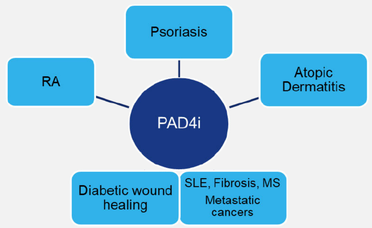
Poster 0104

Novel PAD4 Inhibitors for Treatment of Autoimmune Disorders

Santosh Vishwakarma*, Gurulingappa Hallur, Himanshu Agrawal, Sharad Singh, Annasaheb Kalange, Seema Chikkur Gangadhar, Shuvranshu Praharaj, Nafees Ahmad Quresh, Srikanth Kanagal Gopinath, Krishnakumar V, Sameer Mahmood, Rudresh G, Mohd Zainuddin, Rajendra Kristam, Ishtiyaque Ahmad, Ramachandraiah Gosu, Purra Buchi Reddy, N V S K Rao, Saloni Mehra, Jeyaraj D A, #Dhanalakshmi Sivanandhan, # Sridharan Rajagopal, Takeshi Yura, Saravanakumar Dhakshinamoorthy and Sriram Rajagopal; ***Jubilant Biosys Limited., Bangalore India # Jubilant Therapeutics Inc, USA***

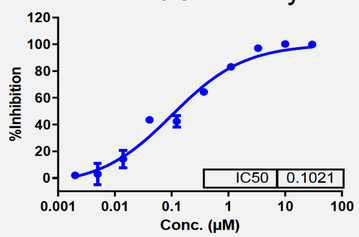
Summary

Citrullination of proteins is catalyzed by a family of enzymes called the peptidylarginine deiminases (PADs). While the citrullinated proteins may have physiological roles in differentiation, development, cell death, and gene regulation, the pathological protein citrullination has been associated with a range of diseases including rheumatoid arthritis (RA), multiple sclerosis, alzheimer's disease, psoriasis and cancer. The lead compound PAD4i showed an IC_{50} of 102 nM against PAD4 in the biochemical assay. It was selective against PAD1,2,3 and 6 enzymes. In the human neutrophil-based assay, the compound showed significant inhibition of H3-Histone citrullination with an IC_{50} of 125 nM. PAD4i showed good oral bioavailability of greater than 50% across species. Treatment with PAD4i showed significant improvement in disease index across the various animal models, with efficacies comparable to the standard of care. In Collagen Induced Arthritis and Imiquimod induced psoriasis models PAD4i showed >70% reversal of clinical score, with no adverse effect. The observed pharmacological benefit was accompanied by reduction of histone citrullination in tissue samples, indicating target engagement.



PAD4 Potency and Selectivity

In vitro Potency

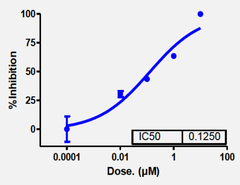


Isoform	IC50, µM
PAD1	>10
PAD2	>10
PAD3	>10
PAD6	>10

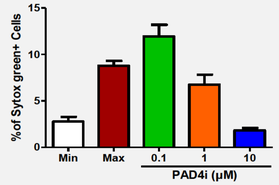
- Compound + Protein (1h incubation)
- Substrate (BAEE 1.5mM) +350 µM CaCl2 (1 h incubation)
- OPA (2.6mM) addition, fluorescence reading.

Modulation of Biomarkers

Cit. H3

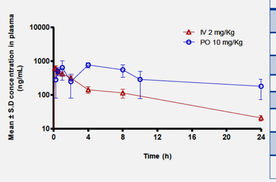


NETosis



Pharmacokinetic properties

Time Vs Plasma concentration profile of PAD4i in male BALB/c mice



PK parameters	Units	Mice 2 mg/kg, IV	Mice 10 mg/kg, PO
$t_{1/2}$	(h)	7	9.6
C_{max}	(ng/mL)	619	772
C_0	(ng/mL)	699	-
AUC_{0-t}	(ng·h/mL)	2956	8691
$AUC_{0-\infty}$	(ng·h/mL)	3166	11210
CL	(mL/min/kg)	10.5	-
V_d	(L/kg)	6.4	-
F	(%)	-	59

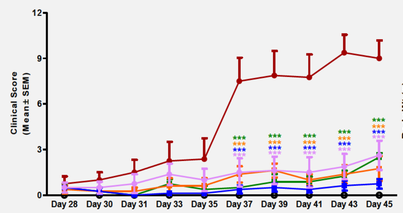
Comparable oral exposure in mice, rat and dogs

Drug-like properties

Parameter/Criteria	PAD4i
Solubility (µM)	72
Met stab H/M (% remain)	>85%
CYP3A4 and CYP2D6 % inhib @ 10 uM	NI/NI
Caco2 (A-B); (B-A) 10-6 cm/sec; Efflux	0.63, 12.6; 20
% protein binding, human	98.5
hERG , % inhibition	30% @30µM
% F, mouse, rat, dog	59, 72, 71
[redacted]	[redacted]

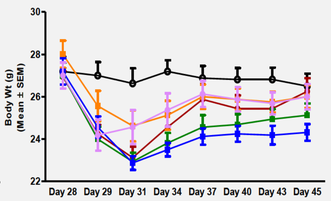
PAD4i Shows Strong Efficacy in CIA Model in Mice

Clinical score

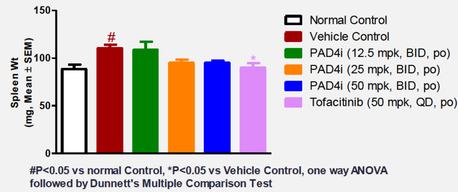


***P<0.001, **P<0.01, *P<0.05 vs Vehicle Control, Two way ANOVA followed by Bonferroni multiple comparisons Test.

Body Weight

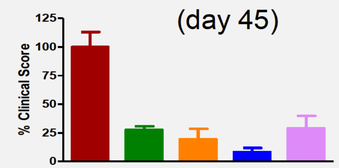


Spleen Weight

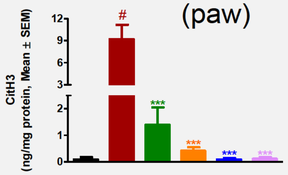


#P<0.05 vs normal Control, *P<0.05 vs Vehicle Control, one way ANOVA followed by Dunnett's Multiple Comparison Test

Clinical Score (day 45)

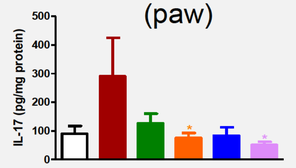


Cit. H3 Level (paw)



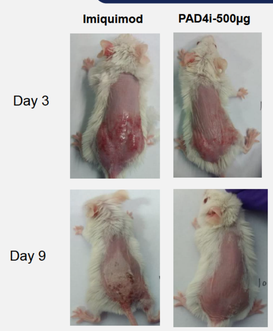
#P<0.001 vs normal Control, ***P<0.001 vs Vehicle Control, one way ANOVA followed by Dunnett's Multiple Comparison Test

IL-17 Level (paw)

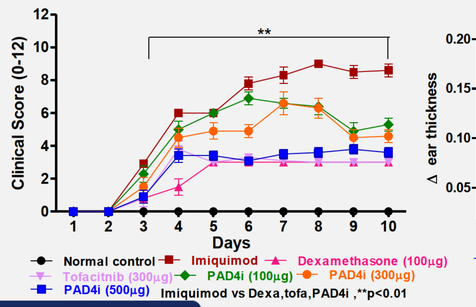


*P<0.05 vs Vehicle Control, one way ANOVA followed by Dunnett's Multiple Comparison Test

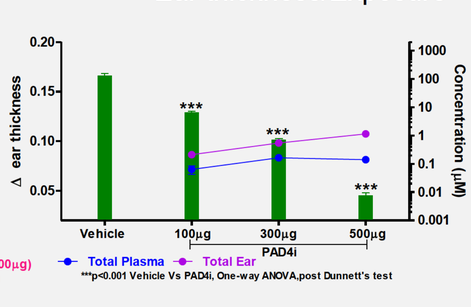
Strong efficacy in Imiquimod induced psoriasis



Clinical Score



Ear thickness/Exposure



***p<0.001 Vehicle Vs PAD4i, One-way ANOVA,post Dunnett's test

Conclusions

- Identified orally available novel, selective small molecule PAD4 inhibitor with no cross-reactivity to other isoforms
- Excellent efficacy demonstrated in CIA , psoriasis, diabetic wound healing, atopic dermatitis models; Clean in CEREP safety panel, hREG; and AMES negative
- Excellent therapeutic margin based on 14 day tox study in rodent

Sponsored by Jubilant Therapeutics Inc, USA; Contact: Dr. Rajiv Tyagi, rajiv_tyagi@jubltherapeutics.com