Identification of a Notch-driven breast cancer stem cell gene signature for anti-notch therapy in an ER+ presurgical window model

Dr. Albain: Merck Oncology provided the compounds MK-0752 and MRK-003 studied in this project, constructed the microarrays from the human tumor biopsies and reimbursed patients for the extra biopsy. The Breast Cancer Research Foundation provided a research grant.

Dr. Zlobin: Nothing to disclose.
Dr. Covington: Nothing to disclose.
Dr. Hilsenbeck: Nothing to disclose.
Dr. Czerianis: Nothing to disclose.
Dr. Lo: Nothing to disclose.
Dr. Robinson: Nothing to disclose.
Dr. Guynor: Nothing to disclose.
Dr. Godellas: Nothing to disclose.

Dr. Bova: Nothing to disclose.
Ms. Czaplicki: Nothing to disclose.
Ms. Busby: Nothing to disclose.
Dr. Stiff: Nothing to disclose.
Dr. Fuqua: Nothing to disclose.
Dr. Osipo: Merck Oncology, Breast Cancer Research Foundation.
Identification of a Notch-Driven Breast Cancer Stem Cell Gene Signature for Anti-Notch Therapy in an ER-Positive Presurgical Window Model


Loyola University Chicago Cardinal Bernardin Cancer Center
Baylor College of Medicine Duncan Cancer Center
Louisiana State University Cancer Center

Background

- Resistance to endocrine therapy (ET) causes mortality, new treatment paradigms needed
- Cancer stem cells drive tumor growth, resistant to ET
- Notch signaling:
  - aids stem cell survival
  - aberrantly increased during ET
  - inhibited by gamma-secretase inhibitors (GSI)
- Adding GSI to ET has major anti-tumor effect in xenografts
- Presurgical window biomarker modulation study completed* to test strategy in humans

*Albain et al. PSABC 2011

This presentation is the intellectual property of the author/presenter. Contact Kathy S. Albain, MD at kathalb@lumc.edu for permission to reprint and/or distribute.
Endocrine Therapy (ET) Plus MK-0752 (GSI)
Presurgical Window Biomarker Modulation Study*
(20 patients, ER+, any age)

Day 1

Core biopsy

Tamoxifen 20 mg
OR Letrozole 2.5 mg PO daily x 14 days

Day 14

Core biopsy

Continue endocrine therapy x 10 days,
ADD
MK-0752 350 mg PO
3 days on
4 days off
3 days on*

Day 25

Definitive surgery

Global gene expression profiling by microarray
Validation by qRT-PCR

Candidate Biomarkers

Working Hypothesis from Preliminary Analyses

ERα

GSI

ET

Notch-1

Cyclin D1
RUNX1

MMP7

NOXA

Proliferation (Ki67), Endocrine resistance


This presentation is the intellectual property of the author/presenter. Contact Kathy S. Albain, MD at kathryn@lumc.edu for permission to reprint and/or distribute.
Objectives

- Identify genes/pathways affected by ET + added GSI from microarray analyses (3 time points)
- Select candidate genes to validate by qRT-PCR
- Confirm ET+GSI suppresses Notch-induced genes/pathways
- Determine genes critical for breast cancer stem cell survival and altered by GSI

Pathway Analyses
qRT-PCR Gene Validation
Key Genes and Pathways Modulated* by Short Exposure to GSI

- Notch signaling
- Cancer stem cells
- Cell cycle & proliferation
- Metastasis
- Fatty acid biosynthesis
- Estrogen signaling
- PI3-kinase/mTOR signaling
- Apoptosis
- Tumor suppression

* Affymetrix 20,000 gene expression microarrays (Albain et al. PSABC 2011)

METHODS qRT-PCR Analysis 33 Genes

This presentation is the intellectual property of the authors/presenter. Contact Kathy S. Albain, MD at kathys@lumc.edu for permission to reprise and/or distribute.
18 of 33 Genes Changed Significantly (FDR<8%)

<table>
<thead>
<tr>
<th>Expression Pattern</th>
<th>Interpretation</th>
<th>N of Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>[graph]</td>
<td>Probable GSI effect</td>
<td>14</td>
</tr>
<tr>
<td>[graph]</td>
<td>GSI effect</td>
<td>0</td>
</tr>
<tr>
<td>[graph]</td>
<td>ET effect, or GSI effect added to ET effect</td>
<td>4</td>
</tr>
<tr>
<td>[graph]</td>
<td>No effect of either ET alone or of added GSI</td>
<td>15</td>
</tr>
</tbody>
</table>

ET, endocrine therapy; GSI, gamma secretase inhibitor

This presentation is the intellectual property of the author(s). Contact Kathy S. Abrams, MD at kablais@tumor.com for permission to reprint and/or distribute.

Notch Signaling

[Graphs showing gene expression over time with statistical significance notes]
Cell line and mammosphere analyses
METHODS
Genes Critical for Stem Cell Survival and Altered by GSI

- Performed mammosphere forming assays
- Measured cancer stem cell survival with/without GSI in 3 ER+ cell lines
- Knocked down or overexpressed 18 genes from the qRT-PCR results in an ER+ cell line (work in progress)
- Done in absence of estrogen (mimicking ET), with/without GSI

GSI Blocks Mammosphere Formation

This presentation is the intellectual property of the author/presenter. Contact Kathy S. Altshuler, MD at kaltshul@lunice.edu for permission to reprint and/or distribute.
GSI Blocks Mammosphere Formation

MCF-7  T47D  ZR-75-1

Vehicle  

GSI  

Which of the 18 genes are necessary for inhibition of stem cell survival?

DAXX Required for GSI Blockade of Mammosphere Formation in Absence of E2

DAXX+  DAXX-low (knockdown)

-E2  

-E2 + GSI  

This presentation is the intellectual property of the author/presenter. Contact Kathy S. Altshuler, MD at labinfo@tumor.md for permission to reprint and/or distribute.
Conclusions

- Discovered genes/pathways affected by ET and ET+GSI from expression arrays of serial biopsies
- Selected 33 genes for validation by qRT-PCR
- Confirmed ET+GSI suppresses 18 Notch and Notch-induced genes
- Identified biomarker gene critical for Notch-regulated stem cell survival, may be predictor of GSI inhibition

---

Critical Cancer Genes and Pathways Significantly Modulated by Short Exposure to GSI during ET

At least one gene (DAXX) is required for GSI inhibition of mammosphere formation
Implications

- Notch regulates 18 genes that promote breast cancer stem cell growth, inhibited by GSI
- Genes could define a signature of anti-Notch GSI therapy efficacy
- DAXX (potentially others) may be predictive marker of GSI inhibition of stem cell survival

Future Plans

- Stem cell renewal inhibition and human xenograft recurrence studies (with GSI+ET) underway
- GSI MK-0752 has promise in optimizing ET and overcoming resistance
- Randomized neoadjuvant trial of ET versus ET+GSI warranted
  - efficacy
  - validate 18 gene signature predicts GSI benefit
  - test biomarker candidates (DAXX expression, others) as predictors of major response
Acknowledgements

- Our patients, who submitted to extra biopsies and postponed their surgery
- Breast Cancer Research Foundation (KSA, CO)
- DOD Fellowship grant BC073237 (KRC)
- Baylor Duncan Cancer Center NCI grant CA125123 (SGH, SAWF, KRC)
- NCI P01CA166009 (LM)