BLU-808: A Potent and Selective Oral Small Molecule Wild-Type KIT Tyrosine Kinase Inhibitor for Allergic Conditions

Poster Number 535

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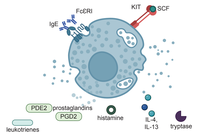
Introduction

- Mast cells (MCs) are involved in multiple allergic diseases including but not limited to the chronic spontaneous and inducible urticarias, allergic rhinoconjunctivitis, allergic asthma, and mast cell activation syndrome (MCAS)[1–5]
- BLU-808 is a potent, selective, orally available, investigational inhibitor of wild-type (WT) KIT (***see poster #698 at AAAAI 2025***) capable of reducing MC activation and eliminating MCs in preclinical models[6]
- By studying the effect of KIT inhibition in CD34+ derived MCs and preclinical models of allergic disease, the utility of KIT inhibitors and the role of MCs in these diseases can be further defined

Mast cells play a known role in Type 2 inflammation

- MCs release mediators that further **activate inflammation**
- Inflammatory responses can lead to **long-term effects** including tissue remodeling
- Targeting **KIT, the regulator of MC survival and differentiation**, is a promising approach to improve disease outcomes



FcεRI, high-affinity immunoglobulin E receptor; KIT, tyrosine protein kinase; IgE, immunoglobulin E; IL, interleukin; MC, mast cell; PDE, phosphodiesterase; PGD, prostaglandin; SCF, stem cell factor. Image generated using BioRender illustration software.

Table 1. BLU-808 is an investigational potent and selective inhibitor of WT KIT

	BLU-808	Key point
Potency		
pKIT cellular IC_{50} (nM)	0.37	WT KIT inhibition in a cellular assay
WT KIT-dependent proliferation IC_{50} (nM)	1.3	
Inhibition of CD63 extracellular expression IC_{50} (nM)	2.7	Blocks human CD34+ derived MC degranulation as measured by surface marker expression
Inhibition of histamine release IC_{50} (nM)	8.6	Blocks human CD34+ derived MC degranulation as measured by histamine release
Selectivity		
S(10) @ 3 µM	0.042	Highly selective across the kinome
PDGFRA/B/FLT3 selectivity[a]	>300x/>400x/>9600x	Selective against key kinases closely related to WT KIT
CSF1R Kd selectivity	>800x	
Brain penetrance (Kp_{uu})	0.021	Peripherally restricted

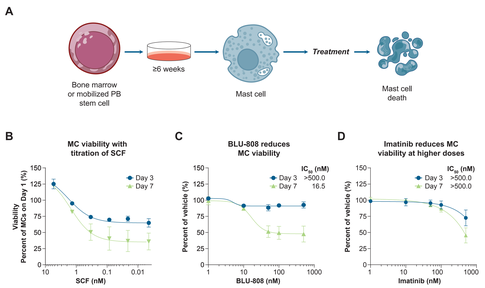
[a]Determined in a cellular assay.
CSF1R, colony stimulating factor 1 receptor; FLT3, FMS-like tyrosine kinase 3; IC_{50}, half-maximal inhibitory concentration; Kd, dissociation constant; Kp_{uu}, unbound brain to plasma partition coefficient; PDGFRA/B, platelet-derived growth factor receptor alpha/beta; pKIT, phosphorylated KIT; S(10) @ 3 µM, selectivity score at a concentration of 3 µM; WT, wild-type.

Methods

- Cell assays to measure KIT-dependent proliferation and stem cell factor (SCF)-mediated KIT phosphorylation were used to assess potency
- Selectivity against the structurally related kinases: platelet-derived growth factor receptor alpha/beta (PDGFRA/B) and FMS-like tyrosine kinase 3 (FLT3) were assessed via cellular assays; colony stimulating factor 1 receptor (CSF1R) and the broader kinome were assessed by KINOMEscan™
- Inhibition of degranulation was evaluated in human CD34+ derived MCs stimulated with immunoglobulin E (IgE) and anti-IgE or in human CD34+ derived MCs stimulated with serum from healthy donors or patients with chronic inducible urticaria (CIndU)
- Survival of human CD34+ derived MCs was assessed by flow cytometry
- Inhibition of asthma-like symptoms was assessed in rodent ovalbumin-induced (OVA-induced) asthma models where a single dose of BLU-808 and/or dexamethasone was administered 3 hours before the final aerosol challenge and 5 hours before enhanced pause (Penh) measurement. Bronchial alveolar lavage fluid (BALF) and pharmacokinetics were assessed for immune cell infiltration the following day, 3 hours after a dose of BLU-808 and/or dexamethasone

Results – *in vitro*

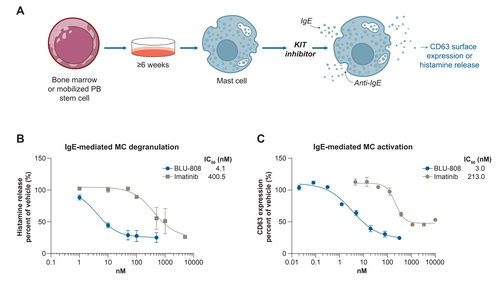
Figure 1. BLU-808 inhibits survival of human CD34+ derived mast cells



Data shown as the mean of at least 2 independent experiments with SEM. Panel A image generated using BioRender illustration software. PB, peripheral blood; SEM, standard error of the mean.

- Human CD34+ derived MCs require SCF, also known as KIT ligand, for survival (**Figure 1B**)
- BLU-808 inhibits human CD34+ derived MC survival (**Figure 1C**) more potently than imatinib (**Figure 1D**) and to the same extent as withdrawal of SCF

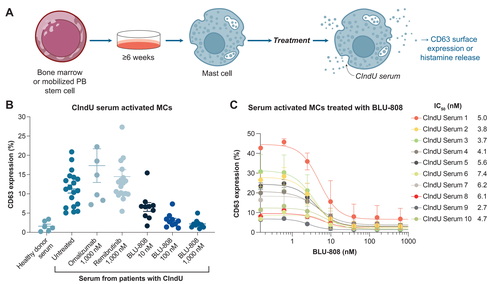
Figure 2. BLU-808 inhibits IgE-mediated activation of human CD34+ derived mast cells at doses that do not impact viability



Data shown as the mean of at least 2 independent experiments with SEM. Panel A image generated using BioRender illustration software.

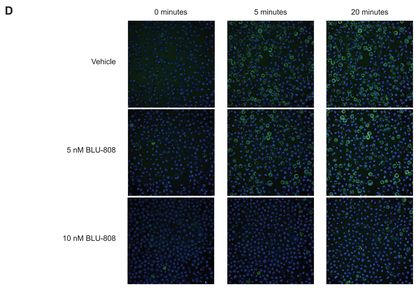
- Potential for activation of human CD34+ derived MCs by IgE was assessed via histamine release and CD63 surface expression assays (**Figure 2A**)
- The ability of BLU-808 to inhibit degranulation as assessed by histamine release (**Figure 2B**) or CD63 surface expression (**Figure 2C**) is greater than that of imatinib

Figure 3. BLU-808 completely inhibits CIndU serum-mediated activation of human CD34+ derived mast cells



Data shown as the mean of at least 2 independent experiments with SEM. Panel A image generated using BioRender illustration software. CIndU, chronic inducible urticaria.

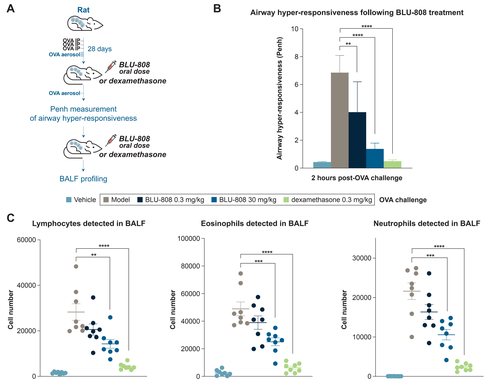
- 19 unique CIndU serum samples collected at times agnostic of symptom or reaction state can induce degranulation in human CD34+ derived MCs (**Figure 3A**)
- While BLU-808 reduces activation to levels observed from healthy donors, omalizumab (anti-IgE antibody) and remibrutinib (bruton tyrosine kinase inhibitor [BTK]) do not inhibit serum-induced degranulation (**Figure 3B**). Omalizumab and remibrutinib are capable of inhibiting IgE-mediated MC activation (data not shown). Each point within a group represents the average MC activation achieved by a single, unique serum
- BLU-808 inhibits human CD34+ derived MC activation by serum from patients with CIndU (**Figure 3C**)



- BLU-808 inhibits degranulation induced by anti-IgE administration as assessed by Avidin-488 cell-periphery staining. Hoechst nuclear staining shows no loss in viability (**Figure 2D**)

Results – *in vivo*

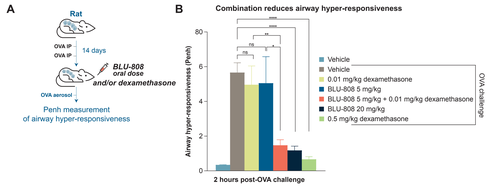
Figure 4. BLU-808 acutely inhibits airway hyper-responsiveness in a preclinical rodent model of allergic asthma



Data shown as the mean for 8 rodents with SEM. Unpaired t-test for significance where **P<0.01; ***P<0.001; ****P<0.0001. Panel A image generated using BioRender illustration software.
BALF, bronchial alveolar lavage fluid; IP, intraperitoneal; OVA, ovalbumin; Penh, enhanced pause.

- BLU-808 inhibits OVA-induced airway hyper-responsiveness in rodents as measured by Penh with a single dose (**Figure 4B**)
- BLU-808 acutely inhibits immune cell infiltration into BALF (**Figure 4C**)

Figure 5. Subefficacious doses of BLU-808 and dexamethasone in an acute setting further reduces airway hyper-responsiveness in a preclinical rodent model of allergic asthma



Data shown as the mean for 8–10 rodents with SEM.
Unpaired t-test for significance where ns, not significant; *P<0.05; **P<0.01; ****P<0.0001. Panel A image generated using BioRender illustration software.

- BLU-808 inhibits OVA-induced airway hyper-responsiveness in rodents as measured by Penh with a single dose; a single subefficacious dose of BLU-808 combines with a single subefficacious dose of dexamethasone to further reduce airway hyper-responsiveness (**Figure 5A** and **5B**)

Table 2. BLU-808 pharmacokinetics in preclinical rodent models of allergic asthma

	BLU-808 (mg/kg)	BLU-808 (Free nM)
Plasma concentration at 3 hours post-dose		
28-day OVA-challenge setting	0.3	0.06 ± 0.0
	30	354.0 ± 20.5
14-day OVA-challenge setting	5	30.6 ± 3.6
	5 with 0.01 mg/kg dexamethasone	40.2 ± 3.7
	20	323.4 ± 33.9

Free nM shown as the mean with SEM.

Conclusions

- BLU-808 is a potent, selective, and orally bioavailable WT KIT inhibitor
- BLU-808 demonstrates potent inhibition of human CD34+ derived MC survival and activation by IgE or serum from patients with chronic CIndU
- In an allergic model of asthma, acute dosing of BLU-808 was able to improve lung function
- In an allergic model of asthma, a single, low dose of BLU-808 was able to combine with a low dose of dexamethasone to improve lung function
- BLU-808 offers a potential best in class MC modulator that provides dosing flexibility. Higher doses block MC survival while lower doses inhibit MC activity without affecting their viability

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