Genetically Engineered Mouse Lung Cancer Models for Pre- and Co-clinical studies

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Ownership Interest (stocks, stock options, or other ownership interest excluding diversified mutual funds): G1 Therapeutics
Comprehensive Preclinical/Translational Lung Experimental Therapeutics Program

New Basic Lung Cancer Genomic and Proteomic Discoveries

- NSCLC lines and Genetically Engineered Cell lines harboring specific oncogenic mutations
- Xenografts and Primary Lung Cancer Transplant Models
- Patient Derived Lung cancer Cell Lines
- Genetically Engineered Mouse Models

Determine best single agent or combination therapy to bring forth to human trials with genetically stratified patients

Preclinical and Co-clinical Trials

Elucidate the mechanism of primary resistance and acquired resistance in the patient samples

Run Human Clinical Trials
Oncogenic Drivers in US Lung Adenocarinomas

Molecular subsets of lung adenocarcinoma

Unknown

KRAS

EGFR

ALK Fusions

HER2

BRAF

PIK3CA

AKT1

MAP2K1

ROS1 fusions

KIF5B-RET

NRAS

Unknown
Genetically engineered mouse models

Target line

Transactivator line

CCSP-rtTA, Tet-op-activating mutations

Allow for turning on and off the oncogenic driver specifically in the lung compartment in adult mouse

Analysis
pCAG-Lox-STOP-Lox conditional transgenic mouse model

Single copy transgene specifically integrated at ColA1 locus by homologous recombination.

Cre mediated transgene activation
Inducible Mouse Lung Cancer Models

- KRAS G12D
- KRAS G12V
- KRAS G12C
- EGFR Del19
- EGFR L858R
- EGFR T790M
- EGFR wild type
- EGFR vIII
- HER2 exon 20 insertion
- HER2 wild type
- BRAF V600E
- p110 exon 20 (H1047R)
- EML4-ALK
- C-MET
- EGFR T790M-Del19/c-MET
- EGFR T790M-L858R/c-MET
- EGFR T790M-Del19
- EGFR T790M-L858R
- EGFR T790M-L858R-C797S
- EML4-ALK F1174L
- EML4-ALK L1196M
Inducible Mouse Lung Cancer Models

- DDR2 mutants
- FGFR 1
- FGFR 2 WT
- FGFR2 W290C
- FGFR2 S320C
- FGFR2 K659N
- FGFR3 WT
- FGFR3 K652E
- FGFR3 S249C
- NRF2 WT
- NRF2 G81S
- NRF2 29H
- SOX2

- IDH1 R132H
- IDH2 R140Q
- IDH2 R172K
- EZH2
- EZH2 Y641N
- RBP2
- LSD2
- KDM5B
- SETDB1
- MINA53
- KDM2a
- JMJD2C
- Histone H3.3 K27M
- Histone H3.3 G34R
Mouse Experimental therapeutics facility

The Lurie Family Imaging Center
DFCI Harbor Campus
Opened August 2009

- MRI
- Optical
- Ultrasound
- PET/CT
- Radio-chemistry
- Image-guided irradiator
- 3,300 cage
- 16,500 animals

Full spectrum of mouse cancer models:
- GEMM
- Primary xenografts
- Cell line orthografts

Full treatment options:
- Expert technical support
- Image-guided XRT

Full spectrum of endpoints:
- Anatomic and molecular imaging
- Conventional metrics
- Histopathology

Expert design and data analysis:
- Physicists
- Cancer Biologists
- Biostatistician
- Clinicians
Genetically engineered mouse models (GEMM)

- Advantages:
  - Driven by clinically relevant genetic changes
  - Tumor arise from normal organs
  - Immuno-competent mice
  - Native Vasculature

Clinical symptoms:

<table>
<thead>
<tr>
<th></th>
<th>3 weeks</th>
<th>5 weeks</th>
<th>7 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>O2 saturation:</td>
<td>99%</td>
<td>97%</td>
<td>54%</td>
</tr>
<tr>
<td>Panting:</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
MRI imaging in NSCLC GEMM studies

EGFR Δexon 19

O2 sat 49%

Carbo/Paclitaxel

O2 sat 58%

BIBW2992

O2 sat 97%

O2 sat 50%
$^{18}$F-fluorodeoxyglucose (FDG) PET imaging

Imaging treatment resistance and efficacy

EML4-ALK

Day 0

Day 1

Day 2

Baseline

Treated with an EGFR inhibitor

Switched to TAE684
Preclinical studies
T790M

• Most patients with lung cancers harboring the EGFR kinase domain mutations initially respond to gefitinib/erlotinib

• Unfortunately, most of the patients relapse with the cancers developing acquired resistance to gefitinib/erlotinib (median duration of response 10-12 months)

• Approximately half of the analyzed relapsed tumors have a secondary T790M mutation in the EGFR gene

• (another 5-15% with c-Met amplification,)
EGFR T790M-Del19 or EGFR T790M-L858R driven lung cancer

**Imaging**

- 0 weeks
- 5 weeks
- 9 weeks
- 16 weeks

**Histology**

100X

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Li et al, Cancer Cell 2007
T790M confers resistance to erlotinib

EGFR L858R: T790M

No FDG-PET response to erlotinib
Erlotinib | HKI-272 | BIBW2992
---|---|---
Control | Before treatment | After treatment
Before treatment | | 
1-wk treatment | | 

L858R | L858R/T790M | L858R/T790M
85% tumor regression | 20% tumor regression | 50% tumor regression

Targeting mutant EGFR in lung cancer

EGFR L858R:

T790M, or

EGFR Δexon19:

T790M

EGFR mutations
(e.g., Δ exon 19, L858R)

EGFR inhibitors
gefitinib (Iressa)
erlotinib (Tarceva)

Acquired resistance
(e.g., T790M mutation)

WZ4002
100-fold higher affinity for T790M mutant EGFR

Development of T790M-specific EGFR inhibitor

Nathanael Gray, Micheal Eck, Pasi Janne, Kwok-Kin Wong

- Use of a novel chemical backbone
- Mutant-selective functional pharmacological screening

PC9GR cells
Del E746_A750/T790M

NIH3T3 cells
Del E746_A750/T790M

WZ4002 inhibits EGFR T790M mutants

Baseline → Treatment day 3

EGFR Δexon19:T790M

Days of Treatment

SUVmax
Is there a difference on WT EGFR in vivo?

Evaluation of EGFR phosphorylation in hair follicle bulb

Liang Chen, Danan Li, Lucian Chirieac, Robert Padera & Kwokk Wong
Co-clinical studies
Preclinical and co-clinical trials

1) Assessment of single agents or combinations in genetically engineered mouse models (GEMMs)/xenografts of genetically distinct and “rarer” cancer types/subsets whereby sizable patient accrual represents major hurdle

2) Testing combinations *in vivo* to determine tolerability, efficacy, and PD.

3) Facilitates prioritization of most attractive combinations for assessment in man

4) Identifying mechanisms of primary and acquired resistance to agents (often even before clinical trials start)

5) Assessment of system impact of agents and combinations (e.g. tumor immune response or impact on tumor stroma and vasculature)

6) Co-clinical and preclinical identification of novel biomarkers

7) Dissect the importance of temporal sequence of genetic alterations in dictating response or resistance to treatment modalities

8) Co-Clinical assessment of novel agents while in phase I and I/II towards rapid stratification of responder and resistant populations
Phase II double-blind, randomized study of selumetinib + docetaxel vs placebo + docetaxel as second-line treatment for advanced KRAS-mutant non-small cell lung cancer (NSCLC)

Pasi A Jänne¹, Alice Shaw², José Rodrigues Pereira³, Gaëlle Jeannin⁴, Johan Vansteenkiste⁵, Carlos Barrios⁶, Fabio André Franke⁷, Lynda Grinsted⁸, Paul Smith⁸, Victoria Zazulina⁸*, Ian Smith⁸, Lucio Crinò⁹

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ClinicalTrials.gov identifier: NCT00890825. This study was sponsored by AstraZeneca
Co-clinical trials: mouse studies to mimic ongoing pivotal human clinical studies

KRAS mutant lung cancer

Taxotere

Taxotere + AZD6244

Kras K12D lung cancer

Taxotere

Taxotere + AZD6244
Co-clinical trials: mouse studies to mimic ongoing pivotal human clinical studies

Response to AZD6244 as a single agent

Kras K12D

Kras K12D : p53-/-

Kras K12D : Lkb-/-
Co-clinical trials: mouse studies to mimic ongoing pivotal human clinical studies

Response to Taxotere as a single agent

- Kras K12D
- Kras K12D : p53-/
- Kras K12D : Lkb-/-
Co-clinical trials: mouse studies to mimic ongoing pivotal human clinical studies

Kras K12D

Kras K12D : p53-/-

Kras K12D : Lkb-/-

Response to Taxotere+AZD6244

22/24

15/23

4/12

p=0.001

Tumor volume change (%)
Kras K12D

Baseline

FDG-PET response

4/4

1 dose

Taxotere+ AZD6244

Kras K12D : p53-/-

Baseline

1 dose

Taxotere+ AZD6244

Baseline

1 dose

Taxotere+ AZD6244

Kras K12D : Lkb-/-

0/4

p=0.006
Change in tumor size at 12 weeks in patients

<table>
<thead>
<tr>
<th>Difference in change in tumor size at 12 weeks, selumetinib + docetaxel vs placebo + docetaxel</th>
<th>-26.0%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>80% CI</td>
<td>-38.34, -13.7</td>
</tr>
<tr>
<td>1-sided p value</td>
<td>0.004</td>
</tr>
</tbody>
</table>

*Least squares means difference treatment comparison
Summary-patients

• First prospective study to demonstrate clinical benefit for patients with KRAS-mutant NSCLC

• Selumetinib 75 mg BID combined with docetaxel 75 mg/m² provided significant improvements in all secondary endpoints (PFS, RR, change in tumor size and APF6); numerical, but not significant, increase in OS (hazards were non-proportional)

• Tolerability findings were as expected based on monotherapy tolerability profiles of selumetinib and docetaxel
  – Toxicity was typically increased with the addition of selumetinib, but some disease-related AEs were improved

• Further investigation of selumetinib combined with docetaxel and other chemotherapies in KRAS-mutant NSCLC is required
  – Clinical activity of the combination could also be affected by dosing order¹ and concurrent tumor suppressor loss² (eg LKB1 and p53)

Summary-mice

- A successful proof of concept study demonstrating the utility of co-clinical trial and predicting human clinical trial outcome
- Loss of Lkb1 or p53 can impact on the responses of the KRAS-mutant NSCLC to treatment
- Reagents and tools to dissect mechanisms of primary response, primary resistance and acquired resistance to selumetinib and docetaxel
- Reagents and tools to perform pre-clinical studies to support and design the next human clinical trial
- Provide guidance to the ongoing analyses of the data from the human clinical trial
Lab Members
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- Oliver Mikse
- Chunxiao Xu
- Esra Akbay
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- Lew Cantley

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- Norman E. Sharpless
- William Kim

UPenn
- Anil Rustgi

Columbia University P&S
- Andrew Kung
Mechanistic studies

immuno-modulation
epigenetics
Tumor immunology for the non-immunologist

Simple

Avoiding immune destruction

Complicated

Weinberg and Lee
Patients with a wide variety of refractory cancers respond to new immunotherapeutics.

Table 2. Clinical Activity of Anti–PD-L1 Antibody in the Efficacy Population.

<table>
<thead>
<tr>
<th>Tumor Type and Dose</th>
<th>No. of Patients</th>
<th>Objective Response</th>
<th>Duration of Response</th>
<th>Stable Disease ≥24 Weeks</th>
<th>Rate of Progression-free Survival at 24 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
<td>% (95% CI)</td>
<td>% (95% CI)</td>
<td>% (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Melanoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.3 mg/kg</td>
<td>1</td>
<td>0 (0–98)</td>
<td>0 (0–98)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>18</td>
<td>6 (0–27)</td>
<td>6.9</td>
<td>0 (0–59)</td>
<td>19 (16–21)</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>17</td>
<td>29 (19–54)</td>
<td>23.5+, 22.9+, 18.2+, 4.1+, 3.5+</td>
<td>3 (0–43)</td>
<td>67 (22–72)</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>16</td>
<td>1 (0–46)</td>
<td>16.6+, 16.6, 2.3</td>
<td>5 (0–59)</td>
<td>44 (19–48)</td>
</tr>
<tr>
<td>All doses</td>
<td>52</td>
<td>17 (8–39)</td>
<td>14 (14–41)</td>
<td>42 (28–36)</td>
<td></td>
</tr>
<tr>
<td>Non–small cell lung cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All patients, 1 mg/kg</td>
<td>11</td>
<td>0 (0–29)</td>
<td>0 (0–29)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>All patients, 3 mg/kg</td>
<td>13</td>
<td>8 (0–36)</td>
<td>2.3+</td>
<td>1 (0–36)</td>
<td>14 (7–66)</td>
</tr>
<tr>
<td>Squamous subtype</td>
<td>6</td>
<td>0 (0–60)</td>
<td>NA</td>
<td>1 (0–81)</td>
<td>50 (1–59)</td>
</tr>
<tr>
<td>Non–squamous subtype</td>
<td>9</td>
<td>11 (0–48)</td>
<td>ND</td>
<td>0 (0–34)</td>
<td>65 (0–53)</td>
</tr>
<tr>
<td>All patients, 10 mg/kg</td>
<td>25</td>
<td>16 (3–36)</td>
<td>16.6+, 12.6+, 9.8, 1.5</td>
<td>5 (20–41)</td>
<td>46 (15–47)</td>
</tr>
<tr>
<td>Squamous subtype</td>
<td>8</td>
<td>13 (0–53)</td>
<td>ND</td>
<td>2 (0–65)</td>
<td>47 (10–83)</td>
</tr>
<tr>
<td>Non–squamous subtype</td>
<td>17</td>
<td>18 (4–43)</td>
<td>ND</td>
<td>3 (4–43)</td>
<td>66 (50–72)</td>
</tr>
<tr>
<td>All patients, all doses</td>
<td>49</td>
<td>10 (3–22)</td>
<td>ND</td>
<td>5 (9–25)</td>
<td>51 (17–57)</td>
</tr>
<tr>
<td>Squamous subtype</td>
<td>13</td>
<td>8 (0–36)</td>
<td>ND</td>
<td>3 (5–34)</td>
<td>43 (3–51)</td>
</tr>
<tr>
<td>Non–squamous subtype</td>
<td>36</td>
<td>11 (3–26)</td>
<td>ND</td>
<td>3 (2–23)</td>
<td>56 (10–42)</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>1</td>
<td>0 (0–98)</td>
<td>NA</td>
<td>0 (0–98)</td>
<td>NA</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>1</td>
<td>0 (0–98)</td>
<td>NA</td>
<td>0 (0–98)</td>
<td>NA</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>16</td>
<td>6 (0–30)</td>
<td>1.3+</td>
<td>3 (4–46)</td>
<td>25 (4–46)</td>
</tr>
<tr>
<td>All doses</td>
<td>17</td>
<td>6 (0–29)</td>
<td>3 (4–43)</td>
<td>22 (1–41)</td>
<td></td>
</tr>
<tr>
<td>Renal cell cancer, 10 mg/kg</td>
<td>17</td>
<td>12 (2–36)</td>
<td>11, 4</td>
<td>7 (11–67)</td>
<td>53 (19–77)</td>
</tr>
</tbody>
</table>

Brahmer et al
Basics of immune checkpoints

Ribas et al
Questions

• Are there genomic features which correlate with response to immunotherapies?
• Do somatic alterations in immunoregulatory genes impact upon the immune microenvironment?
PDL1 Expression in murine KRAS and EGFR mutant lung cancer

Correlation with PDL1 Expression

- p < .05
- p > 0.5

Mutation
- KRAS mut
- EGFR mut 2 week
- EGFR mut 4 week

Log2 Median Centered Expression
Characteristics of EGFR driven lung cancer immune infiltrate
Anti-PD1 therapy is effective in the EGFR mutant model
Effects of anti-PD1 therapy

The figure shows the effects of anti-PD1 therapy on various immune cell populations and cytokine levels.

- **CD3**, **CD8T**, **Treg**, **CD8/CD4**, and **CD8/Treg** cell counts are presented in a bar graph format, with data for Del19 and TD groups.

- **IL-6**, **TGFβ1**, and **PGRN** cytokine levels are also depicted, with similar data for Del19 and TD groups.

Significant changes are indicated by asterisks (*) on the graphs.
EGFR drives PDL1 in cell lines
EGFR inhibition decreases PDL1
Human NSCLC tumors harboring EGFR mutation express PD-L1 (9/12)
Conclusions

• Correlation between EGFR pathway activation and a immunosuppression signature highlighted by upregulation of PD-1, PD-L1, CTLA-4 and multiple tumor promoting inflammatory cytokines

• PD-1 antibody blockade improved survival of EGFR mutant mice harboring lung cancers

• Expression of mutant EGFR in bronchial epithelial cells induced PD-L1, and PD-L1 expression was reduced by EGFR inhibitors in NSCLC with activated EGFR