Decrease in p-PRAS40 plays a role in the synergy between erlotinib and crizotinib in an *EGFR* and *cMET* wild-type squamous NSCLC cell line

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Background

EGF

HGF

EGFR

cMET

PI3K

AKT

mTORc1

Deptor

Raptor

mTOR

mLST8

PRAS40

Proliferation

Survival

Migration
**EGFRamp**

**EGFRmut**

**METamp**

**METex14**

**Background**

**EGFR-TKI**

**MET-TKI**

**RAC1**

**PAC**

**Migration**

**Proliferation**

**Survival**

**PI3K**

**AKT**

**mTOR**

**mTORc1**

**Deptor**

**Raptor**

**mLST8**

**PRAS40**

**Background**
Rationale

- EGFR is overexpressed in squamous NSCLC (∼80%)\textsuperscript{1}
- EGFR-TKIs granted a modest benefit over placebo in unselected squamous NSCLC\textsuperscript{2-4}
- cMET activation is a common resistance mechanism to EGFR-TKIs\textsuperscript{5}

\textbf{Erlotinib + Crizotinib in squamous NSCLC cell lines}

\textsuperscript{1}Hirsch, J Clin Oncol 2003; \textsuperscript{2}Wojtowicz-Praga, Ann Oncol 2012; \textsuperscript{3}Ameratunga, Asia Pac J Clin Oncol 2014; \textsuperscript{4}Soria, Lancet Oncol 2015; \textsuperscript{5}Van Der Steen, Cancer Drug Resist 2018
Erlotinib combined with crizotinib showed strong synergy in LUDLU cell line

Erlotinib + Crizotinib
(72h)
Erlotinib combined with crizotinib reduced LUDLU migration

** = p<0.01
*** = p<0.001
Erlotinib combined with crizotinib induced G2/M arrest and apoptosis

Cell cycle distribution LUDLU (24h)

Apoptosis LUDLU (48h)

* = p<0.05
** = p<0.01
*** = p<0.001
Erlotinib combined with crizotinib reduced the area of spheroids

LUDLU

Control

Erlotinib (10 µM)

Crizotinib (5 µM)

Combination

Day 3

Day 5

Day 7

LUDLU Spheroid assay (Day 7)

Area compared to control (%)

Control

Erlotinib 10 µM

Crizotinib 5 µM

Combination

Treatment

* = p<0.05

* = p<0.05

* = p<0.05
Erlotinib combined with crizotinib decreased the phosphorylation of PI3K/AKT/mTOR pathway proteins.

* = p<0.05  
** = p<0.01
HER3: a link between RAS/MAPK and PI3K/AKT/mTOR pathways

30% of squamous NSCLC overexpress HER3

Engelman, Science 2007; Arteaga, Nat Med 2007; Mishra, Oncol Rev 2018
HER3: a link between RAS/MAPK and PI3K/AKT/mTOR pathways

HER3: a link between RAS/MAPK and PI3K/AKT/mTOR pathways

Engelman, Science 2007; Arteaga, Nat Med 2007; Mishra, Oncol Rev 2018
p-HER3 and p-PRAS40 expression decreased in LUDLU following the combination treatment

**H1703: antagonistic**

**SKMES-1: additive**

**LUDLU: synergistic**

- **p-EGFR**
- **p-cMET**
- **Her3**
- **p-Her3**
- **p-PRAS40**
- **β-actin**
p-HER3 and p-PRAS40 are differently expressed in SCC and ADC

**Squamous cell carcinoma (SCC)**

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<td>SCC</td>
<td>(n=35)</td>
<td>p=ns</td>
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<tr>
<td>ADC</td>
<td>(n=29)</td>
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**Adenocarcinoma (ADC)**

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<td>p=0.0353</td>
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<tr>
<td>ADC</td>
<td>(n=29)</td>
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Conclusions

- Erlotinib combined with crizotinib showed remarkable synergy in the LUDLU squamous NSCLC cell line (EGFRwt, cMETwt)

- p-Her3 and p-PRAS40 have a role in the synergistic effect of erlotinib and crizotinib in LUDLU

- p-Her3 and p-PRAS40 might be used as biomarkers for selecting potential candidates for the combination treatment