

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

Journal Club Presentation 01.12.14

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

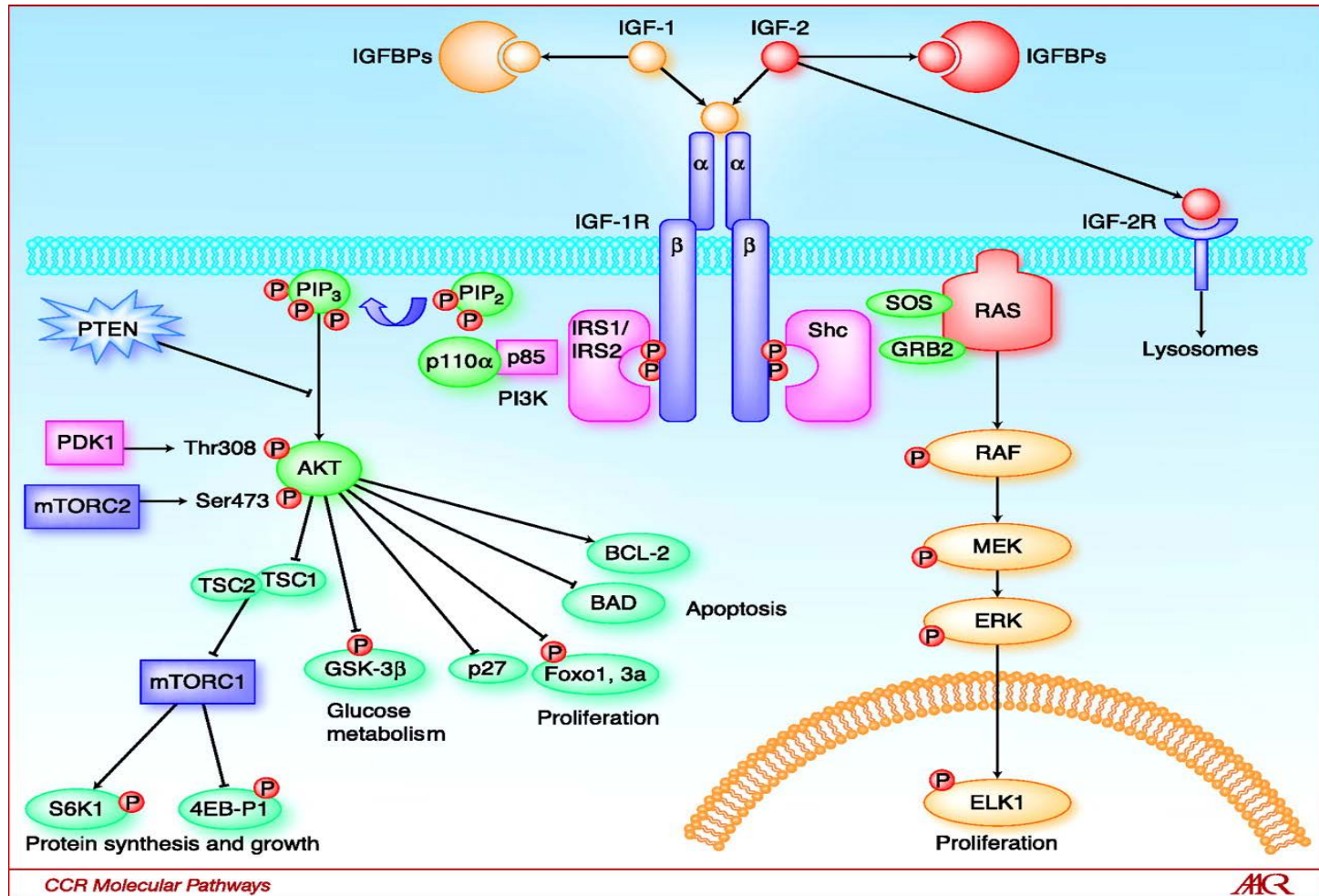
Crizotinib

- 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

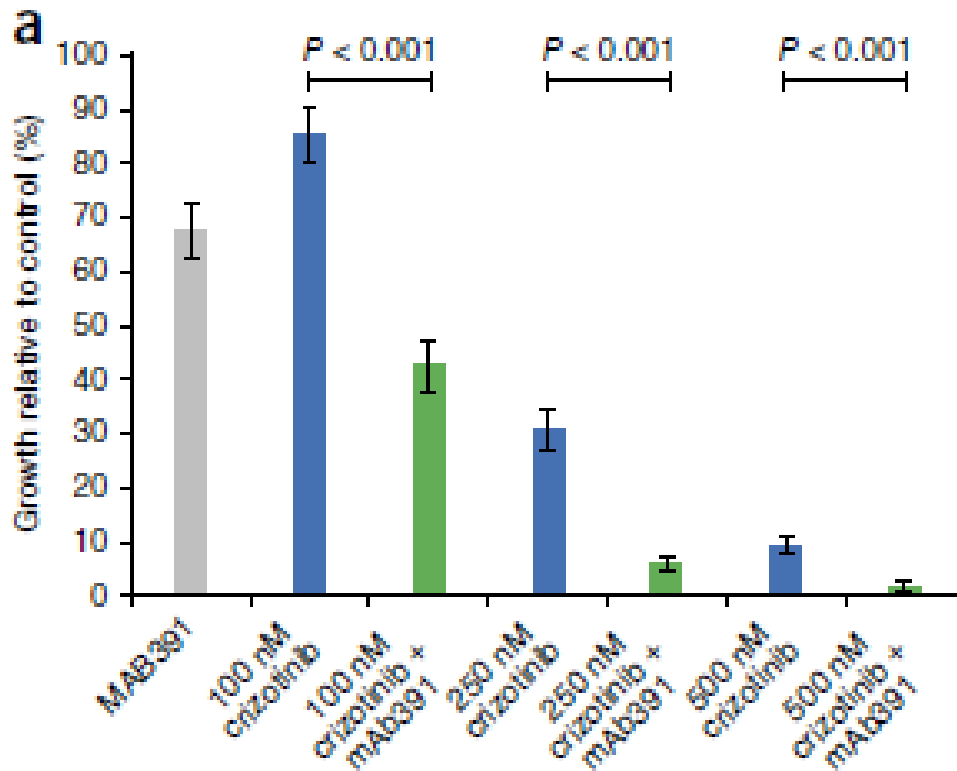
How IGF-1R works



Results

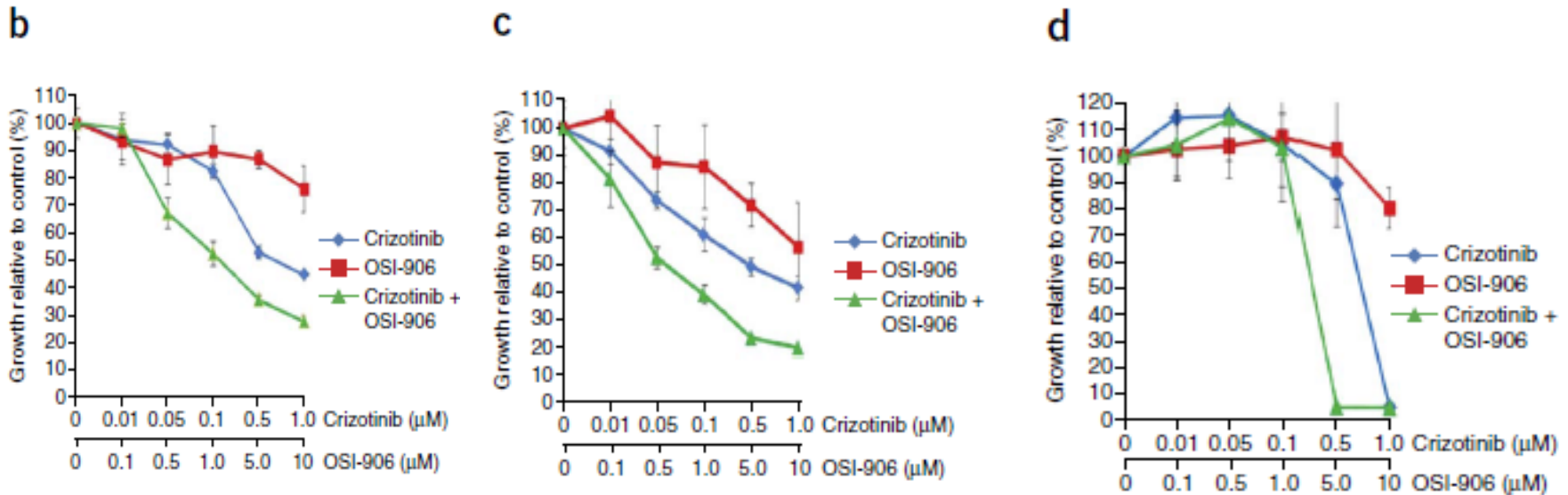
IGF-R1-Inhibition sensitizes

EML4-ALK positive (H3122) cells for crizotinib



Growthinhibition of
H3122 measured by
Soft-Agar-Assay

Combining ALK and IGF-R1 Inhibitors



In H3122 cells

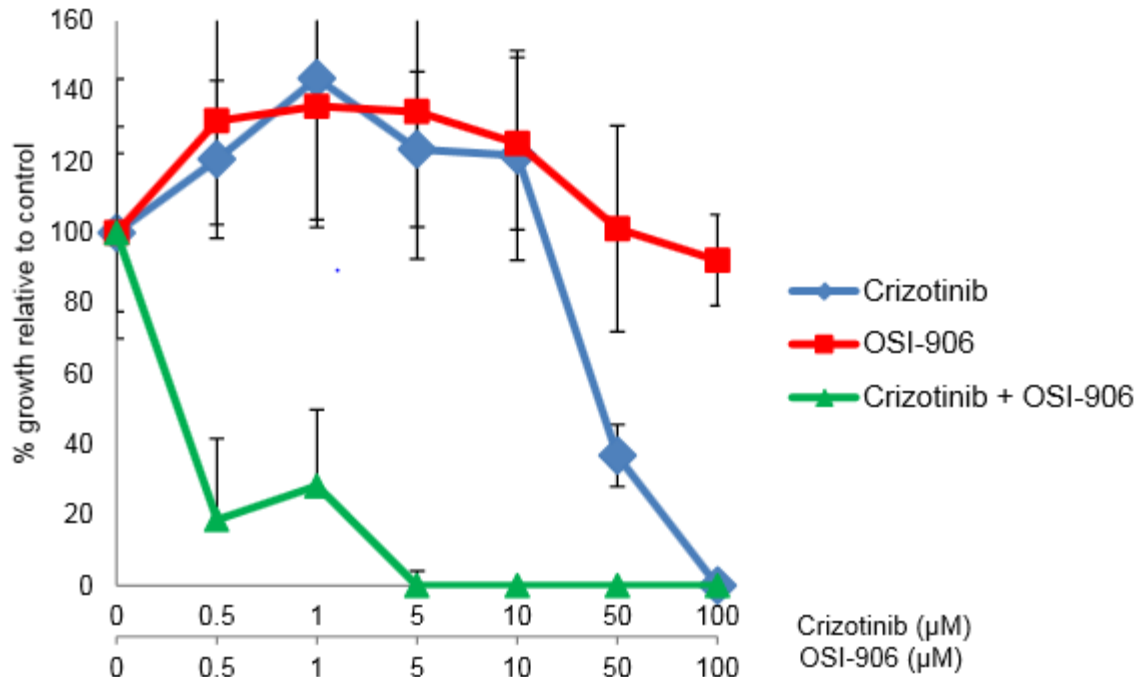
In H2228 cells

STE-1 cells

Assessed by cell Titer Blue Assays

Combining IGF-1R and ALK

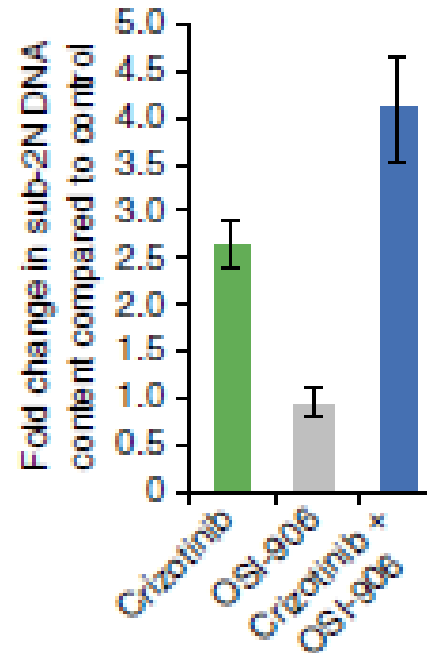
Inhibitors in other cancer cells



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

Combination and Apoptosis

Apoptotic cells
measured by
propidium iodide
stain

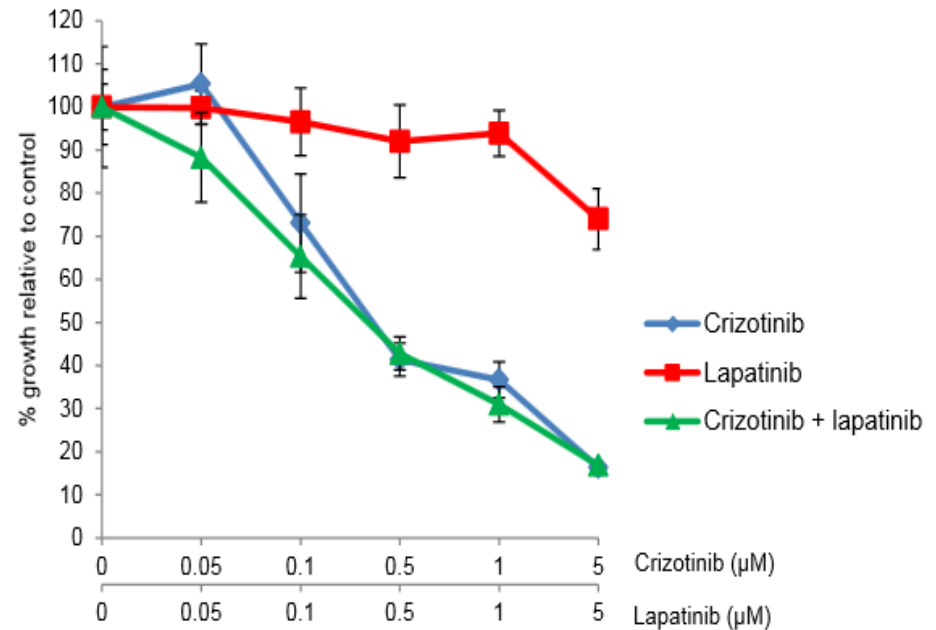
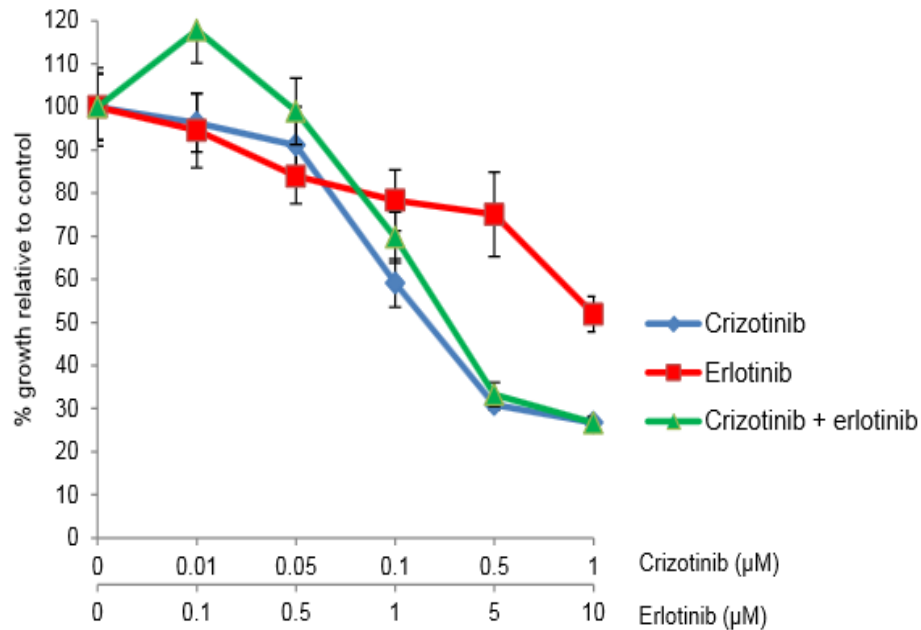


Effects on Downstream-Targets



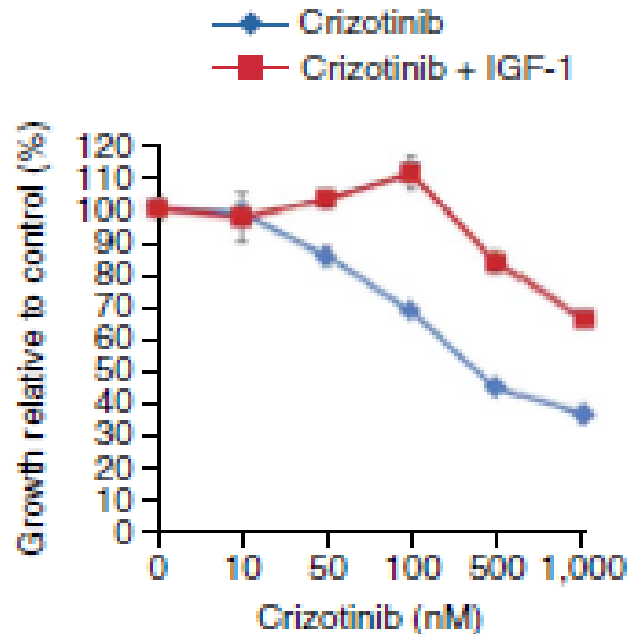
Immunoblot

Not every Tyrosine Kinase Inhibitor is that capable



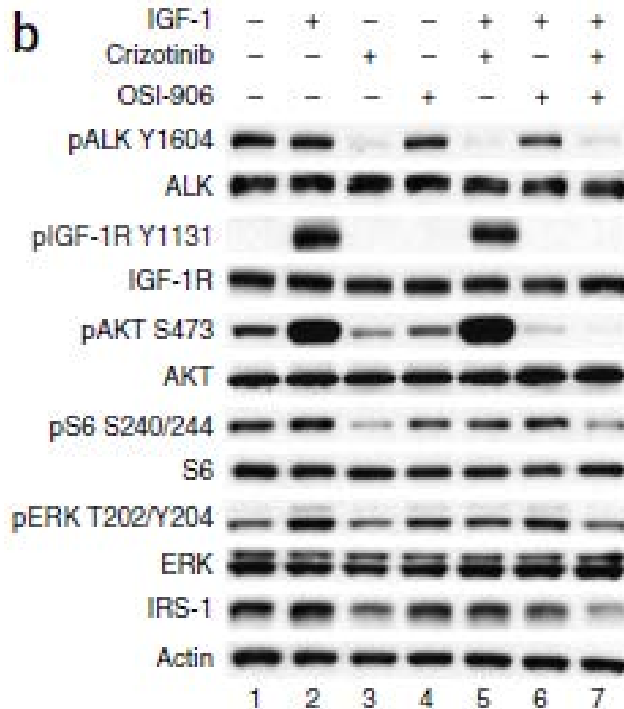
Cell titer blue assays of H3122 cell

IGF-1 induces Crizotinib-resistance



Cell titer blue assay of H3122 cells

It's not what it looks like...



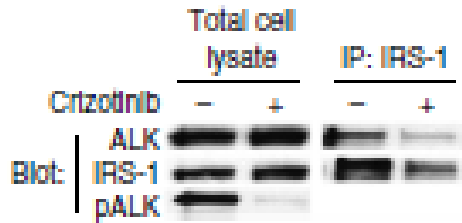
Immunoblot of lysates of H3122 cells

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

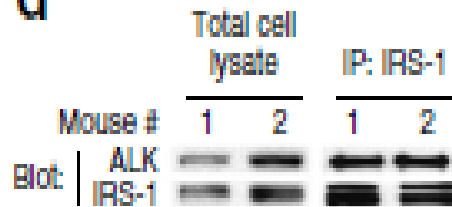
Interaction Between IRS-1 and ALK

c



Immunoprecipitation and Westernblot of lysates from H3122 cells (in vitro)

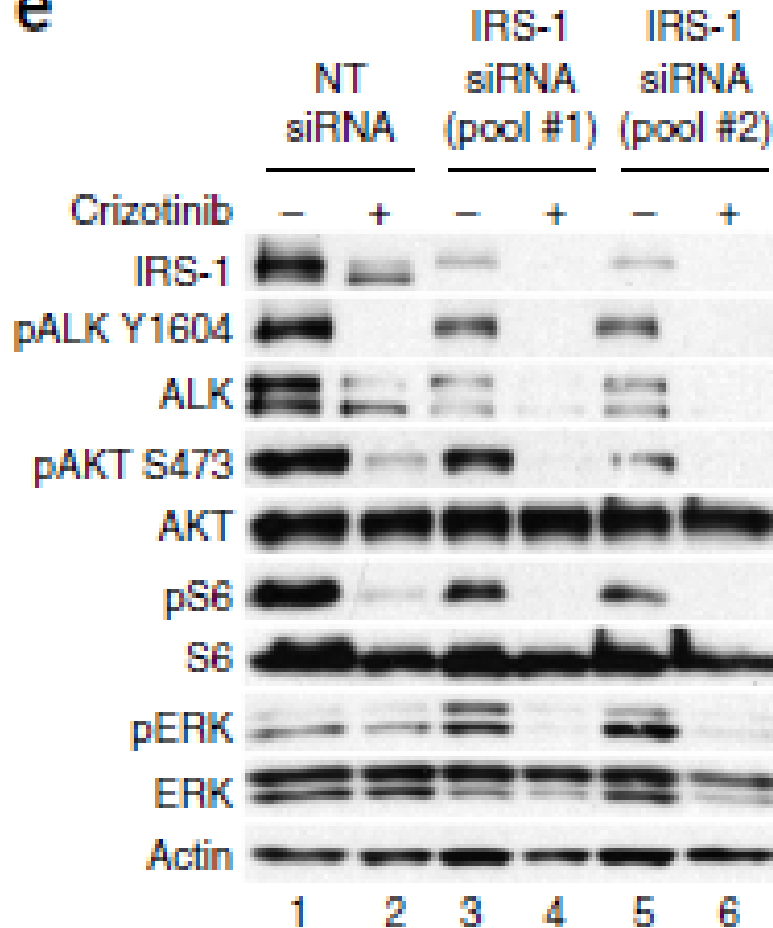
d



Immunoprecipitation and Westernblot of lysates from lung tumor tissue of transgenic mice (EML4-ALK; in vivo)

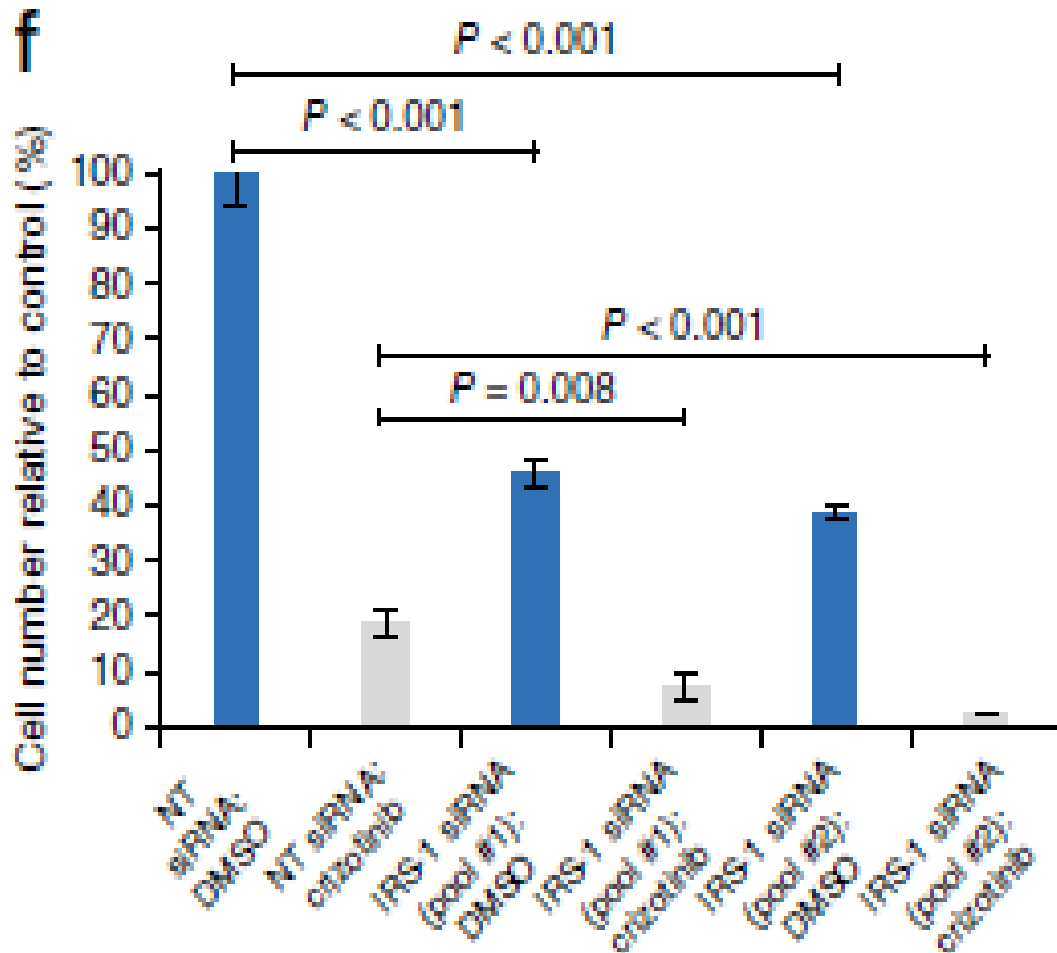
IRS-1 Knockdown

e



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

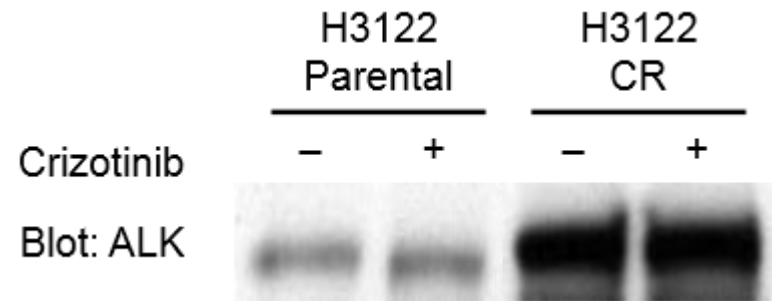
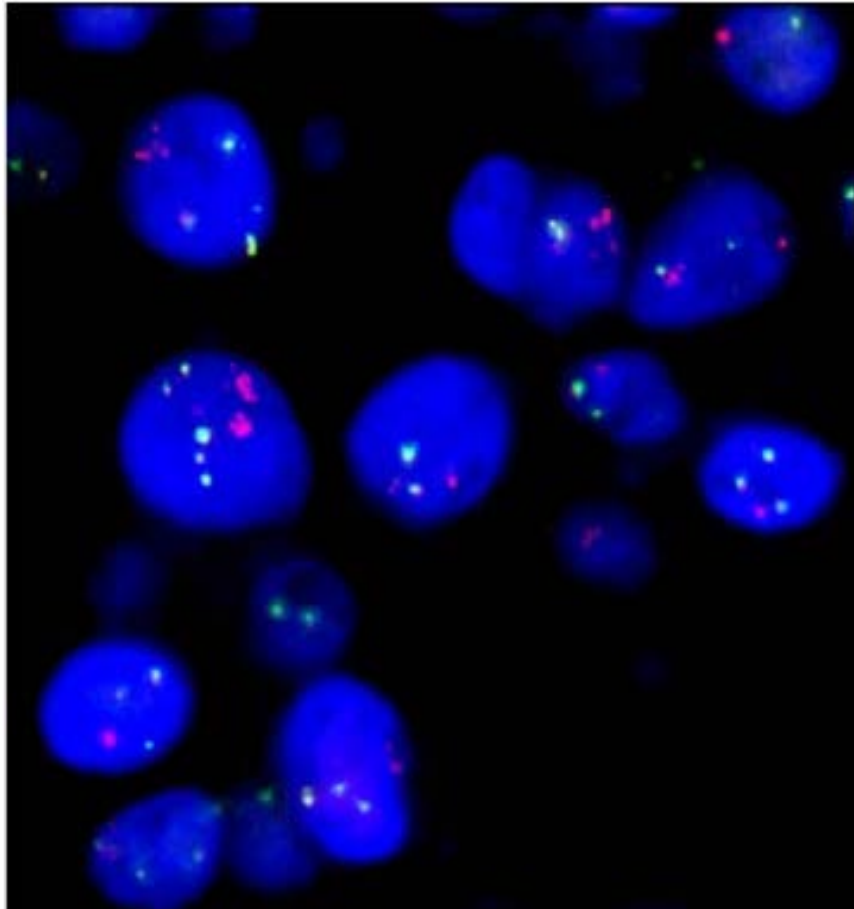
IRS-1 Knockdown



Coulter Counter
cellnumber
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

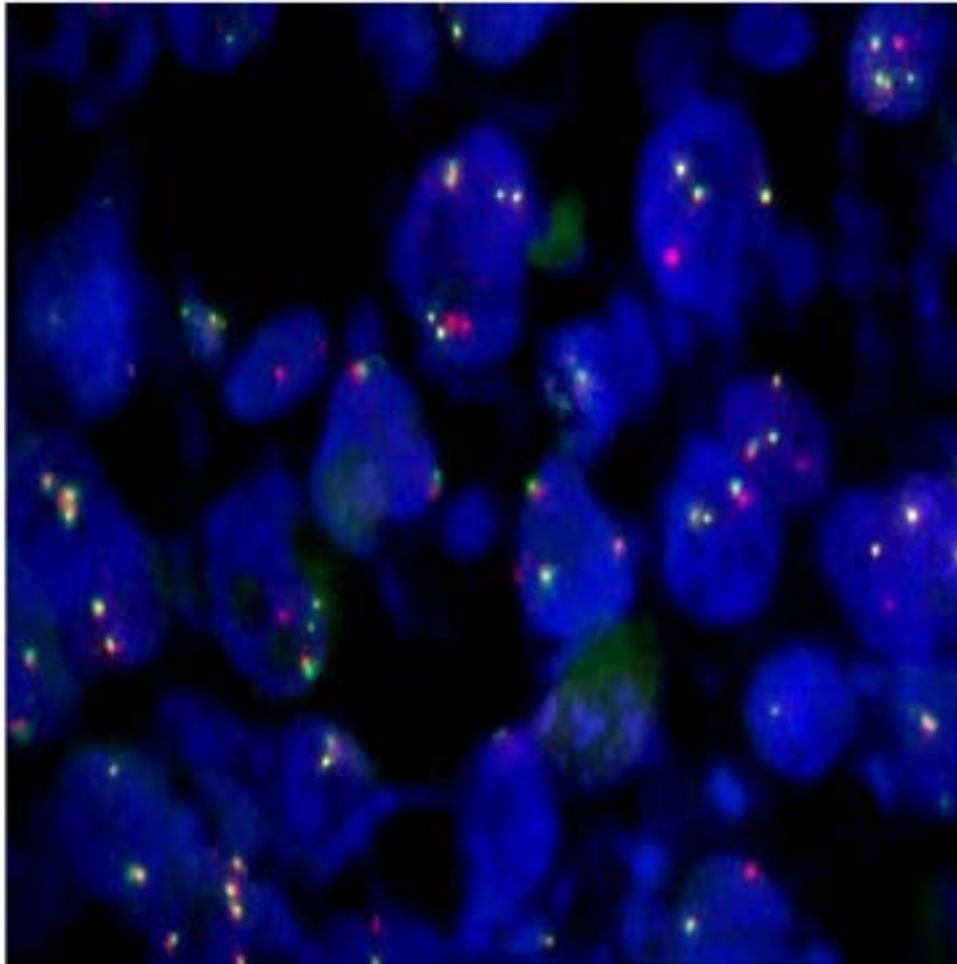


Immunoblot of cell lysates

FISH of H3122-cr cells

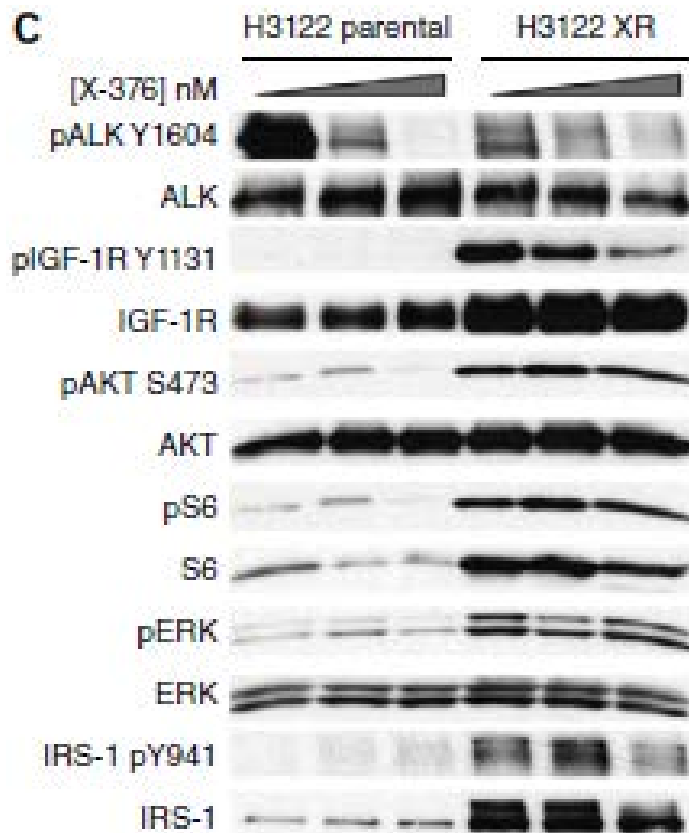


No EML4-ALK Fusion Amplification in H3122-xr cells



FISH of H3122-xr cells
(resistant against X-376)

But downstream phosphorylation remains



Immunoblot from H3122-xr
and H3122 parental
celllysates

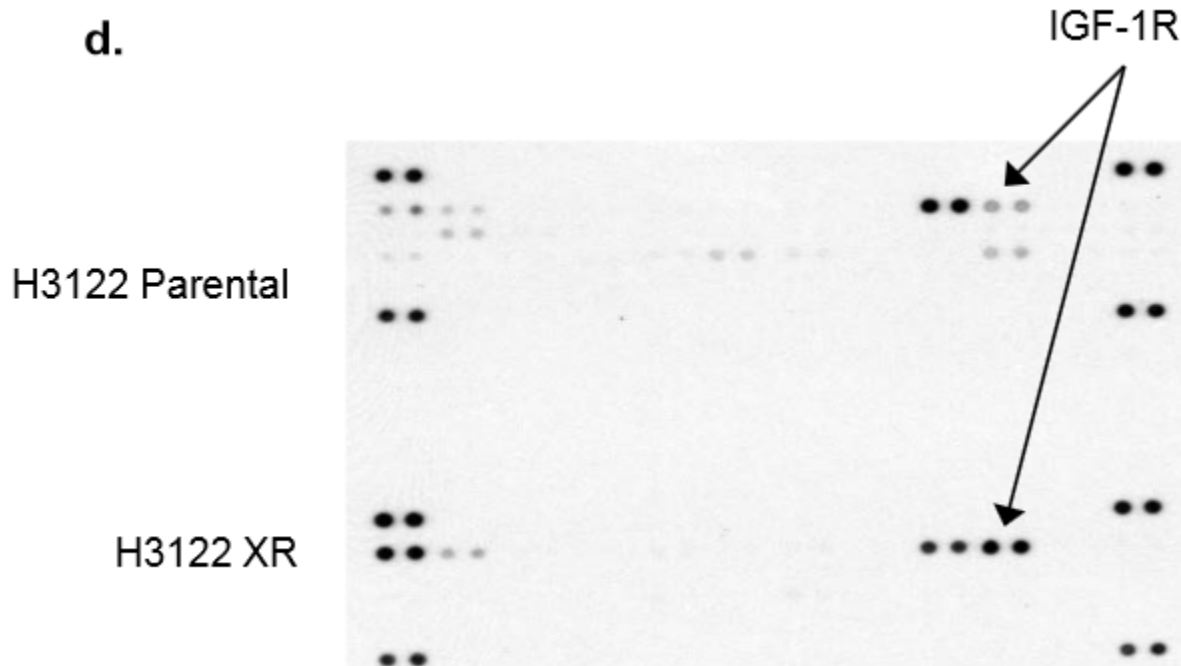


Suggestion:

IGF-1R – IRS-1 pathway has a role in maintaining downstream signaling while ALK inhibition

More IGF-1R In H3122-xr cells

d.



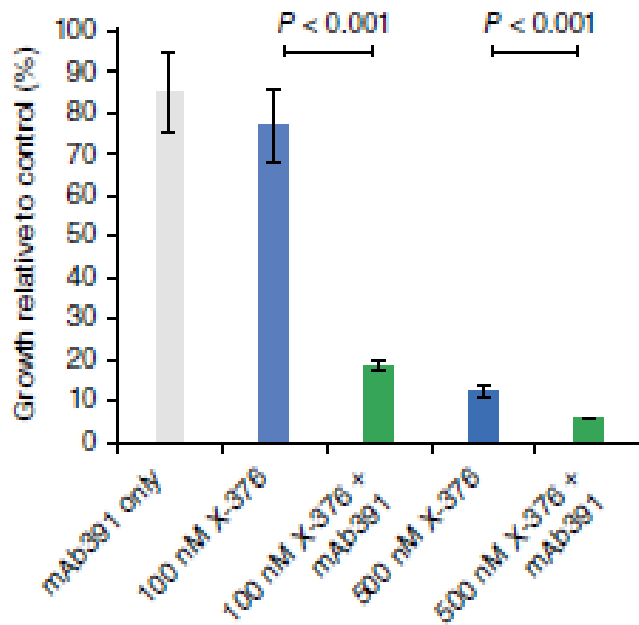
Phospho receptor tyrosine kinase array

Suggestion:

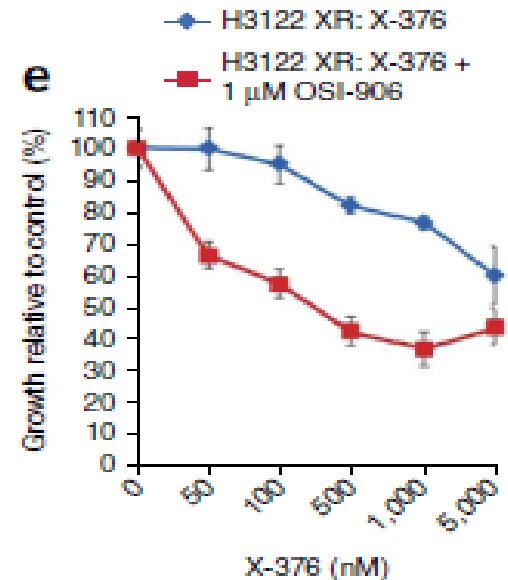
The IGF-1R IRS-1 pathway is a mechanism
by which cells evade ALK-Blockade

IGF-1R inhibition restores ALK-TKI-sensitivity

d

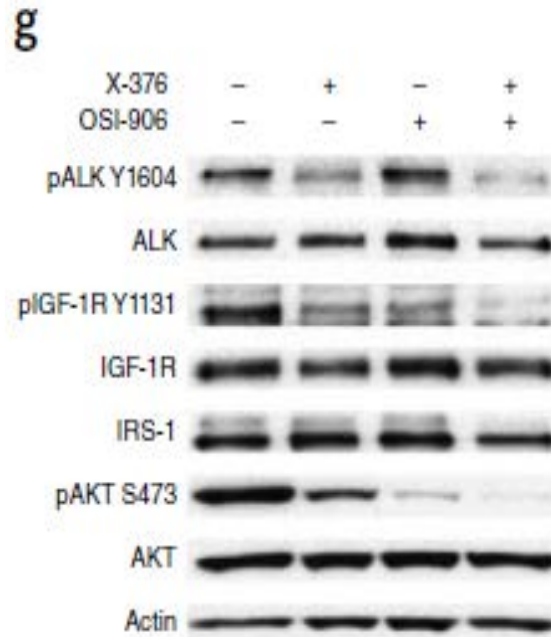


Soft agar assay of H3122-xr cells



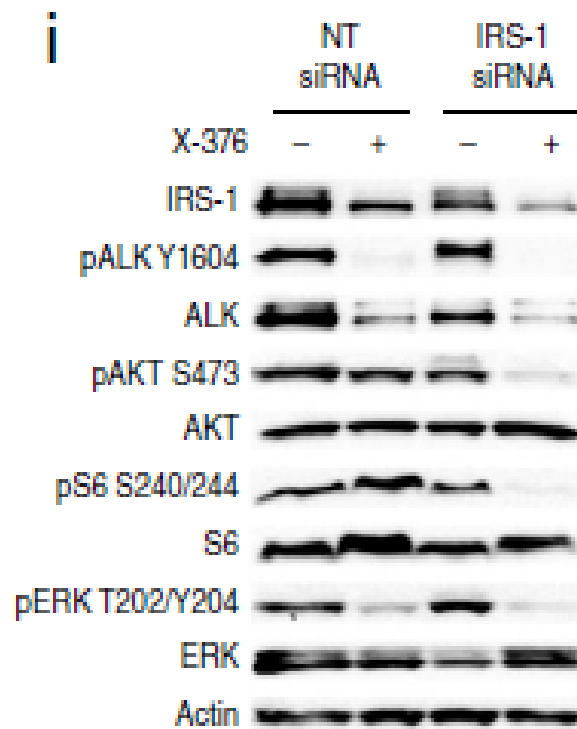
Cell titer blue assay of H3122-xr cells

Combination of ALK- and IGF-1R Inhibition



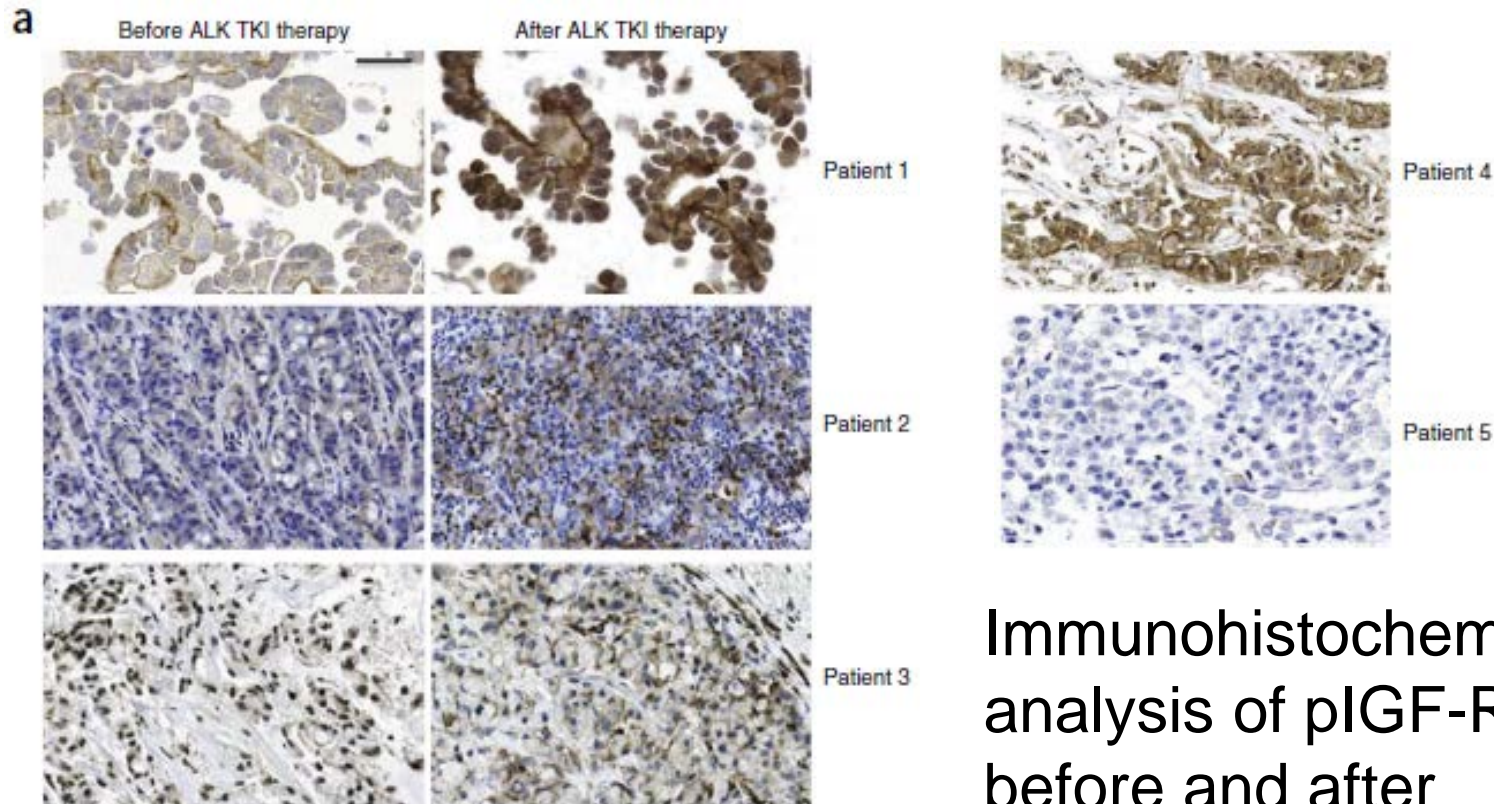
Immunoblot of
H3122-xr
celllysates

Effects of IRS-1 Knockdown



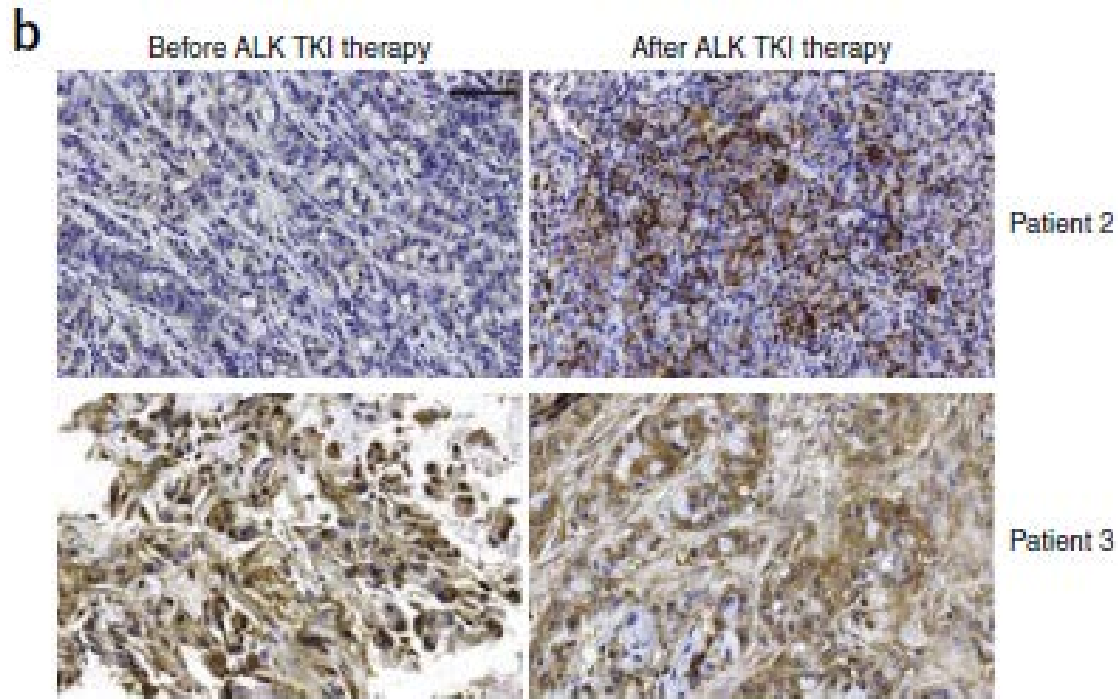
Western-Blot

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

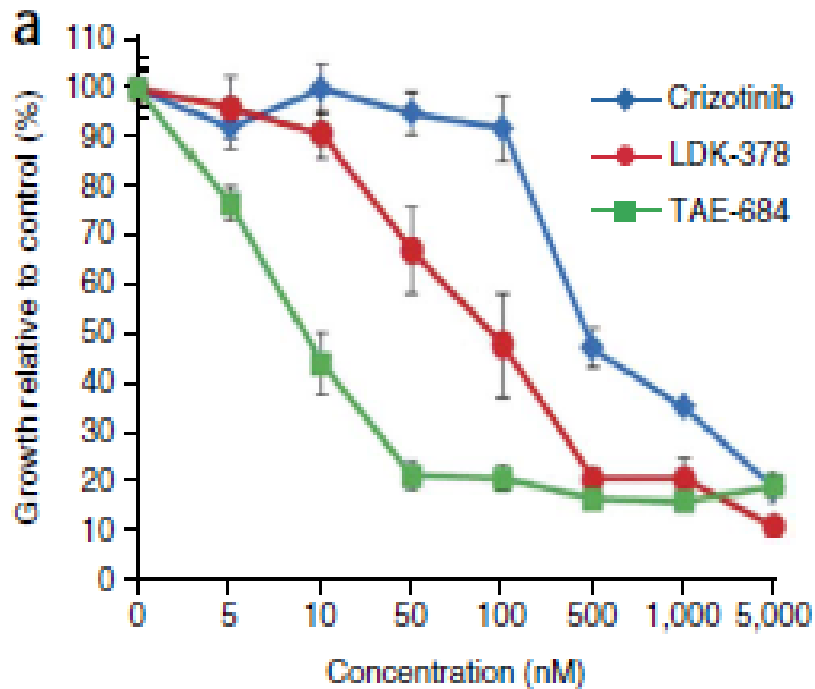


Immunohistochemical analysis of pIGF-R1 before and after Crizotinib-therapy

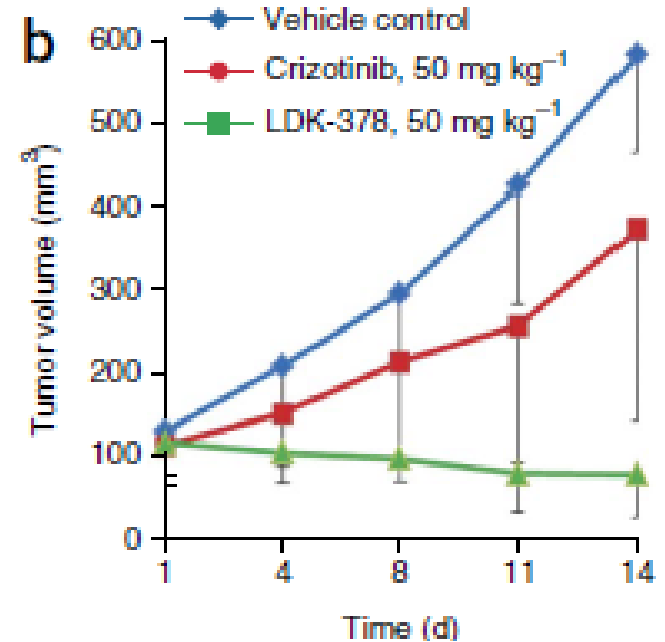
Also IRS-1 Is upregulated In Crizotinib-resistant lung cancer



Immunohistochemical analysis of IRS-1 before and after Crizotinib-therapy

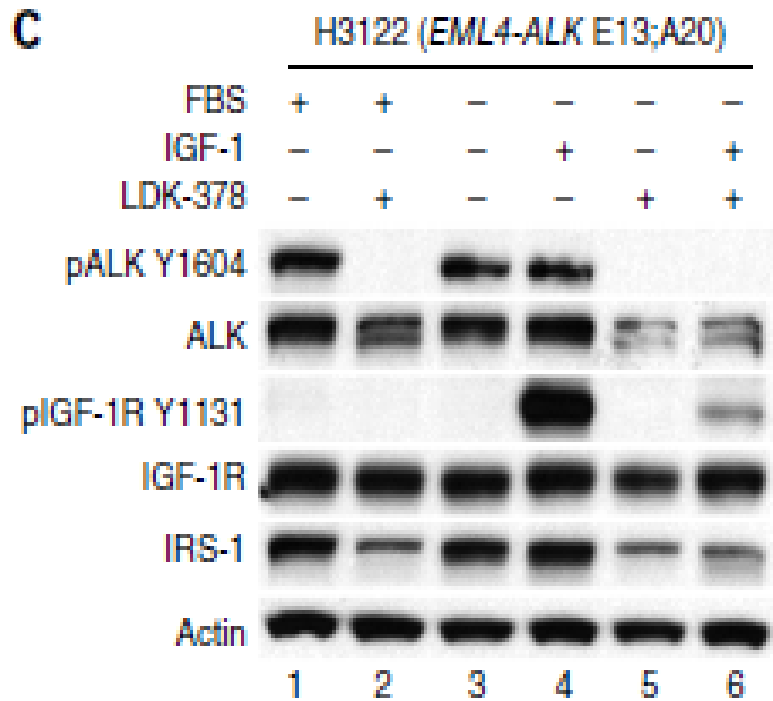


Cell titer blue analysis of H3122 cells

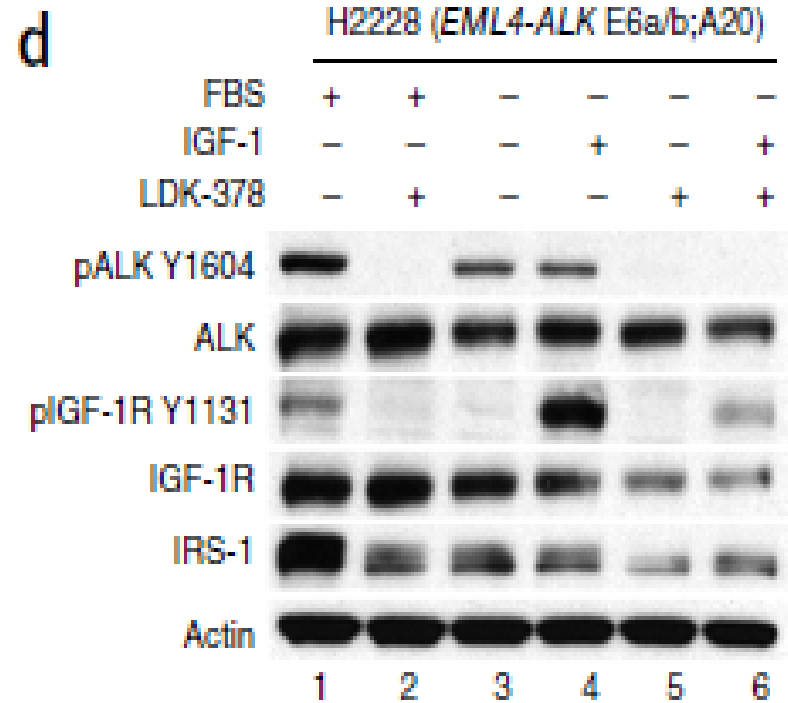


Measurement of tumor volumes in mice

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**



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References

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