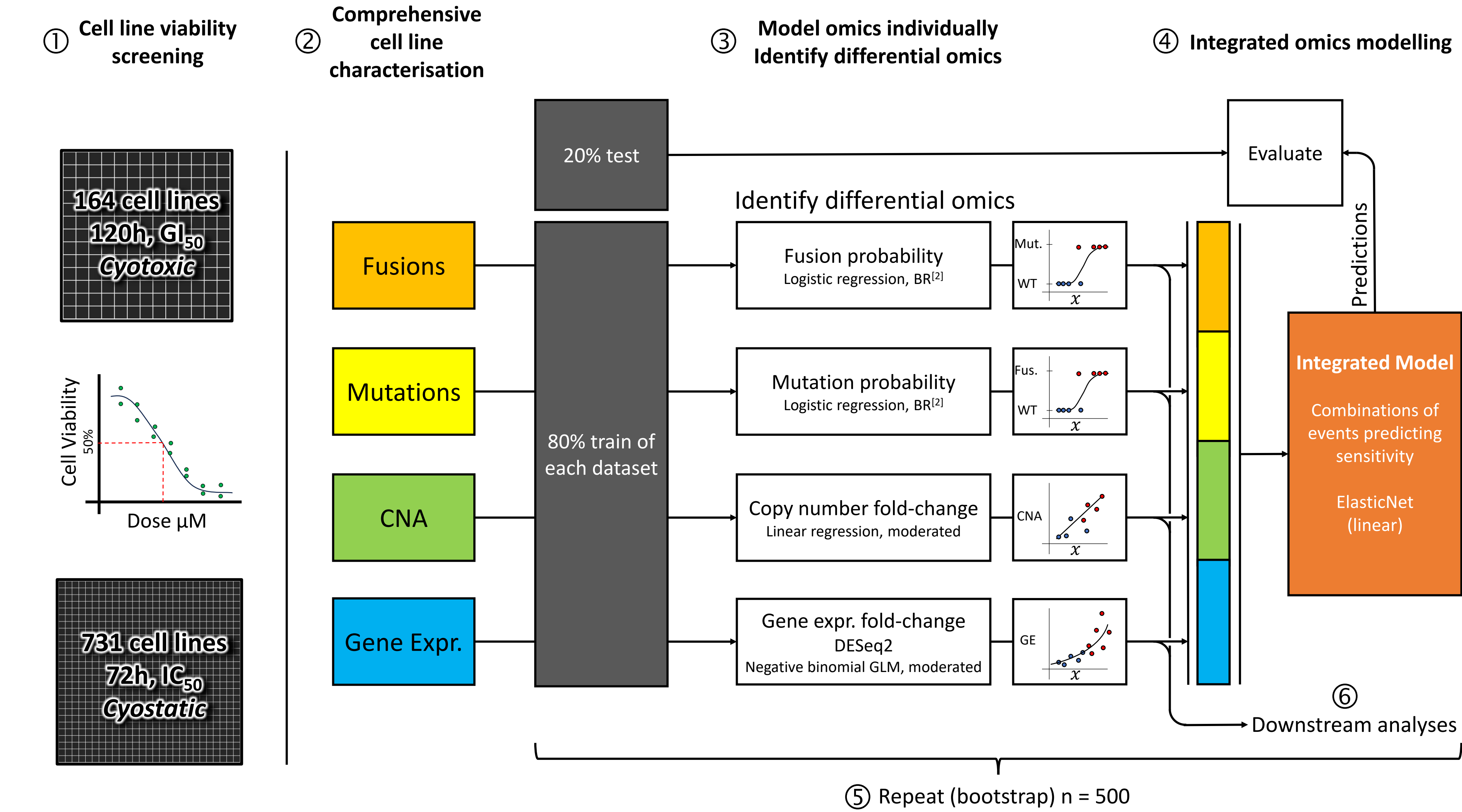


A multi-omics approach to identify biomarkers of response to the novel and selective SRC/YES1 inhibitor NXP900

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Abstract

- Identification of biomarkers of sensitivity or resistance to a compound is a crucial step in preclinical drug development.
- Combined with an understanding of drug mechanism of action, biomarkers of response can identify potential synthetic lethality opportunities, drug combinations, and guide patient stratification in the clinic leading to improved clinical outcomes.
- Integrated analysis of omics can reveal mechanisms underlying drug response. The presented framework integrates four different sources of omics data: gene expression, copy number, fusions, and mutations.
- The analysis framework was validated on known biomarkers of response to approved kinase inhibitors; BCR-ABL fusions to imatinib and dasatinib in myeloid leukaemia.
- The framework was utilised to identify biomarkers of response to the novel and highly selective SRC/YES1 inhibitor NXP900. ERBB4, a secondary target of NXP900, was identified as a potential biomarker of response to NXP900.
- Putative biomarkers are currently being validated in the wet-lab and validated biomarkers are expected to assist with patient stratification in clinical trials.

Methods

731 cell lines were screened by the Genomics of Drug Sensitivity in Cancer (GDSC) and 164 cell lines were screened by Oncolines against NXP900.

Comprehensive cell line characterisation was obtained from the Cancer Cell Line Encyclopedia (CCLE)¹. Whole-genome sequencing data was processed to produce copy number alteration (CNA) and mutation call datasets. Fusion calls and gene expression were produced from RNA-Seq data.

Omics were modelled individually to identify differential events between sensitive and resistant lines. Bias-reduced logistic regression was used to model the probability of binary events (fusion and mutations), linear regression with empirical bayes shrinkage was used to model CNA, and negative binomial GLM with empirical bayes shrinkage (DESeq2) was used to model gene expression data^{2,3}.

Differential events were concatenated and modelled with regularised (Elastic Net) regression to identify combinations of differential events predicting sensitivity. Predictions were compared with a held-out test set to evaluate the performance of the integrated model.

The process is bootstrapped 500-times, to ensure stability of identified biomarkers.

Traditional downstream analyses, such as GSEA and protein-protein association network analysis, were conducted on differential events to identify biological relationships.

Methods validation with FDA-approved compounds

The statistical framework was run on publicly-available cell line screening data from the GDSC. It identifies known biomarkers of response (BCR-ABL1 fusions) to the approved tyrosine kinase inhibitors imatinib and dasatinib, both dual SRC/ABL1 inhibitors (Figure 1.1, 1.2).

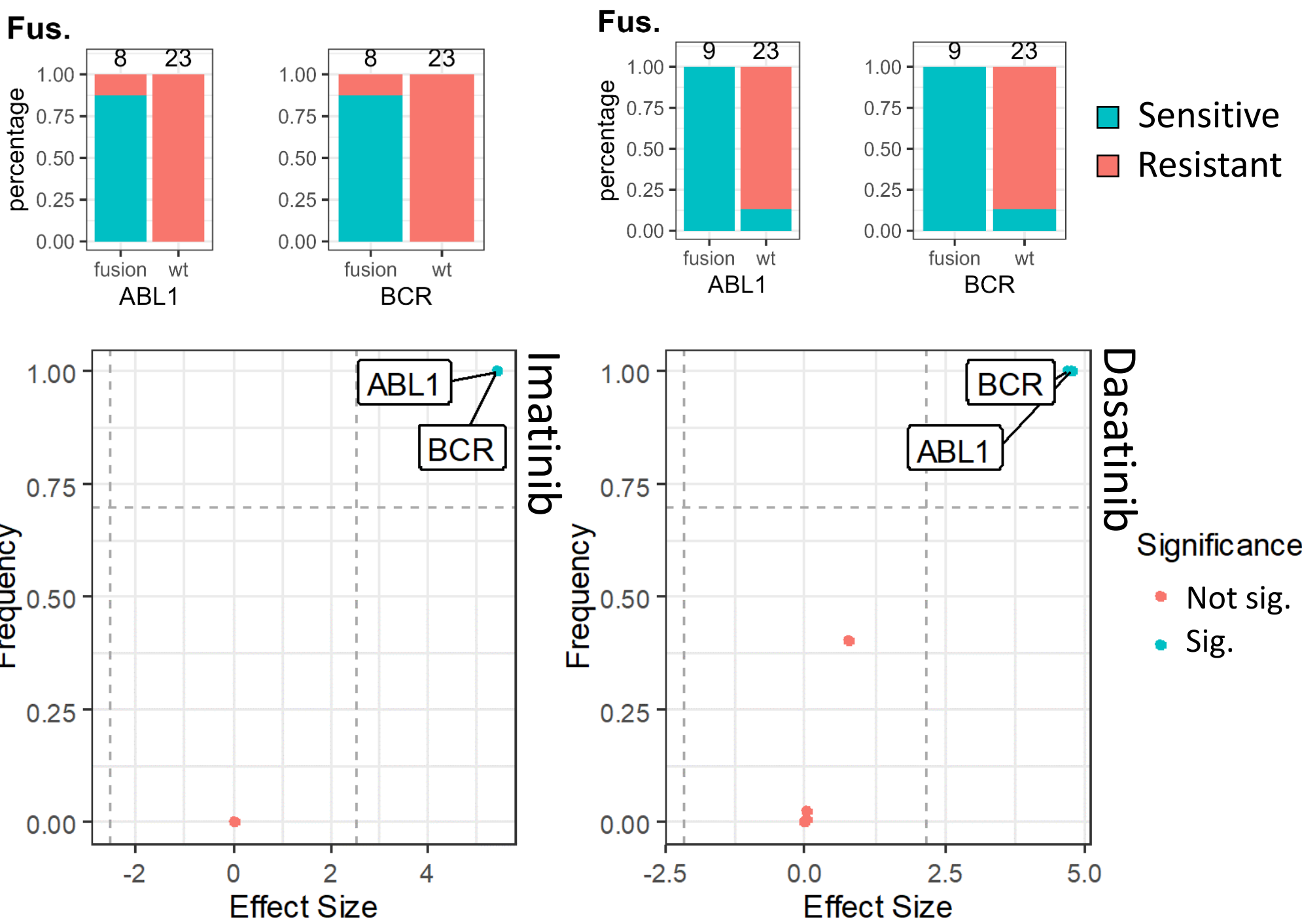


Figure 1.1 (left), Figure 1.2 (right) – The statistical framework identified BCR-ABL1 fusions as the key predictors of sensitivity to imatinib (1.1) and dasatinib (1.2). Frequency shows how often a biomarker is considered significantly associated with response, across bootstrap iterations.

ERBB4 is a secondary target of NXP900

In an extensive kinase screen ERBB4 was identified as a secondary target of NXP900 (Table 2.1, Figure 2.1).

Kinome screen		
Kinase	IC ₅₀ (nM)	Class
YES1	0.47	SFK
SRC	2.44	SFK
HCK	2.77	SFK
ERBB4	26.4	EGFR gene family

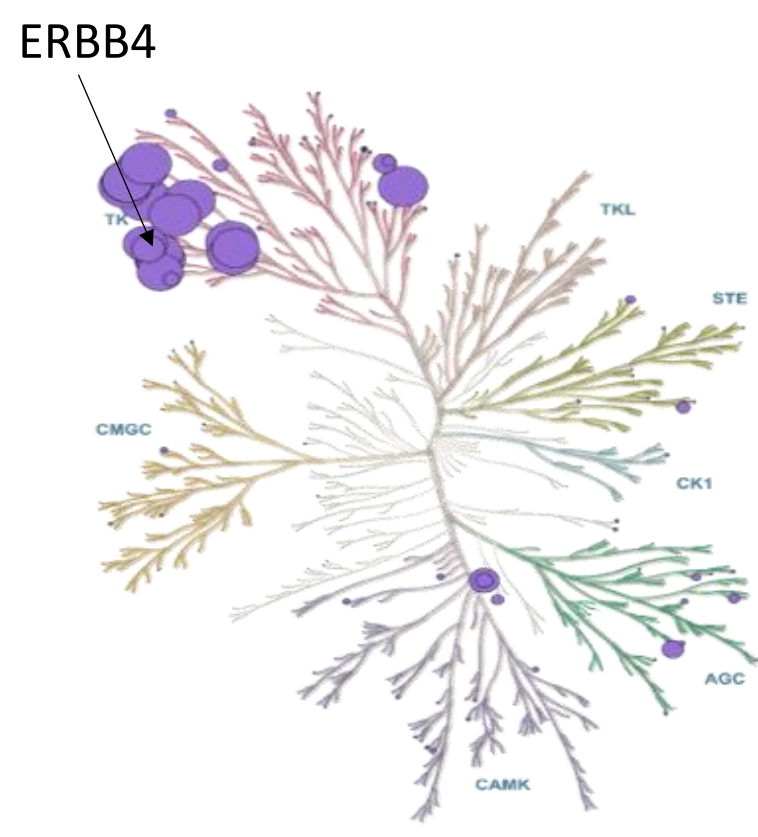


Figure 2.1 (left), 2.2 (right) – Results from a kinome screen against NXP900. (2.1) The table displays activity percentage remaining after treatment with NXP900. (2.2) Graphical representation of the kinome, purple dots show kinases inhibited by NXP900.

Increased ERBB4 expression predicts sensitivity to NXP900 in solid tumours

ERBB4 was identified as one of the key predictors in the multi-omic elastic net model across solid tumours in the Oncolines dataset (Figure 3.1), with increased expression predicting sensitivity (Figure 3.2). Dose-response curves of the highest and lowest ERBB4-expressing cell lines reveal clear differences in sensitivity (Figure 3.3).

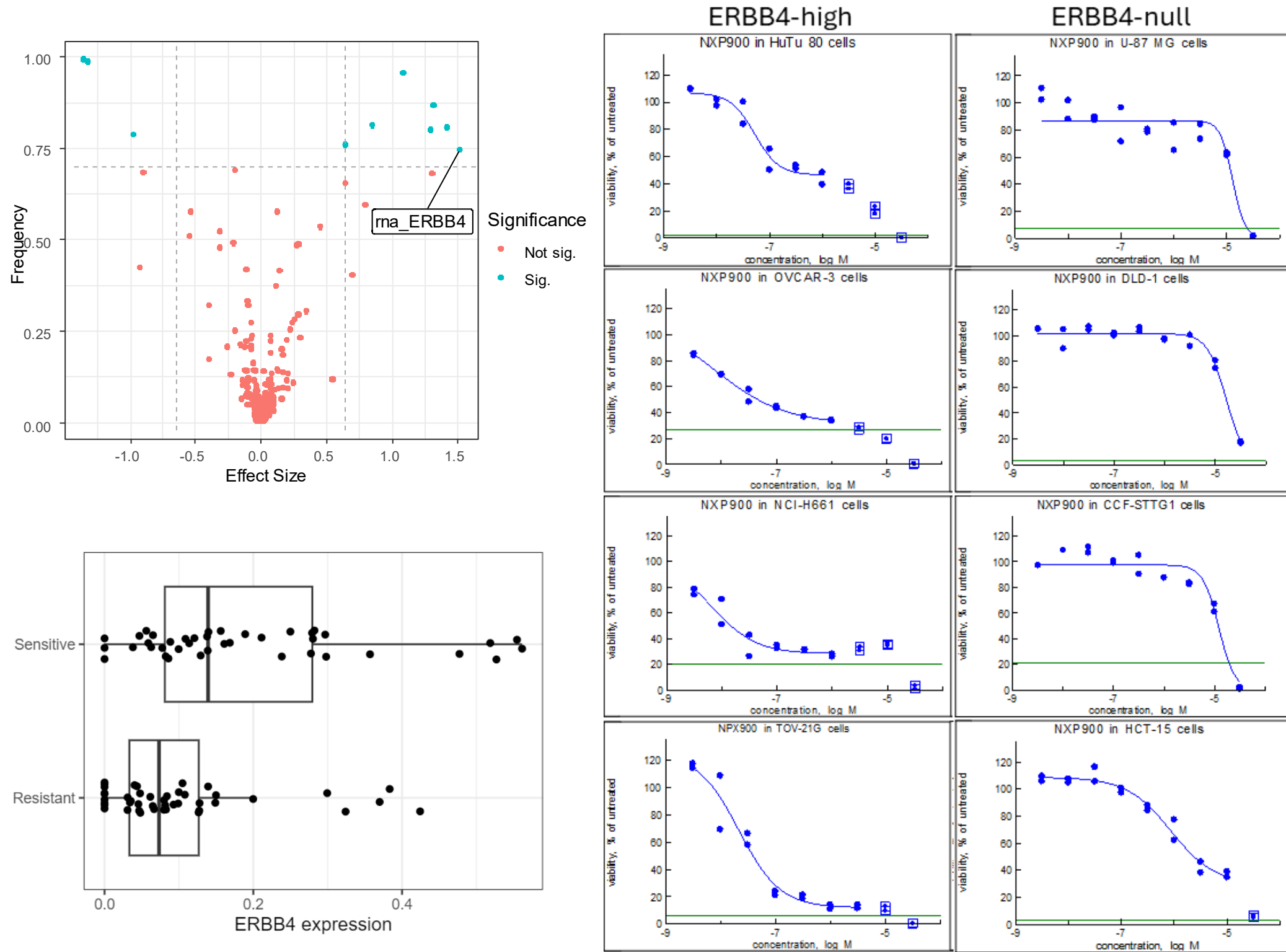


Figure 3.1 (left, top), 3.2 (left, bottom), 3.3 (right) – The statistical framework identified ERBB4 expression as a key predictor of sensitivity to NXP900. (3.1) Volcano plot showing the top predictors of sensitivity to NXP900. ERBB4 is labelled. (3.2) Boxplot showing the difference in expression between sensitive and resistant cell lines. (3.3) Dose-response curves (from Oncolines) showing response to NXP900 in ERBB4-high and ERBB4-null cell lines.

Conclusion

- The statistical framework presented for the identification of biomarkers of drug response has been validated on known biomarkers of approved compounds.
- High ERBB4 expression was identified as a potential biomarker of sensitivity to NXP900.
- Xenograft mouse models of group 4 medulloblastoma, driven by ERBB4/SRC signalling, showed marked response to NXP900.
- High ERBB4 expression, activating mutations and/or copy number alterations have also been identified in lung, colorectal, stomach, head and neck, and ovarian cancers, representing a wide range of potential therapeutic applications for NXP900⁴.

ERBB4/SRC signalling interacts with the PI3K-Akt pathway

ERBB4 has been shown to directly associate with both SRC and PI3K-subunits to promote cell growth, survival and proliferation^{4,5}. Furthermore, SRC association with RTKs, such as ERBB proteins, has been shown to stimulate PI3K-Akt signalling in a SRC-dependent manner via FAK activation⁶.

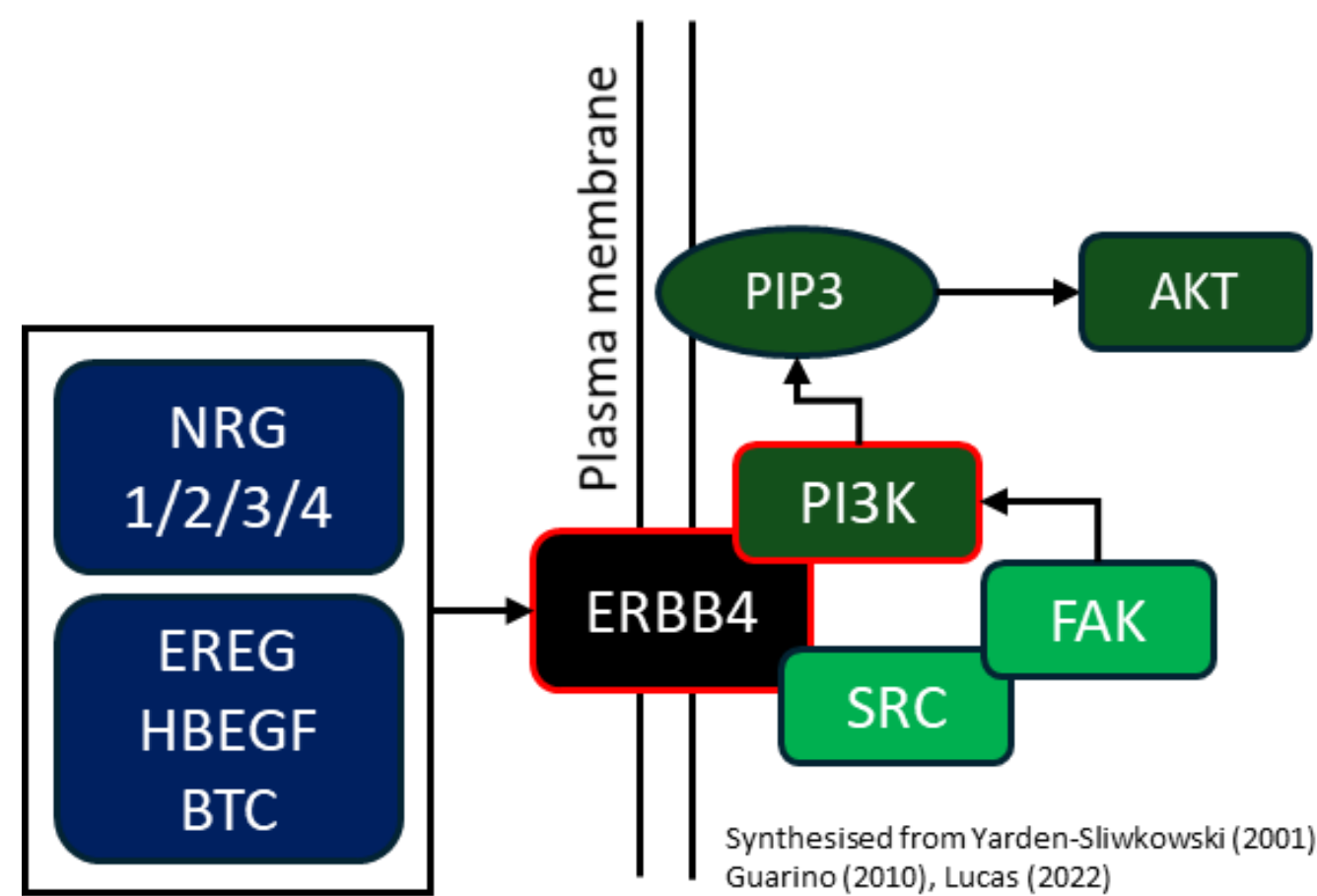


Figure 4 – Regulation and downstream signalling of ERBB4. Red borders show genes that were upregulated in sensitive cell lines.

Gene set enrichment analysis (GSEA) revealed the PI3K-Akt pathway was one of the most enriched gene sets in sensitive cell lines, alongside cell adhesion signalling (adjusted p < 0.05).

NXP900 inhibits ERBB4/SRC-driven medulloblastoma

Several cancers exhibit aberrant ERBB4 signalling. Recently, group 4 medulloblastoma was found to be driven by ERBB4-SRC signalling⁷. When tested in a murine xenograft model of medulloblastoma group 4, NXP900 was highly effective, demonstrating complete inhibition of SRC phosphorylation in vivo, inducing potent tumour growth inhibition, and increased animal survival (Figure 5.1, 5.2, 5.3). Data from collaboration with the Chesler lab at The Institute for Cancer Research, London.

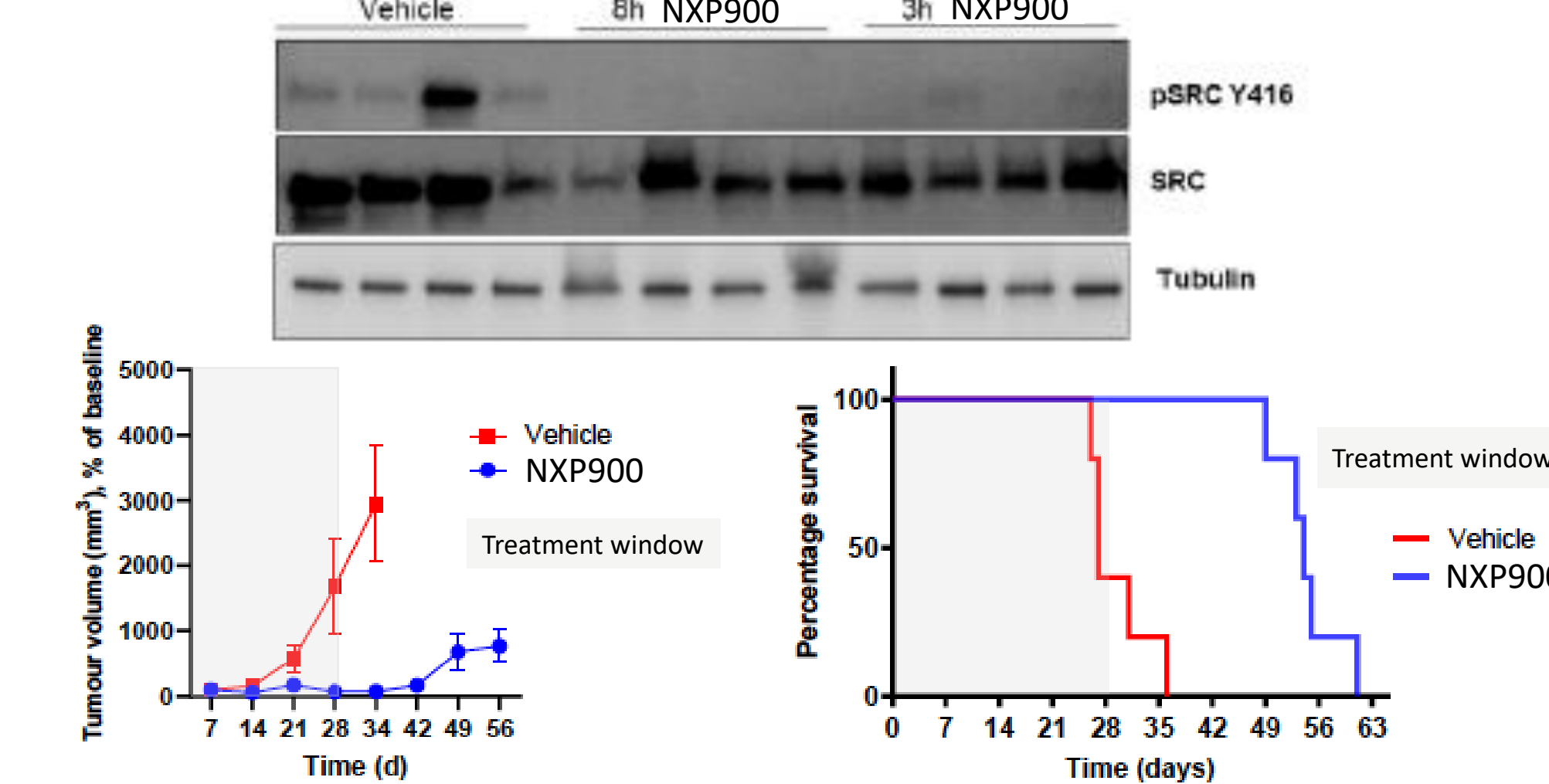


Figure 5.1 (top), 5.2 (left, bottom), 5.3 (right, bottom) – NXP900 potentially inhibits SRC and tumour growth and improves animal survival *in vivo*. (5.1) Western blot of SRC and phospho-SRC with no treatment (vehicle), 3h NXP900, and 8h NXP900 treatment. (5.2) Inhibition of tumour growth with control (vehicle) and NXP900 treatment. (5.3) Survival of mice treated with control (vehicle) and NXP900.

References

- Ghandi, M. et al., 2019. Next-generation characterization of the cancer cell line encyclopedia. *Nature*, 569(7757), pp.503-508.
- Kosmidis, I., Firth, D., 2009. Bias reduction in exponential family nonlinear models. *Biometrika* 96, 793–804.
- Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* 15, 550.
- Lucas, L.M. et al., 2022. The Yin and Yang of ERBB4: Tumor Suppressor and Oncoprotein. *Pharmacol Rev* 74, 18–47.
- Yarden, Y., Slivkowsky, M.X., 2001. Untangling the ErbB signalling network. *Nat Rev Mol Cell Biol* 2, 127–137.
- Guarino, M., 2010. Src signaling in cancer invasion. *Journal of Cellular Physiology* 223, 14–26.
- Forget, A. et al., 2018. Aberrant ERBB4-SRC Signaling as a Hallmark of Group 4 Medulloblastoma Revealed by Integrative Phosphoproteomic Profiling. *Cancer Cell* 34, 379-395.e7.