Overcoming Resistance to EGFR Tyrosine Kinase Inhibitors in EGFR Mutant Lung Cancer

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Oncology for Scientists
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Lung Cancer

• Leading cause of cancer mortality for both men and women in the United States
• More than 220,000 individuals diagnosed annually
• 5 year survival for advanced disease < 15%
• Histologically and molecularly heterogeneous disease
  • contemporary therapy for advanced disease is based on identification & targeting of oncogenic drivers
Histologic and Molecular Subtypes of Non-Small Cell Lung Cancer

Oncogenic Drivers:
- Mutually exclusive mutations
- Reflect tumor cell addiction
Precision Medicine in NSCLC Treatment

• In about 60% of lung adenocarcinomas, a specific oncogenic driver mutation can be identified

• Drugs that target these oncogenic drivers are selectively toxic to malignant cells

• Targeted therapies offer better-tolerated disease modifying treatment options in patient populations defined by relevant oncogene mutations

• Successful implementation requires robust biomarker assays; rapid turn-around time; menu of targeted therapies; access to targeted agents
Precision medicine extends NSCLC survival

Targeted therapy matched to mutations in a tumor extends survival for patients with advanced lung cancer.

N=264

N=313

*all patients had a targetable lesion

Mark Kris, MD
EGFR Structure

- Receptors exist on the surface of cells and contain an extracellular, transmembrane, and intracellular domain.

- Each domain is responsible for a different aspect of signaling
  - Extracellular Domain
    - Ligand binding
    - Receptor dimerization
  - Intracellular Domain
    - Tyrosine kinase
    - Regulatory region

EGFR downstream signaling

- EGF, TGF-alpha, etc
- EGFR
- PI3-K
- AKT
- mTOR
- STAT
- GRB2
- SOS
- RAS
- RAF
- MEK
- ERK
- Gene transcription
- Cell cycle progression
- Cell proliferation
- Inhibition of apoptosis
- Angiogenesis
- Migration, Adhesion, Invasion
**EGFR Gene Mutations in NSCLC**

- Activating *EGFR* gene mutations identified in 15% of lung adenocarcinoma cases in the United States
  - higher rate of mutation in Japan, Korea
- TK domain commonly mutated
  - L858R point mutation in exon 21
  - Exon 19 deletion (near ATP binding site)
  - Mutations stabilize the “activated” conformation of the TK domain
- In mouse models, presence of mutated *EGFR* is necessary and sufficient to induce lung tumors
EGFR Tyrosine Kinase Inhibitors for Treatment of Advanced EGFR Mutant NSCLC

- **Exon 19 del746-750**
- **Exon 21 L858R**

EGFR Tyrosine Kinase Inhibitors

- **Erlotinib**: FDA Approved, First Line Therapy
  - First generation inhibitor
  - Binds reversibly to TK domain of EGFR
  - High rate of initial response (70%)
  - Treatment failure within 1 year
  - Majority of resistance due to EGFR T790M
  - T790M Lies within the ATP/drug binding cleft of EGFR
  - Improves ATP binding, making erlotinib less effective
Osimertinib

- Oral, irreversible inhibitor selective for EGFR sensitizing mutations and the EGFR T790M resistance mutation
- Wild-type sparing (does not bind wildtype EGFR)
- Osimertinib demonstrates efficacy in Phase II AURA2 trial
  - When delivered after EGFR TKI failure, osimertinib has an objective response rate of 70%
  - Complete responses were observed in 3% of patients
  - Responses were durable, average of 11.4 months
- FDA approved for patients with EGFR T790M mutant NSCLC who have progressed on or after EGFR TKI therapy
The Question

• Pre-clinical and epidemiologic studies support a role for vitamin D3 metabolites in reducing lung cancer risk

• Impact of vitamin D3 metabolites on lung cancer survival is variably reported
    • no overall association between 25(OH)D$_3$ and lung cancer mortality in NHANESIII cohort
    • lifetime never smokers with lung cancer significantly less likely to die from their disease if 25(OH)D$_3$ $\geq$44 nmol/L vs. <44 nmol/L

• Might lung cancers that develop in never smokers be preferentially affected by vitamin D3 metabolites?
Vitamin D Metabolism and Signaling Pathway

7-dehydrocholesterol $\rightarrow$ Vitamin D$_3$ $\rightarrow$ UVB $\rightarrow$ Liver CYP2R1 $\rightarrow$ 25-hydroxyvitamin D$_3$ (25D3) $\rightarrow$ Kidney CYP27B1 $\rightarrow$ 1,25-dihydroxyvitamin D$_3$ (1,25D3) $\rightarrow$ lung epithelial cell or lung tumor

Processes Affected by 1,25D3: differentiation & proliferation, growth factor signaling, inflammation, angiogenesis
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NSCLC cells with *EGFR* mutations are preferentially sensitive to $1,25(OH)_2D_3$.

**VDR**

- **VDR$^{High}$**
  - HCC827
  - H1975
  - H292
  - SK-LU-1
  - A549
  - A427
  - H23

- **VDR$^{Low}$**

**Mutation Profile**

**Fold-increase CYP24A1-luc**

- **HCC827**
  - 24h

- **A549**

**%Colonies Rem.**

- **HCC827**
- **A549**

**[1,25(OH)$_2$D$_3$] nM**

- 0.1
- 1
- 10
- 100
- 1000
VDR is expressed in human lung tumors that harbor activating EGFR mutations.

- **A.** VDR IHC

- **B.** VDR H-score

- **C.** Box plot showing difference in VDR H-score between L858R and del 745/6-750

**EGFR TKI activity**

<table>
<thead>
<tr>
<th>EGFR mt</th>
<th>RR (%)</th>
<th>OS (mo)</th>
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<tbody>
<tr>
<td>del746-750</td>
<td>70-100</td>
<td>26-30</td>
</tr>
<tr>
<td>L858R</td>
<td>20-67</td>
<td>8-17</td>
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</tbody>
</table>

Effect of modulating dietary vitamin D3 intake on growth of *EGFR* mutant NSCLC xenografts

HCC827 cells (*EGFR* del746-750)

Week 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14

- N=10
- N=10
- N=10

- 100 IU Vitamin D3/kg diet
- 10,000 IU Vitamin D3/kg diet
Dietary vitamin D3 intake modifies growth of HCC827 human lung tumor xenografts

**Growth rate (d13-d27)**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>P-value</th>
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<tbody>
<tr>
<td>100 IU vs switch</td>
<td>0.011</td>
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<tr>
<td>10,000 IU vs switch</td>
<td>0.359</td>
</tr>
<tr>
<td>100 IU vs 10,000 IU</td>
<td>0.001</td>
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</table>

**Fold-increase in tumor volume**

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>Final TV/Initial TV</th>
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<tbody>
<tr>
<td>100 IU vitamin D3/kg</td>
<td>16</td>
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<tr>
<td>Switch diet</td>
<td>7</td>
</tr>
<tr>
<td>10,000 IU vitamin D3/kg</td>
<td>3</td>
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</table>

**Fraction below 100 mm³ at study termination**

<table>
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<tr>
<th>Diet Group</th>
<th>Fraction</th>
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<tbody>
<tr>
<td>100 IU vitamin D3/kg</td>
<td>2/10</td>
</tr>
<tr>
<td>Switch diet</td>
<td>5/10</td>
</tr>
<tr>
<td>10,000 IU vitamin D3/kg</td>
<td>7/10</td>
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