Inhibition of CDK7 overcomes resistance to CDK4/6 inhibitors in hormone receptor positive breast cancer cells.

PD7-12

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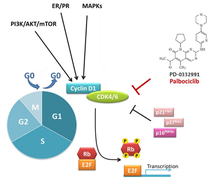
INTRODUCTION

- **CDK4/6 inhibitors have emerged as effective treatments in hormone receptor positive (HR+) metastatic breast cancer (MBC).**
- **Despite the efficacy of the combinations of endocrine therapy (ET) and CDK4/6 inhibitors in the HR+/HER2- MBC setting, the majority of patients eventually acquire resistance to these treatments.**
- **The loss of Rb is one of the mechanisms of resistance to CDK4/6 inhibitors [1]**

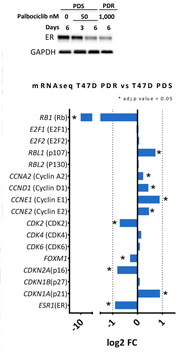


BACKGROUND

Palbociclib-resistant T47D PDR development

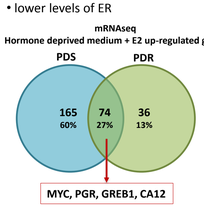
T47D palbociclib-resistant (PDR) breast cancer (BC) model was developed by exposing HR+/HER2- T47D palbociclib-sensitive (PDS) cells to increasing concentrations of palbociclib [2].

ER and cell cycle-related molecules expression levels in T47D PDS and PDR



Compared to T47D PDS cells, at protein and mRNA levels T47D PDR cells are characterized by:

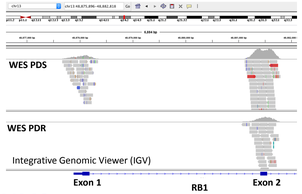
- loss of Rb,
- higher levels of p107, cyclin E1 and cyclin E2
- lower levels of ER



E2 transcriptional regulation is retained in T47D PDR cells for many of the key ER regulated genes.

Modified from
Guarducci C. et al. Npj Breast Cancer (2018) 4:38

The loss of Rb in T47D PDR is due to a deletion in the exon 1 of RB1



Whole Exome Sequencing (WES) in T47D PDS and PDR shows that T47D PDR cells lack exon 1 reads.

AIMS

- **To identify genes that are essential for cell growth in palbociclib-resistant cells with loss of Rb.**
- **To identify significantly essential genes that are targets with available drugs and test the efficacy of these compounds in T47D PDS and T47D PDR cells.**

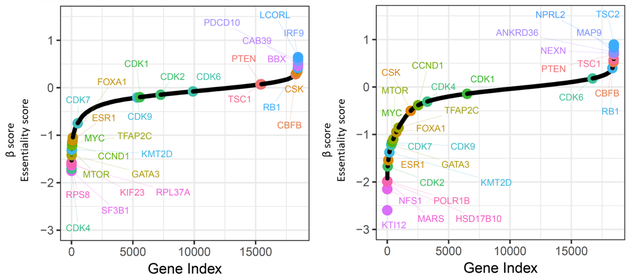
RESULTS

Identification of T47D PDR vulnerabilities

Genome-wide CRISPR-Cas9 knock out screen in T47D PDS and PDR

H1/H2 library (10 single guide RNAs/gene)→more than 18,000 genes targeted.
β-score negative values are associated with genes that are essential for cell growth (negatively selected genes).
β-score positive values are associated with tumor suppressor genes (positively selected genes).

β scores of sgRNA targeted genes in T47D PDS and T47D PDR



Highlighted genes:
Significant genes (β< -0.5, β> -0.5) (FDR< 0.001) and genes of interest

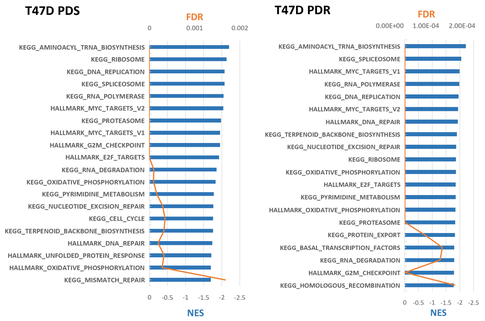
	Genes	T47D PDR		T47D PDS	
		β-score	FDR	β-score	FDR
Genes not essential in T47D PDR	***TSC2***	0.90	< 0.001	0.15	0.76
	PTEN	0.69	< 0.001	0.07	0.72
Genes essential in T47D PDR	***CDK2***	-1.67	< 0.001	-0.14	0.47
	CDK7	-1.21	< 0.001	-0.75	< 0.001
	ESR1	-1.54	< 0.001	-1.11	< 0.001
	MYC	-1.16	< 0.001	-1.13	< 0.001
Palbociclib target genes	***CDK4***	-0.30	0.10	-1.70	< 0.001
	CDK6	0.18	0.36	-0.07	0.74

In T47D PDR we identified:

- 29 genes that are significantly positively selected (among these genes were *TSC2* and *PTEN).*
- nearly 600 genes that are significantly negatively selected (among these genes *were CDK2, CDK7, ESR1* and *MYC).*

***CDK2, CDK7, ESR1* and *MYC* are among the top ranked genes that are essential for T47D PDR cells growth.**

Top 20 datasets enriched in T47D PDS and PDR.

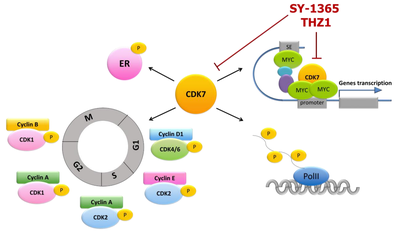


GSEA of T47D PDS and PDR CRISPR screen results ranked by β-score and FDR.

Among the top 20 enriched datasets in T47D PDS and PDR are:

- MYC targets
- RNA polymerase
- E2F targets
- G2/M checkpoint

T47D targetable essential gene: CDK7

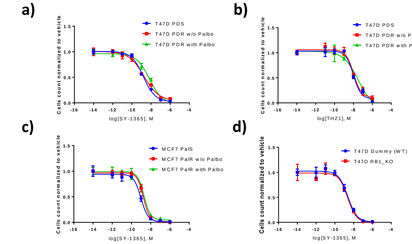


CDK7 is a serine/threonine kinase involved in:

- ER activation
- RNA polymerase II (RNApolII)-mediated RNA transcription.
- Phosphorylation of cell cycle CDKs (CDK activating kinase, CAK)
- Activation of super-enhancer-linked oncogenic transcription in MYC-driven cancers [3].

THZ1 and SY-1365 are covalent selective CDK7 inhibitors.

Concentration dependent activity of two CDK7 inhibitors, SY-1365 and THZ1

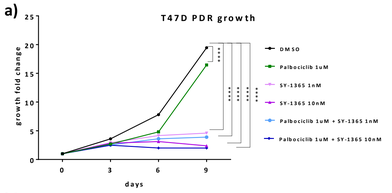


e)

IC50 after 5 days of drugs treatment	SY-1365	THZ1
T47D PDS	1.60 nM	9.93 nM
T47D PDR w/o Palbociclib 1 µM	1.87 nM	10.30 nM
T47D PDR with Palbociclib 1 µM	6.70 nM	32.8 nM
MCF7 PalS	0.96 nM	
MCF7 PalR w/o Palbociclib 1 µM	1.77 nM	
MCF7 PalR with Palbociclib 1 µM	2.03 nM	
T47D Dummy (WT)	2.4 nM	
T47D RB1_KO	2.31 nM	

- SY-1365 was equally effective in T47D PDS and PDR (a, e).
- THZ1 was equally effective in T47D PDS and PDR (b, e).
- The effect of SY-1365 was confirmed in MCF7 HR+ BC palbociclib sensitive (PalS) and resistant (PalR) cells (c, e).
- The loss of Rb in T47D cells (T47D RB1_KO) doesn't affect the sensitivity to SY-1365 (d, e).

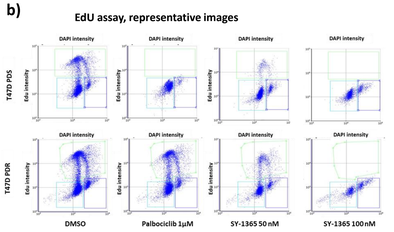
T47D PDS and PDR growth and cell cycle analysis with palbociclib and SY-1365

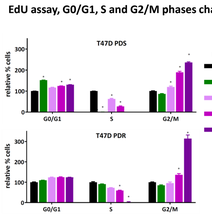


SY-1365 significantly arrests T47D PDR growth (a).

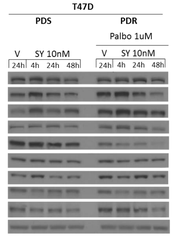
Cell cycle analysis with palbociclib and SY-1365 on T47D PDS and PDR (b) shows that:

- palbociclib strongly reduces the fraction of cells in the S-phase while increasing the fraction of cells in the G0/G1-phase in T47D PDS but not PDR.
- SY-1365 reduces the fraction of cells in the S-phase and increases the fraction of cells in the G2/M-phase in both T47D PDS and PDR.





CDK7 targets modulation in T47D PDS and T47D PDR treated with SY-1365

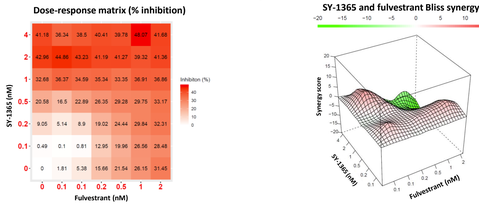


Compared to vehicle (V), long term 48h treatment with SY-1365 (SY) 10 nM in T47D PDS and PDR shows:

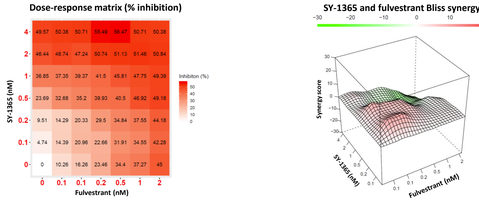
- •↓ levels of p-RNA polII, Ser2
- •↓ levels of p-RNA polII, Ser7
- •↓ levels of ER
- •↓ levels of c-MYC

Fulvestrant and SY-1365 combination studies

T47D PDR without palbociclib treated with escalating doses of fulvestrant and SY-1365



T47D PDR with palbociclib 1µM treated with escalating doses of fulvestrant and SY-1365



SY-1365 and fulvestrant in T47D PDR show synergistic activity (positive synergy score) at low drugs concentrations.

CONCLUSIONS

1. **In Rb-loss palbociclib-resistant T47D cells, cyclin D1, CDK4 and CDK6 are not essential for *in vitro* cell growth.**
2. ***CDK7* and *ESR1* are significant essential genes for *in vitro* cell growth in palbociclib-sensitive and palbociclib-resistant T47D cells.**
3. **The CDK7 inhibitor SY-1365 arrests the cell cycle progression in G2/M phase, and reduces the expression level of CDK7 inhibitor targets in palbociclib-sensitive and -resistant T47D cells.**
4. **In palbociclib-resistant T47D cells, the CDK7 inhibitor SY-1365 and fulvestrant have synergistic activity.**

Our results suggest that CDK7 is a new therapeutic target for the treatment of naïve and palbociclib-resistant HR+/HER2- BC, and support the ongoing clinical investigation of SY-1365 (NCT03134638) in combination with fulvestrant in HR+ BC patients who have progressed through CDK4/6 inhibitors.

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1. Pernas S. et al. *Ther Adv Med Oncol. 2018 Jul 17;10:1758835918786451.*
2. Guarducci C. et al. *Npj Breast Cancer (2018) 4:38.*
3. Chipumuro et al. *Cell. 2014 Nov 20; 159(5): 1126–1139.*

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