Orally bioavailable Cyclin A/B RxL macrocycle has antitumor effect in SCLC and NSCLC cell line derived (CDX) and patient derived xenograft (PDX) models

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Introduction and Background

- CID-078 is an orally bioavailable, passively cell permeable, potent, and selective macrocycle that binds to the hydrophobic patch (HP) of cyclins A and B and blocks RxL-dependent interactions with proteins such as E2F1 with cyclin A-CDK2 and Myt1 with cyclin B-CDK1, providing a novel therapeutic strategy for SCLC and NSCLC patients (Figure 1).
- Previously, we demonstrated that disruption of these interactions selectively drives lethality in cancer cells containing oncogenic alterations that elevate expression of E2F1[1].
- We have further identified a pharmacodynamic (PD) biomarker, separase (ESPL1), a mitosis specific protein which is a direct substrate of cyclin B1-CDK1 and is inhibited by securin and phosphorylation-dependent binding to cyclin B1[2].
- Previously, we demonstrated that cyclin A/B RxL inhibition induces potent antiproliferative activity in SCLC cell line derived xenograft (CDX) and triple negative breast cancer (TNBC) patient derived xenograft (PDX) models[1,3].
- Here we demonstrate single agent efficacy of CID-078 in SCLC and NSCLC PDX models and show that elevated E2F targets and G2M checkpoint hallmark pathway scores and/or elevated levels of E2F1, cyclin B1 and ESPL1 (separase) are associated with response to cyclin A/B RxL inhibition in SCLC and NSCLC xenografts.

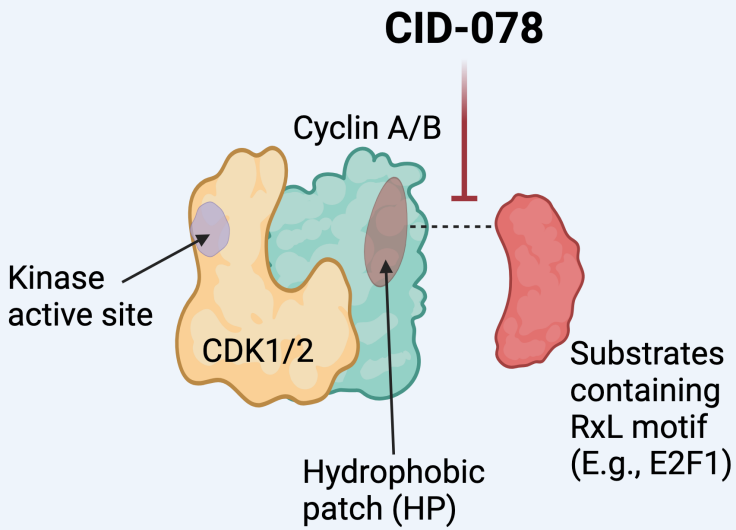
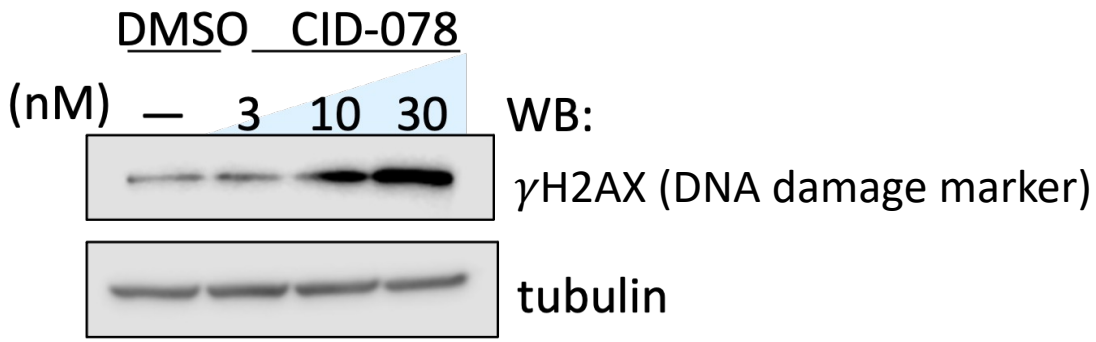


Figure 1. CID-078 disrupts Cyclin A/B substrate engagement.
Created with BioRender.com

[1]Gleason C, Garcia P, Odeh R, et al. Poster #1560 presented at AACR Annual Meeting, April 14-19, 2023, Orlando, FL; [2]Haaß W, Stehle M, Nittka S, et al. *PLoS One* 7(8): e42863 (2012); [3]Dias M, Liu L-F, Molina C, et al. Poster presented at EORTC-NCI-AACR International Conference on Molecular Targets and Cancer Therapeutics, October 11-15, 2023, Boston, MA.

Background: CID-078 induces DNA damage and G_2/M phase arrest in NCI-H446 SCLC cell line

A. Induction of DNA damage



B. Cell cycle profile

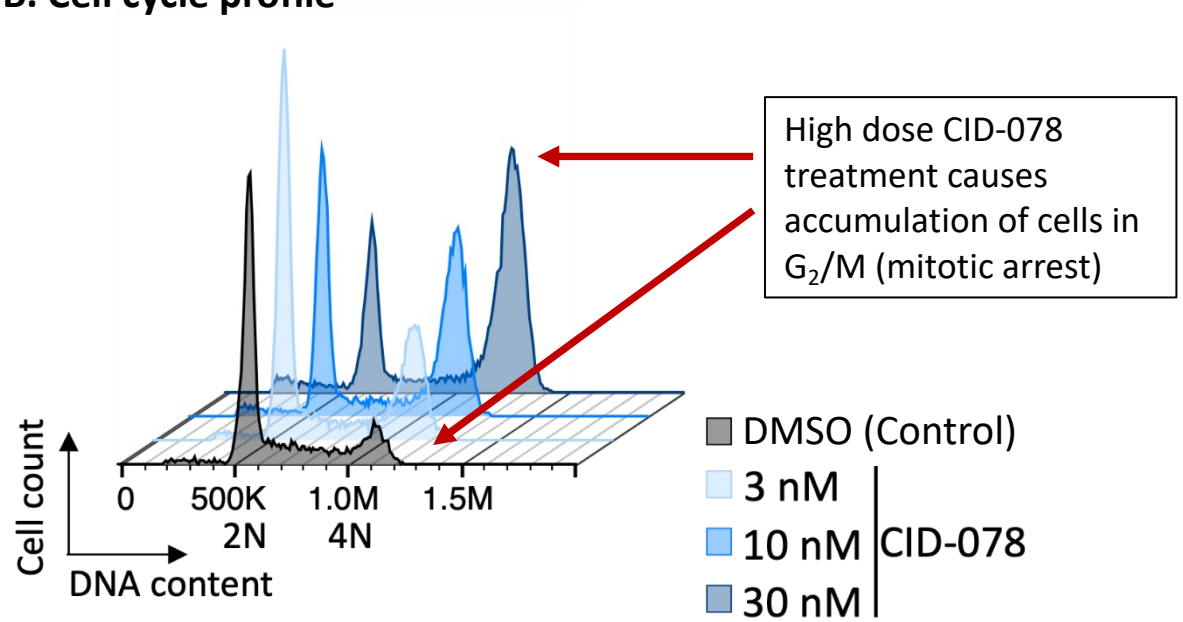
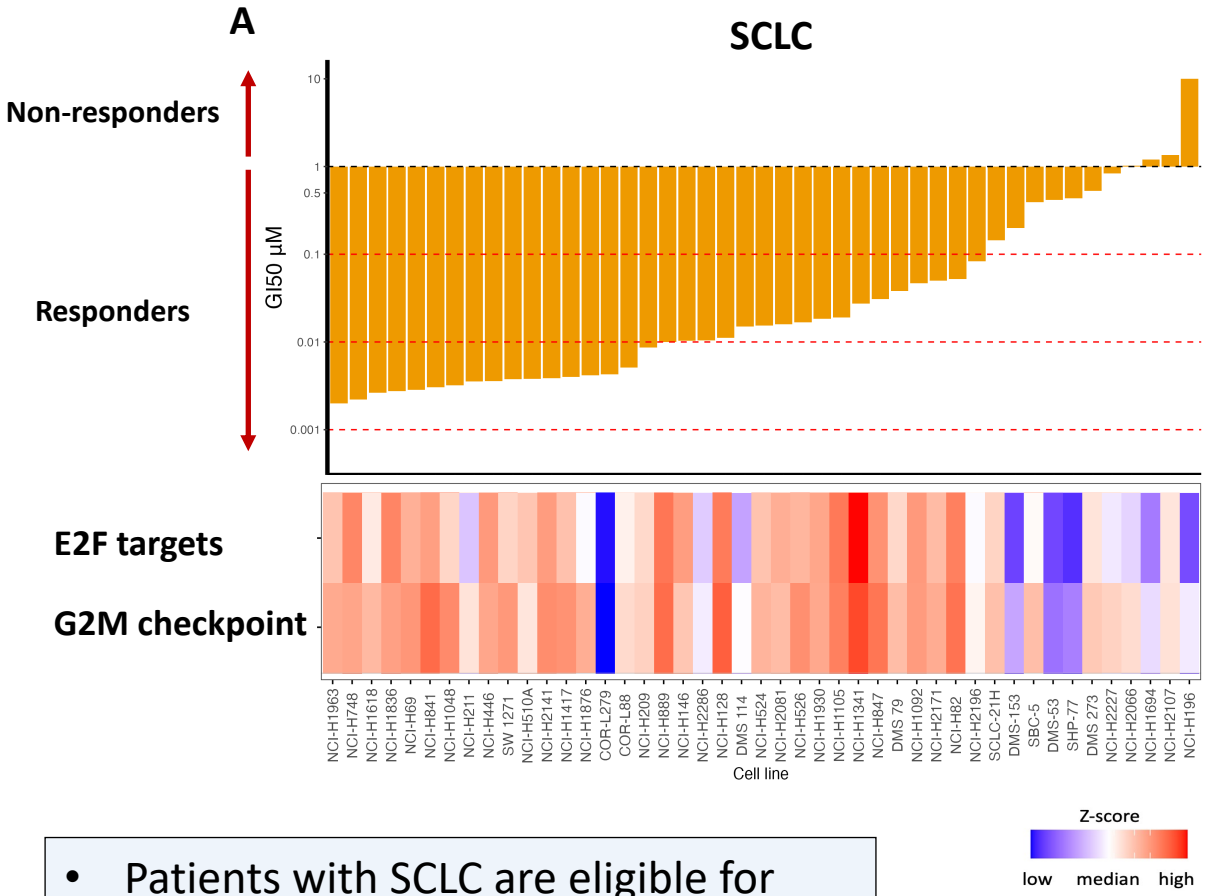


Figure 2. (A) CID-078 induces DNA damage. NCI-H446 cells were dosed with CID-078 at the indicated concentrations for 24h and processed for analysis of γH2AX levels, a marker for activation of the DNA damage response pathway, by Western blot. **(B) Representative flow cytometry plots showing G_2/M phase arrest after treatment with CID-078.** NCI-H446 cells were dosed with DMSO or CID-078 at the indicated concentrations for 24h. DNA content was measured by FxCycle violet.

Higher E2F targets and G2M checkpoint hallmark pathways scores predict favorable response to CID-078 *in vitro*



- Patients with SCLC are eligible for enrollment into CID-AB1-24001

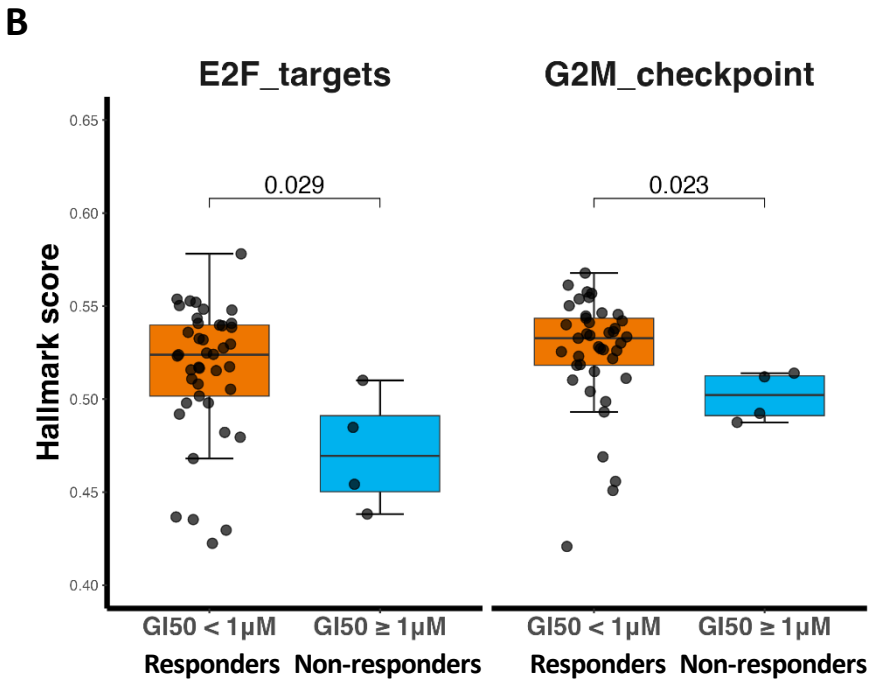
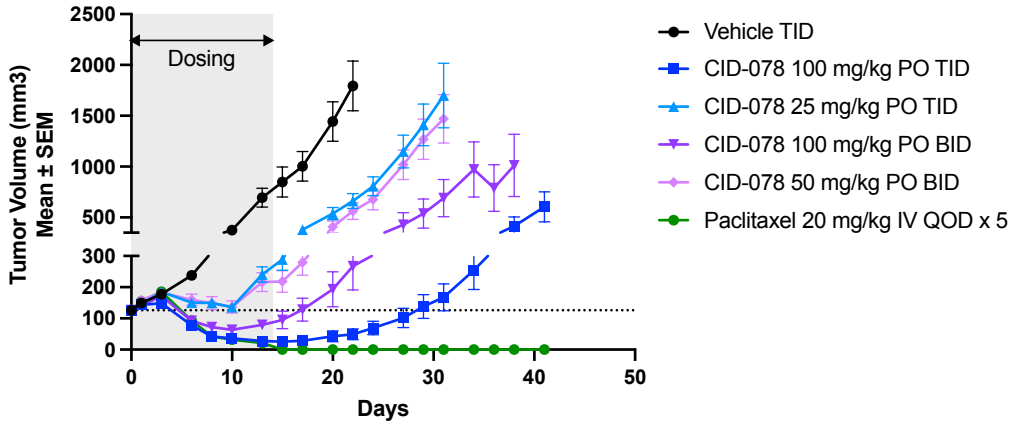


Figure 3. (A) Waterfall plots of sensitivity (as assessed by GI_{50}) and heatmaps of E2F targets and G2M checkpoint hallmark pathways Z-scores of CID-078 tested in 45 SCLC cell lines. **(B)** E2F targets and G2M checkpoint hallmark pathway scores were associated with sensitivity to CID-078 in SCLC cell lines.

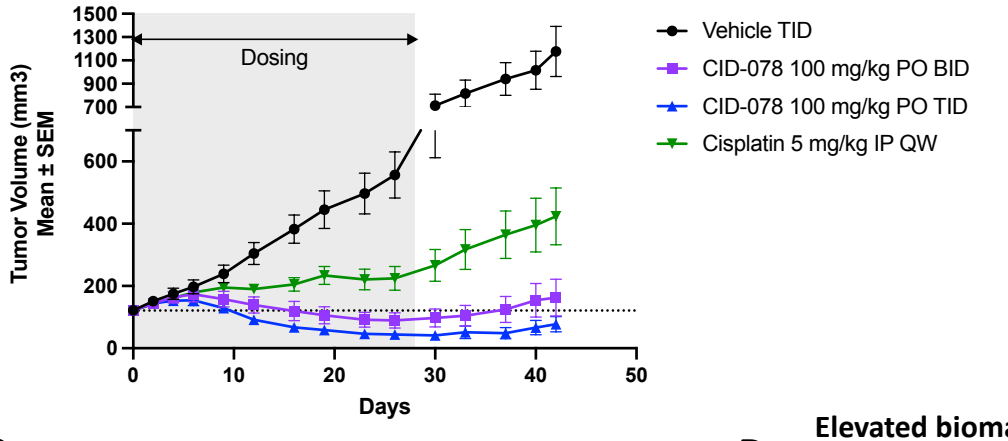
P-values are shown and calculated by the Wilcoxon rank sum exact.

CID-078 demonstrates monotherapy benefit in SCLC models with high E2F targets and G2M checkpoint hallmark pathways scores

A **NCI-H446 (CDX)**



B **LUX083 (PDX)**



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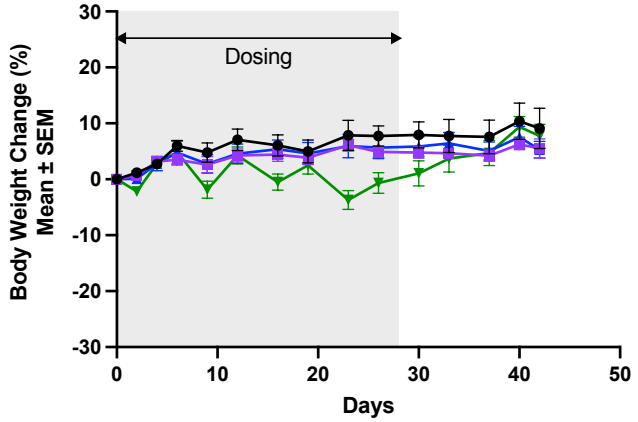


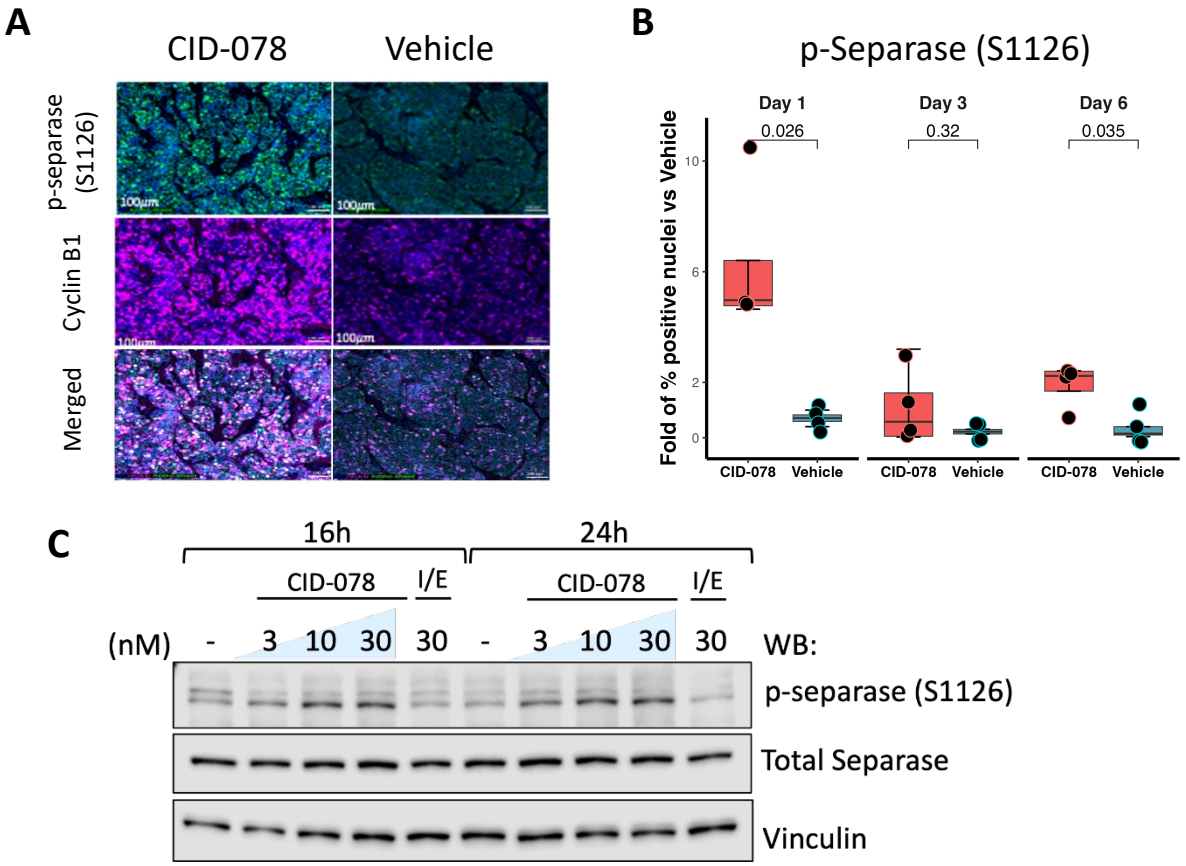
D **Elevated biomarker characteristics are associated with response**

	CDX-H446 SCLC	PDX-LUX083 SCLC
RB1	MUT	WT
E2F targets	High	NA
G2M checkpoint	High	NA
E2F1	High	High
ESPL1	High	High
CCNB1	High	High

Figure 4. *In vivo* SCLC efficacy studies. (A) Mice were inoculated SC with $5x10^6$ NCI-H446 CDX cells or **(B)** fragments of LUX083 PDX tumors. Treatment was initiated when tumors reached 100-200mm^3. CID-078 was administered PO at the doses indicated. **(C)** All treatment regimens were tolerated as assessed by body weight measurements. **(D)** Summary table of biomarker characteristics for each model.

SC, subcutaneous; PO, orally; QD, once daily; BID, twice daily; TID, three times daily; QOD, once every two days; SEM, standard error of mean

Mitosis specific protein p-separase S1126 is a candidate PD biomarker



The role of separase (ESPL1) in the cell cycle[1,2,3,4]

- Separase is a direct substrate of cyclin B1/CDK1
- CID-078 binds to cyclin B1 and leads to inhibitory phosphorylation of separase, resulting in mitotic arrest and apoptotic cell death

Figure 5. Time- and dose-dependent PD effect observed upon CID-078 treatment in the NCI-H446 SCLC model. (A) Representative immunohistochemistry (IHC) images and **(B)** quantification of IHC results of tumors treated with vehicle or 100 mg/kg CID-078 QD x 1 or TID x 3 and 6 show an increase in p-separase (S1126), and co-expression of p-separase (S1126) and cyclin B1 by multiplex IHC analysis. Samples were collected 4 h post final dose except for CID-078 QD x 1, collected 10 h post dose. **(C)** CID-078 treatment leads to a dose-dependent increase in p-separase (S1126)**.** Western blot for p-separase (S1126) in cells dosed with CID-078 for 16 or 24 hours at the indicated concentrations. **(D)** Proposed model for pharmacodynamic effect of CID-078, created with BioRender.com.

I/E, inactive enantiomer of CID-078

[1]Haaß W, Stehle M, Nittka S, et al. *PLoS One* 7(8): e42863 (2012); [2]Pérez de Castro I, de Cárcer G, Malumbres M. *Carcinogenesis*, 28(5):899-912, (2007); [3]Morgan DO. The Cell Cycle: Principles of Control. New Science Press (2007); [4]Holland AJ, Taylor SS. *J Cell Sci*, 119(Pt 16):3325-36, (2006).

High E2F targets and G2M checkpoint hallmark pathways scores in large cell and squamous cell NSCLC predict favorable response to CID-078 *in vitro*

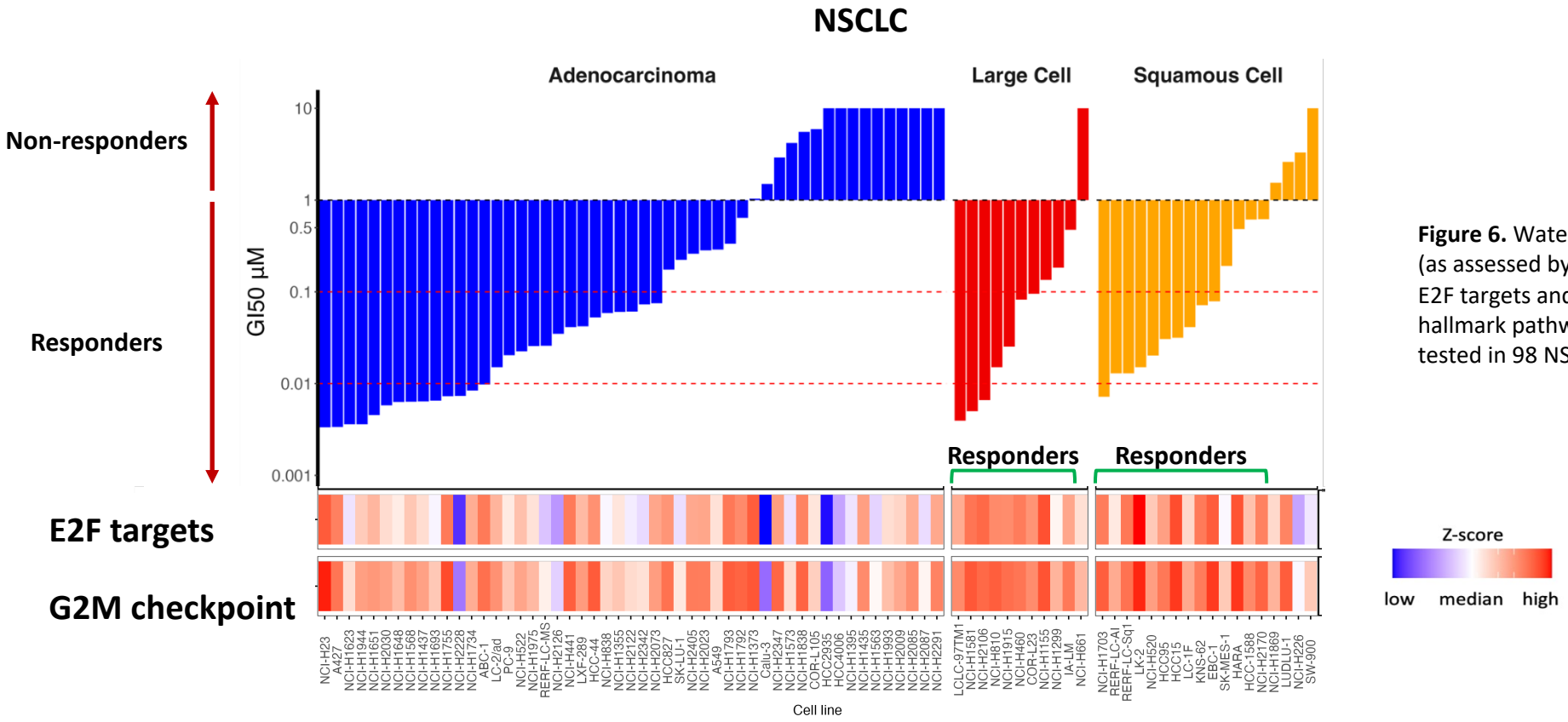
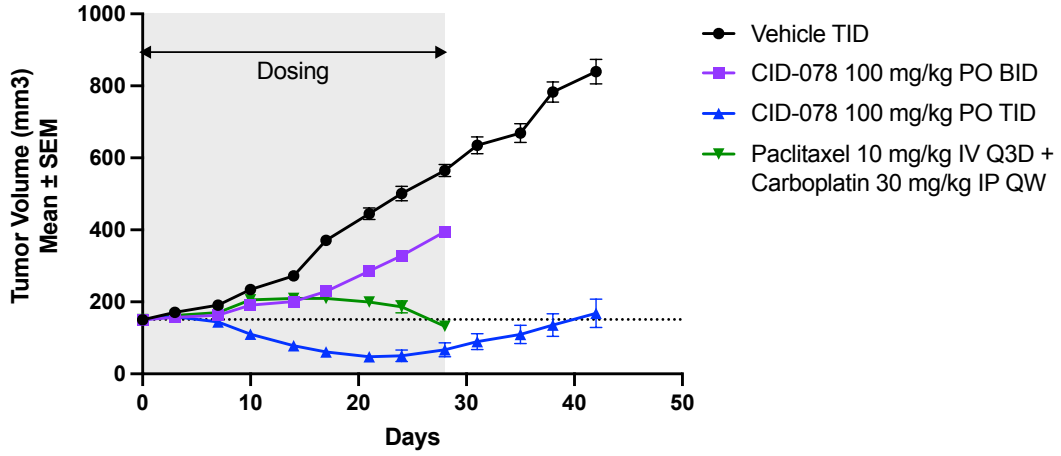


Figure 6. Waterfall plots of sensitivity (as assessed by GI_{50}) and heatmaps of E2F targets and G2M checkpoint hallmark pathways Z-scores of CID-078 tested in 98 NSCLC cell lines.

- Patients with NSCLC are eligible for enrollment into CID-AB1-24001

CID-078 demonstrates monotherapy benefit in NSCLC CDX models with high E2F targets and G2M checkpoint hallmark pathways scores

A



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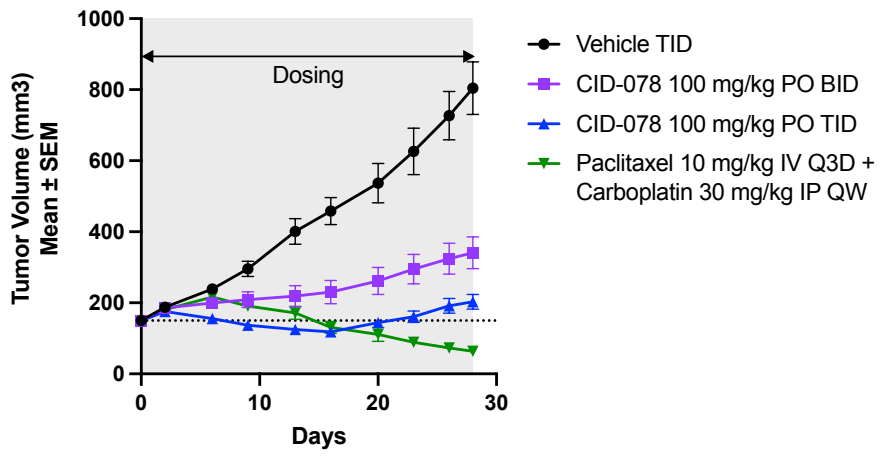


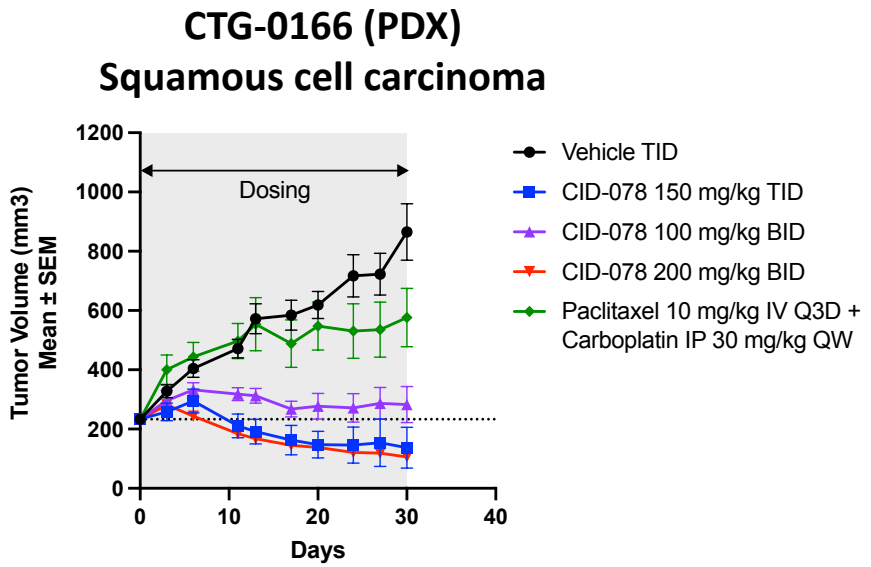
C **Elevated biomarker characteristics are associated with response**

	CDX-H23 Adenocarcinoma	CDX-H2106 Large cell carcinoma
RB1	WT	MUT
E2F targets	High	High
G2M Checkpoint	High	High
E2F1	High	High
ESPL1	High	High
CCNB1	High	Med

Figure 7. ***In vivo*** **NSCLC CDX efficacy studies. (A)** Mice were inoculated SC with 1.5x10^7 NCI-H23 or **(B)** 1x10^7 NCI-H2106 CDX cells. Treatment was initiated when tumors reached 100-200mm^3. CID-078 was administered PO at the doses indicated for each model. All treatment regimens were tolerated as assessed by body weight measurements (not shown). **(C)** Summary table of biomarker characteristics for each model is shown.

CID-078 demonstrates monotherapy benefit in a NSCLC PDX model with favorable biomarker characteristics

A



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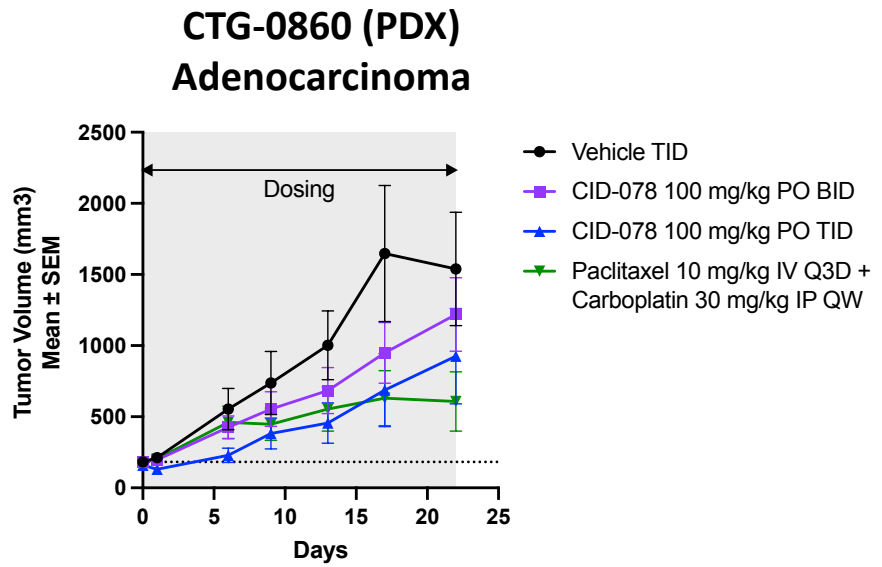

C **Elevated biomarker characteristics are associated with response**

	PDX-CTG-0166 Squamous cell carcinoma (PR)	PDX-CTG-0860 Adenocarcinoma (PD)
RB1	MUT	WT
E2F targets	High	Med
G2M Checkpoint	High	Med
E2F1	High	Low
ESPL1	High	Low
CCNB1	High	Med

Figure 8. *In vivo* NSCLC PDX efficacy studies. Mice were inoculated SC with fragments of **(A)** CTG-0166 or **(B)** CTG-0860 PDX tumors. Treatment was initiated when tumors reached 150-250mm^3. CID-078 was administered PO at the doses indicated for each model. All treatment regimens were tolerated as assessed by body weight measurements (not shown). **(C)** Summary table of best response and biomarker characteristics for each model is shown.

PR, partial response; PD, progressive disease

Conclusions

- CID-078 induced antiproliferative activity in SCLC and NSCLC cell lines (Figures 3 and 6). In the SCLC cell line, NCI-H446, CID-078 induces significant DNA damage and G_2/M phase accumulation that correlates with increased separase S1126 inhibitory phosphorylation (Figure 5).
- Tumor models with high E2F targets and G2M checkpoint hallmark pathways scores and elevated levels of E2F1, cyclin B1 and ESPL1 demonstrate tumor growth inhibition and/or regression when treated with CID-078 dosed at 100 mg/kg BID and TID (Figures 4, 7, and 8).
- In the SCLC tumor model NCI-H446, we observed an increase in p-separase (S1126) and co-expression of p-separase and cyclin B1 following treatment with CID-078 (Figure 5) as well as p-ATM nuclear staining and E2F1 (not shown).
- CID-078 demonstrates potent anti-tumor activity in the SCLC and NSCLC preclinical models evaluated, correlating well with E2F1 and ESPL1 expression and consistent with proposed cyclin A/B RxL inhibition leading to DNA damage (γ-H2AX & pATM).
- CID-078 activity further correlates with an increase in a mitotic phase-specific protein (p-separase S1126) and leads to apoptotic tumor cell death.
- Based on these compelling results, CID-078 may provide a novel therapeutic option patients with SCLC and NSCLC. This hypothesis and the correlation of anti-cancer activity with the proposed biomarkers will be explored in the first-in-human clinical trial, CID-AB1-24001 (NCT06577987).