First-in-class oral macrocyclic cyclin D1-selective inhibitors demonstrate anti-tumor activity in cyclin D1-dependent tumors

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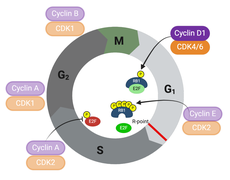
BACKGROUND

D-type cyclins (D1, D2, and D3) form complexes with cyclin-dependent kinase CDK4 or CDK6 to drive cell cycle progression by phosphorylating the retinoblastoma protein (Rb) and enabling G1/S transition. Among these, cyclin D1 is a well-established oncogene, characterized by t(11;14) translocation in mantle cell lymphoma (MCL) and other hematologic malignancies, and by amplification or overexpression in ER$^+$ breast cancer and various solid tumors. These genetic events drive tumorigenesis and therapeutic resistance while rendering tumors particularly dependent on cyclin D1-CDK4/6 activity. Although dual CDK4/6 inhibitors have significantly improved outcomes in ER$^+$ breast cancer, their therapeutic index is constrained by neutropenia resulting from inhibition of the cyclin D3-CDK6 complex, a key regulator of myeloid progenitor survival. To target cyclin D1 specifically while avoiding toxicity of targeting cyclin D3, we leveraged our MXMO™ platform to generate passively permeable and orally bioavailable macrocycle inhibitors that selectivity disrupt the cyclin D1-Rb interaction and demonstrate efficacy and combination potential in cyclin D1-dependent indications.

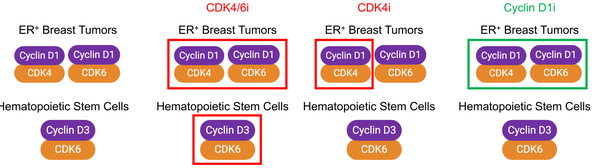
Figure 1. Cyclin D1 is an undrugged "holy grail" oncogene target



Indication	Cyclin D1 Alteration	Prevalence	Est Patients/year (US)
Mantle cell lymphoma	t(11;14)(a13;q32)	95%	6,000
B-cell lymphoma	t(11;14)(a13;q32)	20%	14,000
Multiple myeloma	t(11;14)(a13;q32)	20%	7,000
Breast	Amplification	17%	40,800
Head and neck	Amplification	12%	8,000
Bladder	Amplification	10%	8,000
Gastric	Amplification	15%	4,500
Esophageal	Amplification	7%	1,600
Melanoma	Amplification	5%	5,000
Neuroblastoma	Amplification	20%	800

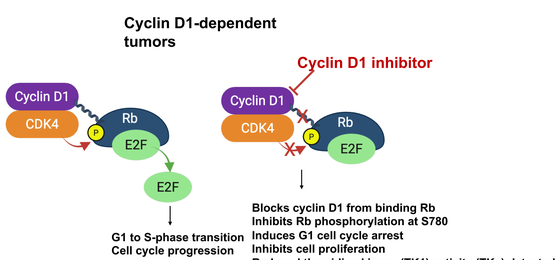
The Cancer Genome Atlas (2018), Lakshman A (2018), Diamantidis (2022)

Figure 2. Rationale for targeting cyclin D1: Achieving "CDK4/6-like" efficacy while reducing hematologic toxicity by sparing cyclin D3



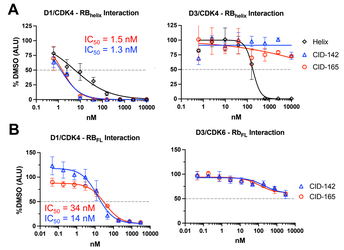
Yang et al., Oncogene 2017, Li et al., Cancer Cell 2018, O'Leary et al., Cancer Discovery 2018 (Paloma-3 trial), Griffiths et al., Nat Cancer 2021, Li et al.,, Cancer Discovery 2022, Haines, Shapiro et al., Oncotarget 2018, Cornell, Shapiro et al., Cell Rep 2019

Figure 3. Mechanism of action of cyclin D1 inhibitors



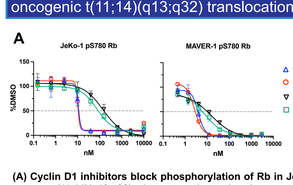
Results

Figure 4. Cyclin D1 inhibitors selectively disrupt the interaction of cyclin D1 with Rb but do not affect Rb binding to cyclin D3



(A) Cyclin D1 inhibitors block binding of C-terminal helix of Rb to cyclin D1 but not D3. Interactions were monitored by AlphaScreen using a GST-tagged 20 amino acid fragment from Rb described by Topacio et. al. and cyclin D1 or D3 complexed with CDK4. **(B) Advanced candidates block binding of full length Rb to cyclin D1/CDK4 but not to D3/CDK6.** HEK 293 cells were transfected with a split luciferase (NanoBiT system) appended to a cyclin D1/CDK4 or D3/CDK6 fusion protein and full length Rb. Cells were treated with compounds for 24 h and luminescence was measured after addition of substrate.

Figure 5. Cyclin D1 inhibitors prevent Rb phosphorylation in MCL cell lines harboring the oncogenic t(11;14)(q13;q32) translocation that drives overexpression of cyclin D1

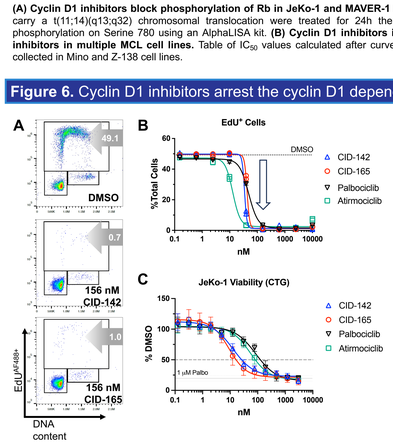


B

Compound	Rb S780 phosphorylation - IC$_{50}$ (nM)			
	JeKo-1	Maver-1	Mino	Z-138
CID-142	11	3.9	12	6.6
CID-165	10	2.9	6.7	4.9
Atirmociclib	75	6.2	13	51
Palbociclib	142	10	54	147

(A) Cyclin D1 inhibitors block phosphorylation of Rb in JeKo-1 and MAVER-1 MCL cell lines. Mantle cell lymphoma lines which carry a t(11;14)(q13;q32) chromosomal translocation were treated for 24h then lysates were prepared and assessed for Rb phosphorylation on Serine 780 using an AlphaLISA kit. **(B) Cyclin D1 inhibitors inhibit Rb phosphorylation better than CDK4/6 inhibitors in multiple MCL cell lines.** Table of IC$_{50}$ values calculated after curve fitting data shown in **(A)** as well as similar data collected in Mino and Z-138 cell lines.

Figure 6. Cyclin D1 inhibitors arrest the cyclin D1 dependent MCL JeKo-1 cell line in G1

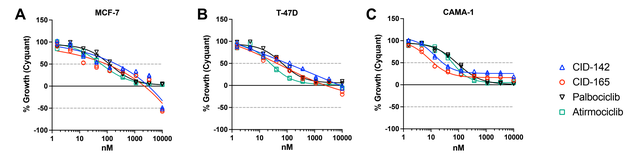


D

Compound	JeKo-1 IC$_{50}$ (nM)	
	EdU	CTG
CID-142	34	26
CID-165	43	16
Atirmociclib	13	81
Palbociclib	49	125

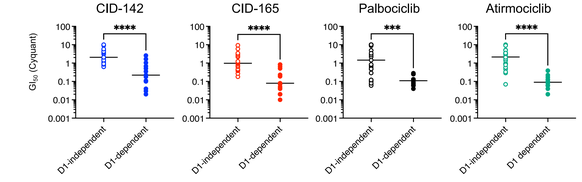
(A) Cyclin D1 inhibitors block entry into S-Phase. JeKo-1 cells were treated for 24h then pulsed with EdU for 1h. Cells were fixed and measured for EdU incorporation and DNA content by flow cytometry. **(B) Cyclin D1 inhibitors prevent EdU accumulation in a dose dependent manner.** EdU labeling was quantitated by flow cytometry and plotted as a function of treatment. **(C) Cyclin D1 inhibitors prevent JeKo-1 cell growth.** Cells were treated for 4d then assessed for growth using CellTiter-Glo. Palbociclib at 1µM was included as a standard for no growth. **(D) Cyclin D1 inhibitors arrest cells in G1 with nanomolar IC$_{50}$.** Table contains values obtained from curve fitting in B & C.

Figure 7. Cyclin D1 inhibitors prevent the proliferation of ER+ breast cancer cell lines



(A-C) Cyclin D1 inhibitors prevent cell growth of D1 dependent ER+ breast cancer cell lines. MCF-7, T-47D, and CAMA-1 cells were treated for 5 days then growth was assessed using Cyquant cell viability assay with Day 0 referenced for "0" growth.

Figure 8. Cyclin D1 inhibitors selectively inhibit cell proliferation in D1-dependent breast and hematologic tumor cell lines



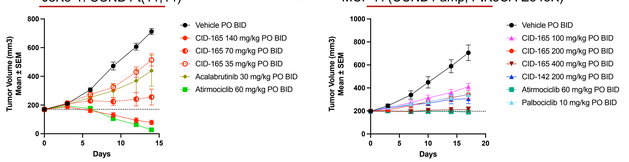
Cyclin D1 dependence and compound potency in D1-dependent vs. D1-independent cell lines. Cell line dependence was defined by DepMap Chronos scores (< –1.4 = D1-dependent, N = 13; ≥ –1.4 = D1-independent, N = 27). Statistical significance was determined using a Mann–Whitney non-parametric test (*** p = 0.006; **** p < 0.0001). Cyclin D1 advanced candidates are highly protein-bound in culture media; therefore, their unbound GI$_{50}$ values are expected to be substantially lower than the total GI$_{50}$ values shown.

Figure 9. Cyclin D1 inhibitor CD34$^+$ myeloid progenitor in-vitro toxicity profiles are superior to Palbociclib suggesting anti-tumor efficacy with a reduced risk of neutropenia can be achieved

	CID-142	CID-165	Palbociclib	Atirmociclib
JeKo-1 EC$_{50}$ nM, u	1.16	1.25	43	29
MCF-7 GI$_{50}$ nM, u	10.8	8.7	88	43
CAMA-1 GI$_{50}$ nM, u	2.5	1.2	58.9	43.4
Myeloid Hematox IC$_{50}$ nM, u	4.3	3.9	6.1	60.6
Myeloid hematox IC$_{50}$/Jeko IC$_{50}$,u	3.7	3.1	0.1	2.1
Myeloid hematox IC$_{50}$/MCF-7 GI$_{50}$,u	0.4	0.4	0.1	1.4
Myeloid hematox IC$_{50}$/CAMA-1 GI$_{50}$,u	1.7	3.1	0.1	1.4

Cyclin D1 compound selectivity in myeloid precursor vs. D1-dependent cancer cell lines. Cyclin D1 inhibitors were tested for 7-day anti-proliferative activity in human myeloid precursor cells (HemaTox Myeloid Kit, CD34$^+$ HSPCs; Stemcell Technologies/Lonza). Unbound IC$_{50}$ values were compared with those from D1-dependent cancer cell lines, with fold-selectivity (myeloid vs. D1-dependent) shown in boxes.

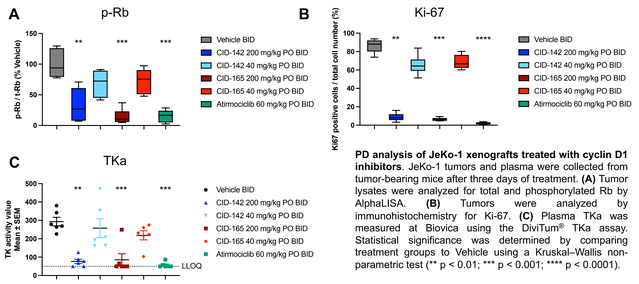
Figure 10. Cyclin D1 inhibitors induce single agent anti-tumor activity in a cyclin D1 translocated MCL tumor model and a cyclin D1 amplified ER$^+$ breast cancer tumor model



Human dose predictions for cyclin D1 advanced candidates range from 100-350 mg BID

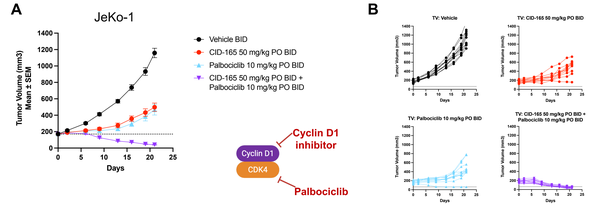
***In vivo* MCL and ER$^+$ breast cancer xenograft studies**. Mice were inoculated SC with 5x10^6 JeKo-1 **(A)** or 1.5x10^7 MCF-7 **(B)** cells. Treatment was initiated when tumors reached 150-250 mm^3. CID-142, CID-165, and controls were administered at the doses indicated (n=9-10 mice/group). All treatment regimens were well tolerated as assessed by body weight measurements (not shown). SC, subcutaneous; PO, orally; BID, twice daily.

Figure 11. Cyclin D1 inhibitors demonstrate dose-dependent pharmacodynamic (PD) responses marked by inhibition of Rb phosphorylation, decreased Ki-67 expression, and suppression of plasma thymidine kinase activity (TKa) in the JeKo-1 MCL tumor model



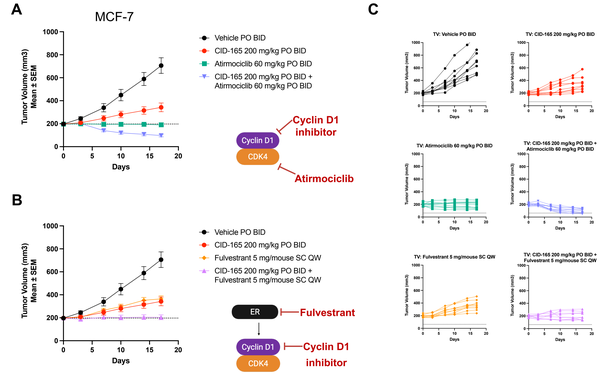
PD analysis of JeKo-1 xenografts treated with cyclin D1 inhibitors. JeKo-1 tumors and plasma were collected from tumor-bearing mice after three days of treatment. **(A)** Tumor lysates were analyzed for total and phosphorylated Rb by AlphaLISA. **(B)** Tumors were analyzed by immunohistochemistry for Ki-67. **(C)** Plasma TKa was measured at Biovica using the DiviTum® TKa assay. Statistical significance was determined by comparing treatment groups to Vehicle using a Kruskal–Wallis non-parametric test (** p < 0.01; *** p < 0.001; **** p < 0.0001).

Figure 12. Cyclin D1 inhibitor shows combination benefit with Palbociclib in a cyclin D1 translocated MCL tumor model



***In vivo* MCL xenograft combination study**. JeKo-1 tumor-bearing mice were treated with CID-165 and Palbociclib as single agents and in combination at the doses indicated (n=10 mice/group). Mean tumor volumes are shown in **(A)** and curves for individual animals are shown in **(B)**. All treatment regimens were well tolerated as assessed by body weight measurements (not shown).

Figure 13. Cyclin D1 inhibitor shows combination benefit with Atirmociclib and Fulvestrant in a cyclin D1 amplified ER$^+$ breast cancer tumor model



***In vivo* ER$^+$ breast cancer xenograft combination study**. MCF-7 tumor-bearing mice were treated with CID-165, Atirmociclib, and Fulvestrant as single agents and in combination at the doses indicated (n=9 mice/group). Mean tumor volumes are shown for Atirmociclib and Fulvestrant combinations in **(A)** and **(B)**. Curves for individual animals are shown in **(C)**. All treatment regimens were well tolerated as assessed by body weight measurements (not shown). QW, once weekly.

CONCLUSIONS

- First-in-class oral cyclin D inhibitor that is isoform-selective for cyclin D1, designed to avoid hematopoietic toxicity associated with cyclin D3 inhibition.
- Potently disrupts cyclin D1-Rb binding with >2,000-fold selectivity over cyclin D3-Rb disruption, inducing phospho-Rb suppression and G1 arrest in D1-dependent models.
- Demonstrates robust anti-tumor activity in cyclin D1-dependent MCL and ER$^+$ breast models, with enhanced efficacy in CDK4-selective, CDK4/6-dual, and ER therapy combinations.
- Superior CD34$^+$ myeloid progenitor in-vitro toxicity profile compared to dual CDK4/6 inhibitor suggesting anti-tumor efficacy with a reduced risk of neutropenia can be achieved.
- On track to achieve Development Candidate milestone by EOY 2025 and IND enabling studies in 2026.