Development of biomarkers – promises and pitfalls

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Pathology-based therapy

cytotoxic
downarrow
target-oriented

cytostatic
The Preclinical Challenge

If you have a tumor and are a mouse, you haven’t got a problem

Experimental animal data can “only” provide us with
• a sound scientific rationale
• toxicity / PK data to move early into clinical trials
• Proof of concept in early clinical trials
Clinical drug development follows new rules

- Endpoints for “conventional” chemotherapy (cytotoxic)
  Phase I: Define “maximum tolerated dose (MTD)”, and move to
  Phase II: Prove efficacy by RECIST criteria
    Empirical approach,
    as long as it works mode of action of less importance

- Endpoints for molecular targeted therapy (usually cytostatic)
  - Frequently the MTD “cannot” be determined
  - Response according to RECIST criteria less meaningful
    It is critical to understand the science behind and develop biomarkers (prospectively)
Potential endpoints of clinical trials

- Clinical endpoint
- Biomarker
  an indicator of normal biologic / pathogenic processes or a pharmacological response to a therapeutic intervention
- Surrogate endpoint
  biomarker intended to substitute for a clinical endpoint
Clinical/Translational Research – “Bi-directional”

‘Bench-to-Bedside’
Discoveries from mechanistic studies from the basic science laboratory (bench)
Translated into practical applications at the bedside and clinic

‘Bedside-to-Bench’
Observations from the bedside and clinic drive mechanistic studies at the bench
(“no biopsy - no trial”)
Case story:
Challenges and pitfalls of clinical biomarker development - Lessons learnt from EGFR inhibitors
Successful biomarker development: Trastuzumab

- Validation of target - biomarker - antibody relationship involved a great deal of effort (initial test suboptimal)
- Once marker was validated only Her-2/neu overexpressing tumors enrolled (20-25% of patients)
  - 470 instead of an estimated 2200 patients
  - Significant benefit demonstrated after 1.6 years instead of >5 years
  - Response rate 50% compared to about 10%
  - Approved also in adjuvant setting
The Importance of Patient Selection: The Trastuzumab Example

- All Breast Cancer Patients
  - Trastuzumab
  - < 10% Response

- Her2+ Breast Cancer Patients
  - Trastuzumab
  - 35-50% Response

Can we do the same for other targeted therapies?
Activating mutations in the EGFR predict responsiveness to gefitinib in NSCLC

- 8/9 gefitinib-responsive and 0/7 resistant tumors exhibited somatic mutations of the TK domain
- EGFR mutations led to enhanced TK activity in response to ligand binding and increased sensitivity to gefitinib

Lessons to be learnt:

- search for biomarkers is a key issue
- treat subgroup of patients earlier / other tumors?
- we are on our way towards personalized medicine?

*Lynch et al., NEJM 350:2129-2139, 2004*
Biomarker-dependent therapy is the way forward for cancer treatment

<table>
<thead>
<tr>
<th>Agent</th>
<th>Cancer type</th>
<th>Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trastuzumab</td>
<td>Metastatic breast cancer</td>
<td>Overexpression/amplification of HER-2</td>
</tr>
<tr>
<td>Imatinib</td>
<td>Chronic myeloid leukemia</td>
<td>BCR/ABL positive</td>
</tr>
<tr>
<td></td>
<td>GIST</td>
<td>Activated C-KIT receptor tyrosine kinase/CD117, exon 9 mutation</td>
</tr>
<tr>
<td>cetuximab</td>
<td>mCRC</td>
<td>EGFR expression: <strong>NO</strong></td>
</tr>
<tr>
<td>panitumumab</td>
<td></td>
<td>KRAS wild-type status</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>(retrospective evaluation)</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Raf, PTEN, PI3K</td>
</tr>
</tbody>
</table>
329 patients with CRC progressed on or within 3 months of *irinotecan-based* chemotherapy

**2:1 RANDOMIZATION†**

- **Irinotecan* + Cetuximab**
  - n = 111
- **Cetuximab**
  - n = 218

**Irinotecan* + Cetuximab**
- n = 56

† Data at start of study indicated combination treatment might be more effective

* Same regimen as previously failed;
** Initially 400 mg/m² IV, then 250 mg/m² IV weekly

### BOND trial

**Correlation response – EGFR expression**

<table>
<thead>
<tr>
<th>% EGFR expressing cells</th>
<th>combination n/N</th>
<th>RR (%)</th>
<th>monotherapy n/N</th>
<th>RR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 10 %</td>
<td>25/109</td>
<td>22.9</td>
<td>4/56</td>
<td>7.1</td>
</tr>
<tr>
<td>&gt; 10 - ≤ 20 %</td>
<td>4/20</td>
<td>20.0</td>
<td>5/16</td>
<td>31.3</td>
</tr>
<tr>
<td>&gt; 20 - ≤ 35 %</td>
<td>6/27</td>
<td>22.2</td>
<td>0/7</td>
<td>0.0</td>
</tr>
<tr>
<td>&gt; 35 %</td>
<td>15/62</td>
<td>24.2</td>
<td>3/32</td>
<td>9.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>EGFR staining intensity</th>
<th>combination n/N</th>
<th>RR (%)</th>
<th>monotherapy n/N</th>
<th>RR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>weak</td>
<td>11/53</td>
<td>20.8</td>
<td>1/21</td>
<td>4.8</td>
</tr>
<tr>
<td>moderate</td>
<td>22/89</td>
<td>24.7</td>
<td>7/55</td>
<td>12.7</td>
</tr>
<tr>
<td>strong</td>
<td>17/75</td>
<td>22.7</td>
<td>4/34</td>
<td>11.8</td>
</tr>
</tbody>
</table>

Cetuximab in EGFR-non expressing CRC by immunohistochemistry (IHC)

- EGFR as determined by IHC does not correlate with sensitivity to cetuximab
  - EGFR negativity should not be used to exclude mCRC patients from cetuximab treatment
  - EGFR positivity in non-mCRC cancer patients is equally non-predictive and should not be used to justify cetuximab treatment in non-colorectal patients
- KRAS gene codes for a protein involved in the EGFR pathway.
- Protein regulates other proteins, downstream in the EGFR signaling pathway associated with tumor survival, angiogenesis, proliferation and metastasis.
- There are different types of the KRAS gene found in tumors coding for:
  1. “normal”, non-mutated KRAS protein – wild-type KRAS
  2. mutated KRAS protein – mutant KRAS (constitutively switched “on”)
KRAS Wild-type: Data Consistency

`CRYSTAL` (n=540)
`OPUS` (n=233)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Response Rate (%)</th>
<th>PFS Estimate</th>
<th>HR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRYSTAL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOLFIRI</td>
<td>43</td>
<td></td>
<td>0.68</td>
<td>0.017</td>
</tr>
<tr>
<td>Cetuximab + FOLFIRI</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FOLFOX</td>
<td>37</td>
<td></td>
<td>0.57</td>
<td>0.016</td>
</tr>
<tr>
<td>Cetuximab + FOLFOX</td>
<td>61</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

However: pG13D mutation NOT predictive
Selective approach to personalizing the treatment strategy in mCRC

Treatment strategy:
Personalizing treatment to maximize success and avoid unnecessary AEs

Chemotherapy

- 40% responders
- 40% no effect on tumor growth
  - 40% waste of medical resources
  - Unnecessary AEs

Add-on selective targeted therapy

- Up to 60% responders

Non responders

- 40% no effect on tumor growth
  - Waste of medical resources
  - Unnecessary AEs
mCRC patients treated with panitumumab or cetuximab, n = 113

<table>
<thead>
<tr>
<th></th>
<th>Mutant KRAS</th>
<th>Wild-Type KRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>**p&lt;0.05 (p=0.011)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>2/34 (6%)‡</td>
<td>22/79 (28%)‡</td>
</tr>
<tr>
<td>Non Responders</td>
<td>32/34 (94%)**</td>
<td>57/79 (72%)**</td>
</tr>
</tbody>
</table>

BRAF mutational status on Wild-Type KRAS tumors

<table>
<thead>
<tr>
<th></th>
<th>Mutant BRAF</th>
<th>Wild-Type BRAF</th>
</tr>
</thead>
<tbody>
<tr>
<td>*p&lt;0.05 (p=0.029)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Responders</td>
<td>0/11 (0%)‡</td>
<td>22/68 (32%)*</td>
</tr>
<tr>
<td>Non Responders</td>
<td>11/11 (100%)*</td>
<td>46/68 (68%)*</td>
</tr>
</tbody>
</table>
**PIK3CA-PTEN** alterations and response to anti EGFR antibodies

<table>
<thead>
<tr>
<th>Mutated KRAS</th>
<th>Wild-Type KRAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>32/109 (29%)</td>
<td>77/109 (71%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Responders</th>
<th>Non Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>2*</td>
<td>20*</td>
</tr>
<tr>
<td>30*</td>
<td>57*</td>
</tr>
</tbody>
</table>

**p<0.01** (p=0.00003)

<table>
<thead>
<tr>
<th>Mutated PIK3CA/PTEN</th>
<th>Normal PIK3CA/PTEN</th>
</tr>
</thead>
<tbody>
<tr>
<td>27/59 (46%)</td>
<td>32/59 (54%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Responders</th>
<th>Non Responders</th>
</tr>
</thead>
<tbody>
<tr>
<td>1**</td>
<td>17**</td>
</tr>
<tr>
<td>26**</td>
<td>15**</td>
</tr>
</tbody>
</table>

* *p<0.05 (p=0.019)

Sartore Bianchi et al., 2010
Molecular redefinition & hypersegmentation of cancer: Somatic Mutations in Lung Adenocarcinoma N=188

Crizotinib in metastatic NSCLC with ALK rearrangement

Incidence: 4-7% (mainly AdenoCa in nonsmokers)
n=82: RR 57% (1CR), SD 33%, 6-mths-PFS-Rate 72%
Metastatic CRC - Molecular predictive markers

- **KRAS mutation (codon 12 & 13) validated for predicting resistance to anti-EGFR**
- **Emerging predictive markers**
  - BRAF mutations: predictive for resistance to anti-EGFR in chemorefractory CRC
    - Early lines of treatment: not validated
    - BRAF mutations mutually exclusive with KRAS mutations
- **Potential markers under investigation for anti-EGFR**
  - PI3K
  - PTEN
  - NRAS
- **Potential markers for chemotherapy toxicity and efficacy:**
  - ERCC1 for oxaliplatin
  - UGT1A1 phenotype (irinotecan)
  - DPD for 5-FU
Biomarkers in Phase 1 Trials: Utility

- Proof of mechanism (drug hits proposed target)
  - Target
  - Pathway
  - Proliferation, apoptosis

- When toxicity may be insufficient to determine active dose/schedule
  - Unlikely to occur at dose/exposure that affects the target
  - Due to off target effects and effects on target are uncertain
  - To target a specific degree of target inhibition to avoid significant toxicity

- When pharmacokinetics may be insufficient to determine active dose/schedule
  - Assay lacking
  - Pharmacokinetics in plasma does not match tissues
Supposed we have a new targeted therapy designed to be effective in patients with marker A:

What type of clinical trials should we design?
Target selection / enrichment designs

• If we are sure that the therapy will not work in marker-negative patients
  AND

• We have an assay that can reliably assess the marker
  THEN

• We might design and conduct clinical trials for marker-positive patients or in subsets of patients with high likelihood of being marker-positive
Enriched patient populations

Example:
Gefitinib in Asian patients with adenocarcinoma of the lung – never / light smokers
• Need not have an assay for the biomarker
• Accrual faster
• Sample size smaller than unselect but larger if not all patients are marker positive
Predictive Marker Study Design

Selection/Enrichment Design
*Only marker+ patients are randomized and/or treated*

- **All patients** → Marker assay →
  - Marker + → New drug
  - Marker - → OFF study
  - Control?

**Questions**
- Does new drug benefit marker negative patients also?
- If no control arm
  - Is good outcome due to better prognosis?
  - Is good outcome due to new drug?
## Prospective versus Retrospective Analysis

<table>
<thead>
<tr>
<th></th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| **Prospective**      | • Fewest numbers of patients  
                       • Study design guaranteed to have sufficient power to show treatment effect in marker groups | • Must know marker to select patients  
                       • Rapid turnaround to determine eligibility |
| **Retrospective**    | • Maximize accrual  
                       • Need not know marker  
                       • Refine marker/assay while trial ongoing  
                       • Allows assessment in marker+/− groups | • Risk of insufficient numbers within marker group(s)  
                       • Collection of samples compromised  
                       • Results may not be generalizable due to bias sampling |
VEGF is the Key Mediator of Angiogenesis

VEGF

Survival

Migration

Proliferation

ANGIOGENESIS

Upstream activators of VEGF synthesis

Downregulation of T cell function

Endothelial cell

Downstream signaling pathways
How to Identify the “Relevant” Biomarker?

Dream: Single Signal Approach

Reality: A lot of redundancy
Use of proteomics as biomarker in cancer

Advantage of protein biomarkers over genomics: they represent functional endpoints

- Monitor disease progression
- Monitor response to therapy (VeriStrat®)
- Detection of a „pattern“ of events that reflect cancer growth
- Assessment of complex biologic processes resulting from therapeutic interventions
Many questions remain (1)

- (New) biomarkers may come up during drug development
- Prospective sampling - retrospective evaluations
- Biomarkers may make frequent diseases (NSCLC, CRC) to rare diseases
- Biomarkers may differ among histopathologically defined cancer types
- Investigator initiated trials are to be encouraged

Academia must seek for grants to help support translational research
Many questions remain (2)

• Do we always need to run large phase III trials in small but well-defined patient populations?
• What if a patient has got two or more mutations that can each be targeted with a drug?
• Combination with chemo? Other „targeted“ drugs?
• Basically combinations are the only way to fight redundancy
What we have before us are some breathtaking opportunities disguised as unresolvable problems

John Gardner, US minister of health, 1965