PMD-026, a First in Class Oral RSK inhibitor, Demonstrates Activity Against Hormone Receptor Positive Breast Cancer with Acquired CDK4/6 Inhibitor Resistance

Abstract #5378

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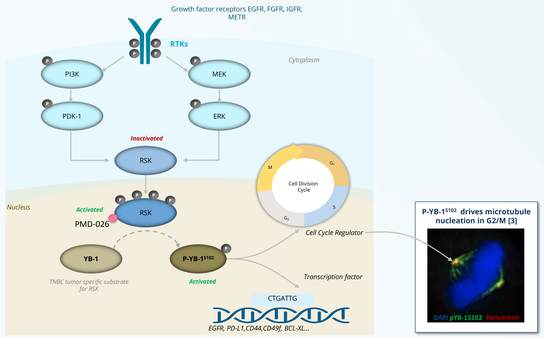
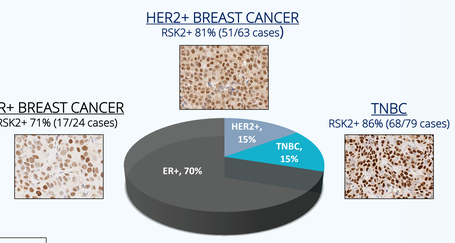
1. Background

PMD-026 is a first in class, reversible, oral small molecule inhibitor of p90 ribosomal S6 kinase (RSK). RSK is the major convergence point for the MAPK, PDK-1 and PI3K pathways, which regulate substrates involved in cancer cell proliferation and drug resistance through the Y-box binding protein (YB-1). RSK phosphorylates YB-1S102, leading to cancer cell proliferation through microtubule assembly at the G2/M phase of the cell cycle. Specifically, both MAPK and PI3K pathways are implicated in resistance to standard of care (SOC) CDK4/6 inhibitors in hormone receptor positive (HR+) breast cancer (BC) [1]. Comparatively, RSK2 has been identified as a major driver in BC. Functional dependency of BC on RSK2 was discovered through unbiased kinome-wide screens across a heterogeneous panel of breast cancer cell lines [2]. RSK2 is expressed in 71% of HR+ primary BC, therefore we hypothesized that disrupting these critical pathways (MAPK and PI3K) with PMD-026 could overcome resistance to CDK4/6 inhibitors palbociclib (Ibrance) and abemaciclib (Verzenio) through cell growth inhibition, apoptosis induction and downstream target inhibition alone and in combination with SOC SERD fulvestrant. In preclinical studies and in a Phase I/Ib clinical trial in metastatic BC (NCT04115306), PMD-026 demonstrated a good safety profile, making it an ideal candidate for the CDK4/6 inhibitor resistant HR+ BC population. Here we present preclinical data on the effect of PMD-026 alone and in combination with fulvestrant on HR+ BC cell lines sensitive and resistant to CDK4/6 inhibitors.



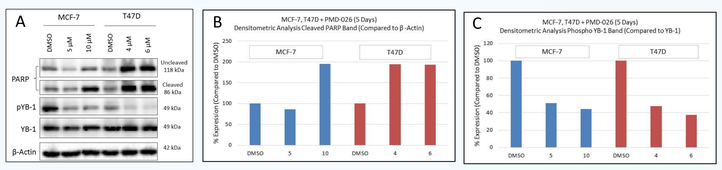
2. RSK2 is activated in 71% of hormone receptor positive (HR+) primary breast cancers

RSK2+ in 82% (136/166 cases) of all breast cancer subtypes



RSK2 expression was determined by immunohistochemistry (IHC). Breast cancer samples from primary tumours were given a score of 0, 1 or 2 based on intensity of activated RSK2 localized in the nuclei. Samples given a score of 1 or higher were categorized to have activated RSK2. Data is based on the optimized RSK2 CDxV1.0 staining method, which is a CAP/CLIA certified assay. Tissues were evaluated by screening tumor tissue microarrays BRC964, BRC1021 (Pantomics), BR487d (US Biomax) and BC8 (SuperBioChips).

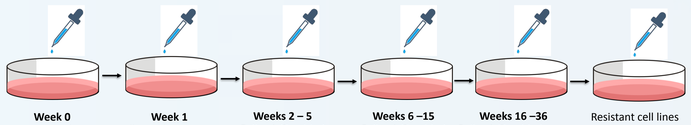
3. PMD-026 promotes apoptosis and inhibits YB-1 phosphorylation in HR+ breast cancer cell lines



MCF-7 & T47D cell lines were treated with PMD-026 at increasing concentrations for 5 days. Following generation of lysates, western blot analysis indicated increased PARP cleavage and decreased YB-1 phosphorylation in the presence of PMD-026 (**A**) . This was confirmed by densitometric analysis of cleaved PARP bands compared to β-Actin bands (**B**) and pYB-1 bands compared to the corresponding total YB-1 bands (**C**). The increased cleavage of PARP and the decrease of pYB-1, a downstream effector, confirms PMD-026 mediated apoptosis and inhibition of RSK activity, respectively. MCF-7 and T47D cell lines represent HR+ breast cancer models of mutant PIK3CA and p53, respectively.

4. Development of CDK4/6 inhibitor resistant HR+ breast cancer models

Schematic – Development of CDK4/6 Inhibitor Resistant Models *in vitro*



Week 0 Parental MCF-7/T47D

Week 1 Palbociclib starting at 0.02 µM added to cultures

Weeks 2 – 5 Palbociclib added once/week at increasing 0.02 µM increments up to 0.1 µM

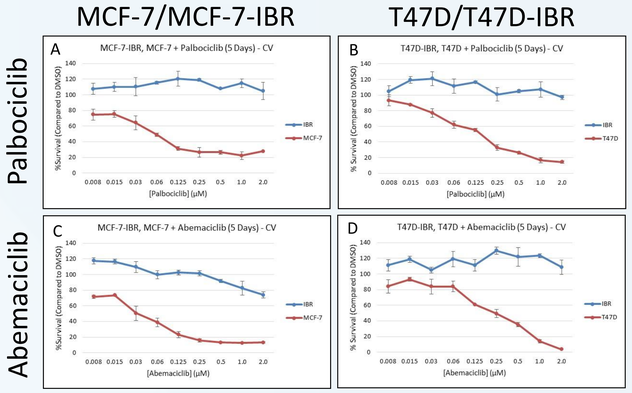
Weeks 6 –15 Palbociclib added once/week at increasing 0.1 µM increments up to 1 µM

Weeks 16 –36 Palbociclib added once every 2 weeks at increasing 0.1 µM increments up to 2 µM

Resistant cell lines continue to be grown in cultures with **2 µM palbociclib**

HR+ BC cell lines **MCF-7** and **T47D** were treated with increasing concentrations of the CDK4/6 inhibitor palbociclib (Ibrance) up to 2 µM for several months. A concentration of 2 µM was selected to establish resistance greater than 10X the half maximal inhibitory concentration (IC_{50}) values of 148 nM and 127 nM for MCF-7 and T47D, respectively [4]. The resistant cell lines, **MCF-7-IBR** and **T47D-IBR**, were subsequently grown in cultures supplemented with 2 µM palbociclib.

5. CDK4/6 resistant models (MCF-7-IBR and T47D-IBR) demonstrate cross resistance to both palbociclib and abemaciclib *in vitro*

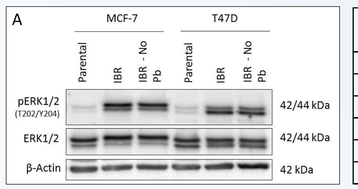


E

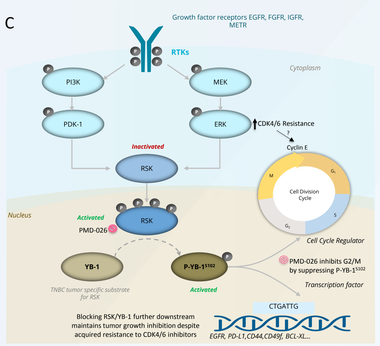
Inhibitor	IC50 Values (5 Days) - CV (µM)			
	MCF-7-IBR	MCF-7	T47D-IBR	T47D
Palbociclib	> 2.0	0.17 ± 0.06	> 2.0	0.19 ± 0.08
Abemaciclib	> 2.0	0.25 ± 0.18	> 2.0	0.27 ± 0.03

- MCF-7 and T47D parental and CDK4/6 inhibitor resistant cell lines were treated with increasing concentrations of palbociclib (**A**, **B**) and abemaciclib (Verzenio) (**C**, **D**) up to 2 µM for 5 days. Following crystal violet staining, growth inhibition analysis determine IC_{50} values for all cell lines tested (**E**).
- MCF-7-IBR & T47D-IBR demonstrated resistance to both palbociclib and abemaciclib with IC_{50} values greater than 2 µM.
- MCF-7 & T47D were sensitive to both palbociclib and abemaciclib with IC_{50} values ranging from 0.17 to 0.19 µM for palbociclib and 0.25 to 0.27 for abemaciclib.

6. CDK4/6 inhibitor resistant cell lines express increased phospho-ERK1/2 compared to parental cell lines

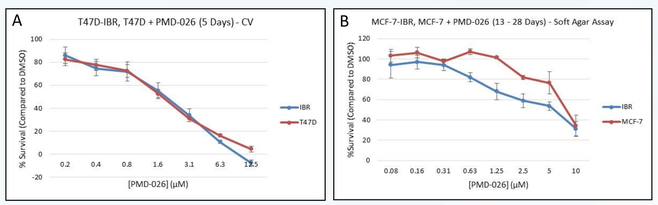


B Lane	% Expression pERK/ERK
MCF-7	100.0
MCF-7-IBR	371.6
MCF-7-IBR No Pb	363.9
T47D	100.0
T47D-IBR	304.5
T47D-IBR No Pb	302.9



- MCF-7 & T47D parental and CDK4/6 resistant cells were grown in culture for 5 days. For resistant cell lines, cultures were grown with (IBR) and without (IBR – No Pb) palbociclib to ensure that signalling changes observed were not due to the presence of palbociclib alone.
- Following generation of lysates, western blot analysis determined expression of phosphorylated and total ERK1/2. β-Actin was used as a loading control (**A**). Increased pERK1/2 in resistant cell lines with and without palbociclib compared to parental lines were detected. (**B**). Increased pERK1/2 expression and reliance on the MAPK pathway in cancers with acquired resistance to CDK4/6 inhibitors present an opportunity for the application of MAPK inhibitors (**C**) [1, 5].

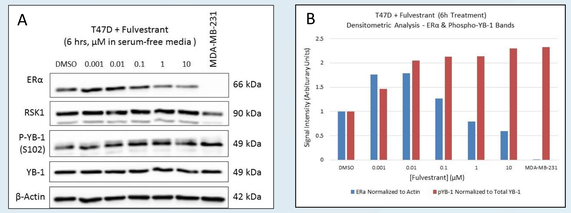
7. PMD-026 inhibits cell growth of parental and CDK4/6 inhibitor resistant HR+ breast cancer cell lines *in vitro*



C IC50 Values - HR+ BC Cell Lines + PMD-026 Monolayer & Soft Agar Assays (µM)			
Monolayer (5 Days)		Soft Agar (13 - 28 Days)	
T47D-IBR	T47D	MCF-7-IBR	MCF-7
3.3 ± 2.4	3.3 ± 1.8	4.7	8.4

- T47D & T47D-IBR cells were treated with increasing concentrations of PMD-026 (0.2 to 12.5 µM) in monolayer for 5 days (**A**). Following crystal violet staining, IC_{50} values were determined to be identical for both cell lines (3.3 µM) (**C**).
- MCF-7 and MCF-7-IBR cells were treated with increasing concentrations of PMD-026 (0.08 to 10 µM) in soft agar for 13 days and 28 days, respectively (**B**). Following counting of colonies in triplicate, IC_{50} values were determined to be 8.4 µM for MCF-7 and 4.7 µM for MCF-7-IBR (**C**).
- These results demonstrate that both parental and resistant cell lines remained sensitive to PMD-026 at similar IC50 values, indicating that CDK4/6 inhibitor resistance does not affect PMD-026 sensitivity.

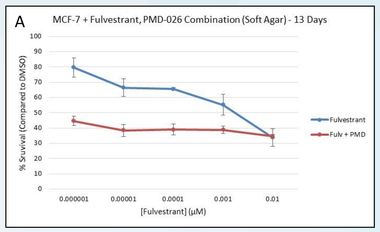
8. Phosphorylated YB-1 remains active in HR+ breast cancer cell line T47D treated with fulvestrant



- T47D cells were grown in serum-free media and then subsequently treated with increasing concentrations of fulvestrant (0 – 10 µM) for 6 hours. The triple negative breast cancer (TNBC) cell line MDA-MB-231 was included as a negative control for ERα expression and a positive control for RSK1 and YB-1 expression.
- Following generation of lysates, western blot analysis and densitometric analysis, it was determined that P-YB-1 remained active in the cells treated with fulvestrant only (**A**, **B**).
- Fulvestrant degraded ERα as expected in a dose-dependent manner, however levels of P-YB-1 remained unchanged and highly activated, suggesting an escape mechanism for proliferation (**A**, **B**).

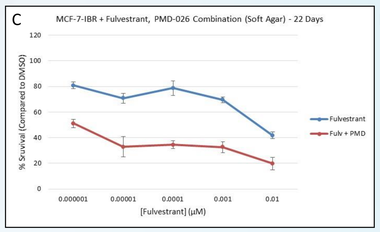
9. PMD-026 synergizes with fulvestrant in parental and CDK4/6 inhibitor resistant HR+ MCF-7 cell lines *in vitro*

MCF-7 + PMD-026 + Fulvestrant Combination



B CDI Calculations - MCF-7 + PMD-026, Fulvestrant (13 Days) - Soft Agar	
[Fulvestrant] (µM)	3 µM PMD-026
0.000001	0.73
0.00001	0.76
0.0001	0.78
0.001	0.92
0.01	1.34

MCF-7-IBR + PMD-026 + Fulvestrant Combination



D CDI Calculations - MCF-7 -IBR + PMD-026, Fulvestrant (22 Days) - Soft Agar	
[Fulvestrant] (µM)	3 µM PMD-026
0.000001	0.79
0.00001	0.58
0.0001	0.55
0.001	0.58
0.01	0.59

MCF-7 (**A**) and MCF-7-IBR (**C**) cells were treated with fulvestrant alone and in combination with PMD-026 (3 µM) for 13 days and 22 days, respectively, in soft agar. Following counting of colonies in triplicate wells, growth inhibition analysis determined coefficient of drug indices (CDI) [6] values ranging from 0.73 to 0.92 and 0.55 to 0.79 for MCF-7 (**B**) and MCF-7-IBR (**D**), respectively. These results indicate that PMD-026 synergizes with fulvestrant in both CDK4/6 inhibitor sensitive and resistant models. Figures are representatives of three separate experiments.

CDI	Interaction
> 1.1	Antagonism
1.10 - 0.95	Additive
0.94 - 0.80	Weak Synergy
< 0.80	Synergy

10. Conclusions

- Activated RSK2 is expressed in ~70% of HR+ breast cancers.
- HR+ BC cells lines with acquired resistance to palbociclib demonstrate cross resistance to abemaciclib.
- CDK4/6 inhibitor resistance does not affect PMD-026 sensitivity.
- PMD-026 synergizes with fulvestrant in parental and CDK4/6 inhibitor resistant HR+ cells.
- Together, these data support the combination of PMD-026 and fulvestrant in HR+ BC with acquired resistance to CDK4/6 inhibitors. Given that the MAPK pathway is upregulated in this setting, the PMD-026 and fulvestrant combination could give patients with HR+ BC and resistance to CDK4/6 inhibitors new hope.

11. References

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