Rationale for co-targeting IGF-1R and ALK in ALK-fusion-positive lung cancer
by Christine M Lovly et al.

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By Christoph Glogner
What is ALK?

- Member of the receptor tyrosine kinase family
  => part of the signal-transduction-cascade
- Located in the p arm of chromosome 2
- Potential to be fused with other proteins after translocations or inversions
  → NPM-ALK t(2:5)
  → EML4-ALK
- Target of Crizotinib
- Target of LDK-378
What about this ALK in lung-cancer?

- In about 4% of NSCLC
- Fusionprotein: EML4-ALK (Inversion of Chromosome 2)
- Mainly in NSCLC of young non-smokers
EML4?

- Echinoderm microtubule associated protein like 4
- Located on the p arm of Chromosome 2
- Strongly expressed in mitosis
- Stabilizes microtubules
Crizotinib

- Aminopyridin
- Inhibitor of ALK
- Oral Therapy of EML4-ALK positive NSCLC
- 7377.56€/month
And IGF-1R?

- Insulin-like Growth Factor Receptor 1
- Potent Anti-apoptotic agent
- Involved in growth, development and differentiation
- Target of MAB291
- LDK-378
How IGF-1R works

IGF-1R binds IGF-1, activating PI3K and AKT, leading to protein synthesis and growth. PTEN inhibits PI3K. AKT can also be activated by mTORC2, PI3K, and GSK-3β. AKT regulates cell growth and metabolism through BCL-2 and BAD. The network involves additional phosphorylation sites and interactions with other proteins like mTORC1 and S6K1, which are crucial for cellular proliferation.
Results
IGF-R1-Inhibition sensitizes EML4-ALK positive (H3122) cells for crizotinib

Growth inhibition of H3122 measured by Soft-Agar-Assay
In H3122 cells
In H2228 cells
STE-1 cells
Assessed by cell Titer Blue Assays
Combinating IGF-1R and ALK Inhibitors in other cancer cells

Effect on SUDHL-1 lymphoma cells (NPM-ALK fusion) measured by cell titer blue assay
Combination and Apoptosis

A poptotic cells measured by propidium iodide stain

![Graph showing fold change in sub-2N DNA content compared to control. The graph has three bars labeled Crizotinib, OSI-906, and Crizotinib + OSI-906. The Crizotinib bar is green, the OSI-906 bar is gray, and the Crizotinib + OSI-906 bar is blue. The y-axis represents fold change, ranging from 0 to 5.0.]
Effects on Downstream-Targets

Immunoblot
Not every Tyrosine Kinase Inhibitor is that capable

Cell titer blue assays of H3122 cell
IGF-1 induces Crizotinib-resistance

Cell titer blue assay of H3122 cells
It's not what it looks like...

Immunoblot of lysates of H3122 cells

There is no direct Cross-talk between IGF-1R and ALK !!
It is suggested that the activation of the IGF-R1 pathway is a compensatory mechanism, when growth is inhibited by ALK-Inhibitors.
Interaction Between IRS-1 and ALK

Immunoprecipitation and Western blot of lysates from H3122 cells (in vitro)

Immunoprecipitation and Western blot of lysates from lung tumor tissue of transgenic mice (EML4-ALK; in vivo)
IRS-1 Knockdown

Immunoblot of STE-1 cells treated with IRS-1 siRNA and controls
IRS-1 Knockdown

Coulter Counter

Quantification of STE-1 cells
Suggestion:
IRS-1 links ALK as well as IGF-R1 to downstream signaling pathways
Amplification of EML4-ALK Fusion in H3122-cr cells

Immunoblot of cell lysates

FISH of H3122-cr cells
No EML4-ALK Fusion Amplification in H3122-xr cells

FISH of H3122-xr cells (resistant against X-376)
But downstream phosphorylation remains

Immunoblot from H3122-xr and H3122 parental cell lysates
Suggestion:
IGF-1R – IRS-1 pathway has a role in maintaining downstream signaling while ALK inhibition
More IGF-1R in H3122-xr cells

Phospho receptor tyrosine kinase array
Suggestion:
The IGF-1R IRS-1 pathway is a mechanism by which cells evade ALK-Blockade
IGF-1R inhibition restores ALK-TKI-sensitivity

Soft agar assay of H3122-xr cells

Cell titer blue assay of H3122-xr cells
Combination of ALK- and IGF-1R Inhibition

Immunoblot of H3122-xr cell lysates
Effects of IRS-1 Knockdown

Western-Blot

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In vivo Upregulation of IGF-1R after ALK-TKI treatment

Immunohistochemical analysis of pIGF-R1 before and after Crizotinib-therapy
Also IRS-1 is upregulated in Crizotinib-resistant lung cancer.

Immunohistochemical analysis of IRS-1 before and after Crizotinib-therapy.
Cell titer blue analysis of H3122 cells

Measurement of tumor volumes in mice
What is LDK-378 able to?

Immunoblot of H3122 celllysates

Immunoblot of H2228 celllysates
LDK-378 is more efficient than Crizotinib due to its ability to inhibit both ALK and IGF-R1.
Summary

- Therapeutic synergism between ALK- and IGF-R-Inhibitors in ALK-TKI-sensitive as well as in ALK-TKI-resistant cells
- IRS-1 is involved in the ALK pathway
- IGF-1R activation seems to compensate effects of ALK-Inhibition
- So Co-Targeting ALK and IGF-1R in ALK positive NSCLC seems to be a promising therapeutic way
Finally Done!
References

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