Screening of NXP900 and dasatinib across 121 cancer cell lines identifies differences in their antiproliferative activity profiles

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Abstract

We have recently reported that the total SRC inhibitor NXP900 binds and locks SRC tyrosine kinase in a closed and inactive conformation, representing a novel mechanism of action for the class of inhibitors (Fig. 1)[1]. This contrasts with the mechanism of the approved dual SRC-ABL tyrosine kinase inhibitor dasatinib. We anticipated these mechanistically distinct inhibitors could induce distinct antiproliferative effects in particular cancer cell lines. Screening of 121 cancer cell lines was undertaken to determine differential signatures of sensitivity to NXP900 and dasatinib (Fig. 2).

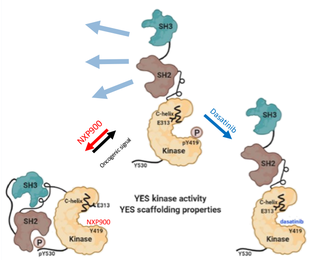

Figure 1 – *NXP900 inhibits SFKs with a novel mechanism-of-action by locking the protein in a closed conformation, preventing both enzymatic and scaffolding functions. This contrasts with dasatinib which binds to the ATP-binding site and allows scaffolding activity.*

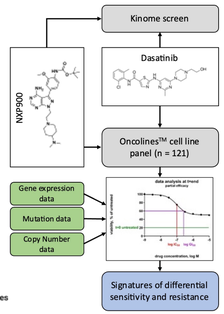

Figure 2 – *NXP900 and dasatinib were screened against the Oncolines™ cell lines panel for sensitivity. Publicly-available omics datasets were integrated to reveal signatures of sensitivity and resistance.*

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 compared to dasatinib.

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

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

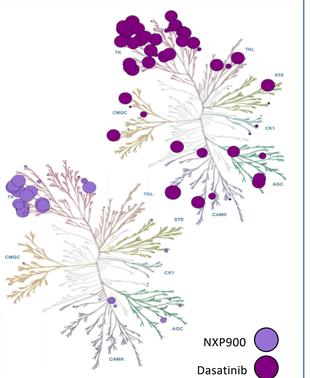

Figure 3 – *Kinome profiling reveals high selectivity of NXP900 when compared with dasatanib.*

Clustering reveals distinct sensitivity groups

Viability assays for NXP900 and dasatinib were performed against the Oncolines™ panel of 121 cell lines; GI_{50} and IC_{50} were calculated. Correlation analysis showed the majority of cancer cell lines responded similarly to both compounds (R = 0.57, p < 0.001). K-means clustering highlighted two distinct groups representing cell lines differentially sensitive to NXP900 (green) *or* dasatinib (red) (Fig. 4).

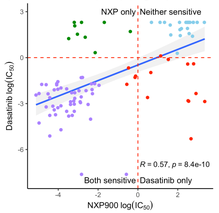

Figure 4 – *K-means clustering (k=4) highlights two groups which correspond to sensitivity to NXP900 or dasatinib only. Blue line represents Pearson's correlation coefficient ± SEM.*

To investigate the cause of this variation, 38 key cancer genes in these cell lines, as identified by Oncolines™, that were mutated, part of a translocation, or had copy number variation were compared between the two groups revealing distinct cancer genes exclusively associated with cell lines sensitive to dasatinib or NXP900 (Fig. 5). Of interest, FAT1 was altered in two cell lines in the dasatinib resistant-NXP900 sensitive group. FAT1 has been shown to regulate a variety of key oncogenic processes and play an important role in the Hippo signalling pathway[3,4].

Figure 5 – *Altered genes exclusively associated with differential sensitivity to NXP900 (green) and dasatinib (red). Size of gene represents frequency of alteration.*

Gene expression and protein levels correlate with sensitivity to NXP900

Basal gene expression of 571 key cancer genes in cell lines was correlated to sensitivity to both NXP900 and dasatinib. Normalised Pearson correlation coefficients (z-scores) for each gene were compared between NXP900 and dasatinib to produce **Δz**-score. Levels of expression, from reverse-phase protein array (RPPA), of 268 proteins were correlated to sensitivity and coefficients compared between NXP900 and dasatinib.

Basal expression of multiple genes was correlated with sensitivity to NXP900, but not dasatinib. Interestingly, expression of multiple genes associated with Hippo signalling were correlated with NXP900 sensitivity, including YAP, WWTR1 (encoding TAZ), and YES1, but not to dasatinib sensitivity.

Phosphorylated YAP and total YAP protein levels correlated with sensitivity to NXP900, but not dasatinib. Additionally, other key proteins known to play key roles in the Hippo signalling pathway and induce alterations in adhesion and migration of cancers, such as Caveolin-1, β-catenin, and α-catenin, were found to be significantly associated with sensitivity to NXP900 but not dasatinib[4,5,6].

EGFR-Ras-MAPK signalling promotes Ajuba protein family phosphorylation which in turn regulates YAP activation[7]. AJUBA gene expression was correlated with sensitivity to NXP900, as well as protein levels of EGFR, further implicating the Hippo signalling network.

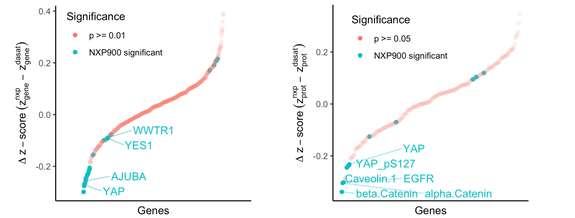

Figure 7 – *Difference in normalized correlation coefficients (z-scores) of sensitivity to NXP900 and dasatinib with basal gene expression (left) and protein levels (right). P-values were adjusted with the FDR method. Blue points represent genes significantly correlated with sensitivity to NXP900, but not dasatinib.*

Differential lineage-specific sensitivity

GI_{50} of the overlapping 96 cell lines were compared between NXP900 and dasatinib (Fig. 6). Of particular interest, the colorectal cancer cell lines SNU-C2B and HCT-116 and the uterine cell line RL95-2 were much more sensitive to NXP900 than dasatinib. In line with the superior potency of dasatinib to ABL, the chronic myelogenous leukaemia cell lines K-562 and KU812 exhibited much higher sensitivity to dasatinib.

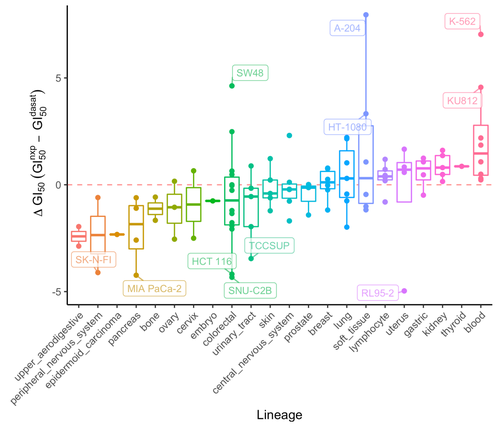

Figure 6 – *Difference between NXP900 and dasatinib log_2GI_{50} across the cancer tissues from which the cell lines originated. Highlighted cell lines have >3 or < -3 difference in log_2GI_{50}.*

Distinct mutation profiles associate with sensitivity

The mutation panel was expanded to include 571 genes with known mutations associated with cancer[8]. GI_{50} from all cell lines with these mutations were compared to those without. Mutations in 2 genes were associated with sensitivity to NXP900, whilst none were significantly associated with sensitivity to dasatinib.

Mutations associated with sensitivity to NXP900 include NF2, aka Merlin, a key regulator of the Hippo signalling pathway[9]. Additionally, mutations to the gene encoding G-protein subunit alpha I2 (GNAI2) were associated with resistance to NXP900. G-protein coupled receptors have also been shown to regulate Hippo signalling[10].

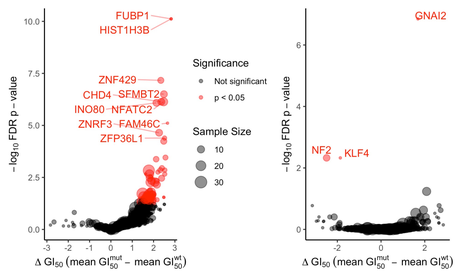

Figure 8 – *Sensitivity to NXP900 (right) and dasatinib (left) in cell lines with mutations to 568 cancer genes were compared to wild-type using a two-sample t-test with FDR adjustment for p-values. Mutations in genes that are significantly associated with a difference in sensitivity are displayed in red. For the dasatinib volcano plot, only the top 10 genes (by p-value) are labelled.*

NF2 mutation is associated with sensitivity to NXP900

The Hippo pathway is an important network that transduces extracellular signals to alter stemness, cellular proliferation, apoptosis, and angiogenesis via YAP/TAZ activation[9,11]. NF2 is a tumour suppressor and upstream regulator of the Hippo pathway shown to be a crucial mediator of contact inhibition and adherens junction formation. Approximately 23% of pleural mesothelioma and 7% of kidney carcinomas have mutations to NF2[8]. Interestingly, RKO and SNU-C2B colorectal and RL95-2 uterine cell lines with NF2 mutation were 3.8-fold, 73-fold, and 140-fold more sensitive to NXP900 than dasatinib.

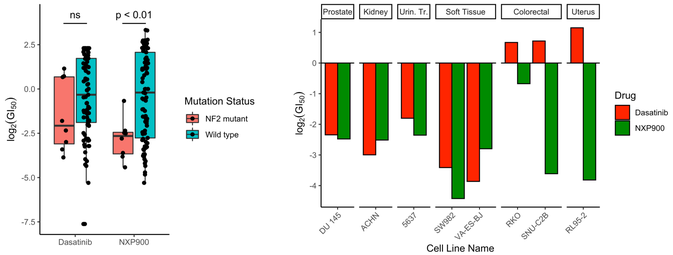

Figure 9 – *NF2 mutations are associated with increased sensitivity to NXP900, but not dasatinib (left). Significance was determined as in Figure 7. NF2 mutant colorectal and uterine cancer cell lines had large differences in sensitivity to NXP900 and dasatinib (right). Lineages are displayed at the top.*

Conclusions

- NXP900 and dasatinib induce SRC inhibition through different mechanisms of action, with NXP900 inhibiting both the catalytic and scaffolding functions of SRC.
- NXP900 has higher selectivity than dasatinib across the kinome and inhibits growth of different cancer lineages.
- Hippo signalling may play a role in sensitivity to NXP900 and dependencies in this network may represent exploitable synthetic lethalities.

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