HORIZON DISCOVERY

Application Note

Secondary Profiling of ALK Inhibition in Cell Lines Harboring Genomic ALK Alterations

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Introduction

Anaplastic Lymphoma Kinase (ALK) is a receptor tyrosine kinase belonging to the insulin receptor superfamily. Alterations in the ALK gene have been shown to have a role in oncogenesis, with chromosomal rearrangements being the most common form of constitutive ALK activation. Two products of such rearrangements are the fusion proteins NPM-ALK and EML4-ALK. NPM-ALK is found in 75% of anaplastic large-cell lymphomas (ALCL), while EML4-ALK is found in a subset of non-small cell lung carcinomas (NSCLC). High level overexpression as a result of gene amplification has also been found to result in constitutively activated ALK in neuroblastoma cell lines[1]. In addition, activating point mutations of the ALK gene have been linked to a subset of neuroblastomas[2] and anaplastic thyroid cancer[3].

A number of cancer cell lines which harbor genomic ALK alterations are highly sensitive to ALK inhibition. The dual ALK/MET small-molecule inhibitor PF-2341066 (Crizotinib) is currently in clinical trials, demonstrating the therapeutic potential of targeted agents towards a subset of tumors with ALK alterations[4]. Here we have profiled ALK inhibitors in a panel of cell lines containing different genomic ALK alterations, in order to characterize their sensitivity to the targeted agents and the effects on downstream signaling.

Secondary profiling, to evaluate the effects of compounds on signaling pathways, improves insight of a compound's mechanism of action and aids the identification of relevant biomarkers. Such information is of enormous value for understanding where to employ targeted agents for maximum benefit in personalized medicine.

Methods

For Western blotting, KARPAS-299 cells were plated into 6- well plates and treated with the inhibitor PF-2341066 for 2h at the concentrations shown. Sample lysate was separated by SDS-PAGE and Western blotting performed.

For proliferation assays, cells were seeded into 96-well plates and incubated overnight. Cells were then treated with inhibitor for 96 h. Cell viability was quantified with alamar blue.

Cell Lines Used

Description	Cancer Type	ALK status
KARPAS-299	ALCL	NPM-ALK positive
MOLM-13	AML	Negative
MV4-11	AML	Negative
NCI-H2228	NSCLC	EML4-ALK positive
NCI-H1650	NSCLC	Negative
Note: All cell lines were sourced from commercial cell banks		

Results and Discussion

Treatment of the NPM-ALK positive ALCL cell line KARPAS-299 showed a clear dose-dependent decrease in phosphorylation of NPM-ALK. In addition, inhibition of the phosphorylation of downstream signaling proteins such as ERK1/2 and STAT3 was also demonstrated.

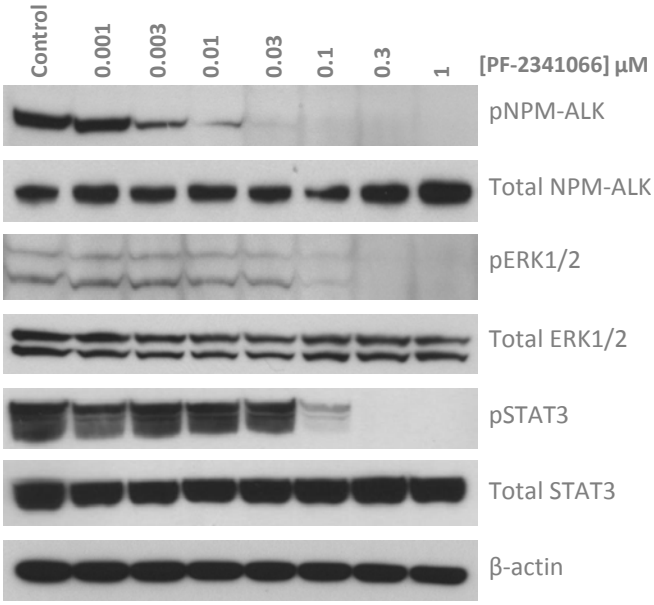

Figure 1. Inhibition of NPM-ALK phosphorylation and downstream signaling proteins in KARPAS-299 cells, following treatment with PF-2341066.

PF-2341066 was further investigated alongside NVP-TAE684, an ALK-specific inhibitor, to evaluate anti-proliferative effects in KARPAS-299 cells. The ALK-specific inhibitor NVP-TAE684 was approximately 10-fold more potent in the KARPAS-299 cell line than PF-2341066, with IC_{50} values of 3 nM and 20 nM respectively.

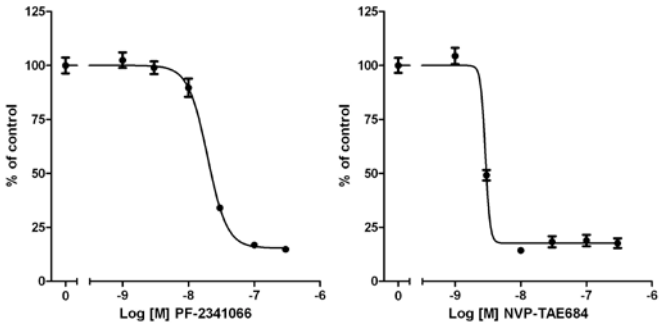

Figure 2. KARPAS-299 cells show sensitivity to the ALK inhibitors PF-2341066 and NVP-TAE684.

For comparison, PF-2341066 was also evaluated in the AML (acute myeloid leukemia) cell lines, MV4-11 and MOLM-13 which do not have constitutively activated ALK. Both cell lines were 10-fold less sensitive to the inhibitor than the KARPAS-299 cells with IC_{50} values of 300 nM and 200 nM respectively, confirming the targeted effects of this compound.

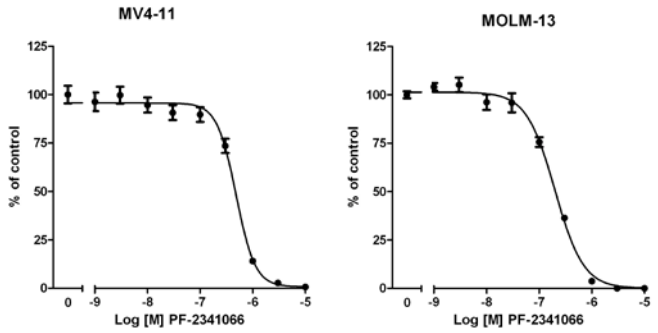

Figure 3. AML cell lines, MV4-11 and MOLM-13 show reduced sensitivity to the ALK inhibitor PF-2341066 compared to NPM-ALK positive cell line, KARPAS-299.

Following evaluation of anti-proliferative effects of ALK inhibition in lymphoma cell lines, the investigation was extended to profile a pair of NSCLC cell lines, NCI-H2228 and NCI-H1650. NCI-H2228 and NCI-H1650 are positive and negative for the EML4-ALK fusion protein, respectively. The NCI-H2228 EML4-ALK-positive cells showed greater sensitivity over the EML4-ALK-negative cell line when treated with both PF-2341066 and NVP-TAE684, again confirming the targeted effects of these compounds in cell lines with activated ALK.

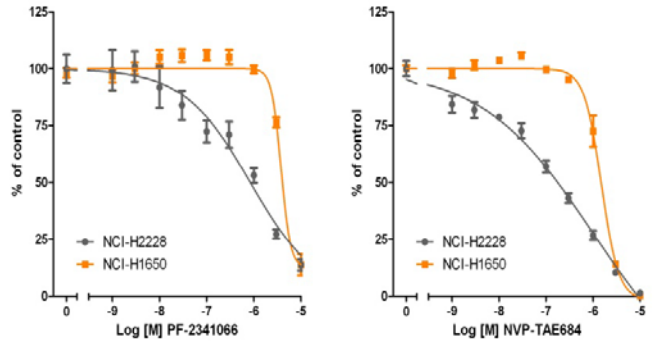

Figure 4. EML4-ALK-positive cells (NCI-H2228) show increased sensitivity to ALK inhibitors compared to EML4-ALK negative cells (NCI-H1650).

Conclusion

We have shown that a number of cancer cell lines which harbor genomic ALK alterations are sensitive to ALK inhibition, with clear anti-proliferative effects seen alongside inhibition of downstream signaling pathways. This study illustrates the value of cell line panels with defined genetic differences in evaluating compounds in a targeted manner.

Horizon Support

Horizon supplies genetically-defined cell lines, custom cell line generation, *in vivo* models, reporter gene assay kits, molecular reference standards and assay development and screening services to organizations engaged in academic research; drug discovery and development; clinical diagnostics; and biopharmaceutical process optimization. Please contact us to learn more about how Horizon can support your work.

X-MAN® cell lines which harbor genomic ALK alterations are currently in development. These include a cell line containing the ALK translocation EML4-ALK.

References

1. Webb *et al.,* Expert Rev Anticancer Ther 2009 (9) p331
2. George *et al.,* Nature 2008 (455) p975
3. Muragan and Xing, Cancer Res 2011 (71) p4403
4. Shaw and Solomon, Clin Cancer Res 2011 (17) p2081

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