Theranostik – Perspektiven für die personalisierte Medizin in der Infektionsdiagnostik

Till Bachmann
University of Edinburgh
Talk Outline

• Personalised Medicine

• Companion Diagnostics

• Infectious Diseases & Antibiotic Resistance

• New Technology Solutions for ID Diagnostics

Not Every Drug Works with Every Patient

<table>
<thead>
<tr>
<th>Therapeutic area</th>
<th>Efficacy rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alzheimer’s</td>
<td>30</td>
</tr>
<tr>
<td>Analgesics (Cox-2)</td>
<td>80</td>
</tr>
<tr>
<td>Asthma</td>
<td>60</td>
</tr>
<tr>
<td>Cardiac Arrhythmias</td>
<td>60</td>
</tr>
<tr>
<td>Depression (SSRI)</td>
<td>62</td>
</tr>
<tr>
<td>Diabetes</td>
<td>57</td>
</tr>
<tr>
<td>HCV</td>
<td>47</td>
</tr>
<tr>
<td>Incontinence</td>
<td>40</td>
</tr>
<tr>
<td>Migraine (acute)</td>
<td>52</td>
</tr>
<tr>
<td>Migraine (prophylaxis)</td>
<td>50</td>
</tr>
<tr>
<td>Oncology</td>
<td>25</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>48</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>50</td>
</tr>
<tr>
<td>Schizophrenia</td>
<td>60</td>
</tr>
</tbody>
</table>
Personalised Medicines

Therapeutic Need

Innovative Medicines

Patient Targeted Therapies

Right Medicine
Right Patient
Right Disease
Right Time
Right Dose
Right Response
Right Price

Keys issues with medicines today are …..

**Efficacy**
(does the patient get better?)

**Safety**
(is the drug safe for the patient?)
Industry’s View

“For every single Pharma product … the biomarker research and the development of potentially companion diagnostics is a standard part of the development process” (Severin Schwan, Roche CEO, June 2007)

Technology Path to PGx

- Assays
- Biomarker
- Drugs

Personalised Medicine
Personalised Medicine - The Beginnings

- **Herceptin/Trastuzumab (Genentech/Roche)**
  - Antibody against Human Epidermal growth factor Receptor 2 (HER2 (erbB2, HER2/neu))

- **HercepTest (DakoCytomation)**
- **Pathway (Ventana/Roche)**
  - Identify which patients overexpress Her2
- **PathVysion (Vysis/Abbott)**
  - Identify which patients have multiple copies of Her2 gene

[Link to Herceptin](http://www.herceptin.com)
[Link to Dako](http://www.dako.com)
[Link to Ventana](http://www.ventanamed.com)
[Link to PathVysion](http://www.abbottmolecular.com/PathvysionHER2DNAProbeKit_5138.aspx)

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Companies in Companion Diagnostics

- ELISA
- Bead Array
- Microarray
- Sequencing
- RT-PCR
- Invader
- In Vivo Virus assay
- Methylation
- IHC
- FISH
- miRNA


Nature Biotechnology 26, 509 - 517 (2008)

Personalised Medicine M&A Deals 2011


Economic Impact of Companion Tests

No CDx

Discounted Cash Flow - No Dx, NPV = $892 (15% dcf)

CDx

Discounted Cash Flow - Companion Dx, NPV = $2,694 (10% dcf)

Companion Diagnostics Development

Scenario 1 – CDx to be developed

Preclinical Phase I Phase 2 Phase 3 Filing Post-Approval

Rx Development

BM Assay Development

Rx & Dx data filed for approval

Diagnostic launched at same time as drug

Companion diagnostic identified and NOT available

Eg. Herceptin

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Chris Chamberlain
Reimbursement is a Key Driver

- Health Technology Assessment (cf UK NICE)

*Hughes NRDD 8: 261 (2009)

To implement this risk-sharing agreement, clinicians are required to measure the levels of serum M protein (SMP; a specific biomarker for tumour load) after a maximum of four cycles of treatment. If the patient has a reduction in SMP of 50% or more, indicating a complete or partial response, treatment will continue and the NHS will pay. If not, the JnJ must rebate the full cost.

Andrew Dillon, Chief Executive of NICE commented, “the NHS will only pay for the drug when it has been proven to work…”

Eddie Blair, Integrated Medicines Ltd 2010
Companies Entering the Infectious Disease CDx Market I

Combining forces against Hepatitis C

Hepatitis C virus (HCV) causes acute and chronic liver diseases that can lead to liver failure, cirrhosis and cancer. There are currently over 185 million people infected with HCV worldwide. Diagnostic tests based on PCR technology can now measure viral load in the blood of patients with chronic hepatitis C infection and determine which of the four different HCV subtypes (genotypes) is present.

The disease can then be treated by pegylated interferon tailored to the appropriate genotype and viral load levels, such as the Roche product Peginteron. The viral load test is also used after a few weeks to check treatment response. In many patients, the virus can be completely eliminated. For chronic hepatitis C patients who do not benefit from interferon-based therapy, several small orally active molecules are in clinical development at Roche.

The focus here is on Hepatitis C subtype 1 infections, which is very difficult to treat. Initial results show that in patients who have not responded to interferons, the new combined treatment can achieve a 95-99% reduction in blood viral load within just two weeks.

<table>
<thead>
<tr>
<th>Hepatitis C virus</th>
<th>Diagnostics</th>
<th>Therapeutics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular test (PCR) to determine levels of circulating HCV viral load</td>
<td>HCV nucleoside polymerase inhibitors (NS5BIBI) prevent replication of hepatitis C virus</td>
<td></td>
</tr>
</tbody>
</table>

* These tests are being developed by Roche Diagnostics.

Companies Entering the Infectious Disease CDx Market II

BIOCARTIS’ MOLECULAR DIAGNOSTICS PLATFORM

Early 2012, Biocartis acquired Phexis’ technology platform to rapid liquid-extended CD4/CD8/HLA-DR molecular diagnostic testing. The platform has been designed for applications in a wide range of patient sample testing, including oncology and infectious disease.

Biocartis’ technology platform is the result of several years of development by the life science research and applied technology group within Philips Healthcare’s Diagnostics division. It has been designed to fully integrate, automated access, multiplexed CD4/CD8/HLA-DR molecular diagnostic testing, which makes a complex and variable sample preparation system. The platform is characterized by ease-of-use, which is a prerequisite for the application of all types of laboratory environments, including the point-of-care setting.

Biocartis develops and commercializes the platform and a menu of tests through strategic partnerships and will distribute variations of the platform at its newly-established and wholly-owned Dutch subsidiary Biocartis BV.

News

Biocartis completes EUR 7.5 million of EHV 1 (now) Series F round Funding

Funds will be used to commercialize a fully automated, compact molecular diagnostic system designed to drive the widespread adoption of personalized medicine and other diagnostic applications including the fight against infectious diseases.

Luzanne/Switzerland — November 17, 2011. have recognized our potential and the progress we have made towards our goal of becoming a fully integrated global diagnostics company commercializing molecular diagnostics systems that will make personalized medicine an everyday reality.

bioMerieux and Janssen Pharmaceuticals (K&J)

Companies Entering the Infectious Disease CDx Market III

January 12, 2012

Qiagen, Max Planck Developing qPCR-Based Test for Active TB Risk in Latently Infected Patients

By Ben Bukus

Qiagen and the Max Planck Institute for Infection Biology this week announced a collaboration to develop a real-time PCR-based molecular diagnostic test to assess the risk of patients with latent tuberculosis developing active TB during their lifetime.

According to Qiagen, the assay will be developed for use on its QIAsymphony molecular testing platform; would ideally comprise two to five gene markers; and would serve as a reflex test following the company’s Quantiferon-TB Gold test, a “pre-molecular” diagnostic assay that can be used to detect latent TB in patients.

In addition, researchers at both Qiagen and Max Planck are hoping that the qPCR-based whole-blood transcription profiling work they will be conducting to develop the test will also help reveal more about the biological pathways involved in the progression of latent TB to active TB.

Companies Entering the Infectious Disease CDx Market IV

Siemens, Illumina Partner on Infectious Disease Testing Using Next-Generation Sequencing

Posted: November 2, 2011

Siemens Healthcare Diagnostics and Illumina have entered into a partnership aimed at setting new standards in the use of next-generation sequencing for the rapid, accurate identification of patients’ infectious disease states and potential treatment paths. Through this agreement, the companies plan to make existing Siemens molecular HIF tests compatible with the recently launched Illumina HiSeq next-generation sequencing platform, with the ultimate goal of introducing breakthrough sequencing-based infectious disease assays for the clinical diagnostics market.

"Next-generation sequencing is a transformational technology that we believe will significantly impact clinical diagnostics over the next five years," said Michael Fehrenbacher, CEO, Siemens Healthcare Diagnostics. "Our partnership with Illumina brings together two innovation leaders to set a new standard of care in the next wave of clinical diagnostics and personalized medicine."

Next-generation genome sequencing (DNA sequencing) is the determination of the precise sequence of nucleotides in a sample of DNA. These data points help provide physicians with deeper insights into patients’ genetic characteristics, including suitability to drug regimens based on a patient’s profile of an infectious disease, source of infection, and insights into treatment directions and personalized medicine status — both current disease state and susceptibility to future disease. According to a recent report in Genetic Engineering & Biotechnology News, Genetic Engineering & Biotechnology News, according to a recent report in Genetic Engineering & Biotechnology News, Genetic Engineering & Biotechnology News, Genetic Engineering & Biotechnology News, Genetic Engineering & Biotechnology News, Genetic Engineering & Biotechnology News, Genetic Engineering & Biotechnology News, Genetic Engineering & Biotechnology News, Genetic Engineering & Biotechnology News. Genentech’s estimates that the next-generation sequencing market could reach $5.8 billion in sales by 2014, with a broader picture putting the figure closer to $3.5 billion.
Not Every Drug Works with Every Bug

The Problem of Antibiotic Resistance

- Growing antibiotic resistance threatens the effectiveness of antibiotics
- Serious infections in humans
- Overuse and misuse of antibiotics leads to resistance
- 2.5m extra hospital days
- 380,000 extra infections, 25,000 extra deaths (4 indications)
- 1.5b € per year extra costs in EU
- Beta-lactams most used antibiotics
- Some resistance rates have more than doubled in the past five years
- Few antibacterial agents with new mechanisms of action under development (gram-)

Antibiotic Resistance Develops Quickly

Antibiotic deployment

Antibiotic resistance observed

- Antibiotic prescribing and consumption varies between European countries.
- Primary care accounts for about 80 to 90% of all antibiotic prescriptions, mainly for respiratory tract infections.


Do beta-lactamase variants matter therapeutically?

**TABLE 2.** Antimicrobial susceptibilities of clinical isolates and KPC variants.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (μg/ml) for the following strain (KPC-2)</th>
<th>Ecoli (KPC-2)</th>
<th>KPS (KPC-4)</th>
<th>KPC (KPC5)</th>
<th>Ecoli (KPC-4)</th>
<th>KPS (KPC-4)</th>
<th>KPC (KPC5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Piperacillin + tazobactam</td>
<td>&gt;256</td>
<td>&gt;64</td>
<td>128</td>
<td>1</td>
<td>&gt;256</td>
<td>128</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>64</td>
<td>&gt;64</td>
<td>128</td>
<td>0.12</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>ND</td>
<td>&gt;64</td>
<td>ND</td>
<td>&lt;0.06</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>ND</td>
<td>0.06</td>
<td>ND</td>
<td>&lt;0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>ND</td>
<td>&gt;64</td>
<td>ND</td>
<td>&gt;0.12</td>
<td>&gt;128</td>
<td>&gt;128</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Imipenem</td>
<td>64</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>0.12</td>
<td>64</td>
<td>64</td>
<td>64</td>
</tr>
<tr>
<td>Meropenem</td>
<td>ND</td>
<td>&gt;32</td>
<td>&gt;64</td>
<td>&lt;0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*“As demonstrated in this study, the KPC variants have different susceptibilities and hydrolytic properties. This may contribute to the difficulty that clinical laboratories have encountered in identifying KPC-producing organisms.”*
Unmet Need in Clinical Microbiology

Pathogen → Drug Resistance → Therapy

Rapid Tests to Enable Prospective Therapy

Diagnostic DNA Microarrays
DPM Microbial DNA Microarray Portfolio

SNP
- Microbial Antibiotic Resistance
  - ESBL (SHV, TEM, CTX-M)
  - OXA, AmpC
  - KPC
  - gyrA/parC
- E. coli Pathogenicity
  - fimH
  - ECOR

Species
- Pathogens
  - Sepsis
  - Fungi
  - Protozoa
  - Gastroenteritis
  - HCV
- Environment
  - Cyanobacteria

Genotyping DNA Microarray Assay

Spotting
- Microgrid II
- Epoxy slides
- Spotted capture probes
- 20 - 25 nt

Sample
- Crude sample
- Pure cultures

DNA extraction
- AE resin

Amplification and Labeling
- PCR
- Cy3-dCTP
- DNase

Hybridization
- 1 h

Imaging & Identification
- Scanarray Express
Antibiotic Resistance: 
**Extended Spectrum Beta-Lactamases**

Carbapenemases

**Class A***
Group 2**

Class C***
Group 1**

Class D***
Group 2d**

Class B***
Group 3**

Cephalosporinases
chromosomal, plasmid encoded
all \( \beta \)-lactams except Carbapenems
no inhibition with clavulanic acid
e.g. AmpC

**Carbapenemases**
chromosomal, plasmid encoded
all \( \beta \)-lactams except Monobactams
no inhibition with clavulanic acid
e.g. IMP, NDM-1

**Cloxacillin (Oxacillin)**
hydrolyzing enzymes
chromosomal, plasmid encoded
e.g. OXA

**plasmid encoded**
inhibition with clavulanic acid
2be broad spectrum \( \beta \)-Lactams
2br inhibitor resistant
e.g. TEM, SHV, CTX-M, KPC

\* older Classification according to Ambler (1980)
** Classification according to Bush, Jacoby and Medeiros (BJM)

---

**SNP Detection Microarray Principle**

allele-specific hybridisation

<table>
<thead>
<tr>
<th>Probe</th>
<th>SNP</th>
<th>Product/Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A: GTTACATGACCTGGATCTCA</td>
<td>A</td>
<td>perfect match</td>
</tr>
<tr>
<td>1G: GTTACATGACCTGGATCTCA</td>
<td>G</td>
<td>perfect match</td>
</tr>
<tr>
<td>1C: GTTACATGACCTGGATCTCA</td>
<td>C</td>
<td>perfect match</td>
</tr>
<tr>
<td>1T: GTTACATGACCTGGATCTCA</td>
<td>T</td>
<td>perfect match</td>
</tr>
</tbody>
</table>

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ESBL Microarray

Specifications
- 3 chip modules
- 14 subarrays
- 155 probe sets
- 2060 spots

ESBL Microarray Testing of Clinical Isolates

Klebsiella pneumoniae carbapenemase (KPC) microarray

- Confers resistance to Carbapenems
- Carbapenems classed as “drug of last resort”
- First reported in North Carolina in 2001 by Yigit et al.
- Found in New York, Israel, China, South America, ...

<table>
<thead>
<tr>
<th>KPC variant</th>
<th>GenBank ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC-2</td>
<td>AJ135887</td>
</tr>
<tr>
<td>KPC-3</td>
<td>AY150941</td>
</tr>
<tr>
<td>KPC-4</td>
<td>F47598</td>
</tr>
<tr>
<td>KPC-5</td>
<td>EU00222</td>
</tr>
<tr>
<td>KPC-6</td>
<td>EU05234</td>
</tr>
<tr>
<td>KPC-7</td>
<td>EU00227</td>
</tr>
<tr>
<td>KPC-8</td>
<td>F234412</td>
</tr>
<tr>
<td>KPC-10</td>
<td>EU00246</td>
</tr>
<tr>
<td>KPC-11</td>
<td>HM00956</td>
</tr>
</tbody>
</table>

KPC Reference Strains for Assay Development

- Escherichia coli Strain KPC-2
- Klebsiella pneumoniae Strain KPC-2
- Klebsiella pneumoniae Strain KPC-3
- Klebsiella pneumoniae Strain KPC-3
- Klebsiella pneumoniae Strain KPC-3
- Klebsiella pneumoniae Strain KPC-2
- Klebsiella pneumoniae Strain KPC-2
- Klebsiella pneumoniae Strain KPC-2
- Klebsiella pneumoniae Strain KPC-2
- Klebsiella pneumoniae Strain KPC-2

urine dilutions 1x10^9 – 1 CFU / ml

→ QIAprep Spin Miniprep Kit (Qiagen)
→ Urine Bacterial DNA Isolation Kit (Norgen)
KPC Microarray Results I

Array 1 (K. pneumoniae, VA 367, KPC-3)

Array readout results:

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Rapid and Sensitive Detection of Carbapenem Resistance Directly from Urine Samples using DNA Microarrays. submitted

Identification Directly from Spiked Urine Samples

Qiagen

LOD 4000 CFU / ml urine

Norgen

LOD 360 CFU / ml urine
### Limit of Detection (LOD)

**Analysis directly from spiked urine samples**

<table>
<thead>
<tr>
<th>Committee</th>
<th>LOD in (cpm / ml urine)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong></td>
<td>16000</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>16000</td>
</tr>
<tr>
<td><strong>K. pneumonia</strong></td>
<td>16000</td>
</tr>
</tbody>
</table>

### Improving the Resolution of SNP Detection

SNP detection process involves hybridization and digestion steps.

- **SNP**:
  - **SNP**
  - **SNP**

- **Discussion**:
  - Enzymatic on-chip enhancement of genotyping DNA microarrays.
PCR amplified KPC-3 positive sample labeled with Cy3 was hybridized to the KPC array and treated with 1:10 dilution of nuclease (left) or a control (1x Taq PCR buffer) (right) at 42ºC for 30 min. Normalized signal intensity data.

⇒ CEL nuclease substantially enhanced SNP discrimination of a KPC microarray tested with PCR products derived from clinical isolates.


Point of Care Testing

Biosensing Platform Project

- Integration Of Nucleic Acid, Protein And Small Molecule Detection On A Rapid, Point-of-care Multi-parameter Platform
- 4 year, $15M funding provided by ITI Scotland and the European Regional Development Fund
- Demonstrator cartridge & instrument
- Microfluidics core capability
- Prototype system designed for flexible development
- Short time-to-result achieved
  - Total TTR of 10 – 15min
  - Sample preparation <2min
  - Plasma to cDNA ~6min
  - qPCR <7min
- Small molecule (antibiotic) detection
- Multiplex and Multiparameter assays
- Novel detection modalities
- Partners
  - ITI-Scottish Enterprise, Glasgow
  - Lab901, Edinburgh, Scotland
  - Division of Pathway Medicine, Edinburgh
  - Axis-Shield Diagnostics, Dundee

Chronic Wound Care Programme

- Development of a an easy-to-use, portable medical device that can be readily applied to diagnose and treat chronic wounds in a clinical environment and in the community.
- University of Edinburgh, Zisy Ltd. research provider
- Aim: Molecular MRSA detection from clinical specimen without PCR
Electrochemical Impedance Spectroscopy (EIS)

- Label free detection
- Small AC potential is applied to an electrochemical cell and the current response measured
- Interrogate different frequencies ranging from 0.1 - 100,000 Hz

Detection of MRSA using EIS

- PCR Product
- PNA Probes

\( R^2 = 0.9920 \)

\( \text{Signal Increase Ratio} \)

\( \text{mecA PCR product / nM} \)

**Enzymatic Signal Amplification to Increase EIS Signal**

- SNP discrimination
- Localised precipitation
- Detection even before 5 min feasible

**Towards NDM-1 Point of Care Testing using EIS**

- 15 min incubation
- Synthetic target
Sample Manipulation to Improve Assay Performance

- Dielectrophoresis (DEP)
- Trapping of bacterial RNA by DEP in order to achieve faster and more sensitive detection

Frequency Dependency of rRNA

Manipulation of rRNA in Real Time


Conclusion and Outlook

• Personalised Medicine is becoming a viable approach in healthcare.
• IVD and Pharma partnering is rising.
• Omics research feeds into constant stream of novel biomarkers.
• Rx demand for Dx creates opportunities for biosensing developments.
• Antibiotic resistance creates an unmet need for therapies of infectious disease.
• New technologies offer advanced possibilities for infectious disease diagnostics.
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