Pre-clinical efficacy in multiple syngeneic models with oral immune checkpoint antagonists targeting PD-L1 and TIM-3

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ABSTRACT

Antibody-mediated blockade of PD1 and PD-L1 has transformed the cancer therapy paradigm by eliciting durable antitumor responses and long-term remissions in a subset of patients with a broad spectrum of cancers. Interestingly, in an endeavor to enhance the response rate, a combination of antibodies targeting PD1 and CTLA-4 has resulted in significantly higher patient response rate. However, such combination has suffered from increased immune-related adverse events (irREs) due to the breaking of immune self-tolerance. Sustained target inhibition as a result of a long half-life (>15-20 days) and >70% target occupancy for months are likely contributing to irAEs observed. Towards addressing these shortcomings, we are developing small molecule agents targeting more than one immune checkpoint pathway to increase the response rate and dosing by oral route with relatively shorter pharmacokinetic exposure for better management of irREs.

Herein we report the pharmacological evaluation of the first-in-class small molecule antagonists capable of targeting both PD-L1 and TIM-3 immune checkpoint pathways. The design hypothesis for generating a dual antagonist is primarily based on the pockets of sequence similarity of PDL-1 and TIM-3 proteins. A focused library of compounds mimicking the interaction of checkpoint proteins was designed and synthesized. Screening and analysis of the resulting library led to the identification of hits capable of functional disruption of the PDL-1 and TIM-3 signaling pathways. Further optimization resulted in compounds displaying equipotent antagonism towards PD-L1 and TIM-3 with desirable physico-chemical properties and exposure upon oral administration.

Potent functional activity comparable to that obtained with an anti-PD1 or anti-TIM-3 antibody in rescuing lymphocyte proliferation and effector functions were observed with lead compound, AUPM-327. AUPM-327 showed selectivity against other immune checkpoint proteins including CTLA-4, VISTA, LAG-3 and BTLA as well as in a broad panel of receptors and enzymes. In syngeneic preclinical models of melanoma, breast and colon cancers, AUPM-327 showed significant efficacy in inhibition of both primary tumor growth and metastasis upon once a day oral dosing with excellent tolerability. Anti-tumoral activity correlated well with drug exposure and activation of CD4+ and CD8+ T cells.

The findings demonstrating the dual inhibition of PD-L1 and TIM-3 pathways resulting in activation of T cells and anti-tumor activities support further development of these orally bioavailable agents.

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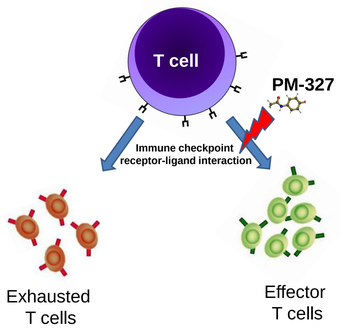
STRATEGY AND APPROACH

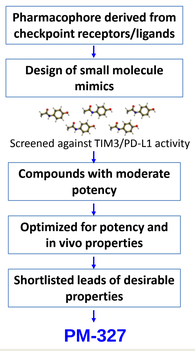
Small molecule immune checkpoint antagonists with the ability to disrupt the PD-L1 and TIM3 checkpoint pathways resulting in:

- Oral bioavailability for the ease of dosing
- Short-acting agents for better management of adverse events
- Targeting of PD-L1 and TIM3 to improve the response rate and with an opportunity to expand patient population beyond those that respond to anti-PD1/PD-L1 therapies

Strategy

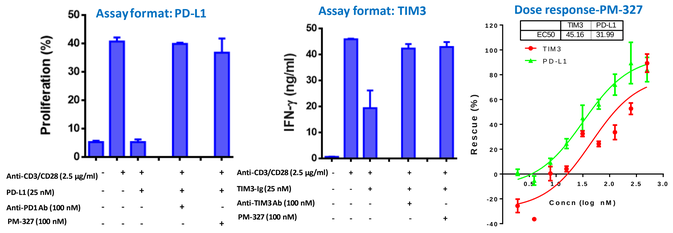


Approach

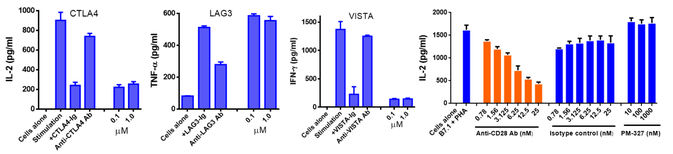


RESULTS

PM-327 exhibits equipotent antagonism against PD-L1 and TIM3

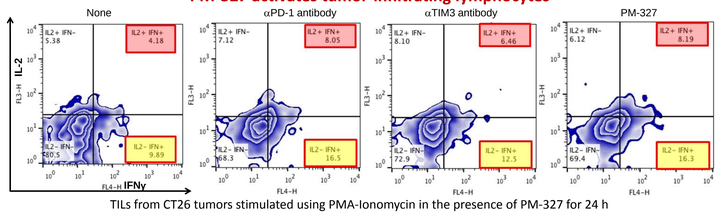


PM-327 does not inhibit other immune checkpoints and B7.1



RESULTS

PM-327 activates tumor-infiltrating lymphocytes



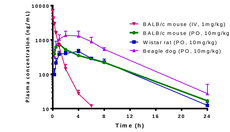
TILs from CT26 tumors stimulated using PMA-Ionomycin in the presence of PM-327 for 24 h

PM-327 exhibits excellent in vitro ADME properties and oral pharmacokinetic profile across species

In vitro ADME profile of PM-327

Assay	Species	Conc (µM)	Remarks
Metabolic stability in liver microsomes	M, R, D, Mo, H	1	Stable across the test species. $T_{1/2}$ is >60 minutes
Metabolic stability in kidney membrane	M, R, Mo, H	1	Stable across the test species. >90% stable at 30 min
CYP450 inhibition using liver microsomes	H	0.005 to 50	No inhibition(IC_{50} >50 uM) of hepatic CYP 1A2, 2C9, 2C19, 2D6 and 3A4 including TDI/MBI
Metabolic stability hepatocytes	M, R, D, Mo, H	1	Stable across the test species. $T_{1/2}$ is >60 minutes
Plasma stability	M, R, D, Mo, H	10	Stable across the test species. >90% stable at 4h.
Plasma protein binding	M, R, H	3	AUPM-327 exhibited low plasma protein binding (5-25%)
Caco2 Permeability assay	H	3,10, 30	Low permeable , non-ppg substrate
CYP Phenotyping (rCYP)	H	1	No tested CYP isoform was responsible for metabolism

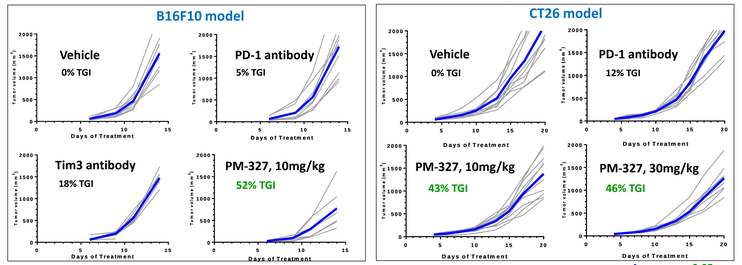
PK profile of PM-327



Parameters	Units	BALB/c Mouse	Wistar Rat	Beagle Dog
$AUC_{0\text{-}LAST}$ (PO)	h*ng/ml	5069	4631	12568
Beta $t_{1/2}$	h	4.5	3.9	3.5
C_{max}	ng/ml	755	481	1450
T_{max}	h	1.0	3.3	2.6
Bioavailability	%F	34.7	24.1	29

Dosed at 10 mg/kg PO

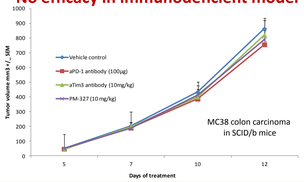
PM-327 shows significant *In vivo* efficacy in multiple syngenic tumor models



Summary of efficacy studies in syngenic models

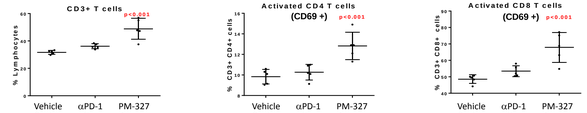
Model	Treatment	Dose (mg/kg)			
		1	3	10	30
CT26 colon carcinoma	PM-327	22	35	43	46
	αPD-1 ab		7		
B16F10 melanoma	PM-327	45	41	52	33
	αPD-1 ab		5		
	αTim3 ab			18	
MC38 colon carcinoma	PM-327	24	33	40	39
	αPD-1 ab		34		

No efficacy in immunodeficient model

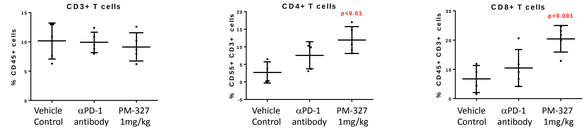


PD modulation in CT26 tumor bearing mice

Cell surface markers in the whole blood



Cell surface markers in tumors



PM-327 shows no off-target inhibition in a 80 panel CEREP analysis

Non peptide receptors						Peptide Receptors						Other	
Adenosine		**GABA**		P2X, P2Y	14, 3	**Cholecystokinin**		**Neurokinin**		**Vasopressin**	
A_1, A_{2A}, A_3	13.4, -19	GABA	-31	**Serotonin**		CCK1, CCK2	-36, -9	NK1, NK2, NK3 (antago)	-4, 8.7	V1a	16
Adrenergic		BZD (Central)	-6	SHT1A, SHT1B, SHT2A, SHT2B, SHT2C, SHT3, SHT5A, SHT6, SHT7	-16 to 15	Cytokines		Neuropeptide Y		**Nuclear receptors**	
α_1, α_2, β_1, β_2	5, -3, 10, 2	CI-Channel	1	**Sigma non-selective**	2	TNFα			-7, -11	GR, PPARγ	1, -12
Benzodiazepine		**Histamine**		**Peptide Receptors**		**Endothelin**		**Neurotensin**		**Ion Channels**	
BZD	7	H1, H2	-8, 0	Angiotensin -II		ETA, ETB	-13, 4	NTS1	2	$Ca2+$, Kv, Skca, $Na2+$	5, -1, 0, 31
Glutamate		**Melatonin**		AT1, AT2,	5, -8	**Galanin**		**Opioid & Opioid like**		**Amine transporters**	
(PCP)	-2	MT1	-11	**Bombesin (BB)**	6	GAL1, GAL2	-0.8, 18	DOP, KOP, MOP, NOP	6, 5, -11, 6	DA, NE, SHT	12, 0, 5
Cannabinoid		**Muscarinic**		**Bradykinin (B2)**	2	**Growth factors**		Somatostatin		Test concentration of PM-327: 10 µM	
CB1	-18	M1, M2, M3, M4, M5	5, 6, 3, 12, -8	**CGRP**	1	PDGF	1.5	sst	-4		
Dopamine		**Prostanoid**		**Chemokines**		**Melanocortin**		**VIP**			
D1, D2S, D3 D4.4, D5	9, 15, -4, 1.6, 0	EP4, IP	-6, 6	CCR1, CXCR2	3, -16	MC4	-12	PACAP, VIP1	5, 2		

PM-327 exhibits excellent safety profile with a >100 fold therapeutic window

Assay	Results
hERG, AMES and micronucleus test	Clean profile
14 day non-GLP tox in mouse	Well tolerated with the expected NOAEL >1000mg/kg
Rat cardiovascular safety studies	No changes in mean arterial pressure, heart rate and cardiac contractility up to maximum tested plasma concentration of 15µg/ml

SUMMARY

We have identified an orally bio-available PD-L1/TIM3 dual antagonist.

- The lead candidate targeting PD-L1 and TIM-3 pathways exhibits desirable potency, DMPK properties including oral bioavailability, shows anti-tumor efficacy in multiple syngeneic tumor models, promotes tumor infiltrating T cell activation and exhibits a desirable safety profile

Flexible oral administration and antagonism of PD-L and TIM3 checkpoint pathways may provide for improved or expanded clinical benefit in cancer patients