S6-05
High levels of APOBEC3B, a DNA deaminase and an enzymatic source of C-to-T transitions, are a validated marker of poor outcome in estrogen receptor-positive breast cancer

Dr. Sieuwerts: Nothing to disclose.
Dr. Burns: Nothing to disclose.
Dr. Look: Nothing to disclose.
Dr. Meijer-Van Gelder: Nothing to disclose.
Dr. Schlicker: Nothing to disclose.
Dr. Heidemann: Nothing to disclose.
Dr. Jacobs: Nothing to disclose.
Dr. Wesseis: Nothing to disclose.
Dr. Willis: Nothing to disclose.
Dr. Leyland-Jones: Nothing to disclose.
Dr. Gray: Nothing to disclose.

Dr. Faekens: Nothing to disclose.
Dr. Harris: Nothing to disclose.
Dr. Martens: Nothing to disclose.
High levels of APOBEC3B, a DNA deaminase and an enzymatic source of C-to-T transitions, are a validated marker of poor outcome in estrogen receptor-positive breast cancer.

1. Mutations are recurrent in a few common and many rare driver genes.
2. Mutations evolve over time (cancer evolution).
3. Mutations are found in a nucleic context !!!!!!
1. Mutations are recurrent in a few common and many rare driver genes
2. Mutations evolve over time (cancer evolution)
3. Mutations are found in a nucleic context !!!!!!
Three lessons learned from Next Generation Sequencing of breast cancer exomes & genomes

1. Mutations are recurrent in a few common and many rare driver genes
2. Mutations evolve over time (cancer evolution)
3. Mutations are found in a nucleic context !!!!!!
A lessons learned from complete NGS of 21 breast cancers
Mutations are found in a nucleic context !!!!!!

5' base
XpCpG → XpTpG

3' base
TpCpA/T → TpT/GpA/T


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Five such mutational signatures in 21 breast cancer


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Five such mutational signatures in 21 breast cancer

Kataegis (Thunderstorm)
Regional Hypermutation of Base Substitutions

- Regional C>T substitutions on chromosome 6 (in CIS)
- Many genomic rearrangements in this region
- Kataegis is often of the signature B and E type!

Signature A is related to B (Tth1111 endonuclease)

Few alterations

TpCpA/C/T → TpGpA/C/T
TpC deamination

Signature E

Signature C

Signature B

XpCpG → XpTpG
mCpG deamination (global)

TpCpX → TpA/G/TpX
TpC deamination

XpCpG → XpG/TpG

Which DNA deaminases are involved?
AID/APOBEC proteins? And which one?

APOBEC family

- APOBEC family contains several members
- APOBEC catalyzes deamination of cytosins (RNA/DNA)
- AID edits hypervariable region in antibody genes in B/T cells
- APOBEC1 edits mRNA in gastrointestinal tissues (& breast cancer!!)
- APOBEC3s restrict foreign DNA (viruses; transposons)

- A3B-preferred motifs are common in breast cancer genomes

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APOBEC3B is over-expressed in breast cancer and expression linked to C-T mutations


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APOBEC3B is causally linked to C-deamination


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Summary of the state-of-the-art

- C to T conversions (C-deamination) are common in breast cancer
- Context suggested involvement of APOBEC family members
- APOBEC3B (not other members) is over-expressed in breast cancer
- APOBEC3B causes C to T conversion in breast cancer cell lines

Is there a relation between APOBEC3B & breast cancer progression?

APOBEC3B & ER-positive breast cancer progression

- mRNA expression of APOBEC3B was determined in selected cohorts:
  1. A retrospective cohort of primary breast cancer, FF specimen (Rotterdam, NL)
     - 633 untreated LNN ER-positive cases (total cohort=1491; Q-RT PCR)
     - Endpoints: DFS, MFS and OS
  2. METABRIC dataset (FF; DASL; n=788+706; Endpoint: DSS)
  3. Publicly available dataset (FF; Affymetrix; n=754; Endpoint: DFS)
  4. The NKI datasets (FF; Agilent; n=181; Endpoint: DFS)
  5. BIG 1-98, a prospective randomized trial (FFPE; DASL; n=1219; Endpoint: BCF1)

- Expression levels (median cut-off) were associated using Cox regression with indicated endpoints (uni- and multivariately)
- Kaplan Meier survival curves are presented
APOBEC3B expression is a prognostic marker in ER-positive breast cancer

Retrospective cohort of primary breast cancer cases (Rotterdam, NL)
-633 untreated LNN, ER-positive cases
- surgery between 1978 and 2000

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<table>
<thead>
<tr>
<th>Uni- and multivariate analysis for DFS</th>
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<tbody>
<tr>
<td><strong>Univariate analysis</strong></td>
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<tr>
<td><strong>P-value</strong></td>
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<tr>
<td><strong>Univariate analysis</strong></td>
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<tr>
<td><strong>P-value</strong></td>
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<table>
<thead>
<tr>
<th>Age (years)</th>
<th>HR (95% CI)</th>
<th>P-value</th>
<th>HR (95% CI)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>&lt; 40</td>
<td>1</td>
<td>P = NS</td>
<td>1</td>
<td>P = NS</td>
</tr>
<tr>
<td>41-55</td>
<td>0.65 (0.46-0.91)</td>
<td>P &lt; 0.01</td>
<td>0.71 (0.50-1.01)</td>
<td>P = NS</td>
</tr>
<tr>
<td>56-70</td>
<td>0.55 (0.38-0.77)</td>
<td>0.49 (0.32-0.73)</td>
<td>0.57 (0.31-1.03)</td>
<td>P = 0.03</td>
</tr>
<tr>
<td>≥ 71</td>
<td>0.49 (0.32-0.73)</td>
<td>0.49 (0.26-0.94)</td>
<td>0.49 (0.26-0.94)</td>
<td>P = 0.01</td>
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<tr>
<th>Menopausal status</th>
<th>P-value</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Premenopausal</td>
<td>1</td>
<td>P = NS</td>
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<tr>
<td>Postmenopausal</td>
<td>0.73 (0.58-0.92)</td>
<td>0.96 (0.80-1.00)</td>
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<table>
<thead>
<tr>
<th>Tumor size</th>
<th>P-value</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>pT1</td>
<td>1</td>
<td>P = NS</td>
</tr>
<tr>
<td>pT2</td>
<td>1.31 (1.03-1.66)</td>
<td>1.18 (0.92-1.51)</td>
</tr>
<tr>
<td>pT3</td>
<td>1.88 (1.08-3.24)</td>
<td>2.06 (1.18-3.33)</td>
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<table>
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<tr>
<th>Tumor grade</th>
<th>P-value</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Poor</td>
<td>1</td>
<td>P = 0.01</td>
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<tr>
<td>Unknown</td>
<td>0.97 (0.75-1.29)</td>
<td>1.07 (0.83-1.40)</td>
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<tr>
<td>Good/Moderate</td>
<td>0.60 (0.43-0.84)</td>
<td>0.65 (0.47-0.91)</td>
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<tr>
<th>Log Pgr mRNA</th>
<th>P-value</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>0.90 (0.85-0.96)</td>
<td>P &lt; 0.01</td>
<td>0.79 (0.56-1.00)</td>
</tr>
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<table>
<thead>
<tr>
<th>APOBEC3B*</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>in 2 quartiles</td>
<td>P &lt; 0.01</td>
<td>P = 0.04</td>
</tr>
<tr>
<td>Low</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>High</td>
<td>1.55 (1.22-1.95)</td>
<td>1.32 (1.02-1.69)</td>
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**APOBEC3B predicts outcome in independent retrospective datasets**

### 1. METABRIC

**METABRIC Discovery set**

- HR = 1.77 (1.35-2.32) P < 0.001

**METABRIC Validation set**

- HR = 1.77 (1.34-2.33) P < 0.001

Retrospective cohort (Canada, UK) of primary breast cancer cases (METABRIC):
- 786 ER+ cases (METABRIC training set)
- 706 ER+ cases (METABRIC validation set)
- 15 yrs follow-up; Endpoint disease-specific survival

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**APOBEC3B predicts outcome in independent retrospective datasets**

### 2. Public Affy and NKI

**Public Affymetrix data KMPict**

- HR = 1.57 (1.23-2.01) P < 0.001

**NKI dataset**

- HR = 1.72 (0.98-3.02) P = 0.054

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**APOPBC3B predicts outcome in independent retrospective datasets**

**2. Public Affy and NKI**

![Graph showing survival probability (DFS) with DFS data from Public Affymetrix KMPlot.](image)

**HR=1.57 (1.23-2.01) P<0.001**

![Graph showing APOPBC3B Affy probe (206535_s_at) Q-RT-PCR.](image)

**APOPBC3B, Rs=0.87**

**APOPBC3B is prognostic in a prospectively collected BIG 1-98 trial**

Prospective Randomized, double-blinded phase III trial (n=6010)
- Postmenopausal women
- HR-positive early breast cancer
- 5 yrs of TAM or LETRO versus
- 2 yrs of TAM or LETRO+3 yrs the other

A nested case-cohort sample of the trial population (n=1,219)
- FFPE tissue samples
- with RNA available
- representative of all four treatment arms
- DASL Illumina microarray HT-12 v4
Endpoint: Breast Cancer Free Interval (BCFI)
(a weighted analysis methods was used to adjust KM estimates and inference)

![Graph showing survival probability (BCFI).](image)

**HR=1.42 (1.16-1.83) P=0.008**

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Conclusions

- C to T conversions (C-deamination) are common in breast cancer
- Context suggested involvement of APOBEC family members
- APOBEC3B (not other members) are over-expressed in breast cancer
- APOBEC3B causes C to T conversion in breast cancer cell lines
- APOBEC3B expression is a independent prognostic marker in ER-positive breast cancer (High level of evidence)
  - In various independent retrospective and in a prospective clinical trial of postmenopausal breast cancer (ER+) (BIG 1-98)
  - From independent centres
  - On various platforms detecting APOBEC3B
  - Using a conservative fixed median cut-off

-> APOBEC3B-induced mutations should be considered for driving breast cancer progression

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APOBEC3s restrict retrovirus replication (e.g., HIV-1)

Harris, Evans & Huiquist, 2013, JBC minireview

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- Kathryn Gray
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- METABRIC dataset
- IBCSG for BIG 1-98 data

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7,042 exomes of 30 cancer types → 21 signatures

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