Poster Number: B162. Abstract Number: 35119

NXP900, a novel YES1/SRC Kinase Inhibitor in Phase 1, Demonstrates Potent Inhibition of Proliferation in Cell Lines Resistant to ALK and EGFR Inhibitors

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Introduction

NXP900 (eCF506) is a novel potent and selective SRC family kinase (SFK) inhibitor, (IC50 of 0.47 and 2.44 nM against YES1 and SRC respectively (Table 1)). NXP900 locks its target into its native "closed" conformation (type 1.5), thereby inhibiting both kinase activity and complex formation with protein partners (Temps et al. Cancer Res. 2021, 81, 5438). In contrast, multi-kinase inhibitors, including dasatinib and bosutinib, block SRC in the active "open" conformation (type 1) promoting the association of SFK and signaling partners via allosteric facilitation (Higuchi et al.) (Figure 1).

Mutant-selective epidermal growth factor receptor (EGFR) inhibitors such as osimertinib, demonstrate activity in the treatment of EGFR-mutant lung cancer. Alectinib an inhibitor of the anaplastic lymphoma kinase (ALK) is also used to treat ALK positive non-small-cell lung cancer (NSCLC). Despite promising activity the efficacy of both agents is limited by acquired resistance. Previous studies have shown that SFK activity can play a role in off-target acquired resistance to both osimertinib and alectinib in NSCLC preclinical models (Ichihara et al.; Yoshida et al).

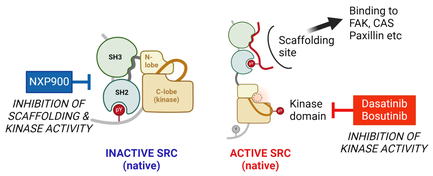
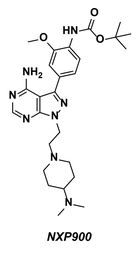


Figure 1. Mechanism of SRC/YES1 inhibition. NXP900 locks SRC in its native inactive conformation inhibiting both catalytic and scaffolding functions. (Created with Biorender)

Table 1. IC_{50} values (nM) calculated for NXP900 and dasatinib in a selection of 6 recombinant kinases.

	IC_{50} values in nM (% kinase inhibition @ top [])	
Kinase	NXP900	Dasatinib
SRC	2.44	< 0.5
YES	0.47	< 0.5
ABL	> 500 (32 %)	< 0.5
RET	> 500 (4 %)	433
KIT	> 500 (4 %)	39
PDGFRα	> 500 (14 %)	9.9



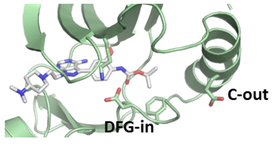


Figure 2. Structure of NXP900 and in complex with SRC (inactive conformation, PDB: 7NG7).

NXP900 sensitivity across lung cancer cell lines

Cell Line	Notable mutations and genomic/proteomic alterations	GI50 (nM)
PC9	EGFR E746_A750 DL	605
NCI-H2172	ERBB4,EGFR	10,000
A-549	KRAS, HER2 high expression	>3,160
NCI-H460*	KRAS Q61H, PIK3CA E545K	27,216
A-427	KRAS	81
NCI-H661	BRAF, FAT1, ROS1	8.4
LC-2/ad	CCDC6-RET fusion	11
NCI-H2228	EML4-ALKv3	15
SW900	KRAS, FAT1	23

* Highly resistant to TAS2940, lapatinib, neratinib, afatinib, osimertinib

Table 2. Sensitivity of lung lineages to NXP900. Cell lines underwent a 120-hour CellTiterGlo viability assay with a 9-point titration of NXP900 from 3.16 nM to 31.6 µM (Oncolines B.V., The Netherlands)

NXP900 in combination with osimertinib reverses resistance in NSCLC cell lines

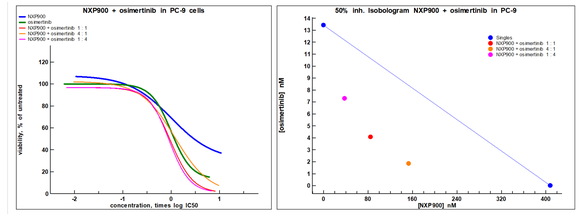
Cell Line	NXP900 GI50 (nM)	Osimertinib GI50 (nM)	NXP900 GI50 (nM) + Osimertinib at 160 nM
PC9	605	62	13
PC9-OR1	4,665	1,508	121
PC9-OR3	826	1,377	43

NXP900 demonstrates potent single agent activity in alectinib sensitive and resistant NSCLC cell lines

Cell Line	NXP900 GI50 (nM)	Alectinib GI50 (nM)	NXP900 GI50 (nM) + Alectinib at 25 nM
NCI-H2228	48.5	76	15
NCI-H2228-ALR1	26	6,460	
NCI-H2228-ALR2	19	6,614	
NCI-H2228-ALR3	30	2,962	
NCI-H2228-ALR5	22	2,158	

Table 3. Alectinib and osimertinib resistant NCLC cell lines were generated by treatment of PC9 or NCI-H2228 with increasing concentrations of, respectively, osimertinib or alectinib (Bertran-Alamillo et al). Cell proliferation assay: Cells were diluted in the corresponding ATCC recommended medium and dispensed in a 384-well plate, depending on the cell line used, at a density of 100 - 6400 cells per well. For each cell line the optimal cell density is used. Cells were treated with the indicated inhibitors for 120 h.

NXP900 demonstrates potent synergy with osimertinib and alectinib across sensitive and resistant cell lines

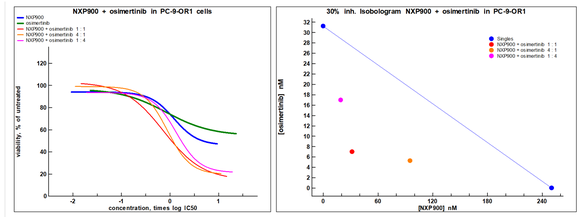


NXP900 + osimertinib in PC-9 cells			
Compound	IC_{50} ratios		
NXP900	1	4	1
osimertinib	1	1	4
	molar ratios		
NXP900	21	83	5.2
osimertinib	1	1	1
Δlog IC_{50}	-0.02	0.08	-0.05
Factor IC_{50} shift	1.05	0.83	1.11

Table 2: Curve shift values

combination experiment	CI_{50}	CI_{75}	CI_{90}	average	st. dev.
NXP900 + osimertinib 1 : 1 in PC-9	0.51	0.27	<0.1	<0.29	*na*
NXP900 + osimertinib 4 : 1 in PC-9	0.51	0.16	<0.1	<0.26	*na*
NXP900 + osimertinib 1 : 4 in PC-9	0.64	0.45	<0.1	<0.40	*na*
average	0.55	0.29	<0.1		
standard deviation	*0.07*	*0.15*	*na*		

Figure 3. Drug combination analysis: NXP900 + osimerinib in PC-9 (NSCLC) cells

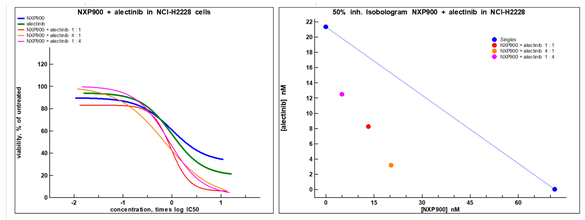


NXP900 + osimertinib in PC-9-OR1 cells			
Compound	IC_{50} ratios		
NXP900	1	4	1
osimertinib	1	1	4
	molar ratios		
NXP900	4.5	18	1.1
osimertinib	1	1	1
Δlog IC_{50}	-0.13	-0.05	0.13
Factor IC_{50} shift	1.36	1.13	0.74

Table 2: Curve shift values

combination experiment	CI_{50}	CI_{75}	CI_{90}	average	st. dev.
NXP900 + osimertinib 1 : 1 in PC-9-OR1	0.05	<0.1	na	<0.07	*na*
NXP900 + osimertinib 4 : 1 in PC-9-OR1	0.11	<0.1	na	<0.11	*na*
NXP900 + osimertinib 1 : 4 in PC-9-OR1	0.02	<0.1	na	<0.06	*na*
average	0.06	<0.1	na		
standard deviation	*0.04*	*na*	*na*		

Figure 4. Drug combination analysis: NXP900 + osimertinib in PC-9-OR1 osimertinib resistant (NSCLC) cells.



NXP900 + alectinib in NCI-H2228 cells			
Compound	IC_{50} ratios		
NXP900	1	4	1
alectinib	1	1	4
	molar ratios		
NXP900	1.6	6.5	0.40
alectinib	1	1	1
Δlog IC_{50}	-0.02	-0.15	-0.07
Factor IC_{50} shift	1.06	1.40	1.17

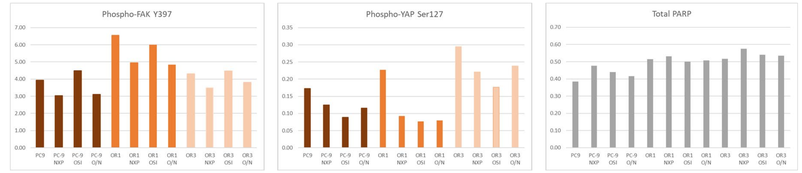
Table 2: Curve shift values

combination experiment	CI_{50}	CI_{75}	CI_{90}	average	st. dev.
NXP900 + alectinib 1 : 1 in NCI-H2228	0.57	0.14	<0.1	<0.27	*na*
NXP900 + alectinib 4 : 1 in NCI-H2228	0.43	0.10	<0.1	<0.21	*na*
NXP900 + alectinib 1 : 4 in NCI-H2228	0.66	0.29	<0.1	<0.35	*na*
average	0.55	0.18	<0.1		
standard deviation	*0.11*	*0.10*	*na*		

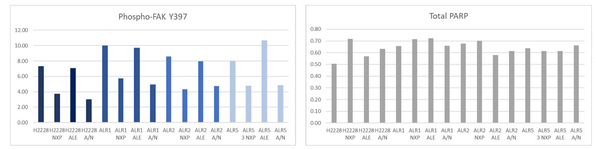
Figure 5. Drug combination analysis: NXP900 + alectinib in NCI-H2228 (NSCLC) cells

Reverse Phase Protein Array (RPPA) reveals compensatory signalling pathways in osimertinib and alectinib drug resistant cell lines that is inhibited by NXP900 and combination treatment

PC9 and osimertinib resistant NSCLC lines:



NCI-H2228 and alectinib resistant NSCLC lines:



**Phospho FAK and Phospho YAP levels represent ratios over total FAK and YAP1 protein levels respectively. Total PARP represents loading control.*

Figure 6. Cells were seeded in 6-well plates for 24 h prior to following treatments: *PC-9 cells - 40 nM NXP900(NXP), 20 nM Osimertinib(OSI) or 40 nM NXP900+20 nM Osimertinib(O/N); NCI-H2228 - 40 nM NXP900(NXP), 25 nM alectinib(ALE) or 40 nM NXP900+25 nM alectinib(A/N).* Protein lysates were prepared at 3 & 24 h (data not shown) following treatments and analysed by RPPA as previously described (Rukhlenko et al.). Key pathway changes in drug treated cells (3 h following treatment) for each cell line is shown.

Conclusions

- Here we demonstrate that NXP900 can potently inhibit cell proliferation of ALK resistant cell lines as a single agent and EGFR resistant cell lines in combination with Osimertinib.
- RPPA analysis reveals upregulation FAKY397 and YAPpSer127 in osimertinb and alectinib resistant NSCLC cells indicative of compensatory activation of the SRC/FAK signalling complex, compensatory signalling is inhibited upon NXP900 and combination treatments.
- Activation of SFK and YAP1 has been shown to be important in the development of resistance to ALK and EGFR treatment.
- We have previously demonstrated that NXP900 potently inhibits YAP1 nuclear localization and induces tumor regressions in squamous models in vivo providing additional proof of concept for targeting solid tumors with YES1/SRC and Hippo pathway alterations, including emerging resistance to ALK and EGFR inhibitors in NSCLC.
- A FIH, Phase1 dose escalation study for NXP900 is ongoing.

References, Contacts and Acknowledgements

References

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Acknowledgments

- With thanks to research grants provided by the Medical Research Council Precision Medicine Doctoral Training Programme and Nuvectis Pharma, Inc.
- Oncolines B.V., The Netherlands, for cell panel testing of NXP900 and combinations.
- NXP900 is licensed to Nuvectis Pharma, Inc.