ESGCT 2018

Comparable Photoreceptor Transduction Efficiency of AAV2tYF, AAV5 and AAV8 in Non-Human Primates

Mark S. Shearman, Chunjuan Song, Guo-jie Ye, Adrian Timmers and Judith Newmark

Applied Genetic Technologies Corporation, Alachua, FL 32615

INTRODUCTION

X-linked retinitis pigmentosa (XLRP) caused by mutations in the retinitis pigmentosa GTPase regulator (RPGR) gene is a primary rod and cone disease affecting ~20,000 patients in the US and EU [1, 2]. Gene replacement therapy using adeno-associated virus (AAV) vectors for gene delivery is effective in preventing photoreceptor degeneration and preserving retinal structure and function in disease animal models [3, 4].

Important considerations in the design and evaluation of an AAV vector include the capsid, promoter, cDNA, and the species of animals in which it is tested [5]. AAV-RPGR vectors packaged in capsids of various serotypes provide structural and functional rescue in naturally occurring RPGR mutant XLPR2 dogs or in RPGR knockout mice [4]. AAV therapeutic vectors packaged in AAV2tYF (a AAV2 capsid variant with three tyrosine to phenylalanine mutations), AAV5, or AAV8 capsids are currently in clinical development for treatment of patients with XLRP. However, data of a direct head-to-head comparison of their transduction efficiency in non-human primate (NHP) photoreceptors is limited or non-existent.

In the present study, we evaluated three AAV capsids, AAV2tYF (*rep*2/*cap*2tYF), AAV5 (2/5) and AAV8 (2/8) for their efficiency in transducing NHP photoreceptors using a hGFP reporter gene driven by the GRK1 promoter.

METHODS

AAV vectors were manufactured by plasmid transient transfection in HEK 293 cells, purified by double iodixanol step gradient centrifugation and formulated in balanced salt solution (BSS) containing 0.014% Tween 20.

The transduction efficiency of AAV vectors in photoreceptors was evaluated in NHPs by subretinal injection of AAV vectors containing a hGFP expression cassette. Cynomolgus macaques received bilateral subretinal injections of 0.1 mL of AAV vector at a concentration of 4.5×10^{11} vg/mL, and were followed for 13 weeks. GFP expression was evaluated by fluorescence intensity captured in fundus fluorescence using Image J software, and by qRT-PCR (3 eyes each with AAV2tYF or AAV8) and immunohistochemistry (4 eyes each with AAV2tYF or AAV5, and 5 eyes each with AAV2tYF or AAV8) post-mortem. The study design is summarized in **Table 1.**

Table 1 NHP Study Design

Group	# of animals	Vector		Dose level		
		Left Eye	Right Eye	vg/mL	vg per eye	Volume
1	4	AAV5-GRK1-hGFP	AAV2tYF-GRK1-hGFP	4.5×10^{11}	4.5×10^{10}	0.1 mL
2	8	AAV8-GRK1-hGFP	AAV2tYF-GRK1-hGFP	4.5×10^{11}	4.5×10^{10}	0.1 mL

RESULTS

Subretinal injection of all three individual AAV vectors was well tolerated. All three vectors were associated with an acute anterior and posterior segment inflammatory response, which rapidly improved over time, such that, by Study Day 29 (or sooner), aqueous cell/flare was no longer observed and vitreous cell scores ranged from none to moderate (2+). There were neither abnormal intraocular pressure-related findings nor abnormal findings in clinical or anatomic pathology. Similar inflammation profiles were observed for all three AAV vectors tested (**Figure 1**).

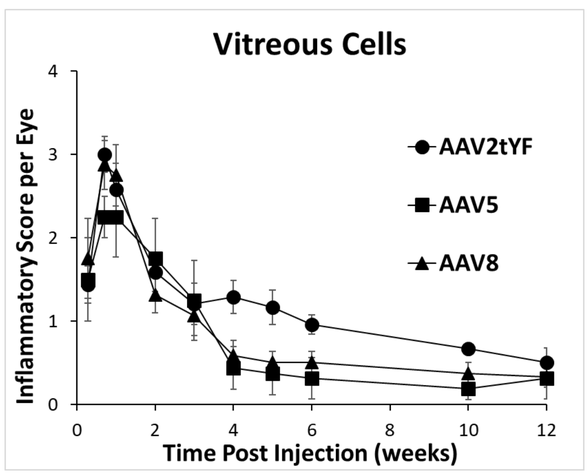
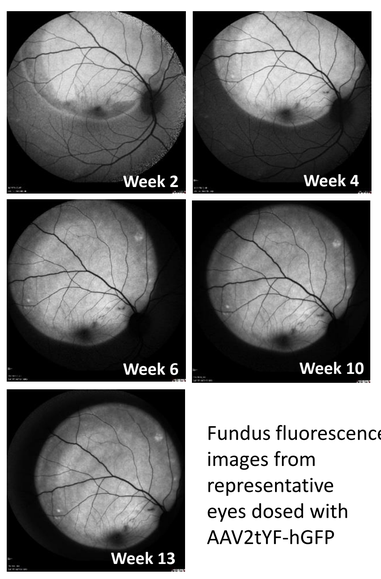


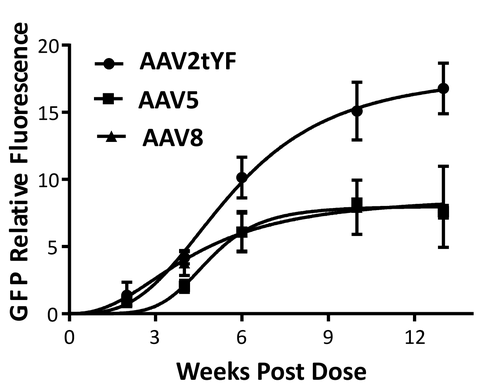
Figure 1 Ocular inflammation findings. Vitreous cells in individual eyes were scored in a standardized fashion as 0, trace (0.5), 1+, 2+, 3+, or 4+. Each symbol represents an average score with SEM from all eyes receiving the same vector through week 12

Quantitative analysis of the GFP fluorescence captured by fundus fluorescence imaging indicated that AAV2tYF outperformed both AAV5 and AAV8 starting at week 4 (**Figures 2 & 3**).

GFP Reporter Fluorescence Analysis



Fundus fluorescence images from representative eyes dosed with AAV2tYF-hGFP



Curve fitting of GFP fluorescence in NHP eyes dosed with AAV2tYF-GRK1-hGFP, AAV5-GRK1-hGFP or AAV8-GRK1-hGFP (4.5×10^{10} vg/0.1 mL; subretinal).

Figure 2. Representative GFP fundus fluorescence and quantitative analysis of the relative fluorescence intensity. AAV2tYF outperformed both AAV5 and AAV8 starting at week 4

RESULTS

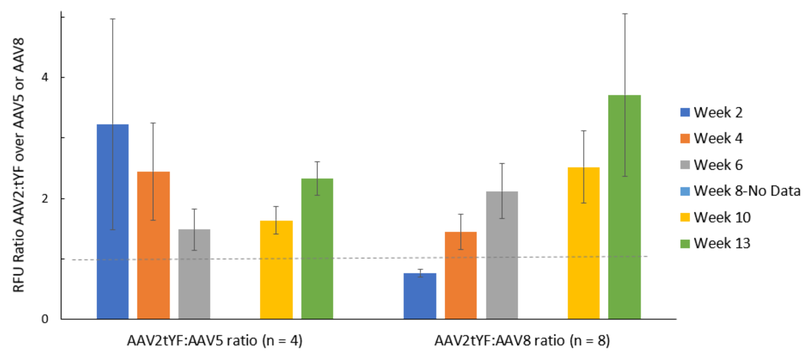


Figure 3. Pairwise ratio of AAV2tYF over AAV5 or AAV8. A ratio of 1 or higher indicates superior performance by AAV2tYF. AAV2tYF outperformed both AAV5 and AAV8 starting at week 4

Three NHPs receiving AAV2tYF-GFP in one eye and AAV8-GFP in the contralateral eye were used for qRT-PCR analysis for GFP mRNA levels. Retinal punches were collected from the bleb area and non-bleb area guided by fundus fluorescence images and processed for qRT-PCR. Data from qRT-PCR analysis showed no statistically significant difference between AAV2tYF and AAV8 at the mRNA level (**Figure 4**).

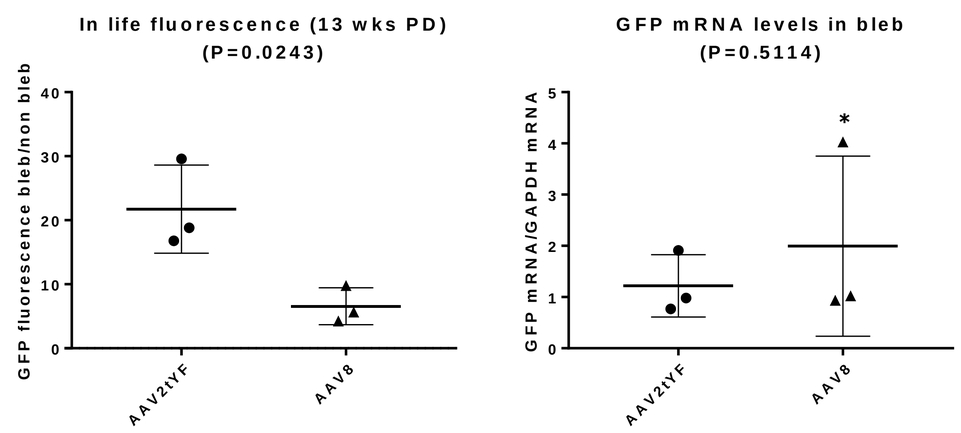


Figure 4. qRT-PCR analysis of GFP mRNA in retinal tissue collected from bleb area at week 13. Analysis of in-life fundus fluorescence images of these animals showed AAV2tYF is superior to AAV8, qRT-PCR data suggested that there is no statistically significant difference between these two capsids. * A spot with intense fluorescence was noted in the subretinal bleb area from one eye (animal 108, OS) injected with AAV8. This spot contributed to high mRNA measurement.

Nine NHPs receiving AAV2tYF-hGFP (9 eyes) in the left eyes and AAV5-hGFP (4 eyes) or AAV8-hGFP (5 eyes) in the right eyes, were used for immunohistochemistry (IHC) assessment for GFP expression. Sections collected from fovea or parafovea areas were stained for hGFP and cone arrestin (CAR, a biomarker for cones). GFP staining (brown) intensity of each section was scored by a pathologist. Sections were scored and averaged for each eye. IHC data suggested that all 3 vectors achieved comparable intensity of GFP staining (**Figure 5**).

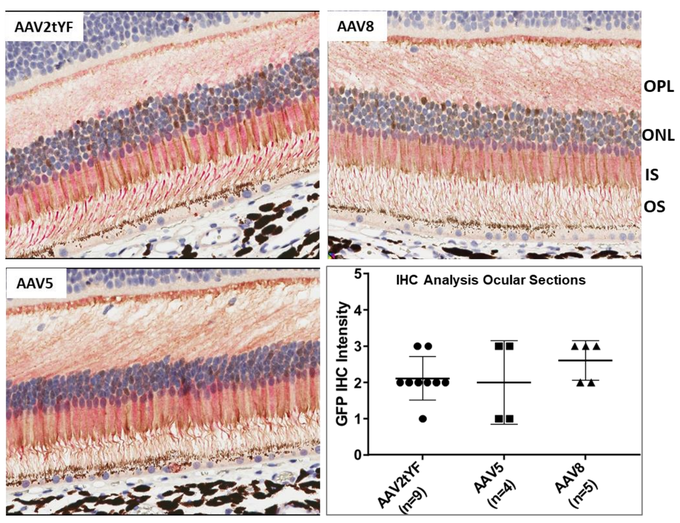


Figure 5. IHC staining for GFP and cone arrestin in eyes receiving AAV2tYF, AAV5, or AAV8-GRK1-hGFP vectors. GFP immunolabeling (brown) was observed in the temporal retina of both eyes in animals administered AAV2tYF, AAV5, or AAV8. The finding was characterized by hGFP labeling of the photoreceptor outer segment, inner segment, the outer nuclear layer, and the outer plexiform layer of rods and cones (cone arrestin, in red), especially cone pedicles and rod spherules. OPL: outer plexiform layer, ONL: outer nuclear layer, IS: inner segment; OS: outer segment

CONCLUSIONS

- Fundus fluorescence images captured in-life through week 13 indicate that AAV2tYF capsid shows a statistically higher GFP fluorescence in the retinal cells compared to AAV5 and AAV8.
- By qRT-PCR, AAV2tYF and AAV8 show comparable expression levels of GFP in retina (no statistical significance).
- Immunohistochemistry assessment indicates that the AAV2tYF capsid shows comparable expression of GFP in photoreceptor cells compared to AAV5 and AAV8 (no statistical significance), and the expression of GFP is photoreceptor cell specific.
- Taken together, detailed evaluation suggests that AAV2tYF was comparable to or superior to both AAV5 or AAV8 in transduction of photoreceptors in NHPs when delivered subretinally. AAV2tYF represents an attractive therapeutic choice for human XLRP gene therapy.

REFERENCES

1. He S, Parapuram SK, Hurd TW, et al. Retinitis Pigmentosa GTPase Regulator (RPGR) protein isoforms in mammalian retina: insights into X-linked Retinitis Pigmentosa and associated ciliopathies. Vision Res. 2008;48(3):366-376.
2. Vervoort R, Lennon A, Bird AC, et al. Mutational hot spot within a new RPGR exon in X-linked retinitis pigmentosa. Nat Genet. 2000;25(4):462-466.
3. Beltran, WA, Cideciyan, AV, Lewin, AS, Iwabe, S, Khanna, H, Sumaroka, A, *et al.* (2012). Gene therapy rescues X-linked photoreceptor blindness in dogs and paves the way for treating RPGR form of human retinitis pigmentosa. *Proc Natl Acad Sci U S A* 109: 2132-2137.
4. Wu Z, Hiriyanna S, Qian H, et al. A long-term efficacy study of gene replacement therapy for RPGR-associated retinal degeneration. Hum Mol Genet. 2015;24(14):3956-3970.
5. Beltran WA, Cideciyan AV, Boye SE, et al. Optimization of Retinal Gene Therapy for X-Linked Retinitis Pigmentosa Due to RPGR Mutations. Mol Ther. 2017;25(8):1866-1880.

agtc

Visionary science for life changing cures.