Precision Medicine: Panacea or False Dawn?

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Worldwide Vice President Early Clinical Development, AstraZeneca
Chair, Translational Medicine, University of Manchester

ESMO
Monday 29th September 2014
Conflicts of Interest

• Employment: Chair in Translational Medicine

• Employment: VP Early Clinical Development

• Shareholder

• Panel Member BMERP: Non-pecuniary

• Scientific advisor: Pecuniary
Challenges to the dawn

1. “Precision Medicine” can be less efficient than “unselected”

2. The requirement for a contemporaneous molecular profile

3. Single molecular aberration trials can be very inefficient
1. Precision Medicine can be less efficient than “unselected”

Assume you had a drug
• which doubled the time to progression (HR=0.5) in biomarker +ve subjects
• had no effect in biomarker –ve subjects
• biomarker +ve subjects comprise 25% of the population
In this scenario, a Precision Medicine approach is a more efficient development route than an unselected approach.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Active</th>
<th>Effect (HR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomarker +ve (25%)</td>
<td>6 mo</td>
<td>12 mo</td>
<td>0.50</td>
</tr>
<tr>
<td>Biomarker –ve (75%)</td>
<td>6 mo</td>
<td>6 mo</td>
<td>1.00</td>
</tr>
</tbody>
</table>

But this assumes we have:
- a perfect selection test (100% sensitive; 100% specific)
- there is no efficacy in the biomarker –ve population

What happens when this is not the case?

| Efficiency over unselected | 8.6 fold | 2.1 fold |

1 median follow-up of 18 months assumed and no screen failures
An imperfect selection/stratification test lessens the efficiency of a Precision Medicine trial.

<table>
<thead>
<tr>
<th>Sens, Spec</th>
<th>PPV</th>
<th>Control</th>
<th>Active</th>
<th>Effect size</th>
<th>N req’d to enter</th>
<th>N req’d to screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>100%,100%</td>
<td>100%</td>
<td>6 mo</td>
<td>12 mo</td>
<td>0.50</td>
<td>117</td>
<td>468</td>
</tr>
<tr>
<td>95%, 75%</td>
<td>56%</td>
<td>6 mo</td>
<td>9.4 mo</td>
<td>0.64</td>
<td>260</td>
<td>613</td>
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<tr>
<td>75%, 95%</td>
<td>83%</td>
<td>6 mo</td>
<td>11 mo</td>
<td>0.55</td>
<td>149</td>
<td>663</td>
</tr>
<tr>
<td>75%, 75%</td>
<td>50%</td>
<td>6 mo</td>
<td>9 mo</td>
<td>0.68</td>
<td>317</td>
<td>845</td>
</tr>
</tbody>
</table>

NB: An Unselected trial required 1000 patients to be screened and entered.
Even a small (one third*) effect in biomarker –ve patients erodes the apparent advantage of a targeted trial

<table>
<thead>
<tr>
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<th>Control</th>
<th>Active</th>
<th>Effect (HR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomarker +ve (25%)</td>
<td>6 mo</td>
<td>12 mo</td>
<td>0.50</td>
</tr>
<tr>
<td>Biomarker –ve (75%)</td>
<td>6 mo</td>
<td>7.5 mo*</td>
<td>0.80*</td>
</tr>
<tr>
<td>All patients</td>
<td>6 mo</td>
<td>8.7 mo</td>
<td>0.69</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Number required to enter</th>
<th>Number required to screen</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td></td>
<td>384</td>
</tr>
<tr>
<td>+ve (25%)</td>
<td>117</td>
<td>468</td>
</tr>
<tr>
<td>Efficiency over unselected</td>
<td>3.3 fold</td>
<td>0.8 fold</td>
</tr>
</tbody>
</table>

* Effect in –ve pts = 1/3 effect in +ve patients
Conclusion:

For Precision Medicine to be a more efficient drug development strategy over an unselected approach we would need to be very confident that (i) we had a very good stratification test and (ii) the untargeted population achieved minimal benefit from treatment.

Understanding these two variables is a key deliverable of the pre-registrational clinical programme.
2. The requirement for a contemporaneous molecular profile

With the advent of ultrasensitive genomic methods

Comes the Advantage of Repeat “Biopsy”

But the insight/challenge that repeat sampling show tumour molecular phenotype is dynamic

And the tumour heterogeneous

Circulating nucleic acid biomarkers: tumour, cfDNA, CTC, miRNA
cfDNA: Current Method Development in MCRC

- Simple, robust, specific and sensitive
  - Automatable
- Transferable to diagnostic laboratory

(i). Can we work with preserved whole blood 96 hrs post draw?
- Avoids on site plasma preparation and variability
- Simple blood draw only

(ii). Can we analyse CTCs as well as cfDNA in the same sample?
- cfDNA from CellSave plasma - combine with GCLP CTC analysis
- Compare to Streck Cell-Free DNA BCT® -

(iii). Can we establish routine sensitive genome wide NGS from cfDNA
- Since levels of cfDNA are low – often at the level of 3 ng (~1000 genomes) need maximum capture efficiency to be representative
- Compare commercial kits and “home-grown”

Courtesy Ged Brady, Dominic Rothwell, Caroline Dive
(i) Can we work with preserved whole blood 96h post draw?

EDTA plasma samples require processing within 4 hrs of collection. Can cfDNA be preserved in CellSave tubes?

- Maintenance of consistent cfDNA levels up to 96 hrs post-draw in CellSave tubes (A)

- Mutational status consistent in both EDTA and CellSave via real-time PCR analysis (B)

- Targeted NGS of EDTA and CellSave cfDNA showed good correlation in 4 SCLC patient samples (C)

**A**

![cfDNA levels](chart)

**B**

![log scale](chart)

**C**

<table>
<thead>
<tr>
<th>SCLC Pts</th>
<th>Sample type</th>
<th>cfDNA input (ng/tube)</th>
<th>TP53:7578212 G&gt;A stopgain COSM99618</th>
<th>TP53:7577022 C&gt;T stopgain COSM99947</th>
<th>TP53:7579312 G&gt;T synonymous COSM45940</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>MUTATION DETECTED</td>
<td>Total coverage</td>
<td>% ALT</td>
</tr>
<tr>
<td>12068</td>
<td>WT gDNA</td>
<td>14.00</td>
<td>0</td>
<td>470.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>EDTA cfDNA</td>
<td>1.35</td>
<td>0</td>
<td>309.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>CellSave cfDNA</td>
<td>2.30</td>
<td>0</td>
<td>480.00</td>
<td>0.70</td>
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<tr>
<td>12071</td>
<td>WT gDNA</td>
<td>20.00</td>
<td>0</td>
<td>189.00</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>EDTA cfDNA</td>
<td>10.35</td>
<td>1</td>
<td>283.00</td>
<td>47.70</td>
</tr>
<tr>
<td></td>
<td>CellSave cfDNA</td>
<td>14.65</td>
<td>1</td>
<td>358.00</td>
<td>47.77</td>
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<tr>
<td>12088</td>
<td>WT gDNA</td>
<td>20.00</td>
<td>0</td>
<td>533.00</td>
<td>0.19</td>
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<tr>
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<td>22.50</td>
<td>0</td>
<td>250.00</td>
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<td>19.45</td>
<td>0</td>
<td>549.00</td>
<td>0.36</td>
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<tr>
<td>12090</td>
<td>WT gDNA</td>
<td>20.00</td>
<td>0</td>
<td>289.00</td>
<td>0.00</td>
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<tr>
<td></td>
<td>EDTA cfDNA</td>
<td>1.50</td>
<td>0</td>
<td>205.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>CellSave cfDNA</td>
<td>0.75</td>
<td>0</td>
<td>350.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Courtesy Ged Brady, Dominic Rothwell, Caroline Dive
Can we analyse CTCs as well as cfDNA in the same sample? : PARSORTIX

- Chip utilising cell size and deformability – epitope independent
- Compatible with blood preservatives – CellSave, Streck Cell-Free DNA BCT®
- Plasma and cells obtained from a single blood sample
- Captured cells can be fixed and stained in the cassette
- Cells can be recovered for external staining and/or genetic analysis

Molecular Analysis

Courtesy Ged Brady, Dominic Rothwell, Caroline Dive
(iii) Can we establish routine sensitive genome wide NGS from cfDNA

**Approach**

**Reference Samples**
- Replicates of SCLC cfDNA – contain known TP53 mutation
- 8 x 0.5 ng input = 165 genomes and 4 x 2.5 ng input = control samples
- Targeted pull-down of x 7 cancer associated genes – NGS analysis to determine pull-down efficiency

**Commercial Kits**
- NEB Ultra
- Microplex (Rubicon)
- KAPA
- HomeGrow – *in development*

<table>
<thead>
<tr>
<th></th>
<th>NEB</th>
<th>Microplex</th>
<th>KAPA</th>
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<tbody>
<tr>
<td>Total Reads (genomewide)</td>
<td>97,904</td>
<td>108,913</td>
<td>107,265</td>
</tr>
<tr>
<td>NRAS</td>
<td>28</td>
<td>38</td>
<td>43</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>27</td>
<td>32</td>
<td>46</td>
</tr>
<tr>
<td>BRAF</td>
<td>37</td>
<td>72</td>
<td>84</td>
</tr>
<tr>
<td>EGFR T790M</td>
<td>27</td>
<td>31</td>
<td>26</td>
</tr>
<tr>
<td>KRAS 63bp</td>
<td>24</td>
<td>38</td>
<td>74</td>
</tr>
<tr>
<td>TP53 E</td>
<td>50</td>
<td>48</td>
<td>47</td>
</tr>
<tr>
<td>TP53 H</td>
<td>27</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>Total 'on target' reads</td>
<td>220</td>
<td>283</td>
<td>339</td>
</tr>
<tr>
<td>Ratio of 'non-target' to 'target' reads</td>
<td>445.02</td>
<td>384.85</td>
<td>316.42</td>
</tr>
<tr>
<td>Fold enrichment</td>
<td>44944</td>
<td>52083</td>
<td>63291</td>
</tr>
</tbody>
</table>

Developing NGS approaches to analyse cfDNA to determine copy number aberrations and Whole Exome Sequencing of patient samples.

Courtesy Ged Brady, Dominic Rothwell, Caroline Dive
Beginning to become Practice Changing:
IRESSA: Application for update to EU label*

ON 16th May 2014, AZ submitted an application for a Type II Variation for IRESSA (gefitinib) regarding use of circulating tumour DNA (ctDNA) for the assessment of EGFR mutation status in advanced NSCLC patients for whom tumour samples are unavailable or unevaluable.

IPASS: PFS in patients is improved by IRESSA in patients where EGFR mutations are identified by ctDNA

IFUM: ORR in patients in response to IRESSA in patients where ctDNA is used to determine EGFR status

Diagnostic performance of ctDNA vs Tumour:
Specificity = 99.8%
Sensitivity = 65.7%
Turnaround = 3-4d


*The type II variation application is subject to review by EMA/CHMP
...and now “Business as Usual” across AZ Oncology Portfolio

• Currently >80% of AZ oncology clinical projects have personalised healthcare strategies

• Targeted/Singleplex assays

<table>
<thead>
<tr>
<th>Drug</th>
<th>Aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iressa</td>
<td>EGFR mutation</td>
</tr>
<tr>
<td>Olaparib</td>
<td>BRCA 1, 2 mutation</td>
</tr>
<tr>
<td>Selumetinib</td>
<td>Kras mutation</td>
</tr>
<tr>
<td>AZD4547</td>
<td>FGFR mutation, fusion, amplifications</td>
</tr>
<tr>
<td>AZD9291</td>
<td>EGFR and T790M mutation</td>
</tr>
</tbody>
</table>

• Multiplex/NGS assays

<table>
<thead>
<tr>
<th>Drug</th>
<th>Aberration</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD1775</td>
<td>P53 mutations</td>
</tr>
</tbody>
</table>

AZ projects

• Currently ranked among the top 5 companies in the PHC field¹,²

¹. Diaceutics Pharma Readiness for Personalized Medicine 2011
². PharmaTimes, April 2011
3. Single molecular aberration trials can be very inefficient

AZ recent experiences in conducting single molecular aberration precision medicine trials in lung cancer: ~23 patients “screened” for 1 “enrolled”

### Iressa- Phase 3 trial

**Attrition factors in EGFR mutation analysis:**
- Archival tissue
- Pathology findings
- Sample tracking issues
- Consent issues

560 patients with archival samples
380 suitable for extraction (60%)
215 DNA sufficient (40%)

**DNA extraction**
EGFR: 26
K-Ras: 12
B-Raf: 0

### AZD4547- Phase 1 trial

**Attrition factors in FGFR amplification analysis:**
- Fresh tissue
- Analyzed

90 patients with fresh samples
71 patients tissue evaluable for analysis
8 patients known biomarker +ve
4 patients enrolled
11% prevalence
50% dropout & clinical screen failure

Experience with archival samples from the ISEL study a double-blind, placebo-controlled Phase III survival study of gefitinib in 2L/3L Stage IIIb/IV NSCLC with 1692 patients in 210 centres, 28 countries

Experience with fresh samples from the AZD4547 open label study in 2L/3L Stage IIIb/IV squamous NSCLC in 19 centres

Thus $23K analytical screening costs (@ $1k/test) per patient enrolled
Stratified Medicine Requires Portfolio Approach
AZ / MedImmune portfolio well placed in Lung Cancer...

Adenocarcinoma

- AZD5363
- Selumetinib (AZD6244)
- AZD9291
- Caprelsa (vandetanib)
- Selumetinib (AZD6244)
- Iressa
- AZD9291
- AZD5363
- AZD2014
- AZD8186
- AZD4547
- FGFR 2/3 mutation
- FGFR1 amplification
- FGFR translocations
- Volitinib
- Met expression
- Met amplification
- Medi-4736
- PDL-1 expression
- PDL-1 amplification

Squamous

- AZD5363
- AZD4547
- Medi-4736
Lung Master Protocol – Friends Of Cancer Research. Squamous NSCLC

‘Master protocol’ aims to revamp cancer trials (2013). Pilot project will bring drug companies together to test targeted lung-cancer therapies.

Master Protocol for squamous cell lung cancer readies for launch (2014). The master protocol is a “truly exciting development, one that will benefit industry and patients,” says US Food and Drug Administration (FDA) Commissioner Margaret Hamburg.
MATRIX National Lung Trial – CRUK
Squamous and adenocarcinoma NSCLC

Genetic Technology Hubs
Cardiff  Birmingham  RMH

Clinical Hubs
ECMC Network

Data Repository

Researchers
Partners
NHS

Gary Middleton (PI)
Birmingham (Sponsor, CTU)
Sanjay Popat (TMG Chair)

Biomarker +ve Patients

Central Pharmacy Hub

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular segment</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>AZD5363</td>
<td>PI3KCA mutation, PIK3CA mutation, AKT1 mutation</td>
<td>4.6% 15.2%</td>
</tr>
<tr>
<td></td>
<td>PIK3CA amp, PTEN null</td>
<td>7.0% 7.9%</td>
</tr>
<tr>
<td>AZD4547</td>
<td>FGFR2/3 mutation, FGFR2/3 mutation</td>
<td>3.3% 4.4%</td>
</tr>
<tr>
<td>AZD2014</td>
<td>LKB1 mutation, TSC1/2 mutation</td>
<td>12.2% 8.9%</td>
</tr>
<tr>
<td>AZD9291</td>
<td>T790M (Her2 amp)</td>
<td>7.5% (5.0%)</td>
</tr>
<tr>
<td></td>
<td>KRAS wild type, NF1, NRAS, HRAS mutation</td>
<td>24.9%</td>
</tr>
<tr>
<td>Selumetinib/docetaxel</td>
<td></td>
<td>24.9%</td>
</tr>
<tr>
<td>MEDI4736</td>
<td>All markers negative (PD-L1 positive)</td>
<td>est. 40%</td>
</tr>
</tbody>
</table>
SAFIR02 Lung Trial – UNICANCER
Squamous and adenocarcinoma NSCLC

**Compound** | **Molecular segment**
--- | ---
AZD5363 | PI3KCA mutation
         | AKT1 mutation
         | PIK3CA amp
         | PTEN loss
         | PTEN mutation
AZD4547 | FGFR1 amplification
AZD2014 | LKB1 mutation
AZD8931 | HER2 mutation
         | HER2 amplification
Selumetinib | KRAS mutation
            | BRAF mutation
Vandetanib | RET mutation
“Basket” Studies by Tumour Type and Region
AstraZeneca

www.clinicaltrial.gov
These studies are more patient efficient...although challenges remain...but the emerging science is promising

- Definition of “biomarker +ve” by NGS: understanding the clinical relevance of variants

- Necessitates a consortia approach

- Flexibility desirable for clinical patient selection decisions imposes statistical challenges

- Intent needs to be clear in the design- to signal search or adaptive with registration intent

- Lack of familiarity to IRB’s

- With multiple drugs, with multiple toxicities and disparate monitoring requirements- can attract regulatory concerns
Acknowledgements

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Ruth March
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Antoine Yver
Max Kirkby
Anne Galer
Kevin Carroll*
Carl Barrett
Simon Hollingsworth
Elaine Kilgour
Iressa Product Team
AZD4547 Product Team

*formerly
Backups
Example Workflow

Approach

- 100 H2009 cells spiked into HNV blood collected in either Streck Cell-Free DNA BCT® (S) or CellSave Preservative tubes (C) and left 96 hours at room temperature
- Remove plasma and process cells on Parsortix device
- Count retrieved spiked cells and white blood cells (WBCs)
- Whole genome amplify (WGA) pools of 40 cells and evaluate using PCR QC

Cell Enrichment

- ‘CTC’ recovery >30%
- Total WBCs <200
- Streck and CellSave comparable

cfDNA

- Streck and CellSave comparable

Molecular Analysis

- Efficient WGA for Streck and CellSave
- Quantitative NGS underway

Considerations for Clinical Use

Single Tube for Plasma and Cells

- Reduces number of blood samples required
- Direct comparison of cfDNA and CTCs

Low WBC contamination (<200)

- Suitable for single cell isolation eg DEPArray
- Direct analysis of entire enriched population possible if assay sensitivity can detect at least 0.5 % tumour component (1 CTC amongst 200 WBCs)

Molecular CTC signature

- Based on:
  - common driver mutations eg KRAS in pancreatic cancer
  - sequence analysis of tumour
  - sequence analysis of CTCs or cfDNA
- Allows epitope independent CTC assessment

Courtesy Ged Brady
Sequenom Targeted Panels:

- High-throughput somatic mutation profiling for disease-specific genes of interest (e.g. Lung Cancer Research has the LungCarta Panel)

- The LungCarta Panel evaluates mutations in 26 oncogenes and tumour suppressor genes (a total of 214 somatic mutations):

  Panels consist of key mutations identified by sequencing discovery studies that affect key pathways in the disease of interest (e.g. lung adenocarcinoma tumours).
Each sample is PCR amplified (using gene-specific primers mentioned earlier), and then dispensed on the MassARRAY and analysed using Mass
Qiagen GeneRead:

- Another PCR-based target enrichment method
- Panels commercially available, include Lung Cancer, Colon Cancer and a Comprehensive Cancer Panel.