

# The effects of the novel SHIP1 activator AQX-1125 on allergen-induced responses in mild-to-moderate asthma

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## Clinical & Experimental Allergy

### Summary

**Background** SH2-containing inositol-5'-phosphatase 1 (SHIP1) is an endogenous inhibitor of the phosphoinositide-3-kinase pathway that is involved in the activation and chemotaxis of inflammatory cells. AQX-1125 is a first-in-class, oral SHIP1 activator with a novel anti-inflammatory mode of action.

**Objective** To evaluate the effects of AQX-1125 on airway responses to allergen challenge in mild-to-moderate asthmatic patients.

**Methods** A randomized, double-blind, placebo-controlled, two-way crossover study was performed in 22 steroid-naïve mild-to-moderate asthmatics with a documented late-phase response to inhaled allergen (LAR). AQX-1125 (450 mg daily) or placebo was administered orally for 7 days. Allergen challenge was performed on day 6 (2 h postdose), followed by methacholine challenge (day 7), and induced sputum collection and fractional exhaled nitric oxide (FeNO).

**Results** AQX-1125 significantly attenuated the late-phase response compared with placebo (FEV<sub>1</sub> 4–10 h: mean difference 150 mL, 20%;  $P = 0.027$ ) and significantly increased the minimum FEV<sub>1</sub> during LAR (mean difference 180 mL;  $P = 0.014$ ). AQX-1125 had no effect on the early-phase response. AQX-1125 showed a trend in reduction of sputum eosinophils, neutrophils and macrophages although this did not achieve significance as there were only 11 paired samples for analysis. There was no effect on methacholine responsiveness or FeNO. Pharmacokinetic data showed AQX-1125 was rapidly absorbed with geometric mean  $C_{max}$  and AUC<sub>0–24 h</sub> values of 1417 ng/mL and 16 727 h ng/mL, respectively. AQX-1125 was well tolerated, but mild GI side-effects (dyspepsia, nausea and abdominal pain) were described in 4/22 subjects on active treatment. These side-effects were mild self-limiting, required no further treatment and did not lead to discontinuation of therapy.

**Conclusion and Clinical Relevance** AQX-1125, a novel oral SHIP1 activator, significantly reduces the late response to allergen challenge, with a trend to reduce airway inflammation. AQX-1125 was safe and well tolerated and merits further investigation in inflammatory disorders.

**Keywords** airway hyperresponsiveness, asthma, induced sputum, inhaled allergen challenge, phosphoinositide-3-kinase, SH2-containing inositol-5'-phosphatase 1

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### Introduction

Inhaled corticosteroids are the cornerstone of anti-inflammatory treatment in asthma [1]. However, many patients remain symptomatic despite high doses of inhaled corticosteroids, even when combined with long-acting  $\beta_2$ -agonists [2, 3]. New asthma treatments

targeting inflammation are therefore needed, and there would be an advantage if oral dosing were possible, as many patients, in particular children and the elderly, can find inhalers difficult to use or have poor inhalation techniques which result in a reduced amount of drug reaching the lung [4].

AQX-1125 is a first-in-class, orally bioavailable, new chemical entity with demonstrated anti-inflammatory activity mediated through SH2-containing

inositol-5'-phosphatase 1 (SHIP1) activation in different cell lineages. SHIP1 is a 145-kDa intracellular protein, predominantly expressed in cells of the haematopoietic lineage including many inflammatory cells [5, 6]. The catalytic function of SHIP1 is to dephosphorylate phosphoinositide 3,4,5-trisphosphate (PIP<sub>3</sub>), the product of phosphoinositide-3-kinases (PI3K; Fig. 1), to phosphoinositide 3,4-diphosphate (PIP<sub>2</sub>).

The selectivity of cellular expression of SHIP1 means that AQX-1125 induces a selective down-regulation of the activity of this pathway only in haematopoietic cells. PIP<sub>3</sub> is a key molecule involved in the regulation of a variety of signal transduction pathways, including the protein kinase B/Akt pathway, calcium signalling, secretion of inflammatory mediators and many others. These pathways regulate vesicular trafficking, cell proliferation, differentiation, protein translation, inhibition of apoptosis, and actin cytoskeletal rearrangement and, hence, cell migration [7–10]. Therefore, activation of SHIP1 activity redirects signalling away from PIP<sub>3</sub>-mediated activation cascades including those that regulate immune activation (Akt phosphorylation) and chemotaxis in the inflammatory cells.

AQX-1125 (administered orally) has been evaluated in several *in vivo* models of airway inflammation in the mouse and rat and markedly reduces the accumulation of inflammatory cells in bronchoalveolar lavage fluid and inhibits the production of multiple inflammatory cytokines [11]. We investigated the pharmacodynamics, safety and pharmacokinetics of AQX-1125 in an allergen challenge study with a double-blind placebo-controlled, crossover design in steroid-naïve asthmatic subjects.

## Methods

### Subjects

Twenty-two steroid-naïve subjects with a diagnosis of asthma of at least 6 months duration were recruited and their demography is shown in Table 1. Subjects were required to be aged 18–55 years and non-smokers for at least 6 months with less than or equal to a 10 pack-year history. At screening, patients were required to have a prebronchodilator forced expiratory volume in 1 s (FEV<sub>1</sub>) ≥ 70% predicted, a positive skin test to either house dust mite, grass pollen or cat dander, and to demonstrate both an early and late asthmatic reaction to one of these allergens to which they were sensitive when given by inhalation. Subjects had normal haematology, biochemistry and creatinine clearance

Table 1. Patient demography

| Parameter                            | Study population (n = 22) |
|--------------------------------------|---------------------------|
| Gender                               |                           |
| M                                    | 20                        |
| F                                    | 2                         |
| Age (Mean ± SD)                      | 31 ± 9                    |
| Race                                 |                           |
| Caucasian                            | 14                        |
| Asian                                | 3                         |
| Black                                | 3                         |
| Other                                | 2                         |
| BMI (Mean ± SD)                      | 24 ± 3                    |
| FEV <sub>1</sub> (L, Mean ± SD)      | 3.63 ± 0.78               |
| FEV <sub>1</sub> (% pred, Mean ± SD) | 92 ± 12                   |

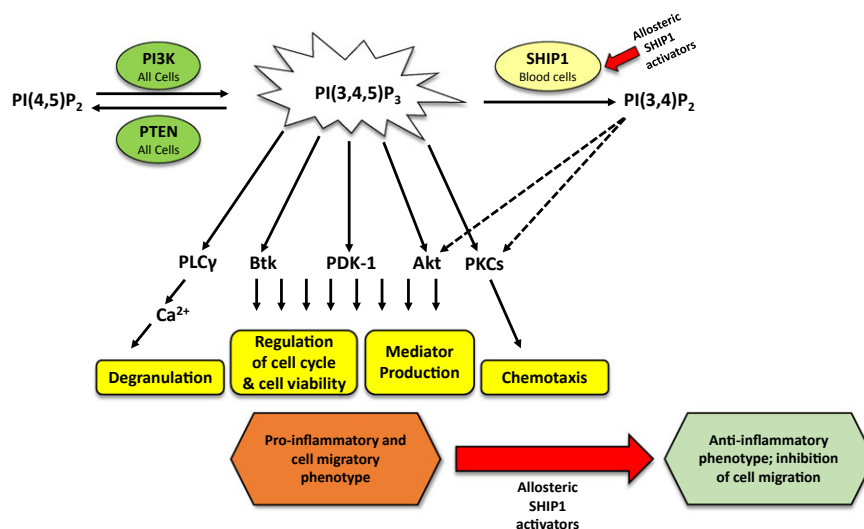


Fig. 1. Regulation of phosphoinositide metabolism by SHIP1 and closely related pathways in cells of haematopoietic origin.

values, and all patients provided written informed consent. The study was given ethics approval by National Research Ethics Service Committee South East Coast – Brighton and Sussex (11/LO/1379) and registered on the EU Clinical Trials Register (EudraCT: 2011-003012-23).

### Study design

This was a single-centre, double-blind, randomized, placebo-controlled, crossover study (study design schematic; Fig. 2). Eligible subjects were randomized to receive 450 mg AQX-1125 orally once daily (in three capsules) for 7 days or matching placebo. The washout period was 14–21 days between treatment periods. Dosing was performed under supervision at the sites on days 1, 6 and 7. On days 2–5, subjects were instructed to take the study medication at the same time of day and were required to complete a diary card to document the time that medication was taken.

Heart rate, blood pressure and ECG were measured at screening, day 1 and follow-up, and FEV<sub>1</sub> and fractional exhaled nitric oxide (FeNO) were measured at screening, on days 1, 6, 7 and FEV<sub>1</sub> at follow-up. On day 6, an inhaled allergen challenge was performed after the 1 h postdose FEV<sub>1</sub> and FeNO measurements. Methacholine challenge was then performed at 24 h after allergen challenge. Adverse events and rescue  $\beta_2$ -agonist use were monitored throughout the study with the aid of diary cards. A placebo-controlled phase was included for each subject, providing a comparison between active drug and no active drug. A 6-day treatment period was considered sufficient to allow the product to have an effect on the efficacy parameters, and the washout period of up to 3 weeks

was included between treatments to ensure all active drug had been eliminated from the body and its effects had vanished before subjects were crossed over to receive the alternative, double-blind product (placebo or AQX-1125).

### Allergen and methacholine challenges

Bronchial challenges were performed as we have previously described [12, 13] using a Mefar Dosimeter (Mefar, Bologna, Italy). Allergen for skin prick tests (Soluprick SQ; Alk Abelló, Reading, UK) was stored at 4°C; each subject was assessed for sensitivity to house dust mite, cat dander, grass pollen, and positive and negative controls. The allergen for inhalation was selected according to the largest skin test weal (positive > 3 mm) and clinical history. Fresh solutions of house dust, grass pollen and cat dander allergen (Aquagen®; Alk-Albello) were made up in 0.9% saline in doubling concentrations from 250 SQ-U/mL to 32 000 SQ-U/mL. At screening, incremental doses of allergen were administered over five inhalations [12] until an early asthmatic response (EAR) was observed, defined as a fall in FEV<sub>1</sub> of  $\geq 20\%$  from the postsaline value, on at least one occasion, between 5 and 30 min after the final concentration of allergen. The late asthmatic response (LAR) was defined as a fall in FEV<sub>1</sub> of  $\geq 15\%$  from the postsaline value, on at least three occasions, two of which must be consecutive, between 4 and 10 h after the final concentration of allergen. During the treatment periods, the total dose of allergen required to cause an EAR and LAR was administered as a single bolus dose derived from the total screening dose used. This bolus dose was used for both treatment

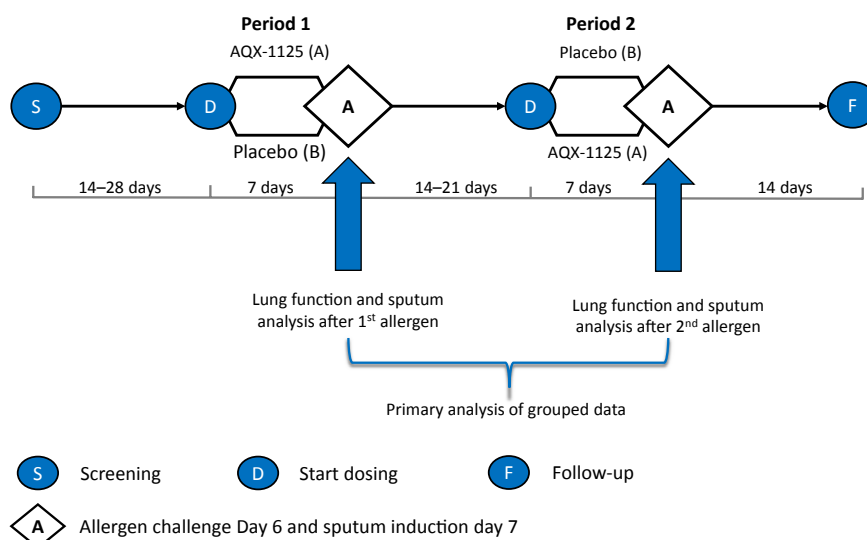


Fig. 2. Flow chart showing study design. Allergen = inhaled allergen challenge.

periods. The minimum FEV<sub>1</sub> between 0 and 4 h (EAR), 4 and 10 h (LAR) and 0 and 10 h (total asthmatic response; TAR) in each treatment period was calculated and analysed using the same method as the primary efficacy parameter. Subjects were administered five inhalations of nebulized phenol-saline (diluent of methacholine) followed by FEV<sub>1</sub> measurements. If the postsaline FEV<sub>1</sub> was  $\leq 10\%$  from baseline, then the subject inhaled doubling concentrations of methacholine from 0.03125 to 32 mg/mL until a  $\geq 20\%$  fall in postsaline FEV<sub>1</sub> was achieved or the highest concentration of methacholine was administered. The provocative concentration required to reduce the FEV<sub>1</sub> by 20% of the postsaline baseline value (PC<sub>20</sub>) was derived by linear interpolation between the lowest concentration that caused a  $> 20\%$  fall and the preceding concentration as described [12].

### Sputum induction

Sputum induction was performed at screening visit 2 and on day 7 of each treatment period postmethacholine challenge [14]. Subjects completed, where possible, three cycles of sputum expectoration. For each cycle, the subject inhaled 4% hypertonic saline solution for 5 min and then encouraged to cough up sputum. Inhalation was performed using an OMRON nebulizer (Milton Keynes, UK). The sputum sample was liquefied using 0.1% dithiothreitol and filtered through 48- $\mu$ m nylon gauze. The sputum sample was washed with phosphate-buffered saline (PBS) and centrifuged at 400 g. The fluid phase was collected and stored for biomarker analysis. The cell pellet was resuspended in PBS, and cells were counted using an improved Neubauer haemocytometer with cell viability measured using trypan blue exclusion method. Induced sputum was analysed for IL-4, IL-5, IL-13 and TNF- $\alpha$  using a multiplexed bead assay system (R&D Systems, Abingdon, UK) measured by Luminex (Texas, USA). Differential cell counts were also performed.

### FeNO

Standardized FeNO measurements were taken using NIOX<sup>®</sup> (Aerocrine, Stockholm, Sweden), with three valid tests being performed to ensure reproducibility. FeNO was recorded on day 1 predose and on days 6 and 7 predose and at 4, 8, 10 and 24 h postallergen challenge.

### Pharmacokinetic sampling and bioanalytical method

Blood samples were collected on day 1 predose and on day 6 predose, 15 min, and 1, 1.5, 4, 8 and 24 h postdose. Plasma concentrations were determined by a validated ultra-high-performance liquid chromatography

tandem mass spectrometry (LC-MS/MS) method and reported in terms of ng/mL of the free base.

### Statistics

Minimum LAR was derived as the minimum FEV<sub>1</sub> value over 4–10 h postallergen challenge. Minimum EAR was derived as the minimum FEV<sub>1</sub> over 0–4 h postallergen challenge. Weighted mean LAR and EAR end-points were derived by calculating the area under the curve (AUC) over the relevant time interval using the linear trapezoidal rule and dividing by the time interval. Given the statistical analysis of previous studies, and the possible dropout rate, this study was appropriately powered [13, 15]. Assuming a 25% treatment response with 80% power and two-sided level of significance 0.05 (5%), 21 evaluable subjects were required. The primary efficacy parameter was the LAR as defined by the baseline-corrected AUC from 4 to 10 h (AUC<sub>4–10 h</sub>) and was calculated using the trapezoidal rule. The EAR and TAR as defined by AUC<sub>0–4 h</sub> and AUC<sub>0–10 h</sub>, respectively, were calculated using the same method as the primary efficacy parameter.

Comparison between treatments for FEV<sub>1</sub> AUC<sub>4–10 h</sub> were made using an analysis of covariance for a crossover design (ANCOVA) using baseline FEV<sub>1</sub> (day 6, postsaline) as a covariate. The FeNO for each time-point and the AUC over the postallergen challenge interval values were analysed using the ANCOVA method described above. For sputum cell counts, PC<sub>20</sub> values and FeNO determinations, statistical analysis was performed on the log<sub>2</sub>-transformed values using an analysis of variance (ANOVA) for a crossover design. All statistical analyses were specified *a priori*. Pharmacokinetic parameters were estimated using non-compartmental methods.

### Results

Forty subjects were screened, of whom 22 subjects were randomized and 21 completed both treatment periods. One subject was withdrawn for a positive drug test after day 1 in treatment period 1.

### Allergen challenge

Allergens used were house dust mite (14 subjects), grass (seven subjects) and cat dander (one subject). The allergen challenges had no significant period or sequence effect. The results are displayed in Table 2 and Fig. 3. Treatment with AQX-1125 inhibited the LAR with a mean difference from placebo in FEV<sub>1</sub> (AUC LAR<sub>4–10 h</sub>) of 54 min L, which was a 20% reduction vs. placebo ( $P = 0.0268$ ). This effect corresponds to a mean weighted improvement of 150 mL during the LAR. A significant treatment effect was also seen in the

Table 2. Allergen challenge on day 6 LAR, EAR and TAR responses. Descriptive statistics and ANCOVA (evaluable populations)

|                                     | AQX-1125 <i>n</i> = 21 | Placebo <i>n</i> = 21 | Difference<br>(AQX-1125 – placebo) | Percentage<br>difference |
|-------------------------------------|------------------------|-----------------------|------------------------------------|--------------------------|
| LAR AUC <sub>4–10 h</sub> (min L)   |                        |                       |                                    |                          |
| Mean (SD)                           | –289 (182)             | –342 (178)            | 53 (100)                           | –20 (41)                 |
| ANCOVA analysis                     |                        |                       |                                    |                          |
| Adjusted mean (SE)                  | –293 (40)              | –347 (40)             | 54 (22)                            |                          |
| 95% CI                              | –378, –209             | –431, –263            | 7, 101                             |                          |
| Probability of treatment effect     |                        |                       |                                    | <i>P</i> = 0.027         |
| EAR (AUC <sub>0–4 h</sub> ; min L)  |                        |                       |                                    |                          |
| Mean (SD)                           | –122 (75)              | –125 (80)             | 3 (91)                             | 136 (345)                |
| ANCOVA analysis                     |                        |                       |                                    |                          |
| Adjusted mean (SE)                  | –120 (17)              | –125 (17)             | 5 (20)                             |                          |
| 95% CI                              | –157, –84              | –162, –88             | –38, 47                            |                          |
| Probability of treatment effect     |                        |                       |                                    | <i>P</i> = 0.83          |
| TAR (AUC <sub>0–10 h</sub> ; min L) |                        |                       |                                    |                          |
| Mean (SD)                           | –411 (240)             | –467 (237)            | 56 (174)                           | –11 (44)                 |
| ANCOVA analysis                     |                        |                       |                                    |                          |
| Adjusted mean (SE)                  | –414 (54)              | –472 (54)             | 59 (39)                            |                          |
| 95% CI                              | –527, –301             | –585, –360            | –23, 140                           |                          |
| Probability of treatment effect     |                        |                       |                                    | <i>P</i> = 0.15          |

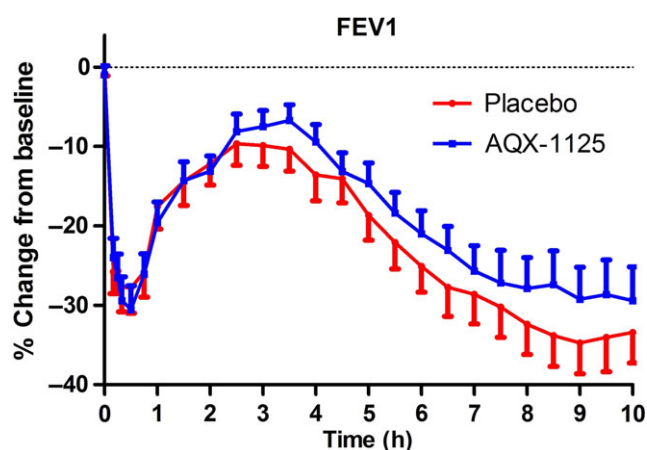


Fig. 3. Mean and SEM FEV<sub>1</sub> values over time after inhaled allergen challenge. The late response between 4 and 10 h after inhaled allergen (LAR<sub>4–10</sub>) is reduced by 20% vs. placebo (*P* < 0.03).

minimum FEV<sub>1</sub> of 180 mL (10%) for the LAR (*P* = 0.0137). No significant treatment effect for EAR or TAR (Table 2 and Fig. 3).

Methacholine reactivity 24 h postallergen challenge was not different after treatment with AQX-1125 compared with placebo, and the PC<sub>20</sub> was 0.38 mg/mL (0.19–0.77) compared with placebo 0.45 mg/mL (0.22–0.91), geometric mean (95% CI), *P* = 0.36, *n* = 18.

#### Induced sputum parameters

Sputum cell counts were evaluated on day 7 (Table 3). None of the sputum cell counts showed a significant treatment effect; however, the ratio AQX-1125/placebo

Table 3. Induced sputum cell count data. Data are from subjects with paired data only (*n* = 11)

| Sputum cell parameter            | Adjusted means |         | AQX-1125/Placebo<br>ratio (SE) |
|----------------------------------|----------------|---------|--------------------------------|
|                                  | AQX-1125       | Placebo |                                |
| Eosinophils (%)                  | 5.5            | 10.2    | 0.53 (1.79)                    |
| Neutrophils (%)                  | 61.1           | 77.0    | 0.79 (0.23)                    |
| Macrophages (%)                  | 1.3            | 3.1     | 0.43 (1.83)                    |
| Eosinophils<br>(cells/mg sputum) | 305            | 950     | 0.32 (2.24)                    |
| Neutrophils<br>(cells/mg sputum) | 5226           | 7176    | 0.73 (0.78)                    |
| Macrophages<br>(cells/mg sputum) | 53             | 287     | 0.19 (2.68)                    |

for both the absolute and differential sputum cell counts for eosinophils, neutrophils, and macrophages favoured active treatment. IL-5 was found to be increased in the AQX-1125 group compared with placebo, 9.56 pg/mL ± 5.24 and 6.39 pg/mL ± 5.95, respectively (mean ± SD; *P* = 0.014). There were no differences between groups for the other biomarkers measured. The sputum data were underpowered as there was paired data available in only 11 subjects. This is a frequent finding in these studies as subjects are often unable to safely undergo sputum induction postallergen challenge procedures.

#### Exhaled nitric oxide

There was no significant treatment effect at any of the time-points and the AUC FeNO (min\*p.p.b.; mean, 95%

CI): AQX-1125 129 (3, 254), placebo 42 (–82, 166;  $P = 0.31$ ).

### Pharmacokinetics

The time to maximum plasma concentration at steady state ( $C_{\max}$ ) indicated that AQX-1125 was rapidly absorbed. The median time to the maximum plasma concentration at steady state ( $T_{\max}$ ) was 1.50 h and ranged from 1.00 to 4.00 h. The mean  $C_{\max}$  and  $AUC_{0-24\text{ h}}$  values were 1508 ng/mL and 17 228 h ng/mL, respectively, and the geometric mean  $C_{\max}$  and  $AUC_{0-24\text{ h}}$  values were 1416.70 ng/mL and 16726.66 h ng/mL. The intersubject variability in exposure parameters was within or close to expected physiological variability. The %CV for  $C_{\max}$ ,  $AUC_{0-24\text{ h}}$  and the minimum plasma concentration at steady state ( $C_{\min}$ ) were 38.62%, 25.73% and 37.73%, respectively. Parameter values are shown in Table 4 (sampling times did not cover enough of the terminal elimination phase to estimate accurately the terminal elimination half-life).

### Safety and adverse events

The majority of the adverse events (AEs) were considered mild with none considered severe. The most common AE was headache which was reported in four subjects on active treatment and in three subjects on placebo treatment. Dyspepsia was the most common gastrointestinal AE that was considered related to treatment. Other AEs considered a result of the allergen challenge included back pain (two subjects on placebo), chest discomfort (two subjects on AQX-1125 and one on placebo) and dyspnoea (two subjects on placebo). One subject was withdrawn from the study prior to the second treatment period (placebo) due to a positive urine drugs of abuse test. There were no changes in any mean laboratory values over time.

### Discussion

AQX-1125 is a novel first-in-class orally available agent that has demonstrated anti-inflammatory properties in both neutrophilic and eosinophilic animal models. This is the first study with AQX-1125 performed in

asthmatics, and we used an allergen challenge model as the drug had demonstrated anti-inflammatory effects in the allergen challenge model in rats. AQX-1125 demonstrated a significant, albeit modest, attenuation of allergen-induced responses compared with placebo. AQX-1125 had no effect on the secondary end-points of methacholine reactivity or FeNO. Although the effects on induced sputum cell counts were not significant, there was a trend towards a favourable treatment effect. Together, these observations indicate the potential for a therapeutic effect and merit the testing of AQX-1125 in further clinical trials.

AQX-1125 activates SHIP1, a modulator of PIP3 signalling for diverse processes including cell growth, activation and immune/inflammatory regulation [7–10]. Cellular PIP3 levels are regulated by its biosynthesis by PI3K and its breakdown by PTEN and SHIP1 (see Fig. 1). The end product of SHIP1 enzyme activity, PI (3,4)P<sub>2</sub>, has its own cellular signalling abilities and biological roles. The restricted tissue expression of SHIP1 to haematopoietic cells allows for pharmacological redirection of PIP3 signalling to PI(3,4)P<sub>2</sub> responses in inflammatory immune cells through SHIP1 activation. SHIP1 activation leads to a decrease in Akt phosphorylation [8, 9, 16]. Akt signalling is involved in several pathways responsible for cell survival, cell proliferation, metabolism and angiogenesis [17]. Previous studies have shown that AQX-1125 attenuates inflammatory mediator production (TNF- $\alpha$ , eotaxin, MIP-1 $\beta$ ) and is a potent inhibitor of leucocyte chemotaxis [11, 18]. The tissue-specific expression of SHIP1 in haematopoietic cells and low degree of homology with SH2-containing inositol-5'-phosphatase 2 (SHIP2) also reduces the likelihood of off-target, off-tissue toxicities. AQX-1125 displays PK properties suited to once daily dosing. The activity of AQX-1125 in preclinical models of allergen challenge, chemotaxis and cytokine release provided the pharmacological rationale for testing in a human allergen challenge study as a proof of concept for its activity in asthma.

The inhaled allergen challenge model is a well-recognized and robust model to assess the therapeutic potential of novel treatments for asthma [12, 19–25] as all of the currently active molecules show activity and there are no false positives. Comparing the magnitude of the

Table 4. Pharmacokinetic parameters for AQX-1125. \*values are median (min–max)

|                          | $C_{\max}$ (ng/mL) | $T_{\max}$ * (h) | $AUC_{0-24\text{ h}}$ (h ng/mL) | $C_{\min}$ (ng/mL) |
|--------------------------|--------------------|------------------|---------------------------------|--------------------|
| <i>n</i>                 | 22                 | 22               | 22                              | 22                 |
| Mean (SD)                | 1507.64 (582.20)   | 1.50 (1.00–4.00) | 17228.31 (4433.15)              | 324.58 (122.47)    |
| Geometric mean           | 1416.70            | –                | 16726.66                        | 267.64             |
| 95% CI of geometric mean | 1211.42, 1656.77   | –                | 14998.14, 18654.39              | 178.55, 401.19     |

effect across different studies should be made with caution as the details of the period of measurement of the late response can vary between studies. In studies where measurements have been made up to 10 h postchallenge, as in the present study, the magnitude of the effect with AQX-1125 being 20% on the LAR is comparable with 25% reduction seen with PDE4 inhibitors [13, 26], leukotriene receptor [12, 21] and CRTH2 [27] antagonists that all drive the inflammatory response through TH2-mediated mechanisms. Targeting the TH2 response with associated reductions in the LAR has also been achieved by inhibiting the cytokine mediators IL-4 and IL-13 by blocking their common receptor with the IL-4 variant pitrakinra [22]. Eosinophils and lymphocytes are key cells mediating these TH2 responses [28] and these haematopoietic cells express SHIP1 [5, 6, 9].

The magnitude of the mean improvement on FEV<sub>1</sub> was 150 mL during the LAR, and a maximum improvement of 180 mL observed at trough FEV<sub>1</sub> is in the range of a clinically meaningful change predicting efficacy in clinical studies [29]. The effects on lung function correlated with nominal reductions in the absolute and differential sputum cell counts for eosinophils, neutrophils and macrophages. However, these data were only obtained in 11 paired subject samples and therefore underpowered to detect a significant difference. The overall trend to show a reduction in sputum inflammatory cells does support an inhibition of TH2-mediated effects as there is a greater effect seen on reduction in sputum eosinophil numbers than neutrophils. It is noteworthy that there is also a reduction in sputum macrophages associated with AQX-1125 treatment compared with placebo.

In the allergen challenge model, the methacholine challenge data frequently result in negative or inconsistent data; inhaled corticosteroids both inhibited [21, 25] or had no effect [23]; montelukast either inhibited the methacholine response [12] or had no effect [21]. In this study, no significant changes were seen in the secondary end-point measurements of methacholine challenge postallergen, or exhaled NO. The study was not powered to show a difference in the secondary parameters and only 8 sets of paired data were obtained for the methacholine challenge. This observation is often made in such studies as subjects' lung function remain hyperresponsive postallergen challenge and further procedures

performed after this procedure, such as methacholine challenge often cannot be performed for safety reasons.

The usefulness of FeNO as a marker of inflammation postallergen challenge also varies with the class of drug that is tested. For example, corticosteroids markedly reduce elevated FeNO [30], but PDE4 inhibitors do not [13, 20]; more variable effects have been reported with the leukotriene inhibitors [12, 31].

AQX-1125 increased the catalytic activity of purified human SHIP1 maximally to 20% at 300  $\mu$ M (96  $\mu$ g/mL) [18]. The EC<sub>50</sub> for inhibition of chemotaxis with human leucocyte subsets with AQX-1125 ranged from 0.002 to 0.76  $\mu$ M (0.64 to 244 ng/mL), depending on the cell type. Once daily dosing in this study resulted in C<sub>max</sub> values of 1507 ng/mL (4.7  $\mu$ M) and 24-h trough levels of 325 ng/mL (Table 4). These values are in the range predicted from the preclinical studies to show pharmacodynamic activity throughout the 24-h time period with once a day dosing. Our study demonstrates the clinical potential for AQX-1125 with its novel mechanism of action as a treatment for allergic asthma and further supports the clinical development of AQX-1125 in inflammatory respiratory diseases.

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## Conflict of interest

BRL has received research funding from Astra Zeneca. PJB has served on Scientific Advisory Boards of Astra-Zeneca, Boehringer-Ingelheim, Chiesi, Daiichi-Sankyo, GlaxoSmithKline, Novartis, Nycomed, Pfizer, Teva and UCB and has received research funding from Aquinox Pharmaceuticals Inc, AstraZeneca, Boehringer-Ingelheim, Chiesi, Daiichi-Sankyo, GlaxoSmithKline, Novartis, Nycomed, Pfizer and Prosonix. He is also a cofounder of RespiVert (now part of Johnson & Johnson) who has discovered novel inhaled anti-inflammatory treatments for asthma and COPD. BOC has received research funding from Astra Zeneca. FYA has no declared conflict of interest. PT, JN and TM are employees of Aquinox Pharmaceuticals Inc. JN was an employee of Aquinox Pharmaceuticals Inc.

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