Targetting placental growth factor attenuates retinal permeability and inflammation in diabetic Akimba mice

Hu TT, De Vriese A, Vanheukelom V, Feyen JHM

ThromboGenics NV, Leuven, Gaston Geenslaan 1, 3001 Heverlee, Belgium

Design of study Therapeutic effect of anti-PlGF in diabetic Akima mice

Purpose Current available therapies for diabetic retinopathy (DR)/diabetic macular edema (DME) suffer from potential side effects and a significant proportion of treated patients do not recover good vision. Thus, there is still an unmet medical need for novel treatments of DME. The diabetic Akimba mouse model was used to investigate whether inhibition of placental growth factor (PlGF) could alleviate retinal permeability and inflammation, two essential DR/DME pathologic hallmarks.

Methods The Akimba mouse ($Ins2^{Akita}VEGF^{+/-}$) is a cross between Akita (type 1 diabetes) and Kimba (transgenic hVEGF-overexpression) mice. Fluorescein angiography (FA) was implemented to analyze retinal permeability. In addition, isolectin-B4 (IB-4) staining was performed on flatmounts to visualize retinal vasculature and presence of monocytes/macrophages. Age-matched wildtype (WT) mice were screened in parallel and Metamorph software (Leica) was used for quantitative analysis.

The *in vivo* efficacy of PlGF inhibition was assessed by treating Akimba males with intravitreal injections of anti-PlGF antibody (PL5D11D4; 36 nM) every other week (w). The inhibitory effect on retinal leakage was monitored via longitudinal FA follow-up: before treatment, 2w and 4w after start of treatment. The impact of PlGF neutralization on inflammatory response in Akimba was investigated on IB-4 stained flatmounts 5w after start of treatment.

Results In comparison to WT, FA analysis and IB-4 stainings revealed that Akimba mice displayed significantly augmented vascular leakage and a pronounced presence of immune cells in the retina. Following anti-PlGF therapy a significant decrease in retinal permeability could be observed 2w and

4w after start of treatment as compared to vehicle control (P<0.05; n=20-27/group). PlGF inhibition also resulted in significant attenuation of inflammation levels in PL5D11D4-injected Akimba mice as compared to vehicle-treated Akimba animals (P<0.05; n=9-17/group).

Conclusions The Akimba mouse is a powerful model for screening next-generation therapeutics against DR-related leakage and inflammation. PlGF inhibition indeed could be identified as a promising integrative therapy for sight-threatening DR/DME.