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Abstract #1038

PMD-026, a First in Class Oral RSK inhibitor, Demonstrates Synergy When Combined with Standard of Care in Breast Cancer Tumor Models

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
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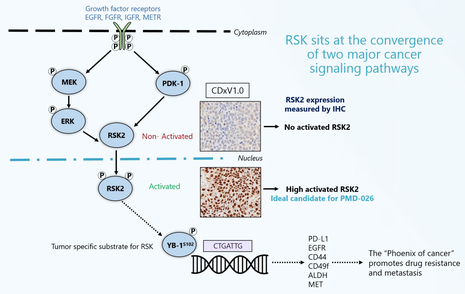
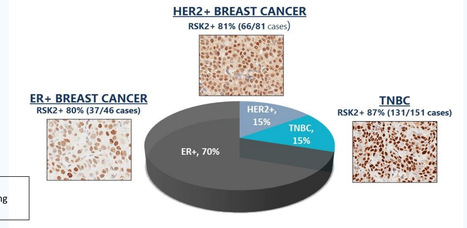
1. Background

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 and PDK-1 pathways, which regulate substrates involved in cancer cell proliferation, drug resistance and inflammation. Specifically, RSK2 has been identified as a major driver in breast cancer (BC). Functional dependency of BC on RSK2 was discovered through unbiased kinome-wide screens across a heterogeneous panel of breast cancer cell lines [1]. Silencing RSK2 by siRNA in BC inhibited growth *in vitro* with induction of apoptosis and suppression of tumor growth in mice [2]. Pharmacological inhibition of RSK2 with RSK inhibitors further validated RSK2 as a triple negative breast cancer (TNBC) target in xenografts in mice [2,3]. In preclinical studies and a Phase I clinical trial in metastatic breast cancer (mBC), PMD-026 demonstrated favorable pharmacological and pharmacokinetic properties and a good safety profile, making it an attractive candidate for combinations with standard of care therapies. Here we present preclinical data on the efficacy of PMD-026 in combination with standard of care (SOC) therapies paclitaxel and doxorubicin in TNBC, as well as fulvestrant in hormone receptor positive (HR+) BC.



2. RSK2 is activated in all breast cancer subtypes



RSK2 expression was determined by immunohistochemistry (IHC). Breast cancer samples from primary tumours, patient derived xenograft (PDx) models and xenografts derived from cell lines 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.

3. PMD-026 induces apoptosis and inhibits growth in BC cell lines *in vitro*

A **BC Cell Lines + PMD-026 (5 Days Treatment)**

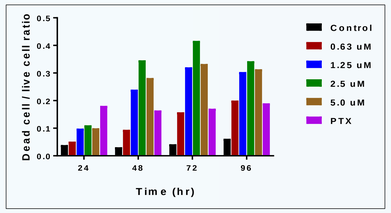
Group	Subtype	Cell Line	Apoptosis	Growth Inhibition
Basal-Like	BL1	MDA-MB-468	Y	Y
	BL1	HCC1937	N	Y
	BL1	HCC3153	Y	Y
	BL1	HCC38	Y	Y
	BL2	HCC1806	Y	Y
	BL2	HCC70	Y	Y
Mesenchymal-Like	MSL	MDA-MB-231	Y	Y
	MSL	MDA-MB-157	N	Y
	MSL	SUM159	N	Y
	MSL	Hs578T	Y	Y
	MSL	MDA-MB-436	N	Y
	M	CAL51	Y	Y
	M	BT549	Y	Y
	M	CAL120	Y	Y
Luminal Androgen Receptor	LAR	MDA-MB-453	N	Y
	LAR	SUM185	Y	Y
	LAR	HCC2185	Y	N
	LAR	MFM223	Y	N
Inflammatory Breast Cancer	IBC	SUM149	Y	Y
	IBC	BCX010	Y	Y
	IBC	FC-IBC-02	Y	Y
ER/PR	ER/PR	T47D	Y	Y
	ER/PR	MCF-7	Y	Y
HER2	HER2	BT474	N	N
	HER2	HCC1954	N	Y
	HER2	SKBR3	Y	Y
Unclassified	U/C	HCC1395	Y	Y
	U/C	BT20	N	N

B

Apoptosis		
Y	20	71%
N	8	Response

Growth Inhibition		
Y	24	86%
N	4	Response

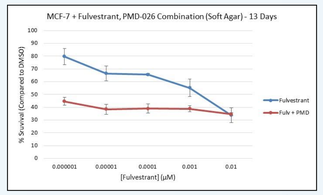
C **SUM149 + PMD-026, Paclitaxel (4 Days Treatment)**



- TNBC cell lines were treated with PMD-026 for 4-5 days. Cell death was determined by caspase-3/7 apoptosis assay and growth inhibition was determined by sulforhodamine B (SRB) proliferation assay (**A**).
- PMD-026 induced apoptosis and inhibited growth in 71% and 86% of BC cell lines, respectively (**B**).
- PMD-026 was more effective at inducing cell death than paclitaxel in the SUM149 TNBC/IBC (inflammatory breast cancer) model (**C**).

4. PMD-026 synergizes with fulvestrant in hormone receptor positive (HR+) breast cancer cell line MCF-7 *in vitro*

PMD-026 + Fulvestrant



CDI Calculations - MCF-7 + Fulvestrant, PMD-026 (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*

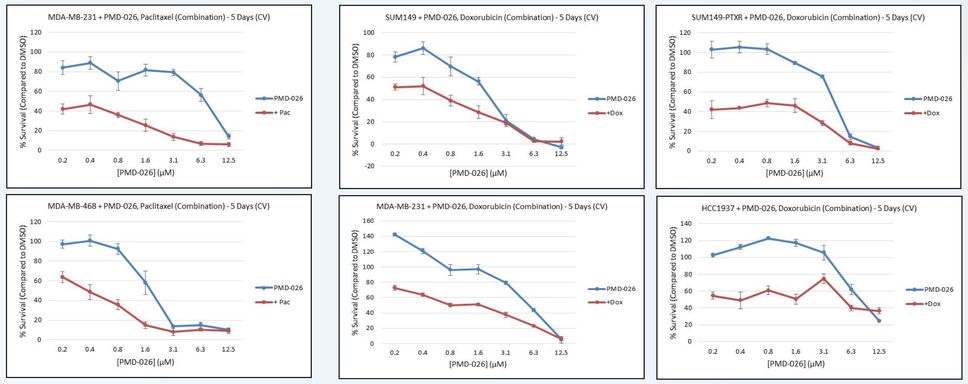
HR+ cell line MCF-7 was treated with PMD-026 alone in combination with fulvestrant for 13 days in soft agar. Following counting of colonies in triplicate wells, growth inhibition analysis determined coefficient of drug indices (CDI) [4] values ranging from 0.73 to 0.92, indicating **synergy**.

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

* CDI values do not accurately estimate synergy when % survival is approaching 0%

5. PMD-026 synergizes with paclitaxel and doxorubicin in TNBC cell lines *in vitro*, including drug resistant model SUM149-PTXR

A: PMD-026 + Paclitaxel | **B: PMD-026 + Doxorubicin**



[PMD-026] (μM)	Paclitaxel CDI Calculations	
	MDA-MB-231 + 0.3 nM Pac	MDA-MB-468 + 0.6 nM Pac
0.2	1.05	0.84
0.4	1.10	0.63
0.8	1.07	0.54
1.6	0.66	0.27
3.1	0.36	0.54
6.3	0.26	1.00
12.5	0.89	1.53*

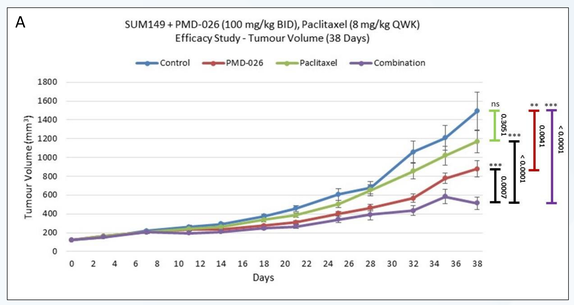
[PMD-026] (μM)	Doxorubicin CDI Calculations			
	SUM149 + 10 nM Dox	SUM149-PTXR + 9 nM Dox	MDA-MB-231 + 7 nM Dox	HCC1937 + 15 nM Dox
0.2	0.92	0.63	0.75	0.79
0.4	0.85	0.64	0.77	0.65
0.8	0.79	0.73	0.76	0.74
1.6	0.71	0.80	0.77	0.64
3.1	1.27	0.58	0.69	1.06
6.3	0.85	0.85	0.78	0.96
12.5	1.00	1.15*	1.75*	2.21*

* CDI values do not accurately estimate synergy when % survival is approaching 0%

TNBC cell lines were treated with PMD-026 alone and in combination with paclitaxel (**A**) or doxorubicin (**B**) for 5 days. Following crystal violet staining, growth inhibition analysis determined CDI values ranging from 0.26 to 0.89 for paclitaxel and 0.58 to 0.92 for doxorubicin, indicating **synergy** for both chemotherapies with PMD-026. SUM149-PTXR are resistant to paclitaxel and cross resistant to most SOC (data not shown).

6. PMD-026 synergizes with paclitaxel in TNBC/IBC xenograft model SUM149 *in vivo*

A

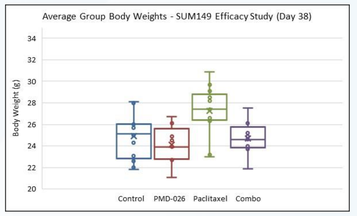


B

SUM149 Study 1 Day 38				
Treatment	Dose	Frequency	%TGI	CDI
Combination	100 mg/kg 8 mg/kg	BID QWK	65.5	0.75
PMD-026	100 mg/kg	BID	41.1	
Paclitaxel	8 mg/kg	QWK	21.7	

SUM149 Study 2 Day 49				
Treatment	Dose	Frequency	%TGI	CDI
Combination	100 mg/kg 8 mg/kg	BID QWK	61.5	0.78
PMD-026	100 mg/kg	BID	43.3	
Paclitaxel	8 mg/kg	QWK	13.2	

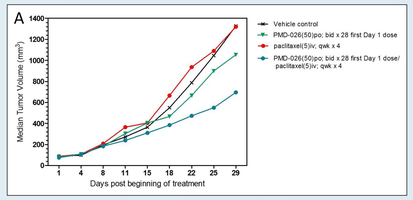
C



- A. PMD-026 synergized with paclitaxel *in vivo* in the SUM149 TNBC/IBC xenograft model after 38 days of treatment. The combination inhibited tumor growth by 66% ($P < 0.0001$), whereas paclitaxel and PMD-026 as single agents inhibited tumor growth by 22% ($P = 0.3051$) and 41% ($P = 0.0041$), respectively.
- B. The synergy of paclitaxel and PMD-026 in SUM149 xenografts was further supported by coefficient of drug indices (CDI) of 0.75 and 0.78 following 38 days and 49 days of treatment, respectively.
- C. After 38 days, the average body weights among the treatment groups did not decrease compared to the control group, indicating that PMD-026 and paclitaxel were well tolerated as single agents and in combination.

7. PMD-026 synergizes with paclitaxel in TNBC xenograft model MDA-MB-231 *in vivo*

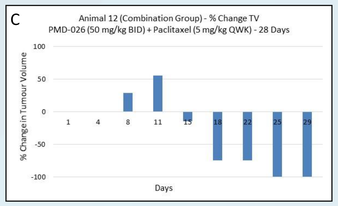
A



B

Study MDA-MB231-e514 Day 28				
Treatment	Dose	Frequency	%TGI	CDI
Combination	50 mg/kg 5 mg/kg	BID QWK	48	0.66
PMD-026	50 mg/kg	BID	21	
Paclitaxel	5 mg/kg	QWK	1	

C



- A. PMD-026 synergized with paclitaxel *in vivo* in the MDA-MB-231 TNBC xenograft model after 28 days of treatment. The combination inhibited tumor growth by 48% ($P = 0.0038$), whereas PMD-026 and paclitaxel as single agents at **suboptimal concentrations** inhibited tumor growth by 21% ($P = 0.29067$) and 1% ($P = 0.7217$).
- B. The synergy of paclitaxel and PMD-026 in this model was further supported by a CDI of 0.66.
- C. One animal in the combination group (Animal 12) achieved complete tumor regression starting at Day 15 of the study.

8. PMD-026 has an additive effect with paclitaxel in refractory metastatic TNBC PDx model BR5015 *in vivo*

A

BR5015 Model Characteristics:
- Recurrent breast carcinoma
- Mutations: BRCA1, BRCA2, EGFR, p53
- Resistance to: AC/T

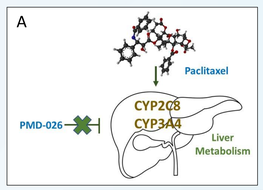
B

Study BR5015 E3275-U1705 Day 18				
Treatment	Dose	Frequency	%TGI	CDI
Combination	100 mg/kg 8 mg/kg	BID QWK	79.66	0.96
PMD-026	100 mg/kg	BID	42.73	
Paclitaxel	8 mg/kg	QWK	62.96	

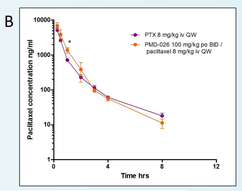
- A. PMD-026 had an additive effective with paclitaxel *in vivo* in the mTNBC BR5015 PDx model after 18 days of treatment. The combination inhibited tumor growth by 79.66% ($P < 0.001$), whereas paclitaxel and PMD-026 as single agents inhibited tumor growth by 42.73% ($P < 0.05$) and 62.96% ($P < 0.001$), respectively.
- B. The additivity of paclitaxel and PMD-026 in this model was further supported by a CDI of 0.96. The absence of synergy is most likely due to the significant efficacy of paclitaxel as a single agent.

9. PMD-026 does not alter metabolism, absorption, distribution or blood levels of paclitaxel

A



B



C

Group	t1/2 (hr)	AUCINF_obs (hr*ng/mL)	Vz_obs (L/kg)	Cl_obs (L/hr/kg)
Paclitaxel	1.98	4671	4.88	1.71
Combination PMD-026 + Paclitaxel	1.64	6422	2.95	1.25

- To address the safety of combining PMD-026 and paclitaxel, drug-drug interactions (DDI) were assessed.
- In cytochrome P450-mediated *in vitro* metabolism assays, PMD-026 showed weak inhibition of Cyp2C8 and Cyp3A4, the main enzymes responsible for paclitaxel metabolism (**A**).
- To understand whether PMD-026 might alter the metabolism of paclitaxel, this potential DDI was assessed *in vivo*.
- Pharmacokinetic analysis of PMD-026 (7 days repeat dosing) combined with paclitaxel (8 mg/kg IV dose Day 1 and Day 7) was evaluated in CD-1 mice, however, PMD-026 did not change the blood levels (**B**) absorption or distribution (**C**) of paclitaxel.

10. Conclusions

- Activated RSK2 is expressed in 85% of BC representing all cancer subtypes (ER+, HER2+ and TNBC).
- PMD-026, a first in class RSK inhibitor, synergizes with SOC therapies paclitaxel and doxorubicin in TNBC models, as well as fulvestrant in HR+ BC .
- The combination of PMD-026 plus paclitaxel was safe, as shown by the metabolism, absorption, distribution, and blood levels of paclitaxel in the presence of PMD-026.
- Overall, these data support adding PMD-026 to SOC therapies in breast cancer, as they demonstrate synergy in combination, improving efficacy without added toxicity. Synergy was observed across multiple drug resistant models, illustrating the breadth of potential for PMD-026 as a catalyst to potentially improve cancer outcomes in the future.

11. References

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