Heterogeneity among early-stage K-Ras driven lung adenocarcinoma predicts tumour aggressiveness and identifies novel therapeutic targets
Introduction: Ras and human cancer

Ras Mutations in Human Tumors (COSMIC, 2013)

<table>
<thead>
<tr>
<th>Tissue/Organ</th>
<th>K-Ras</th>
<th>N-Ras</th>
<th>H-Ras</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOTAL: Samples tested</td>
<td>130,500</td>
<td>56,300</td>
<td>33,500</td>
</tr>
<tr>
<td>TOTAL: Percentage</td>
<td>22%</td>
<td>6%</td>
<td>3%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>58%</td>
<td>1%</td>
<td>0%</td>
</tr>
<tr>
<td>Intestine/Colon</td>
<td>28%</td>
<td>2%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Billiary tract</td>
<td>26%</td>
<td>2%</td>
<td>1%</td>
</tr>
<tr>
<td>Lung</td>
<td>23%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>Ovary</td>
<td>12%</td>
<td>1%</td>
<td>0%</td>
</tr>
<tr>
<td>Endometrium</td>
<td>15%</td>
<td>3%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>All Others</td>
<td>0-7%</td>
<td>0-15%</td>
<td>0-12%</td>
</tr>
</tbody>
</table>
Introduction: lung cancer mutations

- No targeted therapy

- Rudin, C, et al. (2012)

- Sun, JM, et al. (2013)

- Crizotinib
- Erlotinib

P < 0.001

No mutation detected

KRAS 23%

EGFR 18%

EML4-ALK 9%

AKT1
NRAS
MEK1
MET AMP
HER 2
PIK3CA 2%
BRAF 2%

KRAS WT / EGFR +
KRAS WT / EGFR WT
KRAS + / EGFR WT

Months since diagnosis of advanced lung cancer

Overall Survival (%)

Sun, JM, et al. (2013)
Introduction: Ras activation

Mitogens

Ras-GTP

Ras-GDP

GE

PI3K

PI2

PI3K

PTEN

PDK1

translation

survival

cell motility

cell cycle progression

GAP

RAL-GDS

RAL

RHO

p190

TIAM

RAC

GRB2

SOS1/2

EXOCYST

membrane dynamics, vesicle formation

PLD

ERK

MEK

RAF

RAL

RAL-GDS

cell cycle progression

GE

PI2
Targeting MAPK signalling

RTK

Mitogens

K-Ras

N-Ras

Genetic analysis of Ras signalling pathways in cell proliferation, migration and survival

Cell cycle progression
Targeting MAPK signalling

RTK

Mitogens

K-Ras
H-Ras
N-Ras

A-RAF
B-RAF
C-RAF

MEK1
MEK2
ERK1
ERK2

Survival (%)

P30 mice

Mek1Δ/Δ;Mek2+/+
Mek1Δ/Δ;Mek2−/−

4OHT Diet (days)

K-Ras
H-Ras
N-Ras

Mek1+/+;Mek2+/+
Mek1ΔΔ;Mek2−/−

Small Intestine

Colon
Targeting MAPK signalling

A-RAF → B-RAF → C-RAF → MEK1 → MEK2 → ERK1 → ERK2

K-Ras^{G12V}
Targeting MAPK signalling

RTK

Mitogens

K-Ras$^{G12V}$

A-RAF

B-RAF

C-RAF

MEK1

MEK2

ERK1

ERK2

?
Targeting MAPK signalling

RTK → Mitogens → K-Ras\(^{G12V}\) → A-RAF → B-RAF → C-RAF → MEK1 → MEK2 → ERK1 → ERK2

?
Targeting the cell cycle

+ 4OHT

- 4OHT

+ 4OHT

Ther. target
**Target validation: Raf kinases**

C-Raf, but not B-Raf or A-Raf, is essential for initiation of K-Ras\(^{G12V}\) oncogene-driven lung adenocarcinomas, yet dispensable for adult homeostasis.
MAPK: Therapeutic approach

**Diagram:**

- Flp
- ATG
- V12
- TGA
- 3'UTR
- FSF
- RNAPol II
- CreERT
- pA
MAPK: Therapeutic approach

RNAPol II | CreERT | pA

CreERT | 4-OHT

4-OHT

3’UTR

V12

ATG

TGA

Ther. target
K-Ras¹/²FSFG12V

Raf²lox/²lox
PolII²CreERT2

Ad-Flpe infection

K-Ras¹G12V

PET/CT+ tumors

Systemic target ablation

NR
PR
CR

MAPK: Therapeutic approach
**MAPK: Therapeutic approach**

<table>
<thead>
<tr>
<th>TMX treatment (Months)</th>
<th>0</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B-Raf</strong>&lt;sup&gt;+/+&lt;/sup&gt;; <strong>C-Raf</strong>&lt;sup&gt;+/-&lt;/sup&gt;</td>
<td><img src="image1.png" alt="Images of tumors" /></td>
<td><img src="image2.png" alt="Images of tumors" /></td>
</tr>
<tr>
<td><strong>B-Raf</strong>&lt;sup&gt;+/+&lt;/sup&gt;; <strong>C-Raf</strong>&lt;sup&gt;lox/lox&lt;/sup&gt;</td>
<td><img src="image3.png" alt="Images of tumors" /></td>
<td><img src="image4.png" alt="Images of tumors" /></td>
</tr>
<tr>
<td><strong>B-Raf</strong>&lt;sup&gt;lox/lox&lt;/sup&gt;; <strong>C-Raf</strong>&lt;sup&gt;lox/lox&lt;/sup&gt;</td>
<td><img src="image5.png" alt="Images of tumors" /></td>
<td><img src="image6.png" alt="Images of tumors" /></td>
</tr>
</tbody>
</table>

**Fold change of SUV Max**

- **B-Raf**<sup>+/+</sup>; **C-Raf**<sup>+/-</sup>: p<0.0001
- **B-Raf**<sup>+/+</sup>; **C-Raf**<sup>lox/lox</sup>: p<0.0001

**Fold change of the number of tumors uptaking FDG**

- **B-Raf**<sup>+/+</sup>; **C-Raf**<sup>+/-</sup>: p=0.002
- **B-Raf**<sup>+/+</sup>; **C-Raf**<sup>lox/lox</sup>: p=0.002

**Fold change of tumor volume**

- **B-Raf**<sup>+/+</sup>; **C-Raf**<sup>+/-</sup>: p<0.0001
- **B-Raf**<sup>+/+</sup>; **C-Raf**<sup>lox/lox</sup>: p<0.0001
MAPK: Therapeutic Approach

**K-Ras^{G12V}**

- **C-RAF**
  - **MEK1**
  - **MEK2**
  - **ERK1**
  - **ERK2**

**Apoptosis**
**Differentiation**
**Proliferation**

**B-Raf^{+/+};C-Raf^{+/+}**
**B-Raf^{+/+};C-Raf^{lox/lox}**
**B-Raf^{lox/lox};C-Raf^{lox/lox}**

**Ki67^{+} cells (%)**

**pCofilin^{+} cells (%)**

**Active Casp 3^{+} cells (%)**

- *p* < 0.0001
- *p* = 0.0002
- *p* < 0.0001
MAPK: Genetic modelling of kinase inhibition

K (ATP Binding Site)

D (DFG): proton acceptor

D (HRD)

MAPK: Genetic modelling of kinase inhibition

Cre Recombinase

loxP

KD

loxp

Sa

cDNA

P

P

P

Mg^{2+}

D (DFG): proton acceptor

c-Raf D468A

KD

Sa

cDNA

P

P

P

Mg^{2+}

D (DFG): proton acceptor

c-Raf D468A
MAPK: Genetic modelling of kinase inhibition

K-Ras\(^{G12V}\) induction

K-Ras\(^{+/FSFG12V}\)
C-Raf LmLD468A
CreERT2

PET/CT+
tumors

+4OHT
(active Cre)

Systemic
C-RafD468A
induction

Ad-Flpe
infection

?
MAPK: Genetic modelling of kinase inhibition

8 weeks Tamoxifen diet

*C-Raf* LmLD468A/LmLD468A; *Tg.hUBC-CreERT2* +/T show no phenotype after 6 months of Tamoxifen diet.
Overall MAPK signalling is essential for mouse homeostasis and individual mediators are functionally redundant; their function is specialized in K-Ras$^{G12V}$-driven NSCLC.

C-Raf is a valuable therapeutic target for K-Ras$^{G12V}$-driven NSCLC treatment with potentially low systemic toxicity.

Inhibition of C-Raf catalytic activity induces regression of advanced K-Ras$^{G12V}$-driven NSCLC.
Combination therapy: choosing novel targets

Hanahan & Weinberg, Cell 144 (2011)
Combination therapy: choosing novel targets

Assessing therapeutic responses in **Kras** mutant cancers using genetically engineered mouse models

**Experimental Therapeutics, Molecular Targets, and Chemical Biology**

Dual Phosphoinositide 3-Kinase/Mammalian Target of Rapamycin Blockade Is an Effective Radiosensitizing Strategy for the Treatment of Non-Small Cell Lung Cancer Harboring **K-RAS** Mutations

Suppression of non-small cell lung tumor development by the *let-7* microRNA family

**Cancer Research**

The Differential Effects of Mutant **p53** Alleles on Advanced Murine Lung Cancer

Mutations in **BRAF** and **KRAS** Converge on Activation of the Mitogen-Activated Protein Kinase Pathway in Lung Cancer

Requirement for NF-κB signalling in a mouse model of lung adenocarcinoma

Chronic cisplatin treatment promotes enhanced damage repair and tumor progression in a mouse model of lung cancer

Systemic Delivery of Tumor Suppressor microRNA Mimics Using a Neutral Lipid Emulsion Inhibits Lung Tumors in Mice

**A Synthetic Lethal Interaction between K-Ras Oncogenes and Cdk4 Unveils a Therapeutic Strategy for Non-small Cell Lung Carcinoma**

Inhibition of Mammalian Target of Rapamycin Reverses Alveolar Epithelial Neoplasia Induced by Oncogenic K-ras

**c-Raf, but Not B-Raf, Is Essential for Development of K-Ras Oncogene-Driven Non-Small Cell Lung Carcinoma**
Study EARLY lesions to:

- identify the nature of K-Ras-dependent essential mediators in NSCLC
- discover new druggable targets for K-Ras-driven NSCLC treatment
Early stages in NSCLC progression

K-Ras$^{G12V}$ early NSCLC lesions

(BIDMC, Boston)

**Articles**

An oncogenic KRAS2 expression signature identified by cross-species gene-expression analysis

Alejandro Sweet-Cordero¹, Sayan Mukherjee²,³, Aravind Subramanian¹, Han You¹, Jeffrey J Roix¹, Christine Ladd-Acosta², Jill Mesirov¹, Todd R Golub²,³ & Tyler Jacks¹,⁵

$\text{p}<0.0001$
**Ddr1 as a novel therapeutic target**

- DDR1 is up-regulated in NSCLC compared to normal lung tissue, and in metastasis compared to primary tumors

- DDR1 is the most abundantly phosphorylated protein in NSCLC

- DDR1 high expression correlates with poor survival in NSCLC

- DDR1 is DRUGGABLE

- Specific DDR1 inhibitors

Rikova, K et al. (2007)  
Valencia, K et al. (2012)
**Ddr1 as a novel therapeutic target: genetic validation**

**Diagram:**
- **K-Ras^{+/LSL G12V}** and **Ddr1^{-/-}**

**Survival (%)**
- Survival curves for:
  - Ddr1^{+/-}
  - Ddr1^{+/-}
  - Ddr1^{-/-}
- p<0.0001

**Graph:**
- **Months**
- **Survival (%)**
- Comparison of survival rates for different genotypes.

**IHC: TTF1**
- **K-Ras^{G12V};Ddr1^{+/-}** vs. **K-Ras^{G12V};Ddr1^{-/-}**

**Absolute tumor number/lung**
- Comparison of tumor burden for:
  - Ddr1^{+/-}
  - Ddr1^{+/-}
  - Ddr1^{-/-}
- * indicates significant difference (p<0.05).
Ddr1 as a novel therapeutic target: pharmacologic validation

* 7rh inhibitor treatment, 50mg/kg, daily oral administration
DRUG-INSENSITIVE TUMORS

IHC: Ddr1

IHC: KI67

IHC: C3A

DRUG-SENSITIVE TUMORS

*Dr.1 as a novel therapeutic target: pharmacologic validation*
Ddr1 as a novel therapeutic target: in search of a mechanism

Adapted from Borza et al, Matrix Biology (2013)
Ddr1 as a novel therapeutic target: in search of a mechanism

IHC: C3A

2-weeks treatment

% C3A positive cells/tumor

VEHICLE

7rh

CHEMO

7rh+ CHEMO

VEHICLE

7rh

CHEMO

7rh+ CHEMO

**
Drr1 as a novel therapeutic target: in search of a mechanism

**DDR1 Receptor Tyrosine Kinase Promotes Prosurvival Pathway through Notch1 Activation**

Hyung-Gu Kim1, So-Young Hwang1, Stuart A. Aaronson6, Anna Mandinova6, and Sam W. Lee1*1

From the 1Cutaneous Biology Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129 and the 6Department of Oncological Sciences, Mount Sinai School of Medicine, New York, New York 10029
Ddr1 as a novel therapeutic target: in search of a mechanism

K-Ras^G12V;Ddr1^{+/+}

TUMOR

BRONCHIOLES

IHC: Hes1

VEHICLE

CHEMO (2 weeks)

7th (2 weeks)

CHEMO + 7th (2 weeks)

7th (2 months)

% Hes1 positive cells/tumor

- VEHICLE
- CHEMO (2 weeks)
- 7th (2 weeks)
- CHEMO + 7th (2 weeks)
- 7th (2 months)

***
Ddr1 as a novel therapeutic target: in search of a mechanism

Ddr1 as a novel therapeutic target: in search of a mechanism

[Graph and images showing experiment results]

- **% C3A positive cells/tumor**
  - Groups: Vehicle, Chem, Ly-411575, Th+, Chemo, Th+Ly-411575
  - Bars represent mean ± SEM
  - Significance: *p < 0.05, **p < 0.01, ***p < 0.001

- **Average tumor area (mm²)**
  - Groups: Vehicle, Chem, Ly-411575, Th+Ly-411575, Th+LY-411575 + Chemo
  - Bars represent mean ± SEM

- **IHC: C3A**
  - Treatment groups: Vehicle, Chem, Ly-411575, Th+Ly-411575

- **IHC: Hes1**
  - Treatment groups: Vehicle, Chem, Ly-411575, Th+Ly-411575

- **Western Blot:**
  - Proteins: Hes1, Gapdh
**Ddr1 as a novel therapeutic target in aggressive NSCLC**

**GENETIC DELETION OF Ddr1**

- Increase in overall survival
- Decrease in tumor number
- Decrease in tumor size
- Effect in tumor grading

<table>
<thead>
<tr>
<th>$p53^{+/+}$</th>
<th>$p53^{loxp/loxp}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔️</td>
<td>✗</td>
</tr>
<tr>
<td>✔️</td>
<td>✗</td>
</tr>
<tr>
<td>✔️</td>
<td>✗</td>
</tr>
<tr>
<td>✔️</td>
<td>✗</td>
</tr>
</tbody>
</table>

**PHARMACOLOGICAL INHIBITION OF Ddr1**

- Efficacy of 7rh as single agent
- Efficacy of 7rh in combination with chemotherapy
- Efficacy of 7rh in combination with LY-411575

<table>
<thead>
<tr>
<th>$p53^{+/+}$</th>
<th>$p53^{loxp/loxp}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔️</td>
<td>✗</td>
</tr>
<tr>
<td>✔️</td>
<td>✗</td>
</tr>
<tr>
<td>✔️</td>
<td>✔️</td>
</tr>
</tbody>
</table>
Ddr1 as a novel therapeutic target in aggressive NSCLC

**% C3A positive cells/tumor**

<table>
<thead>
<tr>
<th></th>
<th>VEHICLE</th>
<th>7th</th>
<th>CHEMO</th>
<th>LY-411575</th>
<th>7th+CHEMO</th>
<th>LY-411575</th>
<th>CHEMO+LY-411575</th>
<th>7th+LY-411575+CHEMO</th>
</tr>
</thead>
<tbody>
<tr>
<td>% C3A</td>
<td>0</td>
<td>0.5</td>
<td>10</td>
<td>20</td>
<td>35</td>
<td>30</td>
<td>35</td>
<td>40</td>
</tr>
</tbody>
</table>

**IHC: C3A**

**IHC: Hes1**

**Hes1**

**Gapdh**

*VEHICLE* | *LY-411575* | *CHEMO* | *7th+CHEMO* | *LY-411575* | *CHEMO+LY-411575* | *7th+LY-411575+CHEMO*
Is this applicable to human NSCLC?

<table>
<thead>
<tr>
<th>Gene</th>
<th>K-RAS</th>
<th>DDR1</th>
<th>HES1</th>
</tr>
</thead>
<tbody>
<tr>
<td>K-RAS</td>
<td>- -</td>
<td>0.011*</td>
<td>0.025*</td>
</tr>
<tr>
<td>DDR1</td>
<td>- -</td>
<td>- -</td>
<td>0.012**</td>
</tr>
<tr>
<td>HES1</td>
<td>- -</td>
<td>- -</td>
<td>- -</td>
</tr>
</tbody>
</table>

n = 554 patients.  
* Tendency towards co-occurrence (2 < Odds ratio < 10)  
** Strong tendency towards co-occurrence (Odds ratio > 10)
Upon K-Ras\textsuperscript{G12V} expression in lung, there are at least two different types of early lesions based on gene expression profiling (T1 and T2).

T1 signature is closer to normal lung epithelial cells and could help to identify new tumor suppressor genes. T2 overlaps with advanced murine and human NSCLC signature, thus suggesting that the aggressiveness of lesions could be determined early during tumor onset.

By an unbiased approach we identified Ddr1 as a valuable therapeutic target for K-Ras mutated NSCLC.

Genetic ablation or pharmacological inhibition of Ddr1 impairs tumor growth in p53-proficient K-Ras\textsuperscript{G12V} NSCLC.

Combined inhibition of Ddr1 and Notch signaling efficiently triggers an apoptotic response even in p53-deficient K-Ras\textsuperscript{G12V} NSCLC.
Take home message 1

- There are >800 drugs targeting signaling pathways; finding the right drug (or combination of) for personalized treatment is challenging.

- Tumor evolution, heterogeneity and resistance.
Take home message 1

Human NSCLC

Cancer cell line encyclopedia

K-RAS
Take home message 1

Kreso & Dick, Cell Stem Cell 2014

Swanton, Cancer Res 2012
Take home message 2

- DDR1/NOTCH inhibitors

- No targeted therapy

- Crizotinib

- Erlotinib

- No mutation detected

- KRAS 23%

- EGFR 18%

- EML4-ALK 9%

- AKT1

- NRAS

- MEK1/MEKAMP

- HER2

- PIK3CA 2%

- BRAF 2%
Experimental Oncology Group:
Mariano Barbacid
Chiara Ambrogio
Rafael Blasco
Emilie Bousquet
Sarah Francoz
Patricia Nieto

Collaborators:
Ke Ding (GIBG, Guangzhou)
Pierre Dubus (Univ. Bordeaux)
Montserrat Sánchez-Céspedes (Idibell, Barcelona)
Manuel Serrano (CNIO)
Gonzalo Gómez (CNIO)
An elaborate pathway required for Ras-mediated epigenetic silencing

Claude Gazin¹, Narendra Wajapayeey¹, Stephane Gobeli¹, Ching-Man Virbasius¹ & Michael R. Green¹

SampleName

TMR2  TPM2  tropomysin 2 (beta)
CNOT1  CNOT1  DNA (cytosine-5')-methyltransferase 1
ARSA  ARSA  human RNA-directed DNA polymerase (pol) b, RNA component 1
CCTD  CCTD  embryonic reprogramming development
GADD  GADD  x-phosphoinositide dependent protein kinase-1
FZD9  FZD9  FZD9, frizzled family member 9
MARK1  MARK1  MARK kinases, MAPKactivated kinase family member 1
TFF1  TFF1  trefoil factor family member 1
HESR1  HESR1  HESR1 anti-silencing function 1 homolog A (Drosophila)
ADIP1  ADIP1  ADIP anti-silencing function 1 homolog A (Drosophila)
TRPM1  TRPM1  trefoil factor family member 1 (alpha)
RAS  RAS  Ras (GTPase superfamily, member 6)
CTCF  CTCF  CTCF-binding factor (zinc finger protein)
MAK  MAK  MAP kinase 1
DPP10  DPP10  DPP10-like, histone H1 methyltransferase (Drosophila)
RAYN  RAYN  RAYN, RAYN kinase
MAPK1  MAPK1  mitogen-activated protein kinase 1
GRB7  GRB7  Grb7 protein binding tail type information regulation 2 homolog A (Drosophila)
ESR1  ESR1  ESR2 transcription factor 1
LYVE1  LYVE1  secreted frizzled-related protein 1
EGR1  EGR1  Egr1, EGR2,EGR3 signal-regulated kinase 7
KCNQ1  KCNQ1  KCNQ1 trigonum-activated proteins kinase kinase 1
HDAC9  HDAC9  histone deacetylase 9
TRIM37  TRIM37  tripartite motif-containing 37
GLO  GLO  lysoxidase
PLAG1L  PLAG1L  pleomorphic adenoma gene-like 1
PTK2B  PTK2B  PTK2B protein tyrosine kinase 2 beta
VRK1  VRK1  VRK1, VRK2 signal-induced proliferation-associated 1 like 2
SOX14  SOX14  SOX14 (sex determining region X)-box 14
TOP3A  TOP3A  transforming growth factor, beta receptor II (70/80kDa)
CDK7  CDK7  cyclin-dependent kinase 7A (Birchmeier, p15, inhibits CDK4)
SMAD1  SMAD1  SMAD1 and SMAD4 domain containing 1
CCDC6  CCDC6  CCDC6 zinc fingers, coiled coil domain containing 1
PML  PML  PML protein

Combination therapy: validating novel targets (II)
Combination therapy: validating novel targets (II)

IHC: SERPINB5

TSS

CpG

Tumor #1

Tumor #2

Tumor #3

Tumor #4

Tumor #5

Tumor #6

IHC: SERPINB5
Combination therapy: validating novel targets (II)

5-Aza
Entinostat: reversible

8w K-Ras\(^{G12V}\) 3 m

Placebo

5-Aza + Ent.

% Survival

Placebo
5-Aza + Ent.

Age (months)

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

% Survival

Placebo
5-Aza + Ent.