A Novel Eye Drop Formulation of Squalamine For Exudative AMD: Evaluation Of Ocular Distribution And Ocular Safety In Rabbits

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Purpose:
To evaluate the ocular tissue distribution and ocular safety of a novel eye drop formulation of Squalamine, a potent antiangiogenic small molecule inhibitor of multiple growth factors (VEGF, PDGF, bFGF) with previously demonstrated systemic activity in vivo in ocular pathologies and in clinical trials for exudative macular degeneration.

Introduction:
Squalamine, a small molecule aminosterol is an anti-angiogenic compound (Figure 1) which inhibits multiple protein angiogenic growth factors through a unique intracellular mechanism of action by binding to the cell membrane-bound regulatory protein calmodulin and chaperoning it into the cytoplasm (Figure 2) where it becomes unavailable to modulate the downstream signaling of VEGF, PDGF and bFGF.

Figure 1

Unlike other VEGF targeting agents, squalamine does not cause the blockade of the action of endothelial nitric oxide synthase (eNOS) which is linked to producing hypertension, while it does target the MAP kinase cellular proliferative pathway, the p38 inflammatory pathway, as well as VEcadherin and αvβ3 and their downstream signaling pathways which lead to neovascularization.

Squalamine had previously been evaluated in Phase II and III clinical trials in wet AMD as an intravenous infusion where it had demonstrated anti-angiogenic effects. The molecule has now been formulated into an eye drop formulation that has been demonstrated to be safe pre-clinically in rabbits.

Methods:
Male Dutch belted rabbits (n=24) were administered Squalamine eye drops (40 μL) in both eyes, either QD (every 24 hours) or BID (every 12 hours) for 1, 7, and 14 days (n=4/group/dosing regimen). Animals were necropsied and ocular tissues were harvested 24 (+2) or 12(+1) hours post last dosing in the QD or BID groups, respectively. Posterior sclera/choroid, aqueous and vitreous humor, and plasma were assayed for Squalamine concentrations using a validated LC-MS/MS method with a lower limit of quantification (LLOQ) of 10ng/g of tissue. Values below the LLOQ are reported as below quantifiable limits, or BQL.

Results:
Squalamine eye drops, given QD or BID were well tolerated with no adverse clinical effects. Given QD, mean trough concentrations of Squalamine in posterior sclera/choroid were 21.7, 62.6, and 68ng/g in the 1, 7, and 14 day groups, respectively (Figure 3).

Squalamine concentrations in aqueous and vitreous humors were <LLOQ (10 ng/g) in all animals and <LLOQ in plasma (10ng/mL) in 23/24 animals (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Aqueous Humor</th>
<th>Vitreous Humor</th>
<th>Plasma</th>
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<tbody>
<tr>
<td>QD</td>
<td>BID</td>
<td>QD</td>
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<tr>
<td>Day 1</td>
<td>BQL</td>
<td>BQL</td>
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<tr>
<td>Day 7</td>
<td>BQL</td>
<td>BQL</td>
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<tr>
<td>Day 14</td>
<td>BQL</td>
<td>BQL</td>
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Squalamine eye drops proved innocuous and produced no discernible changes in ophthalmological ocular examinations.

Conclusions:
Squalamine eye drops were well tolerated, consistent with previous longer term preclinical studies in which there were no adverse clinical findings or changes in ocular histopathology. Posterior sclera/choroid tissue concentrations of Squalamine given QD or BID exceeded the threshold at which Squalamine is known to inhibit neovascularization in a cell-based model (11 ng/mL). Importantly, as evidenced by trough concentrations in posterior sclera/choroid above the anti-angiogenic threshold level, sustained therapeutically relevant posterior ocular exposure levels were maintained for the duration of a full dosing interval (QD 24h, BID 12h), indicating the ability to consistently remain above the threshold level with continuous QD or BID administration.

Squalamine had rapid uptake, prolonged residence time, and slow tissue clearance when administered QD or BID in the eye drop formulation up to 14 days. Minimal systemic uptake reduces potential systemic safety concerns. The absence of Squalamine concentrations in aqueous humor suggests a passive diffusion mechanism from anterior to posterior sclera and subsequently into the choroid, while also limiting the potential for corneal opacities and deposits which have not been seen in longer term in vivo studies.

These results, consistent with previous preclinical topical data and intravenous clinical studies, warrant the further clinical investigation of Squalamine eye drops to treat neovascular ophthalmic disorders. A Phase II clinical trial of the eye drop formulation in wet AMD patients is planned for the third quarter of 2012.

Commercial Relationships: Irach B. Taraporewala, Ohr Pharmaceutical (I, E, P); Michael J. Elman, Genentech (C), Ohr Pharmaceutical (C); Shalom Z. Hirschman, Ohr Pharmaceutical (C); Samuel I. Backenroth, Ohr Pharmaceutical (I, E, P)