THR-687 at Euretina 2018

Title: Therapeutic effect of the potent integrin antagonist THR-687 in the cynomolgus laser-induced choroidal neovascularization model confirms its potential as novel treatment for sight-threatening retinal pathologies

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Purpose: Integrins play an important role in various biological processes including cell differentiation, adhesion, migration, invasion and proliferation. Given their multifaceted function, integrins are associated with various eye diseases such as diabetic retinopathy (DR) and wet age-related macular degeneration (AMD). Integrins are implicated in main pathologic hallmarks like neovascularization, inflammation and vascular leakage. Targeting integrins has the potential to attenuate these vision-threatening processes, independent of anti-VEGF responsiveness. We investigated a novel integrin antagonist, code THR-687, by characterizing its binding affinity towards several integrins, its anti-angiogenic properties in *in vitro* systems, and its impact in the cynomolgus choroidal neovascularization (CNV) model.

Setting: ThromboGenics N.V., Gaston Geenslaan 1, 3001 Leuven, Belgium

Methods: Competition ELISA assays were used to measure the ability of THR-687 to compete with the binding of integrin receptors to their natural ligands. The ORIS™ cell migration assay was implemented to assess the inhibitory effect of THR-687 on the migration of human umbilical vein endothelial cells (HUVECs) and an *ex vivo* mouse choroidal explant model was used to evaluate the effect of THR-687 on blood vessel outgrowth. In addition, the anti-angiogenic potential of 3 different dose levels of THR-687 (4.5mg/eye (n = 6), 2.25mg/eye (n = 5) and 0.45mg/eye (n = 6)) were investigated in the cynomolgus CNV model. Each experimental group received 3 intravitreal (IVT) injections of THR-687 or its formulation buffer (n = 6) with a weekly interval (Day 1, 8 and 15). Fluorescein angiography was performed to assess the grade of CNV-induced leakage in the laser spots on Day 1 (baseline, before IVT treatment), and toward the end of Week 2 and Week 3 after the start of treatment. The safety profile of THR-687 was evaluated in (non)-GLP toxicology studies, including cytotoxicity, genotoxicity, safety pharmacology, ocular and systemic toxicity.

Results: The small molecule integrin receptor antagonist THR-687 was found to inhibit multiple RGD integrin receptors with IC_{50} values in the single-digit nanomolar range, including, but not limited to, $\alpha_v\beta_3$ (IC_{50} of 7.3 ± 2.7nM), $\alpha_v\beta_5$ (IC_{50} of 1.1 ± 0.6nM) and $\alpha_5\beta_1$ (IC_{50} of 8.2 ± 2.4nM). THR-687 potently attenuated the migration of HUVECs in the ORIS™ system (IC_{50} of 258 ± 65nM) as well as *ex vivo* choroidal vessel sprouting (IC_{50} of 236 ± 86nM). In corroboration, all 3 dose levels of THR-687 significantly inhibited angiogenesis-induced leakage in the cynomolgus CNV model. THR-687 was found to be well-tolerated in safety pharmacology (cytotoxicity and genotoxicity), ocular and systemic toxicity studies indicative of a broad therapeutic window.

Conclusion: THR-687 antagonizes RGD integrins implicated in angiogenesis and vascular permeability ($\alpha_v\beta_3$, $\alpha_v\beta_5$ and $\alpha_5\beta_1$) with single-digit nanomolar affinity. THR-687 exhibited anti-angiogenic activity in *in vitro* endothelial cell migration and choroidal vessel sprouting assays. In addition, IVT administration of THR-687 potently inhibited neovascularization-induced leakage in the cynomolgus CNV model. Extensive toxicology and safety pharmacology studies indicated the good ocular tolerability of THR-687. By targeting

the integrin pathway, THR-687 could result in VEGF-dependent as well as VEGF-independent therapeutic mechanisms. Given its multifaceted mode of action combined with its favorable efficacy and safety profile, THR-687 is a promising drug candidate for the treatment of vision-threatening retinal pathologies such as DR and wet AMD.