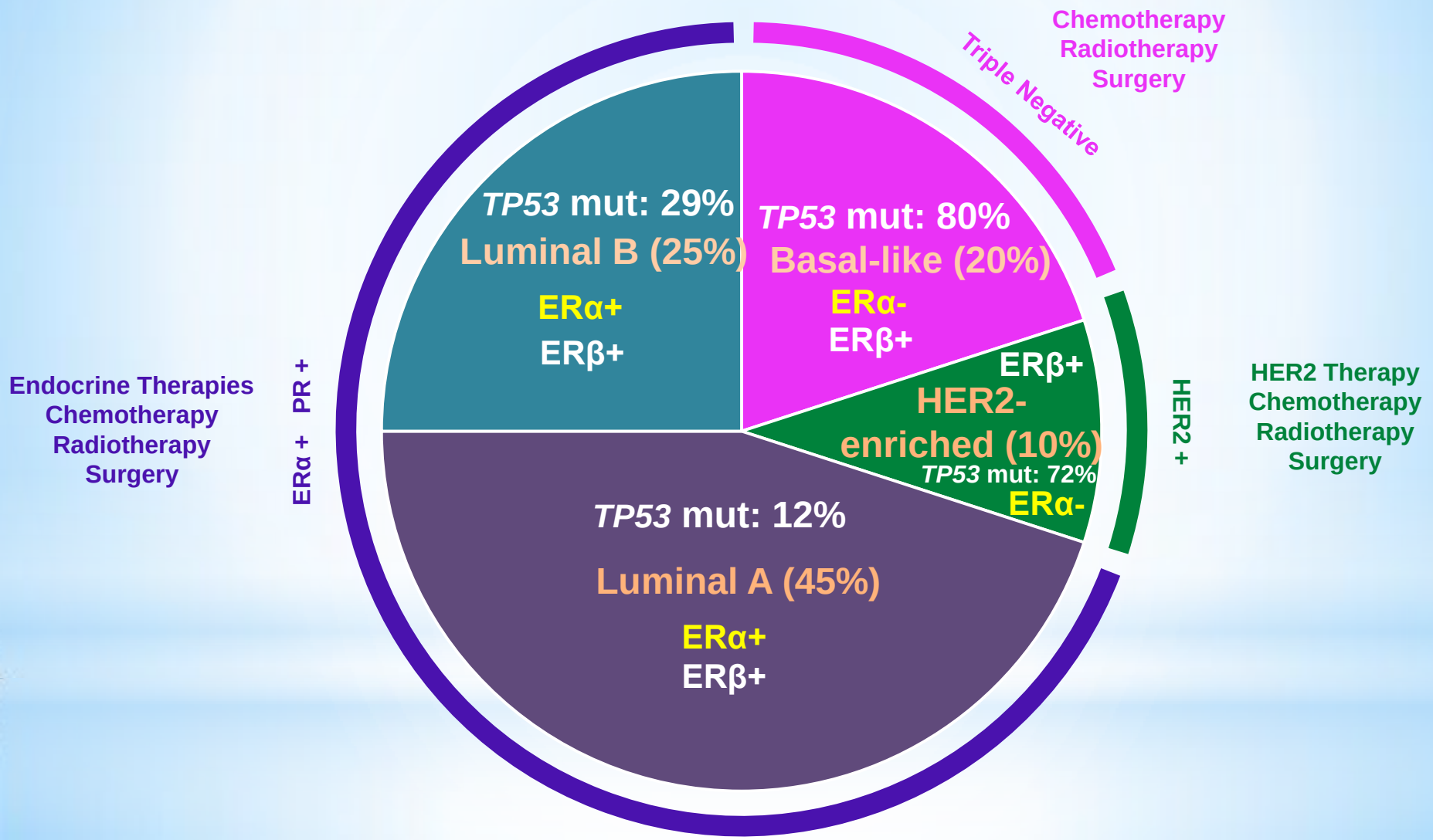
# Breast Cancer
## -From Basic Research to Clinical Trial

**Gokul Das, Ph.D.**

**Department of Pharmacology and Therapeutics**

# Clinical & Intrinsic Molecular Subtypes of Breast Cancer

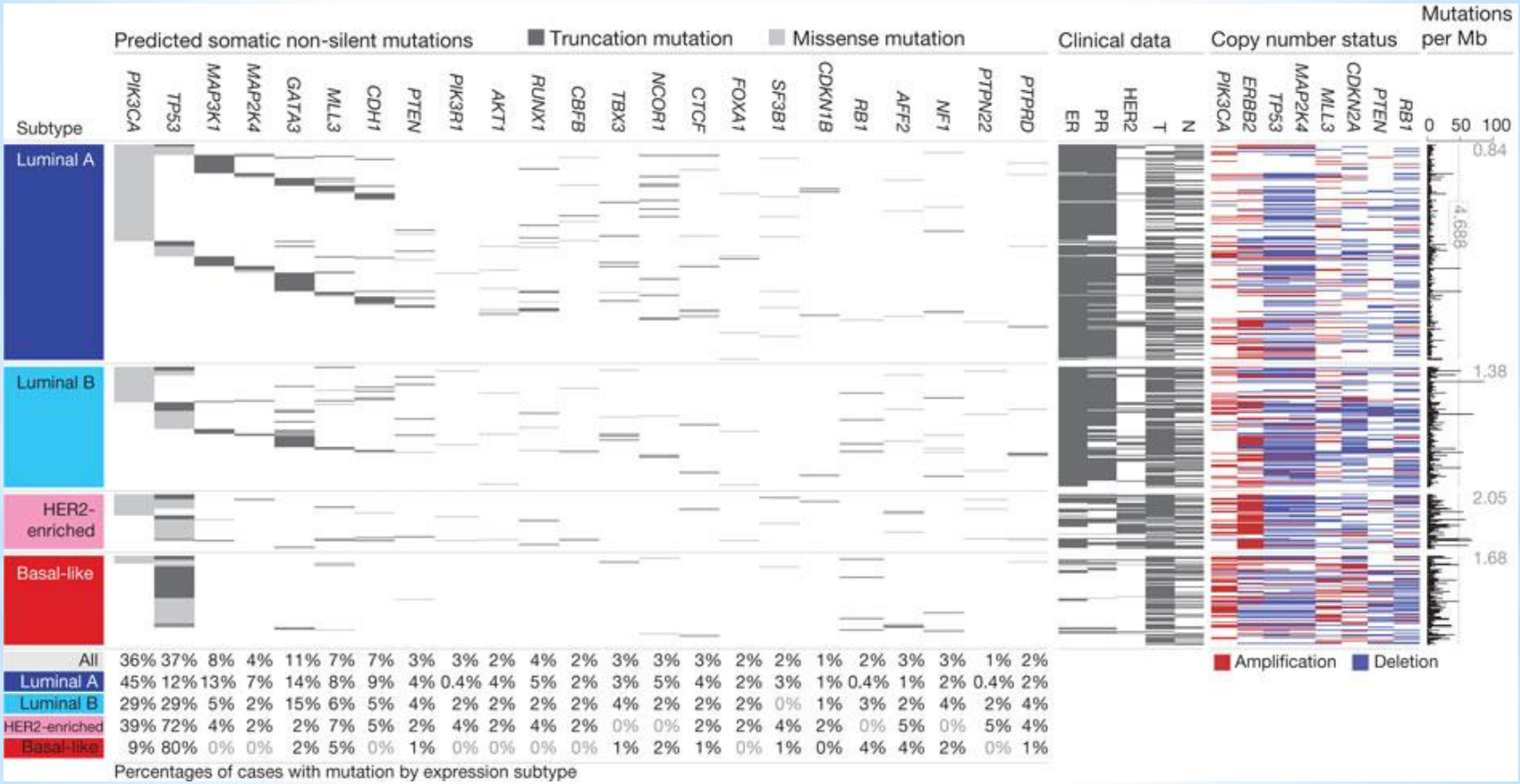



Cancer Genome Atlas Network., *Nature* (2012) & Reis-Filho, *Lancet* (2011).

# Estrogen Receptors (ERs) and p53 in Breast Cancer

- p53 mutations are infrequent in ERα+ breast cancer. 70-80% of tumors have wild type p53. However, p53 is functionally inactive.
- ERα− tumors express mutant p53.
- 80% of triple negative breast cancers (TNBC) express mutant p53 and ERβ.

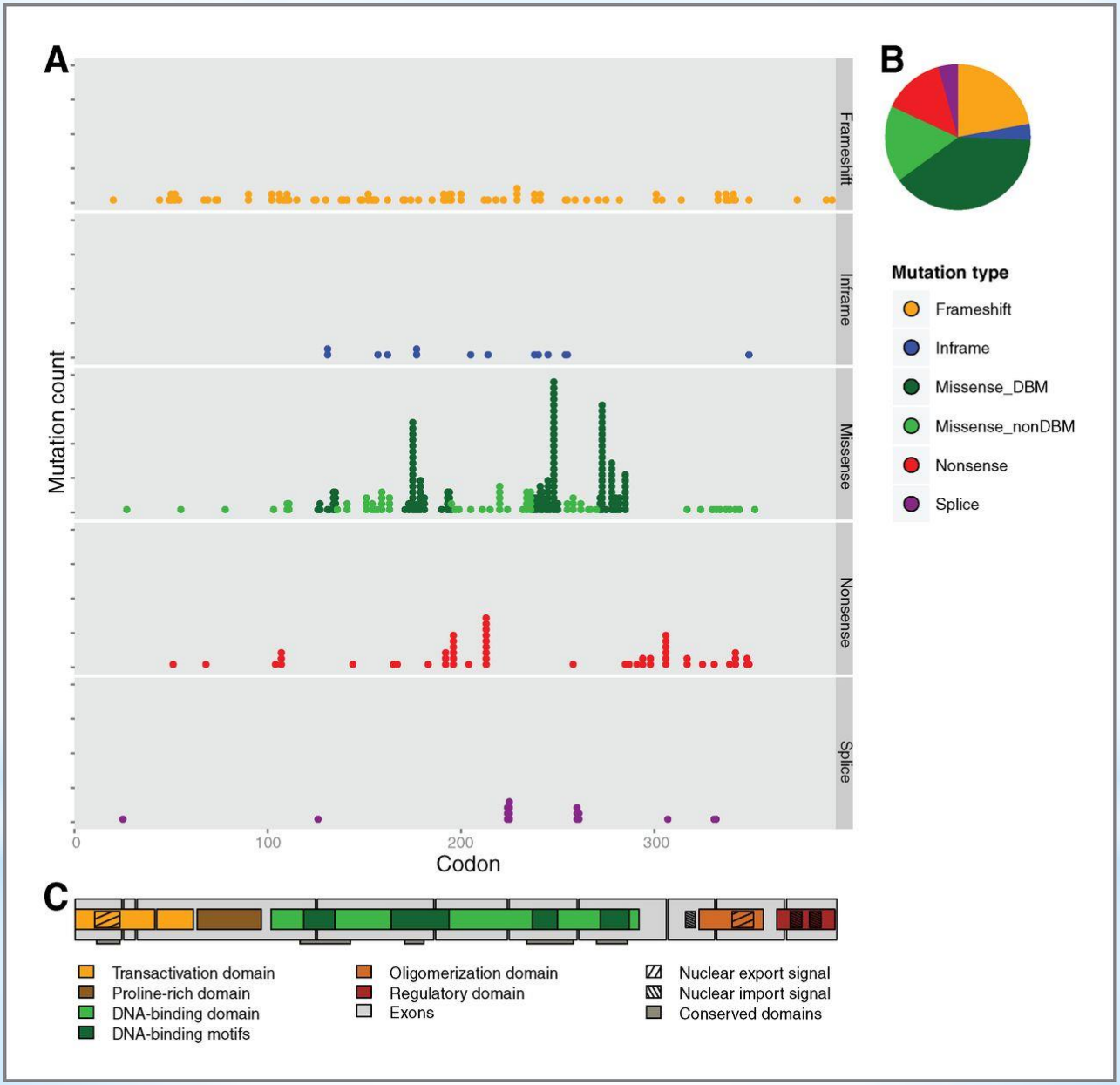
# Significantly mutated genes and correlations with genomic and clinical features




Tumour samples are grouped by mRNA subtype: luminal A (*n* = 225), luminal B (*n* = 126), HER2E (*n* = 57) and basal-like (*n* = 93). The left panel shows non-silent somatic mutation patterns and frequencies for significantly mutated genes. The middle panel shows clinical features: dark grey, positive or T2–4; white, negative or T1; light grey, N/A or equivocal. N, node status; T, tumour size. The right panel shows significantly mutated genes with frequent copy number amplifications (red) or deletions (blue). The far-right panel shows non-silent mutation rate per tumour (mutations per megabase, adjusted for coverage). The average mutation rate for each expression subtype is indicated. Hypermutated: mutation rates >3 s.d. above the mean (>4.688, indicated by grey line).

Koboldt et al. (TCGA), *Nature*, 490:61-70 (2012)

# TP53 mutation spectrum in breast cancer






Laxmi Silwal-Pandit et al. ***Clin Cancer Res*** 2014;20:3569-3580

# TP53 mutation spectrum in molecular subtypes

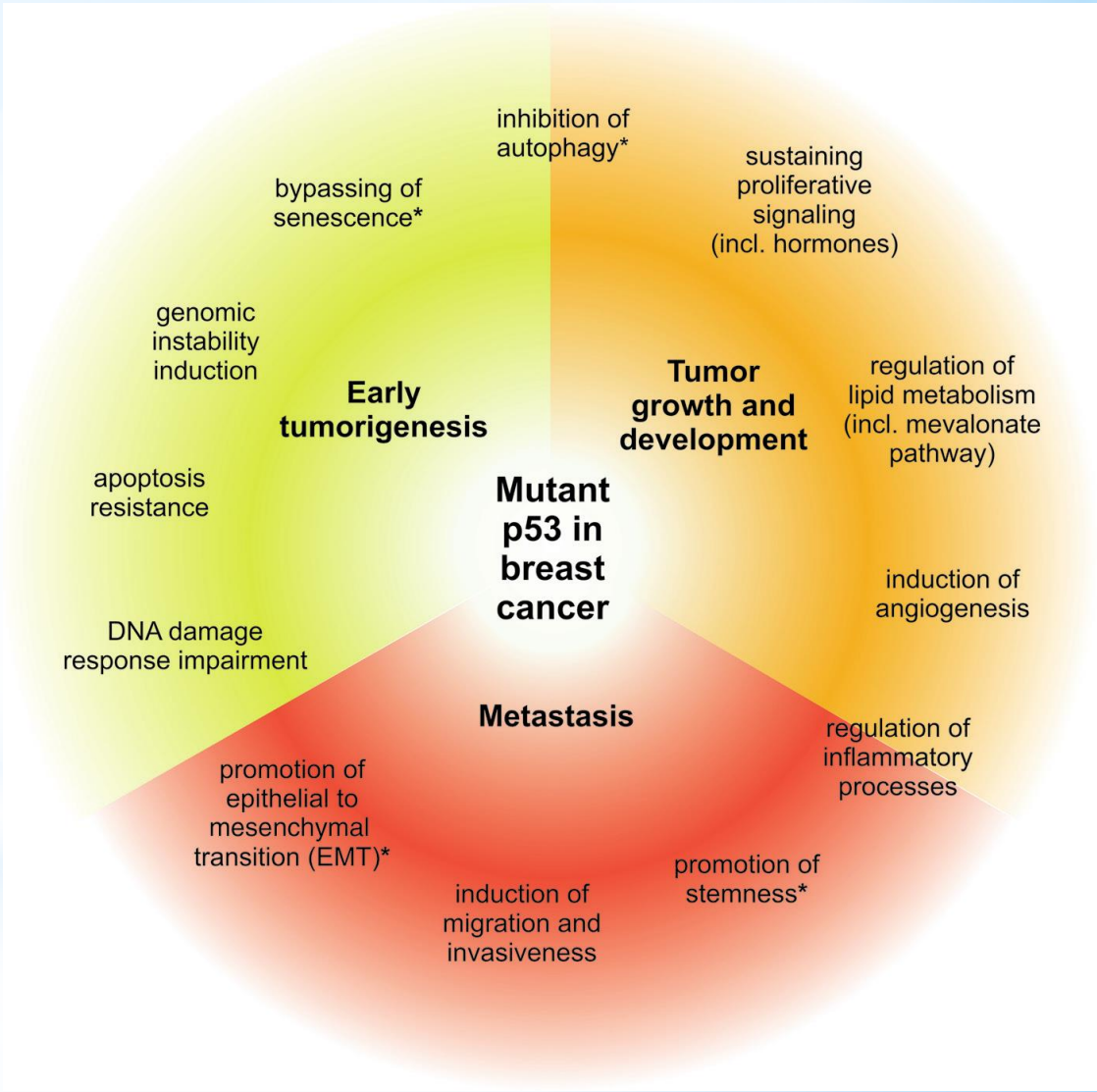

 Laxmi Silwal-Pandit et al. ***Clin Cancer Res*** 2014;20:3569-3580

# Mutant p53 in Breast Cancer

Most TP53 mutations in breast cancer occur in TNBC

* ~80% of TNBC express mutant p53

* Mutant p53 plays a role in tumor growth & metastasis in breast cancer




Walerych, et. al. *Carcinogenesis* 2012

# The Balancing Act

## Estrogen Receptor

Major player in oncogenesis

Induce cell division

Inhibit apoptosis

## p53

Major tumor suppressor

Arrests cell cycle

Activate apoptosis

# Estrogen Receptor Binds to p53

Immunoprecipitation

Chromatin Immunoprecipitation (ChIP)

Electrophoretic Mobility Shift Assay (EMSA)

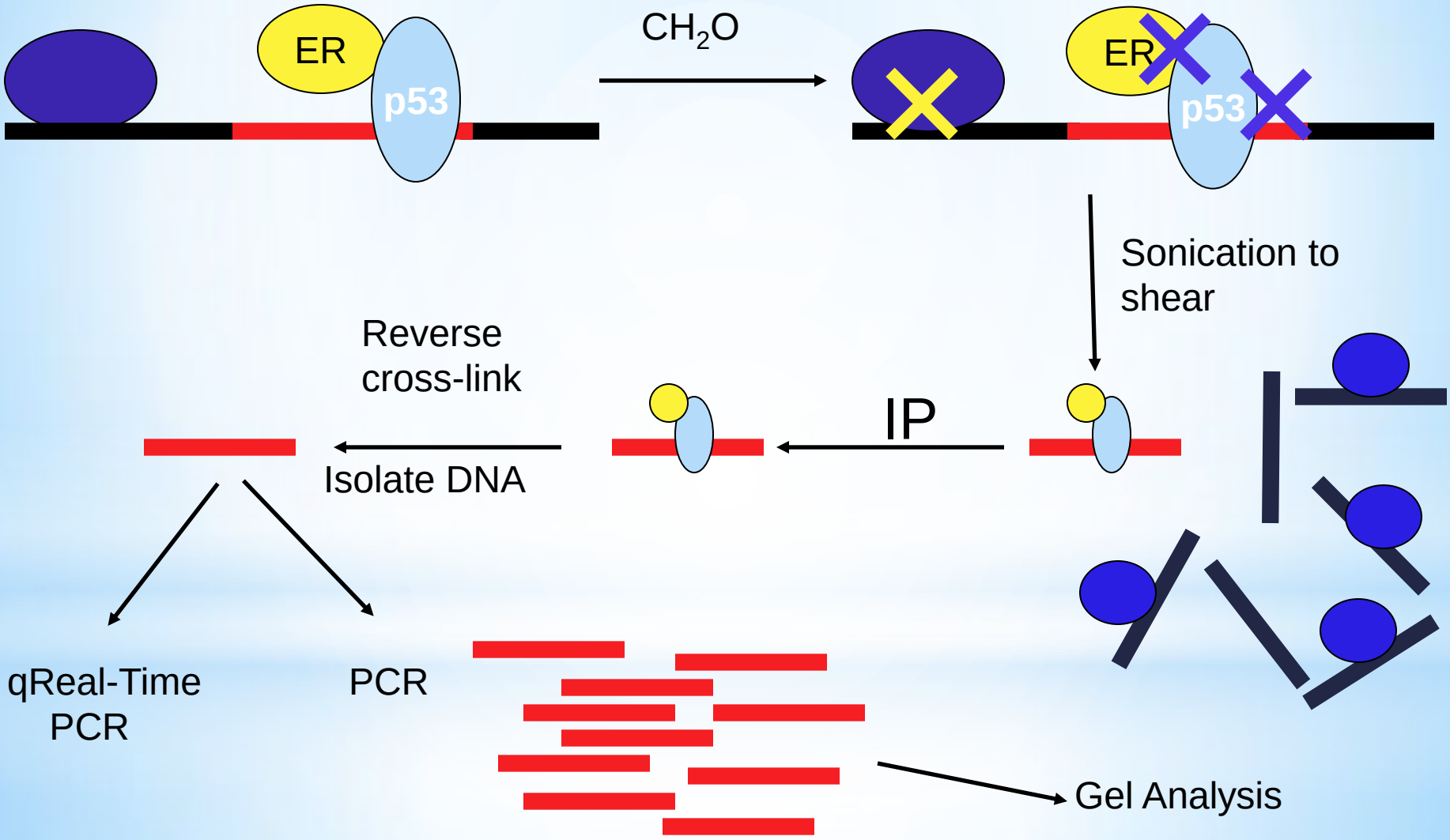
GST Pull-down Assay

Far Western Assay

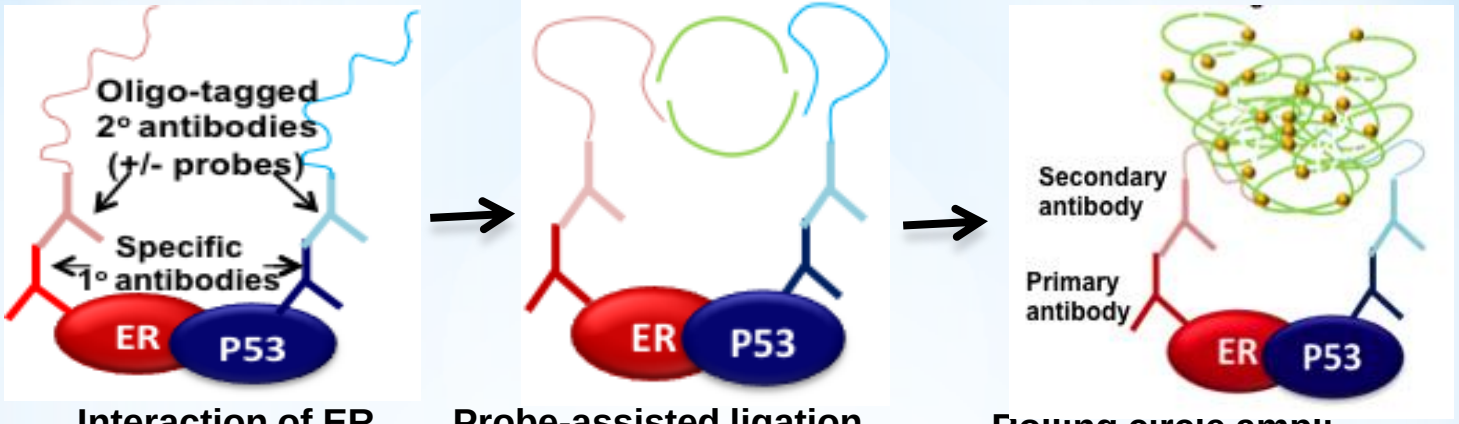
Confocal Microscopy

Proximity Ligation Assay (PLA)

# Chromatin Immunoprecipitation (ChIP) Assay



# ERα Binds to p53 in MCF-7 Luminal Breast Cancer Cells (PLA)

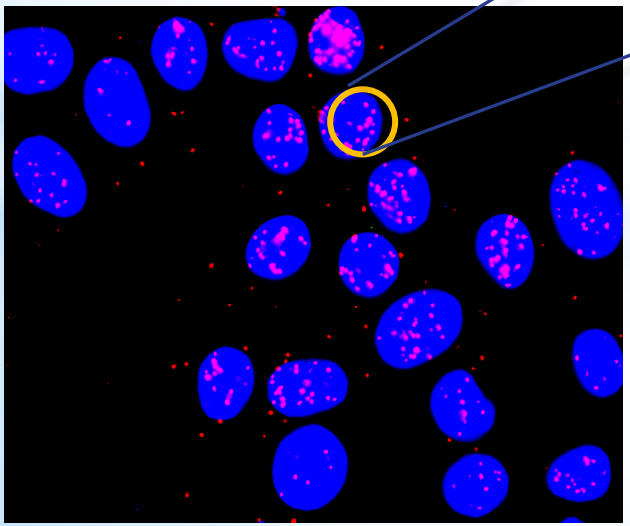



Interaction of ER and P53 allows proximity of +/- probes

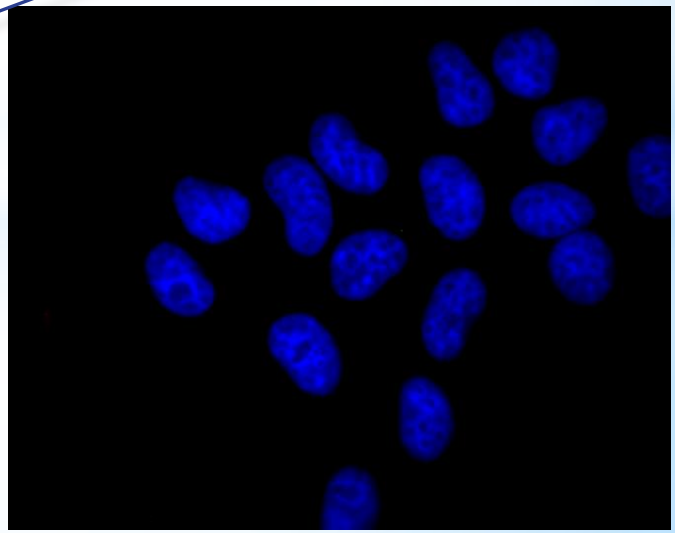
Probe-assisted ligation allows formation of circular template

Rolling circle amplification and Incorporation of fluorophore

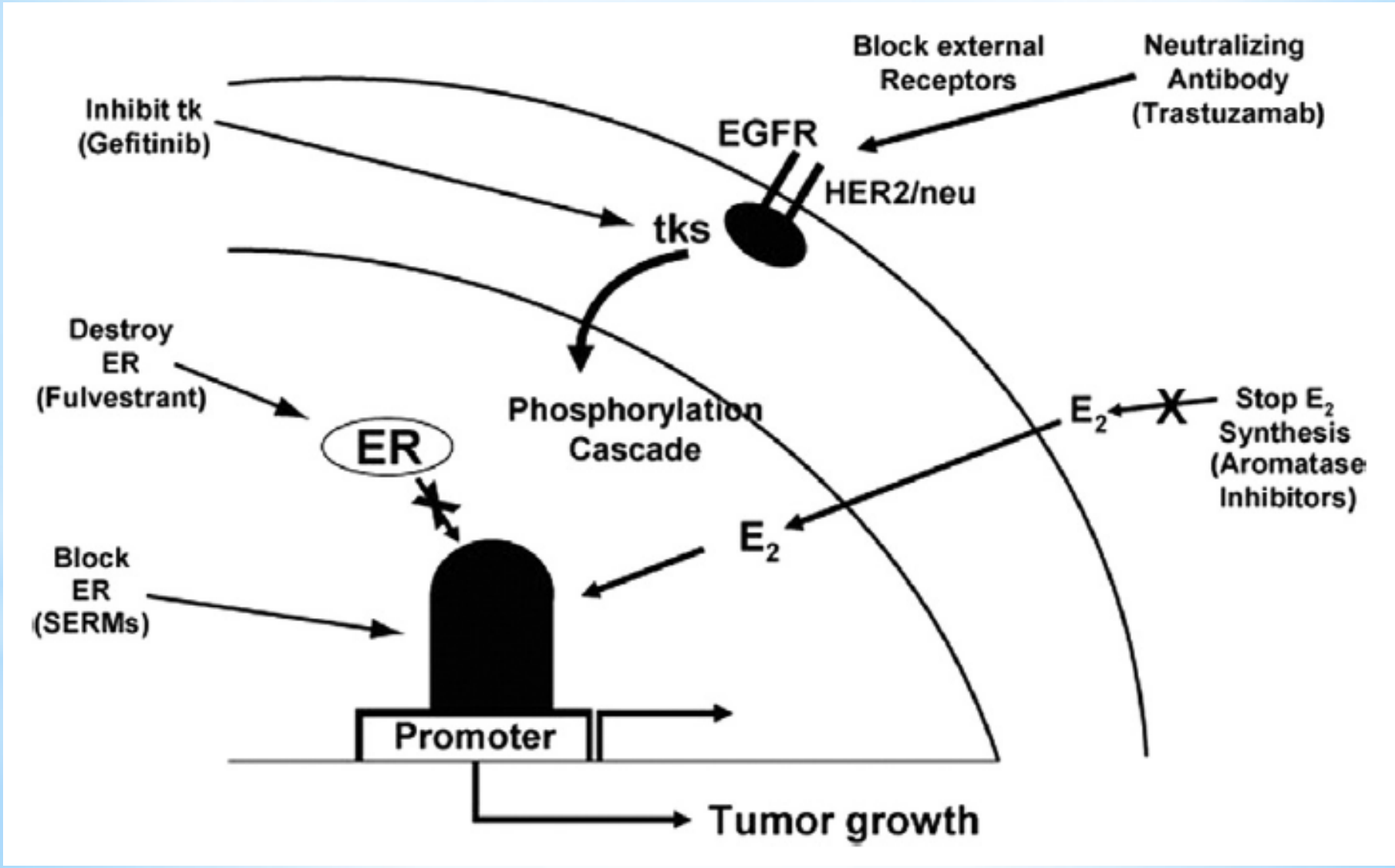
Non-specific siRNA



ERα siRNA

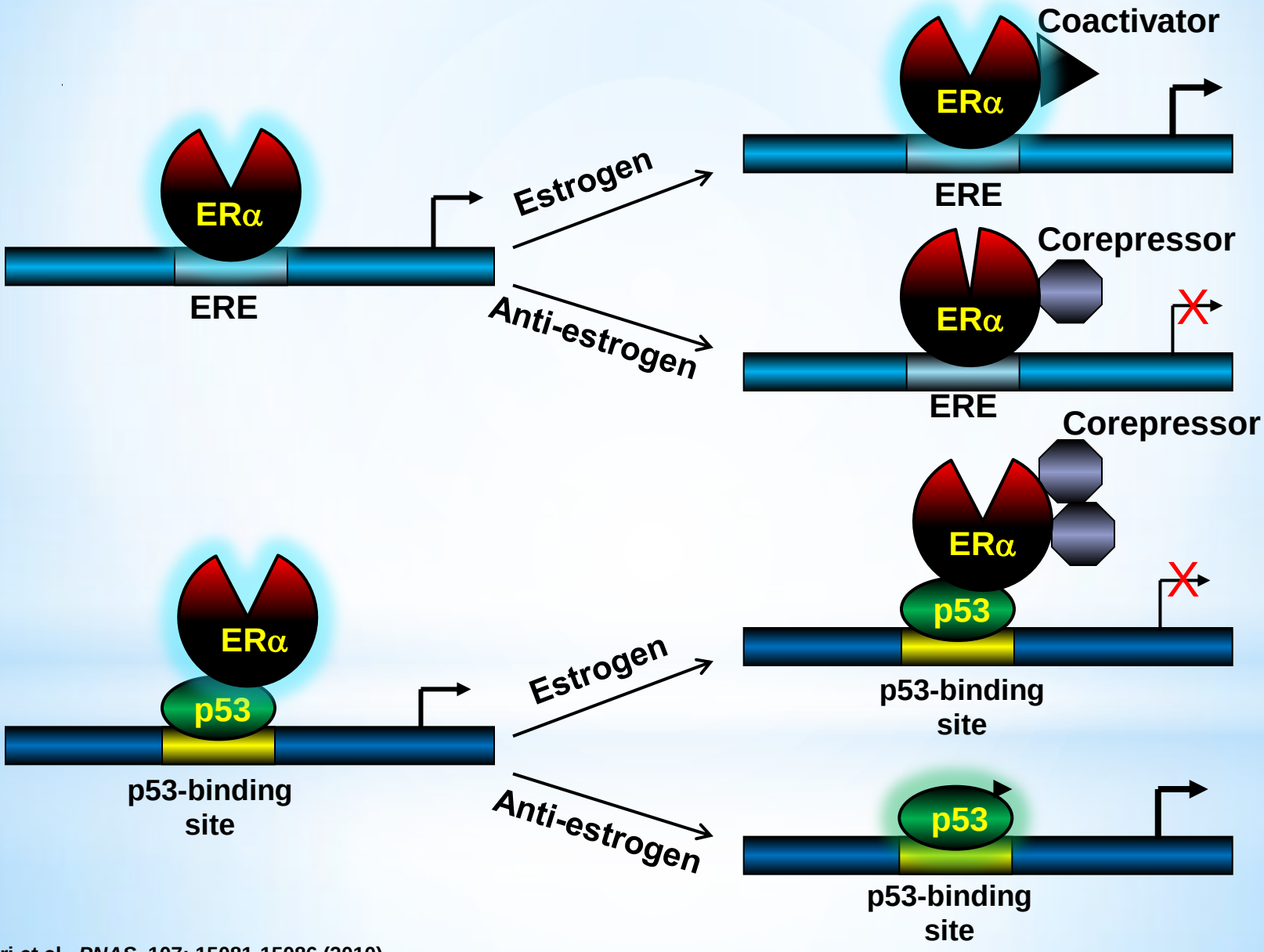


# Therapeutic Strategies that Affect ER Function



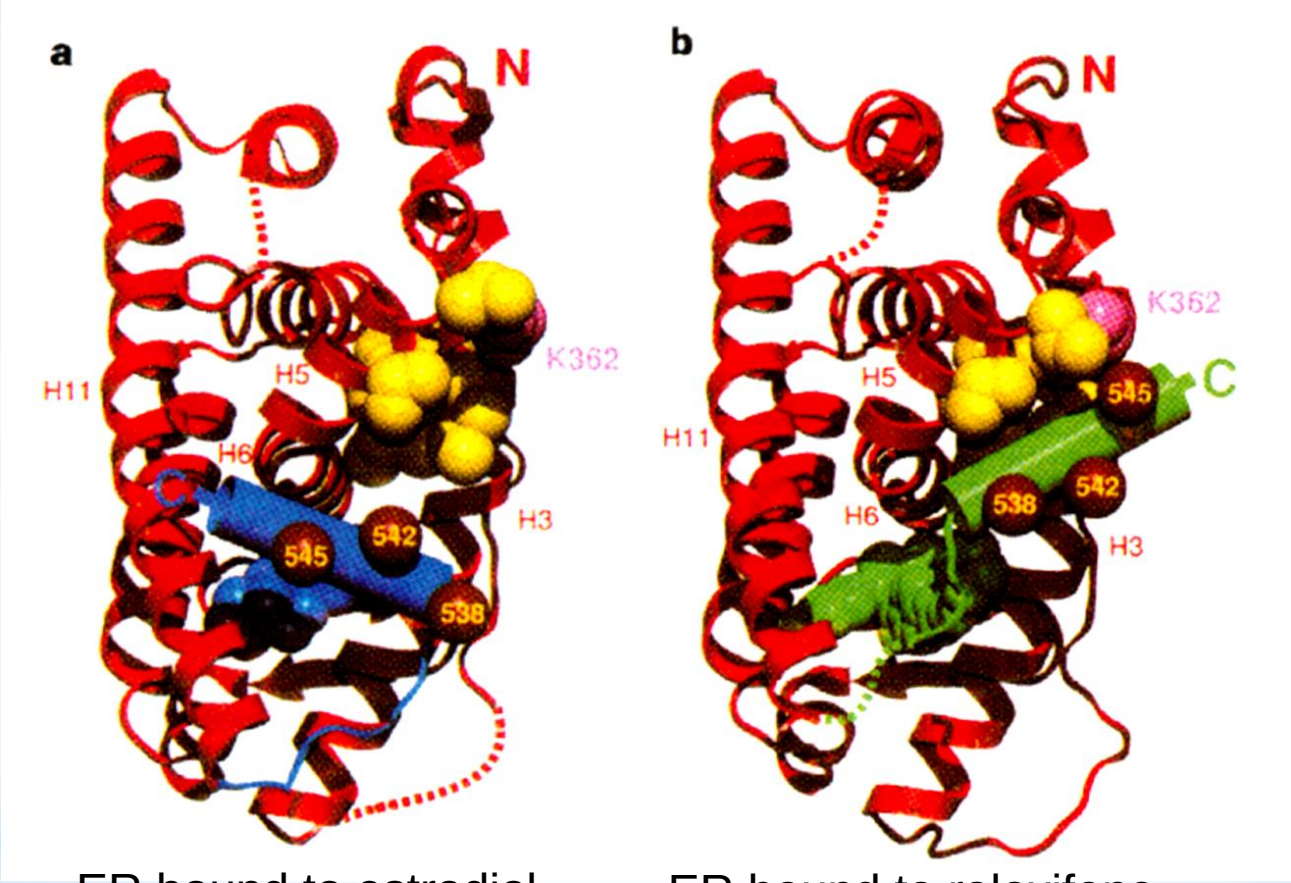

Cordera and Jordan, *Seminars in Oncology*, 2006. 33:631

# Dual Role of ERα in Promoting Oncogenesis




Konduri et al., *PNAS,* 107: 15081-15086 (2010)

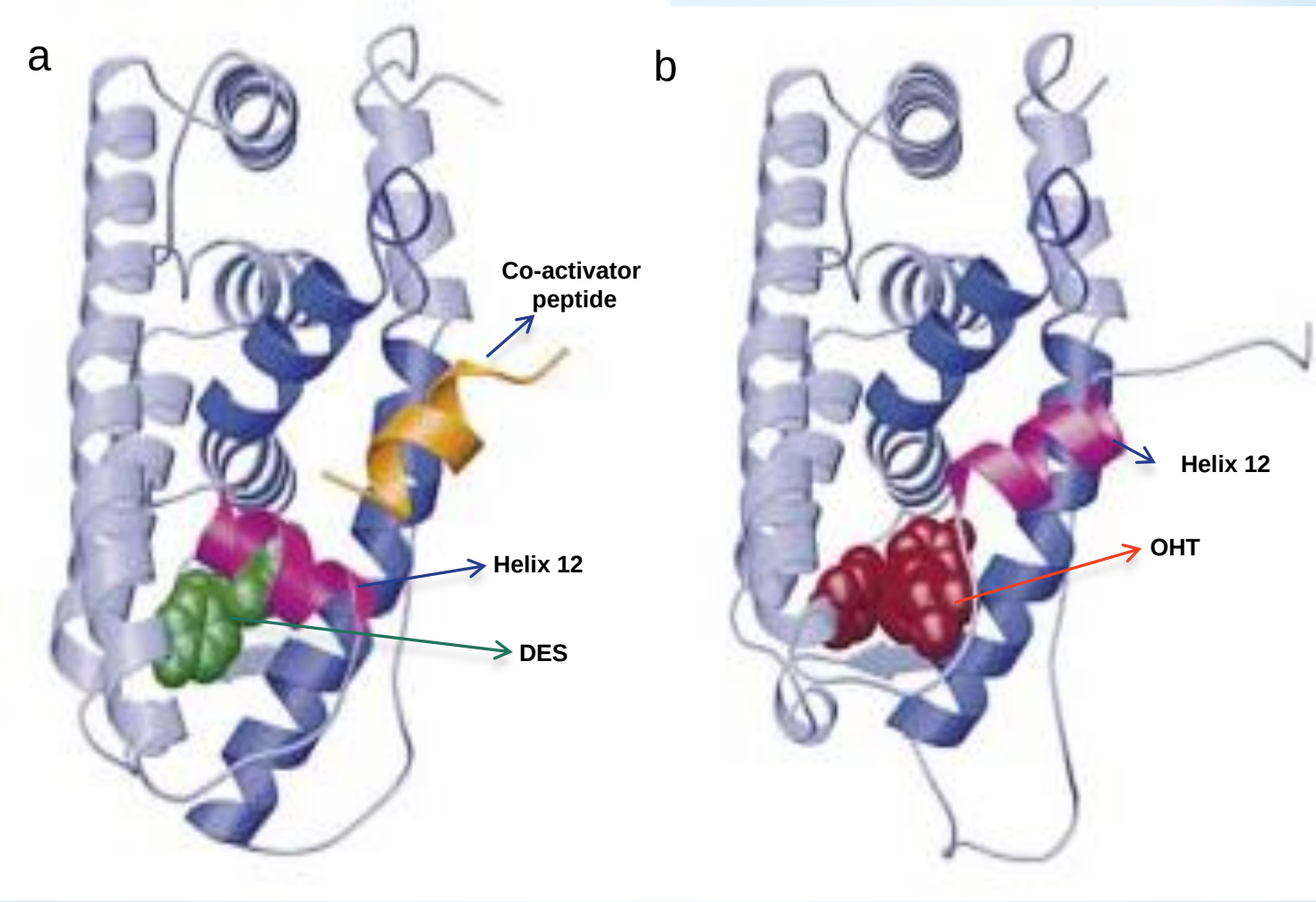
# Structure of ER α in the presence of 17 β Estradiol and Raloxifene




ER bound to estradiol ER bound to raloxifene

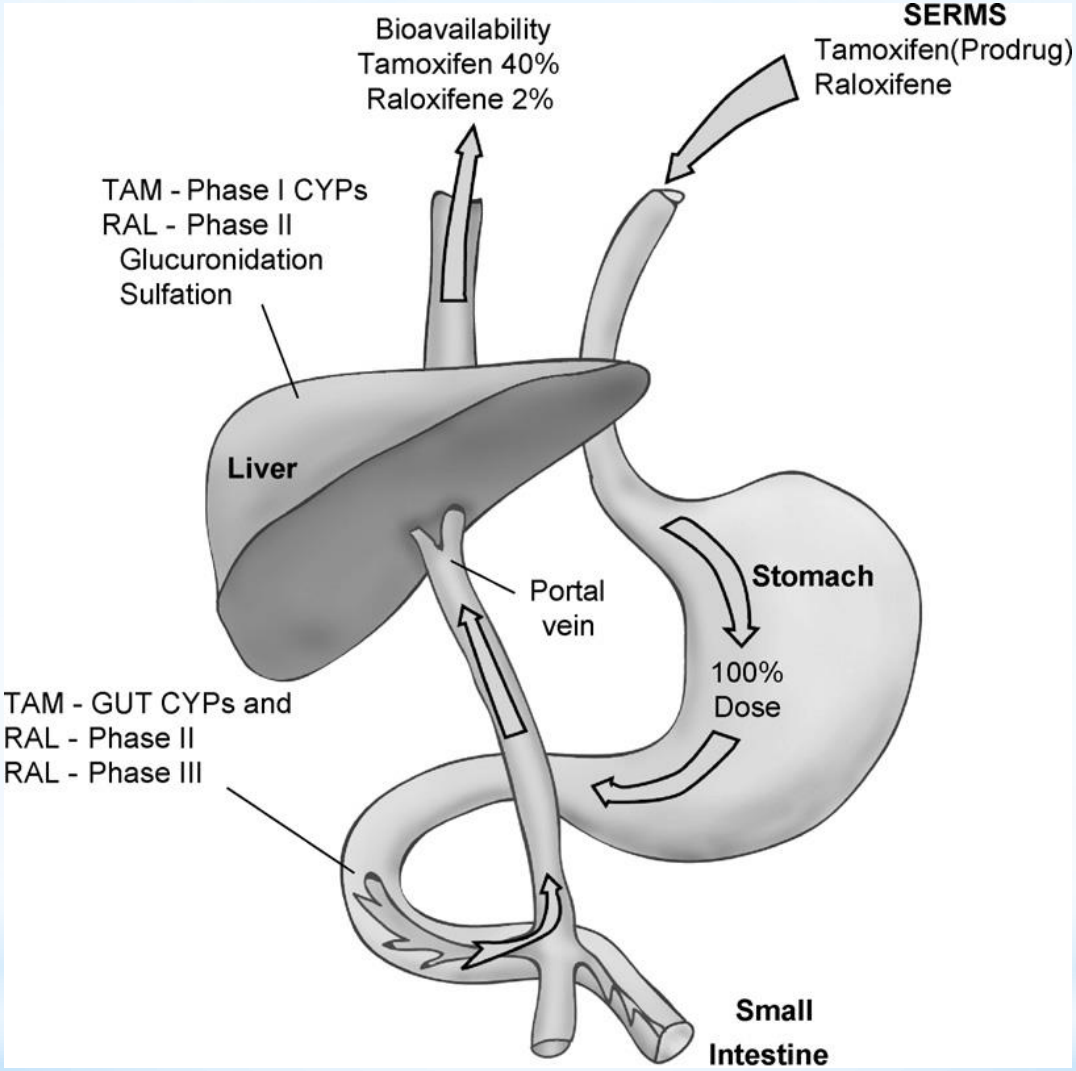
The successful crystallization of the ER ligand binding domain with estradiol (*left; a*) and an antiestrogen raloxifene (*right*; *b*) demonstrated that the bulky side chain moved helix 12 to maintain the jaws open and prevents estrogen action at the activating function 2 site on ER. The key to modulating the SERM ER complex became centered on D351 as a key amino acid that controls the estrogenic and antiestrogenic properties of the complex through interaction with the antiestrogenic side chain. Reproduced with permission from Brzozowski *et al*. Molecular basis of agonism and antagonism in the estrogen receptor.

Brzozowski et. al. Nature 389: 753 - 758 (1997)




Overall Structures of the agonist Diethyl stibestrol (DES -ERα LBD-coacivator peptide complex (a) and antogonist/anti-estrogen 4-hydroxy tamoxifen (OHT) – ERα LBD complex (b). LBD: Ligand binding domain.

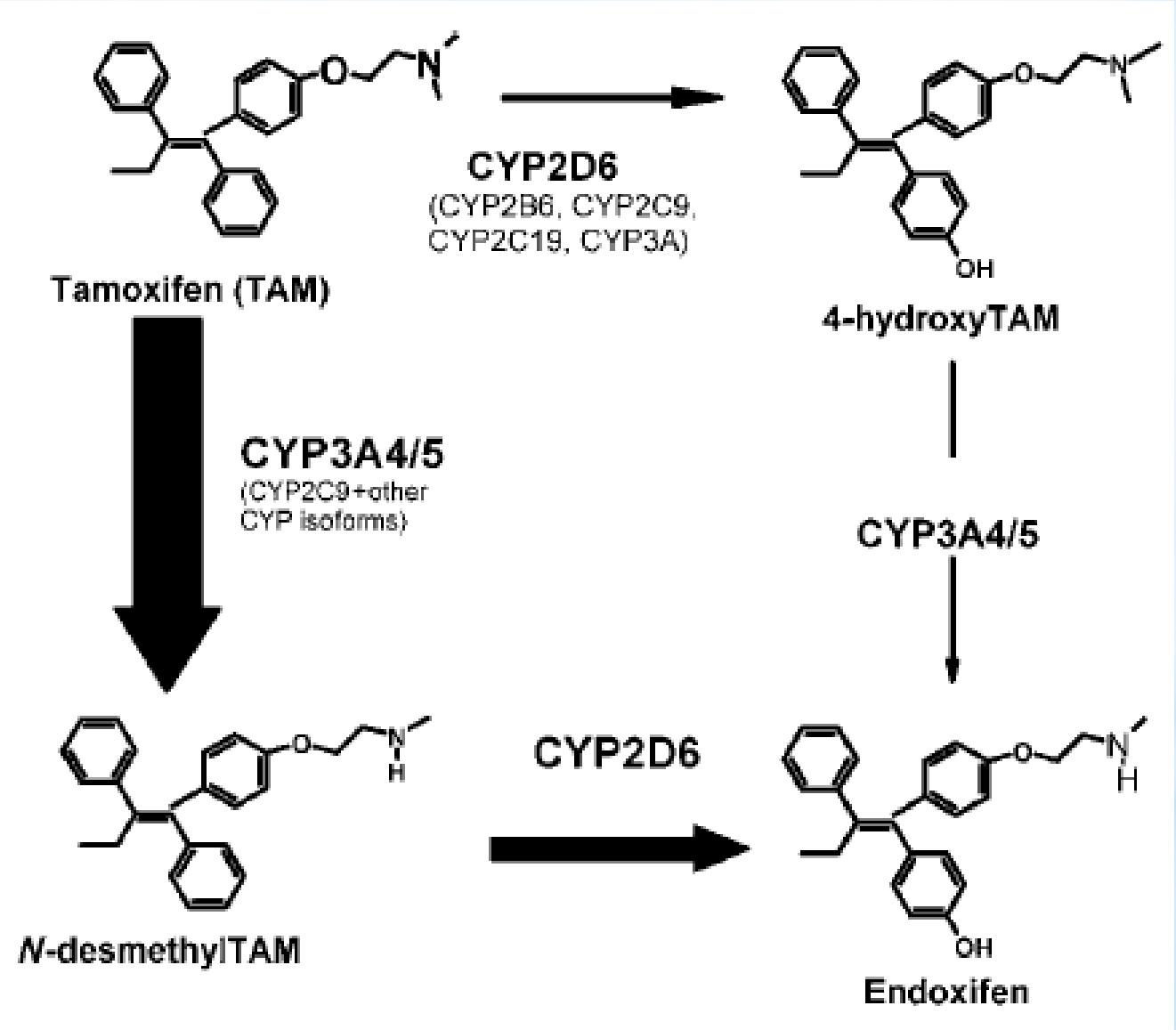
Shia et al., *Cell* 95, 927-937 (1998)




The stylized representation of the absorption of two selective estrogen receptor modulators (SERMS) tamoxifen (TAM) or raloxifene (RAL) into the circulation as bioactive molecules. The polyphenolic SERM raloxifene must transverse phase II and phase III obstacles in the gut and the liver to get into the general circulation. This results in very little of the ingested drug being bioavailable at target sites. In contrast, tamoxifen is extremely lipophilic and 98% protein bound to serum albumin. This extends the duration of action of tamoxifen because phase II metabolism to phenolic compounds is retarded.

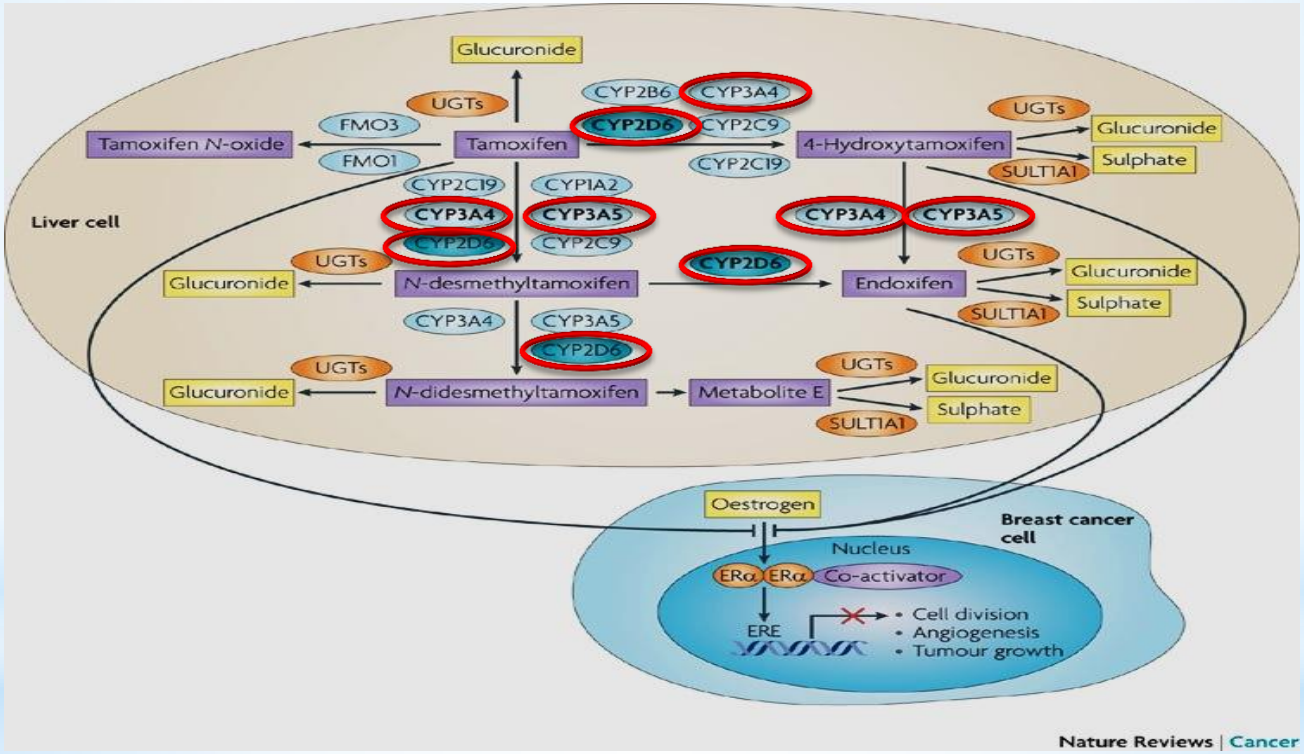
Craig Jordan, *Steroids*, 72: 829-842 (2007).

# Important Metabolic Pathways of Tamoxifen




Goetz et al., *Clinical Pharmacology & Therapeutics*, 2007. 83:160

# Tamoxifen Metabolism



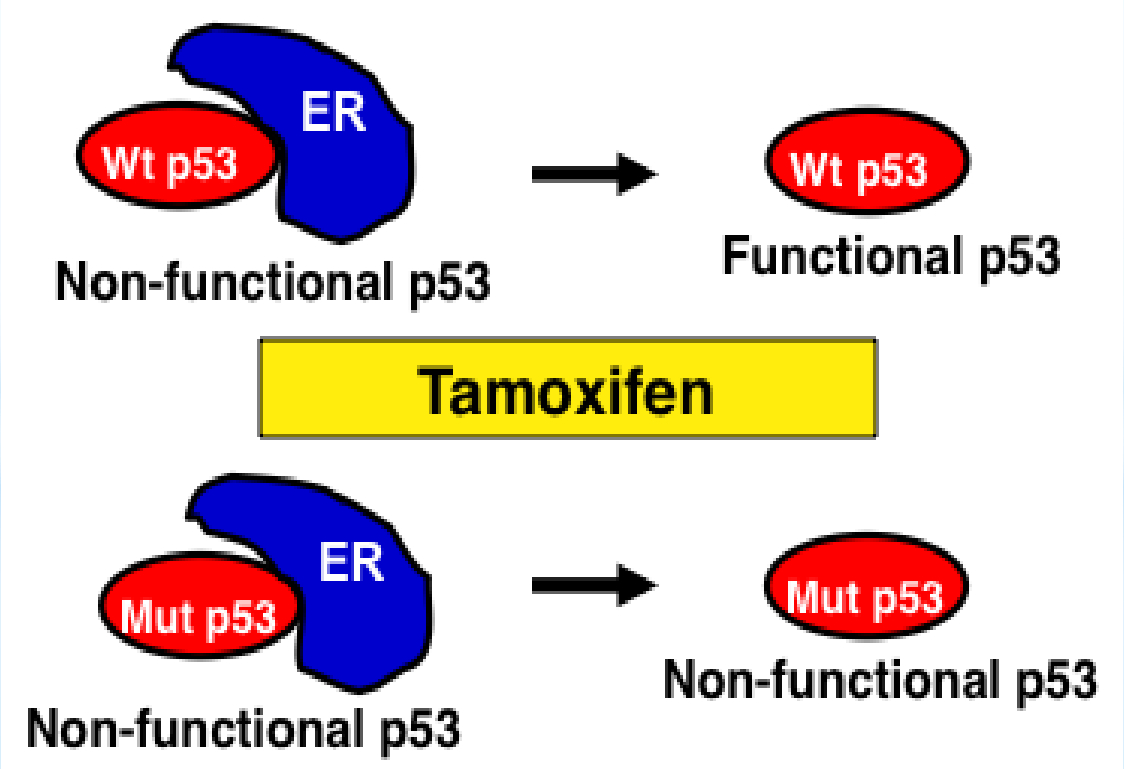

Partial metabolic pathway of tamoxifen and its interaction with oestrogen receptors (ers). The primary pathways of tamoxifen metabolism in the liver are catalysed by cytochrome P450s (CYPs), including CYP3A4, CYP3A5, CYP2C9, CYP2C19, CYP1A2, CYP2B6 and CYP2D6, and flavin-containing monooxygenases (FMOs), including FMO1 and FMO3 (shown in blue). The enzymes that are key for each metabolic pathway are shown in bold. Tamoxifen metabolism to **N**-desmethyltamoxifen is catalysed predominantly by CYP3A4 and CYP3A5, and metabolism to 4-hydroxytamoxifen is catalysed mainly by CYP2D6. The formation of these metabolites accounts for ~92% and ~7% of primary tamoxifen oxidation, respectively. Both of these metabolites are converted to 4-hydroxy-**N**-desmethyltamoxifen (endoxifen)13. Endoxifen formation from **N**-desmethyltamoxifen is almost exclusively catalysed by CYP2D6, and formation from 4-hydroxytamoxifen by CYP3A4 and CYP3A5 . Tamoxifen and its metabolites undergo phase II conjugation reactions, including glucuronidation and sulphation. In a breast cancer cell (shown in blue), oestrogen binds to the ER in the nucleus, leading to phosphorylation and dimerization. The complex recruits co-activators and binds to a specific DNA sequence, called the oestrogen response element (ERE), which is present in oestrogen-responsive genes. Binding of the ER dimer causes transcriptional activation of these genes. Subsequent translation produces proteins that are important for cell division, angiogenesis and survival, leading to sustained breast cancer growth and progression. This function is considered the classic action of ERs. SULT1A1, sulphotransferase 1A1; UGT, uridine diphosphate glucuronosyltransferase.

Hoskins et al., 2009. 9:576-586

# Hypothesis

## Tamoxifen, by relieving functional suppression of wild type p53 by ERα, could re-activate p53

* Tamoxifen may prevent ERα's ability to repress p53 function resulting in the activation of tumor suppressor pathways to prevent disease progression
* This advantage of tamoxifen therapy becomes irrelevant in tumors containing mutant inactive p53, thereby contributing to tamoxifen resistance

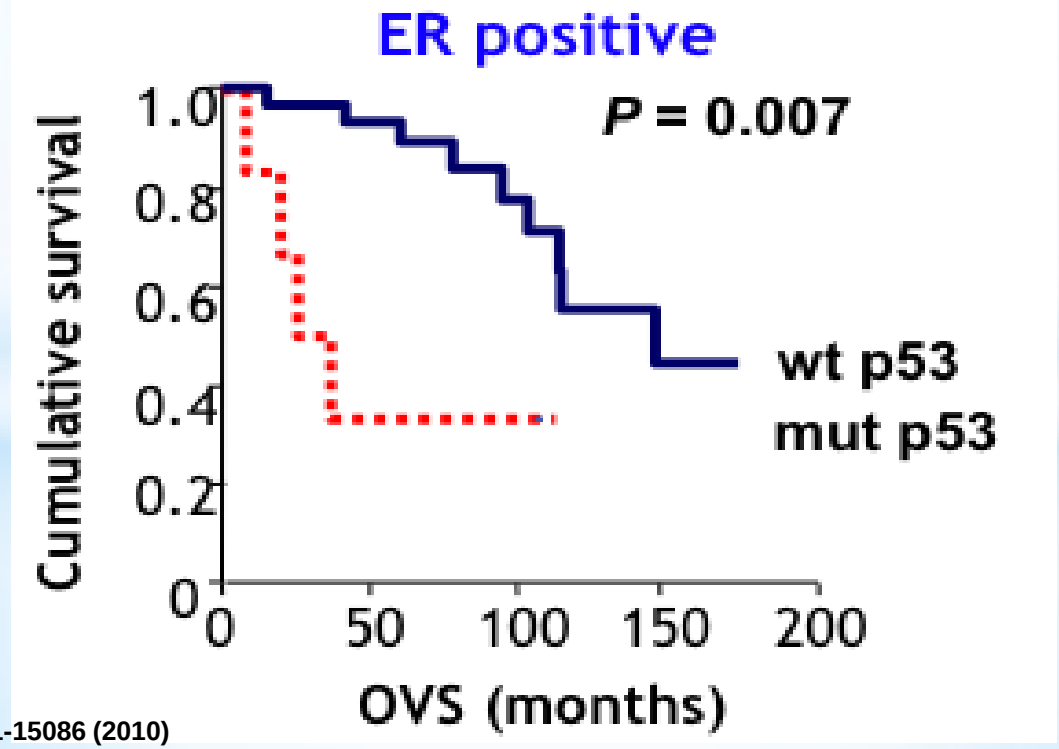



Konduri et al., *PNAS*, 107: 15081-15086 (2010)

# Retrospective Clinical Study

# Human ERα Posiitive Breast cancer Expressing wt p53 is more Responsive to Tamoxifen Therapy (Retrospective Study)

***Breast cancer patients***




Konduri et al., *PNAS,* 107: 15081-15086 (2010)

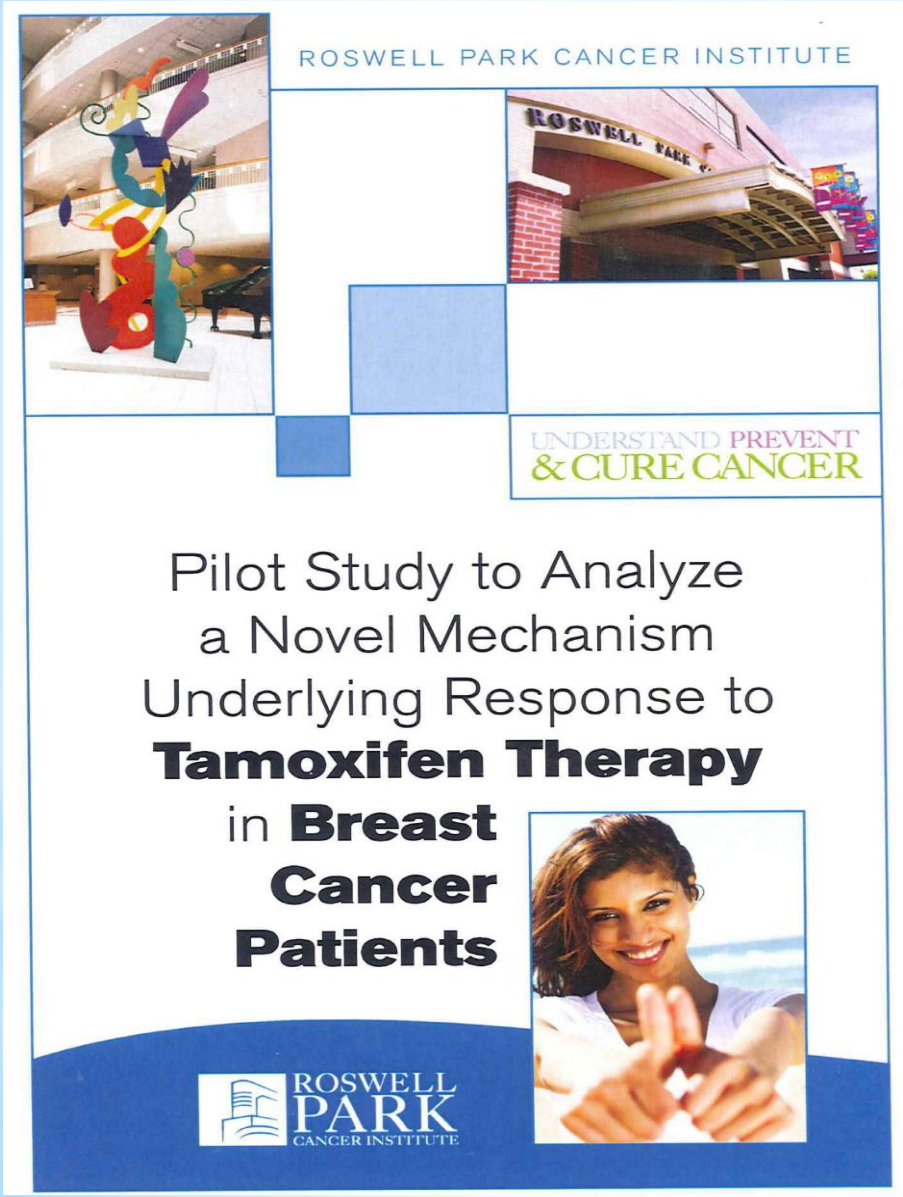
# Prospective Window-of-Opportunity Neo-Adjuvant Phase 2 Clinical Study

# Specific Aims

Investigate the status of ERα-p53 interaction in ERα-positive, p53-wild type breast tumors in untreated patients and examine how tamoxifen therapy impacts this interaction

Determine the effect of reactivation of p53 by tamoxifen on gene expression in breast tumors

# ‘Bench to Bedside’




**NCI Quick-Trials for Novel Cancer Therapies and Prevention: Exploratory Grants**

**PIs:**
**Gokul Das, Ph.D.**
**Swati Kulkarni, M.D.**

**Patients recruited at RPCI and University of Chicago Medical School**

# Eligibility Criteria

**Inclusion Criteria:**

(a) The patient must consent to be in the study and must have signed an approved consent form conforming to institutional guidelines
(b) The patient must be 18 years or older.
(c) Core biopsy should definitively demonstrate invasive carcinoma.
(d) Invasive carcinoma should be ER□ receptor positive
(e) The tumor should be approximately at least 1 cm, to account for variability in imaging and imaging occult disease (physical exam, mammography, ultrasound). We recognize that from time to time because of this variation, there might not be enough tissue available for analysis after surgical excision but this will allow the greatest opportunity to capture as many eligible patients as possible.
(f) Patients in whom surgical excision of the tumor is part of standard of care management
(g) ECOG score of 0 or 1
(h) Negative serum or urine □-hCG pregnancy test at screening for patients of child-bearing potential (this is routinely done if the patient is premenopausal and having surgery)
(i) Consent to participate in DBBR (RPCI only)

**Exclusion Criteria**:

(a) Male patients are not eligible for this study
(b) Female patients with inoperable tumors or women with stage 4 disease diagnosed on CT, PET, PET/CT or bone scan.
(c) Patients with diagnosis by FNA cytology only
(d) Pregnant or lactating women
(e) Prior therapy for breast cancer, including irradiation, chemo- immuno- and/or hormonal therapy
(f) Patients receiving any hormonal therapy, e.g. ovarian hormonal replacement therapy, infertility medications etc., are not eligible
(g) Nonmalignant systemic disease (cardiovascular, renal, hepatic, etc.) that would preclude the patient from being subjected to surgical excision
(h) Psychiatric or addictive disorders that would preclude obtaining informed consent
(i) Patients known or suspected to have hypercoagulable syndrome or with history of venous or arterial thrombosis, stroke, TIA, or pulmonary embolism
(j) Women with non-invasive disease or microinvasion are not eligible.
(k) Women undergoing neoadjuvant chemotherapy are not eligible
(l) women currently on tamoxifen and raloxifene for prevention are not eligible
(m) Patients shall not receive any herbal/alternative therapies such as flaxseed or soy products or black cohosh.
(n) Patients with a known mutation in p53 (Li Fraumeni Syndrome)

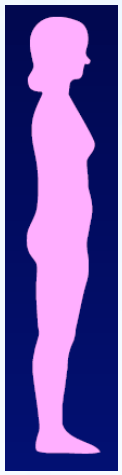
# Prospective Window-of-Opportunity Phase II Clinical Study

**Core Biopsy**:
IHC
ER, p53, and their selected targets

Pharmacogenomics
CYP2D6 SNPs
CYP3A4/5 SNPs

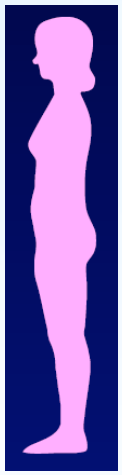
PK/PD
Tamoxifen
Metabolites

N=30



**Patients with ER(+) Luminal Breast Cancer**

No history of BC
No germline p53 muttion
No endocrine Tx



N=23

**Core Biopsy**:
IHC
ER, p53, and their selected targets

Pharmacogenomics
CYP2D6 SNPs
CYP3A4/5 SNPs

PK/PD
Tamoxifen
Metabolites

**No Intervention**

4 weeks
20 mg PO daily
**Tamoxifen**

**Surgery**

**Tumor**

**TMA**

IHC
ER, p53, and their selected targets

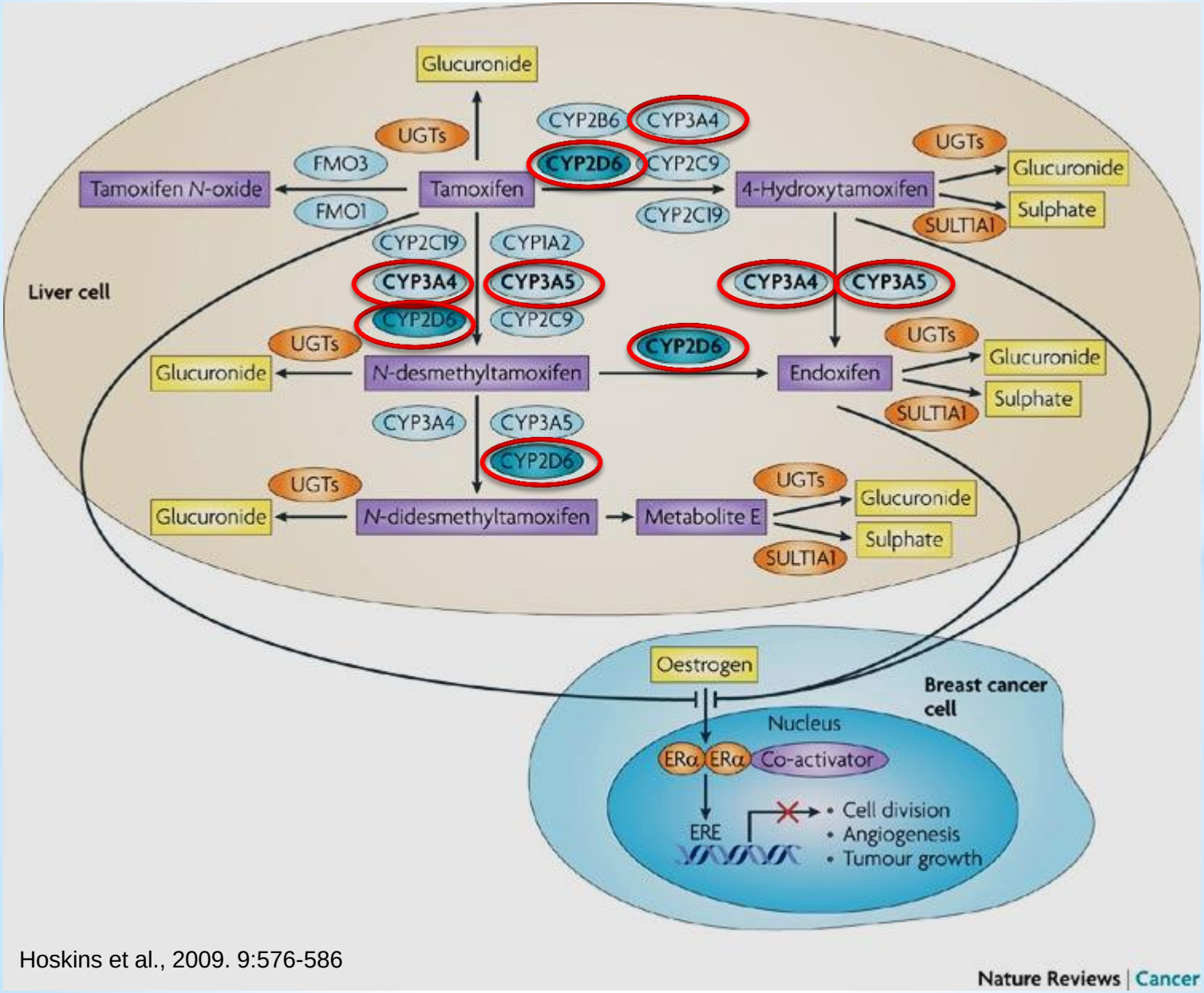
**Intra-operative Core Biopsy:**
Tam and active metabolites

p53 mutation status (NGS)

ER-p53 interaction (PLA)

p53 & ER target gene expression (RNA-seq & RPPA)

# Tamoxifen Metabolism




Hoskins et al., 2009. 9:576-586

Nature Reviews | Cancer

# Novel effect of tamoxifen therapy: disruption of ERα-p53 interaction leading to altered gene expression profile in human breast tumors

Swati Kulkarni[1,2] , Chetan Oturkar[1], Stephen Edge[1], Jianmin Wang[1], John Wilton[1], Wendy Swetzig[1,2], Araba Adjei[1], Robert Bies[1], Alan Hutson[1], Adrienne Groman[1], Carl Morrison[1], Jerry Fetterly[1], Schicha Kumar[1], Helen Cappucino[1] and Gokul Das[1]

Roswell Park Cancer Institute[1] & Northwestern University Feinberg School of Medicine[2]

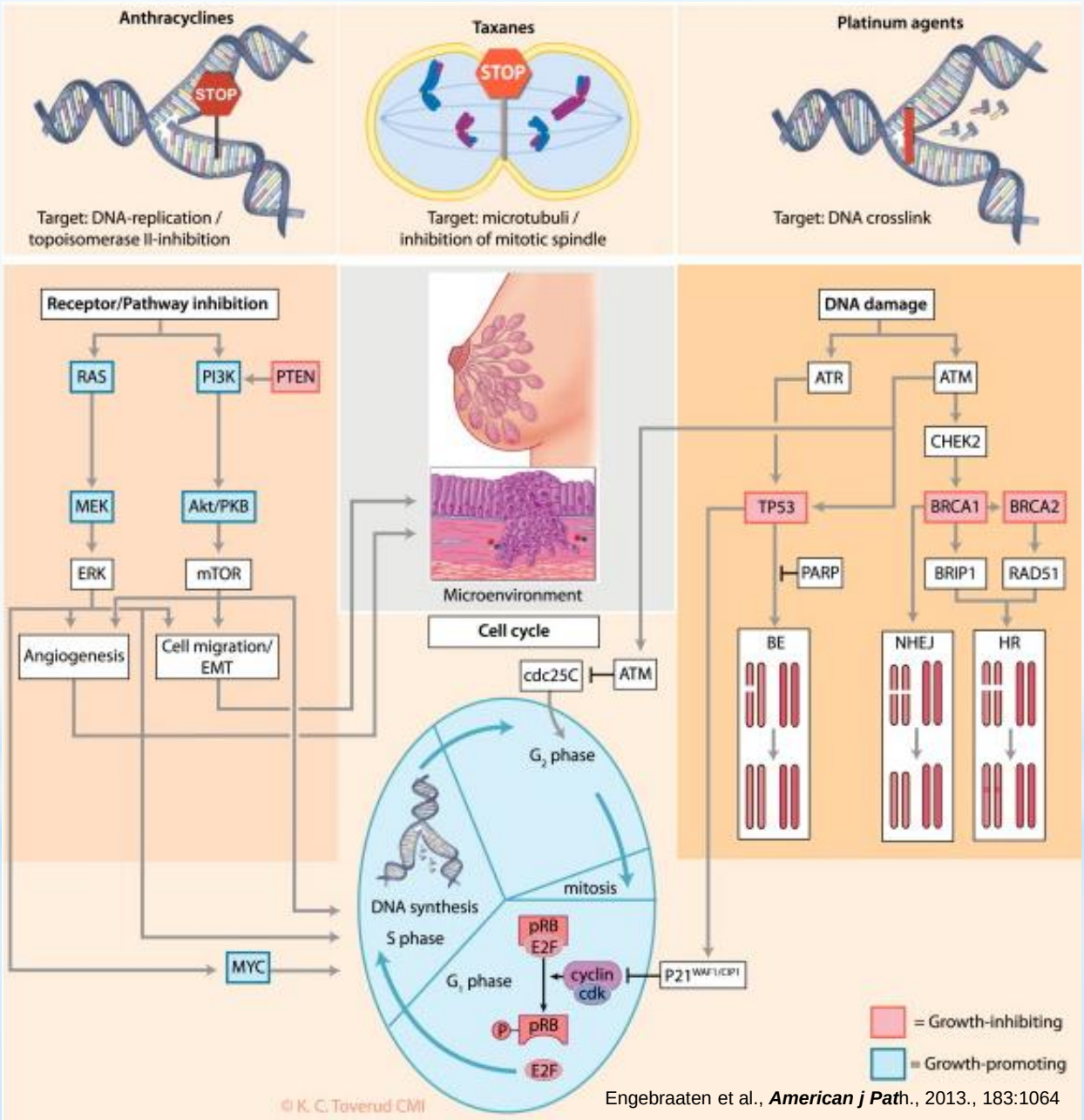
ROSWELL PARK CANCER INSTITUTE

Northwestern Medicine | Robert H. Lurie Comprehensive Cancer Center of Northwestern University

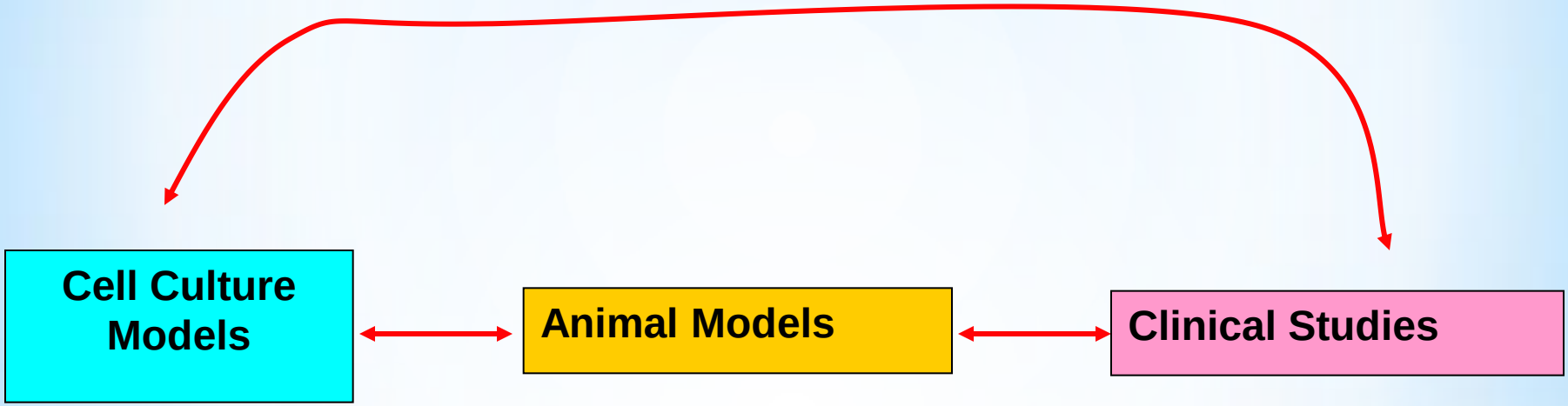
# Conclusions from the Clinical Trial

- Tamoxifen disrupts ER-p53 interaction in luminal breast cancer patient tissues leading to reactivation of p53
- Genes representing several pathways, especially metabolic pathways are differentially expressed in tumors from patients treated with tamoxifen
- provides insight into the mechanism underlying favorable response of wt p53 to TAM therapy,
- Has implications toward stratifying ER+ BC patients to those who will or will not be responsive to TAM therapy.
- The study provides important input into the importance of a therapeutic strategy based on p53 status-dependent stratification of  ER-positive breast cancer

# Currently used Therapies and Potential Molecular Targets in TNBC




Engebraaten et al., ***American j Pat***h., 2013., 183:1064


Cell Culture Models
Animal Models
Clinical Studies

Questions?