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Disclosure Information

Pasi A. Jänne, MD, PhD

I have the following financial relationships to disclose:

Consultant for: Astra Zeneca, Boehringer Ingelheim, Pfizer, Genentech, Roche, Sanofi-Aventis, Clovis Oncology, Chugai Pharmaceuticals, Merrimack Pharmaceuticals

Stockholder in: Gatekeeper Pharmaceuticals

Other: LabCorp - post-marketing royalties from DFCI owned intellectual property on EGFR mutations
Preclinical model systems

Guide clinical drug development

Inform preclinical studies

NSCLC patients

• Prioritize clinical therapies
• Identify resistance mechanisms
• Test novel combination therapies

• Evaluate targeted therapies
• Determine biomarker modulation
• Study clinical drug resistance
# Preclinical Cell Line Models

<table>
<thead>
<tr>
<th>Cell Line Model</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Existing Cell Lines</td>
<td>- Characterized</td>
<td>- Limited number</td>
</tr>
<tr>
<td></td>
<td>- Grow well</td>
<td>- Not all genotypes covered</td>
</tr>
<tr>
<td></td>
<td>- Endogenous context</td>
<td>- May not completely be reflective</td>
</tr>
<tr>
<td></td>
<td>- Used to study efficacy and model resistance</td>
<td></td>
</tr>
<tr>
<td>Ba/F3 or 3T3 Cells</td>
<td>- Uniform background</td>
<td>- Artificial model</td>
</tr>
<tr>
<td></td>
<td>- Compare genotypes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Good for drug screening</td>
<td></td>
</tr>
<tr>
<td>Patient Derived</td>
<td>- Maybe more reflective of clinical scenario</td>
<td>- Resources needed to grow</td>
</tr>
<tr>
<td>Cell Lines</td>
<td></td>
<td>- Not always easy to establish</td>
</tr>
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</tbody>
</table>
### Existing NSCLC Cell Line Models

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cell Lines</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRAS</td>
<td>~ 30</td>
<td>Multiple KRAS genotypes, LKB1 and P53 intact/deficient</td>
</tr>
<tr>
<td>EGFR</td>
<td>8 -10</td>
<td>Mostly Exon 19 deletion; 1 L858R, no exon 20 or rare EGFR genotypes</td>
</tr>
<tr>
<td>ALK</td>
<td>2</td>
<td>H2228 not sensitive in vitro</td>
</tr>
<tr>
<td>HER2</td>
<td>1</td>
<td>Rare HER2 mutation</td>
</tr>
<tr>
<td>ROS1</td>
<td>1</td>
<td>Not very sensitive in vitro</td>
</tr>
<tr>
<td>RET</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>NTRK</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>BRAF</td>
<td>5</td>
<td>1 V600E; 4 non-V600E</td>
</tr>
</tbody>
</table>

Need to develop additional cell lines to reflect diversity of clinical genotypes
Ba/F3 Cell Line Models

Comparison of drug efficacy

Requirement of RET kinase activity for IL-3 independent growth

Enhanced growth rate for EML-ALK F1174L
## Comparison of Animal Models

<table>
<thead>
<tr>
<th>Model</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xenograft</td>
<td>- Well Established&lt;br&gt;- Can use many cell lines&lt;br&gt;- Cheaper</td>
<td>- Somewhat artificial&lt;br&gt;- Not always predictive of clinical outcome&lt;br&gt;- Immunodeficient</td>
</tr>
<tr>
<td>Genetically engineered</td>
<td>- Endogenous model&lt;br&gt;- More reflective of human disease&lt;br&gt;- Intact immune system</td>
<td>- Cost (breeding &amp; imaging)&lt;br&gt;- Tumor latency&lt;br&gt;- Driven by single genetic alteration</td>
</tr>
<tr>
<td>Patient derived xenograft</td>
<td>- Can study both tumor and stroma&lt;br&gt;- Model may reflect clinical scenario&lt;br&gt;- Ability to develop unique models</td>
<td>- Cost&lt;br&gt;- Immunodeficient</td>
</tr>
</tbody>
</table>
Different Types of “Positive” Xenograft data

Drug Resistant Patients

From Patient to In Vitro Model

1. Study heterogeneity of drug resistance
2. Identify novel resistance mechanisms
3. Test Novel Therapeutic Strategies

Primary Cell Line
Primary Xenograft
Design clinical trials
Generation of patient derived cell lines to study drug resistance mechanisms

Baseline
EGFR Exon 19 del

Erlotinib

Chemotherapy

Resistant tumor: EGFR Exon 19 del
Red: MET; Green: CEP 7

Atsuko Ogino
DFCI 81 cells retain MET amplification in vitro

**DFCI 81 Cells**

**Metaphase**

**Interphase**

**Gefitinib**

**PHA665752**

**Gefitinib/PHA665752**

Drug Concentration (µM)

% of control

HCC827 GR Cells

**DFCI 81 Cell line**
DFCI202 - Erlotinib resistant EGFR mutant NSCLC patient derived xenograft - no T790M

DFCI202

Patient

H&E

DFCI202 NSG

P1

DFCI202#4 P1

Lung metastasis

Antonio Calles, Parfulla Gokhale, Sangeetha Palakurthi
DFCI 193 - Erlotinib resistant patient derived xenograft - del 19/T790M

DFCI193
Patient

DFCI193Nx
P1

H&E

DFCI-193#271 P1 xenograft

Antonio Calles, Parfulla Gokhale, Sangeetha Palakurthi
Tumor derived “slice” cultures to study drug resistance mechanisms

Lung Cancer Patient

Surgical Resection or Tumor Biopsy

Subcutaneous Injection

Mouse Xenograft

Slice harvested tumor with Vibratome

Tumor slice in culture medium

Treat tissue culture with inhibitors

Cell Viability Assay
- Examine Signaling Pathways
- Immunohistochemistry
- Heterogeneity of Treatment Response

T=24, 48, 72, 96 hours

Curtis Chong
“Slice” culture using PC9 GR (EGFR del 19/T790M) xenografts

Cell Viability

Histology

Curtis Chong
The use of inducible bitransgenic mouse modeling to examine the *in vivo* role of activating oncogenic mutations in lung tumorigenesis.

**Target line**

- TRE-Pmin CMV
- oncogene

**Transactivator line**

- CCSP
- rtTA

**CCSP-rtTA, Tet-op-activating mutations**

- TRE-Pmin CMV
- oncogene

- rtTA

- Dox

**Analysis**

Kwok Wong
EGFR mutations are oncogenic in vivo and cause lung cancer in mouse models

Ji et al. Cancer Cell 2006
Superior to Efficacy Relative to Clinical Agents in Mouse Models

WZ4002

HKI-272

BIBW-2992

Kwok-Kin Wong, Liang Chen
Impact of genotype on treatment with selumetinib/docetaxel

Chen et al. Nature 2012
Improved PFS with Docetaxel/Selumetinib compared with docetaxel in *Kras G12D* murine model of NSCLC

Chen et al. Nature 2012
Activation of PD1 pathway in EGFR mutant NSCLC

PDL1 expression in human EGFR mutant tumors

Akbay EA et al. Cancer Discovery 2013
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