Diagnosis and monitoring of oncogene addicted cancers

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Guangdong General Hospital & Guangdong Academy of Medical sciences

2015-12-19 Singapore
Management of Lung Cancer

Detection

Diagnosis

Treatment

Monitoring

Heterogeneity in LC pts.

Tissue

Liquid

ALK: ~5%
KRAS: ~15% in Asians, ~30% in Caucasians
EGFR: ~40% in Asians, ~15% in Caucasians
HER2: 3%
ROS1: ~1%
RET: ~1%
BRAF: ~1%
NRAS: ~1%
PIK3CA: ~1%
MET: <5%
Others?

Paradigm of EGFR mutation status vs OS:

L858R
T790M

- Progression with new brain mets
- 6% tumor diameter decrease
- Brain radiation
- Targeted therapy on clinical trial

Detection
Diagnosis
Treatment
Monitoring

ESMO Asia 18-21 December
Singapore 2015
The tissue is the issue. BUT Challenging

- **Challenging:**
  - *Invasive*--high risk, unpleasant, painful;
  - *Selection bias*--Intra-tumoral heterogeneity, primary versus metastase;
  - *Re-biopsy difficult*--monitoring treatment and resistance;
  - *Insufficient*--too small to more molecular analysis;
Liquid biopsy may be the Solution

- Simple and less invasive
- More representative for the overall disease, avoids intra-tumoral and inter-metastatic tumor heterogeneity associated with tissue.
- Performed coherence for monitoring therapy and dynamic changes in the tumor.
Liquid biopsy

- ctDNA
  - Low concentration: average 17 ng/ml plasma in advanced-stage cancers
  - Low proportion: tumor DNA can range between 0.01% and 93%

- CTC
  - range 0~several thousands
Applications of ctDNA
Application 1

Molecular Diagnosis & Predicting Efficacy
FASTACT-2 study

**Screening**

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

**Study treatment**

1. Gemcitabine 1250mg/m² (days 1, 8) + carboplatin AUC=5 or cisplatin 75mg/m² (day 1) + erlotinib 150mg/day (days 15–28); every 4 weeks x 6 cycles, GC-erlotinib (n=226)

2. Gemcitabine 1250mg/m² (days 1, 8) + carboplatin AUC=5 or cisplatin 75mg/m² (day 1) + placebo (days 15–28); every 4 weeks x 6 cycles, GC-placebo (n=225)

**Maintenance phase**

- Erlotinib 150mg/day
- Placebo

PD

IRC = independent review committee

Mok T, et al. ESMO 2012 (abstract 1023)
tEGFR and pEGFR mutation analysis of FASTACT-2 (ASCO 2013)

**TUMOUR SAMPLES**

- 397 (88%) patients consented → 268 (59.4%) samples available → 241 (53.4%) samples analysable

**PLASMA SAMPLES**

- 451 (100%) patients consented → 427 (94.6%) samples available → 427 (94.6%) samples analysable

224 patients with matched tumour and plasma samples

EGFR = epidermal growth factor receptor; tEGFR = tumour EGFR; pEGFR = plasma EGFR

Concordance between tumour and plasma samples using the cobas® 4800 system

- A total of 224 patients had both tumour and baseline plasma samples with available EGFR mutation analysis results
  - sensitivity: \textbf{77\%} (69/90)
  - specificity: \textbf{96\%} (129/134)
  - positive predictive value: \textbf{93\%} (69/74)
  - negative predictive value: \textbf{86\%} (129/150)
  - overall concordance: \textbf{88\%} (198/224)
### Other techniques detecting plasma EGFR mutation

#### ARMS

<table>
<thead>
<tr>
<th>EGFR mutation</th>
<th>serum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>-</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>7</td>
<td>35</td>
</tr>
</tbody>
</table>

**Sensitivity:** 75%; **Specificity:** 97%.
(PS.: Tissue using Sequencing)


#### ddPCR

<table>
<thead>
<tr>
<th>EGFR mutation</th>
<th>serum</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>15</td>
<td>4</td>
</tr>
<tr>
<td>-</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>20</td>
</tr>
</tbody>
</table>

**Sensitivity:** 79%; **Specificity:** 100%.
(PS.: Tissue using ddPCR and Sequencing)


#### PCR/dHPLC

<table>
<thead>
<tr>
<th>EGFR mutation</th>
<th>plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>tissue</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>63</td>
</tr>
<tr>
<td>-</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
</tr>
</tbody>
</table>

**Sensitivity:** 82%; **Specificity:** 90%.

吉非替尼片说明书
请仔细阅读说明书并在医师指导下使用。

【药品名称】
通用名称：吉非替尼片
商品名称：易瑞沙
英文名称：Gefitinib Tablets
汉语拼音：Jifettini Pian

【注意事项】
当考虑本品用于晚期或转移性 NSCLC 患者的一线治疗时，推荐对所有患者的肿瘤组织进行 EGFR 突变检测。如果肿瘤标本不可评估，则可使用从血液（血浆）标本中获得的循环肿瘤 DNA（ctDNA）。

只能使用经论证可用于测定肿瘤或 ctDNA 的 EGFR 突变状态的检测方法，检测方法须稳定、可靠并且灵敏，以避免出现假阴性或假阳性的测定结果。

非吸烟、组织学类型为腺癌、女性或亚裔更可能从本品的治疗中获益。这些临床特点也和较高的肿瘤 EGFR 突变阳性率相关。

4.4 Special warnings and precautions for use
When considering the use of IRESSA as a treatment for locally advanced or metastatic NSCLC, it is important that EGFR mutation assessment of the tumour tissue is attempted for all patients. If a tumour sample is not evaluable, then circulating tumour DNA (ctDNA) obtained from a blood (plasma) sample may be used.
Detect resistant biomarkers
### AURA study (AZD 9291)

- 72 paired FFPET/ctDNA samples
- Detected by cobas and BEAMing

<table>
<thead>
<tr>
<th>Exon 19 deletion assays</th>
<th>Cobas AS-PCR</th>
<th>BEAMing digital PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>82% (22/27)</td>
<td>85% (22/26)</td>
</tr>
<tr>
<td>Specificity</td>
<td>97% (29/30)</td>
<td>97% (29/30)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>L858R assay</th>
<th>Cobas AS-PCR</th>
<th>BEAMing digital PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>88% (21/24)</td>
<td>88% (21/24)</td>
</tr>
<tr>
<td>Specificity</td>
<td>97% (31/32)</td>
<td>97% (31/32)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T790M assay</th>
<th>Cobas AS-PCR</th>
<th>BEAMing digital PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>74% (31/42)</td>
<td>81% (33/41)</td>
</tr>
<tr>
<td>Specificity</td>
<td>70% (16/23)</td>
<td>61% (14/23)</td>
</tr>
</tbody>
</table>

*8 with no T790M tissue result, 16 with no 19del/L858R result

Thress K., et al. ASCO 2014
AURA study (AZD 9291)
Different results between tumor and plasma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tissue cobas AS-PCR</th>
<th>BEAMing dPCR (% mutant)</th>
<th>Plasma cobas AS-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive</td>
<td>Positive (0.021%)</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Positive</td>
<td>Positive (0.048%)</td>
<td>Negative</td>
</tr>
<tr>
<td>3</td>
<td>Positive</td>
<td>Positive (0.064%)</td>
<td>Negative</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
<td>Positive (0.202%)</td>
<td>Negative</td>
</tr>
<tr>
<td>5</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>6</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>7</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>8</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>9</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>10</td>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>11</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>12</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>13</td>
<td>Negative</td>
<td>Positive (0.121%)</td>
<td>Negative</td>
</tr>
<tr>
<td>14</td>
<td>Negative</td>
<td>Positive (0.327%)</td>
<td>Positive</td>
</tr>
<tr>
<td>15</td>
<td>Negative</td>
<td>Positive (0.164%)</td>
<td>Positive</td>
</tr>
<tr>
<td>16</td>
<td>Negative</td>
<td>Positive (0.360%)</td>
<td>Negative</td>
</tr>
<tr>
<td>17</td>
<td>Negative</td>
<td>Positive (0.283%)</td>
<td>Positive</td>
</tr>
<tr>
<td>18</td>
<td>Negative</td>
<td>Positive (0.340%)</td>
<td>Positive</td>
</tr>
<tr>
<td>19</td>
<td>Negative</td>
<td>Positive (0.344%)</td>
<td>Positive</td>
</tr>
<tr>
<td>20</td>
<td>Negative</td>
<td>Positive (0.401%)</td>
<td>Positive</td>
</tr>
<tr>
<td>21</td>
<td>Negative</td>
<td>Positive (1.113%)</td>
<td>Positive</td>
</tr>
</tbody>
</table>

*‘False’ negatives by AS-PCR plasma*

*‘False’ positives by BEAMing*
AURA study (AZD 9291)  
Correlation between T790M status and efficacy of AZD9291

- T790M+:  
  Tissue vs. Plasma: 62% vs. 63%

- T790M-:  
  Tissue vs. Plasma: 26% vs. 29%

<table>
<thead>
<tr>
<th></th>
<th>Tissue</th>
<th></th>
<th>Plasma*</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T790M+</td>
<td>T790M-</td>
<td>T790M+</td>
<td>T790M-</td>
</tr>
<tr>
<td>Response Rate (CR &amp; PR)</td>
<td>62% (26/42)</td>
<td>26% (6/23)</td>
<td>63% (24/38)</td>
<td>29% (10/34)</td>
</tr>
<tr>
<td>Disease control Rate (CR, PR, &amp; SD)</td>
<td>95% (40/42)</td>
<td>70% (15/23)</td>
<td>89% (34/38)</td>
<td>76% (26/34)</td>
</tr>
</tbody>
</table>

*AS-PCR assay

- April 2nd 2014 data cut off  
- Includes confirmed responses & responses awaiting confirmation
TIGER-X: Phase 1/2 Trial of Rociletinib

Key eligibility criteria
- Advanced or recurrent NSCLC with a documented activating EGFR mutation
- Prior treatment with EGFR-directed therapy
- Recent biopsy available or willing to undergo a new on-study biopsy; plasma samples collected
- Phase 2 only
  - Disease progression while on treatment with EGFR-directed therapy
  - T790M-positive biopsy at the time of entering study
  - Treated stable CNS metastases are allowed

Key outcome measures
- Safety
- Tolerability
- PK profile
- ORR
Best Response to Rociletinib (All Doses) in 243 Centrally Confirmed Tissue T790M+ Patients

Presented By Lecia Sequist at 2015 ASCO Annual Meeting
Best Response to Rociletinib (All Doses) in Plasma T790M+ Patients

Presented By Lecia Sequist at 2015 ASCO Annual Meeting
Application 3

Dynamic monitoring both sensitive mutations and resistant biomarkers
FASTACT-2 study

Dynamic mutant DNA change during therapy

Patients treated with GC+P

Patients treated with GC+E

Mutant DNA copy/mL of plasma

Baseline C3 PD

Not detectable 1 10 100 1,000 10,000 100,000
Association between pEGFR mut+ at C3 and PFS/OS (both treatment arms combined)

**PFS**
- C3 mut+
- C3 mut−
  - Median=7.2 months (95% CI: 6.0–7.8)
  - Median=12.0 months (95% CI: 9.6–16.5)
  - HR=0.32 (95% CI: 0.21–0.48); p<0.0001

**OS**
- C3 mut+
- C3 mut−
  - Median=18.2 months (95% CI: 14.2–27.4)
  - Median=31.9 months (95% CI: 23.5–undefined)
  - HR=0.51 (95% CI: 0.31–0.84); p=0.0066

Patients, n
- C3 mut+ 42 42 35 28 14 7 6 4 1 1 1 1 1 0 0 0 0 0 0 0 0
- C3 mut− 80 80 77 65 59 47 40 34 28 13 10 7 3 0

Positive pEGFR at baseline followed by negative pEGFR at C3 is associated with improved outcomes; patients positive at baseline and still positive at C3 experienced worse outcomes.

OS = overall survival
Serial assessment of response and resistance for patients with *EGFR-L858R* mutant lung cancer on a prospective trial

• Qing Zhou, Jin-Ji Yang, Zhi-Hong Chen, Xu-Chao Zhang, Hong-Hong Yan, Jian Su, Hua-Jun Chen, Chong-Rui Xu, Hai-Yan Tu, Wen-Zhao Zhong, Xue-Ning Yang, Yi-Long Wu*

• Guangdong Lung Cancer Institute
• Guangdong General Hospital&
• Guangdong Academy of Medical Sciences
CTONG0901

- Based on a randomized trial (CTONG0901, NCT01024413)
- stage IIIB/IV NSCLC
- ECOG PS 0-2
- *EGFR* Mut+ (exon 19 deletion exon 21 L858R point mutation)
- Primary endpoint: ORR
- Secondary endpoint: OS, PFS, TTP, QOL, biomarker analyses and safety
PFS between two groups

Group A: N = 61, Events = 56, Median (95% CI) = 11.1 (6.6 ~ 15.6), P = 0.035

Group B: N = 19, Events = 19, Median (95% CI) = 7.5 (1.4 ~ 13.6)

Hazard ratio (95% CI) = 0.57 (0.34 ~ 0.97)
Dynamic monitoring of T790M in ctDNA

Application 4

Exploring Novel biomarkers
Acquired EGFR C797S mutation mediates resistance to AZD9291 in non-small cell lung cancer harboring EGFR T790M.


Abstract

Here we studied cell-free plasma DNA (cfDNA) collected from 15 patients with EGFR-mutant lung adenocarcinomas who had developed resistance to the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) AZD9291. The median time from diagnosis to AZD9291 was 6 months. Plasma cfDNA was extracted from seven patients before treatment and subsequently from five patients at disease progression. We found that EGFR T790M mutations were present in the baseline cfDNA of all patients, but that only one patient had an acquired C797S mutation. We then performed droplet digital PCR on serial cfDNA specimens collected from 15 AZD9291-treated patients at baseline, progression, and post-treatment. We found that six patients had acquired C797S mutations, five maintained the T790M mutation but did not acquire the C797S mutation, and four lost the T790M mutation despite the presence of the underlying EGFR activating mutation. Our findings provide insight into the diversity of mechanisms through which tumors acquire resistance to AZD9291 and highlight the need for therapies that are able to overcome resistance mediated by the EGFR C797S mutation.
Mechanisms of acquired resistance to AZD9291 in EGFR T790M positive lung cancer


1Dana-Farber Cancer Institute, Boston, MA, USA; 2AstraZeneca, Gatehouse Park, Waltham, MA, USA; 3Vall d’Hebron University Hospital, Vall d’Hebron Institute of Oncology, Barcelona, Spain; 4Brigham and Women’s Hospital, Boston, MA, USA; 5AstraZeneca, Alderley Park, Macclesfield, UK; 6Winship Cancer Institute, Emory University, Atlanta, GA, USA; 7Yonsei Cancer Center, Yonsei University College of Medicine, Seoul, Korea; 8Gustave Roussy, Paris, France
Results: T790M loss

- 32 of 67 (48%) had no detectable T790M in plasma despite presence of the EGFR-TKI-sensitizing mutation, suggesting overgrowth of an alternate resistance mechanism

- A few patients with loss of T790M had a very low allelic fraction of sensitizing mutation, such that a missed low level T790M cannot be ruled out

Data source: G. Oxnard, C. Paweletz, R. Alden, K. Thress
Results: HER2 amplification

- 15 cases completed plasma NGS after resistance to AZD9291 (4 showing C797S)
- One patient treated at 80 mg had an initial unconfirmed PR (-38%) followed by new liver metastasis
- Whole genome sequencing of resistance cfDNA found high level HER2 amplification

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 weeks (PD)</th>
<th>21 weeks (off tx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L858R</td>
<td>85%</td>
<td>79%</td>
<td>82%</td>
</tr>
<tr>
<td>T790M</td>
<td>42%</td>
<td>0%</td>
<td>1%</td>
</tr>
<tr>
<td>EGFR CNV</td>
<td>6</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>ERBB2 CNV</td>
<td>6</td>
<td>11</td>
<td>32</td>
</tr>
</tbody>
</table>

CNV, copy number variation; PD, progressive disease; PR, partial response; tx, treatment
Results: MET amplification

- 69-year-old female with EGFR-mutant NSCLC metastatic to liver, adrenal, bones who had progression after first-line chemotherapy and subsequent erlotinib

- Resistance biopsy was inadequate for genotyping, but plasma genotyping positive for L858R (26%) and T790M (4%)

- Initiated AZD9291 and responded on the first scan (-40%) but progressed after 24 weeks

- Resistance biopsy undergone for targeted NGS:
  - Positive for L858R, negative for T790M, positive for MET amplification
  - MET protein overexpression also seen on IHC

Pre-AZD9291 plasma genotype:
L858R (26%)
T790M (4%)

Baseline
4 months
6 months

Progression tumor genotype:
L858R
T790M negative
MET amplified

Data source: R. Pillai, S. Ramalingam
IHC, immunohistochemistry; NSCLC, non-small cell lung cancer
Results: BRAF V600E

- 49-year-old male with metastatic NSCLC positive for EGFR exon 19 deletion
- Developed resistance to first-line erlotinib after 11 months, T790M positive biopsy
- Had a confirmed PR to AZD9291 but growth of lung mass, effusion after 5 months
- Targeted NGS of progression biopsy shows exon 19 deletion (8% of reads), no T790M, BRAF V600E (6% of reads)
  - A patient-derived xenograft is in development

Data source: P.A. Jänne, A.J. Redig
ctDNA guides us to see the fact more clearly and further!
Summary

Applications

• Molecular diagnosis & predicting efficacy
• Detect resistance biomarkers
• Dynamic monitoring both sensitive mutation and resistant biomarkers
• Exploring novel biomarkers

Challenges

• More precise techniques false-negative or false-positive results
• Clinical meaning of dynamic monitoring
• Reasonable explanation of results (NGS)
Thanks!