

# Exploring BRD4 as a Potential Target for Cancer Treatment using Computational and Experimental Approaches



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## BACKGROUND

- BRD4 is a member of the BET (bromodomain and extraterminal domain-containing) protein family.
- BRD4 is ubiquitously expressed in human cells and has a role in the promotion of gene transcription, including driving oncogenes in tumour cells.
- In rare and aggressive squamous cell epithelial cancer (NUT midline carcinoma (NMC)), BRD4 can be fused with the NUT oncogene.
- Inhibition of BRD4 using the chemical probe JQ1 shows significant growth inhibition of NMC tumour cell lines.<sup>1</sup>
- Broad spectrum BET inhibitors have also shown preclinical efficacy in other malignancies, including haematological and solid tumours.<sup>2</sup>

## HYPOTHESIS AND AIMS

- Hypothesis:** Targeting the BRD4 protein is a promising therapeutic approach for cancer drug development, which should be evident by examining of the dependency of tumour cell lines to genetic and chemical inhibition of BRD4, combined with assessing its structural druggability.
- Aim:** To critically examine BRD4 as an anti-cancer drug target using *in silico* and *in vitro* approaches for drug discovery.

## RESEARCH DESIGN

### *In silico* Bioinformatic Analysis

Cancer cell lines dependency, sensitivity to BRD4 inhibition, mutations, sequence analysis

DepMap (depmap.org) | COSMIC-3D, Jalview and Clustal Omega

### *In silico* Structural Biology Analysis

Three-dimensional structure-activity relationship of BRD4 inhibitions

UCSF Chimera (structure visualisation) | COOT (ligand fitting) | Gromacs (molecular dynamic simulation)

### *In vitro* Target validation

Cancer cells sensitivity to BRD4 inhibition

Sulforhodamine B growth inhibition assay | Western blotting

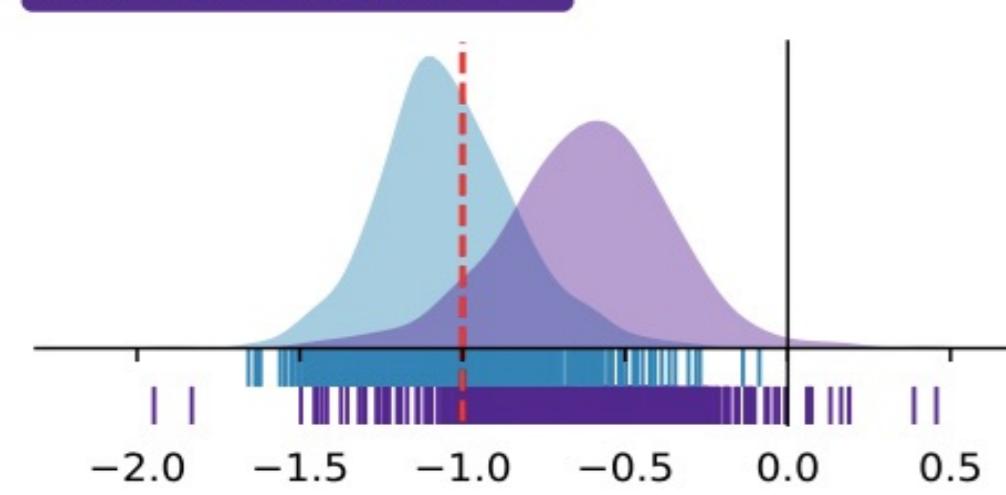
## RESULTS

CRISPR (DepMap 21Q2 Public+Score, CERES): 964/978

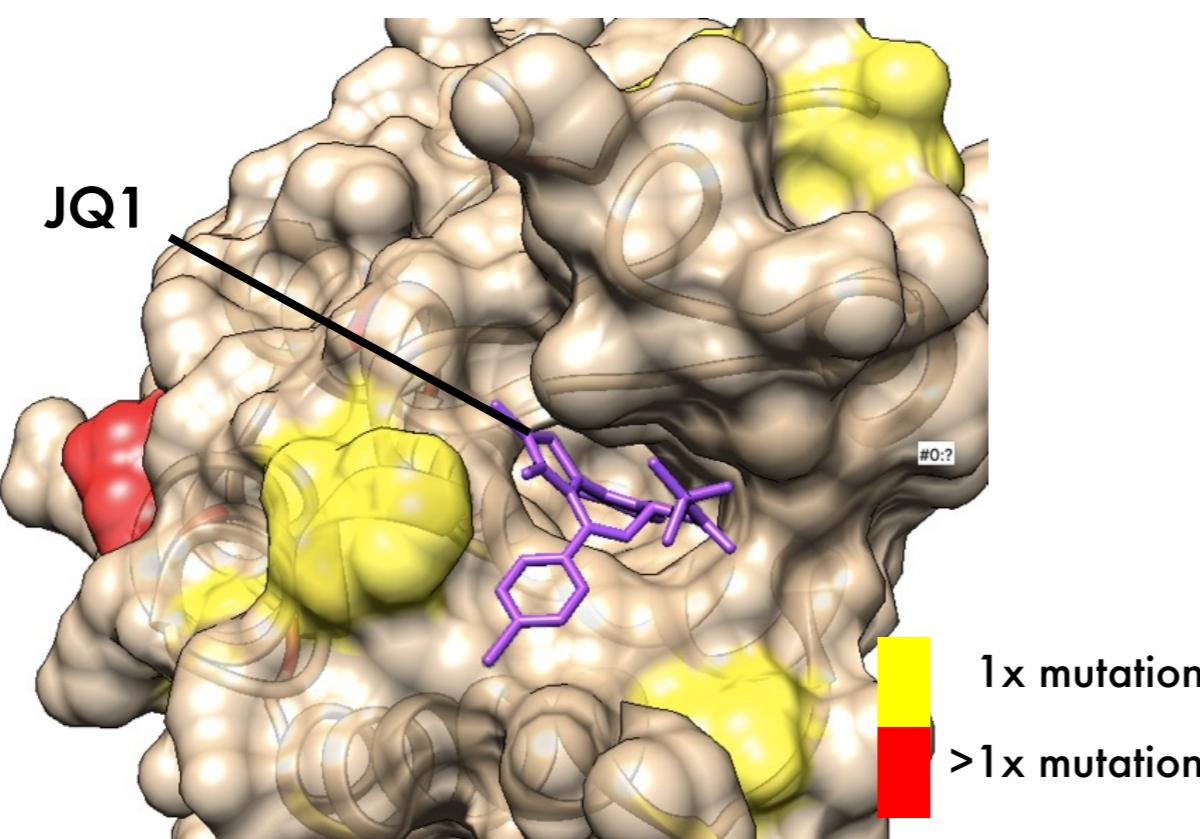
COMMON ESSENTIAL

RNAi (Achilles+DRIVE+Marcotte, DEMETER2) 411/710

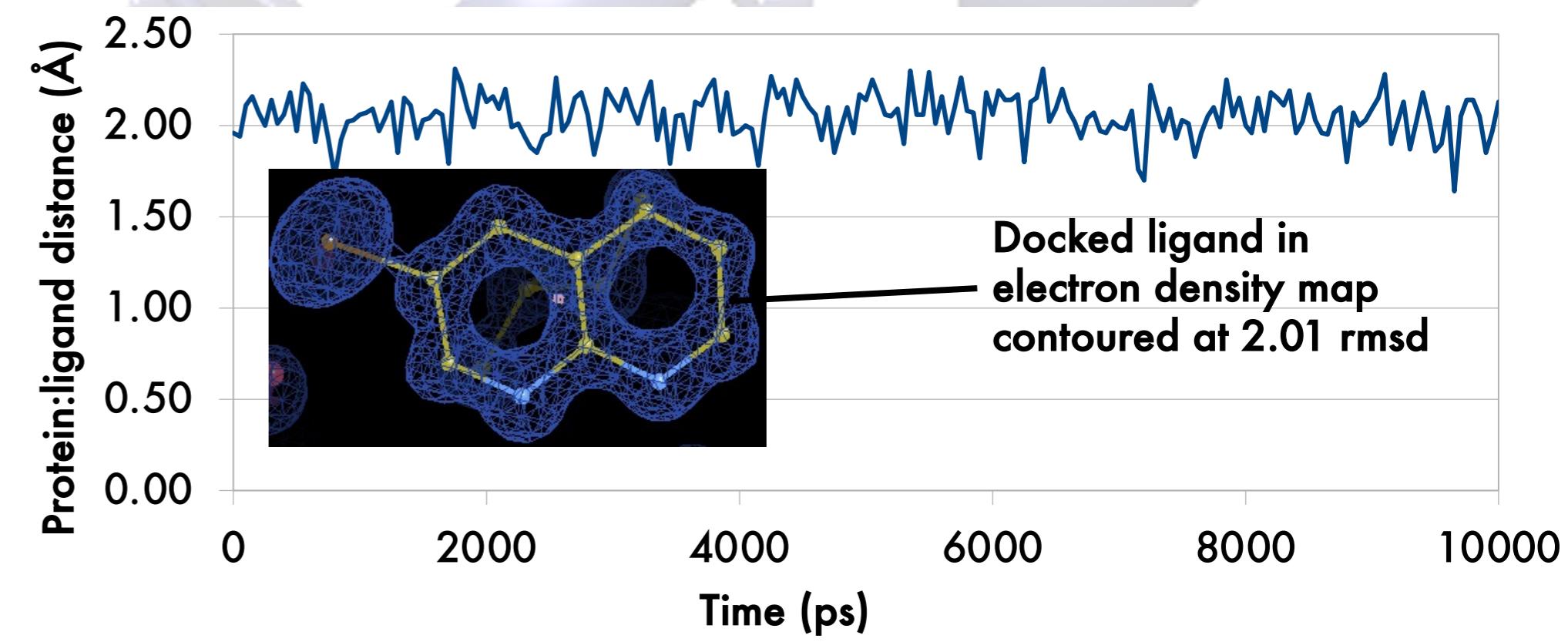
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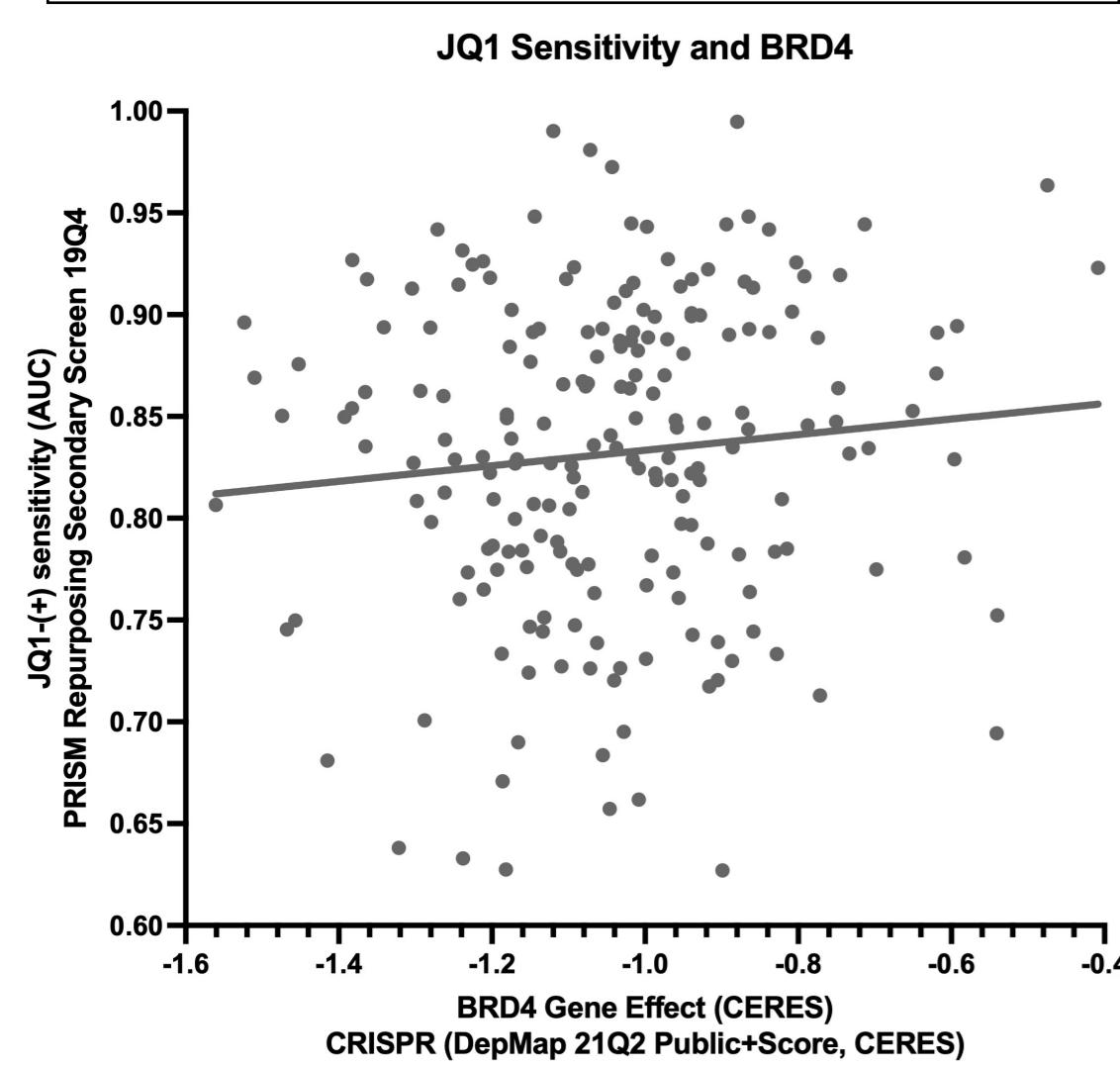
Wide dependency of cancer cell lines to BRD4 suggests active role in cancer cell proliferation, although this might also lead to toxicity upon inhibition.



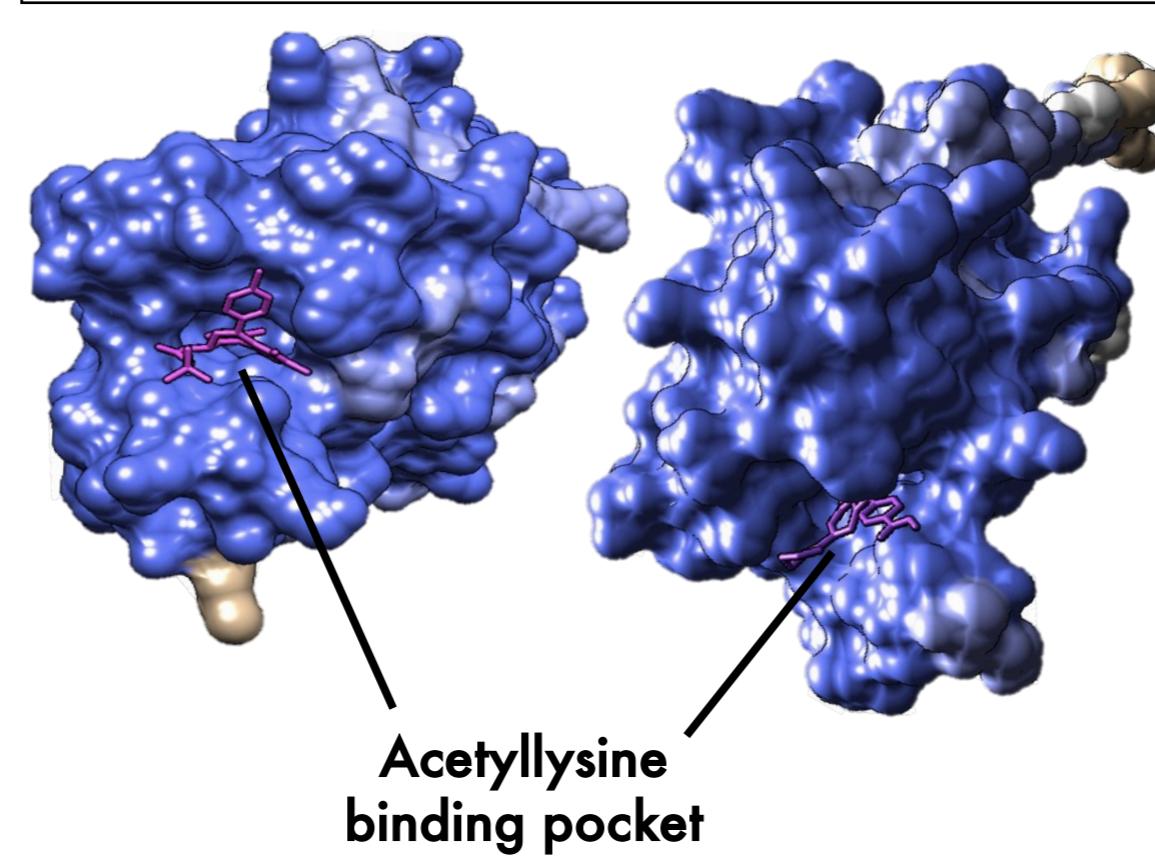
Commonly mutated residues in BRD4 mapped onto 3D structure suggests little likelihood of these interfering with ligand binding sites or known sites for protein-protein interactions



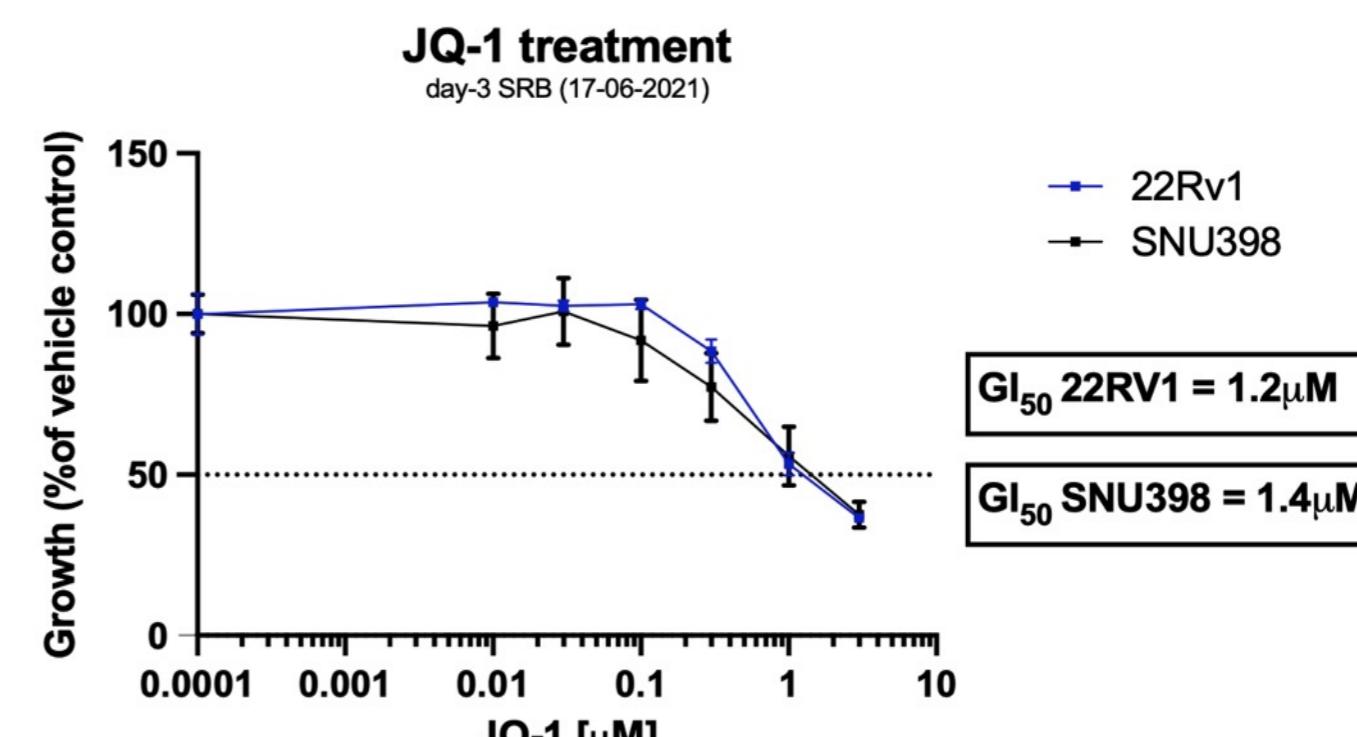
Molecular dynamics simulation showing protein:ligand distance between 1.64 – 2.31 Å, indicating a stable interaction between docked ligand and BRD4. Therefore, confirming a good druggable site in BRD4.



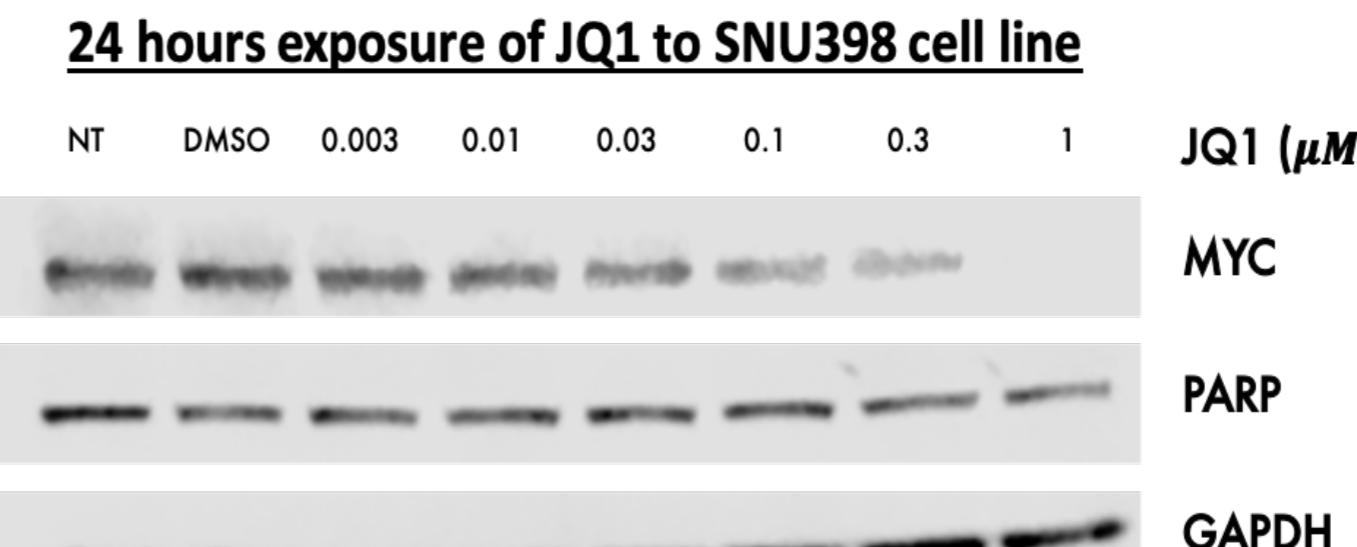
Bioinformatics data suggests no correlation between BRD4 deletion to JQ1 sensitivity. Note that haematological cell lines are underrepresented in drug sensitivity data.



BRD4-BD1 (left) and BRD4-BD2 (right) are highly conserved across species, indicating its critical role in basic cellular stability and function of the protein.



Growth inhibition data (SRB) shows JQ1 has a similar effect on the proliferation of a prostate (22Rv1) and liver cancer (SNU398) tumour cell lines



Western blot results showing JQ1 inhibition to SNU398 can lead to loss of a known oncogenic driver, Myc.

## CONCLUSION

- The *in silico* and *in vitro* data have suggested a high dependency of tumour cell lines on BRD4, a highly druggability binding site, and chemical inhibition to reduce the oncogenic protein Myc, making it a promising target for cancer therapy.
- Broad activity of JQ1 to another target in cancer cells and underrepresentation of haematological cell lines may contribute to lack of sensitivity of JQ1 to BRD4 as shown in bioinformatics data.
- However, further research is still necessary to determine whether specific inhibition of BRD4 may reduce the toxicity.

## ACKNOWLEDGEMENTS

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## KEY REFERENCES

- (1) Filippakopoulos P, Qi J, Picaud S, et al. Selective inhibition of BET bromodomains. *Nature* 2010; 468: 1067–1073
- (2) Xu Y, Vakoc CR. Targeting cancer cells with BET bromodomain inhibitors. *Cold Spring Harb Perspect Med* 2017; 7: 1–17.