# Novel, selective PAD4 inhibitor for treatment of cancer

**D. Sivanandhan***, S. Rajagopal*, G. Chandru*, M. Zainuddin*, M.N. Sadhu*, R. Gosu*, L. Rastelli#, A. Siddiqui , S. Singh, G. Rudresh, S. Mullurwar, S. Kandan, S. Vishwakarma, G. Hallur, R. Trivedi and D. Jeyaraj *Jubilant Therapeutics India Limited, Bangalore, India # Jubilant Therapeutics Bedminster, NJ, Jubilant Biosys Limited, Bangalore, India

**Permanent abstract # 2646**

***Jubilant Therapeutics, Bedminster, NJ***

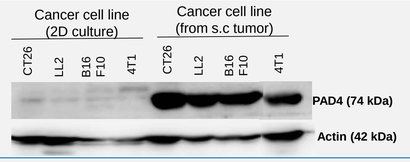
**Abstract Control # 2942**

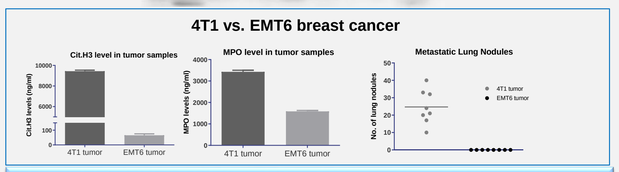
## SUMMARY

Peptidyl Arginine Deiminase 4 (PAD4/PADI4) is an enzyme that converts protein arginine or mono-methylarginine to citrulline. The PAD4-mediated hypercitrullination reaction in neutrophils causes the release of nuclear chromatin to form Neutrophil Extracellular Traps (NETs). NETs have been shown to be associated with several pathological processes including, cancers and other autoimmune diseases. KO studies from literature clearly demonstrate role of PAD4 in cancer progression and metastasis. Therefore, inhibition of PAD4 could be a novel strategy in treating cancer progression and metastasis.

Here, we describe a novel small molecule inhibitor JBI-1044 that is selective for PAD4. JBI-1044 inhibited citrullination of human neutrophils and is not directly cytotoxic to cancer or immune cells in vitro. JBI-1044 shows a dose dependent inhibition of pro-inflammatory cytokines in RAW cells as well as macrophages. Oral administration of JBI-1044 results in strong tumor growth inhibition with a similar inhibition of citrullination and cytokines in tumor and is tolerated well. Exploratory toxicology studies clearly show that JBI-1044 is tolerated well suggesting that JBI-1044 can be developed as a clinical candidate for treatment of cancer.

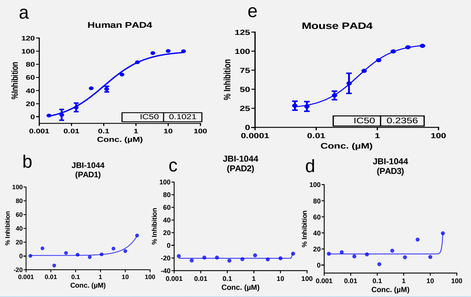
## PAD4, Cit.H3 and MPO expression in cancer



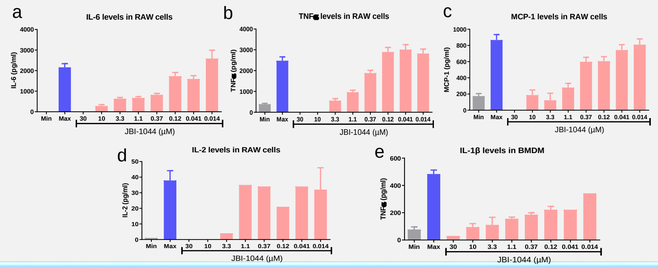

PAD4 level in 2D vs 3D culture was assessed by WB; Cit.H3 and MPO levels were estimated in 4T1 and EMT6 xenograft tumors by ELISA; Ly6G level was estimated by IHC staining

## JBI-1044 is selective for PAD4



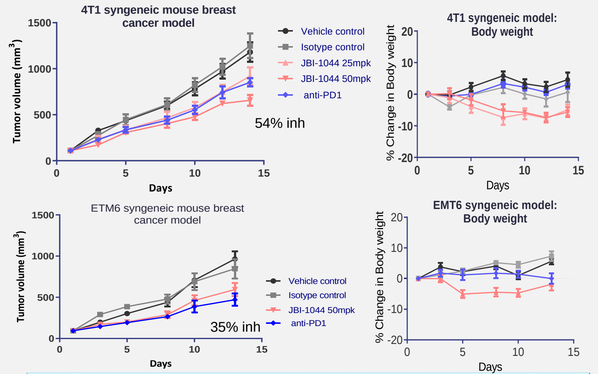

JBI-1044 was screened in human PAD isoforms 1-4 (a-d) and mouse PAD4 (e) isoform in a cell free ammonia release assay

## JBI-1044 modulates pro-inflammatory cytokines



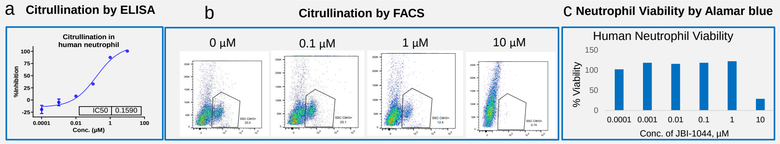

a-d) RAW cells were treated with LPS in the presence or absence of JBI-1044 at indicated various cytokines were estimated by HTRF or ELISA; e) Bone marrow cells were differentiated into macrophages (BMDM) with M-CSF and then treated with LPS; cells were then treated with JBI-1044 for 2 h, then with Nigericin for 3 h and IL-1 β levels were estimated in media by HTRF method

## JBI-1044 is efficacious in 4T1 and EMT6 syngeneic models




JBI-1044 showed stronger efficacy to anti-PD1 antibody in 4T1 model (a) and comparable efficacy in EMT6 (b) model; treatments were well tolerated (b, d)

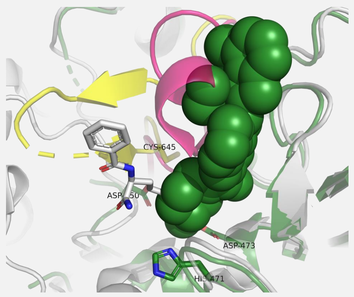
## JBI-1044 inhibits citrullination in human neutrophils and is non-cytotoxic




Neutrophils isolated from human blood was treated with various conc. of JBI-1044 (0 to 10 uM); Citrullination was induced with Calcium ionophore and measured by ELISA (a) or FACS (b); human neutrophils were treated with JBI-1044 for 24h and cell viability was measured by Alamar blue assay (c)

## Co-crystal structure of JBI-1044

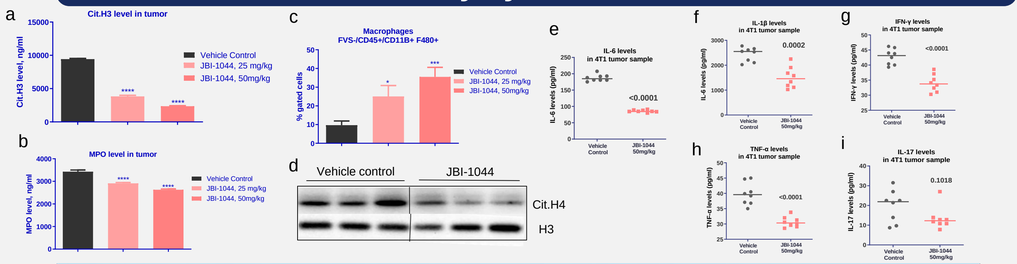
Overlay of PAD4+JBI-1044 onto PAD4+BAA




- JBI-1044 (green) bound to PAD4
- Substrate-mimic BAA (grey) bound to PAD4
- Catalytic residues: Asp-350, His-471, Asp-473, Cys-645 shown in sticks

Conformational change & movement is seen for the region aa630-aa647 from helix (purple) to ß-hairpin (yellow) upon JBI-1044 binding

## JBI-1044 inhibits citrullination, NET formation and pro-inflammatory cytokines in tumor




Dosing with JBI-1044 resulted in significant decreases in Cit.H3 (a, d) and MPO (b) level and increase in macrophages (c) in 4T1 tumor. Expression of pro-inflammatory cytokines IL-6 (e), IL-1β (f), IFN-γ (g), TNF-α (h) ($p<0.0001$) and IL-17 (i) were also inhibited in the tumor

## Conclusions

- PAD4 levels are higher in 3D tumor vs 2D cultures; tumors with high citrullination /NET formation appear to be highly metastatic
- JBI-1044 is a selective orally bioavailable PAD4 inhibitor that inhibits citrullination; not directly cytotoxic to cancer cells or immune cells
- Single X-ray Crystallography determines an unambiguous binding mode for JBI-1044
- Efficacious in 4T1 and EMT6 syngeneic mouse models; inhibits citrullination, NET formation as well as inflammatory cytokines in tumor
- It is well tolerated in toxicology studies and is being further profiled in advanced studies to be developed as a clinical candidate

**Contact:** dhanalakshmi.sivanandhan@jubilanttx.com