# AQX-1125, a Modulator of the SHIP1/PI3K Pathway, Suppresses Chemotaxis and Inflammation

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## Abstract

**Rationale:** AQX-1125, a novel small-molecule allosteric activator of SHIP1, was evaluated for *in vitro* and *in vivo* anti-inflammatory activity.
**Methods:** AQX-1125 was tested in an *in vitro* Boyden chamber chemotaxis assay. The efficacy of AQX-1125 on reducing ear thickness and myeloperoxidase content was evaluated *in vivo* in the phorbol 12-myristate 13-acetate (PMA)-induced ear edema mouse model. AQX-1125 was administered orally for 3 days at 1, 10 and 30 mg/kg prior to application of PMA. The efficacy of AQX-1125 was also tested in a rat ovalbumin-mediated airway inflammation model, administered orally for 4 days at 0.1, 1 and 10 mg/kg before airway challenge. The degree of inhibition of leukocyte infiltration and mediator release in the bronchiolar lavage fluid (BALF) was measured.
**Results:** AQX-1125 is a potent inhibitor of *in vitro* leukocyte chemotaxis. *In vivo*, AQX-1125 at 30 mg/kg significantly reduced ear edema and myeloperoxidase content in the PMA model. At 1 mg/kg, AQX-1125 significantly reduced the total number of leukocytes recovered in the BALF of animals sensitized and challenged with ovalbumin. AQX-1125 also reduced the level of the inflammatory mediators IL-1a and IL-11, in the BALF.
**Conclusions:** AQX-1125 potently inhibits leukocyte chemotaxis *in vitro* and myeloperoxidase (neutrophil) accumulation *in vivo*. AQX-1125 also inhibits leukocyte accumulation and inflammatory mediator release in the BALF in a model of allergic airway inflammation. These data suggest that AQX-1125 has clinical potential for treatment of allergic and inflammatory diseases such as asthma and COPD.

## Introduction

Targeting SHIP1

- ✓ PI3K pathway is one of the most active areas in Biotech/Pharma
- ✓ SHIP1 is an ideal drug target
- ✓ PI3K/SHIP1 pathway plays a key role in regulating cell migration and activation
- ✓ SHIP1 expression restricted to hematopoietic derived cells - limits off target toxicity
- ✓ SHIP1 is a novel target distinct from the extensive PI3K investigations
- ✓ Redirects cellular PI3K signalling, rather than prevent it

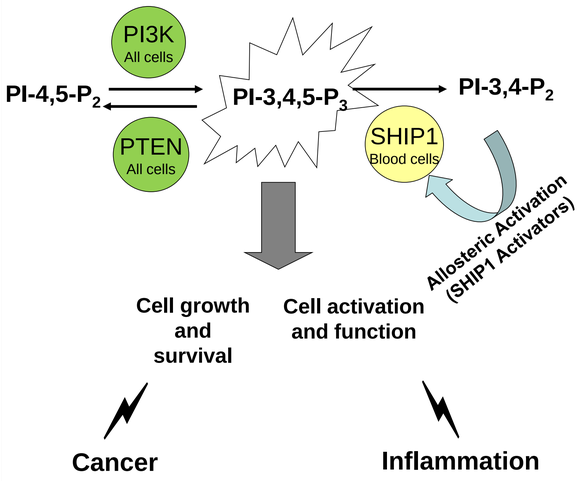
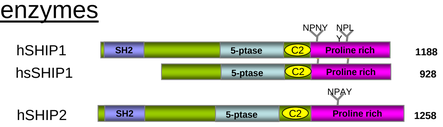



Figure 1. SHIP1 and PI3K signalling. SHIP1 redirects PI3K signalling, PI3K inhibitors block PI3K signalling

## Background

SHIP enzymes

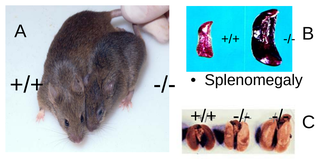



Good homology between human and rodent SHIP1

| | hSHIP2 | mSHIP1 | rSHIP1 |
|---|---|---|---|
| hSHIP1 | 51% | 92% | 91% |
| hSHIP1 C2 domain | 38% | 91% | 90% |

Figure 2. SHIP enzymes – 51% homology between hSHIP1 and hSHIP2, and 38% homology between the hSHIP1C2 and hSHIP2C2 domains. This reduced homology between SHIP1 and SHIP2 confers selectivity

SHIP1 Knockout[1]




- Viable & fertile, ~40% survival by 14 weeks
- Splenomegaly
- Increased GM progenitors
- Infiltration of lungs by macrophages & neutrophils

Figure 3. Phenotype of the SHIP1 knockout mouse. A) small stature, B) splenomegaly and C) inflammatory infiltrate of the lungs.

SHIP1 Activation

Pelorol was the first generation SHIP1 activator isolated. Analogues of Pelorol, AQX-016A and AQX-MN100, were synthesized with greater SHIP1-activating properties[2]. These small molecules activate SHIP1 through an allosteric mechanism, via interaction with the C2 domain, and are anti-inflammatory in cellular and murine models[2].

AQX-1125 is a small molecule next generation SHIP1 activator. It has many of the biological effects of the earlier generation SHIP1 activators, but has more drug-like properties.

## Cellular and *In Vivo* Models

- Akt phosphorylation in T cell lines – Western blotting
- Leukocyte chemotaxis *in vitro*
- PMA-induced ear edema in mice
- Ovalbumin-mediated allergic airway inflammation in rats

## Results

AQX-1125 inhibits Akt phosphorylation in SHIP1-proficient MOLT-4, but not in SHIP1-deficient Jurkats

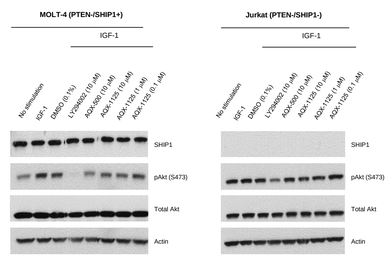
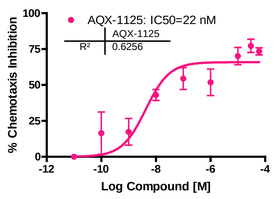



Figure 4. MOLT-4 and Jurkat cells were treated with AQX-1125 for 30 min, followed by stimulation with IGF-1 for 30 min

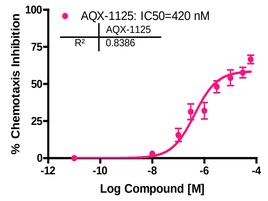
## Results

AQX-1125 inhibits leukocyte chemotaxis

B cells:



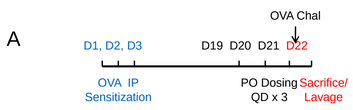

Monocyte:




| Cell Type | Chemokine | Chemokine Receptor | Potency of AQX-1125 |
|---|---|---|---|
| Monocytes | MCP-1 | CCR2 | 288 nM |
| B Cells | BCA-1 | CXCR5 | 28 nM |
| Activated T Cells | IP-10 / I-TAC | CXCR3 | 70 nM / 229 nM |
| Non-activated T Cells | MIP-1α | CCR1 | 33 nM |
| Neutrophils | GRO-α / IL-8 | CXCR1/2 | 30 nM / 73 nM |

Figure 5. Human blood leukocytes were treated with AQX-1125 for 30 min, followed by induction of chemotaxis with chemokines listed.

AQX-1125 inhibits OVA-induced allergic airway inflammation in Brown Norway rats



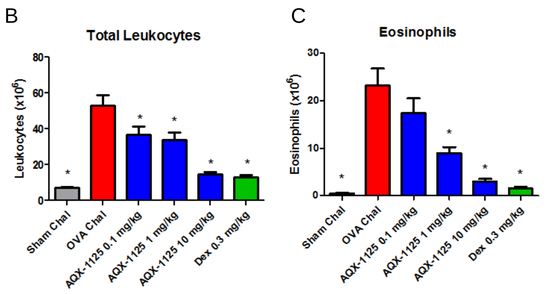




Figure 6. (A) Male Brown Norway rats were sensitized to OVA, followed by oral AQX-1125 administration and OVA challenge. BAL was performed and resulting data shown expressed as mean±SEM of (B) BAL leukocyte and (C) eosinophil counts, *p<0.05 vs OVA.

AQX-1125 inhibits OVA-induced inflammatory mediator content in Brown Norway rat BALF

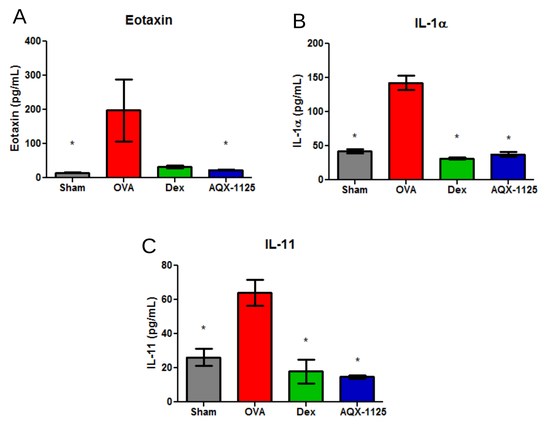



Figure 7. Male Brown Norway rats were sensitized to OVA, followed oral AQX-1125 (10 mg/kg) administration and OVA challenge. BAL was performed and resulting data shown expressed as mean±SEM of (A) eotaxin, (B) IL-1α and (C) IL-11concentrations, *p<0.05 vs OVA.

## Results

AQX-1125 inhibits PMA-induced ear edema and MPO content of the ear

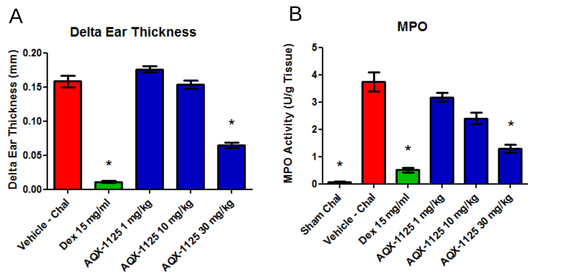
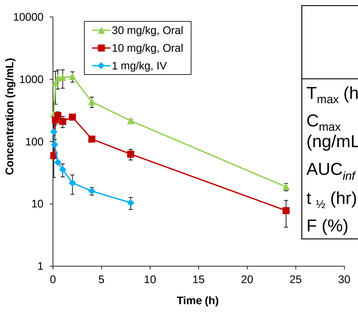



Figure 8. Female ICR dosed orally with AQX-1125 (, 10, 30 mg/kg) were challenged by topical application of PMA to the ear. Six hr post-challenge edema was assessed by measuring the change in ear thickness (A) and tissue MPO content was measured (B), *p<0.05 vs OVA. Data are expressed as mean±SEM.

Pharmacokinetics of AQX-1125 in rats




| | IV 1 mg/kg | PO 10 mg/kg | PO 30 mg/kg |
|---|---|---|---|
| $T_{max}$ (hr) | 0.083 | 1.00 | 1.17 |
| $C_{max}$ (ng/mL) | 144 | 267 | 1226 |
| $AUC_{inf}$ /D | 268 | 176 | 227 |
| $t_{½}$ (hr) | 5.6 | 5.2 | 4.5 |
| F (%) | N/A | 66 | 85 |

Figure 9. Pharmacokinetics of AQX-1125 in male Sprague Dawley rats. AQX-1125 was administered intravenously (IV) or by oral gavage. Plasma concentrations of AQX-1125 were determined at various times. Data are expressed as mean±SD.

## Summary

SHIP1 is a novel drug target which controls PI3K-driven cellular migration and activation. SHIP1's restriction to hematopoietic cells and poor homology with SHIP2 reduces the likelihood of off-target, off-tissue toxicity. AQX-1125, a small molecule with PK properties suited to once per day dosing, inhibits the PI3K pathway through activation of SH2-containing inositol-5'-phosphatase 1 (SHIP1), resulting in a reduction of pAkt in T and B lymphocytes. AQX-1125 has significant *in vivo* anti-inflammatory activity, in models of allergic inflammation. These data indicate that AQX-1125 has significant clinical potential in inflammatory disorders such as asthma.

## References

1. Helgason *et al*. Targeted disruption of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span. Genes & Dev. 1998;12:1610-1620.
2. Ong *et al*. Small molecule agonists of SHIP1 inhibit the phosphoinositide 3-kinase pathway in hematopoietic cells. Blood. 2007;110:1942-1949.

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