P-18

Transduction of Photoreceptor and Pigmented Epithelial Cells Following a Single Subretinal Injection of AAVHSC17 in Minipigs

Jeff L. Ellsworth, Hillard Rubin, and Albert Seymour

Homology Medicines, Inc., Bedford, MA, USA

Adeno-associated viruses isolated from human CD34+ hematopoietic stem cells (AAVHSCs) have shown high-efficiency nuclease-free gene editing as well as gene transfer capabilities. Following I.V. injection in NHPs, AAVHSCs have shown tropism for retinal cells. The current study evaluates the ability of AAVHSCs to transduce retinal cells following localized delivery to the eye. AAVHSC17 packaging a chicken beta actin-promotered self-complementary GFP transgene (AAVHSC17-CBA-scGFP) was prepared by standard procedures. Göttingen minipigs received a single subretinal injection (0.1 mL/eye) in both eyes of either formulation buffer control or AAVHSC17-CBA-scGFP, 1.3×10^{12} vg/eye. Eyes were examined by slit-lamp biomicroscopy and/or indirect ophthalmoscopy following treatment. Ophthalmic examinations were performed pre-study and on days 3, 8, 15, and at sacrifice on day 28 post-dosing. Spectral domain optical coherence tomography (SD-OCT) for GFP autofluorescence was performed once pre-study and on day 28. At sacrifice, animals were perfused with saline followed by 4% paraformaldehyde, tissues were collected and processed for histology. Frozen sections were prepared and analyzed for direct GFP fluorescence and GFP expression by immunohistochemistry. Neither treatment-related ophthalmic changes nor ocular inflammation were observed. SD-OCT images showed regions of GFP autofluorescence that corresponded to the dosing bleb and surrounding tissue in animals treated with AAVHSC17-CBA-scGFP. GFP expression was observed in all retinae, optic nerves, chiasmata, and tracts from animals treated with AAVHSC17-CBA-scGFP. Extensive GFP staining was observed in all retinal layers while staining in optic nerves, chiasmata, and tracts was multifocal, less intense and in filament (axon)-shaped structures. No GFP expression was noted in ocular tissues and brains of animals treated with vehicle alone. These data demonstrate that administration of AAVHSC17-CBA-scGFP by subretinal injection in minipigs was well-tolerated and resulted in GFP expression in all retinal layers. These studies suggest that AAVHSCs may be useful as therapeutic vectors for treating diseases of the eye in humans.