MECHANISMS OF 1ST GENERATION RESISTANCE

Byoung Chul Cho, M.D., Ph.D.
CONFLICT OF INTEREST

Research funding: Novartis, Bayer, AstraZeneca, MOGAM Institute, Dong-A ST

Consulting role: Novartis, AstraZeneca, Boehringer-Ingelheim, Roche, BMS, Yuhan, Pfizer, Eli Lilly
1ST GENERATION EGFR-TKI

- Single agent activity of EGFR TKIs
  - Response rate: 60%-80%
  - PFS 9 – 12 months
  - Superior to Chemotherapy
  - Standard of care as first line therapy
What to do when Gefitinib fails?

- Response Rate ~70%
- PFS is ~10 months

- Repeat biopsy to identify acquired resistance mechanism
- Design subsequent therapy based on mechanisms
# Mechanisms of 1st Generation EGFR TKI

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Frequency</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secondary mutation/Target modification</td>
<td>50%</td>
<td>EGFR (T790M, T263P), EGFR amp, P53</td>
</tr>
<tr>
<td>‘Kinase Switch’ (bypass tract)</td>
<td>5-25%</td>
<td>MET amp / HGF overexpression, AXL overexpression, HER2 amp</td>
</tr>
<tr>
<td>Phenotypic change</td>
<td>5-15%</td>
<td>SCLC transformation (Rb loss), squamous cell Epithelial-mesenchymal transition</td>
</tr>
<tr>
<td>Downstream signal activation</td>
<td>5%</td>
<td>PTEN loss / PIK3CA mutation, BRAF V600E mutation</td>
</tr>
<tr>
<td>Angiogenesis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RTK internalization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug efflux</td>
<td></td>
<td>ABCG2 for gefitinib</td>
</tr>
</tbody>
</table>

Well-documented in human samples

**T790M GATEKEEPER MUTATION**

- The most prevalent mechanism of acquired resistance (50%) to 1\textsuperscript{st} G EGFR TKI
  - Steric hindrance (substitution of Threonine to Methionine at codon 790)
  - T790M increases receptor’s affinity for ATP
- T790M-harboring tumors display slower growth rates than tumors harboring sensitizing mutation ("flare" and "re-response")
- T790M harboring patients has also been found to be associated with a more indolent phenotype

*Oxnard GR. CCR 2011*
Pre-existing versus Evolution

Pre-existing (~10%)

Evolution (~90%)

Early resistance mediated by T790M

Late development of various resistance mechanisms

50 y/o female Exon19del+ lung adenocarcinoma

IF T790M SUBCLONES PREXIST...

Afatinib
IC50 for T790M ~300 nM

Osimertinib
IC50 for T790M ~10 nM
EGFR MUTATION: A MOVING TARGET

- **EGFR^{E19del} amplification** (target \(\uparrow\))
- **EGFR^{T790M} mutation** (steric hindrance, affinity to ATP \(\uparrow\))
- **EGFR^{T263P} mutation** (EGFR/HER2 heterodimerization)
MET amplification activates HER3/AKT Signaling

- Frequency 5-25%
- May be seen concurrently with T790M and SCLC transformation*
- Coupling of MET to HER3 leads to sustained activation of PI3K/AKT signaling
- Clinically meaningful MET amplification vs. poysomy?
- Standardization of screening methods (FISH, aCGH, qRT-PCTR..)?

# MET Inhibitors in Clinical Trials

<table>
<thead>
<tr>
<th>Compound</th>
<th>Modality</th>
<th>Target(s)</th>
<th>Company</th>
<th>Cancer Type</th>
<th>Development Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rilotumumab (AMG 102)</td>
<td>Antibody</td>
<td>HGF</td>
<td>Amgen</td>
<td>Gastric, lung, colon, brain, ovary, renal</td>
<td>2/3</td>
</tr>
<tr>
<td>Ficlatuzumab (AV-299)</td>
<td>Antibody</td>
<td>HGF</td>
<td>AVEO Pharmaceuticals</td>
<td>Lung</td>
<td>1/2</td>
</tr>
<tr>
<td>HuL2G7 (TAK701)</td>
<td>Antibody</td>
<td>HGF</td>
<td>Galaxy Biotech</td>
<td>Solid tumors</td>
<td>1</td>
</tr>
<tr>
<td>Onartuzumab (MetMab)</td>
<td>Antibody</td>
<td>MET</td>
<td>Genentech/Roché</td>
<td>Lung, colon, breast</td>
<td>2/3</td>
</tr>
<tr>
<td>AMG 337</td>
<td>Small molecule</td>
<td>MET</td>
<td>Amgen</td>
<td>Solid tumors</td>
<td>1/2</td>
</tr>
<tr>
<td>INC 280</td>
<td>Small molecule</td>
<td>MET</td>
<td>Novartis/Incyte</td>
<td>Renal, brain, liver, lung, melanoma, head and neck</td>
<td>2</td>
</tr>
<tr>
<td>Tivantinib (ARQ 197)</td>
<td>Small molecule</td>
<td>MET</td>
<td>ArQule/Daiichi-Sankyo/Kyowa Hakko Kirin</td>
<td>Lung, colon, breast, liver, prostate, myeloma</td>
<td>2/3</td>
</tr>
<tr>
<td>Crizotinib (PF-2341066)</td>
<td>Small molecule</td>
<td>MET, ALK</td>
<td>Pfizer</td>
<td>Lung, lymphoma</td>
<td>2/3</td>
</tr>
<tr>
<td>Cabozantinib (XL 184)</td>
<td>Small molecule</td>
<td>MET, VEGFR2, RET, KIT, AXL, FLT3</td>
<td>Exelixis/Bristol-Myers Squibb</td>
<td>Lung</td>
<td>2/3</td>
</tr>
</tbody>
</table>
INC280 PLUS GEFITINIB IN MET-AMPLIFIED EGFR MUTANT NSCLC AFTER PROGRESSION ON EGFR TKI

A) GCN <4

Best % change from baseline

ORR 14%

B) 4 ≤ GCN < 6

Best % change from baseline

ORR (95% CI) 24% (6.8-49.9)

C) GCN ≥ 6

Best % change from baseline

ORR (95% CI) 50% (31.9-68.1)

n/N (%) = 32/40 (80.00%)

n/N (%) = 32/36 (88.89%)

Wu YL, et al ASCO 2016
• TAM family receptor tyrosine kinase
  - Promotes cancer cell proliferation, migration and survival
  - Inhibits proinflammatory cytokine response and immune activation

• AXL overexpression is highly correlated with mesenchymal phenotype

Levin PA et al. JTO 2016
Activation of the AXL kinase causes resistance to Erlotinib in lung cancer


<table>
<thead>
<tr>
<th>Marker</th>
<th>Number positive (%)</th>
<th>Concurrent EGFR T790M mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AXL</td>
<td>7/35 (20)</td>
<td>2</td>
</tr>
<tr>
<td>GAS6</td>
<td>7/28 (25)</td>
<td>1</td>
</tr>
</tbody>
</table>
## AXL INHIBITORS IN CLINICAL TRIALS

<table>
<thead>
<tr>
<th>Agent</th>
<th>Target Protein</th>
<th>Axl IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Clinical Trial</th>
<th>Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cabozantinib</td>
<td>Axl, MET, VEGFR2, RET, Kit, Flt-1,3,4, Tie2</td>
<td>7 nM</td>
<td>NCT01639508</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NCT00596648</td>
<td>1 and 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NCT01708954</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>NCT01866410</td>
<td>2</td>
</tr>
<tr>
<td>Crizotinib</td>
<td>Axl, ALK, MET, RON, ROS1</td>
<td>294 nM</td>
<td>NCT02034981</td>
<td>2</td>
</tr>
<tr>
<td>ASLAN002</td>
<td>Axl, RON, MET, Tyro3, Mer, Flt-3</td>
<td>1.1 nM</td>
<td>NCT01721148</td>
<td>1</td>
</tr>
<tr>
<td>MGCD265</td>
<td>Axl, MET, VEGFR2</td>
<td></td>
<td>NCT00697632</td>
<td>1</td>
</tr>
<tr>
<td>MGCD516</td>
<td>Axl, RET, TRK, DDR2, MET, Kit, VEGFR, PDGFR</td>
<td>14 nM</td>
<td>NCT02219711</td>
<td>1</td>
</tr>
<tr>
<td>BGB324</td>
<td>Axl</td>
<td>14 nM</td>
<td>NCT02424617</td>
<td>2</td>
</tr>
<tr>
<td>BPI-9016</td>
<td>Axl, MET</td>
<td></td>
<td>NCT02478866</td>
<td>1</td>
</tr>
</tbody>
</table>

Levin PA et al. JTO 2016
**SCLC TRANSFORMATION**

- **Frequency 5-15%**
- **Clonal evolution (“harboring original activating EGFR mutation”)**
- **Loss of EGFR expression → resistant to EGFR TKI**
- **Clinical behavior of SCLC**
  - Rapid acceleration of growth rate
  - Initial response to chemotherapy followed by rapid clinical deterioration
- **Sensitive to BCL2 family inhibitor (ABT-263)**

RESISTANT EGFR MUTANT SCLC HAVE GENETIC LOSS OF RB1

Rb staining  | SCLC resistant | NSCLC resistant
---|---|---
Negative | 10/10 – 100% | 1/9 – 11%
Positive | 0/10 – 0% | 8/9 – 89%

Fisher's exact test $P < 0.0001$

HETEROGENEITY

After progression on erlotinib


"Genomic divergence"
CLONAL EVOLUTION

- Adenocarcinoma
- SCLC
- Erlotinib
- Chemotherapy

Timeline

Tumor Burden
It’s like playing an endless game of whack-a-mole

Is combination therapy a solution?
OTHER RESISTANCE MECHANISMS

HER2 Amplification (8-12%)

Epithelial-mesenchymal transition (?)

<table>
<thead>
<tr>
<th>Gene status</th>
<th>HER2 amp⁺</th>
<th>HER2 amp⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>EGFR T790M⁺</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>EGFR T790M⁻</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

More than one mechanism of acquired resistance can exist (~4%)*

A NEW STANDARD OF CARE MAY CHANGE LANDSCAPE OF ACQUIRED RESISTANCE TO 1ST GENERATION EGFR TKI

Combining EGFR-TKI with antiangiogenic agent in EGFR mutant NSCLC


Stahel, et al. ECC 2015
How can we use mechanisms of resistance for therapy?

**Target Mutation / modification**
- EGFR E19del / T790M
- G719S / T263P

**By-pass track**
- MET amplification
- Axl upregulation

**Activation of downstream effectors**
- PIK3CA mutation

**Phenotypic changes**
- SCLC transformation

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1st G EGFR TKI + MET inhibitor
1st G EGFR TKI + Axl inhibitor
1st G EGFR TKI + PI3K inhibitor
3rd G EGFR-TKI
2nd G EGFR TKI

SCLC transformation
BCL2 family inhibitor (ABT-263)

*Modified Garraway Cancer Discovery 2012*
How can we identify additional mechanisms of resistance?
Sanger Sequencing (PCR-based assays) vs. NGS

Response to treatment (CT scans)

Gene rearrangement and copy number variation

“Hotspot mutations”
GENOMIC PROFILES OF TUMORS WITH ACQUIRED RESISTANCE

Pre-EGFR TKI

Sample

Post Progression

Median depth 607x
GENOMIC LANDSCAPE IN POST-TKI TUMORS

T790M = 12/17 (70%)
(None pre-existing)
WHAT COULD BE UNKNOWNS?

- Predominantly genomic mechanisms
  - Epigenome?
  - miRNA?

~30% downregulate Axl mRNA.

Mird-34 and mir-199a/b downregulate Axl mRNA. Mudduluru et al. Oncogene 2011
Summary

- Understanding mechanisms of acquired resistance improve clinical outcomes
  - EGFR T790M gatekeeper mutation
  - Bypass tract (MET, AXL, HER2)
  - SCLC transformation

- Ultra-deep sequencing/RNA-seq may reveal more comprehensive pictures of acquired resistance mechanisms to 1\textsuperscript{st} G EGFR TKIs
HAVE A PLEASANT STAY IN SEOUL!