Rational Clinical Dose Selection of Adeno-Associated Virus-Mediated Gene Therapy Based on Allometric Principles

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Supplementary Materials and Methods

Studies testing three types of vectors expressing FIX as described in Table S2 were primarily identified by searches of the PubMed database. Furthermore, bibliographies of the search results were reviewed manually to retrieve additional preclinical studies published prior to the respective clinical trials. Data were excluded if vectors did not match descriptions in Table S2, or if adequate description of the AAV expression cassette was not provided. Data were also excluded if no plasma FIX concentration or percent FIX activity was reported. In addition, study animals or subjects were excluded if sustained FIX expression was never achieved or was abrogated by immune response. Actual weights of animals/subjects were used whenever available; otherwise, animal weights were estimated based on age and gender; human weights were assumed to be 70 kg. For mice, if no information was given on age and gender, weights were assumed to be

0.02 kg. If multiple vector doses (vg/kg) were tested in one study, then the GEF value and weight averaged across dosing groups were calculated for this study. In the primary analysis on allometry of GEF, mean values of weight and GEF for each species were reported as unweighted average values from all referenced studies. As a supplementary analysis on allometry of GEF, regression was performed on values from individual referenced studies (results shown in Figure S1). Specifically to the human studies on rAAV2-CMV-FIX vector, as subjects from Kay et al. [1] were also included in Manno et al. [2], FIX levels from Manno et al. [2] were used in calculating the GEF, and all FIX levels reported as below the quantification limit were assumed as 0. It was assumed that FIX expression in study animals and subjects entirely resulted from gene transfer.

Figure S1. Allometric scaling of gene efficiency factor for rAAV2-CMV-FIX, rAAV2-hAAT-FIX and scAAV2/8-LP1-hFIXco. Values from individual referenced studies for each species are presented.

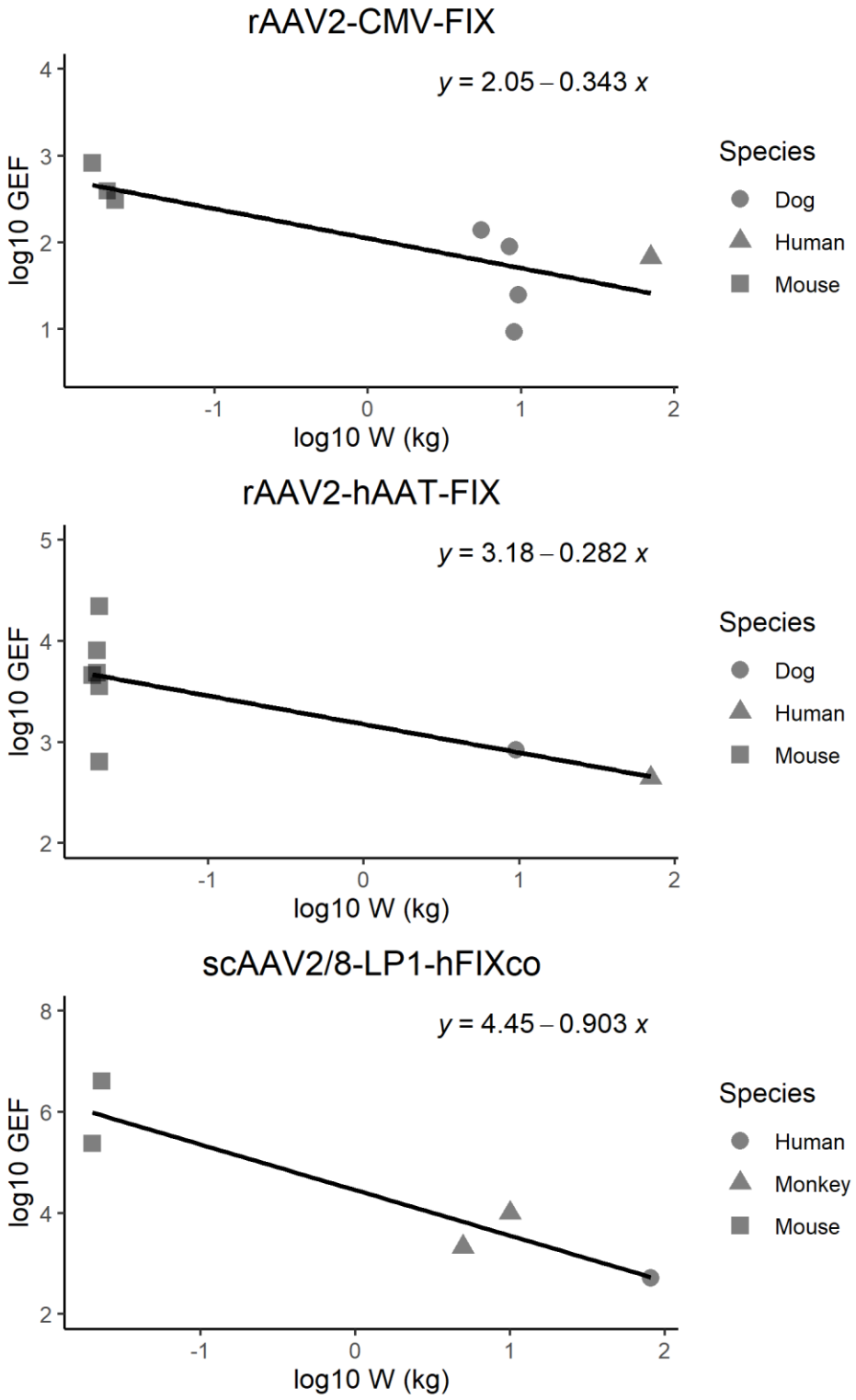

Figure S2. Relationship between log10 gene efficiency factor (GEF) and log10 cellular metabolic rate (Bc) for (A) rAAV2-CMV-FIX, (B) rAAV2-hAAT-FIX and (C) scAAV2/8-LP1-hFIXco.

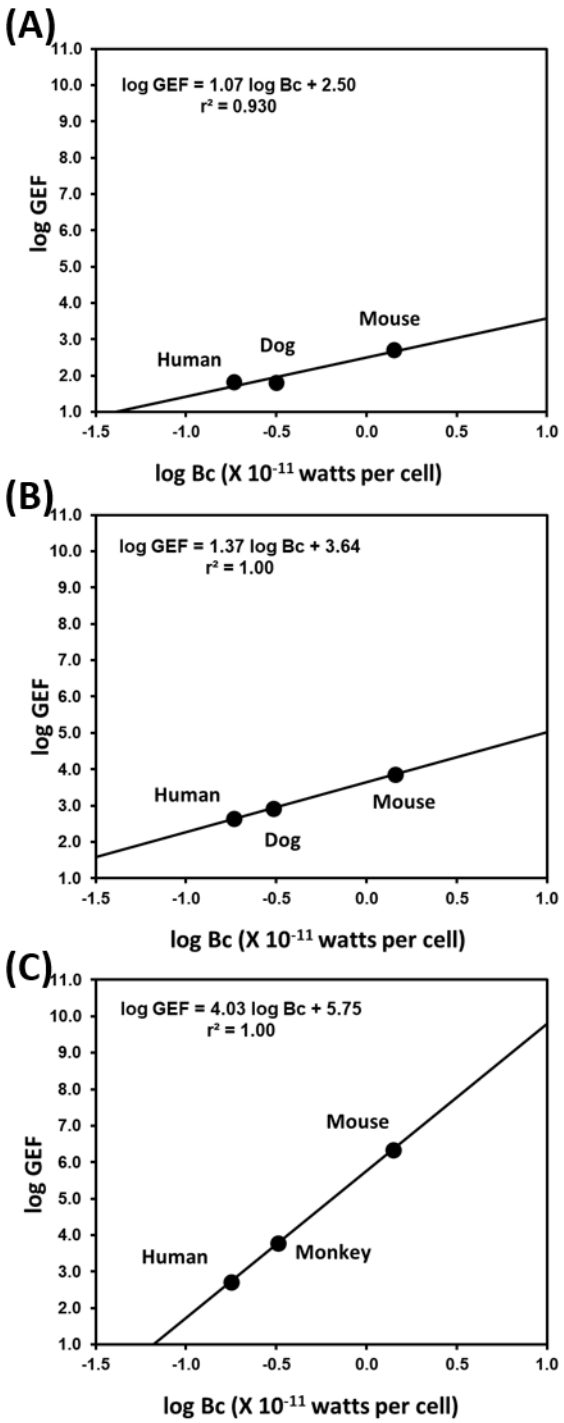

Table S1. Clearance of recombinant factor IX ($CL_{protein}$) used for gene efficiency factor calculations.

Species	$CL_{protein}$ [†] (mL/h/kg)
Mice	34.8 ± 3.1 [6,7]
Dogs	9.96 ± 0.55 [8,9]
Monkeys	8.42 ± 2.00 [7,9]
Humans	7.58 ± 1.84 [10-14]

[†]mean + SD from studies within the same species

Table S2. AAV-FIX vectors used for interspecies allometric scaling.

	rAAV2-CMV-FIX	rAAV2-hAAT-	scAAV2/8-LP1-

		FIX	hFIXco
AAV serotype	AAV2	AAV2	scAAV2/8
Transgene	hFIX or cFIX	hFIX or cFIX	hFIXco
Enhancer/Promoter	CMV early-immediate	ApoE/hAAT	LP1
Intron	1.4-kb truncated hFIX intron I, or a chimeric β-globin/CMV intron	1.4-kb truncated hFIX intron I, or a chimeric β-globin/CMV intron	Modified SV40 intron
Polyadenylation Signal	SV40 Late, hGH or bGH	hGH or bGH	SV40 Late
Route	Intramuscular	Intraportal	Intravascular

AAV: adeno-associated virus; CMV: cytomegalovirus; hFIX: human factor IX; cFIX: canine factor IX; hGH: human growth hormone; bGH: bovine growth hormone; ApoE: apolipoprotein E; hAAT: human α1-antitrypsin; scAAV: self-complementary AAV vector; hFIXco: codon-optimized hFIX; LP1: liver-specific promoter 1.

Table S3. Allometric equations and human prediction for gene expression efficiency factor (GEF) for AAV-FIX vectors.

Vector	Allometric Equation		Human GEF (mole/d/vg)	
	Observed (r^2)[†]	Predicted[‡]	Observed	Predicted[§] (% error)
rAAV2-CMV-FIX	$\log_{10}GEF = -0.268 \log_{10}W + 2.21$ ($r^2 = 0.9304$)	$\log_{10}GEF = -0.341 \log_{10}W + 2.13$	66.8	31.3 (53%)
rAAV2-hAAT-FIX	$\log_{10}GEF = -0.343 \log_{10}W + 3.27$ ($r^2 = 0.9996$)	$\log_{10}GEF = -0.349 \log_{10}W + 3.26$	442	414 (6.4%)
scAAV2/8-LP1-hFIXco	$\log_{10}GEF = -1.01 \log_{10}W + 4.65$ ($r^2 => 0.9999$)	$\log_{10}GEF = -1.00 \log_{10}W + 4.66$	520	557 (7.1%)

[†]Observed allometric equation is obtained by linear regression using three species. Equation is followed by the r^2 in parentheses.

[‡]Predicted allometric equation is obtained by linear regression of preclinical species only (2 species)

[§]Predicted human GEF estimated using predicted allometric equation using mean human weight reported in Tables S4-S6

Table S4. Gene efficiency factor values used for allometric analysis for rAAV2-CMV-FIX vector.

Species	Ref	Dose (vg/kg)	Weight (kg)	Total Dose (vg)	FIX Level (ng/mL)	Animal or Subject ID/Wt	$CL_{protein}$ (mL/h/kg)	$CL_{protein}$ (mL/h)	K_{syn} (ng/d)	K_{syn} (moles/d)	GEF (mole/d/vg)	Study Mean Wt	Study Mean GEF	Overall Mean Wt	Overall Mean GEF
Mouse	[15]	6.25E+11	0.016	1.00E+10	98.3	4 animals per group, estimated weight (4–6 weeks old female mice)	34.8	0.557	1.31E+3	1.44E+13	1.44E+3	0.016	823	0.020	507
		1.25E+13	0.016	2.00E+11	286		34.8	0.557	3.82E+3	4.18E+13	209				
	[16]	4.44E+12	0.0225	1.00E+11	170	4 animals per group, estimated weight (reported median of 20-25 g)	34.8	0.783	3.20E+3	3.50E+13	350	0.023	306		
			0.0225	1.00E+11	140		34.8	0.783	2.63E+3	2.88E+13	288				
			0.0225	1.00E+11	135		34.8	0.783	2.54E+3	2.78E+13	278				
	[17]	2.00E+11	0.02	4.00E+09	19	4 animals in each group; weight is assumed	34.8	0.696	317	3.47E+12	868	0.02	391		
		1.00E+12	0.02	2.00E+10	20		34.8	0.696	334	3.66E+12	183				
		4.00E+12	0.02	8.00E+10	53		34.8	0.696	885	9.69E+12	121				
Dog	[18,19]	1.30E+11	5.7	7.41E+11	2.6	B45	9.96	56.8	3.54E+3	3.88E+13	52.3	9.63	24.6	8.1	65.3
		1.10E+12	9.1	1.00E+13	12	B46	9.96	90.6	2.61E+4	2.86E+14	28.5				
		3.40E+12	20	6.80E+13	17	B93	9.96	199	8.13E+4	8.90E+14	13.1				
		3.00E+12	13.6	4.08E+13	21	B48	9.96	135	6.83E+4	7.47E+14	18.3				
		8.50E+12	4.9	5.02E+13	69	B85	9.96	58.8	9.73E+4	1.07E+15	16.6				
				3.23E+13	39	D31	9.96	37.8	3.54E+4	3.88E+14					
		5.60E+12	4.5	2.52E+13	40	D32	9.96	44.8	4.30E+4	4.71E+14	18.7				
	[20]	1.00E+12	8.4	8.40E+12	34	Wilbur	9.96	83.7	6.83E+4	7.47E+14	89.0	8.4	89.0		
	[21]	3.00E+12	9	2.70E+13	10	M14; estimated WT	9.96	89.6	2.15E+4	2.35E+14	8.72	9	9.16		
			9	2.70E+13	11	M24; estimated WT	9.96	89.6	2.37E+4	2.59E+14	9.59				
	[22]	2.20E+12	5.5	1.20E+13	115	B86	9.96	54.8	1.52E+5	1.66E+15	138	5.5	138		
Human	[1,2]	2.00E+11	70	1.40E+13	10	Subjects B-H; weight is assumed	7.58	531	1.27E+5	1.39E+15	118	70	66.8	70	66.8
				1.40E+13	13.8		7.58	531	1.75E+5	1.92E+15					
		6.00E+11		4.20E+13	16.7		7.58	531	2.12E+5	2.32E+15	63.6				
				4.20E+13	30		7.58	531	3.82E+5	4.18E+15					
				4.20E+13	10.8		7.58	531	1.38E+5	1.51E+15					
		1.80E+12		1.26E+14	20		7.58	531	2.55E+5	2.79E+15	18.4				
				1.26E+14	13.3		7.58	531	1.70E+5	1.86E+15					

Table S5. Gene efficiency factor values used for allometric analysis for rAAV2-hAAT-FIX vector.

Species	Ref	Dose (vg/kg)	Weight (kg)	Total Dose (vg)	FIX Level (ng/mL)	Animal or Subject ID/Wt	$CL_{protein}$ (mL/h/kg)	$CL_{protein}$ (mL/h)	K_{syn} (ng/d)	K_{syn} (moles/d)	GEF (mole/d/vg)	Study Mean Wt	Study Mean GEF	Overall Mean Wt	Overall Mean GEF
Mouse	[23]	1.67E+13	0.018	3.00E+11	8.41E+3	4 animals; estimated weight (6-8 weeks female mice)	34.8	0.626	1.26E+5	1.4E+15	4.61E+3	0.018	4.61E+3	0.019	7.29E+03
	[24]	4.00E+12	0.02	8.00E+10	9.63E+3	3-21 mice; weight is assumed	34.8	0.696	1.61E+5	1.8E+15	2.20E+4	0.02	2.20E+4		
	[25]	1.04E+12	0.0193	2.00E+10	1.06E+3	6 weeks old mice (n=5 or 8) per group; estimated weight	34.8	0.672	1.71E+5	1.9E+14	9.36E+3	0.019	8.05E+3		
		1.04E+13	0.0193	2.00E+11	1.09E+4		34.8	0.672	1.76E+5	1.9E+15	9.63E+3				
		1.04E+14	0.0193	2.00E+12	5.85E+4		34.8	0.672	9.43E+5	1.0E+16	5.16E+3				
	[26]	5.18E+12	0.0193	1.00E+11	2.75E+3	25 8-10-week-old female mice; estimated weight	34.8	0.672	4.43E+4	4.8E+14	4.85E+3	0.019	4.85E+3		
	[27]	1.85E+11	0.02	3.70E+09	4.07	5 mice per group; weight is assumed	34.8	0.696	68.0	7.4E+11	201	0.02	3.57E+3		
		5.50E+11	0.02	1.10E+10	103		34.8	0.696	1.72E+3	1.9E+13	1.71E+3				
		1.65E+12	0.02	3.30E+10	897		34.8	0.696	1.50E+4	1.6E+14	4.97E+3				
		5.00E+12	0.02	1.00E+11	2.82E+3		34.8	0.696	4.71E+4	5.2E+14	5.16E+3				
		1.50E+13	0.02	3.00E+11	9.55E+3		34.8	0.696	1.59E+5	1.7E+15	5.82E+3				
	[28]	5.00E+12	0.02	1.00E+11	350	Average of 2 mice; weight is assumed	34.8	0.696	5.84E+3	6.4E+13	639	0.02	639		
			0.02	1.00E+11	87.1	Average of 3 mice; weight is assumed	34.8	0.696	1.45E+3	1.6E+13	159				
			0.02	1.00E+11	60.9	Average of 3 mice; weight is assumed	34.8	0.696	1.02E+3	1.1E+13	111				
Dog	[29]	1.20E+12	10.2	1.25E+13	590	Brad	9.96	102	1.44E+6	1.6E+16	1.26E+3	9.5	831	9.5	831
		1.60E+12	6	9.70E+12	220	Semillon	9.96	59.8	3.16E+5	3.5E+15	356				
		8.00E+11	12.3	9.60E+12	262	E34	9.96	123	7.70E+5	8.4E+15	878				
Human	[30]	2.00E+12	70	1.40E+14	444	Subject E; weight is assumed; average value from 2-4 weeks	7.58	531	5.65E+6	6.2E+16	442	70	442	70	442

Table S6. Gene efficiency factor values used for allometric analysis for scAAV2/8-LP1-hFIXco vector.

Species	Ref	Dose (vg/kg)	Wt (kg)	Total Dose (vg)	FIX Level (ng/mL)	# of Animals/Animal or Subject ID/Wt	$CL_{protein}$ (mL/h/kg)	$CL_{protein}$ (mL/h)	K_{syn} (ng/d)	K_{syn} (moles/d)	GEF (mole/d/vg)	Study Mean Wt	Study Mean GEF	Overall Mean Wt	Overall Mean GEF
Mouse	31	5.00E+10	0.02	1.00E+09	563	4 mice per group; 7-10 week old male mice; estimated weight	34.8	0.696	9.40E+3	1.03E+14	1.03E+05	0.02	2.38E+05	0.021	2.18E+06
		1.00E+11	0.02	2.00E+09	3.25E+3		34.8	0.696	5.43E+4	5.95E+14	2.97E+05				
		1.25E+12	0.02	2.50E+10	3.66E+4		34.8	0.696	6.12E+5	6.69E+15	2.68E+05				
		5.00E+12	0.02	1.00E+11	1.55E+5		34.8	0.696	2.60E+6	2.84E+16	2.84E+05				
	32	2.00E+11	0.0227	4.54E+09	3.04E+4	N=5-8 per group; 6-8 week male mice; estimated weight	34.8	0.79	5.76E+5	6.31E+15	1.39E+06	0.023	4.11E+06		
		4.00E+10	0.0227	9.08E+08	1.77E+4		34.8	0.79	3.35E+5	3.67E+15	4.04E+06				
		4.00E+09	0.0227	9.08E+07	1.61E+3		34.8	0.79	3.05E+4	3.34E+14	3.68E+06				
		4.00E+08	0.0227	9.08E+06	322		34.8	0.79	6.10E+3	6.67E+13	7.35E+06				
Monkey	33	1.00E+12	4.9	4.90E+12	1.40E+3	M5-sc	8.42	41.3	1.39E+6	1.52E+16	3.10E+03	4.97	2.14E+03	7.5	6.04E+03
			5.7	5.70E+12	800	M6-sc	8.42	48.0	9.21E+5	1.01E+16	1.77E+03				
			4.3	4.30E+12	700	M7-sc	8.42	36.2	6.08E+5	6.66E+15	1.55E+03				
	34	2.00E+12	7.4	1.48E+13	1.61E+4	37103	8.42	62.3	2.41E+7	2.64E+17	1.79E+04	10.0	9.94E+03		
			8.5	1.70E+13	3.00E+3	37102	8.42	71.6	5.15E+6	5.64E+16	3.32E+03				
			11.3	2.26E+13	1.10E+4	6100	8.42	95.2	2.51E+7	2.75E+17	1.22E+04				
		2.00E+11	11.7	2.34E+12	523	561	8.42	98.7	1.24E+6	1.36E+16	5.78E+03				
			15.1	3.02E+12	1.03E+3	7155	8.42	127	3.15E+6	3.45E+16	1.44E+04				
			15.7	3.14E+12	1.49E+3	7150	8.42	132	4.71E+6	5.16E+16	1.64E+04				
		6.00E+10	6.7	4.02E+11	161	6747	8.42	56.4	2.18E+5	2.39E+15	5.94E+03				
			7.3	4.38E+11	213	6802	8.42	61.5	3.14E+5	3.44E+15	7.85E+03				
			6.3	3.78E+11	235	6712	8.42	53.1	2.99E+5	3.27E+15	8.66E+03				
Human	35,36	2.00E+11	80.7	1.61E+13	109	Participants 1-10; used median weight of all patients	7.58	612	1.59E+6	1.74E+16	8.89E+02	80.7	5.20E+02	81	5.20E+02
			80.7	1.61E+13	70		7.58	612	1.03E+6	1.12E+16					
		6.00E+11	80.7	4.84E+13	143		7.58	612	2.10E+6	2.30E+16	4.17E+02				
			80.7	4.84E+13	109		7.58	612	1.59E+6	1.74E+16					
		2.00E+12	80.7	1.61E+14	178		7.58	612	2.61E+6	2.86E+16	2.54E+02				
			80.7	1.61E+14	361		7.58	612	5.29E+6	5.79E+16					
			80.7	1.61E+14	250		7.58	612	3.67E+6	4.02E+16					
			80.7	1.61E+14	334		7.58	612	4.90E+6	5.36E+16					
			80.7	1.61E+14	262		7.58	612	3.84E+6	4.20E+16					
			80.7	1.61E+14	145		7.58	612	2.12E+6	2.32E+16					

Table S7. Animals/subjects excluded from referenced studies for reasons other than mismatch of vector descriptions.

Ref	Excluded Animals/Subjects	Reason for Exclusion
[20]	Wes, Sauvignon	FIX levels were undetectable due to immune response
[19]	B14	FIX level was transiently detectable due to immune response
[2]	Subject A	FIX levels may have been elevated due to coadministration of zidovudine
[27]	The following dosing groups: vg = $1.8x10^{12}$, $1.1x10^{13}$	Doses were known to be outside the linear range of the vector dose response
[30]	Subjects A, B, C, D, G	Maximum FIX activity levels were < 1%
[30]	Subject F	Steady state of FIX was not achieved potentially due to immune response, as a high anti-AAV level was reported
[37]	Dosing group: vg = $6.9x10^{12}$	Low expression levels of AAT
[39]	Dosing group: vg/kg=$4x10^{11}$	Steady state of AAT was not achieved

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