New Directions in Translational Molecular Research

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Focus on Genomics of ER-Positive Breast Cancer:
Translation to the Clinic

- Matthew Ellis
- Jim Dixon
- Obi Griffith
- Ryan Hartmaier
- Peter Schmid
- Alana Welm
- Yibin Kang
“Rediscovery” of ESR1m in Metastatic Tumors Using Next Generation Sequencing

Hormone-independent

Hormone-dependent

Nat Gen Nov 2013
CR & CCR 2014

S Fuqua

Constitutively Active Conformation of Ligand-binding Domain Mutations by Aberrant Hydrogen Bonding

Y537S and D538G: agonist conformation in the absence of ligand

Therefore ESR1m are hormone-independent, resistant to aromatase inhibitors

M Ellis
Transcriptional Activity of ESR1m is Resistant to Tamoxifen

Toy, W. Nat Gen 2013
Similar results in Jeselsohn, R. Can Res 2014 and Segal CCR 2014

Treatment may require a better antiestrogen

ESR1m Hotspot: Response to Fulvestrant

Robinson D. Nat Gen, 2013

Clinical question are they resistant to tamoxifen, but sensitive to fulvestrant?
Patient-derived xenografts—Good for preclinical testing of therapeutics

Next Generation Oral SERD ARN-810 is active in ESR1 Y537S Mutant Mouse Xenograft Models

WHIM20 Y537S

Oral ER degrader

Next Generation Sequencing

Few highly recurrently mutated driver/targetable genes

HER2 mutations 1.5% of breast cancers

ESR1 mutations 0.6% of luminal cancers

www.cbioPortal.org: TCGA Breast (provisional); n=962

J. Reis-Filho
# Whole genome sequencing in a clinical trial

**ARTICLE**

Whole-genome analysis informs breast cancer response to aromatase inhibition

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<table>
<thead>
<tr>
<th>Mutated Tyrosine Kinase</th>
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<th>Mutation(s)</th>
<th>Candidate Drugs</th>
<th>Comments</th>
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<td>G152L, F281K, D419H</td>
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<td>A629V</td>
<td>Nilotinib, Imatinib, and INNO-406*</td>
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</tbody>
</table>

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**HER2 Somatic Mutations**

- Blue circle from Bose et al, Red from Ross et al
- From 8 publications with a total of 1,499 patients
- 45% of patients had mutations at amino acids 519 or 515
- 68% of patients had mutations at amino acids 575-780

Cancer Discovery 3; 1-14 2013

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**M Ellis**
Truncating NF1 mutations drive poor outcome in ER+ breast cancer

NF1m

Conclusions

- Analysis of metastatic breast cancer genomes reveals ESR1 mutations and translocations are an important mechanism for endocrine therapy resistance.
- Pre-clinically estrogen receptor degrading agents overcome ESR1 Y537 mutation driven resistance
- Mutant HER2 tumors can be successfully treated with neratinib but exhibit acquired resistance
  - Pharmacological restoration of RB and TP53 tumor suppressor function is therapeutic when these genes are wild-type.
- Genomic analysis of metastatic disease is an essential perquisite for logical clinical trial development.
- Neoadjuvant endocrine therapy trials should be embraced as the way forward for genome-driven combination studies in the early disease setting - can we get ER+ uCR rate up?
In-depth genomic analysis of ER+ breast cancers during development of endocrine resistance

Breakthrough Labs and Edinburgh Breast Unit Scotland UK and Lineberger Comprehensive Cancer Center University of North Carolina USA

Aromatase Inhibitor Resistance

- Continuous Letrozole Treatment
  - Post menopausal women
  - ER Rich Alfred 7 or 8
  - Large operable or LA
  - Invasive breast cancer

- Non-responder
  - Static

- Non-responder
  - NR

- Responders
  - Continued Response

- Baseline 14-days
  - 3-months
  - 6-months = 3 years

- Predictive Test
  - Fresh tissue at baseline + 14 days
  - 73 patients Edinburgh
  - 44 patients Royal Marsden

- mRNA expression
- Understanding Resistance
  - 17 patients Edinburgh
  - enriched for Non response
  - Multiple biopsies at baseline 14 days + 6m-3y when responding and resistant

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J Dixon
Loss of Mutations in Another Responder

Development of Mutations with Acquired Resistance

J Dixon
Summary

- Molecular subtypes change with response
  - Non responders more likely to be Lum B at start or end
  - Responders more likely Lum A or become Lum A
- Loss in mutations in responding cancers
- New mutations develop in non responding cancers
  - These changes suggest clonal selection is occurring
- Only one ESR1 mutation in this series
  - Responder - fall in MAF of mutation
- Baseline analysis insufficient to predict ET response
  - Need to analyse cancer DURING therapy
- 4 Gene Model (2 Genes AT Dx and 2 at 14d) predicts response to letrozole with high degree of accuracy

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Prognostic effects of gene mutation in estrogen receptor positive breast cancer

Obi Griffith, Nick Spies, Malachi Griffith, Jingxin Luo, Jasreet Hundal, Katie Campbell, Chris Miller, David Larson, Robert Fulton, Richard Wilson, Shuzhen Liu, Samuel Leung, Torsten Nielsen, Elaine Mardis, Matthew Ellis

SABCS, San Antonio, Texas
Wednesday, December 10, 2014
Background

- 632 ER+ patients treated with five years of adjuvant tamoxifen monotherapy with an average 10.4 year follow-up (Nielsen et al CCR 16:5222, 2010)
- 83 genes selected based on meta-analysis of mutation recurrence data from TCGA (2012), Ellis (2012), Shah (2012) + 5 more studies AND breast cancer relevance
- Probes designed to capture complete exon regions for targeted sequencing and analysis

Mutation rates recapitulate TCGA exome-sequencing results

Also: five KRAS G12/13; one BRAF V600E; 28 AKT1 E17K
**Genes significantly associated with RFS and BCSS**

<table>
<thead>
<tr>
<th>Gene</th>
<th>BCSS</th>
<th>RFS</th>
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<tr>
<td>DDR1</td>
<td>0.0001</td>
<td>2.52</td>
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<tr>
<td>FOXC1</td>
<td>0.0029</td>
<td>2.09</td>
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<tr>
<td>TP53</td>
<td>0.0498</td>
<td>1.25</td>
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<tr>
<td>MAP3K1</td>
<td>0.0553</td>
<td>0.64</td>
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<tr>
<td>ARID1B</td>
<td>0.0149</td>
<td>0.59</td>
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<tr>
<td>ERBB3</td>
<td>0.0434</td>
<td>0.36</td>
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<tr>
<td>PK3CA</td>
<td>0.0464</td>
<td>0.77</td>
</tr>
<tr>
<td>SMG1</td>
<td>0.0467</td>
<td>0.57</td>
</tr>
</tbody>
</table>

* FDR < 0.05. Larger studies still needed for some genes.

**ERBB2 kinase domain mutations in 5.0% of samples**

* Bose et al, 2012, Cancer Discovery. Note: many mutations at low variant allele frequency – only detected with depth*
No correlation of ESR1m status and response

Summary

- Excellent sequence coverage possible even from very old FFPE
- Extensive filtering and manual review required (especially in absence of normal)
- Recurrently mutated genes confirmed and novel/enriched hotspots discovered
  - CBFB splice site mutation
- ERBB2 activating and ESR1 resistance mutations possibly more frequent than previously reported
- Several prognostic associations identified
  - Known/expected: PIK3CA, TP53, MAP3K1
  - Novel: DDR1, FOXC1, ARID1B, ERBB3, SMG1, NF1

O Griffith
Identification of Base Pair Mutations and Structural Rearrangements Acquired in Breast Cancer Metastases Including a Novel Hyperactive ESR1-DAB2 Fusion Gene Specifically in Hormone-Resistant Progression

Ryan J. Hartmaier, Shannon L. Puhalla, Steffi Oesterreich, Aju Mathew, Amir Bahreini, Peter Lucas, Nancy E. Davidson

Paried primary and metastasis samples
Summary

- Single nucleotide variants (SNVs) are seldom shared between primary and metastatic disease
- Structural variants (SVs) are frequently retained in metastatic disease
- ESR1 SNVs and SVs gained during metastasis confer resistance to endocrine therapies
  - ESR1 Y537S mutation is undetectable in primary and metastatic disease before endocrine therapy
  - Novel ESR1-DAB2 fusion gene is constitutively active and ligand independent

*I think conclusions about resistance may be premature/controversial*
Preoperative window of opportunity study of the PI3K inhibitor Pictilisib (GDC-0941) plus Anastrozole vs Anastrozole alone in patients with ER+, HER2-negative breast cancer (OPPORTUNE study)


on behalf of the OPPORTUNE study investigators

OPPORTUNE Study Design

- Randomisation (2:1) favouring the combination, stratified by Centre & Grade
- Study dosing once daily for 14 days (+/- 2 days)
  - Anastrozole: 1 mg
  - Pictilisib: initially 340 mg; changed to 260 mg in 08/2012
- Adjuvant therapy as indicated
- 1st analysis of primary endpoint scheduled after 70 evaluable patients;
  2nd analysis after 141 patients focusing on subset analyses and additional biomarkers

P. Schmid
**PIK3CA inhibitor decreased proliferation in Luminal B**

**Summary and Conclusions**

- Addition of the PI3K inhibitor Pictilisib significantly increased the anti-proliferative response to Anastrozole in ER+ early breast cancer
- Subset analyses suggest increased benefit of Pictilisib for patients with Luminal B or highly-proliferative tumours
- PIK3CA mutations or PTEN status were not predictive of response to Pictilisib
PATIENT- DERIVED BREAST TUMOR GRAFTS AS MODELS OF METASTASY / TREATMENT RESPONSE

Alana Welm, PhD
Oklahoma Medical Research Foundation / Stephenson Cancer Center

PATIENT- DERIVED BREAST TUMOR XENOGRAFTS (PDX)

- Expand tissue supply by serial transplantation

BREAST PDX MODELS PREDICT THERAPY RESPONSE/RESISTANCE (RETROSPECTIVE, PROOF-OF-PRINCIPLE)

Paclitaxel, Herceptin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Disease Status</th>
<th>Duration</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCI-005</td>
<td>Heavily treated metastatic disease: ER+PR+HER2+</td>
<td>Stable disease – 8 months</td>
<td></td>
</tr>
<tr>
<td>HCI-006</td>
<td>Progressive disease</td>
<td></td>
<td>Death</td>
</tr>
<tr>
<td>HCI-007</td>
<td></td>
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</tbody>
</table>

HCI-005 – heterogeneous responses to paclitaxel
Origin of Metastatic Traits in Breast Cancer

Are Poor-Prognosis Tumors the Result of Driver Oncogenic Events During Tumor Initiation?

Y Kang
Identifying Genomic Alterations Associated with Poor Outcome by Computational Biology

\[ NS_i = \sum_{j=1}^{n} w_j ES_j \]

A Recurrent Poor Prognosis Amplon in 8q22

\[ \text{Not amplified (n=60)} \]
\[ \text{8q22 amplified (n=29)} \]

\[ \text{Log rank p=0.008} \]

Y Kang
Summary

- Deep sequencing is revealing new driver genes and potential new therapeutic targets
- Frequent mutations in HER2, ESR1, and PI3KCa
- However mutations are not “simple” predictive factors
- Human in mouse approach may provide therapeutic opportunities
- Metastasis is complex, but current technologies are starting to reveal therapeutic targets to treat metastasis