Oral Immune Checkpoint Antagonists Targeting PD-L1/VISTA and PD-L1/TIM3 for Cancer Therapy

P. G. Sasikumar, N. S. Sudarshan, N. M. Gowda, R. K. Ramachandra, D. S. Samiulla, T. Chandrasekhar, S. Adurthi, J. Mani, R. Nair, S. Singh, A. A. Dhudashia, Chandranath D. N. and M. Ramachandra*

Aurigene Discovery Technologies, Bangalore, India

AURIGENE Accelerating Discovery

CURIS™

ABSTRACT

Recent successes in achieving highly durable clinical responses with antibodies to immune checkpoint receptors such as CTLA4 and PD1 have transformed the outlook for cancer therapy. While these antibody-based therapies show impressive clinical activity, they suffer from the shortcomings including the need to administer by intravenous injection, failure to show response in majority of patients and immune-related adverse events (irAEs) 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 may be factors contributing to irAEs observed.

We sought to discover and develop small molecule immune checkpoint antagonists capable of targeting PD-L1 and another immune checkpoint pathway. We reasoned that such therapeutic agents will be amenable for oral dosing, likely show greater response rate due to dual antagonism and allow better management of irAEs due to a shorter pharmacokinetic profile.

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 checkpoint protein(s) signaling depending upon the pockets of sequence similarity of interacting proteins. Further optimization resulted in compounds targeting PD-L1/VISTA or PD-L1/TIM-3 with desirable physico-chemical properties and exposure upon oral administration. .

The ability of compounds to disrupt specific immune checkpoint pathways was confirmed by functional studies. Identified lead compounds exhibit potent activity when tested in assays to rescue lymphocyte proliferation and effector functions inhibited by respective ligands/proteins. In a panel of functional assays, the selected lead compounds showed selectivity against other immune checkpoint pathways including CTLA4, LAG3 and BTLA. Lead compounds exhibited sustained immune PD in vitro and in vivo suggesting that drug efficacy may extend beyond drug clearance. Lead compounds exhibited significant efficacy in syngeneic pre-clinical tumor models of melanoma, breast carcinoma and colon cancers upon once a day oral dosing. In repeated dose toxicity studies, the most advanced compound, AUPM-170, a dual antagonist of PD-L1 and VISTA, was well tolerated at >100x of the efficacious doses.

The data demonstrating the inhibition of PD-L1 and another immune checkpoint pathway (VISTA or Tim3) resulting in activation of T cells and anti-tumor activities support further development of these orally bioavailable agents. IND-enabling studies with one of the lead compounds, AUPM-170, are underway towards advancing it to the clinic.

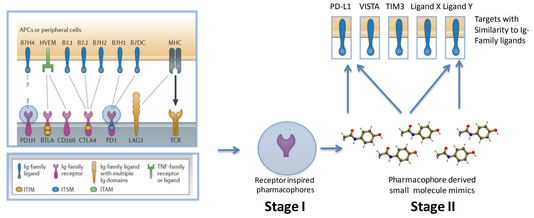
*Murali Ramachandra, Ph. D
Aurigene Discovery Technologies Limited
Email: murali_r@aurigene.com
Phone: +91- 80-71025313
Website: www.aurigene.com

OBJECTIVES

Small molecule immune checkpoint antagonists with the ability to disrupt the PD-1/PD-L1 checkpoint pathway plus one or more related pathways. Advantages include:

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

Our approach



RESULTS

First clinical candidate from our approach: AUPM-170/CA170 (PD-L1/VISTA dual antagonist)

In vitro Pharmacology	Primary functional assay -Mouse (EC_{50} nM)	Rescue of proliferation mediated by PD-L1 /L2	17/13nM
	Primary functional activity in human system - ligand/receptor specific (EC_{50} nM)	Rescue of proliferation mediated by PD-L1 /L2	15/23nM
		Rescue of IFNγ release mediated by PD-L1 /L2	43/140nM
		Rescue of IFNγ release mediated by VISTA-Fc	37nM
	Additional profiling in tox species	Rescue of IFNγ release : Mouse (EC_{50} in nM)	21/20nM
		Rescue of IFNγ release : Monkey (EC_{50} in nM)	53/73nM
	Selectivity analysis	Selectivity against TIM-3, CTLA4, LAG3, BTLA, CD28 demonstrated	
DMPK	Stability in serum at 10μ M (% remaining at 4h)	Mice /Rat/Human	100
	Metabolic stability in liver microsomes (at 1 μM)	Mice/Rat/Dog/Moneky/Human	T1/2 >90 min
	Plasma protein binding (% bound)	Mice/Rat/Human	76/67/67
	CYP inhibition (IC_{50} μM)	3A4, 2C9, 2C19, 2D6	>100
	PK in CD-1 mice	Clearance ((mL/hr/kg)	928
		AUCi iv(0-inf) (3MPK) (hr*ng/mL)	3201
		AUC PO (0-t) (10MPK) (hr*ng/mL)	6943
		Cmax (ng/ml)	1428
		% F	65
In vivo Pharmacology	PK-PD in monkeys	Oral or IV administered CA-170 increases IFN-γ expression in ex vivo stimulated monkey PBMCs in a manner that correlates with plasma drug concentration.	
	B16F10 Melanoma	Oral dosing significantly reduced the number of B16/F10 melanoma lung metastases relative to vehicle and showed comparable efficacy to a blocking anti-PD-1 comparator antibody.	
	MC38 Colon carcinoma	Oral dosing significantly reduced the growth rate of implanted mouse MC38 colon carcinoma tumors relative to vehicle and showed comparable efficacy to a blocking anti-PD-1 comparator antibody. Did not show anti-tumor efficacy when subcutaneous tumors were grown in immune deficient SCID-Beige mice	
	CT26 Colon carcinoma	Oral administration of AUPM170 at 10mpk in combination with cyclophosphamide results in statistically significant tumor growth inhibition	
Safety	CEREP receptor, ion channel and enzyme panel	No significant inhibition at the tested concentration of 10 μM	
	hERG, AMES and micronucleus test	Clean profile	
	28 day GLP tox in mouse and monkey	Well tolerated with the expected NOAEL >1000mg/kg	

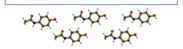
Identified orally bio-available dual antagonist of PD-L1 and VISTA pathways; Completed IND-enabling studies towards advancing it to the clinic

RESULTS

Strategy for lead identification of TIM3/PD-L1 dual antagonists

Compound Selection

Pharmacophore derived small molecule mimics



Screened against TIM3/PD-L1 activity

Compounds with moderate potency

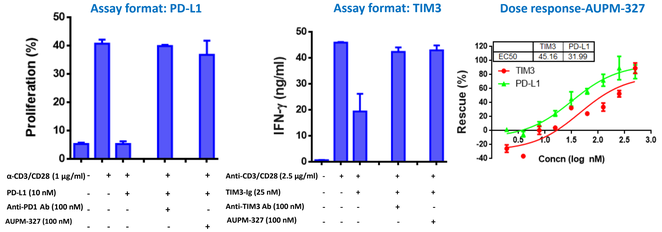
Optimized for potency and in vivo properties

Shortlisted leads of desirable properties

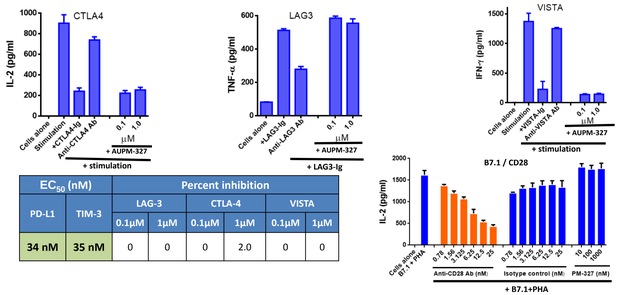
Profile of shortlisted leads

Study			PM-349	PM-362	PM-370	PM-396	PM-327
Primary functional assay - Tim3	Rescue of Tim3 effect in mouse splenocytes (EC_{50} in nM)		114	27	**50**	**30**	**35**
Primary functional assay PD-L1	Rescue of PD-L1effect in mouse splenocytes(EC_{50} in nM)		25	39	29	62	**34**
Selectivity	Against VISTA, CTLA4 and LAG3		☑	☑	☑	☑	☑
ADME/PK	Serum stability	Mice (% remaining at 4h)	>95	>95	>95	>95	**90**
	PK in CD-1 mice	Clearance (mL/hr/kg)	1183	1183	1373	2058	**970**
		AUCi iv(0-inf) (3MPK) (hr*ng/mL)	2530	2503	2184	1458	**3083**
		AUC PO (0-t) (10MPK) (hr*ng/mL)	2043	1583	1904	3114	**7413**
		Cmax	830	1420	1593	816	**1564**
		% F	29	19	26	64	**72**

Profile of the advanced lead AUPM-327



AUPM-327 does not inhibit other immune checkpoints

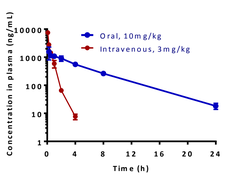


EC_{50} (nM)		Percent inhibition					
PD-L1	TIM-3	LAG-3		CTLA-4		VISTA	
		0.1μM	1μM	0.1μM	1μM	0.1μM	1μM
34 nM	35 nM	0	0	0	2.0	0	0

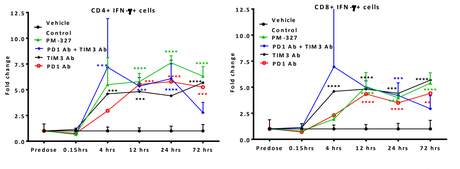
No inhibition of other checkpoints tested and no off target effect on B7.1

Exhibits excellent oral PK that correlates with sustained immune PD *in vivo*

PK profile of AUPM-327



Immune PD profile of AUPM-327

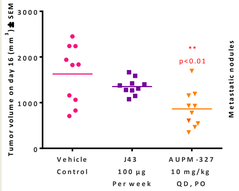


* p value: 0.05; ** p value: 0.005; p value: 0.0001

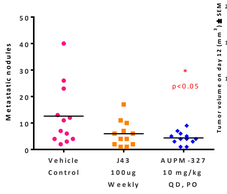
AUPM-327 shows good PD modulation comparable to anti-TIM3 antibody and a combination of anti-PD1 antibody and anti-TIM3 antibody

Anti-tumor efficacy of AUPM-327

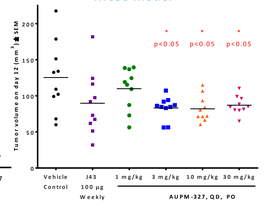
CT26 colon carcinoma model



B16/F10 lung metastasis model



Dose response in MC38 model



AUPM-327 shows significant anti-tumor efficacy in multiple syngenic mouse models

SUMMARY

We have identified orally bio-available immune checkpoint antagonists.

- The first candidate from our approach targeting PD-L1 and VISTA pathways has completed IND-enabling studies and will be advancing to the clinic soon
- We have now identified a lead candidate targeting PD-L1 and TIM-3 pathways that exhibits desirable potency, DMPK properties including oral bioavailability, and anti-tumor efficacy in multiple syngeneic tumor models

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