Investigating the cyclin A/B RxL inhibitor CID-078 in pediatric cancers with RB1 loss and high E2F1

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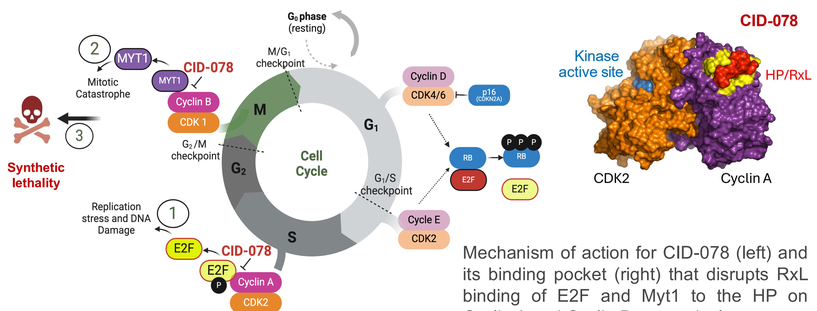
1. BACKGROUND AND DRUG MECHANISM

- The **cyclin-dependent kinase (CDK)-RB-E2F axis** forms the core transcriptional machinery driving **cell cycle progression**. **Alterations in RB1** or other key components occur in many cancers, leading **to increased oncogenic E2F activity**. **E2F downregulation** at the end of S phase relies on the interaction between the cyclin's **conserved hydrophobic patch (HP)** and the **RxL motif** found on E2F and other cyclin/CDK substrates. **Disrupting** this **cyclin A/E2F RxL interaction** leads to **E2F hyperactivation and synthetic lethality** in E2F-driven tumors.

- **CID-078** is a **novel**, **orally bioavailable**, **cell-permeable**, **potent and selective macrocycle that disrupts the RxL binding of E2F and Myt1 to the HP on cyclin A2-CDK2 and cyclin B1-CDK1.** CID-078 induces cell cycle arrest at G2/M phase leading to apoptosis.

- CID-078 has demonstrated **potent and selective antitumor activity** across multiple preclinical **E2F-driven cancer models**, including neuroblastoma[1], Triple Negative Breast Cancer (TNBC)[2], ER+/HER2 Breast Cancer[3], and Small Cell Lung Cancer (SCLC)[4], which harbor **CDK/RB/E2F pathway alterations** leading to **E2F hyperactivation**[5]. These findings are consistent with the **proposed mechanism of action of CID-078**.



Mechanism of action for CID-078 (left) and its binding pocket (right) that disrupts RxL binding of E2F and Myt1 to the HP on Cyclin A and Cyclin B respectively.

2. AIMS

- To perform preclinical efficacy testing of CID-078 in nine pediatric cell lines.

- To analyse preliminary correlations between CID-078 sensitivity and *RB1*, *E2F1*, and *CDKN2A/B* biomarkers.

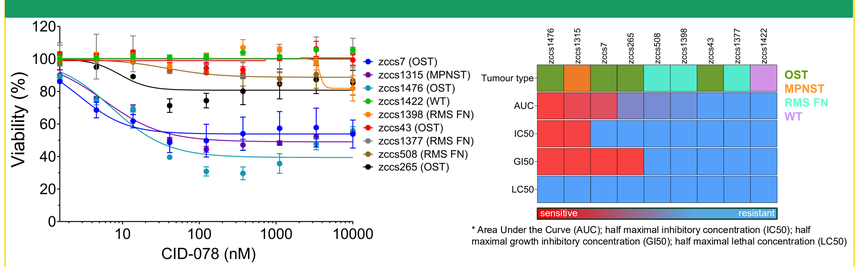
- To identify the proportion of pediatric cancer patients harbouring *RB1*, *E2F1*, and *CDKN2/B* alterations that may be targetable with CID-078.

3. PATIENT COHORT AND METHODS

- **ZERO Childhood Cancer Precision Medicine Program (ZERO)** performs comprehensive molecular profiling (whole genome sequencing, RNA-sequencing and methylation profiling) and *in vitro* high-throughput drug screening on matched patient samples where available for all children (≤ 18 years) with cancer in Australia, regardless of disease type[6,7].
- We obtained **9 cells lines from ZERO**, that either had ***RB1* alterations**, **increased *E2F1* expression**, or ***CDKN2A/B* loss** to assess potential sensitivity to CID-078 *in vitro* through cell viability assays.

ZERO ID	Tumour Type	*RB1* status	*CDKN2A/B* Status	E2F1 expression (TPM)	Median E2F1 (TPM)
zccs7	Osteosarcoma (OST)	Intragenic deletion & 1 copy loss	wt	21.01	5.10
zccs265		Germline SNV	wt	11.72	
zccs43		wt	wt	23.78	
zccs1476		wt	*CDK4* amplification & high expression	9.83	
zccs1377	Embryonal rhabdomyosarcoma (RMS FN)	wt	wt	12.89	6.42
zccs1398		wt	wt	8.01	
zccs508		wt	*CDKN2A* 1 copy loss	7.63	
zccs1422	Wilm's tumour (WT)	wt	wt	9.42	6.34
zccs1315	Malignant peripheral nerve sheath tumour (MPNST)	wt	*CDKN2A/B* biallelic loss	3.3	4.26

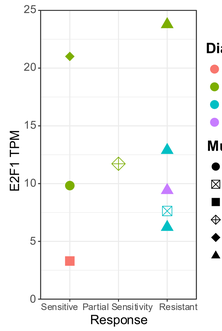
4. CID-078 *IN VITRO* EFFICACY RESULTS



* Area Under the Curve (AUC); half maximal inhibitory concentration (IC50); half maximal growth inhibitory concentration (GI50); half maximal lethal concentration (LC50)

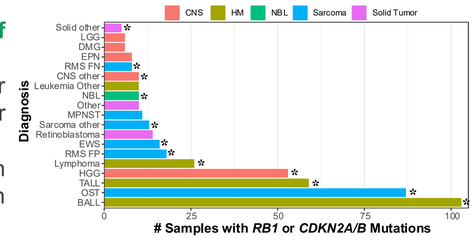
One-third of the samples (3/9) demonstrated **high sensitivity** with substantial growth inhibition and **1** sample showed **moderate sensitivity**. However, this effect attenuated at higher doses in the sensitive samples.

5. MOLECULAR CORRELATION



Preliminary findings suggest:
- **biallelic loss** of ***RB1*** or ***CDKN2A/B*** indicative of **sensitivity**
- expanded cohort required to determine if ***E2F1 mRNA expression*** in **absence** of ***RB1*** or **CDK alterations** indicative of sensitivity
- further testing required to determine if drug efficacy is correlated with diagnosis

From the **ZERO cohort of 2941 tumors:**
- **18%** of samples harbour either ***RB1* alterations** or ***CDKN2A/B* loss**
- **12** diagnosis have high *E2F1* mRNA expression (indicated by *)



6. CONCLUSIONS AND FUTURE DIRECTIONS

- In this small cohort, **3 of the 4 Osteosarcoma** cell lines demonstrated **moderate** to **high sensitivity** to **CID-078**.
- **Expanded cohort** encompassing additional diagnosis needs to be conducted to confirm **biallelic loss** of ***RB1*** or ***CDKN2A/B*** is indicative of **response** to CID-078 in pediatric cancer
- Our preclinical findings support CID-078 as a **promising therapeutic candidate** and emphasise the potential benefit of **combination therapy** to enhance efficacy.

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