Uncovering the molecular mechanisms which predict sensitivity to a novel SRC kinase inhibitor NXP900 to inform personalized healthcare strategies

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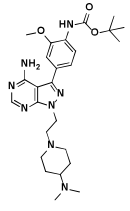
Abstract

Despite the overwhelming evidence for the role of SRC in the progression, and resistance mechanisms of many solid malignancies, SRC inhibitors have so far demonstrated modest clinical benefit in solid tumours. Here we report the development and characterisation of a small molecule —NXP900— with a novel mechanism of SRC inhibition.

The aim of this study was to perform extensive cancer cell line panel screening to inform clinical development and identify predictive biomarkers of sensitivity for this novel class of SRC kinase inhibitor.

NXP900 is a novel SRC inhibitor

We have developed and characterised the first small molecule —NXP900— 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 culture and in vivo.



NXP900

Figure 1. Structure of NXP900

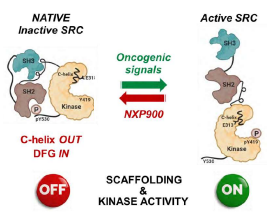


Figure 2. Mechanism of SRC inhibition. NXP900 locks SRC in its native inactive confirmation

	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.

NXP900 displays high selectivity across the kinome

To determine the kinome-wide activity profile of NXP900 (0.5 µM), an enzymatic inhibition screen was performed by Carna Biosciences, against 326 wildtype and mutant kinases. Only 22 of them showed decreased activity below 50% (Figure 3).

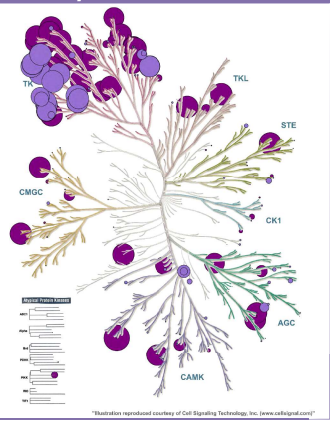


Figure 3. Kinome profiling reveals high selectivity of NXP900 when compared with dasatanib. Circles identify kinase targets of NXP900 (0.5µM, lilac) and dasatinib (1µM, deep purple), size represents percent inhibition. Dasatinib data from [2].

NXP900 displays subtype specific patterns of sensitivity

Extensive cancer cell line panel profiling was performed with Oncolines™ to provide insight into tissue-dependent compound responses. It has revealed clear patterns of sensitivity. Among the most sensitive tissues are cervix and ovarian, head and neck, colorectal, pancreatic, prostate, and certain subtypes of lung and breast cancer, including triple negative breast cancer.

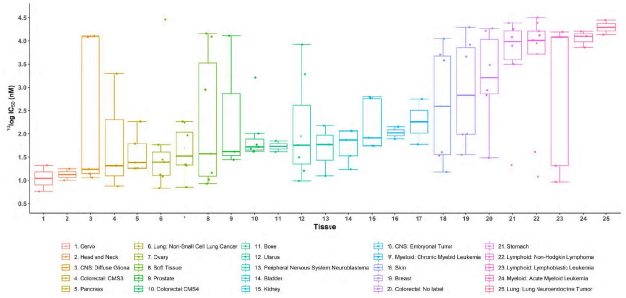


Figure 4. NXP900 ^{10}logIC$_{50}$ distribution across the cancer tissues from which the cell lines originated.

Pan cancer squamous carcinomas are also associated with high sensitivity to NXP900, the biology driving this sensitivity is currently under investigation.

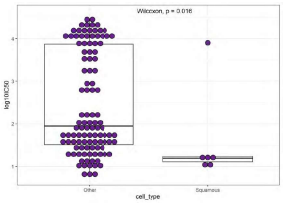


Figure 5. Box and dot plot demonstrating significant sensitivity of pan cancer squamous lines to NXP900, p<0.05

Genetic drivers of sensitivity to NXP900

To determine genetic drivers of sensitivity, we investigated transformations in 38 important cancer genes are statistically associated with shifts in compound sensitivity across the panel of 102 cell lines. While no genes reach significance, we believe due to the limited number of cell lines in the panel, clear trends emerge and genes of interest are being followed up. FAT1 is of interest given its high prevalence in squamous carcinomas. FAT1 loss-of-function mutations has also previously been implicated in SRC activation and promotion of tumour stemness and metastasis [3].

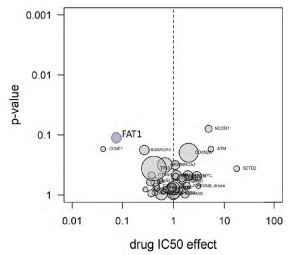


Figure 6. Volcano plot showing how genetic transformations in 38 important genes are statistically associated with shifts in compound sensitivity (as measured by ^{10}logIC$_{50}$). A shift to the left represents increased sensitivity. Size of circles represents number of cell lines with alteration (minimum of cell lines per alteration).

FAT1 mutation may drive squamous sensitivity

Although none are significant after multiple testing corrections, we explored the top gene associated with increased sensitivity- FAT1 deleterious mutations (Figure 7). FAT-1 is altered (mutations with known significance) in 3% of patients across all cancer types and is highly prevalent in squamous carcinomas, particularly cutaneous and head and neck (Figure 7).

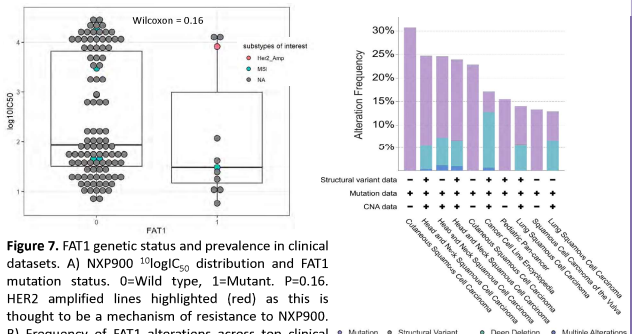


Figure 7. FAT1 genetic status and prevalence in clinical datasets. A) NXP900 ^{10}logIC$_{50}$ distribution and FAT1 mutation status. 0=Wild type, 1=Mutant. P=0.16. HER2 amplified lines highlighted (red) as this is thought to be a mechanism of resistance to NXP900. B) Frequency of FAT1 alterations across top clinical studies and cancer types (data from cbioportal).

ARID1A mutation and clear cell carcinoma

Although not included in the 38 cancer driver gene analysis we note that ARID1A mutations appear to sensitise to NXP900 in a subset of cancer subtypes including ovarian clear cell carcinoma where it is particularly prevalent.

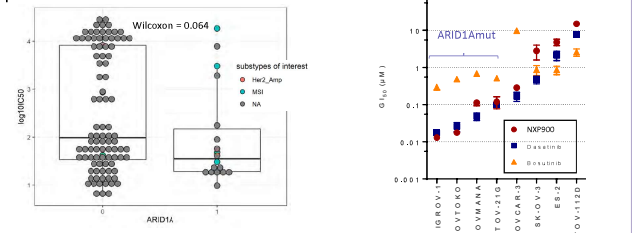


Figure 8. ARID1A mutation status and NXP900 sensitivity. A) Across Oncolines™ cell panel. 0=Wild type, 1=Mutant. B) GI50 values across in-house ovarian clear cell carcinoma panel. First 4 lines are ARID1A mutant ovarian clear cell carcinoma.

Conclusions

These studies demonstrate the identification of cancer cell lines which exhibit high sensitivity to NXP900 and which can be grouped into discrete subtypes based on gene mutation status, transcriptomics and histotype. Our results provide an indication of patient subgroups which may exhibit optimal therapeutic response to NXP900 and provide data to guide further preclinical, biomarker and clinical development.

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Contact

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