Workshop
Roche Diagnostics and Biomarker Development

Joachim Eberle
Head of R&D, Roche Centralized Diagnostics

Biomarkers and Roche

Making a Diagnostics test

Current Programs
Why is Roche interested in Biomarkers?

- **Patients & Clinicians**
  - early detection can increase chance of cure
  - patients can be selected to maximize benefit and minimize toxicity
- **Payers**
  - optimized therapy allows more efficient use of straining healthcare budgets
  - increased cost benefit per patient to reach "threshold"
- **Regulatory**
  - patient selection may be essential to gain regulatory approval
- **Pipeline & Positioning**
  - allows us to make better development and portfolio decisions

Treating patients that will benefit - not treating those that will not

---

Biological markers or “Biomarkers”
*Tests measuring a person’s health status*

- **Risk Assessment / Predisposition**
  Gene setup predisposes for disease - may or may not result in actual development of the disease

- **Screening / Early Detection**
  Discriminate “healthy” from asymptomatic “disease” state. Often used to screen large populations

- **Prognostic**
  Once disease state established, predict the probable course of the disease (“slow” vs. “fast” progressors)

- **Patient Stratification / Therapy selection**
  Predict the likely response to a drug to discriminate “responders” from “non-responders”

- **Therapy Monitoring**
  Monitor efficacy of treatment or recurrence of disease
Potential use of Biomarkers in Oncology

Biomarkers and Roche

Making a Diagnostics test

Current Programs
Roche Diagnostics R&D Strategy

*Drive growth through high-value products & services*

- Identification of **new markers** through initiatives in **Genomics** and **Proteomics**
- Development of tests with **differentiated clinical utility**
- Development of existing platforms
  - markers
  - systems
  - services

---

Roche Oncology Biomarker Program

*For all clinical candidates, throughout their lifecycle*

**Pharma**
- Biology & clinical expertise
- Health economics
- Commercialization of drugs

**Diagnostics**
- Development of prototype & commercial assays
- Assessment new technologies

- Exploration mode-of-action mechanisms
- Biomarker-guided development of new drugs
- Predictive Biomarkers for drug selection/ differentiation
- Sample sharing

**Our competitive edge:**
- sharing core expertise & development of targeted drugs
Biomarker projects in support of Oncology projects

<table>
<thead>
<tr>
<th>Ph I</th>
<th>Ph II</th>
<th>Ph III / Market</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pan-CDK Inhibitor (R547)</td>
<td>Omnitarg (R1273)</td>
<td>Herceptin</td>
</tr>
<tr>
<td>Ocrolizumab (R1594)</td>
<td>Range of candidate response markers</td>
<td>- HER2 expression, gene copy number and exploratory candidate markers</td>
</tr>
<tr>
<td>MAI (R1530)</td>
<td></td>
<td>Tarceva</td>
</tr>
<tr>
<td>Epothilone D (R1492 &amp; R1645)</td>
<td>Exploratory pharmacodynamic and predictive response markers for all programs</td>
<td>- EGFR gene copy number, expression and mutations</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xeloda</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MabThera</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Avastin</td>
</tr>
</tbody>
</table>

Research assays | Validated assay | In-vitro Diagnostic assay (if applicable)

Roche Oncology Biomarker Program

Two pathways to maximise marker detection and validation

Genomics
RNA/DNA

Proteomics
Peptides/Proteins

Biomarker candidates
- Technology (target) feasibility study
- Tumor tissue, serum, urine, CSF etc.
- Assay robustness evaluation
- Technology implementation
- IVD development
### Research program based on clinical need, medical value and technology match

<table>
<thead>
<tr>
<th>Cancer site</th>
<th>Screening</th>
<th>Classification</th>
<th>Relapse prediction</th>
<th>Therapy prediction</th>
<th>Monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>G</td>
<td>G</td>
<td>P</td>
<td>P</td>
<td>G</td>
</tr>
<tr>
<td>Colon</td>
<td>G</td>
<td>P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood / bone marrow</td>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bladder</td>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td>P</td>
</tr>
<tr>
<td>Lung</td>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td>P</td>
</tr>
</tbody>
</table>

#targets:  
- TaqMan/ PCR: 1-10  
- Microarrays: 1000 - 100,000  
- Elecsys: 1-3  
- IMPACT (Protein Array): Up to 20

### From diagnostic marker candidate to a product

#### Three development phases

<table>
<thead>
<tr>
<th>Phase</th>
<th>Description</th>
<th>Duration</th>
<th>Candidates</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Identifying Candidates</td>
<td>2 Years</td>
<td>100 candidates</td>
</tr>
<tr>
<td>II</td>
<td>Prototype Development</td>
<td>1-1.5 Years</td>
<td>20 candidates</td>
</tr>
<tr>
<td>III</td>
<td>Product Development</td>
<td>3 Years</td>
<td>~5 potential markers</td>
</tr>
</tbody>
</table>

**Genomics**
- TaqMan/ PCR: 1-10
- Microarrays: 1000 - 100,000
- Elecsys: 1-3
- IMPACT (Protein Array): Up to 20

**Proteomics**
- Test development
- Platform
- Manufacturing
- Clinical trials
- Registration
- Marketing
Proteomics: From marker discovery to validated marker

**Discovery**
- Comparison of diseased and healthy tissue: marker candidate

**Validation**
- Raise antibodies
- Western blots (tissue lysates)
- Immunochemistry
  - Prototype ELISA
    - marker in blood?
    - sensitivity, specificity

→ Panel A + Panel B

The composition of sample banks is the most critical factor during the validation process

**Panel A:**
- 50 diseased patients compared to 50 healthy individuals

- +
  - discontinue validation

**Panel B:**
- 1,600 samples (statistically relevant subgroup formation)
  - Age and gender matched
  - All disease stages
  - Relevant disease controls according to prevalence
  - Specificity controls (diseases not within the indication area)
  - Healthy controls
Our competitive advantage: a well-defined sample bank covering different stages

- Clinical samples have to be collected in intended indication before prototype assay development starts
- Serum and plasma samples collected. For specific indications other body fluids like urine, SF, CSF etc. are meaningful
- Clinical data are collected according to standardized CRFs
- A positive ethic committee approval and informed consents are mandatory
- Approx. 1,000 different samples are necessary for each indication due to statistical requirements

Biomarkers and Roche
Making a Diagnostics test
Current Programs
Overview: blood-based screening test programs
Dual development genomic and proteomic targets

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Methylated DNA markers</th>
<th>Protein markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>colorectal</td>
<td>22 candidate markers</td>
<td>&gt; 12</td>
</tr>
<tr>
<td>breast</td>
<td>discovery underway</td>
<td>&gt; 13</td>
</tr>
<tr>
<td>prostate</td>
<td>&gt; 20 candidate markers</td>
<td>no program</td>
</tr>
<tr>
<td>lung</td>
<td></td>
<td>&gt; 90</td>
</tr>
</tbody>
</table>

Colorectal cancer (CRC) screening
Highly curable when diagnosed in early stage

<table>
<thead>
<tr>
<th>Location</th>
<th>Stage at first diagnosis:</th>
<th>5-year survival:</th>
</tr>
</thead>
<tbody>
<tr>
<td>colon</td>
<td>Stage I + II</td>
<td>55 %</td>
</tr>
<tr>
<td>small intestine</td>
<td>Restricted to gut</td>
<td>90 %</td>
</tr>
<tr>
<td>rectum</td>
<td>Stage III</td>
<td>28 %</td>
</tr>
<tr>
<td></td>
<td>Regional lymph node</td>
<td>55 %</td>
</tr>
<tr>
<td></td>
<td>involvement</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stage IV</td>
<td>17 %</td>
</tr>
<tr>
<td></td>
<td>Distant metastases</td>
<td>5 %</td>
</tr>
</tbody>
</table>

Less than 40 % CRC detected at early stages

Urgent need for screening markers in body fluids indicating early stage disease
Current CRC screening algorithms

Issues with sensitivity, specificity &/ or compliance

Risk population (> 50 yrs or symptoms or family history)

FOBT (fecal occult blood test)

Sensitivity* 25-50 % (number of true CRC positives detected by the assay)

Specificity* 80-95 % (number of true negatives correctly identified)

Colonoscopy

Sensitivity close to 100 %

Confirmation by Biopsy

Specificity 100 %

unsatisfactory as first-line screening assay

- highly invasive
- poor acceptance
- not useful as general screening method


CRC Results: Final diagnostic test most likely will be a combination of markers

Receiver Operator Curves (ROC) of different markers:

- Identified differences between "healthy" and diseased tissue
- Differences could be confirmed in serum using prototype ELISA
- Combinations of markers increases sensitivity
- Continue validation of additional markers
- Clinical validation underway

AUC = area under the curve

Roche Oncology Event, June 19, 2006
Current standards in diagnosis of leukemia
Varying reproducibility rates of gold standards

- No single test currently sufficient to establish diagnosis
- Current tests subjective, with up to 7 days turnaround
- Lack standardization & automation - need highly skilled staff

Genomics program: Leukemia microarray
Improved diagnosis enables better treatment

- Heterogeneous disease caused by genetic abnormalities
- Distinction between chronic, acute and sub-classifications essential for successful treatment
  - ~20 subgroups

AmpliChip Leukemia
- single assay for sub-classification of major leukemia classes
- potential to replace other methods/technologies
- research program ’05/’06
**MILE Study**

*Compare Clinical accuracy of microarray test with standard leukemia laboratory methods*

Gold standard panel of tests
- Morphology
- Cytogenetics
- Immunophenotyping
- Cytchemistry
- FISH
- PCR

Microarray-based gene expression profile

* The MILE study (Microarray Innovations in Leukemia*) is conducted in collaboration with the European Leukemia Network and US participants

n = 4000 patients

**Marker set distinguishes >20 subclasses**

*All clinically relevant subclasses identified, accuracy ~96%*

<table>
<thead>
<tr>
<th>No.</th>
<th>Class</th>
<th>Subclass</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>mature B-ALL with t(8;14)</td>
<td>ALL, T-lineage, immature (Pre)</td>
</tr>
<tr>
<td>2</td>
<td>Pro-B-ALL with t(11q23)/ MLL</td>
<td>ALL, T-lineage, immature (Pre)</td>
</tr>
<tr>
<td>3</td>
<td>c-ALL/Pre-B-ALL with t(9;22)</td>
<td>ALL, T-lineage, cortical</td>
</tr>
<tr>
<td>4</td>
<td>CML</td>
<td>ALL, T-lineage, mature</td>
</tr>
<tr>
<td>5</td>
<td>ALL with t(12;21)</td>
<td>mutat IgVH</td>
</tr>
<tr>
<td>6</td>
<td>ALL with t(1;19)</td>
<td>unmutat IgVH</td>
</tr>
<tr>
<td>7</td>
<td>ALL with hyperdiploid karyotype</td>
<td>ZAP-70 positive</td>
</tr>
<tr>
<td>8</td>
<td>c-ALL/Pre-B-ALL with t(9;22)</td>
<td>ZAP-70 negative</td>
</tr>
<tr>
<td>9</td>
<td>AML with t(8;21)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>AML with t(15;17)</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>AML with inv(16) t(16;16)</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>AML with t(11q23)/ MLL</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>AML with normal karyotype + other abnormalities</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>AML complex aberrant karyotype</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>PLL</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>CML</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>No call</td>
<td></td>
</tr>
</tbody>
</table>

Data will be used to design custom microarray for application of gene expression profiling for diagnosis & sub classification of leukemia
Summary

- Biomarkers are becoming a requirement in future healthcare
- Biomarker discovery is similar to drug development in timelines and success
- New assays most likely a combination of markers
- Roche has extensive joint program for all drugs throughout their lifecycle
- Our strategy should provide Roche competitive edge in developing innovative products and services

Appendix
MILE study inter-lab data
High inter- and intra-laboratory reproducibility

Data from the training phase (proficiency testing) using all genes represented on HG-U133 Plus 2.0 array

High correlation and concordance ensures high quality data

New Cancer marker launches

<table>
<thead>
<tr>
<th>Year</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Research use</td>
<td>Leukemia microarray (classification)</td>
<td>AmpliChip p53 test (mutational analysis)</td>
<td>Bladder cancer monitoring test</td>
</tr>
<tr>
<td></td>
<td>Research use</td>
<td>Breast relapse prediction test (p53)</td>
<td>Screening test for colorectal cancer</td>
<td>Lymphoma (classification)</td>
</tr>
<tr>
<td></td>
<td>Impact (Protein Array)</td>
<td>Screening test for prostate and breast cancer</td>
<td>Breast cancer therapy (p53)</td>
<td>Screening test for prostate and breast cancer</td>
</tr>
</tbody>
</table>

Launch dates are estimates only; US launches may be later than indicated