Introduction to Molecular Pathology

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Rudolf Virchow
1821 - 1902

Institut für Pathologie – Charité Berlin
The main goal of diagnostic pathology is to extract from the patient’s tissue as many information as possible by applying classical, immunological and molecular techniques.

But it should not be forgotten that the methodological results have to be interpreted by an experienced pathologist who is the one to bring together the diagnostic, prognostic and predictive information and to assign them to the disease of the individual patient.
Predictive Molecular Pathology and Personalized Medicine

A prerequisite of personalized medicine is the capability to predict *pre-therapeutically* the response of individual tumors to certain (targeted) drug.

For this prediction one needs reliable and reproducible biomarker and predictive assays.

This is the current challenge of predictive molecular pathology.
Prediction is difficult, especially about the future

Niels Bohr, 1885-1962

2010
Thinking back to infectious diseases and seeing the current development in cancer – HER2, KRAS, BRAF, EGFR, c-MET etc. - we shouldn’t be too pessimistic.

The predictive power of tissue based analyses is underestimated.
Predictive tissue-based biomarkers for targeted therapies

FDA / EMA-approved drugs associated with eligibility tests* (selection)

- Trastuzumab → metastatic breast cancer, overexpression/amplification of HER2
- Cetuximab → metastatic colorectal cancer, overexpressing EGFR/wild-type KRAS
- Panitumumab → colorectal cancer with wild-type KRAS (mutation excluded)
- Gefitinib → non-small cell lung cancer with mutated EGFR
- Erlotinib → non-small cell lung cancer with mutated EGFR
- Crizotinib → non-small cell lung cancer with mutated EML4-ALK
- Nimotuzumab → metastatic colorectal cancer (still experimental)
- Lapatinib → metastatic breast cancer overexpression HER2/neu (?)
- Vemurafenib (PX4032) → malignant melanoma with mutated B-RAF
- Imatinib → CML, bcr/abl – positive (activated PK)
- Imatinib → GIST with activated c-kit receptor tyrosine kinase/CD117, exon 9 mut
- Rituximab (+ CHOP), Y90-Ibritumomab, I131-Tositumomab → NHLymphoma with CD20
- Gemtuzumab-Ozogamicin → AML with CD33 (> 60 yrs.), mal. melanoma
- Tamoxifen+/− chemo → ER+/HER2− breast cancer, mutation pattern - multigene assays

*Strongly suggested by FDA's Drug-Diagnostic Co-Development Initiative

Already now, in 35% of all tumors a predictive molecular test is appropriate. Notably, prediction of tumour response is exclusively tissue-based.

All these substances have been developed on the basis of histologically characterised human tissue.

This underlines the importance of biobanks.

*Strongly suggested by FDA's Drug-Diagnostic Co-Development Initiative
Multidisciplinary cooperation enables personalised oncology

Cohorts: Clinical Trials Tumour Registries

Patient

Oncologist

Targeted therapies

Tumour board

Sampling

pre-analytic

Results

Predictive biomarkers

Radiology
Endoscopy
Surgery

Pathology

Diagnosis

IHC

In situ hybridization

PC

Finally, in addition to physician and health care provider education, public education will be key to empowering broad interest and participation in personalized genomic medicine by patients and their families.

What is the irreplaceable role of anatomic pathology in the procedure of molecular biomarker analysis?
Recommendations for sample preparation and molecular analysis

**Surgery**
- Tumour resection

**Surgical Pathology**
- Grossing
- Paraffin embedding
- Histological evaluation
- Manual microdissection

**Molecular Pathology**
- DNA isolation
- Amplification
- Detection

**Oncological treatment**
Tumor Entities Important in Predictive Molecular Pathology

- Colon cancer
- NSCLC
- Malignant melanoma
- Breast cancer
- Upcoming challenges
Invasive colorectal cancer
EMA/FDA asked for a pre-therapeutic eligibility test.

Example: therapeutic anti-EGFR antibodies,

e.g. panitumumab
cetuximab
Met. Colon-Ca: Wild-type/mut KRAS/BSC

<table>
<thead>
<tr>
<th>Events N (%)</th>
<th>Median (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>115/124 (93)</td>
<td>12.3</td>
</tr>
<tr>
<td>114/119 (96)</td>
<td>7.3</td>
</tr>
<tr>
<td>76/84 (90)</td>
<td>7.4</td>
</tr>
</tbody>
</table>

HR = 0.45 (95% CI: 0.34–0.59)
Stratified log-rank, P < 0.0001

Mutant KRAS is constitutively active – 40% of patients*

Panitumumab and cetuximab inhibit ligand binding, dimerisation, activation of the receptor and the signalling pathway

Activation of
• Proliferation
• Angiogenesis
• Malignant phenotype

Relevance of different types of (K)RAS and BRAF mutations
## Treatment efficacy - according to mut status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>KRAS/ BRAF wild-type</th>
<th>KRAS mutation codon 12</th>
<th>KRAS mutation codon 13</th>
<th>BRAF mutation</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All patients</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of patients</td>
<td>79 (54%)</td>
<td>41 (28%)</td>
<td>9 (6%)</td>
<td>17 (12%)</td>
<td></td>
</tr>
<tr>
<td>ORR (%)-(95% CI)</td>
<td>59 (47-71)</td>
<td>47 (32-63)</td>
<td>66 (35-88)</td>
<td>57 (33-79)</td>
<td>0.61</td>
</tr>
<tr>
<td>DCR (%)-(95% CI)</td>
<td>92 (83-97)</td>
<td>97 (82-98)</td>
<td>100 (70-100)</td>
<td>79 (52-93)</td>
<td>0.22</td>
</tr>
<tr>
<td>Response not assessable</td>
<td>15</td>
<td>5</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Median PFS (ms)</td>
<td>8</td>
<td>5.8 (4.4-7.1)</td>
<td>9.9 (7.9-12.0)</td>
<td>4.2 (1.4-7.0)</td>
<td>0.058</td>
</tr>
<tr>
<td>95% CI</td>
<td>6.6-9.3</td>
<td>4.4-7.1</td>
<td>7.9-12.0</td>
<td>1.4-7.0</td>
<td></td>
</tr>
<tr>
<td>Median OS (ms)</td>
<td>23.5</td>
<td>18.9 (12.6-25.1)</td>
<td>26.2 (24.3-28.1)</td>
<td>13.0 (7.7-18.3)</td>
<td>0.032</td>
</tr>
<tr>
<td>95% CI</td>
<td>17.7-29.4</td>
<td>12.6-25.1</td>
<td>24.3-28.1</td>
<td>7.7-18.3</td>
<td></td>
</tr>
</tbody>
</table>

Table Legend: ORR: overall response rate; DCR: disease control rate; CI: confidence interval; PFS: progression-free survival; OS: overall survival. Percentages based on non-missing data. P-values ORR and DCR: chi-square-test, p-values PFS and OS: log rank.

Modest, DP et al.: Int J Cancer 2011, accepted preprint
Institut für Pathologie – Charité Campus Mitte
Overall survival in the AIO KRK 0104 –trial

Subgroups in the AIO 0104 trial
- KRAS/BRAF wild-type: OS = 23.5 months
- KRAS mut. codon 12: OS = 18.9 months
- KRAS mut. codon 13: OS = 26.2 months
- BRAF mutation: OS = 13.0 months

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. at risk</th>
<th>0</th>
<th>12</th>
<th>24</th>
<th>36</th>
<th>48</th>
<th>60</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>79</td>
<td>60</td>
<td>38</td>
<td>24</td>
<td>16</td>
<td>7</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Codon 12</td>
<td>41</td>
<td>26</td>
<td>16</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Codon 13</td>
<td>9</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>17</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Prognostic role of BRAF in stage II and III resected colon cancer

Results of the translational study on the PETACC-3, EORTC 40 993, Sakk 60-00 Trial, N= 1307; BRAF-mutated= 103 (7.9%)

Consequence => Therapy with BRAF-inhibitor

Problem: no efficacy


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We report that blockade of the epidermal growth factor receptor (EGFR) shows strong synergy with BRAF(V600E) inhibition. We find in multiple BRAF(V600E) mutant colon cancers that inhibition of EGFR by the antibody drug cetuximab or the small molecule drugs gefitinib or erlotinib is strongly synergistic with BRAF(V600E) inhibition, both in vitro and in vivo. Mechanistically, we find that BRAF(V600E) inhibition causes a rapid feedback activation of EGFR, which supports continued proliferation in the presence of BRAF(V600E) inhibition. Melanoma cells express low levels of EGFR and are therefore not subject to this feedback activation. Consistent with this, we find that ectopic expression of EGFR in melanoma cells is sufficient to cause resistance to PLX4032. Our data suggest that BRAF(V600E) mutant colon cancers (approximately 8–10% of all colon cancers2,3,5), for which there are currently no targeted treatment options available, might benefit from combination therapy consisting of BRAF and EGFR inhibitors.
Treatment efficacy - according to mut status

In CRC ca. 55% are KRAS wild type.

Out of these only ca. 50% (i.e. 25% of all CRC) respond to EGFR antibodies.

Why?

How can they be detected or stratified?
Mutations in KRAS and NRAS genes in colorectal cancer

When the rare mutations are added they represent 17.5 % of all CRC and they are associated with resistance!
Three Cellular RAS Genes Encode
Four Highly Homologous 21 kD Proteins

20020408 Trial RAS (Exon 4) Analysis
PFS in Patients with WT KRAS Exon 2 mCRC

20020408 Trial RAS (Exon 4) Analysis
PFS in Patients with WT RAS* mCRC


*WT KRAS and NRAS exons 2, 3, and 4
20020408 Trial RAS (Exon 4) Analysis
PFS in Patients with MT RAS* Exon mCRC


*MT in any KRAS and NRAS exons 2, 3, and 4
For these types of tumors a therapy with TKIs should be considered if the molecular prerequisites are proven.
NSCLC: Past and Current Landscape

1999 Histology-driven selection

- Adenocarcinoma
- Squamous-cell carcinoma
- Large cell carcinoma

2012 Targeting oncogenic drivers

- NO MUTATION DETECTED
- KRAS 22%
- EGFR 17%
- EML4-ALK 7%
- DOUBLE MUTANTS 3%
- BRAF 2%
- PIK3CA
- HER2
- MET AMP
- MEK1
- NRAS
- AKT1
- NRAS
- MET AMP
- HER2
- PIK3CA
- BRAF 2%
- DOUBLE MUTANTS 3%

Actionable driver mutations identified in 54% of lung adenocarcinoma tumours

LCMC, Lung Cancer Mutation Consortium

Kris MG, et al. Presented at ASCO 2011; Abstract CRA7506
Molecular Screening in NSCLC

- **KRAS** mutation testing
  - KRAS+ (15–30%)
    - Insensitive to EGFR TKIs

- **EGFR** mutation testing
  - EGFR+ (10%)
    - Sensitive to EGFR TKIs

- **ALK** fusion gene
  - ALK+ (3–5%)
    - Sensitive to ALK inhibitors

- Other mutations? **FGFR1**, **HER-2**, **BRAF**, **MEK1**, **AKT1**, **PI3K/mTOR**, etc

- **MEK1**, mitogen activated protein kinase kinase 1;
- **AKT1**, v-akt murine thymoma viral oncogene homolog 1;
- **mTOR**, mammalian target of rapamycin;
- **TKI**, tyrosine kinase inhibitor.


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Targeted Therapy in NSCLC

EMA/FDA: kinase inhibitors can be approved only in combination with a diagnostic eligibility test.

Example:
- therapeutic anti-EGFR
- kinase inhibitors

Gefitinib
(Iressa, Astra Zeneca)

Mok et al., N Eng J Med., 2009
EGFR-mutations and EGFR tyrosine-kinase-inhibitors

**EGFR-mutations**
- NSCLC (10-15%)

tyrosine-kinase-inhibitors interfere with activated receptors and the corresponding pathway

stimulation of:
- growth
- angiogenesis
- malignant phenotype

activating mutation of EGFR

Kinase-Domäne

KRAS (wild type)

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Summary

577 cases* included,

174 cases no sufficient PCR product for exon 20
35 cases have less than 30% tumor.
=> 368 specimen sequenced.

Based on the experience of >3000 cases it is strongly recommended to test all 4 exons

<table>
<thead>
<tr>
<th>Exon</th>
<th>Cases</th>
<th>3,8</th>
<th>25,0</th>
<th>40-45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 21</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exon 18</td>
<td>6</td>
<td>10,7</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Exon 20</td>
<td>4</td>
<td>7,2</td>
<td></td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

EMA (FDA): Therapeutic kinase inhibitors have been approved only in combination with a diagnostic eligibility test.

Examples:

- **Xalkori** – mutEML4-Alk (Crizotinib, Pfizer)
- Gefitinib – mutEGFR (Iressa, Astra Zeneca)
- Erlotinib – mutEGFR (Tarceva, Roche)
Rapid Responses Seen In Some Patients

Tumour responses to crizotinib by patient

**Study A8081001**
N=116

**PROFILE 1005**
N=240

Best objective response according to RECIST:

- PD
- SI
- PR
- CR


*Mature population, excluding those with early death, indeterminate response and non-measurable disease*
EML4-ALK Fusion in NSCLC

Chromosom 2

ALK
Anaplastic lymphoma kinase
Exon20

EML4
Exon13

11 variants

20 13
20 20
20 6a, b

Modified according to Soda et al. nature 448:561 (2007).
Anaplastic lymphoma kinase (ALK)

Exon 20

Exon 13

EML4

Alk

EML4
Malignant Melanoma

50% of all malignant melanomas exhibit a BRAF-Mutation

*Total V600 mutation rate for BRIM-3 (cobas® 4800 BRAF V600 Mutation Test); 9.9% of the cobas-positive cases subjected to retrospective Sanger sequencing had V600K mutations
Vemurafenib phase I overall survival: Updated KM estimates (08. 2011)

Extension cohort landmark
Estimated survival: 1 year = 50%, 2 years = 38%

<table>
<thead>
<tr>
<th></th>
<th>Median OS (month)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose escalation</td>
<td>25.2</td>
</tr>
<tr>
<td>Extension</td>
<td>13.8</td>
</tr>
<tr>
<td>WT or sub-therapeutic</td>
<td>4.18</td>
</tr>
</tbody>
</table>

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Vemurafenib inhibits V600 mutated BRAF kinase

Response to BRAF-inhibitors is given only if a BRAF mutation is present.

This has to be tested prior to the therapy.
Vemurafenib inhibits V600 mutated BRAF kinase

Cellular Proliferation

BRAFV600mut

MEK

ERK

ATP

RTK

50-60%* of melanomas

• Constitutive
• Not responsive to normal regulatory signals

VEMURAFENIB (PLX4032, RG7204, RO5185426)

Cellular Survival

*Total V600 mutation rate for BRIM-3 (cobas® 4800 BRAF V600 Mutation Test); 9.9% of the cobas-positive cases subjected to retrospective Sanger sequencing had V600K mutations.
Presented in Vienna at ESMO 09/2012:

Flaherty (NEJM, 2012):

OS from 5.8 months with monotherapy to 9.9 months with combinational targeted therapy.

Goals of Combination:
1. Synergy in combination
2. Prevent/overcome potential monotherapy resistance
3. Potentially decrease incidence of BRAFi-induced hyper-proliferative skin lesions
Next Steps in Molecular Pathology –
Multigene Assays in Breast Cancer

• **Multi-gene analyses**, predictive molecular pathology and response to chemotherapy in breast cancer

• The development of new multi-gene assays (2nd generation) aimed to answer the following clinical question

„*Which patient with ER+ and Her2 neg. breast carcinoma will show a good prognosis when treated by endocrine therapy only?*“
The supervised approach of classifying tumors has identified prognostic signatures.

Probably the most promising and clinically useful area for the application of genetic analysis is the prediction of response to treatment, including chemotherapy, hormonal therapy, and radiation.

The **prognostic/predictive** gene-expression profiles can be used in clinical practice.
A new molecular predictor of distant recurrence in ER-positive HER2-negative breast cancer adds independent information to conventional clinical risk factors.

Following the EPclin-based predictive data 96% of the low-risk patients do not show-up with metastases after 10 years.
Stratification by EndoPredict\textsubscript{clin}®

1702 Patientinnen in ABCSG 6 & 8

nach S3-Leitlinien

248 Pat. „low risk“*

5,3 % Metastasen

w/o Endopredict these patients may have received CTx

1.371 Pat. „intermed. risk“*

840 EPclin „low risk“

4,5 % Metastasen

83 Pat. „high risk“*

531 EPclin „high risk“

44,5 % Metastasen

4,5 % Metastasen

19,7 % Metastasen

*nach S3-Leitlinien
The EndoPredict Score identifies late distant metastases in ER+/HER2-breast cancer patients


for the

Austrian Breast and Colorectal Cancer Study Group
EndoPredict: Zeigt frühe und späte Metastasen an

**0 – 5 years**

- EPclin low
- EPclin high

P(\text{LogRank}) < 0.001
Hazard ratio: 4.82 (3.12 - 7.44)

numbers at risk:

- 1066
- 1029
- 682
- 636
- 572
- 373

**> 5 years**

- EPclin low
- EPclin high

P(\text{LogRank}) < 0.001
Hazard ratio: 5.11 (3.48 - 7.51)

numbers at risk:

- 642
- 298
- 150
- 32
- 356
- 173
- 101
- 21

EPclin low: 64% with >98% DMFS @> 5 years

Dubsky et al., SABCS 2012
GEICAM 9906-Studie: EP ist prognostisch in prä- und postmenopausalen Patientinnen

Martin et al., SABCS 2012
How often therapy is changing due to test results?

- Endopredict tests during 1st y at Charité n=167
- Results based on 130 patients, retrospektiv evaluated

Berit Müller, 2012
A look into the future, exemplified by a current case
Institut für Pathologie – Charité Berlin

**Up-coming Molecular Diagnostic**

- **Histological Diagnosis**
  - Metastasized neuro-endocrine carcinoma, grade 3

- **Standard Sequential Molecular Diagnostics**
  - KRAS
  - BRAF
  - EGFR exons 18, 19, 21
  - cKIT
  - usw.
  - no mutations

- **Parallel Molecular Diagnostics**
  - IonAmpliseq* Cancer Panel in 46 gene (total 604 loci).
  - other relevant mutations
  - ????

*Ion Torrent
If a nucleotide, for example a C, is added to a DNA template and is then incorporated into a strand of DNA, a hydrogen ion will be released. The charge from that ion will change the pH of the solution, which can be detected by our proprietary ion sensor.
Up-coming Molecular Diagnostic

**histological diagnosis**
- metastasized neuro-endocrine carcinoma, grade 3

**standard sequential molecular diagnostics**
- KRAS
- BRAF
- EGFR exons 18, 19, 21
- cKIT usw.

**parallel molecular diagnostics**
- IonAmpliseq* Cancer Panel in 46 gene (total 604 loci).
- ABL
- APC
- ALK
- KRAS
- BRAF

**treatment options**
- Iressa ➞ EGFR mut exon 20
- FGFR-inhibitor ➞ FGFR2 mut
- sorafinib/sufitinib ➞ FGFR3
- cKIT
- KDL mut
- 604 further loci……
Personalized medicine is based on a “combined morphological-molecular pathology report” including classical morphology (HE/IHC/FISH) and diverse molecular analyses – to do this in a fast and reliable manner will be the future challenge of pathology.