Overview of trials testing tumour molecular profiling:

lung cancer as a model

Pr Jean-Charles SORIA
The aim of molecular profiling trials

How can molecular profiling help the oncologist understand the biology in each patient in order to better treat him?
General goals of tumour molecular profiling

- Tumour molecular profiling can help decipher cancer biology at the individual level and identify:
  - **Oncogenic drivers and predictors of efficacy**
  - Lethal subclones & intratumor heterogeneity
  - Mutagenesis processes & DNA repair defects
  - Dialogue between cancer cells and immune system
Challenges of tumour molecular profiling

- Various models of implementation in the clinical setting
- The optimal technology is yet to be universally adopted
- The optimal setting for analysis (metastatic vs locoregional vs resected) is still debated
- Best patient population to enroll (refractory, sensitive...) TBD
- Access to therapies (and notably combinations) is a problem
Co-existing mutations: a major challenge?

Co-existing mutations are associated with resistance

Lefebvre & Yu Cancer Pharmacogenomics and Targeted Therapies 2014
Partial perspective of molecular profiling trials in lung cancer

- 2006: BATTLE-1
- 2007: BATTLE-2
- 2008: SAFIR02, IFCT1301
- 2009:
- 2010:
- 2011:
- 2012:
- 2013:
- 2014:
- 2015:

**METASTATIC**

- SPANISH ERCC1-trial
  - 1 to 3 Gene-candidates
  - Multiple Gene-candidates
- Large panels-NGS

**IIIB**

- Lung-MAP
- RTOG 1306

**RESECTED**

- TASTE-IFCT0801
- TRACERX
- ALCHEMIST

Many other adjuvant trials
GILT: the 1st prospective customized trial

- **August 2001**
- **October 2005**
- **N = 444 patients**
- **24 sites (Spain)**
- **PI: R Rosell**

### Control arm vs. Docetaxel / cisplatin

- **ORR**
  - Control: 39.3%
  - Guided: 51.2%

But:
- No PFS or OS difference...
- 17% rate of screen-failures
- Prospective FFPE analysis with microdissection and RT-PCR...
BATTLE1

Biomarker-integrated Approaches of Targeted Therapy for Lung Cancer Elimination

- Platform for integrated translational research
  - Clinical trial program
  - Novel trial design
  - Biomarker discovery

- Scientific Hypotheses
  - Real time biopsies are possible to more accurately reflect aberrant signaling pathways of lung cancer
  - Matching targeted agents with abnormal pathways will improve disease control in lung cancer patients
  - 8-week disease control is an acceptable surrogate for efficacy (OS) in patients with pretreated lung cancer

E. Kim et. al, Cancer Discovery 2011
**BATTLE 1 Schema**

- **Umbrella Protocol**
- **Core Biopsy**
  - **Randomization:** Equal → Adaptive
  - **Biomarker Profile**
    - EGFR
    - KRAS/BRAF
    - VEGF
    - RXR/CyclinD1

**Primary end point:** 8 week Disease Control (DC)

Kim E et al AACR 2010
**BATTLE-1 Timelines**

- **Mid 2004:** Concept Developed
- **Mid 2005:** Grant Submitted
- **April 2006:** Grant Approved
- **Nov 2006:** Trials Activated - 1st pt
- **Oct 2009:** Trials completed accrual!

Courtesy WK Hong
Assessment of BATTLE-1 Trial

- Successful completion of a *prospective*, biopsy-driven, study in lung cancer
  → This is now an *acceptable* approach!

- Patients are guided toward more effective personalized treatments (Adaptive design)

- Traditional way to identify biomarkers
  → *Retrospective* analysis of patient archives sample

- The *new way*
  → *Prospective* biomarkers evaluation
  → Unprecedented biospecimen resources for discovery

Courtesy WK Hong
BATTLE-2 Trial

Protocol enrollment
Biopsy performed

Stage 1: (n=200)
Adaptive Randomization
KRAS Mut status

EML4-ALK Fusion or EGFR Mut exclusion

Stage 2: (n=200)
Refined Adaptive Randomization
“Best” discovery markers/signatures

Statistical modeling and biomarker selection

Biomarkers:

- Protein expression (IHC): p-AKT (Ser473), PTEN, HIF-1α, LKB1

- Mutation analysis (Sequenom): PI3KCA, BRAF, AKT1, HRAS, NRAS, MAP2K1 (MEK1), MET, CTNNB1, STK11 (LKB1)

- mRNA pathways activation signatures: Affymetrix®

- Protein profiling – RPPA (n=174)

- NGS-Foundation Medicine

- RNA sequencing

Primary endpoint: 8-week disease control (N = 400)

Courtesy V Papadimitrakopoulou
# Updated Accrual 05/30/2014

<table>
<thead>
<tr>
<th>Trial activation</th>
<th>MDACC: June 2011</th>
<th>Yale: August 2012</th>
<th>Total by Site</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDACC</td>
<td>Yale</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patients screened</td>
<td>604</td>
<td>70</td>
<td>674</td>
<td></td>
</tr>
<tr>
<td>Patients consented &amp; registered</td>
<td>276</td>
<td>56</td>
<td>332</td>
<td></td>
</tr>
<tr>
<td>Biopsies performed</td>
<td>234</td>
<td>41</td>
<td>275</td>
<td></td>
</tr>
<tr>
<td>Screen Failures</td>
<td>105</td>
<td>27</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Patients Randomized</td>
<td>176</td>
<td>32</td>
<td>208</td>
<td></td>
</tr>
<tr>
<td>Patients Treated</td>
<td>171</td>
<td>29</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Tx Erlotinib (Arm 1)</td>
<td>17</td>
<td>5</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Tx Erlotinib/MK-2206 (Arm 2)</td>
<td>36</td>
<td>6</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Tx MK-2206/AZD6244 (Arm 3)</td>
<td>64</td>
<td>11</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Tx Sorafenib (Arm 4)</td>
<td>54</td>
<td>7</td>
<td>61</td>
<td></td>
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<tr>
<td>Primary endpoint reached</td>
<td>161</td>
<td>26</td>
<td>187</td>
<td></td>
</tr>
</tbody>
</table>

| Courtesy V Papadimitrakopoulou |
MOST FREQUENT GENOMIC EVENTS BY Targeted NGS (181 genes FMI)

Courtesy WK Hong and V Papadimitrakopoulou
# Primary endpoint BATTLE-2

## Response

<table>
<thead>
<tr>
<th>8 week response</th>
<th>Arm1</th>
<th>Arm2</th>
<th>Arm3</th>
<th>Arm4</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR</td>
<td>1(5.0%)</td>
<td></td>
<td>3(4.3%)</td>
<td>3(4.9%)</td>
<td>7(3.7%)</td>
</tr>
<tr>
<td>SD</td>
<td>6(30.0%)</td>
<td>18(50.0%)</td>
<td>34(48.6%)</td>
<td>25(41.0%)</td>
<td>83(44.4%)</td>
</tr>
<tr>
<td>PD</td>
<td>13(65.0%)</td>
<td>18(50.0%)</td>
<td>33(47.1%)</td>
<td>33(54.1%)</td>
<td>97(51.9%)</td>
</tr>
<tr>
<td>Non Evaluable</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>0</td>
<td>13</td>
</tr>
</tbody>
</table>

## 8-wk DC

<table>
<thead>
<tr>
<th>8 week disease control</th>
<th>E(1)</th>
<th>E+M (2)</th>
<th>M+A (3)</th>
<th>S</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>8wk DC</td>
<td>7(35.0%)</td>
<td>18(50.0%)</td>
<td>37(52.9%)</td>
<td>28(45.9%)</td>
<td>90(48.1%)</td>
</tr>
<tr>
<td>No 8wk DC</td>
<td>13(65.0%)</td>
<td>18(50.0%)</td>
<td>33(47.1%)</td>
<td>33(54.1%)</td>
<td>97(51.9%)</td>
</tr>
<tr>
<td>P (Fisher’s exact test)</td>
<td>.40</td>
<td>.20</td>
<td>.44</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

 Courtesy V Papadimitrakopoulou
Design of the SAFIR02 lung-IFCT1301 trial

NGS Array CGH: genomic alterations

Therapeutic phase

Targeted therapy according to genomic alteration (6 different therapies)

Standard of care

Genomic alteration

R

mNSCLC EGFR-negative ALK-negative

No alteration: follow-up

Sponsor: UNICANCER-IFCT
Partnership: AstraZeneca & French Charity Foundation ARC

PI: JC Soria
Co-PI: F Barlesi
Biopsy metastatic site: Next generation sequencing Array CGH

Chemotherapy 4-6 cycles

No alteration

Followed up but not included

SAFIR 02 lung-IFCT1301

Molecular alteration Excluding EGFR+/ALK+

No PD

R 2:1

Targeted therapy According to Molecular alteration

Pemtrexed if Non-SCC

EGFR TKI if SCC

metastatic NSCLC first line chemotherapy

N= 650

All histologies

Ethics approval sept 2013; ANSM approval oct 2013
SAFIR 02 lung-IFCT1301 data interpretation challenge

Good et al. Genome Biology 2014, 15:438
LUNG-MAP

Patient Registration Consent → Tumor Collection → Genomic Screening → Assign treatment Arm by marker → Randomization → Treatment

- Interim Endpoint: PFS
- Primary Endpoint: PFS/OS

NGS/IHC (Foundation Medicine) → Investigational Targeted Therapy

Standard of Care Therapy

Squamous cell carcinoma 30%
Lung MAP Will be Run Throughout the US- 500+ sites
Molecular read-out

Foundation Medicine NGS test platform (CLIA/CAP)

1) DNA extraction
2) Library construction: selected cancer genes
   - Based on FM T5 NGS platform
   - Implemented as “mask” of T5 content and classification rules on called alterations
   - Rules determine biomarker positive/negative status
3) Analysis pipeline
   - Illumina HiSeq 2500

4) Master protocol CTA
   - Based on FM T5 NGS platform
   - Implemented as “mask” of T5 content and classification rules on called alterations
   - Rules determine biomarker positive/negative status

Classification rules (preliminary)

Non-NGS biomarkers:

Supplementary assays
- MET IHC (+)
- PIK3CA mutation
- CCND1 amplification or CDKN2A/B deletion, and RB1 wild-type
- FGFR1/2/3/4 amplification, mutation or fusion

Non-match arm
- All assays (-)
- Anti-PD-L1 Ab
- PI3K inhibitor
- CDK4/6 inhibitor
- FGFR inhibitor

PIK3CA mutation
- PI3K inhibitor

FGFR1/2/3/4 amplification, mutation or fusion
- FGFR inhibitor

PI3K inhibitor

CDK4/6 inhibitor

FGFR inhibitor

Courtesy R Herbst
Lung-MAP Sub-Studies for Treatment

Patients with squamous cell lung cancer

Tumor sample analyzed

Sub-Study A
- Tumor has none of the changes listed here
  - Arm A1: 50% Chemo-therapy
  - Arm A2: 50% MEDI 4736

Sub-Study B
- Tumor DNA has PIK3CA gene mutation
  - Arm B1: 50% Chemo-therapy
  - Arm B2: 50% Pictilisib

Sub-Study C
- Tumor DNA has CCND1, D2, CDK4 gene amplification
  - Arm C1: 50% Chemo-therapy
  - Arm C2: 50% Palbociclib

Sub-Study D
- Tumor DNA has FGFR gene amplification, mutation or fusion
  - Arm D1: 50% Chemo-therapy
  - Arm D2: 50% AZD 4547

Sub-Study E
- Tumor contains high levels of c-Met protein
  - Arm E1: 50% Erlotinib
  - Arm E2: 50% Rilotumumab + Erlotinib
Individualized Combined Modality Therapy for Stage III NSCLC
RTOG 1306/Alliance 31101

**Stratification**

<table>
<thead>
<tr>
<th>Mutation Type</th>
<th>Weight Loss (in prior 6 mos.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. EGFR</td>
<td>1. ≤ 5%</td>
</tr>
<tr>
<td>2. ALK</td>
<td>2. &gt; 5%</td>
</tr>
</tbody>
</table>

**EGFR TK Mutation Cohort**

**Arm 1**: Erlotinib, 150 mg/day for 12 weeks
Concurrent chemotherapy and radiation, 64 Gy

**Arm 2**: Concurrent Chemotherapy and radiation, 64 Gy

*Courtesy E Vokes*
TASTE-IFCT0801: design

- TAilored post-Surgical Therapy in Early stage NSCLC
  - is a prospective, randomized, and customized trial
  - incorporating ERCC1 IHC status and EGFR mutational status

Stage II and IIIA (non-N2) NSCLC patients with non-SCC histology were allowed. This French national-wide initiative (IFCT) recruited 150 pts in 3 years.

Wislez et al. JCO 2014
150 pts were randomized between May 2009 and July 2012 across 29 centers
TASTE-IFCT0801: Conclusions

- This adjuvant trial met its primary end point
  - for its phase II component
  - demonstrating the feasibility of a national biology-driven trial in the adjuvant setting.

- Safety data demonstrated an excellent tolerability profile for cisplatin-pemetrexed (as compared to cisplatin-
  navelbine).

- The phase III component was canceled due to the unexpected unreliability of the ERCC1 IHC read-out.

- ERCC1 IHC read-outs need to be refined before a prospective phase III trial is launched.
**ALChEMIST** _Adjuvant Lung Cancer Enrichment Marker Identification Sequencing Trial_

<table>
<thead>
<tr>
<th></th>
<th>ALCHEMIST SCREEN Component A151216</th>
<th>ALK+ E4512</th>
<th>EGFR-mutant A081105</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target Registry</td>
<td>Registry</td>
<td>ALK+</td>
<td>EGFRmut</td>
</tr>
<tr>
<td>Prevalence All comers</td>
<td>~5%</td>
<td>~10%</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6000-8000</td>
<td>336</td>
<td>410</td>
</tr>
<tr>
<td>Primary Endpt DFS-OS</td>
<td>--</td>
<td>DFS-OS</td>
<td>OS</td>
</tr>
<tr>
<td>Power</td>
<td>--</td>
<td>80%</td>
<td>85%</td>
</tr>
<tr>
<td>One-sided α</td>
<td>--</td>
<td>0.025</td>
<td>0.05</td>
</tr>
<tr>
<td>HR</td>
<td>--</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>Adjunct</td>
<td>Extended sequencing for additional targets (TCGA) ; correlation with local testing</td>
<td>Peripheral screening for ALK; RTPCR to identify fusion partners</td>
<td>Targeted sequence and kinome analysis; PRO and QOL</td>
</tr>
</tbody>
</table>
ALChEMIST data flow

1. Consent & Register
   - Pre-op Cohort
   - Post-op Cohort

2. Collect local test info
   - CLIA-approved Lab Marker Analysis
     - EGFR activating mutation
     - ALK+ E4512

3. Screen Neg.
   - A081105

4. 5 year follow-up cohort

5. A151216 Registry
6. Sequencing Database
7. E4512 Trial Database
8. A081105 Trial Database
**TRACERx Tracking Lung Cancer Evolution through Therapy/Rx**

**Clinical**
- Biopsy (diagnosis)
- Surgery

**Functional imaging**
- Adjuvant chemotherapy
- Biopsy (progression)

**Molecular Pathology**
- UCL-AD
- 48 gene panel
- Analyses of multiple regions of the tumour to assess genomic instability
  - FACS – DNA ploidy analysis
  - IHC – Tumour grade/nuclear pleomorphism
  - FISH – chromosomal instability

**Next generation sequencing**
- From multi region primary and metastatic samples:
  - Whole exome & whole genome ‘skim’ sequencing
  - Somatic point mutations, indels, structural changes
  - Construct phylogenetic tree of the tumour sectors
  - Compare ITH/genomic stability indices to drug resistance and outcome

**Technology Development**
- Plasma samples for ctDNA
- Track Cancer Evolution

**Integration of clinical and genomic data**

---

**UCL-AD**

---
UCL-AD 35 Gene NGS Screen*

FFPE Tissue Block

Tissue curls sectioned (under clinical conditions) x4 10µm curls in total.

Samples extracted, prepped and sequenced for mutations (DNA) and Translocations/CNV/Amp (RNA) on Ion Torrent.

EGFR, KRAS, BRAF, ALK, HER2, MET, IDH1, IDH2, PTCH1, ROS-1, KIT, NRAS, PIK3CA, PTEN, TP53, TMPRSS-ERG, H-RAS, MEK1, AKT1, PDGFRA, FGFR1, FGFR2, FGFR3, FGFR4, SMO, HER2, PIK3R1, DDR2, MYC, RB1, CTNNB1, ABL1, MPL1, RET1

*Integrated Cancer Panel Report

• Mutation
• Sequence Change
• Protein Change
• Percentage Change
• Coverage
• Translocation
• Copy Number Variation/Amplification

*Please note 34 genes listed, 35th gene under consideration/development
Evolution of tumour molecular profiling in lung cancer

- Trials have moved from metastatic to resected disease
- Molecular read-outs have been enriched
- Access to targeted therapies is now encompassed in many designs
  - Better collaboration with pharma companies
- The latest generation of trials is randomizing against SOC
- Multiple challenges remain to be solved
- But technological opportunities are enormous...
Applications of Liquid Biopsy

Monitoring & Early Detection

Brain cancer DNA blocked by blood-brain barrier

MULTIPLE TUMOR TYPES

Breast cancer
Pancreatic cancer
Colon cancer

Many tumors release DNA fragments that circulate in the bloodstream

ctDNA & TUMOR CELL ANALYSIS

ctDNA

Detection of Resistance Mutations

Targeted therapy
Response to therapy
Selective pressure
Resistance mutations

ctDNA of resistance mutations collected in blood sample

Courtesy A Bardelli
Lung Cancer Patient in the near future

Lung nodule/metastases

Tumor Biopsy/ blood

MOLECULAR PORTRAIT

Diagnostic

Cancer? Yes/No

Prognosis

Chemotherapy sensitivity

Targeted therapy sensitivity

Immunotherapy

PD I/PDL I

OX40

Other Pathways

...
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Gustave Roussy

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   C Swanton

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Yale Cancer Center

   R Herbst