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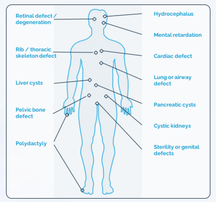
Expression levels of Bardet-Biedl Syndrome (BBS) genes change during retinal development showing an age depended dynamic regulation. Implications for determining gene therapies correct minimum effective doses (MEDs) and threshold safety levels for retinal gene therapies; the case of AXV-101 (BBS1)

Victor Hernandez Hernandez1[1,2], Nertiyan Elangkovan[2], Monica Freitas[2], Marian Seda[2], Rakhi Kesavan Nair Rema[2], Jeshmi Jeyabalan Srikaran[2], Natalie Northcott[2], Rachael Pearson[4], Phil Beales[2,3]

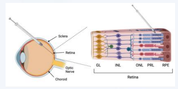
[1]Life Sciences, Brunel University London, Uxbridge, Greater London, United Kingdom; [2]Axovia Therapeutics Ltd., London, Greater London, United Kingdom; [3]GGM, University College London, London, United Kingdom, United Kingdom; [4]Centre for Gene Therapy and Regenerative Medicine, King's College London, London, United Kingdom

Introduction

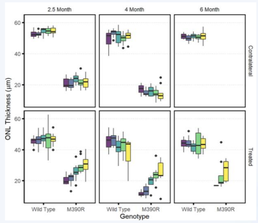
BBS is a monogenic recessive ciliopathy with multiple organs affected including rod-cone dystrophy as a cardinal features leading to BBS patients becoming blind before they reach adulthood. We have designed AXV-101, an AAV9 vector expressing a codon-optimised human BBS1 (hCOBBS1). We have previously demonstrated that AXV-101 halt the retinal degeneration in *Bbs1* M390R mutant mice when injected subretinally in young (P7-9 days after birth) pups.



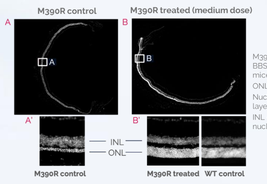
Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder associated with primary cilia dysfunction. BBS is a Mult syndromic disorder, with manifestations such as polydactyly, truncal obesity, intellectual delays and retinal dystrophy. The latest implies a progressive visual loss, with cone-rod distropy starting that develops into complete bindness before adulthood in most cases. More than 20 genes have been associated with BBS and *BBS1* is the most common mutated gene, accounting for more than 40% of patients in western populations, being the BBS1 missense M390R mutation the most common allele found in BBS1 patients



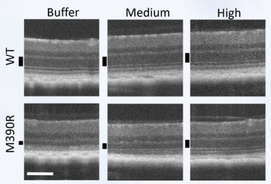
***Bbs1* M390R mutant and Wild-type (WT)** and mice were dosed with AXV-101 at P7-9 subretinally unilaterally with three doses of AXV-101



Longitudinal analysis of the Outer Nuclear Layer (ONL) thickness. We demonstrated that we can rescue the retinal degeneration with no adverse effects in dosed WT littermates. Longitudinal Optical Coherence Tomography (OCT) show that AXV-101 rescue the photoreceptors layer (ONL) thickness 6-months after dosing in a dose dependent manner. Medium and high doses (5E9 and 1e10 vg/eye) keeping the ONL layers and in some cases at a similar levels as treated wild-types. Dosed WT littermates show no adverse effects on ONL.



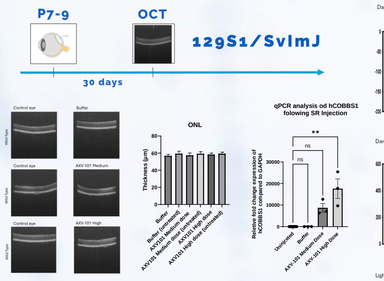
Histopathology showed the photoreceptor rescue, thickness and reorganisation of the retina layers in *Bbs1* M390R treated animals. Regions of interest shown in B, display increased ONL thickness and INL with cell organisation rescued. (A', scale bar = 50 µm).



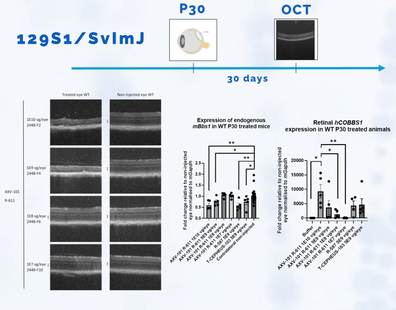
Bbs1 M390R images 6 months retinas after dosing showing how the medium and high doses sustain the ONL compared with the buffer treated,. Observe how the ONL is not affected in WT animals.

Results

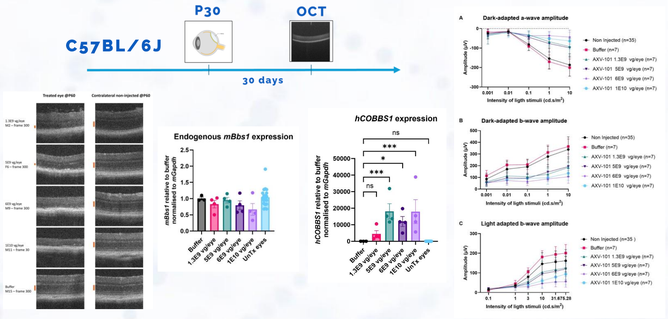
Here we demonstrate that the same **levels of transgene BBS1 overexpression** that proves to rescue retinal degeneration in *Bbs1* M390R mutant mice and are safe for WT littermates at P7-9 days, can cause adverse effects in older WT animals and how it is linked to the tolerability level of *COBBS1* overexpression. We tested the AXV-101 unilaterally overexpression in WT males and females of different mouse strains and review retinal health and BBS1 overexpression 30 days after dosing.



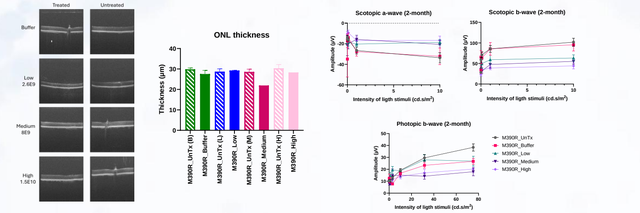
Bbs1 M390R background mouse strain is 129S1/SvImJ. Dosing at P7-9 WT animals show no adverse effects when dosed with 1E10 or 5E9 vg/eye measured by OCT. High levels of COBBS1 transgene expression were found and those are dose depended. Electroretinogram (ERG) also show normal Scotopic and Photopic retinal functions with no impact on BBS1 overexpression.



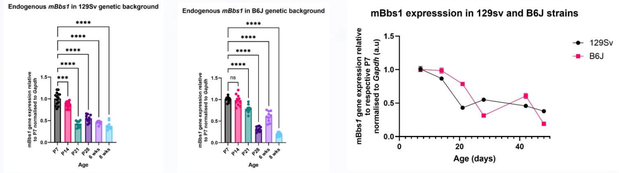
We assessed toxicity of **AXV-101 in P30 dosed animals** with four vector doses of AXV-101 R-611 batch at 1E10, 5E9, 1E8 and 1E7 vg/eye. We also tested two extra different batches of AXV-101 with 5E9 vg/eye (R-587 and T-CEPHEUS-103). ERG analysis demonstrated that both scotopic (rod) and photopic (cone) responses in treated eyes resulted in dose-dependent reduction in retinal function. OCT imaging provided additional insights into degree of loss of retinal structural integrity in eyes treated at 1E10 vg/eye and 5E9 vg/eye. Moreover, *hCOBBS1* transgene showed dose dependent increase in expression in treated eyes, clearly showing dose depended on activity of the transgene. However, endogenous *mBbs1* gene expression in the treated eyes showed a dose dependent reduction, with lowest endogenous *mBbs1* expression at AXV-101 R-611 1E10 vg/eye dose. These data indicate that subretinal overexpression of administration of AXV-101 at P30 cause retinal toxicity and disrupt retinal function in WT 129S1/SvImJ mice.



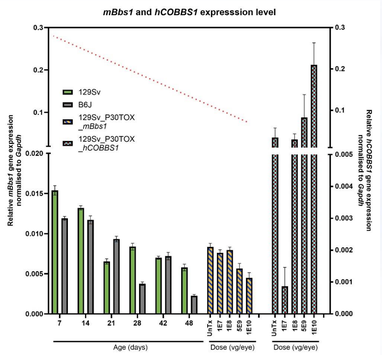
To test if mouse strain had an impact n the phenotypical readouts of AXV-101 overexpression, we dosed **P30 WT C57BL/6J** mice 1.3E9, 5E9, 6E9 and 1E10 vg/eye. We observed dose depended retinal toxicity by OCT and ERG similar to the ones observed in the 129S1/SvImJ mice. Levels of endogenous *Bbs1* mouse expression decrease when the AXV-101 dose is increased also increasing the levels of COBBS1 transgene expression. This demonstrate that regardless of the mouse strain the increased of the BBS1 transgene impact of the health of the photoreceptors



We also dosed *Bbs1* M390R mutant animals at P30 and review 30 days later. In this study we even increased the dose 50%, up to 1.5E10 vg/eye. OCT analysis did not show any histological adverse effect with retinal layers and ONL levels the same than the ones observed in control *Bbs1* M390R animal groups. There is also no toxicity observed on the function of the rods and cones by ERG. The whole cohort is still being analyse to detect rescue at 6 months. These confirms that the toxicity effects observed in dosed WT animals is depended on the overexpression of BBS1.

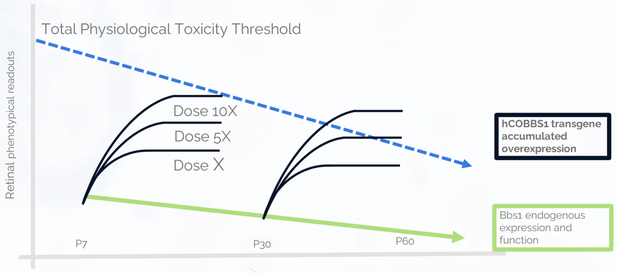


Endogenous *Bbs1* expression levels change with age. To undercover why early dosing did not have an impact in WT animals we analysed the endogenous expression of *Bbs1* from P7 to P60 in 129s and BL6 mouse lines. We discovered that there is a decrease of the physiological expression of Bbs1 over time. This result explains that the levels of BBS1 that cells are needed and able to cope in WT animals is lower in older animals.



Summary of the *Bbs1* endogenous expression and COBBS1 overexpression in 129S1/SvImJ P30 dosed WT mice.
We observed that *Bbs1* expression is physiologically downregulated from P7 to P48 up to 61% and 81% of initial expression in 129Sv and B6 strains respectively. When P7-9 animals were dosed with AXV-101, no reduction of the ONL thickness or drop on the retinal responses in ERGs for any dose was observed and *Bbs1* expression remained at the same levels than untreated animals. Animals dosed at P30 with 1E7 vg/eye and 1E8 vg/eye doses had similar *Bbs1* expression, with no from COBBS1 transgene expression. However, 5E9 vg/eye and 1E10 vg/eye dosed groups, *Bbs1* expression is inversely reduced to *COBBS1* transgene expression levels.

Physiological Toxicity Threshold
Differences between P7 and P30 Wild-type mice dosings



Model describing the BBS1 tolerability thresholds. For BBS1, age and genotype impact on the safety levels. The capability to absorb overexpression in a WT background is reduced with age, making it not the best relevant genotype to calculate safety. Our data demonstrates that to calculate the minimum effective and safe doses for first in human clinical trials, we should be very careful about analysing results obtain in wild-type models. Toxicological Studies should be designed with that in mind to avoid incorrect data and waste of time and resources, specially when using large species. Understanding the physiological regulation of a specific gene is detail is necessary to interpret the results an to define the tolerability physiological thresholds..

Conclusions

AXV-101 has demonstrated efficacy, safety and durability to halt retinal degeneration in the *Bbs1* M390R animal model. We have observed that the same doses that are able to restore functionality of the photoreceptors in Bbs1 mutant mice in P7-9 and are tolerated in WT animals when injected at P7-9, prove to impact retinal structure and function when WT retina are injected at P30. We have also demonstrated that this is due by the physiological differences in age and gene expression of Bbs1.
These have implications about how to interpret the results when using non-mutant animal models to measure AAV transgene expression tolerability thresholds and how this can impact on decisions on minimum effective doses selections.