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NXP900, a novel YES1/SRC kinase inhibitor demonstrates inhibition of YAP1 nuclear localization and potent single agent anti-tumor activity in esophageal squamous cancer models

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Introduction

Background: NXP900 (eCF506) is a novel potent and selective SRC family kinase (SFK) inhibitor, (IC_{50} 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 ([1]Temps 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 ([2]Higuchi 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 (Table 1). This unprecedented mechanism of action results in highly potent and selective pathway inhibition, in cell culture and *in vivo*. Crosstalk between YES1 and the Hippo pathway suggests that NXP900 may have therapeutic potential in cancers with Hippo pathway alterations. YES1 and Hippo pathway alterations are prevalent in several squamous cancers including esophageal (ESCC), lung, head and neck, and cervical ([3]Maehama et al. Cancer Sci. 2021, 112, 51-60).

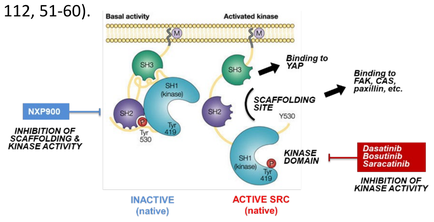
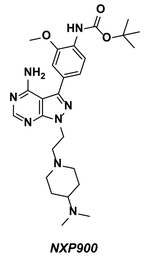


Figure 1. Mechanism of SRC inhibition. NXP900 locks SRC in its native inactive conformation inhibiting both catalytic and scaffolding functions.

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



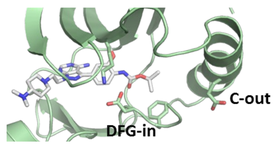
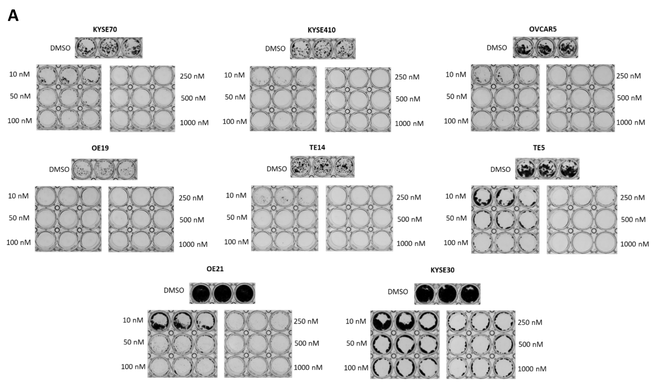


Figure 2. Structure of NXP900 and in complex with SRC (inactive conformation, PDB: 7NG7).

Human esophageal squamous cell carcinoma (ESCC) colony formation & cell viability assays



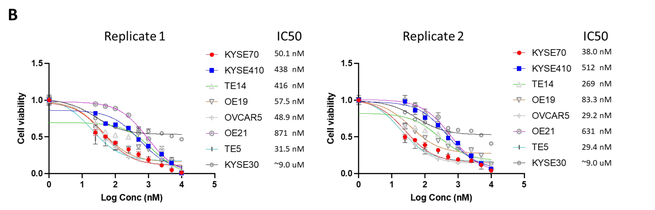


Figure 3. A. ESCC colony formation assay: 500 cells (KYSE70, OE21, KYSE410, KYSE30, OVCAR5) or 1500 cells (OE19, TE5, TE14) were seeded in 24 well plates in triplicates; and treated with NXP900 for 14 days followed by staining with crystal violet. **B. MTS viability assay:** On Day 1, 2000 cells per well were seeded in 96 well plates. On Day 2, the cells were treated with different concentrations of NXP900 in triplicates. After 48 hrs (Day 4), media was removed, and the cells were treated for a second time with NXP900 in fresh media. On Day 5, MTS reagent was added, and the plates were read at 490 nm using a microplate reader.

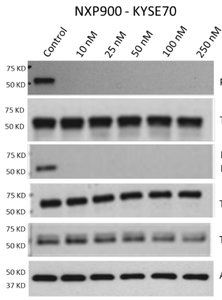


Figure 4. Inhibition of SFK activity. NXP900 significantly inhibited the activating phosphorylation of SRC family kinases in KYSE70 cells in vitro 24hrs post-treatment.

YAP subcellular localization assay

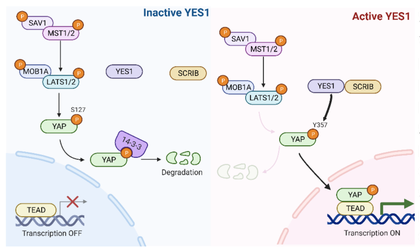


Figure 5. The SFK member, YES1 activates YAP by several mechanisms including phosphorylation (Y357)*.

SRC/YES1 activity crosstalks with the Hippo signalling pathway and Yes-associated protein (YAP) stabilization to promote nuclear translocation, gene transcription and tumor progression[4]. An *in vitro* high-content YAP localisation assay showed NXP900 reduced the proportion of high nuclear YAP expressing KYSE140 and KYSE70 cells (Figure 6).

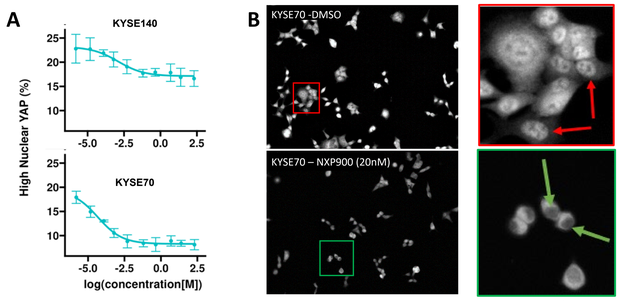
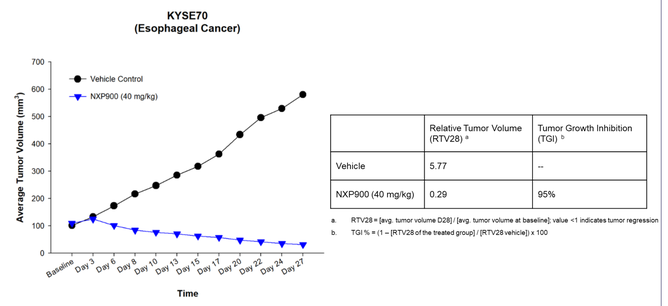


Figure 6. A Dose dependent reduction in high nuclear YAP by NXP900 after 24 hrs treatment *in vitro*. **B.** Representative examples of high (Red) and low (Green) nuclear YAP cells used for image-based classification by IN Carta® (Molecular Devices) software.

In Vivo efficacy study



	Relative Tumor Volume (RTV28) [a]	Tumor Growth Inhibition (TGI) [b]
Vehicle	5.77	--
NXP900 (40 mg/kg)	0.29	95%

a. RTV28 = [avg. tumor volume D28] / [avg. tumor volume at baseline]; value <1 indicates tumor regression
b. TGI % = (1 – [RTV28 of the treated group] / [RTV28 vehicle]) x 100

Figure 7. *In Vivo* Efficacy study was performed in CD1 nude mice. Xenograft tumors were generated by subcutaneous implantation on the right lower flank of the thigh at a cell density of $2x10^6$ to $1x10^7$ "KYSE70" cells/mouse. Mice were treated QD orally with vehicle (citrate buffer 3 mM) and NXP900 (40 mg/kg) for 28 days.

Bioinformatics of esophageal cancer cell line panel data (DepMap and Sanger GDSC)

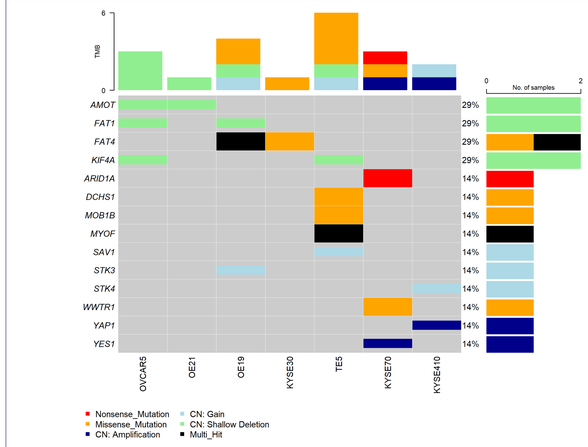


Figure 8. Bioinformatics analysis demonstrating patterns of mutations, deletions and copy number amplifications in Hippo pathway modulators across squamous esophageal cancer cell lines displaying high sensitivity to NXP900 in this study (analysis performed on data from DepMap).

Conclusions

NXP900 is a novel and potent and selective YES1/SRC kinase inhibitor with subnanomolar IC50 against YES1. NXP900 potently inhibits YAP1 nuclear localization and cell proliferation in a panel of ESCC cell lines and bioinformatics analysis indicates *in vitro* sensitivity to NXP900 is associated with Hippo pathway alterations across squamous esophageal cell lines. NXP900 induces significant tumor regressions in a KYSE70 xenograft model providing substantial proof of concept for targeting solid tumors with YES1 and Hippo pathway alterations. An IND for NXP900 has been cleared by the FDA and phase 1 clinical studies have been initiated.

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References

1. Temps C., Lietha D., Webb E.R., et. al. A conformation selective mode of inhibiting SRC improves drug efficacy and tolerability. Cancer Res. 2021. doi: 10.1158/0008-5472.CAN-21-0613.

2. Higuchi M, Ishiyama K, Maruoka M, et al. Paradoxical activation of c-Src as a drug-resistant mechanism. Cell Rep. 2021 Mar 23;34(12):108876. doi: 10.1016/j.celrep.2021.108876.

3. Maehama T, Nishio M, Otani J, et al. The role of Hippo-YAP signaling in squamous cell carcinomas. Cancer Sci. 2021 Jan;112(1):51-60. doi: 10.1111/cas.14725.

4. Hsu PC, Yang CT, Jablons DM, et al. The Crosstalk between Src and Hippo/YAP Signaling Pathways in Non-Small Cell Lung Cancer (NSCLC). Cancers (Basel). 2020 May 26;12(6):1361. doi: 10.3390/cancers12061361.

* Adapted using BioRender.