New Strategies on Horizon

Tony Mok MD
Dept of Clinical Oncology
The Chinese University of Hong Kong
TKI is forever

Addressing the difference

A liquid profile

Man and his mice
Strategy #1
Addressing the differences
Classic concept of cancer

Normal cell division

Cell damage—no repair

Cell Suicide or Apoptosis

Cancer cell division

First mutation

Second mutation

Third mutation

Fourth or later mutation

Uncontrolled growth
What if the cancer is not homogenous?
A Biopsy Sites

R1 (G3)
R2 (G3)
R3 (G4)
R4 (G1)
R5 (G4)
R6 (G1)
R7 (G4)
R8 (G4)
Hilum

10 cm

B Regional Distribution of Mutations

<table>
<thead>
<tr>
<th>Ubiquitous</th>
<th>Shared primary</th>
<th>Shared metastasis</th>
<th>Private</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

M2b
M2a
M1
R4
R9
R8
R5
R3
R2
R1
Pre M
Pre P
Early finding of intratumor heterogeneity in lung cancer

- Twenty-one patients with recurrent EGFR mutation positive lung cancer
- Surgical specimens were retrieved from archive
- Using laser capture microdissection and analyzed 50–60 areas from each tissue
- Fifteen tissues consisted only of cells with EGFR mutations
- Six tissues contained both mutated and non-mutated cells.

### Heterogeneous Distribution of EGFR Mutations Is Extremely Rare in Lung Adenocarcinoma

Yasushi Yatabe, Keigo Kato, and Toshio Miyauchi

#### Primary

<table>
<thead>
<tr>
<th>Exon 19Del</th>
<th>Exon 20Ins</th>
<th>G719X</th>
<th>L858R</th>
</tr>
</thead>
<tbody>
<tr>
<td>34</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

#### Lymph Nodes

<table>
<thead>
<tr>
<th>Exon 19Del</th>
<th>Exon 20Ins</th>
<th>G719X</th>
<th>L858R</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>G719X</th>
<th>L858R</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>L858R</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
</tr>
</tbody>
</table>
EGFR Mutation Heterogeneity and the Mixed Response to EGFR Tyrosine Kinase Inhibitors of Lung Adenocarcinomas

Zhi-Yong Chen, a Wen-Zhao Zhong, a Xu-Chao Zhang, a Jian Su, a Xue-Ning Yang, a Zhi-Hong Chen, a Jin-Ji Yang, a Qing Zhou, a Hong-Hong Yan, a She-Juan An, a Hua-Jun Chen, a Ben-Yuan Jiang, a Tony S. Mok, b Yi-Long Wu a

Screening for EGFR mutations by direct sequencing in consecutive lung cancer patients

- n = 307,1 (yr 2006.11-2011.5)

Only one sample

- n = 276.5

Patients with paired sample

- n = 306

Eligible for heterogeneity analysis

- n = 180 cases

Primary lesions at different time points

- n = 55

Primary tumor and metastatic lymph nodes

- n = 49

Multiple pulmonary nodules

- n = 41

Primary tumor and matched distant metastases

- n = 35

Synchronous

- n = 40

Metachronous

- n = 140

- No systematic therapy n = 43
- Chemotherapy n = 59
- TKI therapy n = 38

- SCLC
- Nonadenocarcinoma
- Absence of primary tumor
- Insufficient tumor cells

- n = 126
A.

<table>
<thead>
<tr>
<th></th>
<th>Frequency (%)</th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>A: Primary lesions at different time points (n=55)</td>
<td>90.9%</td>
<td>5</td>
</tr>
<tr>
<td>B: Primary tumor and metastatic lymph nodes (n=49)</td>
<td>89.8%</td>
<td>5</td>
</tr>
<tr>
<td>C: Multiple pulmonary nodules (n=41)</td>
<td>75.6%</td>
<td>10</td>
</tr>
<tr>
<td>D: Primary tumor and matched distant metastases (n=35)</td>
<td>85.7%</td>
<td>5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Heterogeneity</th>
<th>Homogeneity</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>B</td>
<td>5</td>
<td>44</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>31</td>
</tr>
<tr>
<td>D</td>
<td>5</td>
<td>30</td>
</tr>
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</table>
## Comparison of *EGFR* mutation status between primary tumor and metastases

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of specimen</th>
<th>Detection technology</th>
<th>Metastatic tumor</th>
<th>Discordant rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schimid</td>
<td>96</td>
<td>Sequencing</td>
<td>Ly</td>
<td>6.3% (6/96)</td>
</tr>
<tr>
<td>Kalikaki</td>
<td>25</td>
<td>Sequencing</td>
<td>Ly</td>
<td>8.8% (7/80)</td>
</tr>
<tr>
<td>Kalikaki</td>
<td>25</td>
<td>Sequencing</td>
<td>Sk, Lu, TW, Br, AG, Li, Bo</td>
<td>28.0% (7/25)</td>
</tr>
<tr>
<td>Matsumoto</td>
<td>19</td>
<td>Sequencing</td>
<td>Br</td>
<td>0 (0/6)</td>
</tr>
<tr>
<td>Park</td>
<td>101</td>
<td>Sequencing Heteroduplex analysis</td>
<td>Ly</td>
<td>11.9% (12/101) 16.8% (17/101)</td>
</tr>
<tr>
<td>Gomez-Roca</td>
<td>49</td>
<td>IHC</td>
<td>-</td>
<td>32.7% (16/49)</td>
</tr>
<tr>
<td>Badalian</td>
<td>11</td>
<td>IHC</td>
<td>Bo</td>
<td>54.5% (6/11)</td>
</tr>
<tr>
<td>Rao</td>
<td>51</td>
<td>IHC</td>
<td>Ly</td>
<td>10.6% (5/47)</td>
</tr>
<tr>
<td>Watzka</td>
<td>39</td>
<td>IHC</td>
<td>-</td>
<td>30.8% (12/39)</td>
</tr>
<tr>
<td>Italiano</td>
<td>30</td>
<td>IHC/FISH</td>
<td>AG, Bo, Br, Lu, ST</td>
<td>33.3% (10/30) 26.9% (7/26)</td>
</tr>
<tr>
<td>Bozzetti</td>
<td>31</td>
<td>FISH</td>
<td>Li, Pl, Ab, Ri, Sk, Ly</td>
<td>32.1% (9/28)</td>
</tr>
<tr>
<td>Daniele</td>
<td>28</td>
<td>FISH</td>
<td>Br, AG</td>
<td>22.9% (8/35)</td>
</tr>
<tr>
<td>Monaco</td>
<td>40/366</td>
<td>FISH</td>
<td>Ly, Pl, Br, PF, Li, Bre</td>
<td>32.5% (11/34)</td>
</tr>
<tr>
<td>Fang</td>
<td>35</td>
<td>Taqman RT-PCR</td>
<td>Ly, Br</td>
<td>31.5% (11/35)</td>
</tr>
</tbody>
</table>

*References:*
Strategy towards heterogeneity
FASTACT-2 (MO22201; CTONG0902) study design

### Screening

- Previously untreated stage IIIB/IV NSCLC, PS 0/1 (n=451)

### Study treatment

- **Group 1**: Gemcitabine 1,250mg/m² (d1, 8) + carboplatin AUC=5 or cisplatin 75mg/m² (d1) + erlotinib 150mg/day (d15–28); q4wks x 6 cycles; GC-erlotinib (n=226)

- **Group 2**: Gemcitabine 1,250mg/m² (d1, 8) + carboplatin AUC=5 or cisplatin 75mg/m² (d1) + placebo (d15–28); q4wks x 6 cycles; GC-placebo (n=225)

### Maintenance phase

- Erlotinib 150mg/day

### Primary endpoint

PFS with IRC confirmation

### Secondary endpoints

- subgroup analyses
- OS in all patients and subgroups
- ORR
- duration of response
- TTP
- NPR at 16 weeks
- safety
- QoL

NSCLC = non-small cell lung cancer; PS = performance status; PD = disease progression; AUC = area under the curve; q4wks = every 4 weeks; IRC = independent review committee; OS = overall survival; ORR = objective response rate; TTP = time to progression; NPR = non-progression rate; QoL = quality of life
Updated primary endpoint: PFS in ITT population (27 Jun 2012)

PFS Probability

Months

1.0
0.8
0.6
0.4
0.2
0

Erlotinib
Placebo

HR=0.57 (95% CI 0.47–0.69)
p<0.0001
OS in ITT population (27 Jun 2012)

OS Probability

Months

HR=0.79 (95% CI 0.64–0.99)
p=0.0420
Strategy #2

TKI is forever
5cm

PR at 2cm

Symptomatic PD at 4cm

0m  6m  12m  18m
5cm

PR at 2cm

RECIST PD At 2.6cm

Molecular resistance

Symptomatic PD at 4cm
ASPIRATION: To optimize treatment duration

Advance stage NSCLC with EGFR Mutation

EGFR TKI

PD By RECIST

PD By doctor Discretion*

PFS 1

PFS 2

*Doctor Discretion: Symptomatic progression, multiple progression Threat to major organ…etc

PI: K Park
TKI Resistance after ASCO 2012

Oncogenic driven cancer with tumor response to TKI

- Oligo-Progression
  - Local therapy + continuation of TKI

- Systemic Progression
  - Systemic therapy
    - Targeting the resistant gene
    - Chemotherapy
    - Chemotherapy + TKI
Treatment of TKI Resistance

Oncogenic driven cancer with tumor response to TKI

Oligo-Progression

Local therapy + continuation of TKI

Systemic Progression

Systemic therapy

Targeting the resistant gene

Chemotherapy

Chemotherapy + TKI
Local Therapy in Acquired Resistance: MSKCC

- 18/184 pts/7+ yrs underwent local therapy for extracranial PD
  - CNS PD excluded
- From time of local therapy
  - Median TTP: 10 months
  - Median time to new systemic Rx: 22 months
  - Median OS: 41 months

Yu, ASCO 2012, Abst#7527
Local treatment to oligo-progression plus continuation of TKI

- Colorado University collection of 65 patients with oncogenic driven cancer (EGFR mutation or ALK positive)
- All received EGFR TKI or Crizotinib
- PFS 1 defined as <4 sites of progression
  - Local ablative therapy offered to all sites of involvement and continue TKI
- PFS 2 defined as from time of local therapy to second progression

ASCO 2012 Abst 7526
### PFS of patients treated with LAT and continuation of TKI therapy

<table>
<thead>
<tr>
<th>Site of first progression</th>
<th>Number of patients</th>
<th>PFS1 (months)(95% CI)</th>
<th>PFS2 (months)(95% CI)</th>
<th>Site of 2nd progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS</td>
<td>10</td>
<td>10.9 7.3 – 18.3</td>
<td>7.1 1.7 – 11.3</td>
<td>2 (20%) no prog</td>
</tr>
<tr>
<td>eCNS†</td>
<td>15</td>
<td>9.0 6.5 – 13.8</td>
<td>4.0 2.7 – 7.4</td>
<td>4 (27%) no prog</td>
</tr>
<tr>
<td>All patients</td>
<td>25</td>
<td>9.8 8.8 – 13.8</td>
<td>6.2 3.7 – 8.0</td>
<td>6 (24%) no prog</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site of 2nd progression</th>
<th>3 (30%) CNS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 (50%) eCNS</td>
</tr>
<tr>
<td></td>
<td>8 (53%) eCNS</td>
</tr>
<tr>
<td></td>
<td>12 (48%) eCNS</td>
</tr>
</tbody>
</table>

† Includes 3 patients who progressed systemically (eCNS) and simultaneously within the CNS
Future Prospective Study?

Oncogenic driven cancer with tumor response to TKI

PD by RECIST <4 sites of PD

Randomized

Local therapy + continuation of TKI

Chemotherapy

Primary endpoint: PFS
Secondary endpoint: OS, RR, QOL
Treatment of TKI Resistance

Oncogenic driven cancer with tumor response to TKI

Oligo-Progression

Local therapy + continuation of TKI

Systemic Progression

Systemic therapy

Targeting the resistant gene

Chemotherapy

Chemotherapy + TKI
Chemo/Erlotinib vs. Chemo Alone at Progression after Acquired Resistance

- N = 78 retrospective review of outcomes
  - chemo alone (N = 44) or
  - chemo/erlotinib (N = 34)

- RR 18% (chemo) vs. 41% with chemo/erlotinib)

- No differences in PFS or OS between these two strategies

Goldberg, ASCO 2012, Abst#7524
IMPRESS: Chemotherapy with or with gefitinib at progression

Advance stage NSCLC with EGFR Mutation

Gefitinib

PD By RECIST

Gefitinib + Alimta/Platinum

Alimta/Platinum

Primary endpoint: PFS

Co-PI: Soria J; Mok T
Strategy #3
A liquid profile
Somatic Mutations Known Oncogenes in Lung Adenocarcinoma
N=188 Tumors and 623 Genes


Genomic profiling from plasma DNA?
Characteristics of circulating DNA

- Fragmented DNA at about 140 to 170 bp
- Only few thousands of amplifiable copies per ml of blood
- Circulating tumor DNA (ctDNA) may contain loci of mutated gene (driver or non-driver oncogene)
- Quantity of ctDNA could be related to tumor volume

Gormally et al Mutat Res 635: 105, 2007
Detecting one mutated gene at a time

Mixture of molecules

Digital PCR
Microfluidics Digital PCR
L858R mutation

mutant-specific probe

wild-type-specific probe
Droplet-based digital PCR

- Each micro-droplet contains a fragment of DNA.
- TaqMan reaction takes place within each droplet.
- About 20,000 droplets per tube.

Implied 20,000 single gene sequencing.
Reconstruction of fetal chromosome from maternal plasma

Noninvasive prenatal diagnosis of fetal chromosomal aneuploidy by massively parallel genomic sequencing of DNA in maternal plasma

Rossa W. K. Chiu\textsuperscript{a,b}, K. C. Allen Chan\textsuperscript{a,b}, Yuan Gao\textsuperscript{c,d}, Virginia Y. M. Lau\textsuperscript{a,b}, Wenli Zheng\textsuperscript{a,b}, Tak Y. Leung\textsuperscript{e}, Chris H. F. Foo\textsuperscript{f}, Bin Xie\textsuperscript{c}, Nancy B. Y. Tsui\textsuperscript{a,b}, Fiona M. F. Lun\textsuperscript{a,b}, Benny C. Y. Zee\textsuperscript{f}, Tze K. Lau\textsuperscript{e}, Charles R. Cantor\textsuperscript{g,1}, and Y. M. Dennis Lo\textsuperscript{a,b,1}
Common chromosomal aberrations in hepatocellular carcinoma (HCC)

May be we can detect similar chromosomal aberration from plasma?

Tornillo et al. J Pathol 2000
Detecting multiple mutated gene at a time

RESEARCH ARTICLE

CANCER GENOMICS

Noninvasive Identification and Monitoring of Cancer Mutations by Targeted Deep Sequencing of Plasma DNA

Tim Forshew,1,2,3* Muhammed Murtaza,1,2,3* Christine Parkinson,1,2,3,4* Davina Gale,1,2,3* Dana W. Y. Tsui,1,2,3* Fiona Kaper,4† Sarah-Jane Dawson,1,2,3 Anna M. Piskorz,1,2 Mercedes Jimenez-Linan,3,5 David Bentley,6 James Hadfield,1 Andrew P. May,4 Carlos Caldas,1,2,3,7 James D. Brenton,1,2,3,7‡ Nitzan Rosenfeld1,2‡
Tagged-amplicon deep sequencing (TAm-Seq)

- 48 primer pairs
- ~6 kb genomic target
  - TP53
  - PTEN
  - EGFR
  - BRAF
  - KRAS
  - PIK3CA

Forshew et al. Sci Transl Med 2012
Breast cancer

The graph illustrates the fraction of mutant alleles over time. The x-axis represents time in days, ranging from 0 to 500. The y-axis shows the fraction of mutant alleles on a logarithmic scale, ranging from $10^{-3}$ to $10^{-1}$. The graph shows significant variation in the fraction of mutant alleles for different genes over time. The vertical line labeled 'PD' indicates a period of disease progression.
Strategy #4
Man and his mice
Patient Derived Xenograft (PDX)
Transdisciplinary Approach to Optimizing Drug Development and Personalizing Therapy

iGXT Platform™
integrated GEMM • Patient-Derived Tumor Xenograft • Trials/Technology

CAPR: Center for Advanced Preclinical Research; JAX: Jackson Laboratories; UCDCC: UC Davis Comprehensive Cancer Center

Courtesy of Dr. David Gandara
JAX Lab NSG Mouse Model: PDX Host

NOD-scid Gamma Null (NSG) Mouse Model

• Profoundly immune deficient
  – Lack mature T and B cells
  – No functional NK cells
  – Deficiencies in cytokine signaling
  – NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup>tm1Wjl</sup> Tg(CMV IL3, CSF2, KITLG)1Eav/MloySzJ availability

• High take rate of human tumors
  – 2-3 mm<sup>3</sup> tumor fragments (optimal)
  – Cell pellets from pleural effusion, ascites
  – FNA from IR or EBUS

• Transportability
  – Over-night shipment of tumor specimens for implantation facilitates multi-institution collaboration

Courtesy of Dr. David Gandara
UCD-JAX-WEST NSG Resource: Development Process from Patient → PDX host

Patient biopsy at UC Davis

- Patient tumor
  - P0
    - N=1-10
    - Cryorecovery
    - P1
      - N=10-15
      - Freeze P1 fragments derived from P0 tumors for future use
  - P1 or P2 tumors on drug study
Comparative Characterization of NSCLC Patient Tumors (PTs) & Patient-Derived Xenografts (PDXs) in NSG Mice (JAX-WEST & UC Davis)

1. Histo-Morphologic Evaluation:
   - FFPE
   - IHC & Morphoproteomics

2. Clinically Applicable Molecular Biomarkers:
   - Mutations: EGFR and K-RAS
   - Fusion oncogene: EML4-ALK fusion variants
   - mRNA levels of ERCC1, RRM1 and TS
   - Genotyping for “driver” mutations

3. Genome-wide Molecular Profiling:
   - CNV/SNP and gene expression arrays
   - Next generation sequencing (Illumina)
   - miRNA and epigenetic profiling

4. Targeted in vivo drug testing at JAX

5. Characterization of cancer stem cells in mediating resistance to therapy

From T. Li & P. Mack, UC Davis

Director: Neal Goodwin

EGFR activating MT+ Models (Examples)

- **LG628**
  - EGFR aMT+ (E19del) Sensitive
  - Regimens “A, B, C, D”

- **LG631**
  - EGFR aMT+ (L858R) Sensitive
  - Regimens “A, B, C, D”

- **LG703**
  - EGFR aMT+ (L858R + MET) Acquired Resistance
  - Regimens “A, B, C, D”

- **LG651**
  - EGFR aMT+ (L858R + T790M) De novo Resistance
  - Regimens “A, B, C, D”

- **LG807**
  - EGFR aMT+ E19del+T790M Resistant
  - Regimens “A, B, C, D”

Molecular Characterization of Models

Selected Examples
Examples: EGFR expression levels in Lung Cancer PDXs

LG651: Primary erlotinib resistance

LG703: Acquired erlotinib resistance

LG0551 (SQ) (KRAS)
LG567 Adeno (KRAS)
LG542 Adeno (KRAS)
LG0552 Adeno (KRAS)
LG0556 Adeno (WT)
LG645 Adeno (WT)
LG0812 (WT)
LG0821 (WT)
LG591 Adeno (EGFR)
LG651 Adeno (EGFR)
LG703 Adeno (EGFR)
LG807 Adeno (EGFR)
LG0796 Adeno (MT UNK)
LG1023 (UNK)
LG978 (LCNE)
LG0627 (SC)
LG756 (SC) (LG627 SC)
LG476 (SQ)
LG476 (SQ P0)
LG830 (SQ)
LG0551 (SQ) (KRAS)
LG567 Adeno (KRAS)
LG542 Adeno (KRAS)
LG0552 Adeno (KRAS)
LG0556 Adeno (WT)
LG645 Adeno (WT)
LG0812 (WT)
LG0821 (WT)
LG591 Adeno (EGFR)
LG651 Adeno (EGFR)
LG703 Adeno (EGFR)
LG807 Adeno (EGFR)
LG0796 Adeno (MT UNK)
LG1023 (UNK)
LG978 (LCNE)
LG0627 (SC)
LG756 (SC) (LG627 SC)
Summary

• Addressing the difference
  – Tumor heterogeneity exists and may account to diversity in treatment outcome

• TKI is forever
  – Long term TKI for the concept of oncogenic addiction

• A liquid profile
  – Feasible to study genome profile from plasma DNA

• Man and his mice
  – In-vivo real time drug sensitivity testing method
Blind leading the blind