The Pathologist in Drug Development

Chris Womack

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• Honorary Professor, Faculty of Medical and Human Science, University of Manchester.
We are committed to delivering great medicines to patients through innovative science and excellence in development and commercialisation.

Our vision

To be a global biopharmaceutical business delivering great medicines through innovative science and excellence in development and commercialisation.

Latest news

21 March 2013

AstraZeneca outlines strategy to return to growth and achieve scientific leadership

Investor Day

AstraZeneca’s Investor Day briefing for institutional investors and analysts was held on Thursday, 21 March.

Read details about the event
AstraZeneca is an R&D-focused biopharmaceutical company dedicated to improving global healthcare.

- World’s 5th largest pharma company
- 2012 sales of $28 billion
- >30,000 sales and marketing employees
- R&D activity in key disease areas
- Strong emerging markets presence

2012 AstraZeneca annual report; dollar values are sales
Global R&D footprint is anchored by three strategic centers

Gaithersburg, MD, USA

Cambridge, UK

Mölndal, Sweden
R&D therapy areas and medicines

Cardio-Metabolism  Oncology  Respiratory/inflammation

Biologics  Small Molecules  Immuno-therapies  Protein engineering

Infection & Vaccines  Neuroscience

Core TAs

Opportunity-Driven
Drugs don’t work in all patients

“The vast majority of drugs - more than 90 per cent - only work in 30 or 50 per cent of the people”

*Allen Roses (GSK)*

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Effectiveness</th>
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<tbody>
<tr>
<td>Hypertension Drugs</td>
<td>10-30%</td>
</tr>
<tr>
<td>Heart Failure Drugs</td>
<td>15-25%</td>
</tr>
<tr>
<td>Anti Depressants</td>
<td>20-50%</td>
</tr>
<tr>
<td>Cholesterol Drugs</td>
<td>30-70%</td>
</tr>
<tr>
<td>Asthma Drugs</td>
<td>40-70%</td>
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</tbody>
</table>

- **Hypertension Drugs**: 10-30% ACE Inhibitors
- **Heart Failure Drugs**: 15-25% Beta Blockers
- **Anti Depressants**: 20-50%
- **Cholesterol Drugs**: 30-70% Statins
- **Asthma Drugs**: 40-70% Beta-2-agonists
Pharmaceutical R&D productivity challenges

• In 2009 only 24 drugs were approved by FDA of which 17% were truly innovative

• Only 8% of drugs of selected drug candidates are launched

• Phase 2 and 3 attrition rates 66% and 30% respectively

• Average cost to bring a new drug to market ~ $1.8 billion (includes cost of testing failed drugs)

• Discovery and Development of a new drug ~ 13.5yr

Risk example

- Research
- Early Development
- Late Development
- Regulatory assessment
- Commercialisation

Phase I
- FTIM
- EOP1

Schedule: 6 choices
Dose: 3 choices
Tumour: 160 choices

2,880 opportunities to get it wrong

Phase I Multiple Ascending Dose Patients
Conceptual challenges in oncology

- Cancer is a heterogeneous disease
  - How to select patients for a targeted therapy?

- Cancer cells acquire mutations in multiple targets/pathways
  - How to test rationally based combination therapies?

- Cancer cells deregulate growth controls used in normal cells
  - How to ensure safety of targeted therapies?
Biomarkers - measuring things in human samples

- Pre-disposition
- Screening
- Diagnostic
- Prognostic
- Predictive
- Pharmacological
- Surrogate

Sample types

USEFUL

- Informs risk/benefit ratio when there is a decision to be made.
- Does so in a better/faster/earlier/cheaper way than existing approaches.
- Generally applicable: sample and technology must be available/accessible.
- Has known identity (i.e.,)

Sample quality

Now science driven

Top 5 Abuses

1. Indecent Cryoprotectant Exposure
2. Rack Ghettos
3. Method Inconsistency
4. Intemperate Zones
5. Bad Sample Hygiene

http://scientistsagainstsampleabuse.org/
## Transforming industry approach to biomarkers

<table>
<thead>
<tr>
<th>Old view</th>
<th>New opportunities</th>
<th>Future outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>No robust hypothesis for patient selection</td>
<td>Genetic alterations define driver mutations that can be tested as exploratory predictive markers</td>
<td>Better companion diagnostic tests</td>
</tr>
<tr>
<td>Biopsies not possible</td>
<td>Changing mindset of investigators</td>
<td>Go/No Go decisions early in drug development</td>
</tr>
<tr>
<td>Biomarkers not quantitative and not decision making</td>
<td>New methods allow quantification</td>
<td>Target inhibition can be measured to establish POM/POP</td>
</tr>
<tr>
<td>Biomarkers yield limited information</td>
<td>Multiplex assays provide pathway analysis</td>
<td>Feedback mechanism and MOA better defined</td>
</tr>
<tr>
<td>Biopsies at relapse are not beneficial for patients</td>
<td>New molecular approaches can define new treatment options for patients</td>
<td>New targets identified and new combinations defined</td>
</tr>
</tbody>
</table>
Sequential samples - new insights

Histological analyses of repeat tumour biopsies from 37 patients with drug-resistant NSCLCs carrying EGFR mutations

- All drug-resistant tumours retained their original activating EGFR mutations
- Known mechanisms of resistance including (EGFR T790M/MET)
- Genetic changes including EGFR amplification and PIK3CA mutations
- Five (14%) transformed from NSCLC into SCLC (sensitive to SCLC treatments)
- Serial biopsies from three patients revealed that genetic mechanisms of resistance were lost in the absence of the continued selective pressure of EGFR inhibitor treatment - sensitive to a second round of treatment with EGFR inhibitors

Sequist LV et al Science Translational Medicine 2011;3(75):2-6
Biomarker activity supporting drug development

What can we measure, in what, how and why?

- **Right target/efficacy**
  - Cell lines
  - Xenografts
  - Explants
  - Human “biobank” samples
  - Clinical trial samples
    - archive
    - prospective
    - paired
    - relapse

- **Questions:**
  - Target present?
  - Target relevant?
  - Target active?
  - Target hit?
  - Downstream effect?
  - Phenotypic effect?
  - Clinical effect?

- **Right tissue/right exposure**
  - DNA
  - RNA
  - Protein

- **Right safety**

- **Right patients**

- **Right commercial**
### Pharmacodynamic markers

<table>
<thead>
<tr>
<th>Question</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does it hit the target in man?</td>
<td><strong>Proof of mechanism (PoM)</strong></td>
</tr>
<tr>
<td></td>
<td>e.g. enzyme inhibition, receptor blockade</td>
</tr>
<tr>
<td>Does it have an effect on the disease phenotype?</td>
<td><strong>Proof of Principle (PoP)</strong></td>
</tr>
<tr>
<td></td>
<td>e.g. increased cell death markers (apoptotic markers)</td>
</tr>
<tr>
<td>Does this result in a beneficial clinical effect?</td>
<td><strong>Proof of Concept (PoC)</strong></td>
</tr>
<tr>
<td></td>
<td>e.g. tumour size reduction</td>
</tr>
</tbody>
</table>

Progressive reduction of uncertainty about effects

Increasing level of confidence about outcomes

No guarantee of success: rather staged risk management
PoM/PoP biomarker example - MEK inhibitor

Growth Factor → ras → raf → MEK → ERK → TF

Cell Proliferation

pERK
Ki-67

Biomarker “tool kit”: improving existing platforms

Refine…
• Speed
• Accuracy
• Reproducibility

FOXO3a/FOXO1: 1/40 CST 2497 (Lot 2), secondary detection with Rabbit Envision (Dako) - “old” vs “new” vial

• Validation

To be sure, to be sure . . . the problem of antibody validation
Biomarker “tool kit”: improving existing platforms

• Multiplex
• Quantitate
• Automate

• New insights

Systematic Analysis of Breast Cancer Morphology Uncovers Stromal Features Associated with Survival

Andrew H. Beck,1,2* Ankur R. Sangoi,1,3 Samuel Leung,4 Robert J. Marinelli,5 Torsten O. Nielsen,4 Marc J. van de Vijver,6 Robert B. West,1 Matt van de Rijn,1 Daphne Koller7+


…Cross platform correlation
Quantitate and automate: application of immunohistochemistry and digital image analysis

Preclinical
- Cell lines
- Xenograft
- Primary explant

Translational
- Clinical tissues
- Multi-tumour

Clinical
- Clinical trials
- Paired biopsies

PD
PoM/PoP
PK/PD/efficacy modelling

Expression
Heterogeneity
Tumour types

PoM/PoP

Requirement for robust, reliable and quantitative output for biomarkers
Xenograft model

BT474 (breast): pAKT473

- Modulation of biomarker expression in response to compound
Modulation of pAKT473 in BT474 xenograft model

Manual mark-up and application of digital image analysis

Control (1h)

150mg/kg (1h)

Image analysis output

Scoring key:  0+  1+  2+  3+
Modulation of pAKT473 in BT474 xenograft model

Genie™ mark-up and application of digital image analysis

Manual mark-up vs. Genie™ mark-up

DIA output for manual mark-up

DIA output for Genie™ mark-up

% positive pixels

Vehicle 1h 6h 150mg 1h 6h 100mg 1h 6h

Vehicle 1h 6h 150mg 1h 6h 100mg 1h 6h
Dunning rat model of prostate cancer

Quantification of nuclear AR expression in *tumour*

Vehicle

Treated

Scoring key: 0+, 1+, 2+, 3+ (Aperio nuclear algorithm)

Image analysis output
Dunning rat model of prostate cancer

Comparison of digital image analysis output with pathologist visual scoring

DIA output

**Nuclear AR**
- % positive nuclei

**Pathologist score**
- Nuclear AR
- Cytoplasmic AR

![Graphs showing comparisons between DIA output and pathologist scores for Nuclear and Cytoplasmic AR across different treatment groups (Vehicle, 50mg, 100mg) for Gp 1 to Gp 3.](image-url)
Oncology drug discovery and development

Application of digital image analysis to preclinical studies

Pattern recognition software (Genie™) effectively segments tumour, stroma and necrosis in preclinical xenograft models

Genie™ mark-up of a xenograft TMA using a single classifier trained to recognise tumour/necrosis

<table>
<thead>
<tr>
<th>‘Typical’ models</th>
<th>‘Atypical’ models</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCIH1975</td>
<td>NCIH460</td>
</tr>
<tr>
<td>A2780</td>
<td>A431</td>
</tr>
<tr>
<td>MES-SA</td>
<td>HX147</td>
</tr>
<tr>
<td>Calu-6</td>
<td>A549a</td>
</tr>
<tr>
<td>PC9</td>
<td>A549b</td>
</tr>
<tr>
<td>NCIH526</td>
<td>PC3</td>
</tr>
<tr>
<td>Colo205</td>
<td>C6</td>
</tr>
<tr>
<td></td>
<td>MDAMB435</td>
</tr>
<tr>
<td></td>
<td>MDAMB231</td>
</tr>
<tr>
<td></td>
<td>A375</td>
</tr>
<tr>
<td></td>
<td>NCIN87</td>
</tr>
<tr>
<td></td>
<td>A375M</td>
</tr>
<tr>
<td></td>
<td>U118MG</td>
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MVD analysis of Calu-3 stroma only, using Genie™ classifier

CD31  Genie™ mark-up  MVD in stroma
DIA of preclinical pharmacodynamic studies

Time savings due to automated pattern recognition

• Integration of pattern recognition software has enabled accurate automated region of interest selection

• Dynamic biomarker quantification in region of interest only

• Reduced subjectivity

• Reduced time to study report delivery by 30%

![Graph showing time savings](chart.png)

Time savings based on:
• Average number of sample/study = 35
• Average number of images/study = 105 (35 samples x 3 biomarkers)
• 5 minutes per image for manual markup
• 20 seconds per image for GENIE classifier selection.
Oncology drug discovery and development
Clinical trial: paired biopsies
Peritoneum

Pre-dose          Post-dose

% pAKT473 positive pixels
Block ID
1996 peritoneum pAKT473 (Genie)
Percent Negative
Percent Strong Positive
Percent Medium Positive
Percent Weak Positive

Mean % pAKT473+
Block ID

Genie™ + DIA output for tumour only

Staining intensity  
- negative  
- weak  
- medium  
- strong

Pathologist score

Average H-score

- Pre
- Post

Tumour
Oncology drug discovery and development

Clinical trial: paired biopsies
Colorectal

Genie™ + DIA output for tumour only

Staining intensity
- negative
- weak
- medium
- strong

Pathologist score

Average H-score

Pre    Post

Mean % pAKT473+
Oncology drug discovery and development

Clinical trial: paired biopsies
Breast

- Genie™ failed to distinguish accurately areas of tumour from sweat glands
- Tumour was annotated manually prior to image analysis
Oncology drug discovery and development

PoM biomarker (pAKT473) expression in paired biopsies

<table>
<thead>
<tr>
<th>DIA output</th>
<th>Pathologist Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slides in triplicate (% positive pixels)</td>
<td>Group means (mean %+)</td>
</tr>
</tbody>
</table>

- **Breast**
- **Small bowel**
- **Colorectal**
- **Pancreas**
- **Peritoneum**
- **Breast**

**Slides in triplicate**

**Pathologist Score**

**Mean H-Score**
Still a role for “traditional” histopathology?

Phase II randomized placebo-controlled study of olaparib (AZD2281) in patients with platinum-sensitive relapsed serous ovarian cancer (PSR SOC)

Histopathology of lung cancer

- No 1 cause of cancer related death
- 5 year survival in advanced cancer is <5%
- Cytotoxic chemotherapy has been the standard of care in patients with advanced cancer

Molecular pathology of lung cancer

… poised for transformation with combination and personalised therapies
What is Personalised Healthcare?

Delivering the right treatment to the right patient

- Successful therapy involves more than the drug alone
- Diagnostic tools from clinical indicators to molecular diagnostics
  - Select patients for treatments
  - Optimise treatment
- This is not easy...
- ...but helps deliver better outcomes to patients, payers and healthcare providers
The number of therapeutics linked to a diagnostic for treatment selection, toxicity or monitoring continues to grow.

Launch timeline of targeted therapeutics with required or recommended PharmDx currently on the label

Source: FDA, PMC, Companies web site, L.E.K analysis
Companion diagnostics

Developing the assay

- What is the “intended use” of the assay?

- Ensure assay meets design requirements
  - Analytical validation
  - Clinical validation
  - Manufacturing

- Diagnostic partner engagement

Cummings et al., British Journal of Cancer 2010;103:1313–1317
Cummings et al., Drug Discovery Today 2010;15:816-825
Developing the assay

Phase I

Phase II

Phase IIb

Phase III

Development Phase

Optimisation & Stability

Establishment of Assay Performance

Verification of Assay Performance

Validation Phase

Validate Design

Feasibility Review

Prototype Assay

Development Review Design Lock

Verification

Validation Review PMA Submission
Regulatory approval

- Companion diagnostics considered high risk and therefore likely to need to meet the highest regulatory hurdles in the US [Class III, Pre Market Approval (PMA) required]

- Regulatory approvals across multiple territories required
The real world

Key considerations

• Diagnostic must be available at time of drug launch
• Strategies to drive test adoption
• Strategies to remove barriers to testing
• Reimbursement for diagnostic testing
Pathology Teaching - Prostate

Purpose
This is an introduction prior to separate e-based self learning and self assessment. Following the self-assessment you will be confident that you can:
- Recognise tissue originating from the prostate gland
- Distinguish neoplastic from non-neoplastic tissue
- Identify prostatic adenocarcinoma
- Gleason grade prostatic adenocarcinoma

...so that you are able to primary read human tissue slides and digital images. Competency will be assessed by a medically trained pathologist prior to sign-off and annual review.
Training and shared experience

Three lessons from Industry

1. Understanding technical and tumour variability is essential for validating biomarkers.

2. Technology, both in communication and image analysis has the potential to greatly assist in molecular pathology research and potentially, in the clinic.

3. Developing new treatments for patients is a truly multidisciplinary activity and histopathologists have skills and knowledge that can make a positive contribution to this process.

Histopathology in Industry - Dr Guy Betts
Histopathology trainee NW Deanery
Pathologist attributes

- Diagnostic expertise
- Technology expertise
- Research expertise
- Custodians/gatekeepers of samples - archive/prospective
- Integral part of clinical teams
- Teachers
- Networks/Colleges/Societies
If Pathologists didn't exist

where would the answers come from?

Pathology is the study of the nature and causes of diseases. It underpins every aspect of medicine, from diagnosis to monitoring, and is vital for research.

Without pathologists, any answers found would be questionable to say the least. Every medical test, a case of trial and error. Exactly the kind of words you don't want to hear when it comes to your health. Watch the videos and see for yourself.
The future ...

**Tissue MS**

Sample Preparation for Direct-Tissue MALDI MS Analysis

1. Frozen tissue sample is cut and thaw-mounted onto a sample plate; additional sections are cut and stained for histology
2. Matrix is regionally deposited on the sample
3. Each spot is analyzed on a MALDI TOF MS instrument
4. Computer algorithms are used to process each spectrum
5. Software classifies non-tumor and tumor spectra

**CTCs**

<table>
<thead>
<tr>
<th>Negative</th>
<th>Light</th>
<th>Moderate</th>
<th>Heavy</th>
</tr>
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**NGS**

NanoString/Pro

Cost/Gb vs Platform Throughput (Gb/Run)

- Sanger
- PacBio
- Ion 314
- Ion 316
- Ion 318
- Illumina MiSeq
- (Oxford Nanopore)
- (Ion Proton)
- SOLID
- Illumina HiSeq

$1000 Genome (Reagents Only)
Are we ready?
Acknowledgements

Patients

Teams

Materials:
Andrew Hughes, Carl Barrett, Marie Cumberbatch, Neil Smith, Tony Nash, Maria Orr and Ruth March.

And … Thank You