Somatic Mutation Detection in Cancer

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Major Breakthroughs in Cancer Treatment

- Targeted drugs are given to patients with specific molecular alterations
- Diagnostic test indicates the efficacy of the targeted drugs
  - ‘companion diagnostics’
- Examples of Biomarkers are:
  - Protein (over)expression
  - Genetic alterations: mutations, gene amplification, fusion genes
  - Epi-genetic alterations: promoter methylation

<table>
<thead>
<tr>
<th>Genetic alteration</th>
<th>Cancer type</th>
<th>Drug</th>
<th>Approved</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 amplification (1986)</td>
<td>Breast cancer</td>
<td>Herceptin</td>
<td>1998</td>
</tr>
<tr>
<td>BCR-ABL fusion (1973)</td>
<td>CML</td>
<td>Gleevec</td>
<td>2001</td>
</tr>
<tr>
<td>EML4-ALK fusion (2007)</td>
<td>Lung Cancer (NCSLC)</td>
<td>Crizotinib</td>
<td>2011</td>
</tr>
<tr>
<td>BRCA1/2 mutation</td>
<td>Serous ovarian cancer</td>
<td>Olaparib</td>
<td>2014</td>
</tr>
<tr>
<td>Immunotherapy</td>
<td>Many types</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
# Molecular diagnostics @NKI-AVL

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH Amplifications</td>
<td>HER2, MDM2, MYC, MET</td>
</tr>
<tr>
<td>FISH Translocations</td>
<td>ALK, BCL2, BCL6, MYC, EWS, FUS, ROS, RET</td>
</tr>
<tr>
<td>Fragment analysis</td>
<td>MSI, ERBB2, EGFR</td>
</tr>
<tr>
<td>HRM</td>
<td>KRAS, BRAF, EGFR</td>
</tr>
<tr>
<td>MLPA</td>
<td>Promotor methylation, CNVs</td>
</tr>
<tr>
<td>Sanger sequencing</td>
<td>KRAS, NRAS, EGFR, PIK3CA, BRAF, KIT, PDGFRA, TP53, PTEN</td>
</tr>
<tr>
<td>qPCR</td>
<td>MDM2</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>EWS, FUS, SSX1, SSX2, etc</td>
</tr>
<tr>
<td>NGS - CNVseq</td>
<td>CNVs</td>
</tr>
<tr>
<td>NGS - 178 gene panel</td>
<td>Alterations in 178 genes</td>
</tr>
<tr>
<td>NGS - TSACP panel</td>
<td>Hot spots in 48 genes</td>
</tr>
</tbody>
</table>

![Images of DNA and RNA sequences and reports]
Molecular Diagnostics for personalized medicine

NGS TSACP

NRAS

PIK3CA

TP53

PTEN

ALK

Sequenom

NGS 178 DNA/RNA

ERBB2

EGFR

KRAS

KIT

PDGFRA

BRAF

PIK3CA

TP53

PTEN

ALK

Sequenom

NGS TSACP

NRAS

PIK3CA

TP53

PTEN

ALK

Sequenom

NGS TSACP

DNA/RNA
Challenges in Cancer Pathology

1) Sample quantity and quality

- Formalin Fixed Paraffin Embedded (FFPE) material
  - Low quality and highly fragmented DNA due to fixation
  - Various fixation protocols
- Tumor tissue characteristics
  - Low tumor cell percentages, Tumor heterogeneity
- Limited amounts of material
  - Biopsies, metastatic lesions
Challenges in Cancer Pathology: other bottle necks

2) TAT
   • Current maximum 10 days, aim <5 days

3) Complex diagnostic routings
   • Combination of different techniques

4) Detection of RNA fusions to guide therapy
   • For example ALK fusions in lung cancer, crizotinib (ALK/MET tyrosine kinase inhibitor)
   • RT-PCR and FISH, specificity, material, logistic issues
   • Labor intensive

5) Life time of a validated test is short
   • New genes, drugs, trails, biomarkers, sample types
Illumina TruSeq Amplicon Cancer Panel (TSACP)

Routine NGS test to detect hotspot mutations in 48 genes and amplifications
Illumina TruSeq Amplicon Cancer Panel (TSACP)

- Amplicon PCR
- 212 amplicons covering hotspots in 48 genes
- >35 kb target sequence
- Optimized for DNA from Formalin-Fixed Paraffin-Embedded (FFPE) tissues
- Sequencing on the MiSeq

<table>
<thead>
<tr>
<th>Gene 1</th>
<th>Gene 2</th>
<th>Gene 3</th>
<th>Gene 4</th>
<th>Gene 5</th>
<th>Gene 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL1</td>
<td>EGFR</td>
<td>GNAS</td>
<td>MLH1</td>
<td>RET</td>
<td></td>
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<tr>
<td>AKT1</td>
<td>ERBB2</td>
<td>HNF1A</td>
<td>MPL</td>
<td>SMAD4</td>
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<tr>
<td>ALK</td>
<td>ERBB4</td>
<td>HRAS</td>
<td>NOTCH1</td>
<td>SMARCB1</td>
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<tr>
<td>APC</td>
<td>FBXW7</td>
<td>IDH1</td>
<td>NPM1</td>
<td>SMO</td>
<td></td>
</tr>
<tr>
<td>ATM</td>
<td>FGFR1</td>
<td>JAK2</td>
<td>NRAS</td>
<td>SRC</td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>FGFR2</td>
<td>JAK3</td>
<td>PDGFRA</td>
<td>STK11</td>
<td></td>
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<tr>
<td>CDH1</td>
<td>FGFR3</td>
<td>KDR</td>
<td>PIK3CA</td>
<td>TP53</td>
<td></td>
</tr>
<tr>
<td>CDKN2A</td>
<td>FLT3</td>
<td>KIT</td>
<td>PTEN</td>
<td>VHL</td>
<td></td>
</tr>
<tr>
<td>CSF1R</td>
<td>GNA11</td>
<td>KRAS</td>
<td>PTPN11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTNNB1</td>
<td>GNAQ</td>
<td>MET</td>
<td>RB1</td>
<td></td>
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</tr>
</tbody>
</table>
Validation of Illumina TruSeq Amplicon Cancer Panel

Workflow
- DNA input >100 ng (Quantus), 24 samples per run
- Illumina’s Somatic variant caller
- Data analysis Cartagenia’s Onco bench: >200x & VAF >10%

Sensitivity for SNV mutations 96.4% (95% CI 0.91-0.99)
- 114 SNV mutations in samples that were previously tested in dx
  - 110/114 SNVs detected
  - 4/114 SNVs with variant allele frequencies (VAF) < 10% were not reported

Sensitivity for indels is not sufficient:
## Diagnostic yield TSACP using COSMIC mutations

<table>
<thead>
<tr>
<th>Gene</th>
<th>mutations in COSMIC (all)</th>
<th>COSMIC mutations (all) in TSACP target</th>
<th>DIAGNOSTIC YIELD</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT1</td>
<td>494</td>
<td>443</td>
<td>90%</td>
</tr>
<tr>
<td>BRAF</td>
<td>37,417</td>
<td>37,229</td>
<td>99%</td>
</tr>
<tr>
<td>EGFR</td>
<td>16,445</td>
<td>15,808</td>
<td>96%</td>
</tr>
<tr>
<td>FGFR3</td>
<td>3,401</td>
<td>2,489</td>
<td>73%</td>
</tr>
<tr>
<td>ERBB2</td>
<td>514</td>
<td>288</td>
<td>56%</td>
</tr>
<tr>
<td>KIT</td>
<td>6,805</td>
<td>6,311</td>
<td>93%</td>
</tr>
<tr>
<td>KRAS</td>
<td>31,329</td>
<td>31,244</td>
<td>100%</td>
</tr>
<tr>
<td>NRAS</td>
<td>3,904</td>
<td>3,848</td>
<td>99%</td>
</tr>
<tr>
<td>PDGFRa</td>
<td>1,244</td>
<td>942</td>
<td>76%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>7,943</td>
<td>7,436</td>
<td>94%</td>
</tr>
<tr>
<td>PTEN</td>
<td>3,311</td>
<td>1,495</td>
<td>45%</td>
</tr>
<tr>
<td>TP53</td>
<td>19,922</td>
<td>18,830</td>
<td>95%</td>
</tr>
</tbody>
</table>

+ Low accuracy for indels >18 bp
Gene amplification detection with the TruSeq Amplicon Cancer Panel

- TSACP 48 gene hot spot panel was designed to detect SNVs, smaller indels
- Gene amplification clinically relevant
- Detection of CNVs is possible using the coverage data
- Codecz
  - Simultaneous Detection of Clinically Relevant Mutations and Amplifications for Routine Cancer Pathology (Hoogstraat et al., J Mol Diagn. 2015)
- Adapted Codecz for use on TSACP data
- Validated MET, EGFR and ERBB2 amplification detection
  - Sensitivity for high level amplifications is high
  - To discriminate between low level amplifications or polysomy additional tests are needed
Diagnostic routing
Molecular diagnostics for lung cancer

A typical case
Molecular diagnostics for Lung cancer (NSCLC)

Male, 70,
- NSCLC, stage IV
- Pleural biopsy

Diagnostics (differential diagnosis, therapy choice)
- Adenocarcinoma, TCP: 80
- ALK IHC: negative
- (hot spot) mutations in AKT1, BRAF, DDR2, EGFR, KRAS, MEK1, NRAS, PIK3CA: negative
- Fragment analysis: Activating EGFR mutation
- NGS: Activating EGFR mutation

Treatment
- Chemo therapy (Cisplatin pemetrexed), progressive
- EGFR TKI (erlotinib/gefitinib)
- Progressive
- Biopsy

Diagnostics (resistance)
- NGS: EGFR T790M resistance mutation
- FISH (MET): negative
- IHC/SISH (ERBB2): negative
- HRM (EGFR T790M): EGFR T790M resistance mutation

Treatment
- third generation EGFR TKIs?
Molecular diagnostics for Lung cancer (NSCLC)
‘A typical case’

Male, 70,
• NSCLC, stage 4
• Pleural biopsy

Diagnostics (differential diagnosis, therapy choice)
• Adenocarcinoma, TCP: 80
• ALK IHC: negative
• (hot spot) mutations in AKT1, BRAF, DDR2, EGFR, KRAS, MEK1, NRAS, PIK3CA: negative
• Fragment analysis: Activating EGFR mutation
• NGS: Activating EGFR mutation

Treatment
• Chemotherapy (Cisplatin, pemetrexed)
• EGFR TKI (erlotinib/gefitinib)
• Biopsy

Diagnostics (resistance)
• NGS: EGFR T790M resistance mutation
• FISH (MET): negative
• IHC/SISH (ERBB2): negative
• HRM (EGFR T790M): EGFR T790M resistance mutation

Diagnostic Challenges
• TAT
• Different molecular aberrations involved (amplifications, SNV, indels)
• Limited availability of tissue samples

Treatment
• third generation EGFR TKIs?
Liquid biopsies

Circulating tumor DNA (ctDNA)
Circulating tumor DNA / liquid biopsy

Bettegowda et al., Science transl Med 2014 vol6 issue 224
An example
Lung cancer, Digital PCR Bio-Rad

EGFR T790M detected in blood
BRCAness: the phenotypes that some sporadic tumors share with familial-\textit{BRCA} cancers

‘actionable phenotype’
BRCA deficiency leads to chromosomal instability

3 pathways to repair DNA double-strand breaks
- non-homologous end joining (NHEJ), single-strand annealing (SSA) and homologous recombination (HR)

Cells that lack BRCA1 or BRCA2:
- have a defect in HR
- show inappropriate repair of double-strand breaks via NHEJ, SSA (error-prone)
- this leads to Chromosomal instability and Cancer

Venkitaraman, Cancer Susceptibility and the Functions of BRCA1 and BRCA2, Review, Cell 2002
arrayCGH to detect chromosomal instability

BRCA1-mutated tumors
- More chromosomal aberrations
- More breaks
- Less (focal) high level amplification
- Specific aberrations

Sporadic tumors

3.6 K BAC platform (1Mb spacing)

And many others
BRCAness

• The specific pattern (‘signature’) of chromosomal instability that can be seen in tumours from patients with a germline mutation in BRCA

• BRCAness is also seen when BRCA is inactivated through somatic mechanisms
  - somatic \textit{BRCA1/2} mutation
  - \textit{BRCA1} promotor methylation

• BRCAness can be detected with functional tests
  - ‘BRCA1-Classifier’ and a MLPA (NKI-AVL)
    • Identify patients with a high likelihood of having a germline \textit{BRCA1} mutation.
    • Classification of VUSses in \textit{BRCA1}
  - Formation of RAD51 ionizing radiation induced foci (Naipal et. al., Clin Cancer Res. 2014)
BRCAness status is relevant for therapy choice

• In breast cancer
  – BRCA1-mutated breast cancer patients benefit from high-dose alkylating agents (which induce DNA double-strand breaks)

• In ovarian cancer
  – PARP inhibitors (Olaparib) approved as a therapy
  – For tumors with BRCA mutations (germline, somatic)
  – that have been treated with three or more prior lines of chemotherapy
Exploiting DNA-repair defect of BRCA1/2 mutated cancer: PARPi

Sonnenblick et al. Nat Rev Clin Oncol. 2015
PARP inhibitors and Ovarium Carcinoma

Ovarian cancer is fifth-leading cause of death among women in the US. Most deaths (~70%) are of patients presenting with advanced stage, high-grade serous ovarian adenocarcinoma. Standard of care: Surgery + platinum- and taxane-based chemotherapy, overall five-year survival of 31%. ~13% of OvCa is attributable to germline mutations in BRCA1/2.

- TCGA study ovarian cancer
  - 489 high-grade serous ovarian adenocarcinoma’s
  - Homologous recombination defective in about half
  - ~1/3 has alteration in BRCA
    - 17% germline BRCA1/2 mutations
    - 4% somatic BRCA1/2 mutation
    - 11% BRCA1 promoter methylated

![BRCA altered cases, N = 103 (33%)](TCGA, Nature 2011)
Molecular diagnostics for lung cancer

A not so typical case
Longkanker

Vrouw 38yr EGFR exon 19del+
‘Deze vrouw vanochtend gezien.
Bizar mooie radiologische respons.
Tumor van 6 cm in de hals (niet mooi op de CT te zien) volledig verdwenen. Het klierpakket in de oksel (zie plaatje PPT) is ook verdwenen.
Indrukwekkend en zeer dankbaar.’
Summary NGS in cancer pathology

• Current
  – Diagnostic NGS panels implemented in many molecular diagnostic laboratories in NL
  – WES, WGS not (yet?) first line diagnostics

• Future
  – Somatic BRCA testing
  – ctDNA
  – Fusion gene detection
  – Sensitivity (low frequency resistance mutations)
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