Breast Cancer
-From Basic Research to Clinical Trial

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Clinical & Intrinsic Molecular Subtypes of Breast Cancer

- Basal-like (20%)
  - TP53 mut: 29%
  - ERα+: 80%
  - ERβ+: 72%

- HER2-enriched (10%)
  - TP53 mut: 72%
  - ERα-:
  - ERβ+:

- Luminal A (45%)
  - ERα+:
  - ERβ+:

- Luminal B (25%)
  - TP53 mut: 29%
  - ERα+:
  - ERβ+:

Endocrine Therapies
Chemotherapy
Radiotherapy
Surgery

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).

TP53 mutation spectrum in breast cancer

A

B

Mutation type
- Frameshift
- Inframe
- Missense_DBM
- Missense_nonDBM
- Nonsense
- Nonsense
- Splice

C

Transactivation domain
Proline-rich domain
DNA-binding domain
DNA-binding motifs
Oligomerization domain
Regulatory domain
Exons
Nuclear export signal
Nuclear import signal
Conserved domains
TP53 mutation spectrum in molecular subtypes

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

ER → CH₂O → ER

Reverse cross-link
Isolate DNA

qReal-Time PCR
PCR

Gel Analysis

Sonication to shear

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

ERα siRNA
Therapeutic Strategies that Affect ER Function
Dual Role of ERα in Promoting Oncogenesis

Structure of ER α in the presence of 17 β Estradiol and 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.

Overall Structures of the agonist Diethyl stibestrol (DES -ERα LBD-coactivator peptide complex (a) and antagonistic/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.

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 monoxygenases (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). 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

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

Prospective
Window-of-Opportunity
Neo-Adjuvant
Phase 2 Clinical Study
Specific Aims

Investigate the status of ER$\alpha$-p53 interaction in ER$\alpha$-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.
Pilot Study to Analyze a Novel Mechanism Underlying Response to Tamoxifen Therapy in Breast Cancer Patients

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

Pls:
Gokul Das, Ph.D.
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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

Patients with ER(+) Luminal Breast Cancer

No history of BC
No germline p53 muttition
No endocrine Tx

N=30

No Intervention

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)

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

Pharmacogenomics CYP2D6 SNPs CYP3A4/5 SNPs

PK/PD Tamoxifen Metabolites

N=23

4 weeks 20 mg PO daily Tamoxifen

Patients with ER(-) HER2 Breast Cancer

No history of BC
No germline p53 muttition
No endocrine Tx

N=23

No Intervention

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

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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

Engebretsen et al., American J Path., 2013., 183:1064
Questions?