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Targeting PKMYT1 to Preferentially Kill Tumor Cells that Have CCNE1 Amplifications

Samantha S Hodge, Natalie Hill, Kayla Walzer, Ritu Kushwaha, Yihong Guan, Jeremy Fournier, Ester Fernàndez-Salas

Arcus Biosciences, Inc.; 3928 Point Eden Way, Hayward, CA 94545 (USA)

Background

- Cyclin E1 (CCNE1) amplification occurs in ovarian (19%), gastric (12%), endometrial (11%) and lung (9%) cancers and is associated with shorter disease-free survival and overall survival [1-3]
- Patients with CCNE1 amplification respond poorly to CDK4/6 inhibitors, PARP inhibitors, and platinum agents [3-5]
- CCNE1 amplification deregulates the G1/S checkpoint and causes premature entry into S phase
- CCNE1-amplified cells depend on the G2/M checkpoint to ensure genomic stability before mitosis
- PKMYT1, a member of the WEE1 family of enzymes, phosphorylates and inhibits CDK1 at T14, and to a lesser extent Y15, to regulate the G2/M checkpoint and prevent entry into mitosis
- PKMYT1 inhibition was previously identified as synthetic lethal with CCNE1 amplification [6]

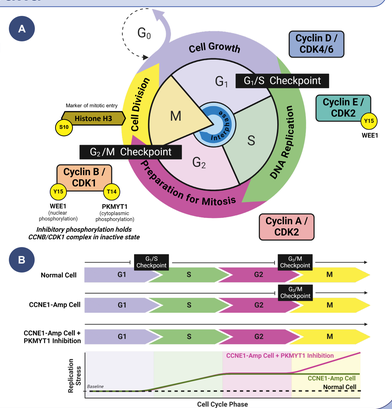


Figure 1. PKMYT1 inhibition is synthetic lethal with CCNE1 amplification

A. Cell cycle schematic. PKMYT1 phosphorylates CDK1 at pT14 to enable the G2/M checkpoint and prevent entry into mitosis.

B. Schematic of replication stress following PKMYT1 inhibition. PKMYT1 inhibition in CCNE1-amplified cell lines results in entry into mitosis, catastrophic DNA damage and replication stress, and cell death.

CCNE1 Overexpression Disrupts the Cell Cycle

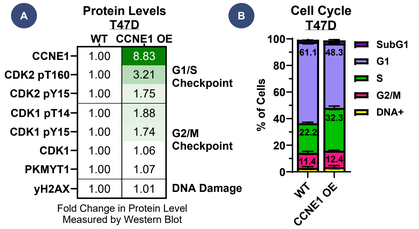


Figure 2. CCNE1 overexpression (OE) in T47D cells increases progression from G1 to S phase and dependence on G2/M checkpoint

A. CCNE1 OE cells have increased phosphorylation of G1/S checkpoint proteins (CDK2 pT160 (activating phosphorylation), CDK2 pY15 (inhibitory phosphorylation)) and G2/M checkpoint proteins (CDK1 pT14, CDK1 pY15 (both inhibitory phosphorylations)). No changes in basal CDK1, PKMYT1, or γH2AX levels were detected.

B. Overexpression of CCNE1 decreases the percentage of T47D cells in G1 and increases the percentage of cells in S phase, consistent with CCNE1 OE dysregulating the G1/S checkpoint.

CCNE1 Overexpression Increases Sensitivity to PKMYT1 Inhibition

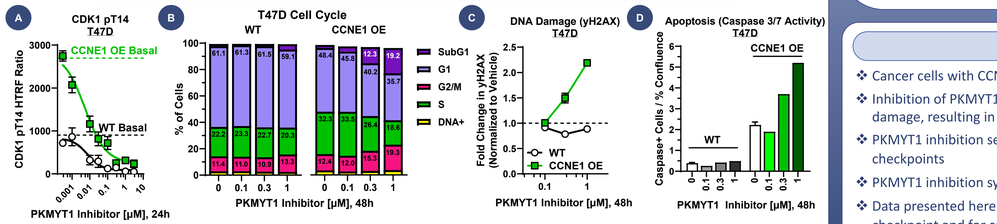


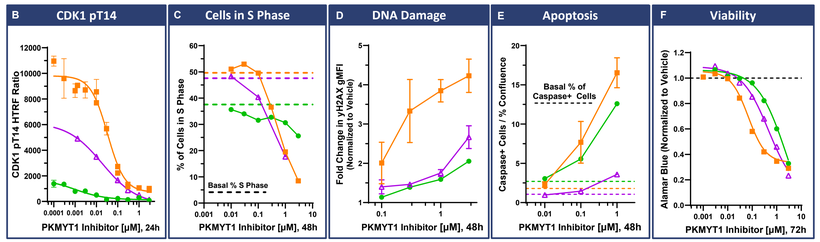
Figure 3. PKMYT1 inhibition disrupts the cell cycle and increases DNA damage and apoptosis in CCNE1 overexpressing T47D cells

A-D. PKMYT1 inhibition: (A) decreases CDK1 pT14 levels in both T47D WT and CCNE1 OE cells, **(B)** decreases G1/S phases and increases SubG1 (apoptotic) and G2/M phases in CCNE1 OE cells. No changes observed in WT cells, **(C)** increases γH2AX in CCNE1 OE cells. No changes observed in WT cells, and **(D)** increases caspase3/7 activity in CCNE1 OE cells. No increases observed in WT cells.

PKMYT1 Inhibition Decreases CDK1 pT14, Disrupts the Cell Cycle, and Induces DNA Damage and Cell Death in CCNE1-Amplified Cancer Cells

A

Cell Lines	Lineage Sub-type	CCNE1 Status	CCNE1 CN	Other Alterations
HCC1569	Breast IDC	CCNE1amp	7	BRCA2, TP53, RB1 null, PTEN, PTCH1
OVCAR3	HGSOC	CCNE1amp	7	TP53, RB1 del, PIK3R1, PPP2R1A
HCC1806	Breast DCIS	CCNE1amp	7	TP53, p16 del



G

HCC1569 + PKMYT1 Inhibitor

	Ctrl	6h 0.01	6h 0.1	6h 1	24h 0.01	24h 0.1	24h 1	48h 0.01	48h 0.1	48h 1 [μM]
CDK1 pT14	1.0	0.7	0.5	0.2	0.9	0.4	0.1	0.5	0.3	0.1
Histone H3 pS10	1.0	1.0	3.5	5.0	2.1	2.4	0.8	0.7	0.6	0.1
γH2AX	1.0	0.9	5.3	14.5	2.3	23.2	46.3	1.4	13.6	14.4
ATR	1.0	0.9	1.4	1.7	1.1	1.2	0.9	1.4	0.8	1.4
ATR pS428	1.0	1.1	1.0	2.2	0.9	1.0	1.0	1.0	0.7	0.5
CHK1	1.0	1.2	1.0	1.1	0.9	0.8	0.9	1.0	0.9	0.5
CHK1 pS345	1.0	1.2	3.7	5.3	2.5	5.1	8.1	1.4	4.7	6.9
DNA-PKcs	1.0	2.2	1.9	1.2	1.2	0.8	1.2	0.6	0.2	0.2
DNA-PKcs pS2056	1.0	0.6	1.1	2.8	1.4	8.1	36.8	0.9	14.0	16.3
Cleaved Caspase 3	1.0	1.1	0.8	0.9	0.5	2.0	3.8	1.7	10.3	29.3
Cleaved PARP	1.0	0.8	0.6	0.7	0.8	1.7	5.6	1.0	4.1	13.2

Lower Level — Higher Level

Fold Change in Protein Level Normalized to Control

Figure 4. PKMYT1 inhibition decreases CDK1 pT14, disrupts the cell cycle, and increases DNA damage and apoptosis in CCNE1-amplified cancer cells

A. Lineage, CCNE1 copy number (CN) and other alterations of CCNE1-amp cell lines.

PKMYT1 inhibition:

B. decreases CDK1 pT14,

C. decreases cells in S phase,

D. increases γH2AX levels,

E. increases caspase3/7 activity, and

F. decreases viability (AlamarBlue) in CCNE1-amp cells.

G. In HCC1569 cells, PKMYT1 inhibition decreases G2/M checkpoint inhibition (CDK1 pT14), increases entry into mitosis (Histone H3 pS10), increases DNA damage response (γH2AX, CHK1 pS345, DNA-PKcs pS2056) and increases apoptosis (cleaved caspase 3, cleaved PARP).

Conclusions

- Cancer cells with CCNE1 amplification/overexpression are dependent on the G2/M checkpoint to maintain genomic stability
- Inhibition of PKMYT1 in cells expressing high levels of CCNE1 leads to premature entry into mitosis and accumulation of DNA damage, resulting in increased apoptosis and cell death
- PKMYT1 inhibition selectively kills CCNE1-amplified cancer cells while sparing non-tumorigenic cells that have intact G1/S checkpoints
- PKMYT1 inhibition synergizes with chemotherapy (Gemcitabine, SN38) to further inhibit CCNE1-amplified cell growth
- Data presented here provides a rationale for targeting PKMYT1 in CCNE1-amplified cancers that are dependent on the G2/M checkpoint and for combining PKMYT1 inhibition with standard of care chemotherapy

Contact Information: shodge@arcusbio.com **Poster available at** https://arcusbio.com/our-science/publications/

[1] cBioPortal, [2] Yao et al. (2022) Scientific Reports, [3] Zhao et al. (2019) BMC Cancer. [4] Nakayama et al. (2009) Cancer, [5] Etemadmoghadam et al. (2009) Clin Cancer Res, [6] Gallo et al. (2022) Nature, [7] Ianevski et al. (2022) Nucleic Acids Research

PKMYT1 Inhibition Selectively Kills CCNE1-Amplified Cancer Cells While Sparing Non-Tumorigenic Cells

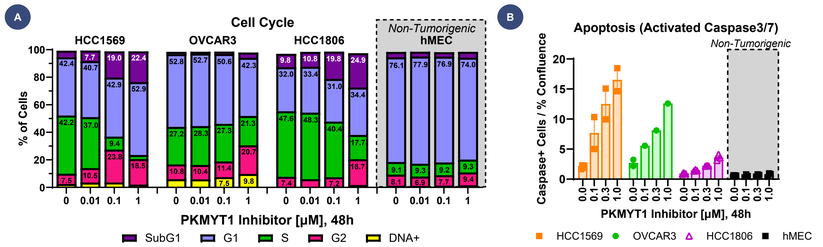


Figure 5. PKMYT1 inhibition selectively disrupts the cell cycle and increases apoptosis in CCNE1-amplified cells while sparing non-tumorigenic cells

PKMYT1 inhibition:

A. decreases G1/S phases and increases SubG1 (apoptotic) and G2/M phases in CCNE1-amp cells. No changes observed in non-tumorigenic cells (hMEC = primary human mammary epithelial cells).

B. increases caspase 3/7 activity in CCNE1-amp cell lines, but not in non-tumorigenic cells.

PKMYT1 Inhibition Synergizes with Chemotherapy in CCNE1-Amplified Cells

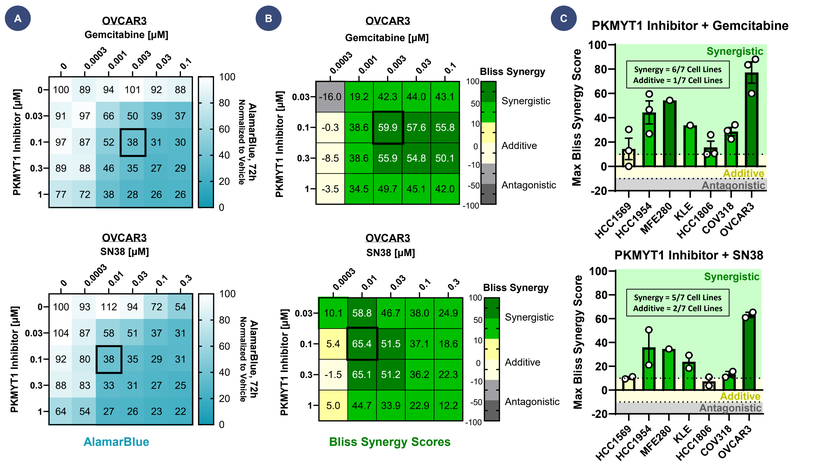


Figure 6. PKMYT1 inhibition and chemotherapy (gemcitabine, SN38) synergize in CCNE1-amplified cell lines to inhibit cell growth

A. PKMYT1 inhibition and gemcitabine or SN38 combine to inhibit CCNE1-amp OVCAR3 growth beyond that of either compound as a single agent.

B. Synergy observed between PKMYT1 inhibition and gemcitabine or SN38 in OVCAR3 cells. Synergy calculated using Bliss Synergy Score [7]. Black squares denote combination that generates maximum bliss synergy score.

C. Max Bliss Synergy scores of PKMYT1 inhibition combined with gemcitabine or SN38 in CCNE1-amp cell lines. Synergy with gemcitabine (Bliss Score >10) observed in 6/7 CCNE1-amp cell lines. Synergy with SN38 observed in 5/7 CCNE1-amp cell lines. HCC1569, the only cell line not to exhibit synergy between PKMYT1 inhibition and gemcitabine, is highly sensitive to single agent PKMYT1 inhibition.