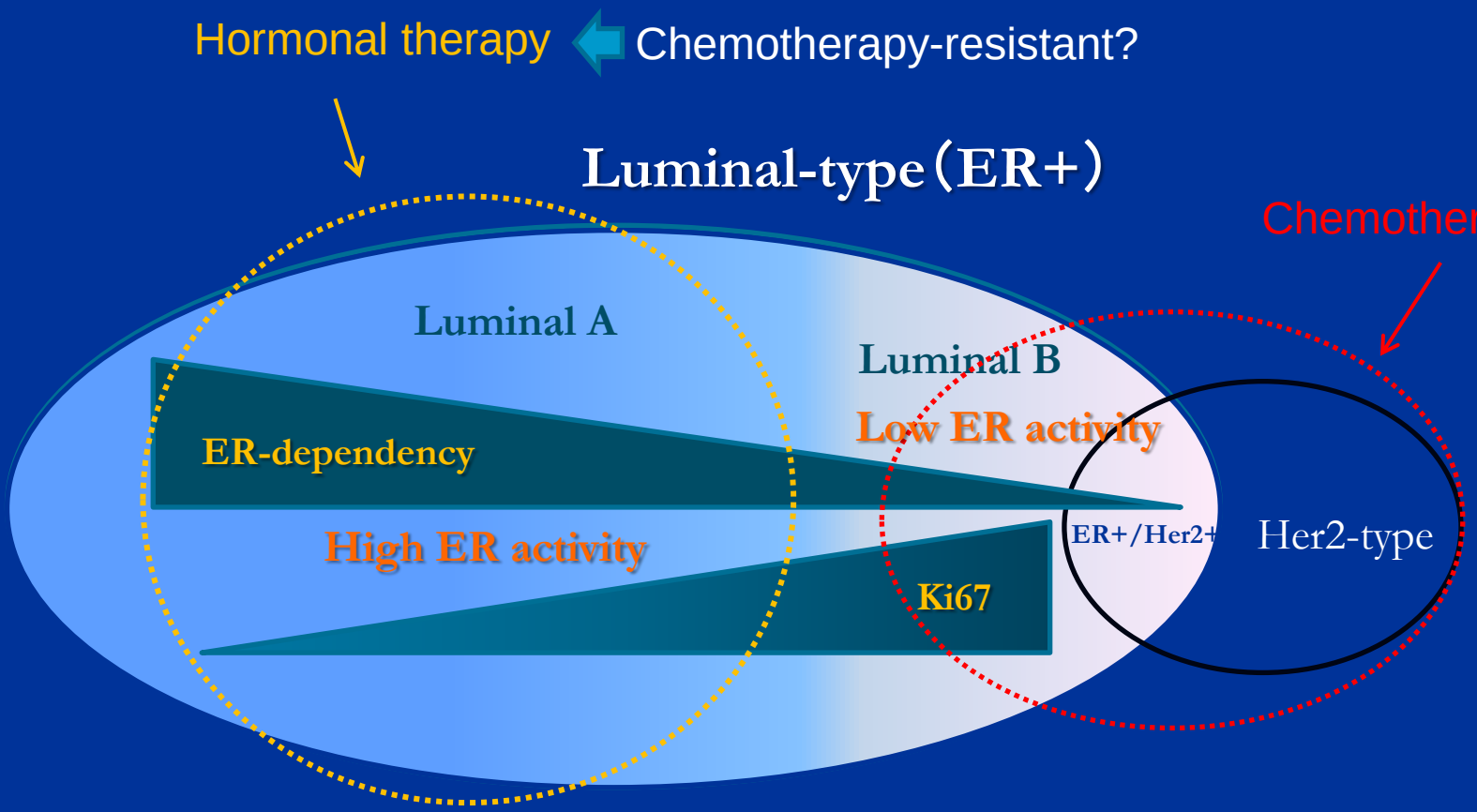
mRNA Expression of ER-related Genes in the ERE-GFP Analyzed Cancers and Its Relation with ER-activity and ER-protein

		n	ER	PgR	HER2	Efp	EGR3	HDAC6	IGFBP4	IGFBP5
ER Protein	Positive	42	0.0208	0.0224	0.1215	0.6733	0.8195	0.4093	0.1665	0.8501
	Negative	13								
	Unknown	2								
ER Activity	Positive	31	0.2839	0.0616	0.7816	0.2813	0.5949	0.2397	0.4101	0.3675
	Negative	26								





Heterogeneity of Luminal-type Breast Cancer

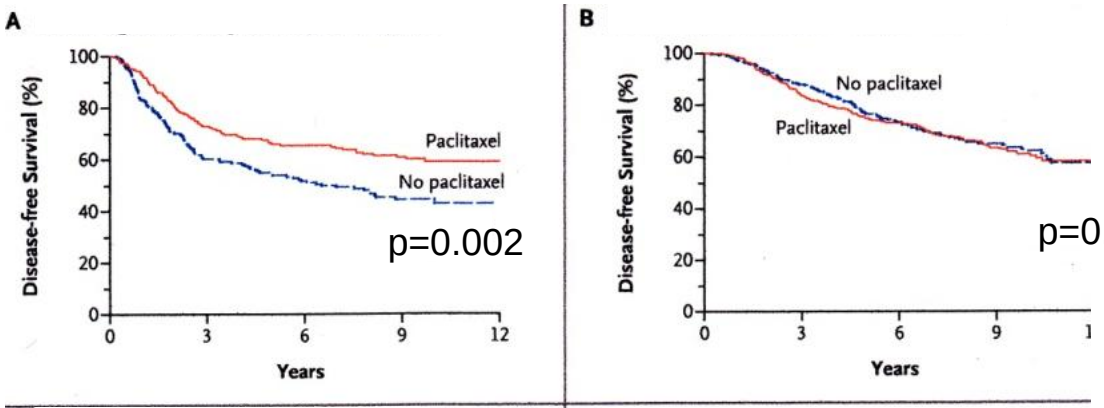


- Expression status of ER is not consistent with ER activiry.
- In the luminal-type breast cancer, high ER activity cases shows low Ki67 expression, and corresponds to Luminal A subtype. They have high ER-dependency and probably efficacious to hormonal therapy.

ERa Expression and Response to Paclitaxel

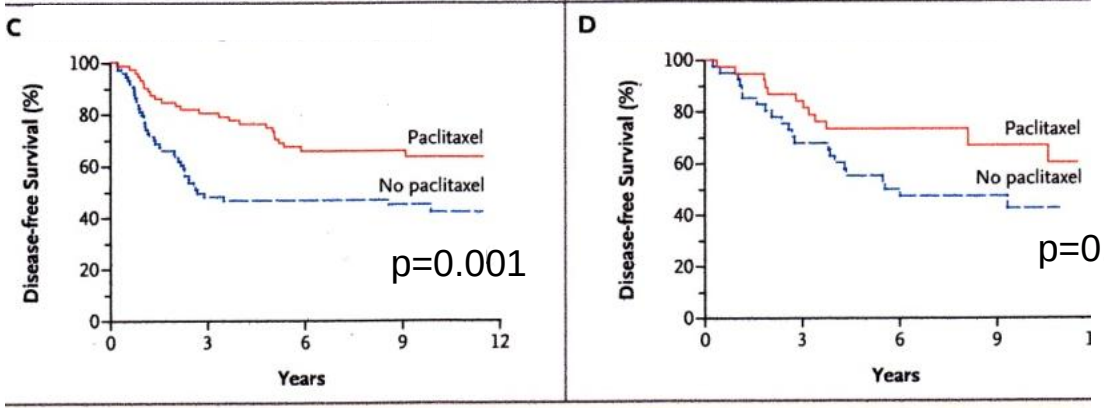
AC vs AC+PAC

ER-/Her2-



ER+/Her2-

ER-/Her2+



ER+/Her2+

Disease-free survival among patients treated with or without paclitaxel according to estrogen receptor status and HER2 expression.

Hayes DF, et.al ; N Engl J Med. 2007 Oct 11;357(15):1496-506.

Clinical Response to Taxanes in NAC-treated Patients

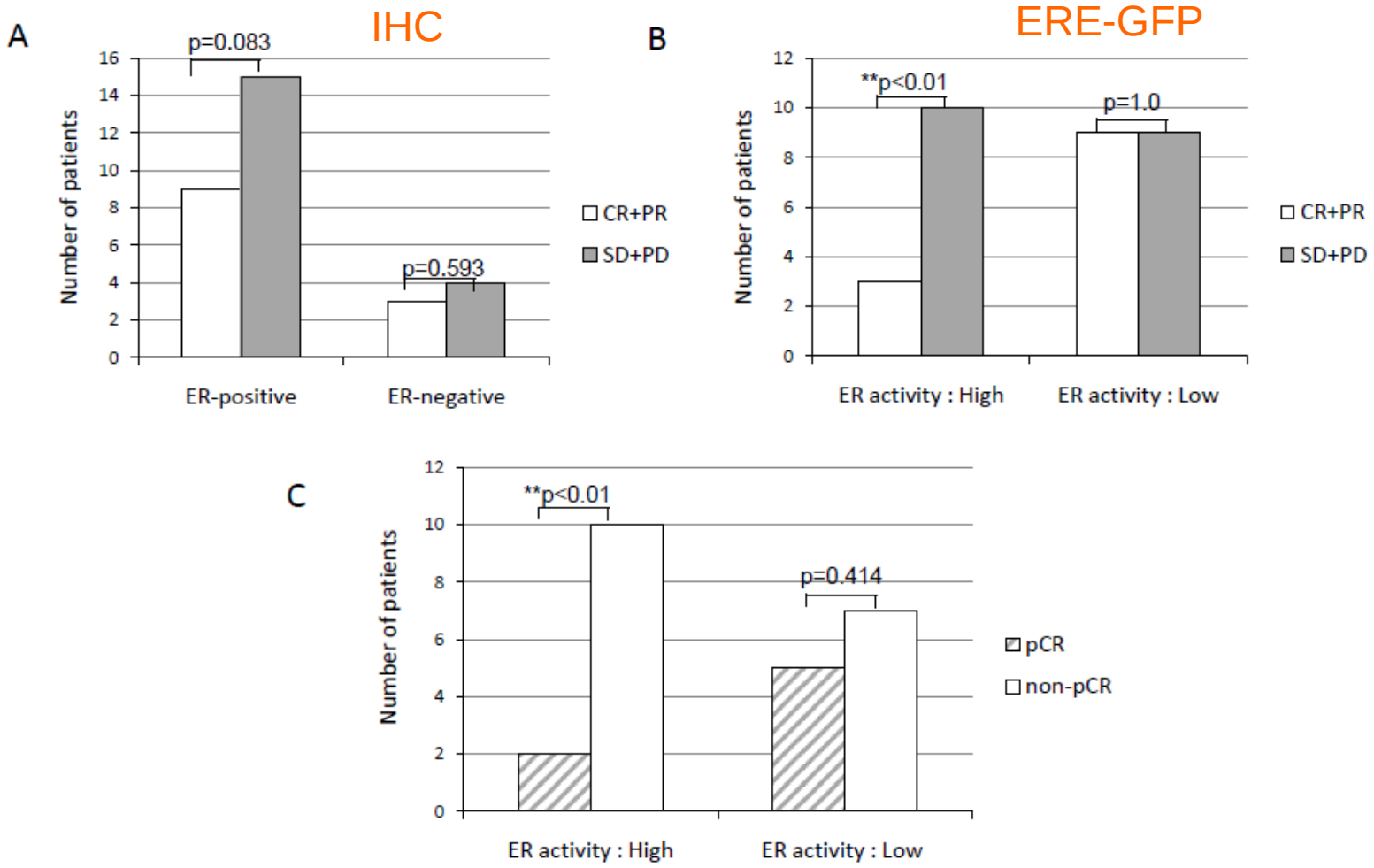
Characteristic	cCR rate of patients No. /Total (%)		cCR+cPR rate of patients No. /Total (%)	
ER				
-positive (n=126)	5/126 (4.0)	*p=0.028	54/126 (42.9)	*p=0.051
-negative (n=64)	8/64 (12.5)		37/64 (57.9)	
HER2				
-positive (n=71)	6/71 (8.5)	p=0.498	36/71 (50.7)	p=0.601
-negative (n=119)	7/119 (5.9)		65/119 (54.6)	
Total (n=190)				

cCR = Clinical complete response

Neoadjuvant chemotherapy: July, 2006 ~ January, 2008 (n=190)

Breast cancer Res. Treat. in press, 2011

Clinical Evaluation and ER Expression of Activities in Pre-NAC Breast Cancer

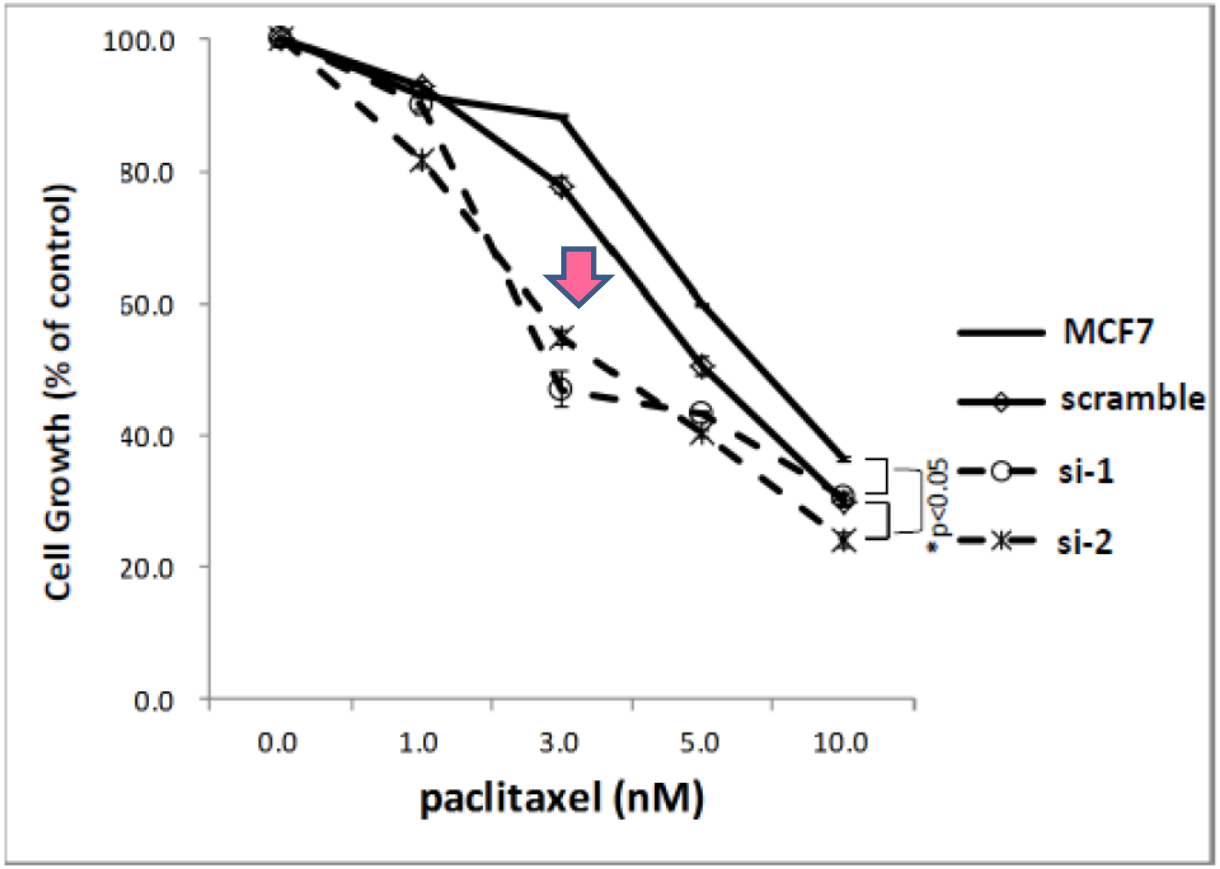


ERE−GFP in pathological response

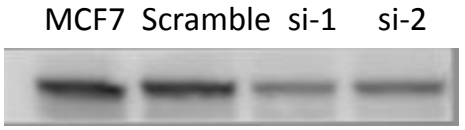
Breast cancer Res. Treat. in press, 2011

ERα Expression and Sensitivity to Paclitaxel in MCF-7

A



B



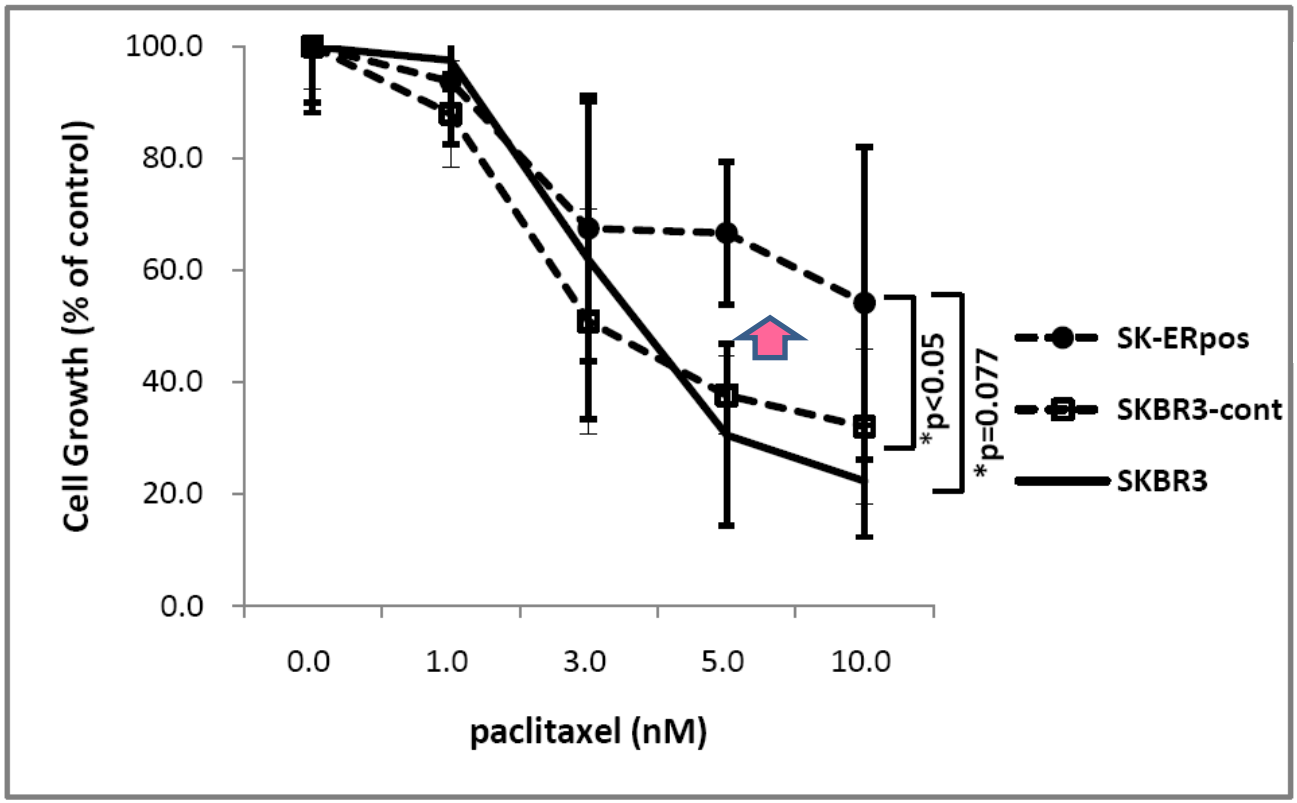
C

IC50 Values (nM)	
MCF7	6.66
Scramble	5.09
si-1	2.80
si-2	3.57

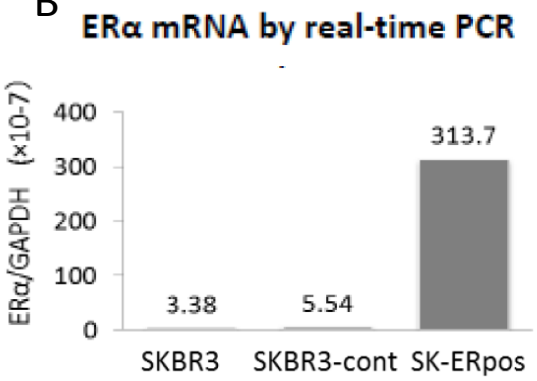
Breast cancer Res. Treat. in press, 2011

Ectopic ERα Expression Reduced Sensitivity to Paclitaxel in SKBR3 Cells

A



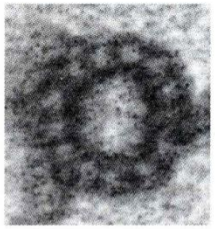
B

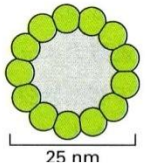


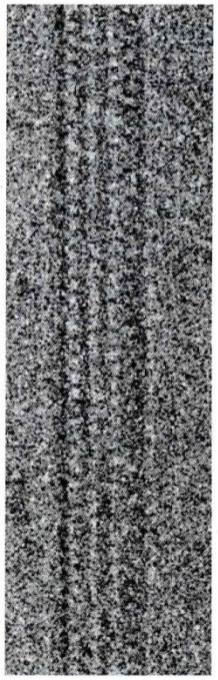
C

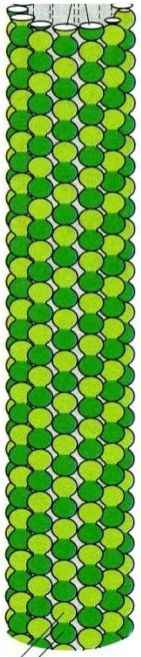
IC50 Values (nM)	
SKBR3	3.60
SKBR3-cont	3.08
SK-ERpos	> 10

Estrogen and HDAC6 Induces the Cell-motility by Tubulin Deacetylation in MCF-7 Cells









Molicules of tubulins

Inhibition of cell division

Estrogen

ER function

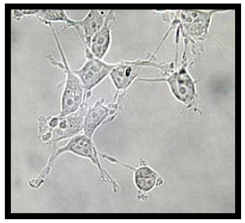
HDAC6

***Deacetylation* of α-tubulin**

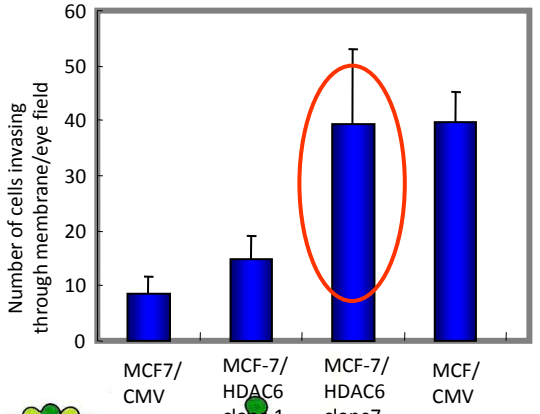
Microtube formation

paclitaxel

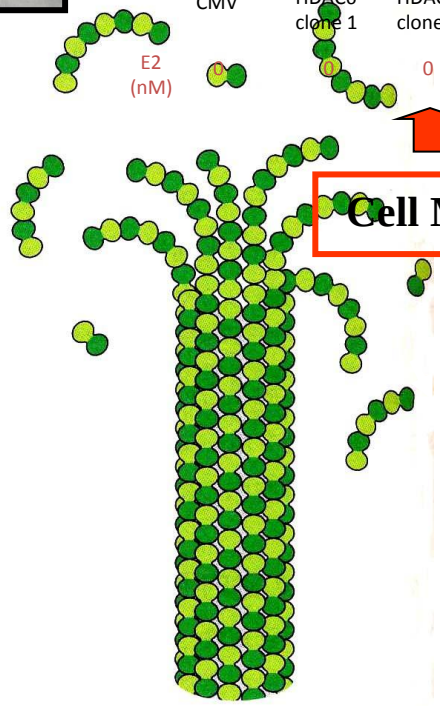
MCF7/HDAC6 clone7



Cell mortility assay



Cell Motility

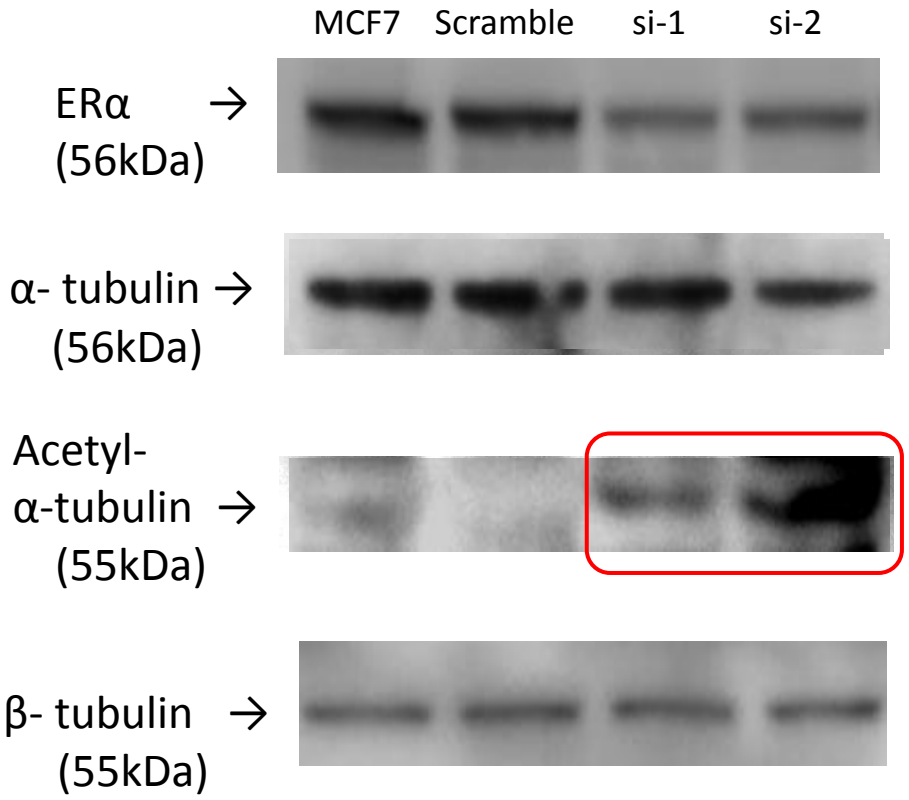


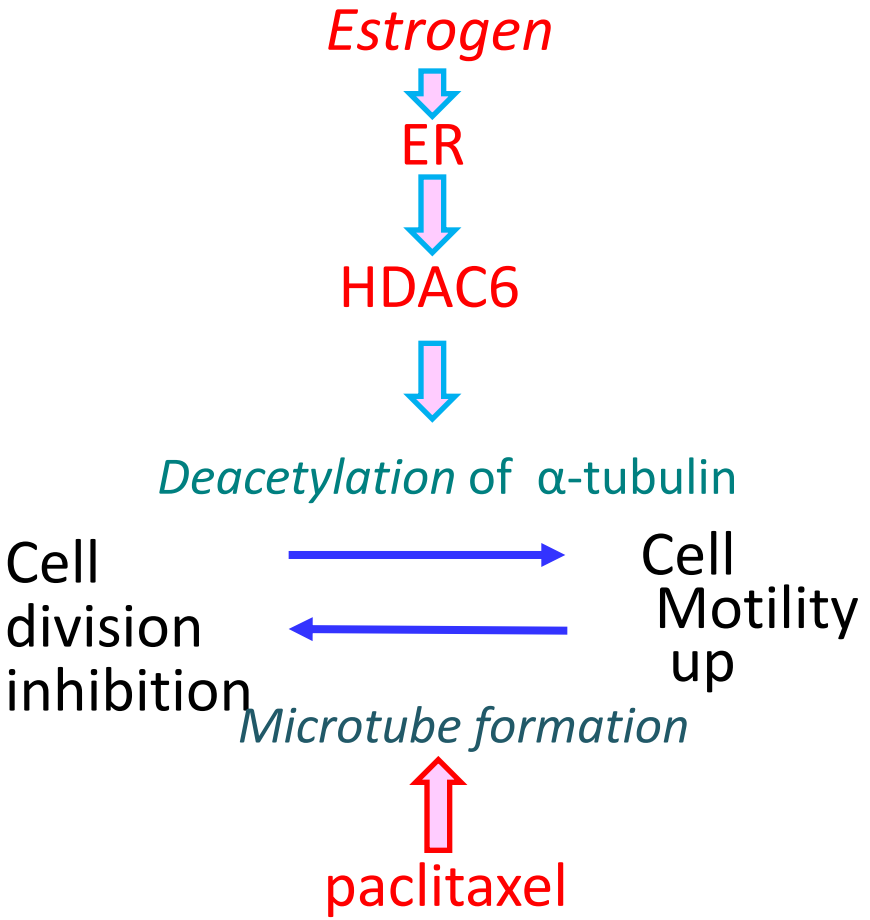
Destabilization of tubulin

Oncogene. 24; 4531-4539,2005

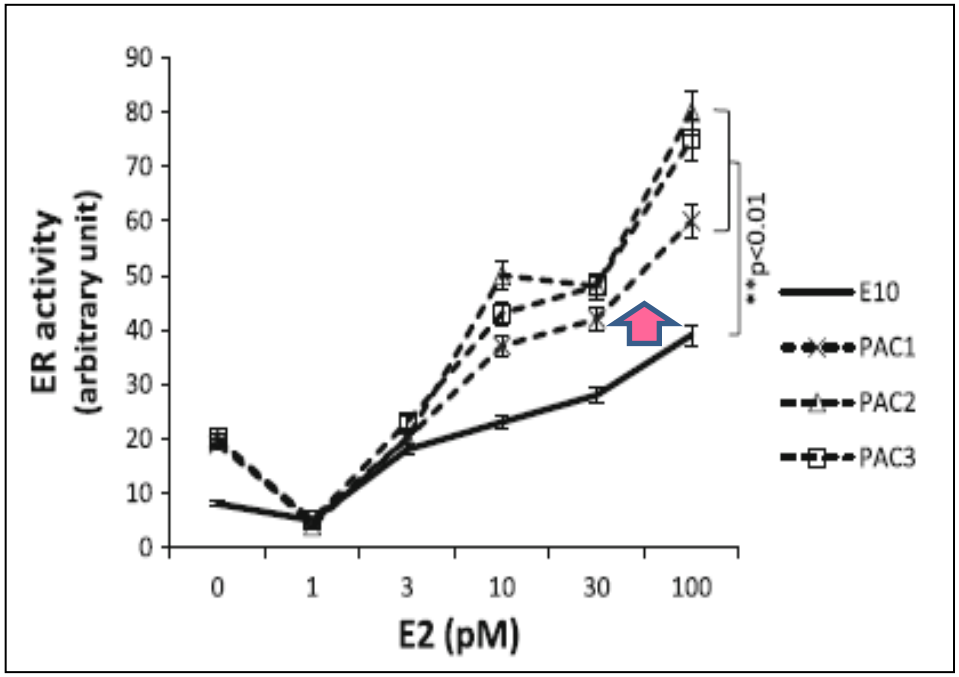
Acetylation of Tublin in ER-nockdown MCF-7 Cells

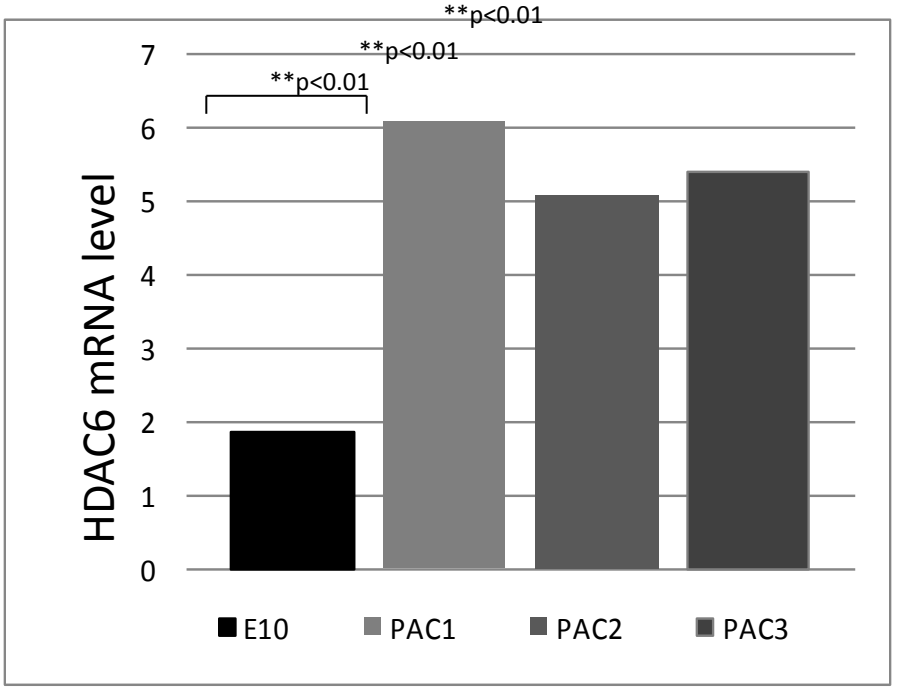
A





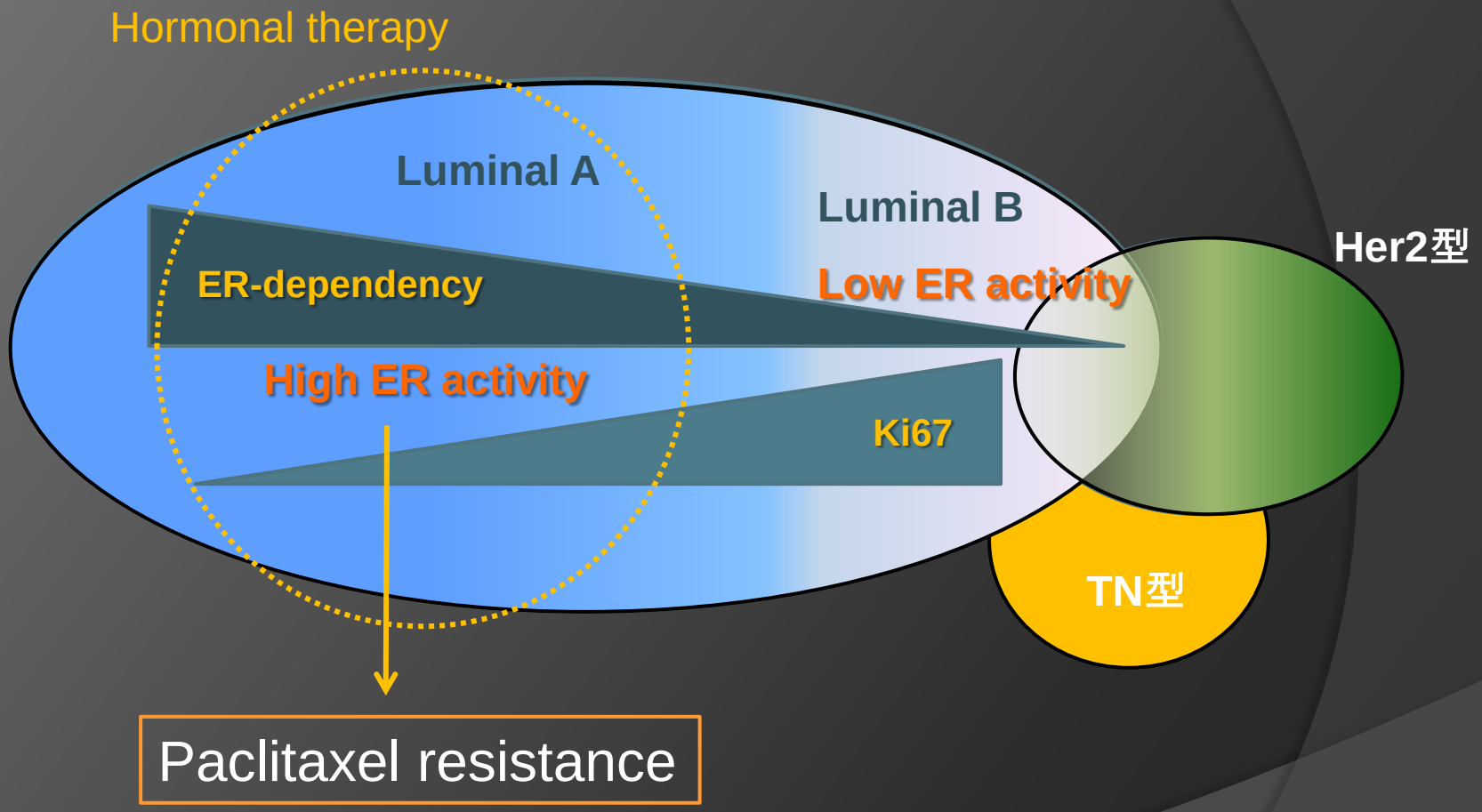
ER activity and HDAC6 expression in Paclitaxel-resistant MCF-7 cells



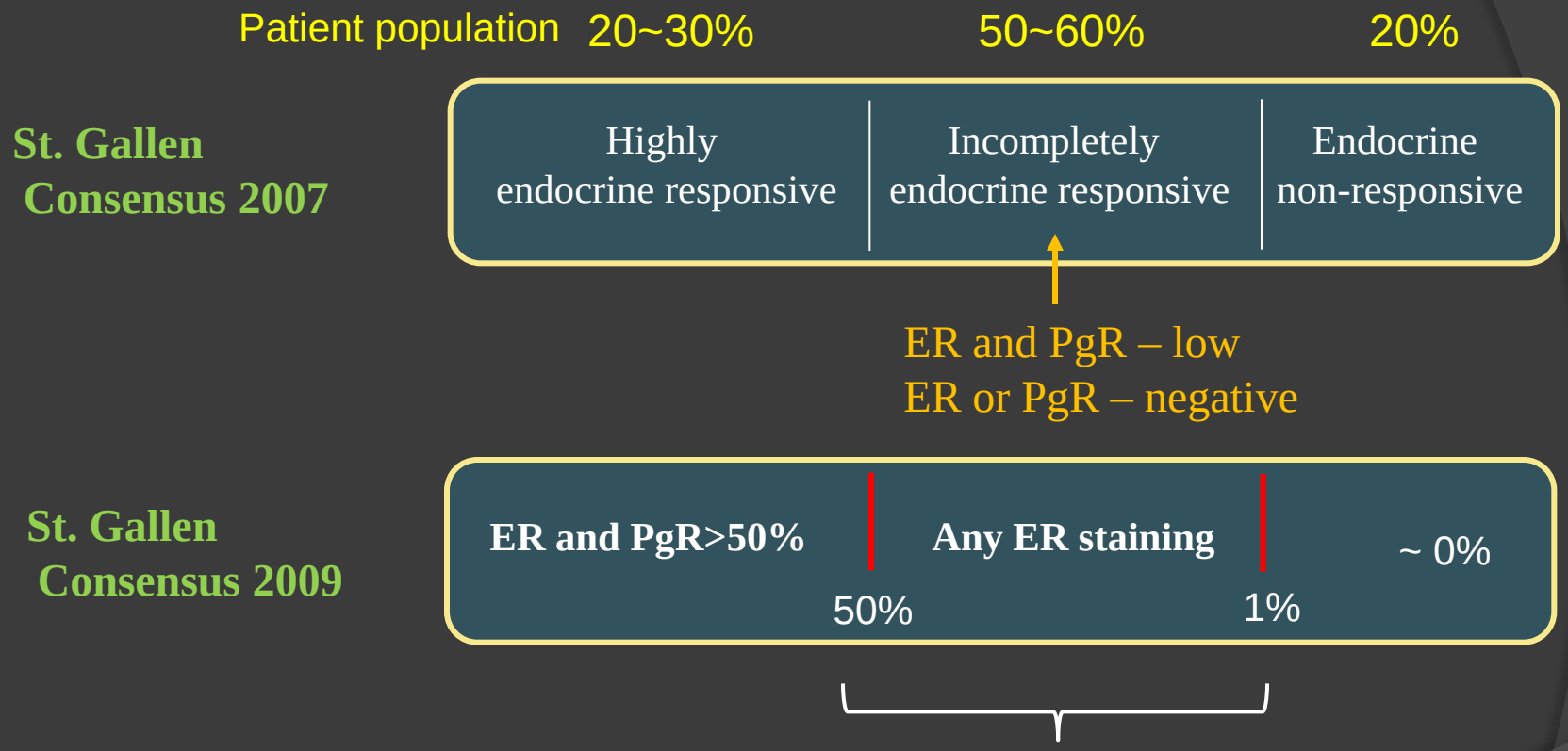


Breast cancer Res. Treat. in press, 2011

Heterogeneity of Luminal-type Breast Cancer

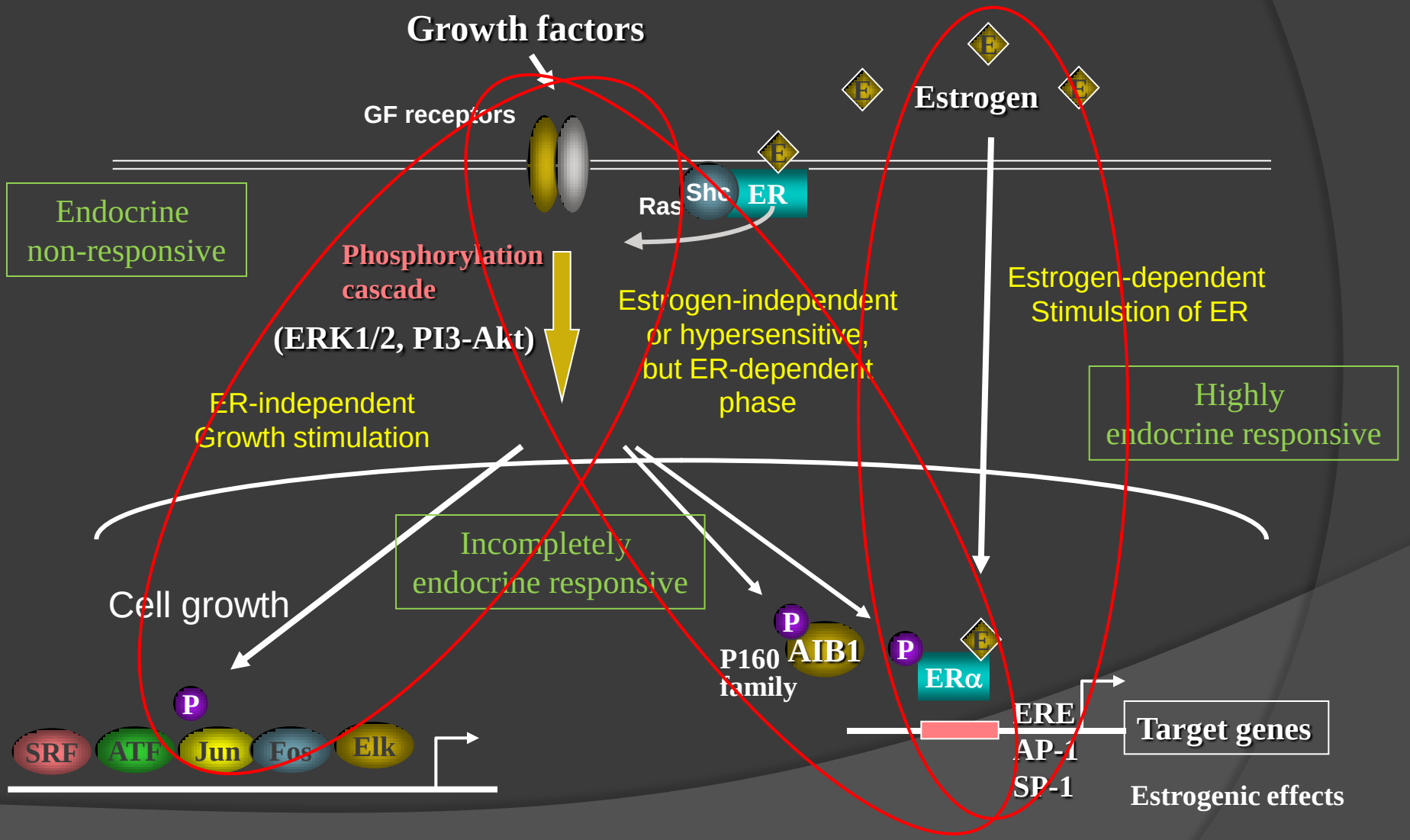


Classification of Hormonal Response



Intracellular estrogen signaling pathway in individuals

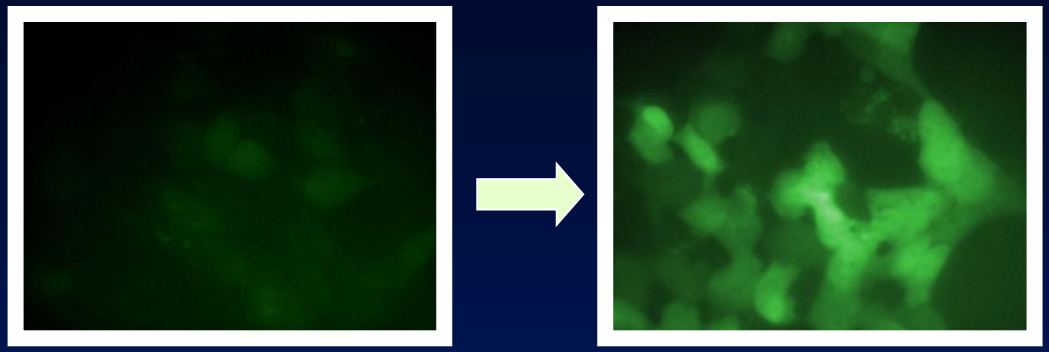
Multiple Estrogen Signaling Pathway in Breast Cancer Cells



MCF7-E10

Parental cells

T47D-TE8



None + Estrogen

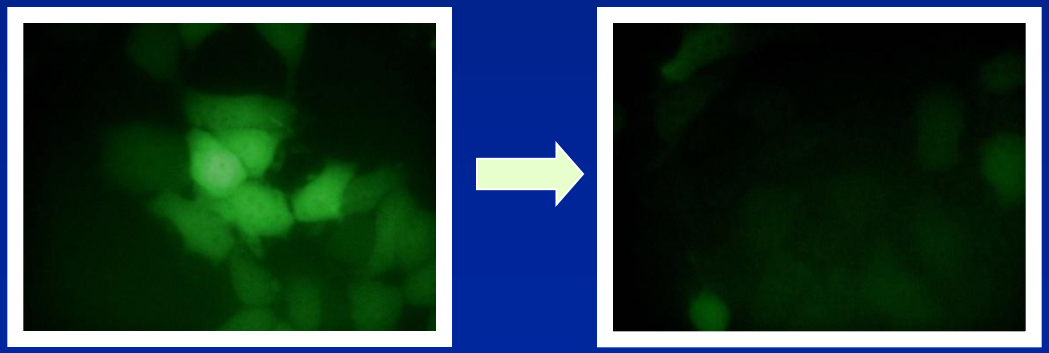
Cancer Res. 65: 4653-4662, 2005

Lomg term estrogen-deprivation culture of ERE-GFP transfected MCF-7 cells (MCF-7-E10)

Select the GFP-positive colony

Depletion resistant

E2-independent but ER-dependent activation



None + Fulvestrant

Established Cell Lines for AI-resistant Models

Type Ⅰ

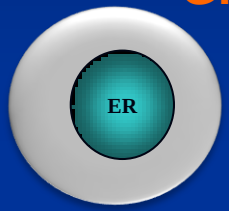
GFP+

ER-high
Ligand-independent activation

A1, A2, C5

Type Ⅱ

GFP -

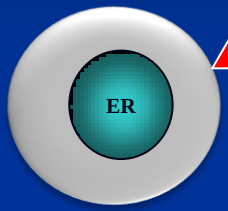


ER-low
Phosphorylation-high

A4, C7, K2

Type Ⅲ

GFP -



GF-dependent

U3, U8, U23, U25

Type Ⅳ

GFP+

Androgen-dependent

G1T, R6T

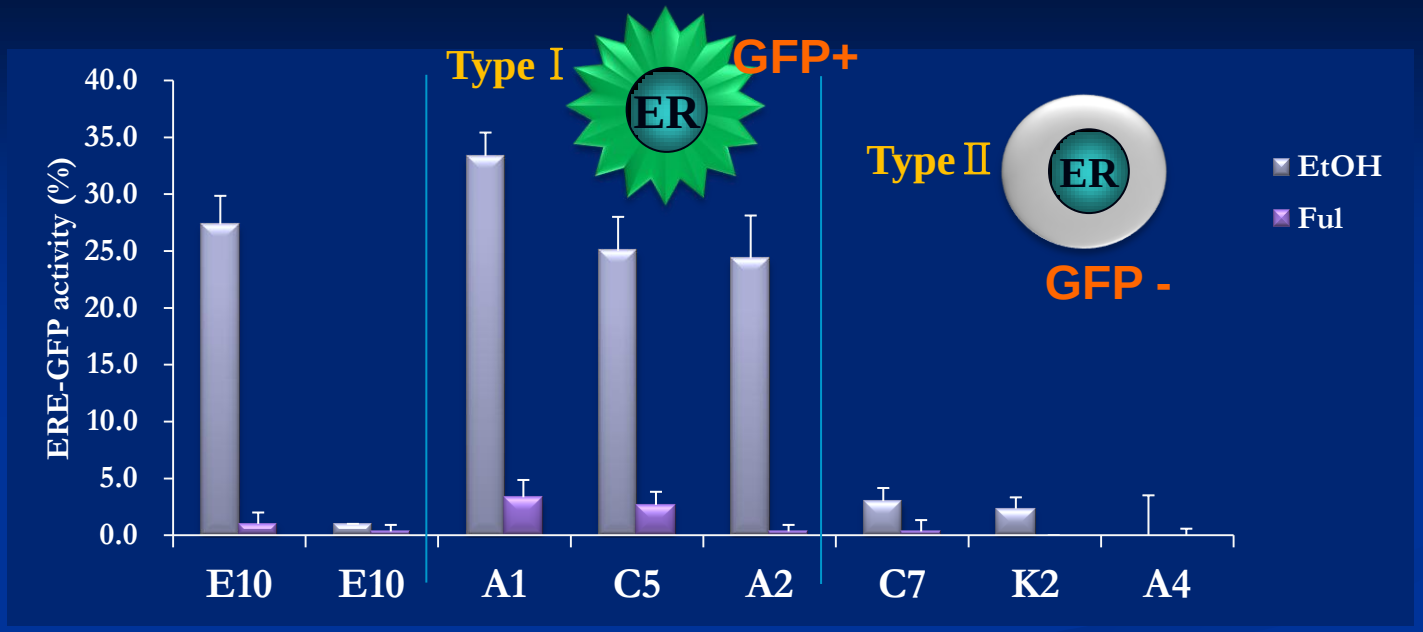
Type Ⅴ

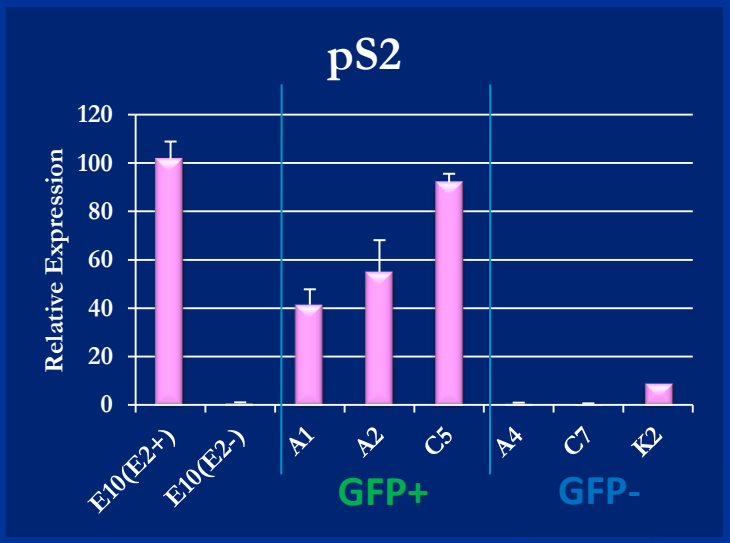
GFP+

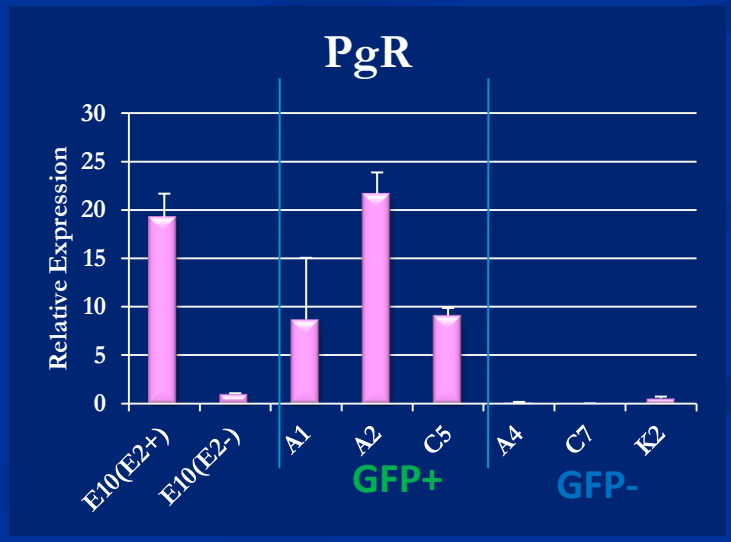
AI-resistant

LR2, LR3

ER Activity of EDR-MCF7-E10 Cells







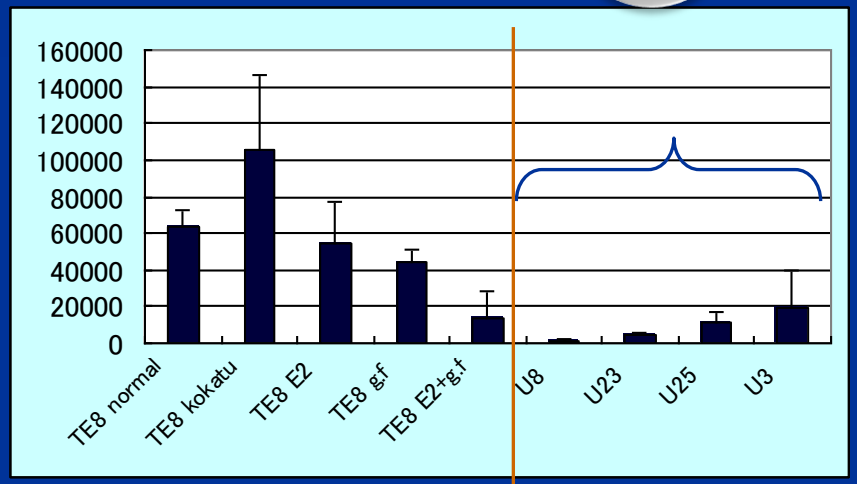
Fujiki et al. in preparation.

Expression of ER and Her2 in EDR-T47D-TE8 (TypeⅢ)

Type Ⅲ GFP -

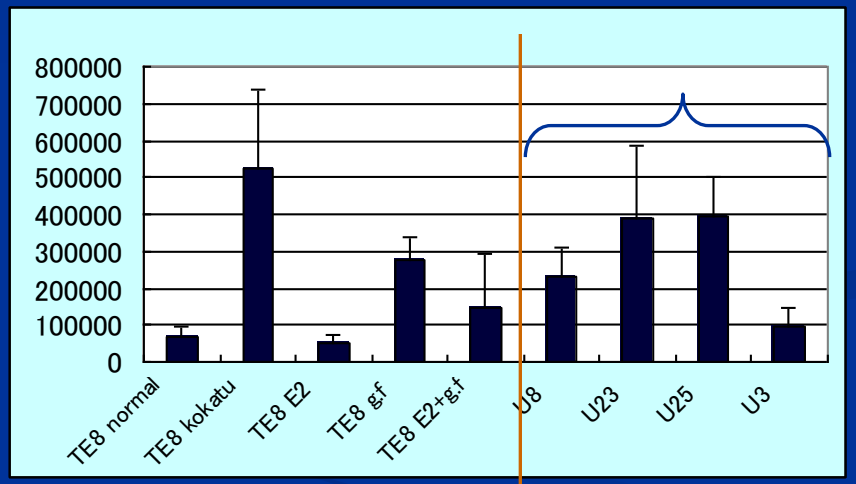
ERα

RT-PCR



HER2

RT-PCR

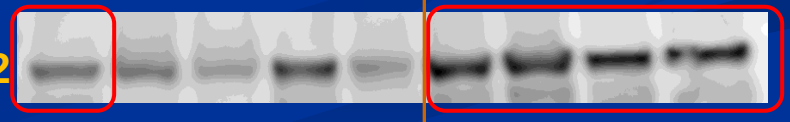


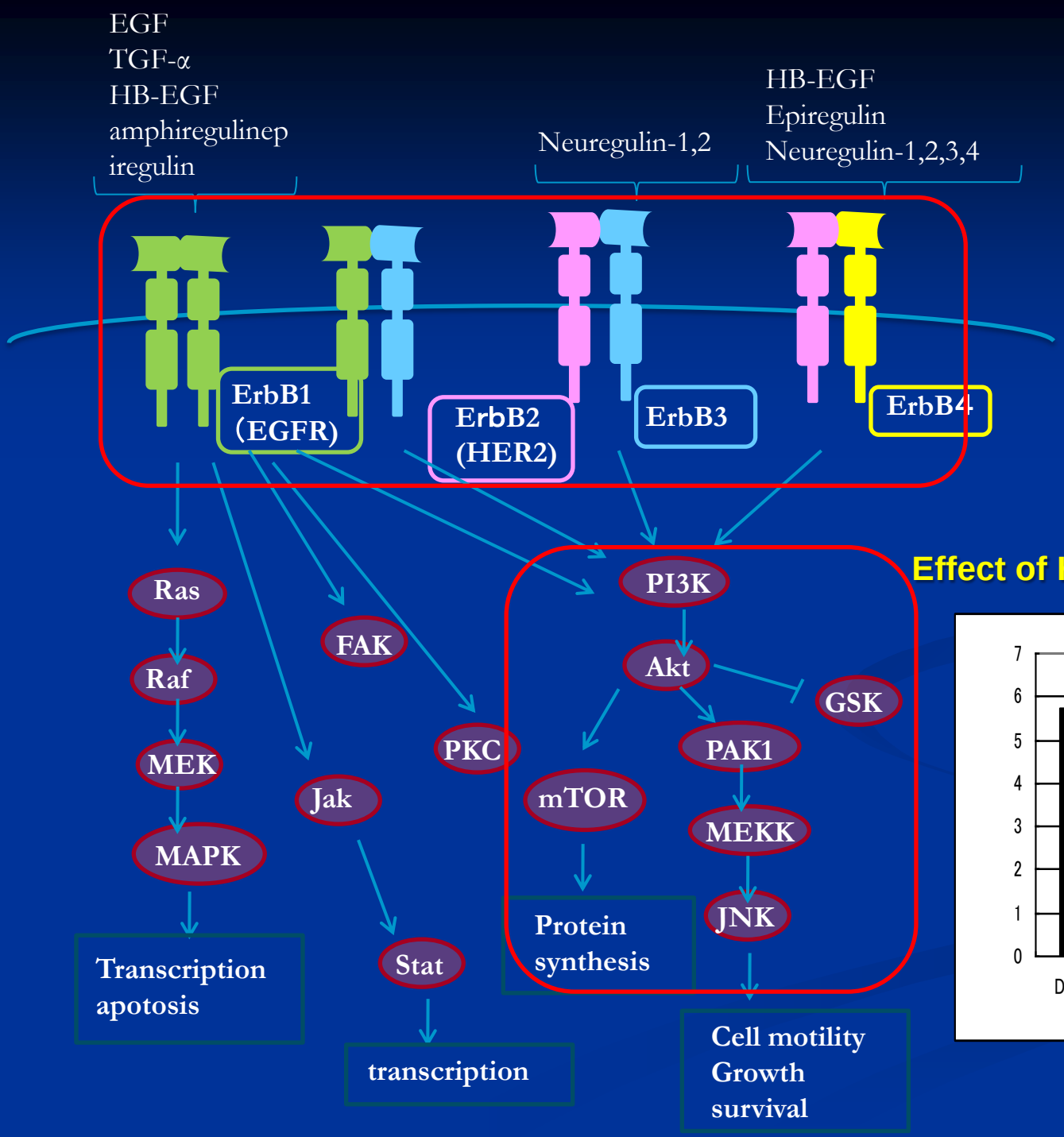
Western blot

ER

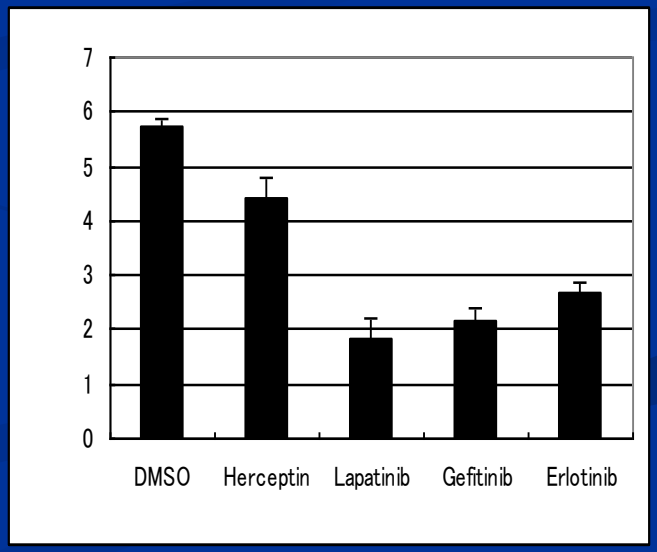
Western blot

HER2

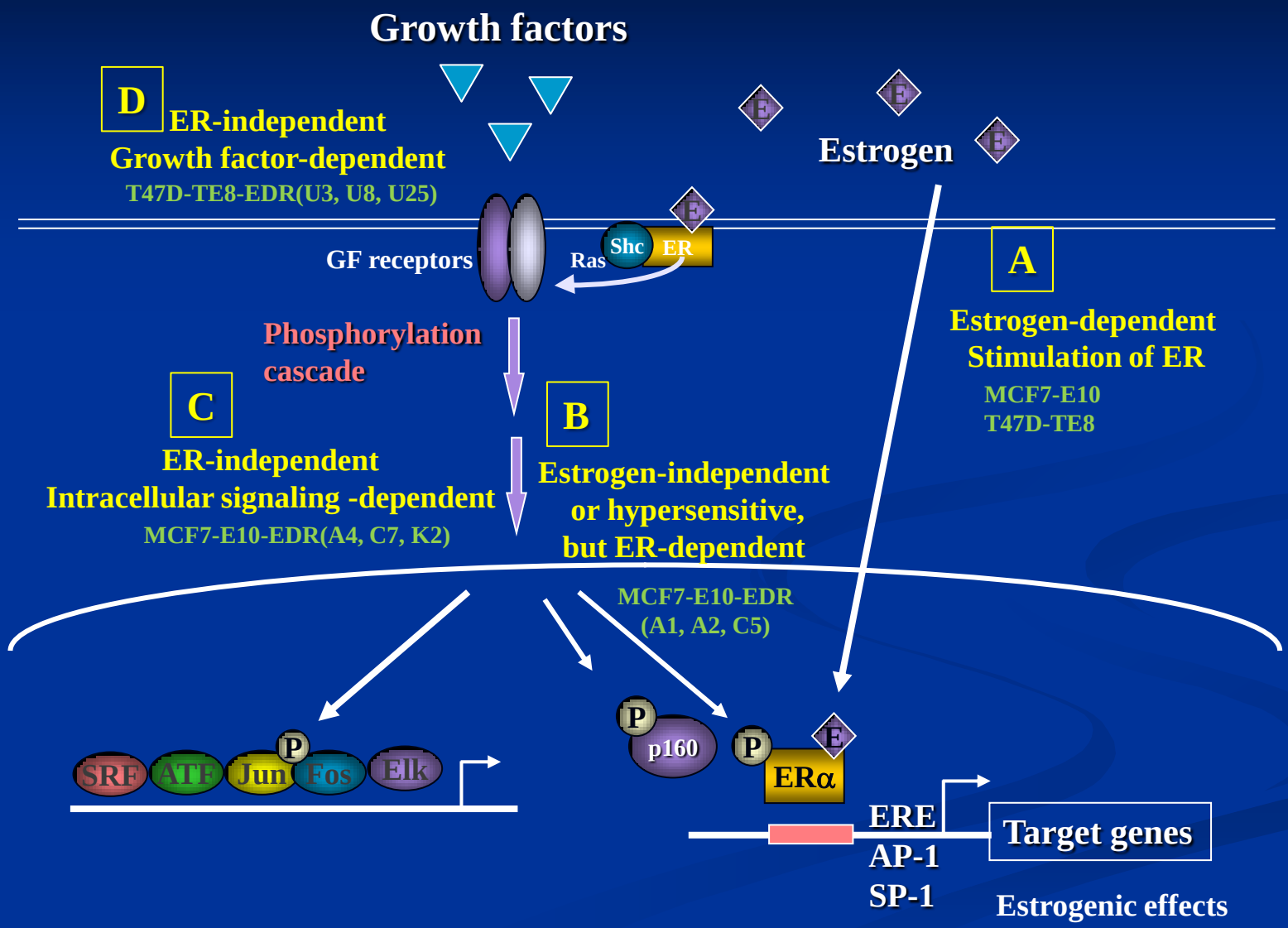




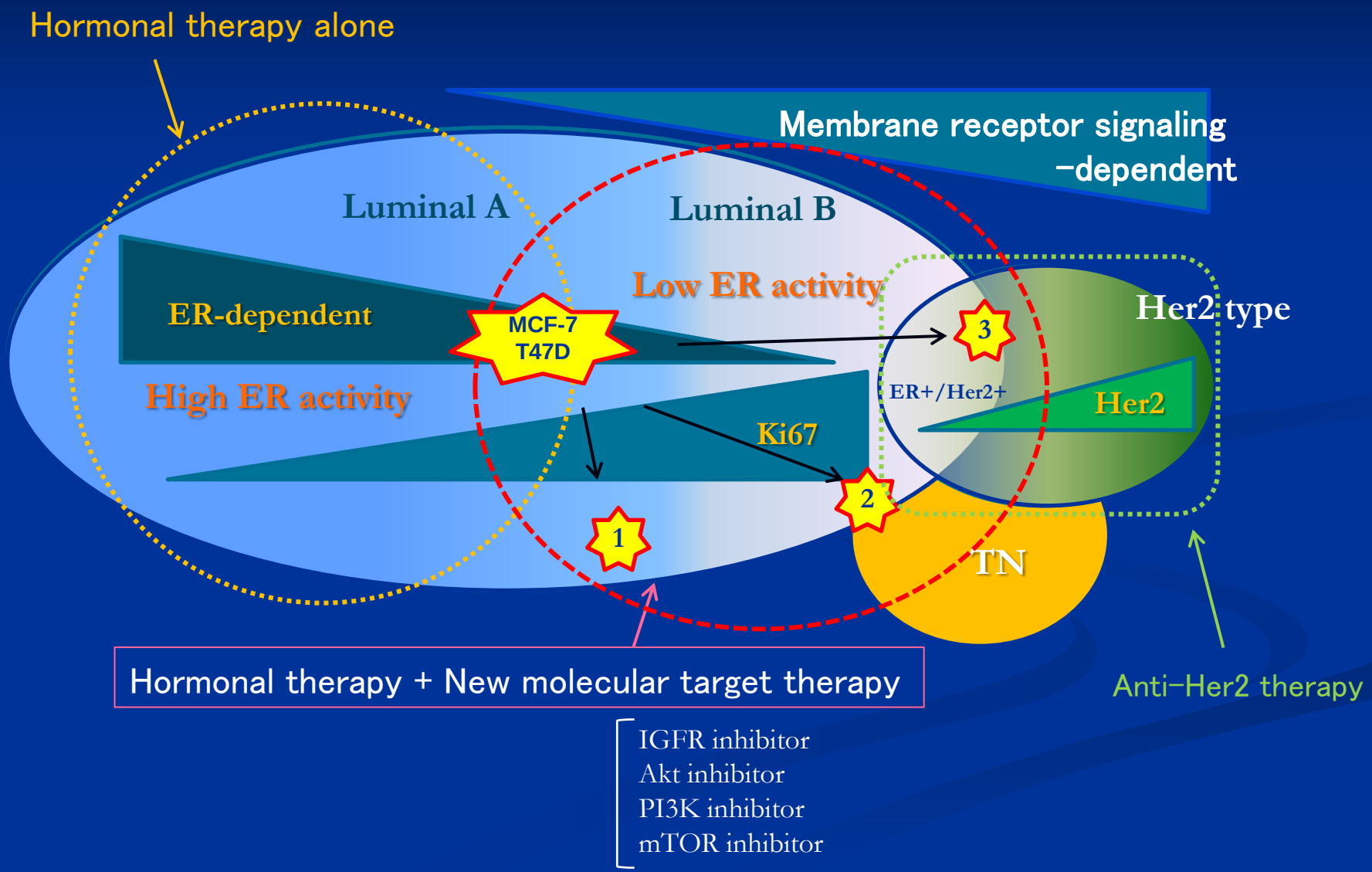
Effect of Her family-targeting drugs



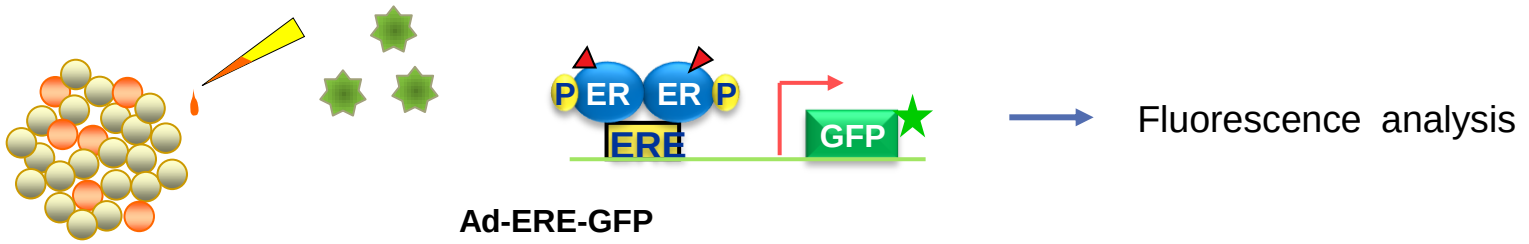
Estrogen Signaling Pathway in Breast Cancer Cells



Flexibility of Luminal-type Breast Cancer and Possible Eligibility of New Molecular targeting Therapy



ER activity in Breast Cancer Mammosphere



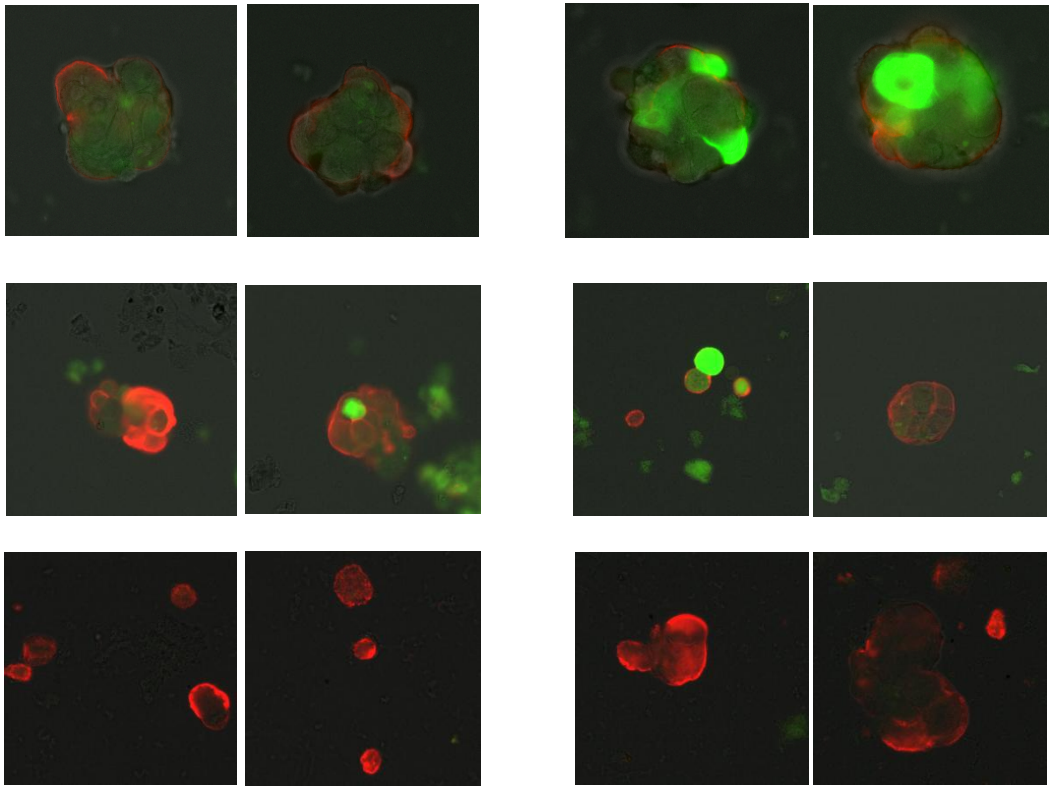
Ad-ERE-GFP

GFP (Green) , CD44 (Red)

Pt91
Subtype : Luminal A
Metastasis : (+)
Spheroid formation : 228
CD44+/CD24-/Hoechst- : 0%

Pt94
Subtype : Luminal A
Metastasis : (+)
Spheroid formation : 62
CD44+/CD24-/Hoechst- : 1.6%

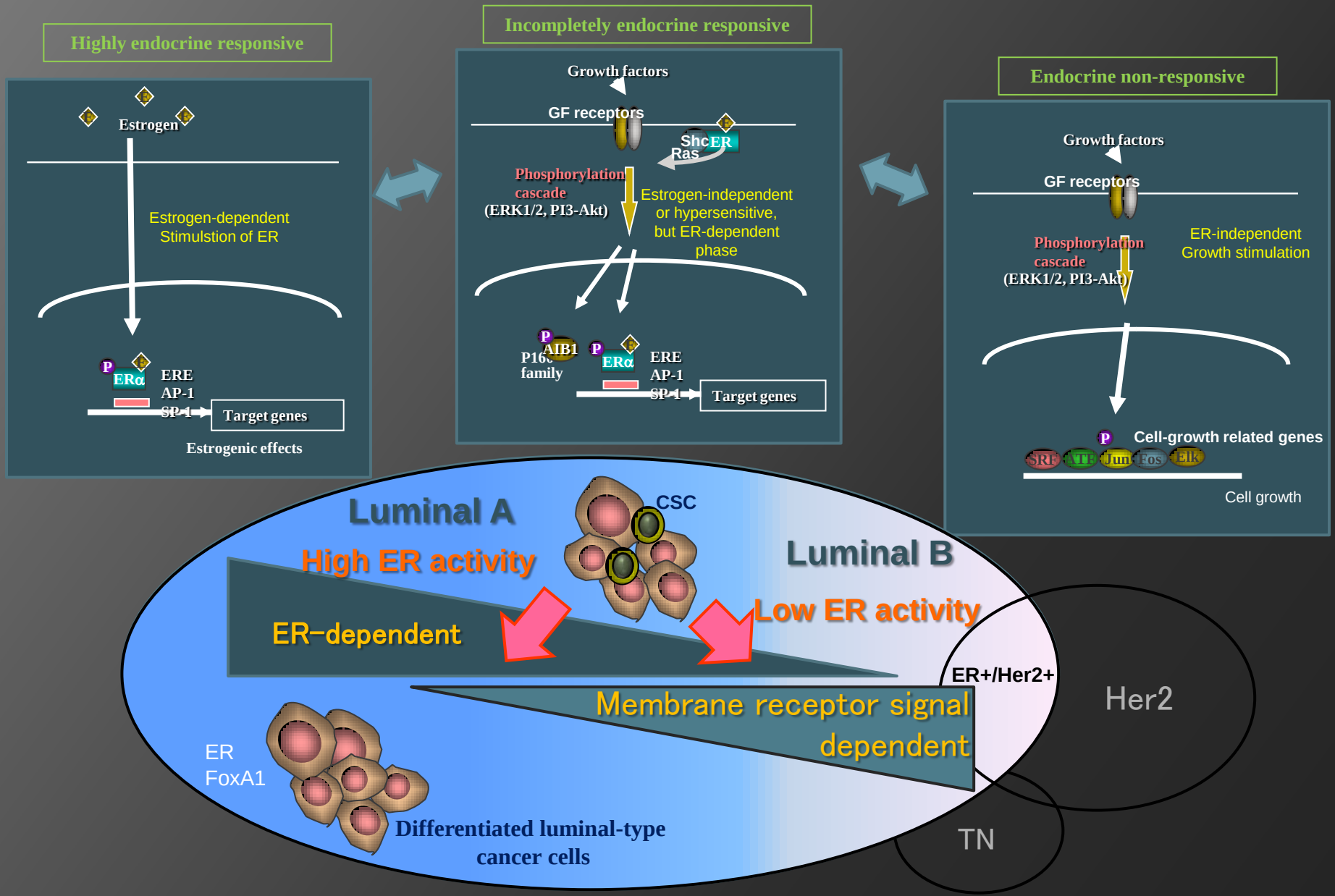
Pt101
Subtype : Basal-like
Metastasis : (-)
Spheroid formation : 173
CD44+/CD24-/Hoechst- : 1.3%



EtOH

+ E2

Heterogeneity of Intracellular Signaling in Luminal-type



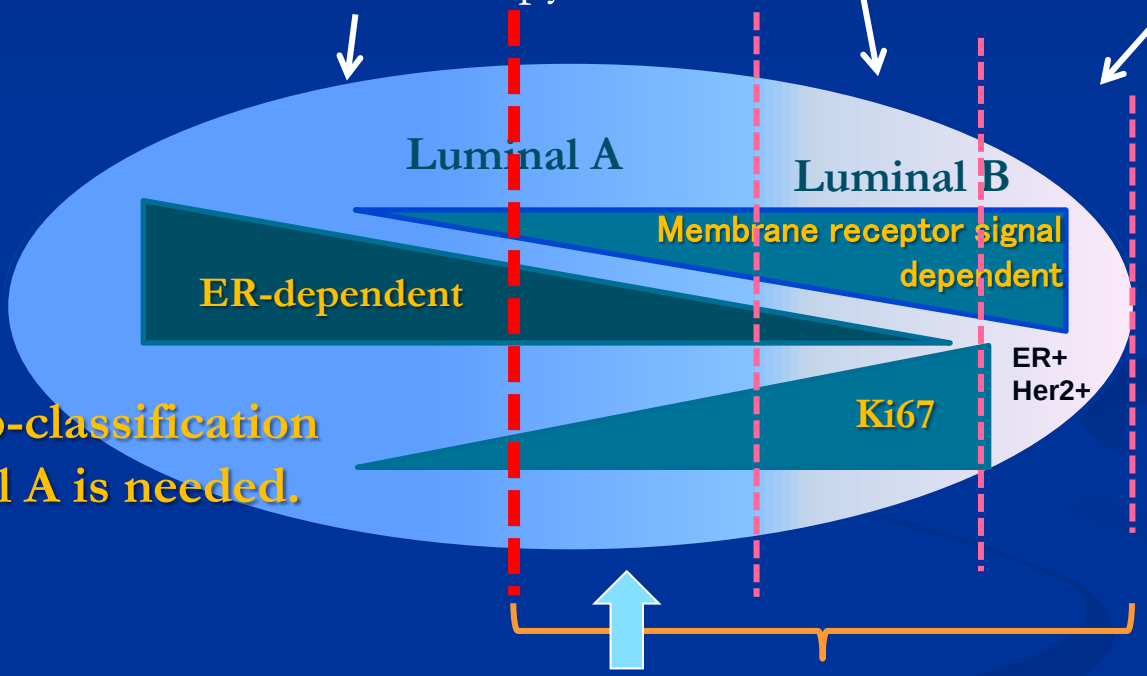
Classification of Luminal type for Theraputic Managment

Present eligibility

Luminal B（Her2+）: Chemotherapy + Anti-Her2 therapy

Luminal B(Ki67-high): Chemotherapy + Hormonal therapy

Luminal A: Hormonal therapy alone



Further sub-classification of Luminal A is needed.

Hormonal therapy + New molecular targeting therapy

For example, AI+Lapatinib+IGFR-I (Akt-I, mTOR-I)

Collaborators

Tohoku University

Hiromi Konno
Chiyuki Uematsu
Tatsuyuki Gohno
Chika Tazawa
Toshifumi Niwa
Takako Ito
Toru Higuchi

Natsu Fujiki
Megumi Endo
Miho Tanaka
Yuri Shinagawa
Toru Hanamura
Ayaka Inaba
Nozomi Sato

Department of Molecular and Functional Dynamics

Saitama Cancer Center

Yuri Yamaguchi
Yuko Seino
Hiroyuki Takei
Emi Tokuda
Masahumi Kurosumi
Hanako Oba

Reserch Institute for Clinical Oncology