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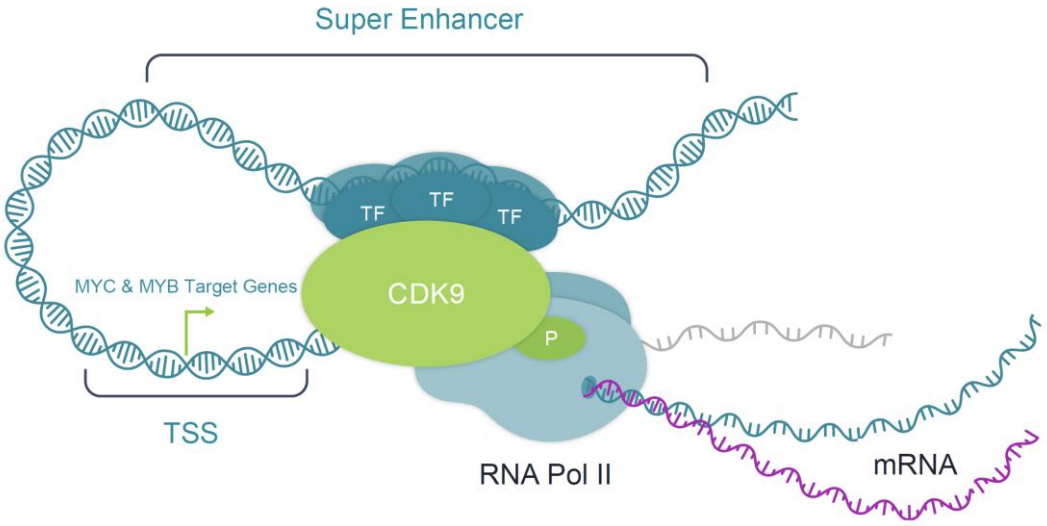
PRT2527 is a potent and selective CDK9 inhibitor that demonstrates anti-cancer activity in preclinical models of hematological malignancies and solid tumors with *MYC* amplification

Yang Zhang, PhD,[1] Liang Lu, PhD, [1] Min Wang, PhD, [1] Dave Rominger, BS, [1] Stefan Ruepp, PhD, [1] Kirsten Gallagher, BS, [1] William Gowen-MacDonald, BS, [1] Chaofeng Dai, PhD,[1] Miles Cowart, MS, [1] Andrew Combs, PhD, [1] Carlo Catapano, MD, PhD, [2] Bruce Ruggeri, PhD, [1] Peggy Scherle, PhD, [1] Kris Vaddi, PhD, [1]

[1] Prelude Therapeutics, Drug Discovery, Wilmington, DE

[2] Institute of Oncology Research, Switzerland

Introduction



- CDK9 phosphorylates RNA Pol II and regulates transcription
- Regulates expression of several immediate early genes driving oncogenesis and resistance (i.e. *MYC, MYB, MCL1*)
- Non-selective CDK9 inhibitors have demonstrated clinical activity in multiple tumor types but poor tolerability
  - Lack of selectivity and potency vs other CDK9 inhibitors is believed to contribute to low therapeutic window

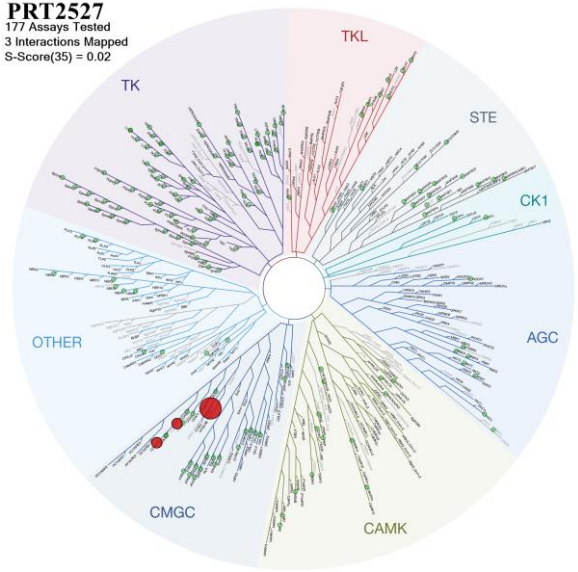
PRT2527 is a Potent and Selective CDK9 Inhibitor

A

Compound		AZD4573	KB0742	VIP152**	PRT2527
Biochemical* IC_{50} (nM)	CDK9	1.9	483	16	0.98
Proliferation* IC_{50} (nM)		11	915	84	18
Plasma* IC_{50} (nM)		192	986	923	198
Fold Selectivity CDK9 *vs* Other Isoforms	CDK1	23x	>20x	371x	73x
	CDK2	35x	>20x	147x	340x
	CDK3	2x	>20x	37x	35x
	CDK4	53x	>20x	38x	250x
	CDK5	37x	>20x	>600x	>1000x
	CDK6	79x	>20x	296x	>1000x
	CDK7	150x	>20x	>600x	>1000x

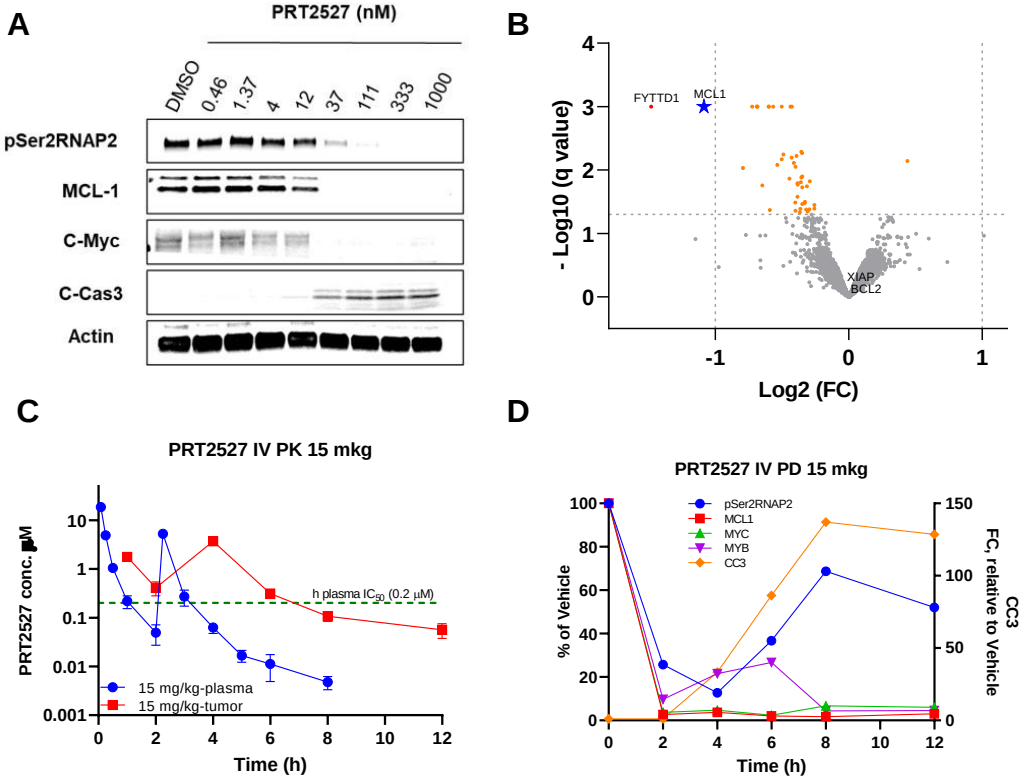
*Internal data; biochemical assay at 1 mM ATP, H929 CTG proliferation assay
**VIP151 was formerly BAY151 and licensed to Vincera by Bayer

B



- PRT2527 is a potent CDK9 inhibitor with a biochemical IC_{50} value of 0.98 nM at 1 mM ATP and a protein binding adjusted potency of 198 nM determined by a human plasma In Cell Western (ICW) assay
- PRT2527 is highly selective among CDK family members and across 177 kinases. Biochemical IC_{50} values for each CDK members were determined at 1 mM ATP and fold selectivity over CDK9 was derived. Kinome screening was conducted with 200 nM PRT2527 at 1 mM ATP

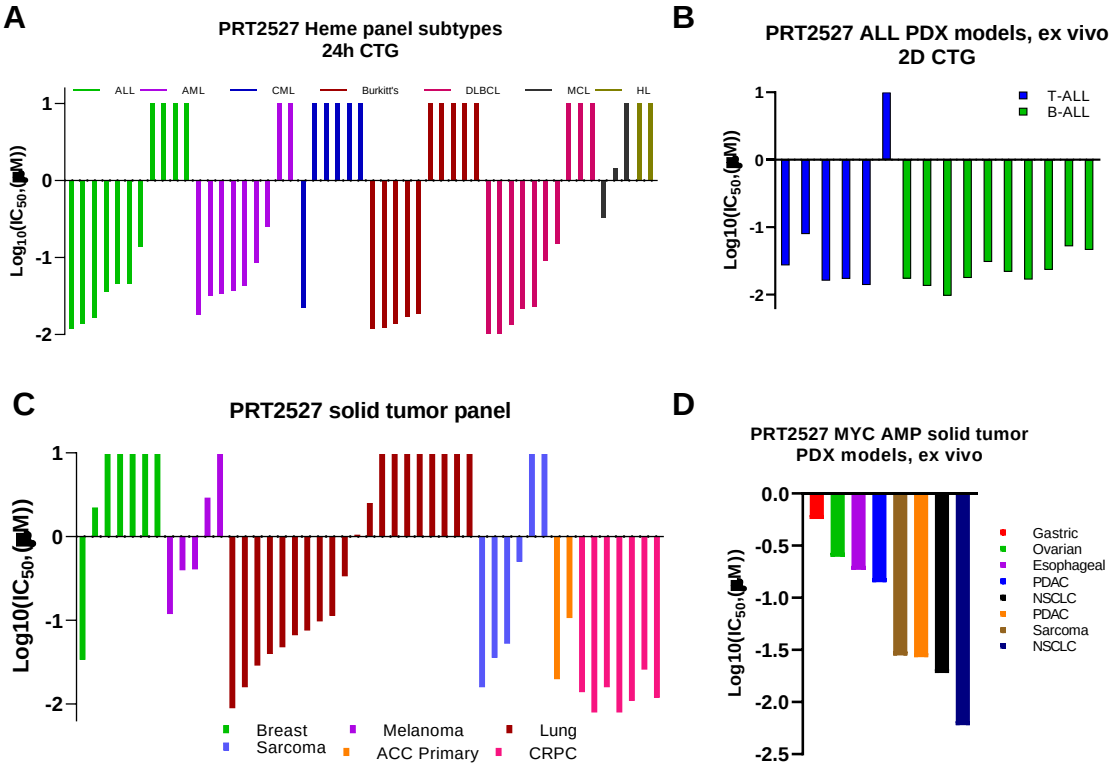
PRT2527 Treatment Depletes MCL1, MYC, and Induces Apoptosis *In Vitro* and *In Vivo*



MV-4-11 AML model

- PRT2527 depletes pSer2RNAP2 and onco-proteins with short half-lives such as MCL1, MYC, and induces apoptosis in a dose-dependent manner in MV-4-11 cells after 4 hours treatment (**A**). MCL1 was identified as one of the major down-regulated proteins in an unbiased differential proteomic analysis (**B**. MV-4-11 cells, 4 hours treatment).
- IV administration of PRT2527 at 15 mg/kg q2h in mice achieved coverage above human plasma IC_{50} for 6 hours in tumor (**C**, red line), temporally depleted pSer2RNAP2, MCL1, MYC, and induced apoptosis as measured by cleaved caspase 3 activation (**D**) in tumor tissue. Study was conducted in a MV-4-11 subcutaneous xenograft model.

PRT2527 Induces Cell Death in Hematological and Solid Tumor Models *In Vitro* and *Ex Vivo*

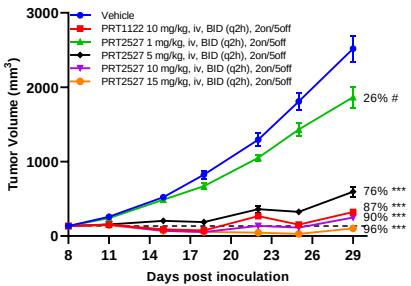


PRT2527 anti-proliferative potencies in hematological (**A**) and solid tumor cell lines (**C**) was were determined via 1-day and 2-day assays, respectively. For primary cultures of heme (**B**) and solid tumors with *MYC* amplification (**D**), anti-proliferative IC_{50} values were determined through a 2-day proliferation assay.

ACC: adenoid cystic carcinoma
ALL: acute lymphoblastic leukemia
AML: acute myeloid leukemia
CML: chronic myelogenous leukemia
CRPC: castrate-resistant prostate cancer
DLBCL: diffuse large B-cell lymphoma
HL: Hodgkin's lymphoma
NSCLC: non-small cell lung cancer
PDAC: pancreatic ductal adenocarcinoma

PRT2527 is Efficacious in CDX and PDX Models of Heme and Solid Tumors with MYC Alterations

A



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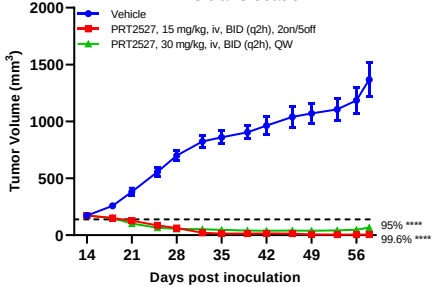

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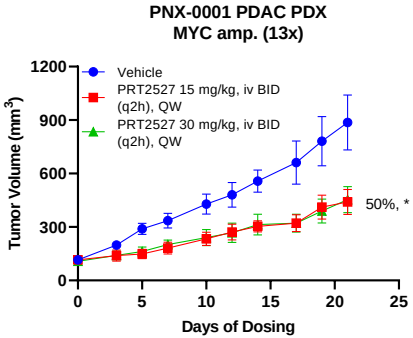

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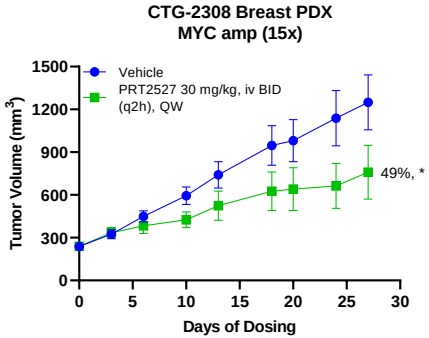
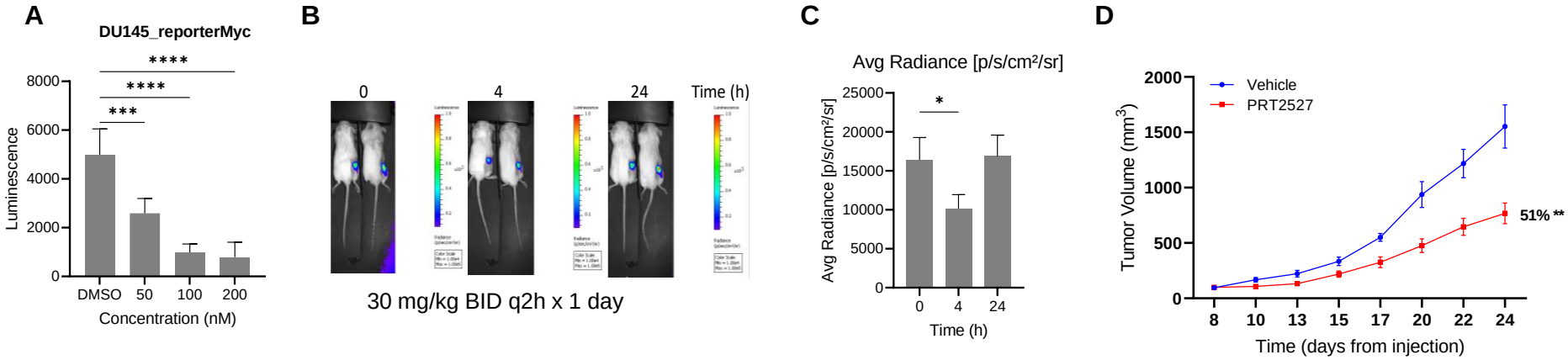


E

Model #	Tumor type	MYC alter.	TGI (%)
SU-DHL-4	TH-DLBCL/CDX	Translocation	126
PNX-0223	DH-DLBCL/PDX	Translocation	84
PNX-0019	Esophageal/PDX	Amp. (36x)	49
CTG-2271	NSCLC/PDX	Amp. (23x)	63
ACCX9	ACC/PDX	Overexpression	50

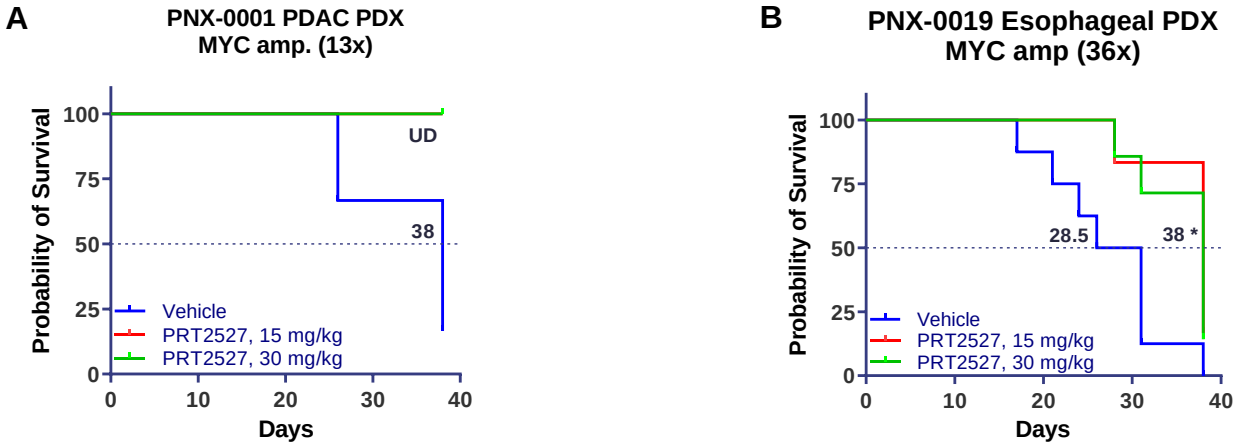
- Intermittent IV administration of PRT2527 (2on/5off or QW) inhibited tumor growth in AML MV-4-11 model (**A**) and DH-DLBCL Karpas-422 model carrying *MYC* translocation (**B**). PRT2527 was also efficacious in various PDX models with *MYC* amplification (**C**, **D**). PRT2527 efficacy in additional CDX and PDX models bearing *MYC* translocation or amplification are summarized in Table

PRT2527 Reduces MYC Transcriptional Activity in a CRPC MYC-Reporter Model *In Vitro and In Vivo*



- PRT2527 inhibited MYC transcriptional activity in a CRPC MYC-DU145 model *in vitro* (**A**, 24h) and *in vivo* (**B, C**). MYC-DU145 cells carry a firefly luciferase reporter gene controlled by a MYC responsive element. MYC transcription activity fully recovered 24h after IV administration (**C**).
- Intermittent IV administration of PRT2527 (15 mg/kg q2h, QW) significantly inhibited tumor growth of a CRPC PDX model (LuCap 35) *in vivo* (**D**). **: $P < 0.01$; ***: $P < 0.001$; ****: $P < 0.0001$

Significant Survival Benefit of PRT2527 in Responsive Pancreatic and Gastroesophageal PDX Models with *MYC* Amplification



- Intermittent IV administration of PRT2527 (15 or 30 mg/kg QW) significantly extended median survival of mice bearing PDX models of PDAC (**A**) and gastroesophageal (**B**) with *MYC* amplification.

Conclusions

- PRT2527 is a potent and highly selective CDK9 inhibitor
- PRT2527 treatment depletes oncogenic drivers with short half-lives such as MYC and MCL1 and induces apoptosis
- Intermittent IV administration of PRT2527 demonstrated strong efficacy in hematological malignancies and solid tumors models with *MYC* amplification and prostate cancer with AR dependence
- PRT2527 IND enabling studies completed and PRT2527 is expected to enter the clinic in Q4 for heme and solid tumors with MYC amplification