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In vivo studies demonstrate differences in target inhibition and anticancer efficacy between NXP900 and dasatinib in ovarian clear cell carcinoma model

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Introduction

We have developed and characterized the first small molecule —NXP900 (eCF506)— that locks SRC in its native inactive conformation, thereby inhibiting both enzymatic and scaffolding functions[1] (Figure 1 and 2). Further, NXP900 exhibits a unique target selectivity profile with 1000 fold selectivity for SRC over ABL kinase (Table 1). This unprecedented mechanism of action results in highly potent and selective pathway inhibition, in cell culture and *in vivo*.

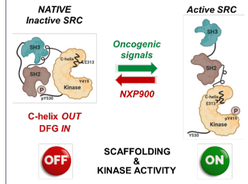


Figure 1. Mechanism of SRC inhibition. NXP900 locks SRC in its native inactive conformation.

	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

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

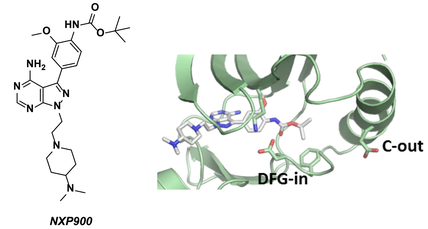


Figure 2. Structure of NXP900 and in complex with SRC (inactive conformation, PDB: 7NG7).

NXP900 displays high selectivity across the kinome

To compare the kinome-wide activity profile of NXP900 (0.5 µM) and dasatinib (1 µM), an enzymatic inhibition screen was performed against 326 wildtype and mutant kinases and NXP900 activity was compared to previous data on dasatinib[2].

NXP900 and dasatinib exhibit overlapping but distinct kinome inhibition profiles; NXP900 shows greater selectivity across the kinome compared to dasatinib (Figure 3).

Of the major kinase families, NXP900 is selective for tyrosine kinases especially SRC-family kinases (SFKs).

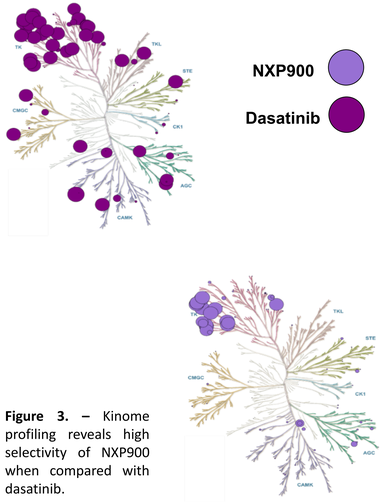


Figure 3. – Kinome profiling reveals high selectivity of NXP900 when compared with dasatinib.

Pharmacodynamic assay

Pharmacodynamic (PD) assays for NXP900 and dasatinib were performed in CD1 nude female mice (n=15, 3 mice per group); Xenograft tumors were generated by subcutaneous implantation of 0.1 million TOV-21G cells (ATTC CRL-11730) with Matrigel bilaterally. Mice were treated QD orally with vehicle (citrate buffer 3 mM), dasatinib 30 mg/kg and NXP900 at 20, 40 and 80 mg/kg; all compounds dissolved in vehicle. Once the tumors were measurable, mice were treated with the compounds and sacrificed 3 and 24 hours after the third dose of treatment and tumors were excised for further analysis. IHC analysis of the tumors demonstrated a clear difference in the expression of p-SRC (Y419) between dasatinib and NXP900 treatment showing substantially less p-SRC expression in NXP900 treated tumors. Moreover, p-SRC expression remained inhibited 24hrs after last dose of NXP900 compared to dasatinib indicative of prolonged pharmacodynamic effect due to inhibiting SRC in its closed conformation (Figure 4 and 5).

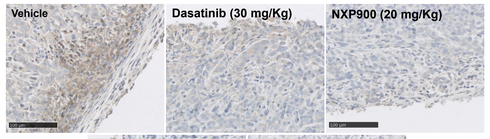


Figure 4. IHC sections from TOV-21G PD study. Tumours excised **3 hours** after the 3rd dose of treatment.

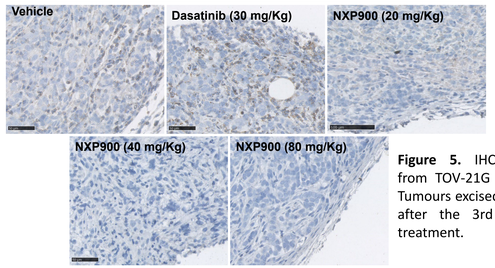
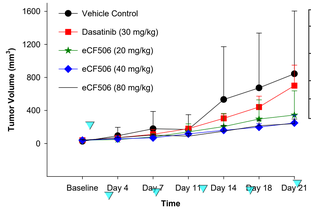


Figure 5. IHC sections from TOV-21G PD study. Tumours excised **24 hours** after the 3rd dose of treatment.

In Vivo efficacy study

In Vivo Efficacy study was performed in CD1 nude female mice (n=25, 5 mice per group); Xenograft tumors were generated by subcutaneous implantation of 0.1 million TOV-21G cells (ATTC CRL-11730) with Matrigel bilaterally. Mice were treated QD orally with vehicle (citrate buffer 3 mM), dasatinib 30 mg/kg and NXP900 at 20, 40 and 80 mg/kg for 21 days; all compounds dissolved in vehicle (Figure 6).



	Contr	Das-30	eCF-20	eCF-40	eCF-80
Baseline (SD)	26 (10.9)	39 (12.4)	40 (11.6)	40 (10.1)	40 (7.9)
Day 21 (SD)	845 (759.9)	701 (248.4)	345 (293.1)	249 (95.4)	241 (5.6)
TGI (%)	--	45	73	80	81

TGI % = (1 – [RTV21 of treated] / [RTV21 vehicle]) x 100

Figure 6. Tumor growth plot and TGI values.

YAP subcellular localisation assay

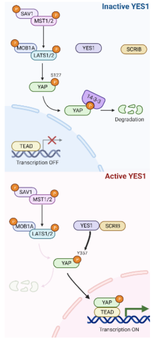


Figure 7. The SFK member, YES1 activates YAP by several mechanisms including phosphorylation (Y357)*.

SRC/YES1 activity crosstalks with the Hippo signalling pathway and Yes-associated protein (YAP) stabilization and nuclear localization to promote tumor progression[3]. An *in vitro* high-content YAP localisation assay showed NXP900 reduced the proportion of high nuclear YAP expressing TOV-21G cells (Figure 8/9).

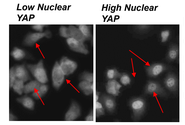


Figure 8. Representative examples of high and low nuclear YAP cells used for image-based classification using IN Carta® (Molecular Devices) software.

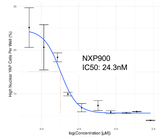


Figure 9. Dose dependent reduction in high nuclear YAP by NXP900 after 24 hrs treatment.

Discussion

Current type I SRC inhibitors in late-stage clinical development or approved (e.g. bosutinib, saracatinib and dasatinib) bind to the catalytic domain of SFK members in their active conformation. While this binding mode inhibits the catalytic activity of SFKs, they are still free to interact with binding partners such as Focal Adhesion Kinase (FAK) and Yes-associated protein (YAP) and signal through their scaffolding function. Higuchi et al[4] reported that type I SRC inhibitors such as dasatinib enhance SRC–FAK complex formation contributing to paradoxical activation of the SRC-FAK signalling complex. In this study we show in a TOV-21G ovarian clear cell carcinoma model with high YES1 expression[5], NXP900, in contrast to dasatinib treatment, results in sustained inhibition of SRC activity *in vivo* with no evidence of paradoxical activation. NXP900 further inhibited *in vivo* tumour growth and nuclear localization of YAP in TOV-21G cells in a dose dependent manner.

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* Adapted using BioRender.