

NXP900, a Novel YES1/SRC Kinase Inhibitor in Phase 1 Dose Escalation, Demonstrates potent Synergy with ALK Inhibitors in ALK Resistant Cell Lines

Asier Unciti-Broceta,¹ Neil O. Carragher¹ and Enrique Poradosu²

¹Edinburgh Cancer Research, CRUK Scotland Centre, Institute of Genetics and Cancer, University of Edinburgh, UK. ²Nuvectis Pharma Inc. 1 Bridge Plaza, 2nd Floor, Fort Lee, NJ, 07024, USA.

Introduction

NXP900 (formerly known as eCF506) is a novel potent and selective SRC family kinase (SFK) inhibitor (IC₅₀ of 0.47 nM against YES1). NXP900 locks its target into its native “closed” conformation (type 1.5 inhibitor), thereby inhibiting both kinase activity and complex formation with protein partners (¹Temps et al. Cancer Res. 2021, 81, 5438-5450). In contrast, multi-kinase inhibitors, including dasatinib and bosutinib, block SRC in the active “open” conformation (type 1 inhibitors) promoting the association of SFK and signaling partners via allosteric facilitation (²Higuchi et al. Cell Rep 2021, 34, 108876). Further, NXP900 exhibits a unique target selectivity profile with 1000-fold selectivity for SRC/YES1 over ABL kinase. This unprecedented mechanism of action results in highly potent and selective pathway inhibition, in cell culture and *in vivo*. Anaplastic lymphoma kinase (ALK) inhibitors are clinically effective for the treatment of NSCLC patients whose tumors are driven by the expression of ALK fusion genes. Unfortunately, the development of resistance to ALK inhibitors is nearly universal and arises through a variety of bypass mechanisms including SFK activation, YES1 amplification and amplification of Yes-associated protein 1 (YAP1), which suggest that NXP900 may have therapeutic potential in cancers with acquired resistance to ALK inhibitors.

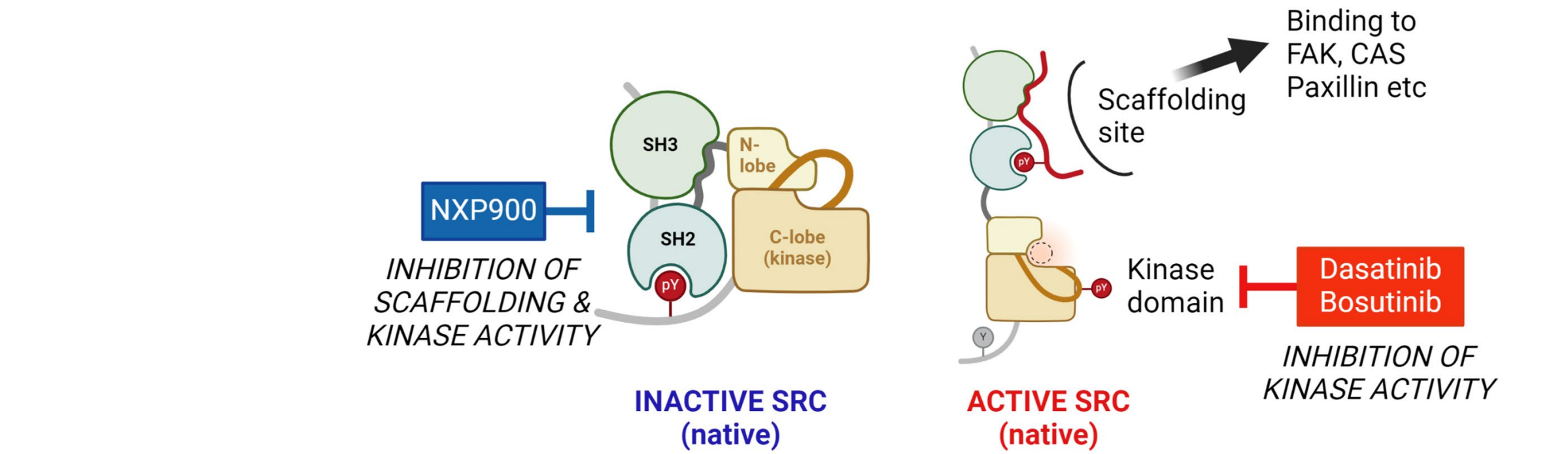


Figure 1. Mechanism of SRC inhibition. NXP900 locks SRC in its native inactive conformation inhibiting both catalytic and scaffolding functions (Created with Biorender).

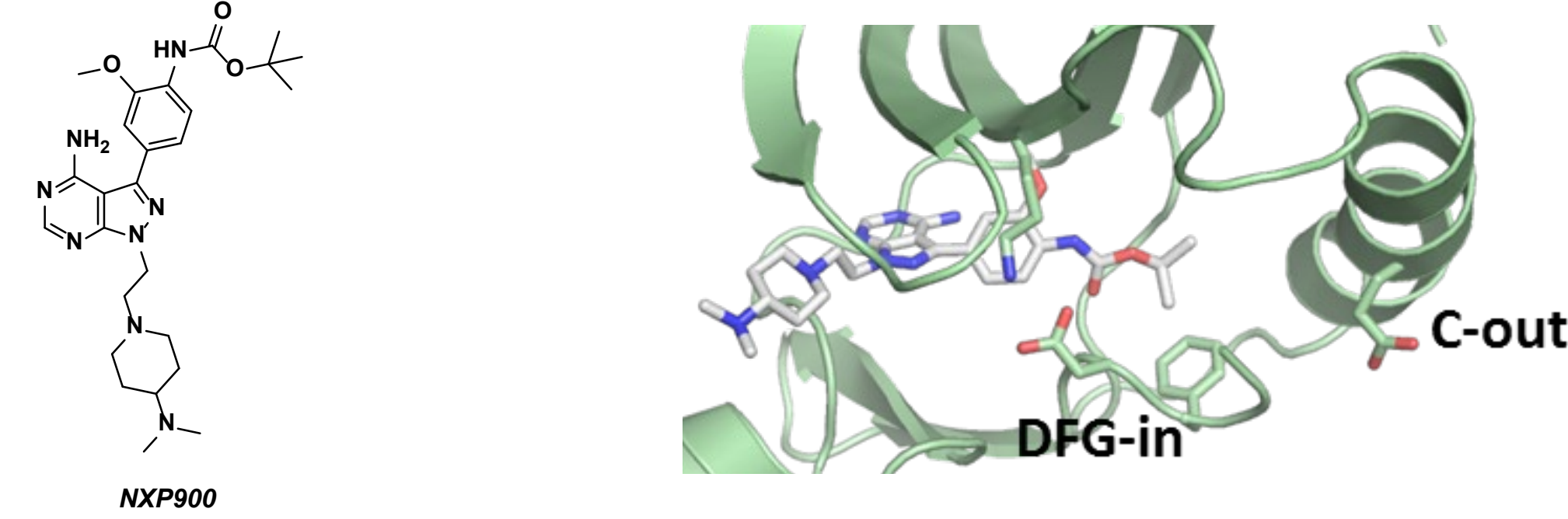


Figure 2. Structure of NXP900 and in complex with SRC (inactive conformation, PDB: 7NG7).

Acquired resistance to ALK inhibitors in ALK-fusion cell lines increases sensitivity to NXP900

A. NXP900 vs alectinib and lorlatinib single agent: Inhibition of proliferation in crizotinib resistant cells

Cell line	Alectinib	Lorlatinib	NXP900
NCI-H3122	364	1.9	14,347
NCI-H3122_CriR1	777	3.1	73
NCI-H3122_CriR3	2,101	47	56

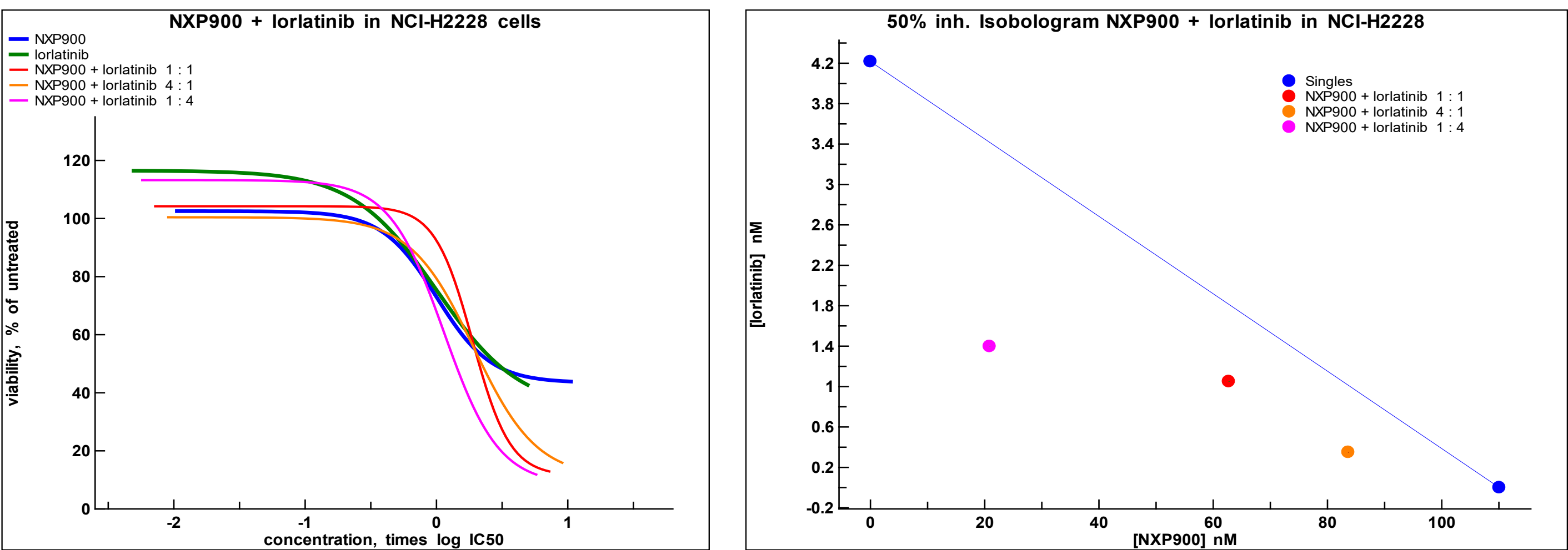
B. NXP900 vs alectinib and lorlatinib single agent: Inhibition of proliferation in alectinib resistant cells

Cell line	Alectinib	Lorlatinib	NXP900
NCI-H2228	76	1.3	82
NCI-H2228_AleR1	6,460	801	26
NCI-H2228_AleR2	6,614	172	19
NCI-H2228_AleR3	2,962	421	30
NCI-H2228_AleR5	2,158	323	22
NCI-H2228_AleR14	>31,600	8,583	44
NCI-H2228_AleR20	>31,600	5,141	30

Figure 3. Alectinib resistant NCLC cell lines were generated by treatment of NCI-H2228 (EML4-ALKv3) by selecting colonies that grew while exposed to one high dose of alectinib (similarly as reported in ³Garcia-Roman 2024). Crizotinib resistant cell lines were generated by treatment of NCI-H3122 (EML4-ALKv1) with increasing concentrations of crizotinib (⁴Bertran-Alamillo et al). Cell viability assay (Oncolines B.V., The Netherlands): All cell lines were cultured in RPMI 1640 and dispensed in a 384-well plate, depending on the cell line used, at a density of 100 – 6,400 cells per well. For each cell line the optimal cell density is used. Cells were treated with the indicated inhibitors for 120 h and underwent ATPlite viability assay with a 9-point titration for each inhibitor. Results are GI₅₀ (nM).

- No secondary mutations in the ALK fusion gene were observed using whole exome sequencing indicating that acquired resistance to crizotinib, alectinib and lorlatinib in these cell lines is driven by a bypass mechanism.
- Acquired resistance to crizotinib and alectinib resulted also in resistance to lorlatinib in the NCI-H3122 and NCI-H2228 in most resistant clones and increases cells sensitivity to NXP900 compared to its potency in the parental, ALK inhibitor-sensitive cell lines.
- NXP900 demonstrates potent single agent activity in ALK fusion driven cell lines with acquired resistance to crizotinib, alectinib and lorlatinib expressing different variants of ALK.

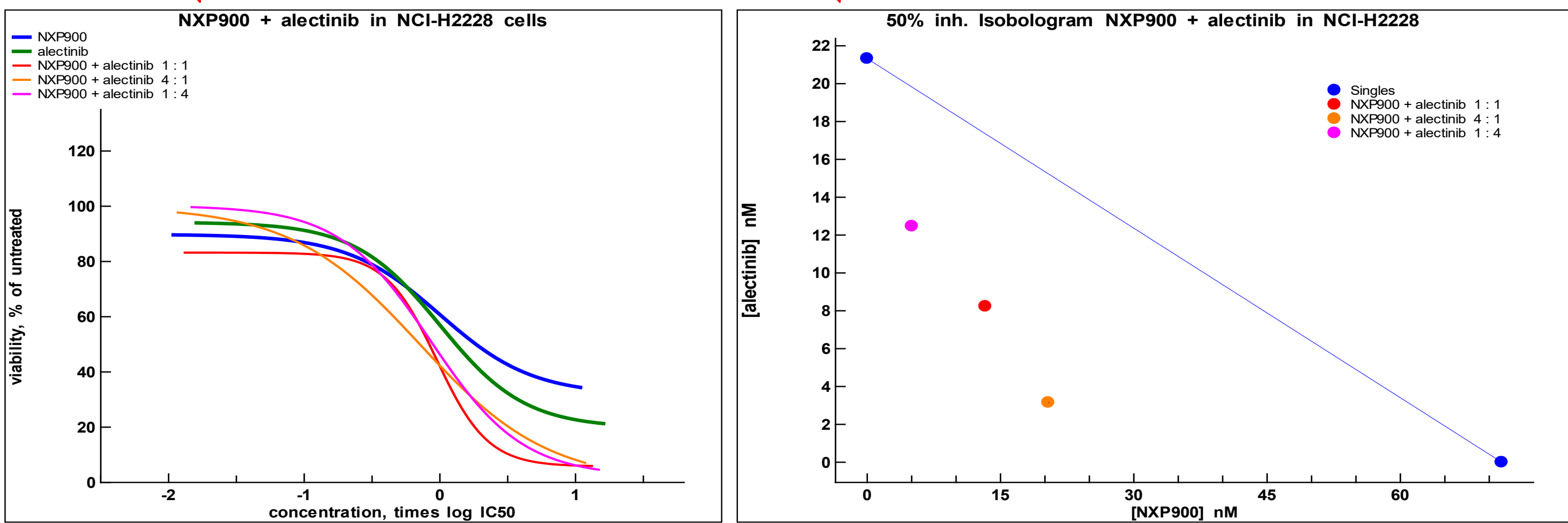
NXP900 is synergistic with lorlatinib and alectinib in ALK-fusion cell lines



NXP900 + lorlatinib in NCI-H2228 cells				
Compound	IC ₅₀ ratios			
	1	4	1	4
NXP900	1	1	1	4
lorlatinib	1	1	1	4
molar ratios				
NXP900	59.72	238.89	14.93	
lorlatinib	1	1	1	
Δlog IC ₅₀	0.28	0.28	0.06	
Factor IC ₅₀ shift	0.53	0.52	0.88	

combination experiment	CI ₃₅	CI ₅₀	CI ₆₅	average	st. dev.
NXP900 + lorlatinib 1 : 1 in NCI-H2228	1.35	0.82	0.03	0.73	0.66
NXP900 + lorlatinib 4 : 1 in NCI-H2228	1.20	0.84	0.01	0.68	0.61
NXP900 + lorlatinib 1 : 4 in NCI-H2228	0.79	0.52	0.04	0.45	0.38
average	1.11	0.73	0.03		
standard deviation	0.29	0.18	0.02		

Figure 4. Drug combination analysis: NXP900 + lorlatinib in NCI-H2228 cells



NXP900 + alectinib in NCI-H2228 cells				
Compound	IC ₅₀ ratios			
	1	4	1	4
NXP900	1	1	1	4
alectinib	1	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	

combination experiment	CI ₃₅	CI ₅₀	CI ₆₅	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 cells

CONCLUSIONS

- Here we demonstrate that NXP900 as a single agent potently inhibits cell proliferation of ALK fusion driven cell lines that are resistant to crizotinib, alectinib and lorlatinib.
- NXP900 is highly synergistic with lorlatinib and alectinib in ALK fusion driven cell lines.
- Synergy studies in cells resistant to alectinib and lorlatinib are ongoing.
- Mechanisms of acquired resistance to ALK inhibitors include SRC activation, YAP1 and YES1 gene amplification. It has been previously demonstrated that NXP900 potently inhibits YAP1 nuclear localization and induces tumor regression in squamous models *in vivo* with YES1 gene amplification or hippo pathway activation.
- SRC/YES1 are key signalling pathway mediators contributing to both primary and acquired resistance to other anti-cancer agents including HER2 targeting agents⁵, indicating a potential for NXP900 to address acquired resistance to targeted therapy in a broader setting including NSCLC, breast and gastric cancer.
- Altogether, these novel data suggest that NXP900 may have therapeutic potential in NSCLC in combination with ALK inhibitors both in first and subsequent lines of therapy. NXP900 is currently in a phase 1 dose escalation (NCT05873686).

Acknowledgements and Contact

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Asier Unciti-Broceta, University of Edinburgh: asier.ub@ed.ac.uk
Neil Carragher, University of Edinburgh: n.carragher@ed.ac.uk
Enrique Poradosu, Nuvectis Pharma Inc.: eporadosu@nuvectis.com

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