NGS for oncogenetics

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Hereditary cancer

- 5-10% of patients with breast, or colon cancer have an inherited predisposition = inherited cancer syndrome

20% of patients with (epithelial) ovarian cancer

- Predisposition < mutated gene
- Predisposition may be passed via germ-line and inherited in family
NGS for oncogenetics

• Large, multiple gene panels available for testing
  – Tumor DNA (somatic cells)  => Precision therapy
  – Patient DNA (constitutive/germ line cells)  => 2ary prevention in patient
                                               => prevention in family relatives

• Areas of uncertainty
  – Gene level  :  penetrance
  – Genetic variant/mutation level :  VUS

• Tumor driving, somatic mutation may be present in patient’s germ-line
 => need strategy for prevention in patient’s family relatives
Large, multiple gene panels

We can test (*):
• All genes (whole exome/whole genome) 20000
• All genes involved in disease (mendeliome) 4000
• All cancer driver genes 500?
• All genes for one disease (gene panel) 5 to 100’s

(*) Current tests = DNA sequencing of protein-coding portions of genes pending whole genome sequencing
Dropping cost of DNA sequencing

Cost per Raw Megabase of DNA Sequence

Moore's Law

NIH National Human Genome Research Institute

genome.gov/sequencingcosts
**Increasing costs of DNA tests**

- Preparing patient’s DNA (library)
- DNA sequencing
  - Bioinformatics analyses for relevant DNA variants
  - Medical interpretation
  - ISO15189 certification/accreditation
Increasing costs of DNA tests

Preparing patient’s DNA (library)

DNA sequencing

• Bioinformatics analyses for relevant DNA variants
• Medical interpretation
• ISO15189 certification/accreditation
Testing cancer patients for an inherited predisposition

- Blood sample => germ line DNA analysis
- If inherited predisposition is suspected
  - Breast / ovarian cancer
  - Colorectal / endometrial cancer
  - Multiple Endocrine Neoplasia
  - others
To test or not to test?

Guidelines: see www.BeSHG.be > guidelines

- HBOC (Hereditary Breast and Ovarian Cancer Syndrome)
- HNPCC Lynch syndrome (pending)
- Cystic fibrosis
- others
Benefits of mutation identification

- Identify risk of cancer in other organ (ovary) for secondary prevention (BRCA; PTEN, and all syndromic BC genes)
- Refine risk of recurrence (CHEK2, ATM)
- Precision drugs (olaparib in BRCA carriers)
- Individualized therapy (avoid radiotherapy in TP53)
- Trace risk in relatives > 1ary / 2ary prevention
Testing cancer patients for an inherited predisposition: how large should a gene panel be?

AREAS OF UNCERTAINTY:

• **Penetrance** of the gene defect
  Penetrance = probability of disease if mutation present

• **Clinical significance** of the patient’s variant in the gene
  Disease causing mutation >< normal variant, harmless (polymorphism)

Large multigene panels include genes with unknown penetrance, and identify variants of unknown significance (VUS)
### GENETIC VARIANTS

<table>
<thead>
<tr>
<th>VARIANT</th>
<th>Frequency</th>
<th>Penetrance (functional effect)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mutation</td>
<td>Rare</td>
<td>High</td>
</tr>
<tr>
<td>VUS</td>
<td>Rare</td>
<td>? ?</td>
</tr>
<tr>
<td>Polymorphism</td>
<td>Frequent</td>
<td>Low or null</td>
</tr>
<tr>
<td>« Rare polymorphism »</td>
<td>Rare</td>
<td>Low or null</td>
</tr>
</tbody>
</table>

**VUS** = variant of uncertain clinical significance: currently impossible to tell if high penetrance (phenotype-causing, mutation) or low/null penetrance (« rare polymorphism »)

VUS classification will require time:
- epidemiology of mutation and/or
- functional data (bioinformatics, machine learning approach)
ACCE criteria for genetic tests
Center of Disease Control, USA

✓ Analytical validity

✓ Clinical validity

✓ Clinical utility

✓ Ethical, Legal & Social issues

You bet! CLIA and ISO15189

Which gene variants (which alleles) cause disease risks?

How much risk?
CLINICAL VALIDITY:
Which variants cause disease risk?

• We will eventually need epidemiological evidence of causality for each mutation i.e., each genetic variant, each allele.

• Class of variant may indicate functional effect on gene
  – Truncating variant (premature stop codon) < missense variant
CLINICAL VALIDITY:
Which variants cause disease risk?

• We will eventually need epidemiological evidence of causality for each mutation i.e., each genetic variant, each allele

• Class of variant may indicate functional effect on gene
  – Truncating variant (premature stop codon) vs missense variant

Current functional interpretation of variants:
✓ Disease-causing (mutation)
✓ Non-disease causing (polymorphisms, frequent or rare in population)
✓ VUS (variants of uncertain clinical significance)
CLINICAL UTILITY
magnitude of the risk caused by gene variant

Limitations in clinical utility of test:
- VUS
- RR of various alleles in one gene may differ
- RR is higher in patients with strong family histories
  => overestimated in individual patients (Evans et al 2014 JMG)

Strong family histories select for:
- Modifier genes
- Lifestyle, hormonal factors, reproductive history
CLINICAL UTILITY

magnitude of the risk caused by gene variant

- High penetrance genes / alleles
  Disease incidence > 4 x normal
  Cumulative risk, lifetime * > 32%

- Moderate penetrance genes / alleles
  Disease incidence 2 – 4 x normal
  18 – 32 %

- Low penetrance genes / alleles
  Disease incidence <2 x normal
  < 18 %

* Baseline = 9%

Easton et al 2015 NEJM
Clinical utility depends on allele penetrance

If mutation explains risk only partially, caveat:

- Undue alarm in mutation carrier
- False reassurance in non-carrier

Clinical utility depends on allele penetrance

If mutation explains risk only partially, caveat:
- Undue alarm in mutation carrier
- False reassurance in non-carrier


Easton et al. 2015, NEJM
Phenocopies
chance occurrence of BC in non-carrier
# Recommendations of the Belgian College of Human Genetics

## Clinically useful, 2016

**Non syndromic BC**
- BRCA1
- BRCA2
- PALB2
- TP53
- CHEK2 (mutation c.1100delC)

**Syndromic BC**
- STK11 (Peutz Jeghers)
- NF1 (neurofibromatosis)
- CDH1 (lobular BC + linitis plastica)
- PTEN (Cowden)

## Research Only
- BARD1
- BRIP1
- RAD51C
- RAD51D
- MRE11A
- RAD50
- NBN
- FAM175A
- ATM
- CHEK2 (other mutations)
- XRCC2
- MEN1
Testing tumor DNA for pathway analysis
Cancer Genetics

- **In tumors** (tumor DNA = somatic DNA):
  Molecular pathology; tumorigenesis routes.
  - Genetic
  - Epigenetic

- **In patients** (constitutional DNA = germ-line DNA):
  Cancer risk profiling, from inherited mutations/polymorphisms
Somatic mutation may be present in the germ line

Driver mutations in tumors of some cancer patients are also present in all other cells
  – e.g., BRCA1/2 mutations in ~20% epithelial ovarian cancer
    = 15% constitutive + 5% tumor only

• These patients are at higher risk for other tumors
  – e.g., breast cancers and/or ovarian cancers

• Constitutive change is present in germ line
=> at-risk relatives need genetic counseling in view of presymptomatic genetic testing
Germline BRCA mutations are frequent in ovarian carcinoma

<table>
<thead>
<tr>
<th>Category</th>
<th>No. (%) positive for mutations in</th>
<th>Total no. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BRCA1</td>
<td>BRCA2</td>
</tr>
<tr>
<td>Tumor histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>81</td>
<td>52</td>
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<tr>
<td>Endometrioid</td>
<td>18</td>
<td>8</td>
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<tr>
<td>Mucinous</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Clear cell</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Carcinosarcoma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Brenner</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Not specified</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

S. Zhang et al. / Gynecologic Oncology 121 (2011) 353–357
BRCA mutations (germ line + somatic) in 15%-25% ovarian cancer tissue

- Somatic BRCA1/2 mutation in 19% of ovarian cancers unselected for type (n=235; Hennessy et al 2012 JCO)
  - 23% in high-grade serous tumors
  - Among tumors with mutations, 20-40% are somatic only

- Germline BRCA1/2 mutations in 15% of (non-mucinous) ovarian carcinoma (n=1001; Alsop et al. 2012 JCO)
  - Better progression-free and better overall survival
  - 44% had no reported family history of breast or ovarian cancer

- Beyond BRCA1/2, homologous recombination defect in up to 50% high-grade serous ovarian adenocarcinomas (TCGA research network 2011 Nature)
Most BRCA1/2 mutations found in ovarian carcinomas are also present in germline.

If mutation is present in germline, it may be inherited and present in other family members!

=> Need for integrated clinical work up and patient trajectories from oncology to pathology to genetics.
If BRCA1/2 mutation present in germline

- Genetic counseling is indicated in FAMILY MEMBERS
- Presymptomatic genetic testing in young adult relatives at-risk
  - Asymptomatic 20-25 years old
  - With psychological support and coaching
  - Pre-test and post-test counseling
  - If mutation present, start prevention of BREAST and OVARIAN cancer

Breast cancer
Ovarian cancer

Ovarian cancer patients with germline BRCA mutation: 44% had no reported family history of breast or ovarian cancer

Alsop et al. 2012 JCO
Test ovarian carcinoma DNA and blood DNA (= germ line) for BRCA1/2 mutation

- In every patient with high grade serous epithelial ovarian carcinoma

- Blood DNA analysis allows for exon duplication / deletion analysis (10-15% of all BRCA1/2 mutations)

- Blood DNA analysis goes beyond BRCA1/2
  - Multigene panel of 26 genes, including 5 validated, high penetrance genes

- Some mutations are somatic only
  - Test tumor DNA + blood DNA in parallel
Patients with epithelial ovarian carcinoma, serous, high grade

Test tumor + blood for BRCA1/2

- ONCOLOGIST; OBGYN
- With patient’s informed consent including family issues

80% 20%

- No mutation
- Mutation found
  - 5% Somatic only
  - 15% Somatic + germline

- No genetics consult needed

Refer to genetics clinic for genetic counseling
NGS for oncogenetics 2016-17: conclusions

• Variants of Uncertain Clinical Significance (VUS) limit clinical validity of test
  – Genetic evidence must be collected over the coming years
• Penetrance limit clinical utility of test
  Lower penetrance means higher unexplained risk in patient
  – Undue alarm, false reassurance
  – Education of lay public, genetic counseling (pretest)
• Hence too large multi gene panels are not recommended
• Sequencing is cheap but medical genetic testing is expensive
• Tumor DNA tests allow for precision therapy: eg, BRCA1/2 in ovarian
• Many cancer-driving mutations in tumors are also present in germ line => need for integrated patients trajectory onco/pathol/genetics
Testing strategy, riziv inami coverage

Specific syndrome

- **Gene panel**, sequenced with 100% accuracy.
  
  => Confirm / exclude known diagnosis (NPV!)

Semi-specific

- Mendeliome + CORE GENES, for which sequencing completed to 100% accuracy

Non-specific syndrome

- All genes (exome), whatever their individual sequencing accuracy.
  
  => **Include** any diagnosis

**Covered by riziv inami**

**Not covered by riziv**
## Remboursement INAMI échelonné des analyses génétiques

<table>
<thead>
<tr>
<th>76 EUR</th>
<th>152 EUR</th>
<th>350 EUR</th>
<th>547 EUR</th>
<th>1350 EUR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Simple test</strong>&lt;br&gt;One or a few mutations, analysed in one or 2 PCR reactions or with (simple) kits</td>
<td><strong>Simple test</strong>&lt;br&gt;One or a set mutations, analysed in one or 2 PCR reactions and/or sequencing reactions, or with (more elaborate) kits</td>
<td><strong>Exceptional category, for simple testing, not related to a specific disease.</strong>&lt;br&gt;- Less than 10 amplicons OR - Deletion/ duplication analysis (as the only test for a specific diagnosis) OR - Simple PCR-based test which are in some cases complemented with Southern blot OR - Dynamic mutation OR - Diagnostic confirmation by sequencing (specific cases, e.g. very rare conditions, parental confirmation)</td>
<td><strong>Complex test</strong>&lt;br&gt;- Between 10 and 39 amplicons, or between 1.5 and 9kb coding sequence per gene or per package of genes (equals per diagnosis)* OR - Sets of dynamic mutations</td>
<td><strong>Complex test</strong>&lt;br&gt;- 40 or more amplicons per gene or more than 9 kb coding sequence per gene or per package of genes (equals per diagnosis)* OR - Sequence capture results</td>
</tr>
<tr>
<td><strong>Exceptional category, for simple tests (O and P categories), until BELMOLGEN advises to move them to O)</strong></td>
<td><strong>(Default category for complex tests (Q, R and S categories), until BELMOLGEN advises to move item to R or S)</strong></td>
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*Testing includes mutation analysis plus deletion/ duplication analysis*

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+ liste limitative des tests remboursables, mise à jour 1x/an