AAV KP1 Efficiently Transduces Human Cell Lines in vitro and Mouse Liver but not Non-Human Primate Liver after Intravenous Injection

Zhong-Dong SHI*, Yingyu ZHANG, Lucas Van GORDER, and Hongbin XU

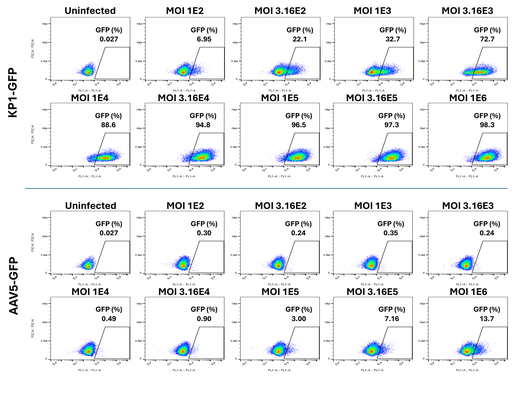
Frontera Therapeutics, Inc. Bedford, MA; *Correspondence: Zhongdong.Shi@fronteratherapeutics.com

Introduction

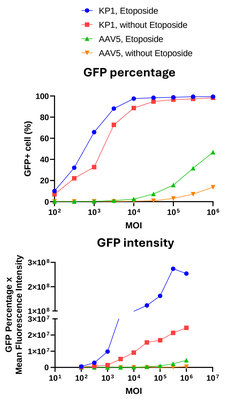
To date, 7 AAV gene therapy drugs have been approved including Glybera (2012), Luxturna (2017), Zolgensma (2019), Upstaza (2022), Roctavian (2022), Hemgenix (2022), and ELEVIDYS (2023). Despite the remarkable achievements, limited tissue transduction has prevented AAV from broader applications. To realize the full potential of AAV vectors, numerous work has been focused on improving cell transduction by modifying the capsids or screening for novel capsids. However, oftentimes novel capsid variants screened from in vitro cell models or mouse models cannot be translated to human patients. KP1 capsid was identified by directed evolution in human pancreatic islets in vitro and has been shown to transduce both mouse and human hepatocytes very efficiently in mice with partially humanized livers (Pekrun et al., 2019). To evaluate whether KP1 is a good liver-targeting capsid, we compared transduction efficiencies of KP1 and AAV5 in cultured cells, in mice and in NHPs - the closest animal models to humans. For in vitro cell and NHP studies, the transgene for both KP1 and AAV5 is CBA-GFP (scAAV); for mouse study, the transgene is HLP-FVIII-SQ (ssAAV). In vitro, KP1-GFP vector transduced 10-1000X better than AAV5-GFP in both 293T cells (data not shown) and human hepatoma Huh-7 cells. In wild-type mice, 5E12 vg/kg of KP1-FVIII-SQ induced higher level of human FVIII-SQ antigen expression than AAV5-FVIII-SQ did at 6E13 vg/kg. The data suggest that KP1 is much more potent than AAV5 when targeting mouse liver. Lastly, we compared KP1 and AAV5 in NHPs by systemically injecting 4E12 vg/kg of each AAV-GFP vector in African green monkey NHPs. The bioanalytical data showed that KP1-GFP vector had lower GFP vector copy, mRNA copy, and protein expression in NHP liver, pancreas and heart compared to AAV5-GFP vector. In summary, KP1 transduces exceptionally well in both human cell lines in vitro and mice liver. However, it shows very poor liver targeting in NHPs compared to AAV5 when injected intravenously. Our observation is consistent with other reported studies in rhesus and cynomolgus monkeys showing KP1 has low liver transduction (Pekrun et al., 2022; Catalyst Biosciences, 2020). In addition, KP1 has higher seroprevalence than AAV5 in human samples tested.

Result

KP1 transduces Huh-7 cells more efficiently than AAV5

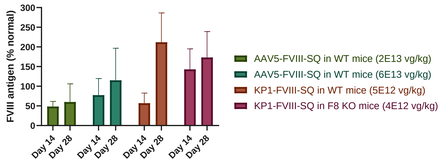


Flow cytometry analysis of KP1 and AAV5 GFP vector transduction in Huh-7 cells at various MOIs without presence of etoposide



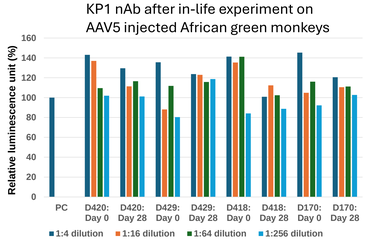
Quantification of KP1 and AAV5 vector transduction efficiency

KP1 transduces mouse liver more efficiently than AAV5

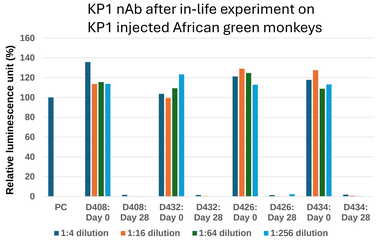


In WT mice, at 4 weeks post-injection of AAV-FVIII-SQ, 5E12 vg/kg of KP1 gave higher level of FVIII-SQ antigen expression than AAV5 at 6E13 vg/kg. The data suggest that KP1 is much more potent than AAV5 when targeting mouse liver.

KP1 iv injection induces neutralizing antibody formation in NHPs



Four NHPs (monkey ID: D420, D429, D418 and D170) were injected with AAV5 at 4E12 vg/kg



Four NHPs (monkey ID: D408, D432, D426 and D434) were injected with KP1 at 4E12 vg/kg

KP1 seroprevalence in human samples

Human seroprevalence rate:
50% in 40 human samples (majority are African Americans) are KP1 Nab positive and 20% are AAV5 NAb positive. This data indicates that KP1 has higher seroprevalence in human than AAV5.

KP1 gives much lower vector copies in NHP liver and pancreas after iv injection

Liver VG

Vector	Animal ID	L.L.L. Lobe (h)	L.L.L. Lobe (p)	L.R.L. Lobe (h)	L.R.L. Lobe (p)	L.Q.L. Lobe (h)	L.Q.L. Lobe (p)	L.C.L. Lobe (h)	L.C.L. Lobe (p)	AVG (vg/ug)	stdev	Mean	stdev
AAV5-GFP	D420	3.08E+06	1.74E+06	1.11E+06	3.28E+06	3.06E+06	6.44E+06	6.42E+06	7.20E+06	4.04E+06	2.32E+06	4.43E+06	1.67E+06
	D429	4.56E+06	1.27E+06	1.88E+06	2.26E+06	5.46E+06	3.84E+06	2.89E+06	4.47E+06	3.33E+06	1.48E+06		
	D418	3.47E+06	5.90E+06	3.43E+06	9.27E+06	7.85E+06	5.13E+06	6.94E+06	1.31E+07	6.89E+06	3.23E+06		
	D170	1.84E+06	1.46E+06	2.34E+06	6.01E+06	5.30E+06	5.25E+06	1.88E+06	3.53E+06	3.45E+06	1.83E+06		
KP1-GFP	D408	2.91E+04	3.08E+04	7.77E+04	1.26E+05	2.15E+05	4.47E+04	2.21E+05	1.02E+05	7.80E+04		3.06E+05	3.38E+05
	D432	3.04E+05	3.13E+05	4.81E+05	4.98E+05	7.33E+05	1.28E+06	1.82E+06	1.03E+06	8.07E+05	5.35E+05		
	D426	4.38E+04	4.28E+04	1.86E+05	6.60E+04	1.11E+05	1.02E+05	1.73E+05	1.02E+05	1.03E+05	5.39E+04		
	D434	1.17E+05	1.35E+05	1.36E+05	1.94E+05	3.01E+05	4.41E+03	4.53E+05	3.65E+05	2.13E+05	1.48E+05		

Left lateral lobe (hilar and peripheral); Right lateral lobe (hilar and peripheral); Quadrate lobe (hilar and peripheral); Caudate lobe (hilar and peripheral)

Pancreas VG

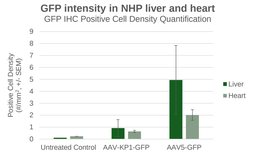
Capsid	Animal ID	Pancreas (VG/ug)		
		VG/ug	Mean	stdev
AAV5-GFP	D420	5.96E+04	6.49E+04	4.59E+04
	D429	2.40E+04		
	D418	4.60E+04		
	D170	1.30E+05		
KP1-GFP	D408	5.61E+03	1.77E+04	2.95E+04
	D432	1.45E+03		
	D426	6.19E+04		
	D434	1.87E+03		

KP1 gives lower transduction in liver, pancreas and heart after iv injection in NHPs

GFP mRNA copies

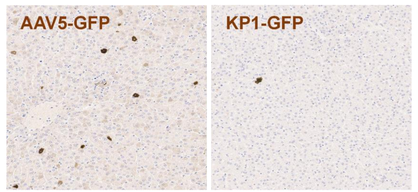
Vector	Sample ID	Liver	Heart	Pancreas
AAV5-eGFP	D420	7177	3542	8
	D429	2977	120	8
	D418	11274	2748	14
	D170	2705	4640	16
KP1-eGFP	D408	241	494	9
	D432	1814	617	4
	D426	167	3610	7
	D434	227	179	4

KP1 gives lower GFP mRNA expression in the liver, heart and pancreas of NHPs. GFP mRNA copy number per ug cDNA were quantified by RT-qPCR.



GFP-positive cell density was slightly higher in the AAV5-GFP group compared to the KP1-GFP group.

GFP IHC in NHP liver



Representative GFP IHC staining images from liver slices shows significantly higher and broader GFP protein expression in the African green monkey liver injected with AAV5-GFP than KP1-GFP. Brown staining and blue staining are GFP protein and nucleus, respectively.

Conclusion

Our study shows that KP1 capsid transduces both human cell lines (Huh-7 and 293T) in vitro and mice liver very well. However, it has very poor liver targeting in NHPs (African green monkey) compared to AAV5 capsid when injected intravenously. We also show KP1 has weaker pancreas and heart transductions in NHPs compared to AAV5. Our observation is consistent with other reported studies in rhesus and cynomolgus monkeys (Pekrun et al., 2022; Catalyst Biosciences, 2020). The underlying cause of dramatic transduction difference in human cell lines, mouse liver and NHP liver remains to be understood and whether KP1 can efficiently transduce hepatocytes in human patients remains unknown.

Acknowledgement and Reference

Acknowledgement: We thank Jessica Nguy for conducting in vitro transduction assays. We thank Trang Nguyen, Pengcheng Zhou, Tim McKee, Shama Pilankar, and Chinmay Patkar for their support in the NHP study and/or bioassays.

Reference:

Pekrun K, et al., 2019. Using a barcoded AAV capsid library to select for clinically relevant gene therapy vectors. JCI Insight, 4(22):e131610.

Pekrun K, et al., 2022. Using recombinant adeno-associated viral vectors for long-term expression of a hyperactive human Factor IX mutant in hemophilic mice and comparison of AAV-LK03 and AAV-KP1 in nonhuman primates. ASGCT2022.

Catalyst Biosciences, 2020. Combination of a novel chimeric AAV capsid and potency enhanced FIX variant for hemophilia B gene therapy. World Federation of Hemophilia.