CID-078, an orally bioavailable cyclin A/B-RxL inhibitor elicits anti-tumor activity in neuroblastoma models

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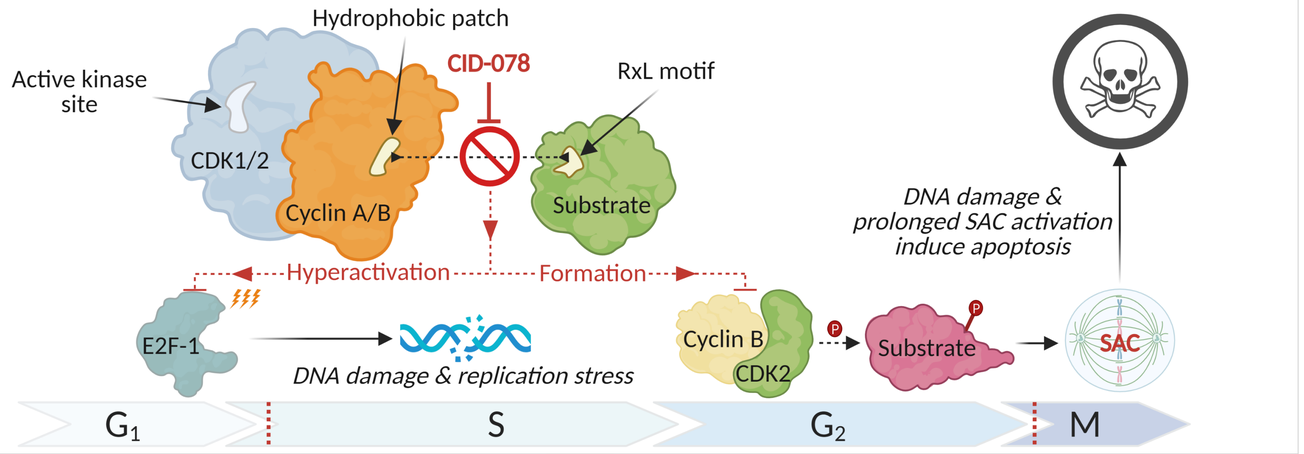
Background

Neuroblastoma (NB) is characterized by frequent deregulation of the G1 cell cycle checkpoint. A hallmark of NB is elevated oncogenic E2F activity driven by alterations in the CDK-RB-E2F axis, which is associated with poor survival. Drugs that selectively target these aberrant cell cycle mechanisms therefore hold great potential to enhance NB treatment strategies. CID-078 is a clinical-stage, orally bioavailable, and cell-permeable macrocyclic inhibitor. It selectively disrupts protein interactions between cyclin A2–Cdk2, cyclin B1–Cdk1, and their cell cycle substrates by targeting the RxL binding motif, a critical interaction site. This disruption has been shown to induce DNA damage via E2F1 hyperactivation, promote the formation of neomorphic cyclin B–CDK2 complexes, and activate the spindle assembly checkpoint (SAC), ultimately leading to synthetic lethality in various E2F-driven models of adult cancers[1]. Here, we present the preclinical evaluation of the anti-tumor effects of CID-078 in multiple *in vitro* models of NB.

Mechanism



1. Neuroblastoma cell lines show sensitivity to CID-078

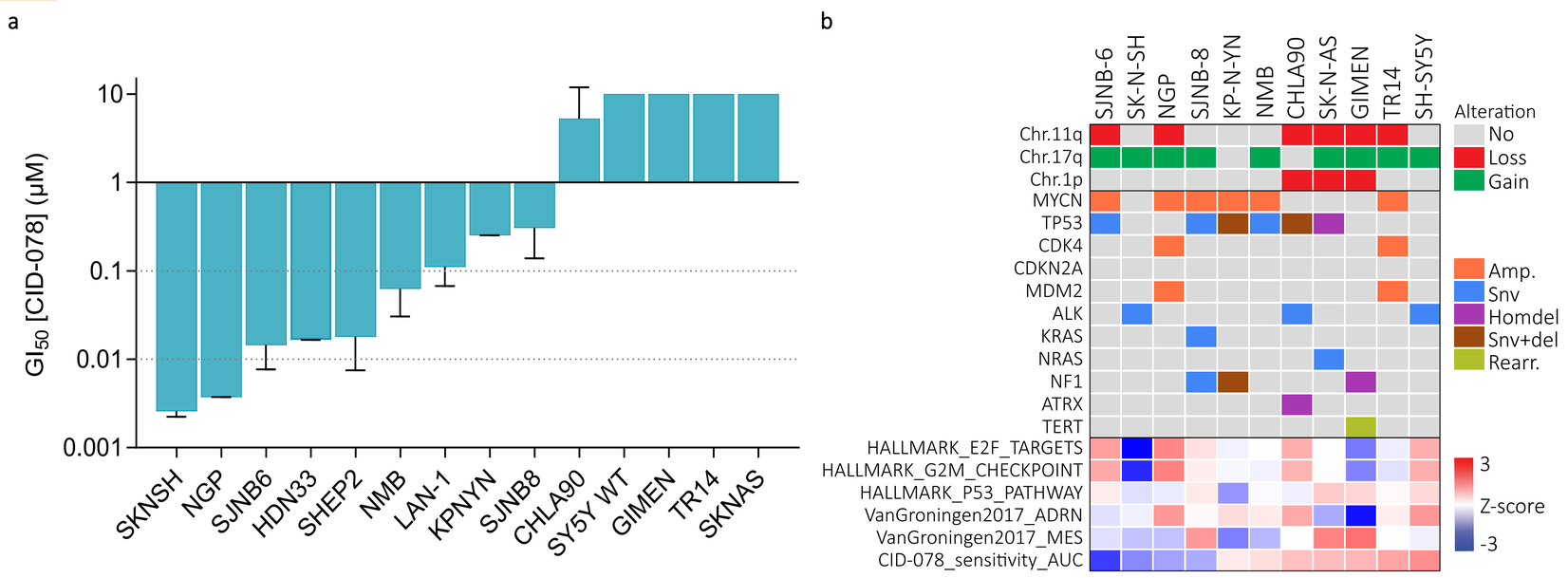


Figure 1 - a. Waterfall plot of *in vitro* response to CID-078 in a panel of 14 neuroblastoma cell lines. Cells were treated for a total of 72 hours after which cell growth was analyzed by MTT assay. Plotted absolute GI^{50} values are averages of at least two independent experiments. **b.** Heatmap describing chromosomal alterations, genomic alterations, Hallmark pathway scores, ADRN signature score, MES signature score, and CID-078 sensitivity in a selection of screened cell lines. From left to right: high CID-078 sensitivity to low CID-078 sensitivity based on area under the curve. Hallmark scores were calculated using the Gene Set Variation Analysis method[2]. Signature scores were calculated using the gene signature from Van Groningen et al. 2017[3]. All data was plotted using the R2 Bioinformatics Platform[4].

2. *CDKN2A* inactivation sensitizes SH-SY5Y

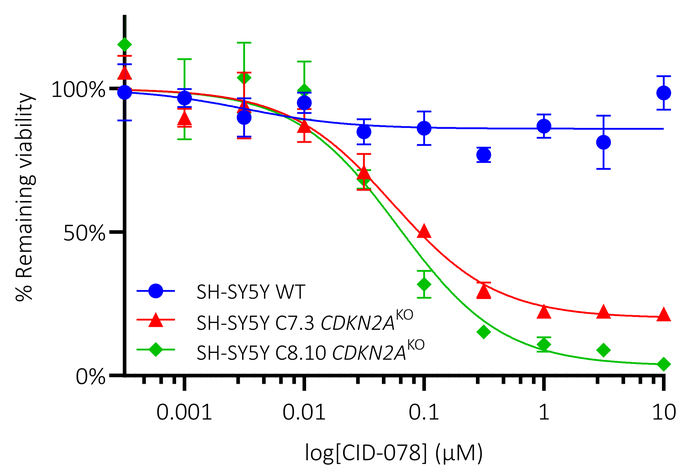


Figure 2. Drug response curve displaying CID-078 sensitivity of SH-SY5Y wild-type cells (GI^{50} >10 µM) and two SH-SY5Y clones in which *CDKN2A* was inactivated with CRISPR-Cas9 (GI^{50} = 55 nM & 6 nM). Cells were treated for 72h after which growth was measured with MTT assay. Data is representative of two experiments.

Results

3. Patient-derived organoid models display high CID-078 sensitivity

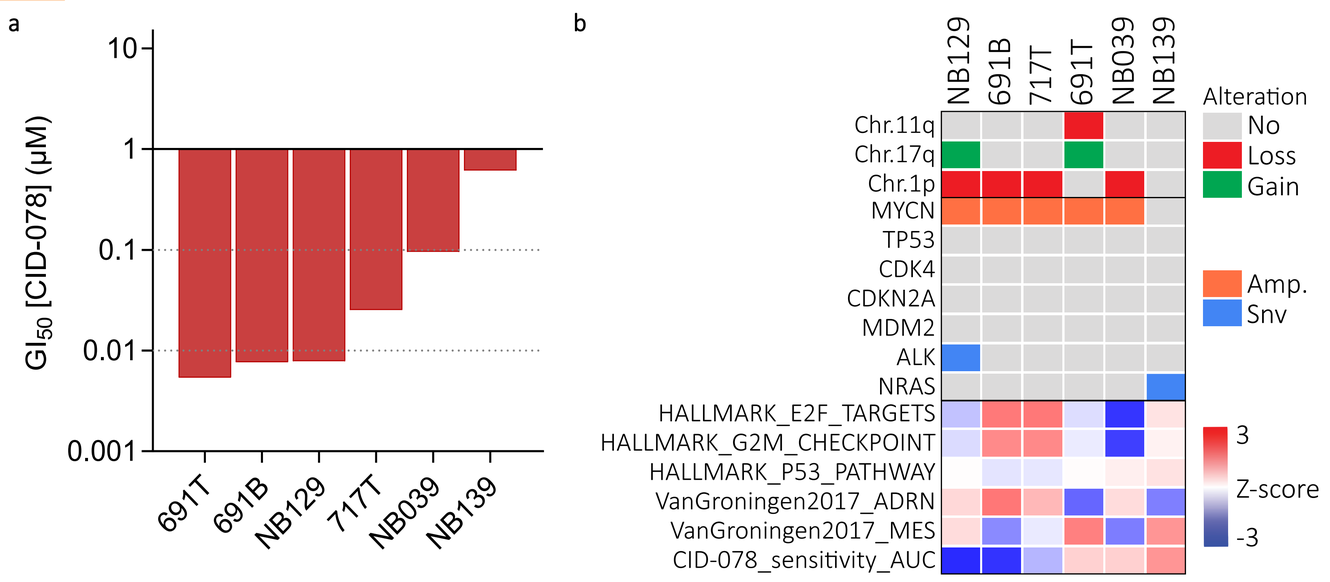


Figure 3 - a. Waterfall plot of *in vitro* response to CID-078 in a panel of 6 patient-derived organoid models. Cells were treated for a total of 120 hours after which cell growth was analyzed by CellTiterGlo assay. Plotted absolute GI^{50} values are of a single experiment. **b.** Heatmap describing chromosomal alterations, genomic alterations, Hallmark pathway scores, ADRN/MES signature scores, and CID-078 sensitivity in a selection of screened models. High E2F target pathway score appears to correlate with drug sensitivity. From left to right: high CID-078 sensitivity to low CID-078 sensitivity based on area under the curve. Scores were calculated and plotted as described below figure 1.

4. CID-078 induces DNA damage, G2/M arrest, SAC activation

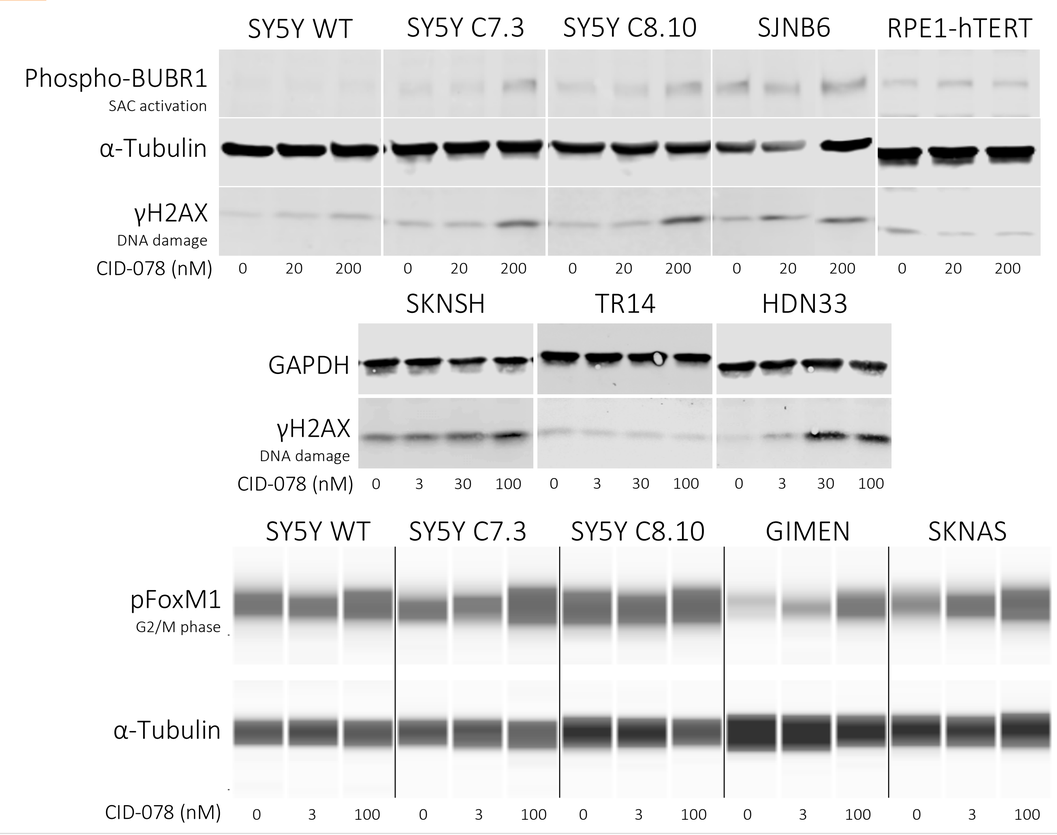
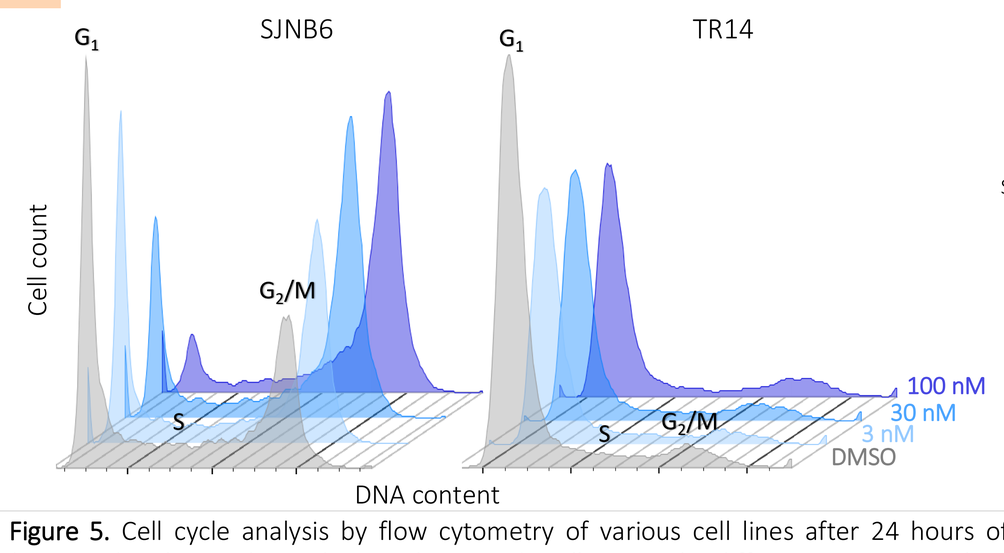


Figure 4. Immunoblot analysis of various cell lines after 24h of CID-078 treatment. SY5Y C7.3 and SY5Y C8.10 are clones with *CDKN2A* inactivation. hTERT-RPE1 cells are included as a healthy control line with intact, non-aberrant cell cycle regulation.

5. CID-078 treatment induces cell cycle arrest in G2/M phase



	%G2/M increase	CID-078 AUC
SY5Y C7.3	52	205
TR14	75	706
SY5Y C8.10	126	77
SY5Y WT	130	918
SHEP2	169	288
HDN33	180	729
SKNAS	279	553
GIMEN	317	583
NGP	382	133
SJNB6	455	38
NMB	458	388
SKNSH	632	93

Figure 5. Cell cycle analysis by flow cytometry of various cell lines after 24 hours of CID-078 treatment. SJNB6 (sensitive) is shown alongside TR14 (resistant) to illustrate the differences in G2/M-phase arrest observed across the tested cell line panel. The table shows the percentage increase in the G2/M-phase population in cells treated with 100 nM CID-078 compared to the DMSO control, alongside compound sensitivity (AUC) after 72 hours of treatment.

Summary

Our preclinical evaluation demonstrates that CID-078 shows strong anti-tumor activity across multiple *in vitro* NB models. Many models exhibited high sensitivity, consistent with the rationale of targeting deregulated cell cycle pathways in NB.

Due to NB's genomic heterogeneity, identifying a single predictive biomarker remains challenging. However, CDKN2A deletion emerged as a potential marker, as its inactivation sensitized SH-SY5Y cells to CID-078. This suggests that CDKN2A-deleted cells exhibit enhanced sensitivity to CID-078, which is potentially mediated by increased E2F activity. Additional biomarkers such as MYCN amplification are under investigation.

Mechanistic studies confirm CID-078's mode of action in NB cells, showing induction of DNA damage, spindle assembly checkpoint (SAC) activation, and G2/M arrest. FACS analysis further demonstrated G2/M-phase cell cycle arrest, which is consistent with SAC engagement.

Overall, these findings support CID-078 as a promising therapeutic candidate for NB, with potential biomarker-driven applications.

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References

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3. Van Groningen, T. *et al*. Nat Genet 49, 1261–1266 (2017).
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