Precision Medicine Based on Genomics in Breast Cancer

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Summary

- Current genomics tools
- Precision medicine
- Massively parallel sequencing
- Delivery of precision medicine
Current genomics tools
Molecular subtypes of breast cancer

- **Additional molecular subtypes**
  - Claudin-low
    - approx 60-70% TN phenotypes
  - Molecular subtypes of TNBC
    - Basal-like I, Basal-like II, Mesenchymal, Mesenchymal stem-like, Immunomodulatory, and Luminal androgen receptor (molecular apocrine)
  - METABRIC subtypes
    - 10 subtypes

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First generation prognostic signatures are associated with chemotherapy response

<table>
<thead>
<tr>
<th>Recurrence Score</th>
<th>≤18</th>
<th>&gt;18 and &lt;31</th>
<th>≥31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prognosis</td>
<td>Good</td>
<td>Intermediate</td>
<td>Poor</td>
</tr>
<tr>
<td>Endo benefit</td>
<td>High</td>
<td>Undetermined</td>
<td>Low</td>
</tr>
<tr>
<td>Chemo benefit</td>
<td>Negligible</td>
<td>Undetermined</td>
<td>High</td>
</tr>
</tbody>
</table>

Fan et al. NEJM 2006; Sotiriou et al. JNCI 2006; Reis-Filho & Puztai. Lancet 2011
15 years of microarray analysis

• ER+ and ER- negative tumours
  – Fundamentally different diseases

• The outcome of ER-positive cancers can be predicted by proliferation-related genes

• The prognosis of ER-negative breast cancers is determined by immune response-related genes

• Microarrays did not result in ways to define the best therapy for individual patients
Precision Medicine

The use of genomic, epigenomic, exposure, and other data to define individual patterns of disease, potentially leading to better individual treatment.

National Academy of Sciences (NAS), 2011
Breast Cancer Patient Management

“Precision medicine”-based breast cancer patient therapy

Haber DA, Gray NS, Baselga J. Cell 2011
Precision medicine is now possible

Development of targeted treatments
- Small molecule inhibitors
- Monoclonal antibodies

Massively Parallel Sequencing (NGS)
- Tumour genomes

Metzker et al. Nat Rev Genet 2010
Genetic changes identified by NGS

Meyerson et al. Nat Rev Genet 2010
Oncogene ‘addiction’ as the basis for predictive markers

Oncogene addiction:

“…cancer cells are often "addicted to" (that is, physiologically dependent on) the continued activity of specific activated or overexpressed oncogenes for maintenance of their malignant phenotype.”

I. Bernard Weinstein
Oncogene ‘addiction’

- **HER2** amplification
  Breast and gastric cancer
- **KIT** mutation
  Gastrointestinal stromal tumours
- **BCR-ABL** fusion
  Chronic myeloid leukaemias
- **EGFR** mutations and/or amplification
  NSCLC
- **EML4-ALK** fusion
  NSCLC
- **BRAF** mutation (V600E)
  Melanoma

Activated through genetic hits
Inhibition is selectively lethal
Breast cancer massively parallel sequencing analysis
Inter-tumour genetic heterogeneity

A few highly recurrent mutations in breast cancer

Distinct subtypes have different repertoires of mutations, but no highly recurrently mutated gene is subtype specific.

Few highly recurrently mutated driver genes...

**HER2** mutations
1.5% of breast cancers

**ESR1** mutations
0.6% of luminal cancers

cbioportal.org; TCGA Breast (provisional); n=962
Have we found all drivers in breast cancers?

Lawrence et al. Nature 2014
Exome analysis of 101 breast cancers

No driver genetic aberrations in a subset of breast cancers

Methods to identify significantly mutated genes in breast cancer focus on highly recurrently mutated genes

- Rare driver genes can be missed
  - *ESR1* mutations
    - 0.6% of luminal tumours
  - *HER2* mutations
    - Approx 1.5% of breast cancers

TCGA. Nature 2012
And even when we believe we know the drivers...

**TP53 mutation**

Chemotherapy sensitivity

Gluck et al., BCRT 2012 (PMID:21373875)

**AKT3 E17K activating mutation**

AKT inhibitors

**RAD17 + RAD50 loss**

Chemotherapy + PARPi sensitivity

Weigman et al., BCRT 2012 (PMID:22048815)

**INPP4B deletion, FBXW7 fusion**

PIK3CA inhibitors, mTOR inhibitors, PIK3CA/mTOR inhibitors

**A7 Lung Metastasis**

How do we prioritise them?

Courtesy Chuck Perou
Intra-tumour genetic heterogeneity

1. Tracking heterogeneity/bottlenecks
2. Tumour sampling bias
3. Drivers of heterogeneity
4. Drivers of disease – actionable mutations

Adapted from Swanton C, Cancer Res 2012
Intra-tumour genetic heterogeneity: Darwinian evolution model

- Tumour cell with mutation 1
- Tumour cell clone with mutations 1+2
- Tumour cell clone with mutations 1+3

Selective pressure
Resistance to therapy
Metastasis
HER2 intra-tumour heterogeneity
2%-3% of HER2+ cancers

HER2 Immunohistochemistry

Dual colour CISH

Amplified

Non-Amplified
Somatic mutations associated with HER2 intra-tumour heterogeneity

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Potential driver mutations present in both HER2-negative and HER2-positive components</th>
<th>Potential driver mutations restricted to the HER2-negative component</th>
<th>Potential drivers within regions whose amplification was restricted to the HER2-negative component</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>TP53 (P152L)</td>
<td></td>
<td>FAM83A, MDM4</td>
</tr>
<tr>
<td>T2</td>
<td>NP</td>
<td>NP</td>
<td>BRF2, FGFR1, ZNF703, RAB11FIP1, LSM1, DDHD2, WHSC1L1, PPAPDC1B, EEFIA2, ERLIN2, BAG4</td>
</tr>
<tr>
<td>T3</td>
<td>TP53 (E258D)</td>
<td>ATRX (splice site dinucleotide substitution)</td>
<td>YWHAZ, MYC, FAM83A</td>
</tr>
<tr>
<td>T4</td>
<td>ARID1A (R1446*)</td>
<td></td>
<td>BRF2, ZNF703, RAB11FIP1, ERLIN2</td>
</tr>
<tr>
<td>T5</td>
<td>TP53 (E286D)</td>
<td></td>
<td>IKBKβ, CAMK1D</td>
</tr>
<tr>
<td>T6</td>
<td>TP53 (R273H), PIK3CA (H1047R)</td>
<td>HER2 (T767M), ETV5 (E60K)</td>
<td></td>
</tr>
<tr>
<td>T8</td>
<td>PIK3CA (H1047R), CBFB (splice site)</td>
<td>BRAF (P403S), XRCC1 (S236F)</td>
<td>PHGDH</td>
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<tr>
<td>T9</td>
<td>TP53 (R282G), PIK3CA (H1047R), MAP2K4 (R110G), M1D2 (R2015M)</td>
<td></td>
<td>LMX1B</td>
</tr>
<tr>
<td>T10</td>
<td>TP53 (S94fs)</td>
<td></td>
<td>CBX3, RAD21</td>
</tr>
<tr>
<td>T11</td>
<td>TP53 (G187E192delLAPPQ)</td>
<td>NRP1 (R762H)</td>
<td></td>
</tr>
<tr>
<td>T12</td>
<td>TP53 (T195N), KIT (A755T)</td>
<td>FANCD2 (L1394F)</td>
<td>DSN1</td>
</tr>
<tr>
<td>T13</td>
<td>TP53 (S240I)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MOSCATO trial: implementation of Next Generation Sequencing in high volume phase I center

- Monocentric
- Target Accrual = 900 patients

Max 21 calendar days

Presented by: Antoine Hollebecque et al., ASCO 2013; Courtesy Fabrice Andre
Patients included
N=339

Patients Biopsied
N=295

Screen Failure N=44 (13%)
- Clinical deterioration (++)
- Biopsy technically impossible (++)
- Withdraw consent (n=2)

NGS
→ 90%

CGH + NGS
→ 80.5%

Actionable Target
N=127 (43.1%)

No Actionable Target
N=168 (57%)

Treatment matched to the Target
N=65 (22.0%)

No Treatment
N=62 (21%)
Take Home Messages

• Breast cancers display complex genomes
• Few highly recurrently mutated genes
• Large number of genes rarely mutated
• No common denominator for each subtype
• Highly recurrent drivers have been identified
• Drivers of rare subtypes and of metastasis and resistance yet to be fully characterised
Take home messages

• Not all drivers have been identified
  – Drivers of metastatic disease
  – Drivers of resistance to specific agents

• Beginning to understand
  – Intra-tumour genetic heterogeneity
Approaches for the delivery of precision medicine
Approaches for massively parallel sequencing and therapy decision making

- Whole genome sequencing
- Targeted capture sequencing
- Whole exome sequencing
- Whole exome sequencing + RNA sequencing
How deep should we sequence in clinical decision making?

- Higher depth – greater accuracy

- Mutations found in at least 10% of cancer cells
  - Typical sample: approx 50% of tumour cell content
  - At least 5 reads supporting a mutation

<table>
<thead>
<tr>
<th></th>
<th>Pure sample 100% tumour cells</th>
<th>Sample with 50% stroma 100% of tumour cells</th>
<th>Sample with 50% stroma 10% of tumour cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>100x</strong></td>
<td>50 reads</td>
<td>25 reads</td>
<td>2 – 3 reads</td>
</tr>
<tr>
<td><strong>200x</strong></td>
<td>100 reads</td>
<td>50 reads</td>
<td>5 reads</td>
</tr>
<tr>
<td><strong>500x</strong></td>
<td>250 reads</td>
<td>125 reads</td>
<td>12 – 13 reads</td>
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</tbody>
</table>
Whole genome sequencing

• All somatic genetic aberrations
  – Mutation calls
    • some uncertainty for SNVs
    • still problematic for indels
  – Fusion gene identification: not trivial
  – Validation with orthogonal methods is required

• Still expensive
  – Usually low depth: 30x to 100x

• Computer power and army of bioinformaticians
What are we trying to achieve?

• Targeted capture sequencing is an excellent option

• If we believe that
  – i) breast cancers are driven by a limited constellation of known driver mutations, fusion genes and copy number aberrations
  – ii) we can target the functional impact of each mutation
Mutation signatures and genomic scars are not identified

Alexandrov et al. Nature 2013
Mutation signatures and genomic scars are not identified
If we go with exome sequencing instead

- Mutations in coding regions and some 3’ and 5’ UTRs

**MAST1 and MAST2**
Robinson et al. Nat Med 2011

~6% of all breast cancers

**NOTCH1 and NOTCH2**
Robinson et al. Nat Med 2011

~25% of TNBCs

Fusion genes cannot be identified reliably
• Excellent approach, but...
• What do we do with the incidental findings?
Take Home Messages

• Sequencing for therapy decision making
  – Dependent on the use intended

  – For enrollment in clinical trials
    • Targeted capture sequencing (including selected intronic regions)

  – For patients in the metastatic setting after multiple lines of therapy
    • Targeted capture sequencing (including selected intronic regions)
    • Exome + RNA seq

  – Whole genome sequencing – unjustified at present
Breast Cancer Patient Management

Size
Grade
Type
Lymph Node metastasis
Vascular Invasion

HER2
HER2
ER, PR and HER2

Clinical
Pathology

Proteomics/metabolomics

Precision medicine-based breast cancer patient therapy
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