Correlative Studies in Clinical Trials

Workshop #3

Ming Tsao and Lois Shepherd
August 2011
Correlative Studies in Clinical Trials can be:

- **Integral** – essential for randomization either as defining the allocation or population, or as a stratification factor.
  - eg. BR.10 – ras as a stratification factor
  - MAC.12 – OncotypeDx
  - MA.31 – HER2 +

- **Integrated** – defined in the protocol and mandatory – usually to better understand the treatment or toxicity profile.
  - eg. MA.32 – glucose and insulin

- **Retrospective** – studies which are usually done after the final analysis of a clinical trial which make use of the treatment regimens, outcomes, toxicity, quality of life.
Translational Research Initiatives

- In 1997 a Correlative Science/Tumour Bank Committee was convened to bring together pathologists, basic and clinical scientists, statisticians, epidemiologists to explore the concept of tumour banking for future research purposes.

- A decision was taken to prospectively consider the inclusion of banking diagnostic FFPE tissue on all new Phase III trials and to attempt to collect retrospectively, material on older trials, to facilitate discovery and validation studies.

- Over time, this has expanded to include collection as appropriate on Phase I and II studies as well as other biospecimens.
As a result the NCIC CTG has created...

• A national resource of clinical trial associated FFPE diagnostic material from many disease sites – breast, lung, colon, pancreas, ovary, prostate, endometrium, CNS

• A frozen tissue bank of NSCLC

• Virtual frozen breast bank

• Serum, plasma, urine, DNA, bone marrow on a growing number of studies
Growing number of Derivatives:
- TMAs
- DNA
- RNA
Protocols are essential:

- standardized protocols for collection, processing, shipping, storage
- laboratory manuals, labels, shipping specifications, safety issues all must be specified - “preanalytical”
- SOPs essential
Biobanks come in lots of shapes and forms

- REB approved
- “Accreditation” – CTRNet, CAP, OLA
- Adherence to ISBER Guidelines
- SOPs
Research/investigative studies for integral/integrated markers should be done in a GLP environment.

- CLIA certified, CAP, OLA
- Use of diagnostic, validated assays
- Retrospective studies in research laboratories may not meet this standard
Recent Publication or Guidelines

- REMARK
Some of our Successes ...
JBR.10 Adjuvant chemotherapy for resected non small cell lung cancer

Conducted with ECOG, CALGB and SWOG
JBR.10 - Study Design

Stratified by Nodal
* N0
* N1
*Ras
* Neg
* Pos
* UNK

Observation Only

Cisplatin Vinorelbine

BR.10 Snap-Frozen Tumor Bank

Randomise
Randomized: 482

Consented to RAS mutation analysis: 482

Sample collected: 452

RAS genotype: 450

Consent for other studies: 445

Frozen samples available: 169

FFPE blocks only: 184

Unstained slide only: 92

With FFPE blocks: 159

TMA: 331
What were the results?

- Chemotherapy improved the overall 5 year survival by **15% (69% vs 54%)**
- The risk of death was decreased by **31%**
- Toxicity was acceptable and changes in quality of life were relatively modest
- Elderly patients (>65) had a similar benefit as younger patients
- *ras* mutations were an adverse prognostic factor
- Adjuvant chemotherapy suggested a survival advantage in wild-type *ras* patients but the test for interaction was not significant
Vinorelbine plus Cisplatin vs. Observation in Resected Non–Small-Cell Lung Cancer

Timothy Winton, M.D., Robert Livingston, M.D., David Johnson, M.D., James Rigas, M.D., Michael Johnston, M.D., Charles Butts, M.D., Yvon Cormier, M.D., Glenwood Goss, M.D., Richard Inclucet, M.D., Eric Vallieres, M.D., Willard Fry, M.D., Drew Bethune, M.D., Joseph Ayoub, M.D., Keyue Ding, Ph.D., Lesley Seymour, M.D., Ph.D., Barbara Graham, R.N., Ming-Sound Tsao, M.D., David Gandara, M.D., Kenneth Kesler, M.D., Todd Demmy, M.D., and Frances Shepherd, M.D., for the National Cancer Institute of Canada Clinical Trials Group and the National Cancer Institute of the United States Intergroup JBR.10 Trial Investigators

ABSTRACT
Adjuvant Chemotherapy for NSCLC
“The Smoke Clears”

On the basis of the data reported..., the controversy surrounding adjuvant chemotherapy for resectable NSCLC is over.

Additional research will enable us to select those patients most likely to benefit from adjuvant chemotherapy, to customize the therapy on the basis of the biology of the tumour....
KRAS Mutation and NSCLC

- **RAS** mutations were the first transforming genes (oncogenes) identified in human cancer cells.

- **RAS** mutations occur in 15-20% NSCLC, with >90% involving **KRAS**.

- In 1990, KRAS mutation was first reported as a prognostic marker in lung adenocarcinoma (Slebos RJC, et al. NEJM 1990;323:561-5)
The role of RAS oncogene in survival of patients with lung cancer: a systematic review of the literature with meta-analysis


**NSCLC (n=2631)**
HR: 1.40; CI 95% 1.18–1.65

**Adenocarcinoma (n=1170)**
HR: 1.50; CI 95% 1.26–1.80
RAS Mutation Analysis on JBR.10 tumor samples

Randomized: 482

Consented to RAS mutation analysis: 482

Sample collected: 452

RAS analysis successful: 450

Wild type: 333 (74%)

Mutant: 117 (26%)
RAS Mutant Patients had Slightly but not Statistically Significant Poorer Survival

JBR.10: Mutant Ras Patients Had Little Benefit from Adjuvant Chemotherapy

Interaction P value = 0.29
(Insufficient evidence to say that the differences seen between mutant and wild type patients are statistically significant)

HR 0.69 (95% CI 0.49-0.97; p=0.03)
HR 0.95 (95% CI 0.53-1.71; p=0.87)

LACE-BIO Project

- Lung Adjuvant Chemotherapy Evaluation - Biomarker Consortium
- JBR.10, IALT, ANITA, CALGB 9633
- Meta- or pooled analysis of promising prognostic/predictive biomarkers
- Total number of patients with samples available for marker studies: 1400-1700
<table>
<thead>
<tr>
<th>marker</th>
<th>Prognostic</th>
<th>Predictive</th>
</tr>
</thead>
<tbody>
<tr>
<td>b-tubulin</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>p27, p16, cyclin E</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Mucin</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>p53 mutation</td>
<td>No</td>
<td>Yes (Sqcc)</td>
</tr>
<tr>
<td>p53 IHC</td>
<td>No</td>
<td>NO</td>
</tr>
<tr>
<td>KRAS</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Bax</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Intense Lymphocytic infiltrate</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>
LACE-Bio Pooled Analysis of the Prognostic and Predictive Value of KRAS Mutation in Completely Resected Non-Small Cell Lung Cancer (NSCLC)


on behalf of the LACE-Bio Collaborative Group
## Pooled Analysis of KRAS Mutation in LACE-Bio

<table>
<thead>
<tr>
<th>Trial</th>
<th>Patients in Trial</th>
<th>Patients with sample for KRAS analysis</th>
<th>ADC</th>
<th>SCC</th>
<th>Others</th>
<th>KRAS Mutation Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANITA</td>
<td>840</td>
<td>143 (17%)</td>
<td>33 (30%)</td>
<td>56 (51%)</td>
<td>21 (19%)</td>
<td>22/110 (20%)</td>
</tr>
<tr>
<td>IALT</td>
<td>1867</td>
<td>783 (42%)</td>
<td>223 (31%)</td>
<td>408 (57%)</td>
<td>87 (12%)</td>
<td>98/718 (14%)</td>
</tr>
<tr>
<td>JBR10</td>
<td>482</td>
<td>452 (94%)</td>
<td>210 (47%)</td>
<td>156 (34%)</td>
<td>84 (19%)</td>
<td>113/450 (25%)</td>
</tr>
<tr>
<td>CALGB 9633</td>
<td>344</td>
<td>343 (&gt;99%)</td>
<td>136 (53%)</td>
<td>85 (33%)</td>
<td>37 (14%)</td>
<td>70/258 (27%)</td>
</tr>
<tr>
<td>LACE-Bio</td>
<td>3533</td>
<td>1721 (48.7%)</td>
<td>602 (39%)</td>
<td>705 (46%)</td>
<td>229 (15%)</td>
<td>303/1536 (19.7%)</td>
</tr>
</tbody>
</table>

ADC: adenocarcinoma; SCC: squamous cell carcinoma
# Pooled Prognostic Value of KRAS Mutation

<table>
<thead>
<tr>
<th></th>
<th>Deaths/All patients</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall Survival</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>602/1233</td>
<td>1</td>
<td></td>
<td>0.09</td>
</tr>
<tr>
<td>Mutant</td>
<td>149/303</td>
<td>1.18</td>
<td>0.97-1.44</td>
<td></td>
</tr>
<tr>
<td><strong>Disease-Free Survival</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wild type</td>
<td>679/1233</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant</td>
<td>167/303</td>
<td>1.15</td>
<td>0.96-1.39</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Inter-trial heterogeneity: p=0.60 for OS and p=0.30 for DFS
### Predictive Value of Benefit from Adjuvant Chemotherapy (Disease-Free Survival)

<table>
<thead>
<tr>
<th></th>
<th>Chemotherapy (Deaths/Patients in Group)</th>
<th>Control (Deaths/Patients in Group)</th>
<th>Hazard ratio CT vs. Surgery [95% CI]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KRAS wild-type</strong></td>
<td>329/621</td>
<td>350/612</td>
<td>0.86 [0.74-0.99] p=0.045</td>
</tr>
<tr>
<td>(n=1233)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>KRAS mutated</strong></td>
<td>85/155</td>
<td>82/148</td>
<td>0.92 [0.67 - 1.24] p=0.57</td>
</tr>
<tr>
<td>(n=303)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Test for interaction KRAS * Treatment**  p=0.70

Interaction HR (95% CI): 1.07 [0.76 ; 1.51]

Between trial heterogeneity: p=0.41
CONCLUSION

- *KRAS* mutation cannot be used to select or exclude patients from cisplatin-based adjuvant chemotherapy

- *KRAS* mutation is only weakly prognostic, and
Prognostic and Predictive Gene Signature for Adjuvant Chemotherapy in Resected Non–Small-Cell Lung Cancer

Chang-Qi Zhu, Keyue Ding, Dan Strumpf, Barbara A. Weir, Matthew Meyerson, Nathan Pennell, Roman K. Thomas, Katsuhiko Naoki, Christine Ladd-Acosta, Ni Liu, Melania Pintilie, Sandy Der, Lesley Seymour, Igor Jurisica, Frances A. Shepherd, and Ming-Sound Tsao
Gene expression profiling

mRNA extracted from 133/166 frozen tissue >20% tumour cellularity
62 OBS / 71 ACT

A 15 gene signature selecting greatest separation of good and poor prognosis subgroups in the OBS patients identified

<table>
<thead>
<tr>
<th>Probe Set</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>201243_s_at</td>
<td>ATP1B1</td>
<td>ATPase, Na+/K+ transporting, beta 1 polypeptide</td>
</tr>
<tr>
<td>203147_s_at</td>
<td>TRIM14</td>
<td>Tripartite motif-containing 14</td>
</tr>
<tr>
<td>221591_s_at</td>
<td>FAM64A</td>
<td>Family with sequence similarity 64, member A</td>
</tr>
<tr>
<td>218881_s_at</td>
<td>FOSL2</td>
<td>FOS-like antigen 2</td>
</tr>
<tr>
<td>202814_s_at</td>
<td>HEXIM1</td>
<td>Hexamethylene bis-acetamide inducible 1</td>
</tr>
<tr>
<td>204179_at</td>
<td>MB</td>
<td>Myoglobin</td>
</tr>
<tr>
<td>204584_at</td>
<td>L1CAM</td>
<td>L1 cell adhesion molecule</td>
</tr>
<tr>
<td>202707_at</td>
<td>UMPS</td>
<td>Uridine monophosphate synthetase</td>
</tr>
<tr>
<td>208399_s_at</td>
<td>EDN3</td>
<td>Endothelin 3</td>
</tr>
<tr>
<td>203001_s_at</td>
<td>STMN2</td>
<td>Stathmin-like 2</td>
</tr>
<tr>
<td>210016_at</td>
<td>MYT1L</td>
<td>Myelin transcription factor 1-like</td>
</tr>
<tr>
<td>202490_at</td>
<td>IKBKAP</td>
<td>Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein</td>
</tr>
<tr>
<td>206426_at</td>
<td>MLANA</td>
<td>Melan-A</td>
</tr>
<tr>
<td>205386_s_at</td>
<td>MDM2</td>
<td>Mdm2, transformed 3T3 cell double minute 2</td>
</tr>
<tr>
<td>219171_s_at</td>
<td>ZNF236</td>
<td>Zinc finger protein 236</td>
</tr>
</tbody>
</table>
The 15-Gene Signature is Prognostic in Observation Patients (Stages IB and II)

HR, 15.02 (95% CI 5.12-44.04) p<0.0001
Signature is Prognostic in Observation but not in Chemotherapy Treated Patients

**JBR.10, observation (n=62)**

- **Low Risk**
  - Percentages at different time points:
    - 0 years: 100%
    - 3 years: 71%
    - 6 years: 54%
    - 9 years: 31%

- **High Risk**
  - Percentages at different time points:
    - 0 years: 100%
    - 3 years: 31%
    - 6 years: 22%
    - 9 years: 9%

**HR 15.02 (95% CI 5.12-44.04) p<0.0001**

**JBR.10, chemotherapy (n=71)**

- **Low Risk**
  - Percentages at different time points:
    - 0 years: 100%
    - 3 years: 78%
    - 6 years: 53%
    - 9 years: 35%

- **High Risk**
  - Percentages at different time points:
    - 0 years: 100%
    - 3 years: 28%
    - 6 years: 19%
    - 9 years: 8%

**HR 1.15 (95% CI 0.56-2.37) p=0.6942**
Validation of 15-gene Signature in the NCI Director’s Challenge
Stage IB-II Patients without Adjuvant Chemotherapy

HR 3.21 (95% CI 1.69-6.11)
p=0.0002
Not Prognostic in the DCC’s Stage I-II Patients with Adjuvant Chemotherapy

HR 3.21 (95% CI 1.69-6.11)  
P=0.0002

HR 1.10 (95% CI 0.47-2.53)  
P=0.8294

DCC, no adjuvant (n=169)

DCC, adjuvant chemo (n=41)
Chemotherapy Benefits JBR.10 High Risk but Not Low Risk Patients

Interaction $p = 0.0001$

**High risk**

- HR 0.33 (95% CI 0.17-0.63)  
  $p=0.0005$

**Low risk**

- HR 3.67 (95% CI 1.22-11.06)  
  $p=0.0133$

Interaction $p = 0.0001$
A novel 15-gene signature may identify early stage non-small cell lung cancer patients who are most likely to benefit from chemotherapy after complete surgical resection.

If validated by further testing, the signature may improve the current method for deciding which patients should receive adjuvant chemotherapy.
NCIC CTG BR.26: A double blind placebo controlled trial of PF-804 in patients with incurable stage IIIB/IV NSCLC after failure of standard therapy for advanced of metastatic disease
PF-00299804 (PF804)

- Selective, irreversible inhibitor of HER family of tyrosine kinases
- HER receptor inhibition via irreversible covalent modification of ATP-binding site
- Overcomes resistance to gefitinib/erlotinib in T790M+ve tumours
- Oral, RP2D 45mg continuous oral dosing daily
HER Biology

PF-00299804 (inhibits ErbB1, ErbB2, ErbB4)
Study Overview: NCIC CTG BR.26

Design

Randomised double blind placebo controlled trial

Advanced/Metastatic NSCLC
After failure of standard therapy

2:1 randomisation

PF-804
45 mg PO daily

Placebo
45 mg PO daily

Stratification factors: Centre, ECOG PS, Tobacco use, Best response to prior EGFR TKI, Weight Loss, Ethnicity
NCIC CTG BR.26

- **Primary Endpoint**
  - Overall survival

- **Secondary endpoints**
  - OS in *K-Ras* WT patients
  - OS in *EGFR* mutation +ve patients
  - Progression Free Survival
  - Objective RR
  - Time to Response and response Duration
  - Toxicity
  - Quality of life
  - Economic evaluations
  - Correlation of tumour and blood markers with outcomes
Study Overview: Statistical Design

• Randomised double blind placebo controlled trial

• **Sample Size n= 720 patients**
  – placebo arm estimated median OS of 4 months
  – 90% power to detect 33% improvements with PF-804
  – 1-sided 2.5% significance test

• **Interim analysis**
  – For futility
  – Performed at approximately 200 deaths

• **Accrual Aims**
  – 30 patients per month
  – 720 patients accrued over 2 years
BR.26 correlative analyses
Background

- Biomarkers have the potential to inform which patients most likely to benefit from a therapy
  - Save toxicity for those unlikely to benefit
  - Reduce societal costs if ineffective in a subset
  - Allow identification of subset for whom other therapies can and should be developed

- EGFR pathway has well characterised biomarkers of interest
  - EGFR gene mutation / copy number
  - KRAS mutations
  - Acquired EGFR mutations predictive of resistance
NCIC CTG BR.21: Survival According to Updated EGFR Copy Number

EGFR FISH Low Copy

- Placebo vs. Erlotinib
- Interaction P value = 0.35
- Hazard ratio, 0.80 (95% CI, 0.49-1.29)

EGFR FISH High Copy

- Placebo vs. Erlotinib
- Interaction P value = 0.004
- Hazard ratio, 0.43 (95% CI, 0.23-0.78)

NCIC CTG BR.21: Survival According to Updated KRAS Mutation Status

**KRAS Wild Type**

- Erlotinib
- Placebo

HR = 0.69 (0.49, 0.97)

**KRAS Mutation**

- Erlotinib
- Placebo

HR = 1.67 (0.62, 4.50)

Interaction P value = 0.09

*Zhu et al. J Clin Oncol, 2008*
Survival According to Updated EGFR Mutation Status

**A** Exon 19 Deletions and L858R Mutations

- Hazard ratio: 0.55 (95% CI, 0.25-1.19)
- Interaction P value: 0.47

**B** Wild-Type EGFR and Indeterminate Variants

- Hazard ratio: 0.74 (95% CI, 0.52-1.05)
- Interaction P value: 0.09

First-line gefitinib vs. carboplatin / paclitaxel patients with adenocarcinoma (IPASS)

Progression-free survival in EGFR mutation positive and negative patients

**EGFR mutation positive**
- Gefitinib (n=132)
- Carboplatin / paclitaxel (n=129)
- HR (95% CI) = 0.48 (0.36, 0.64)
- p<0.0001
- No. events gefitinib, 97 (73.5%)
- No. events C/P, 111 (86.0%)

**EGFR mutation negative**
- Gefitinib (n=91)
- Carboplatin / paclitaxel (n=85)
- HR (95% CI) = 2.85 (2.05, 3.98)
- p<0.0001
- No. events gefitinib, 88 (96.7%)
- No. events C/P, 70 (82.4%)

Treatment by subgroup interaction test, p<0.0001

ITT population
Cox analysis with covariates

C/P Gefitinib
BR.26 Correlative Sciences

• **Strong rationale to evaluate these biomarkers prospectively in BR.26**

• **To evaluate a biomarker requires adequate proportion of samples from the clinical trial participants**
  
  – BR.21 clinical trial included an optional tissue consent
  – Only a subset of tissues were available
  – Some available tissue not sufficient to yield data
  – Small proportion of samples severely compromises the biomarker analysis and impacts on the clinical utility of the biomarker

• **Therefore, tissue collection mandatory in BR.26**

  – But, recognising difficulty of collecting tissue in practice, inclusion of evaluation of biomarkers from blood samples
BR.26 Correlative Studies

• Prospectively planned analyses to evaluate prognostic and predictive biomarkers
  – Secondary endpoints:
    • OS in patients with baseline \textit{EGFR} gene mutation positive tumours
    • OS in patients with baseline \textit{KRAS} WT tumours

• Additional prognostic / predictive assays planned
  – serum \textit{EGFR} extracellular domain (ECD), serum \textit{HER2} ECD, SNPs, \textit{E-cadherin} ELISAs, \textit{TGF-\(\alpha\)} and \textit{HGF}

• Only prognostic / predictive biomarkers will be evaluated
  – NO testing for inherited diseases

• Collection of plasma to validate a blood based biomarker assay
## BR.26 Samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Collection Time Point</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
</tr>
<tr>
<td><strong>Tissue (Archival or Fresh sample)</strong></td>
<td>✓</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td>✓</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td>✓</td>
</tr>
<tr>
<td><strong>Blood for DNA/RNA</strong></td>
<td>✓</td>
</tr>
</tbody>
</table>