

**OMTM, Volume 10**

**Supplemental Information**

**Toxicology Study of Intra-Cisterna Magna**

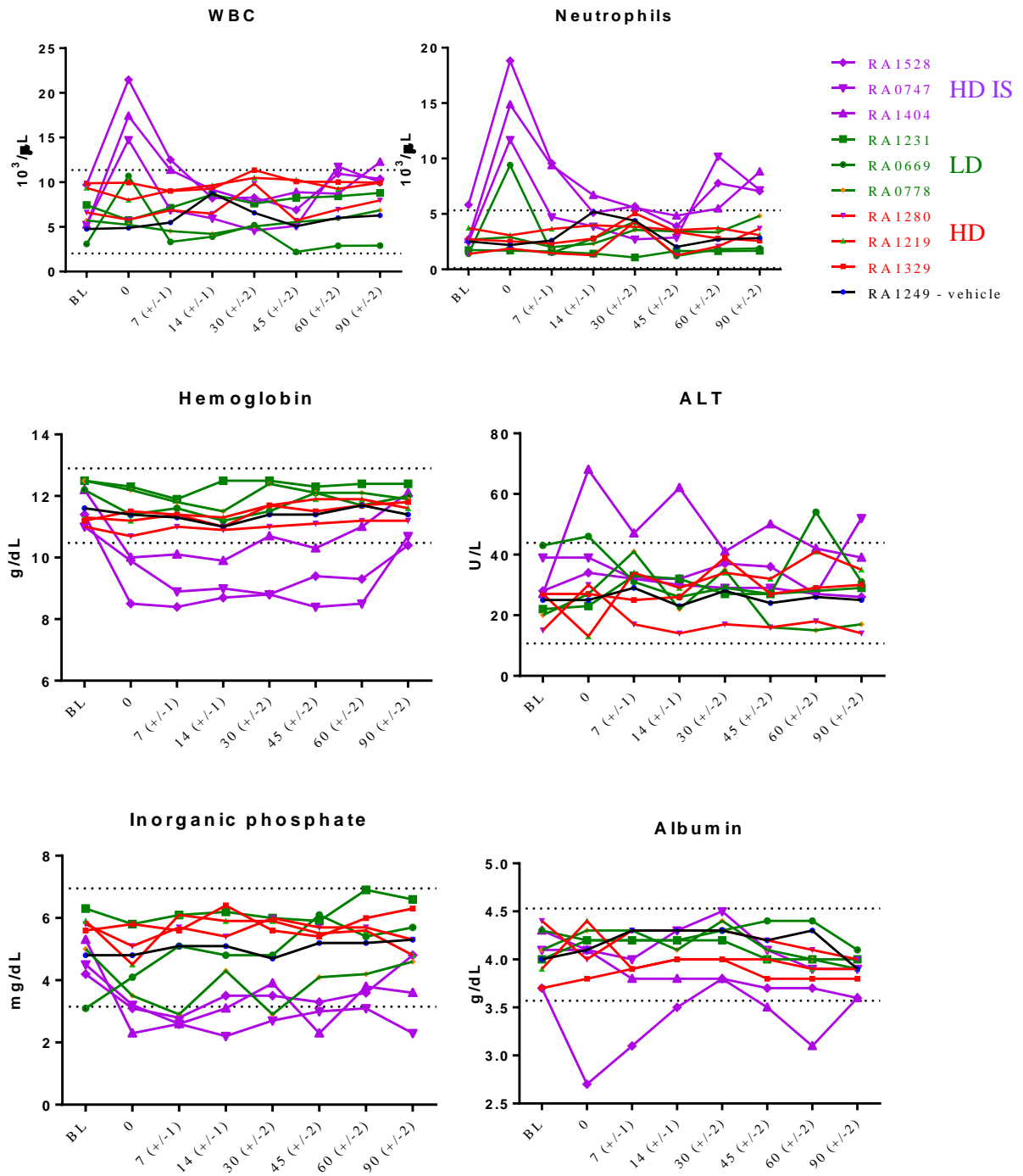
**Adeno-Associated Virus 9 Expressing Human**

**Alpha-L-Iduronidase in Rhesus Macaques**

**Juliette Hordeaux, Christian Hinderer, Tamara Goode, Nathan Katz, Elizabeth L. Buza, Peter Bell, Roberto Calcedo, Laura K. Richman, and James M. Wilson**

**Figure S1. Blood anomalies induced by the IS regimen.** WBC counts, neutrophils, hemoglobin, ALT, phosphate, and albumin levels were transiently modified in at least one IS animal, after the onset of IS and before vector dosing, without any relation to intrathecal AAV9.hIDUA administration. Dotted lines represent the baseline average plus or minus two standard deviations. Black = vehicle control; red = HD; green = LD; purple = HD IS.

Figure S1.



**Table S1. Animal weights, study RGX150210p**

Animal ID	Group		BL	D0	D3	D7 (+/-2)	D14 (+/-2)	D21 (+/-2)	D30 (+/-2)	D45 (+/-2)	D60 (+/-2)	D90 (+/-2)	D120 (+/-2)	D150 (+/-2)	D180 (+/-2)
RA1492	1A	HD D14	4.51	4.49	4.5	4.58	4.7	NA	NA	NA	NA	NA	NA	NA	NA
RA1292			5.49	5.54	5.66	5.48	5.51	NA	NA	NA	NA	NA	NA	NA	NA
RA0502			5.19	5.22	5.26	5.34	5.46	NA	NA	NA	NA	NA	NA	NA	NA
RA1470	1B	HD D90	4.93	5.05	5.05	4.92	5	5.27	5.6	5.91	5.75	5.9	NA	NA	NA
RA1514			4.41	4.33	4.39	4.4	4.64	4.41	4.39	4.86	4.95	4.65	NA	NA	NA
RA1287			5.36	5.44	5.49	5.66	5.55	5.71	5.6	5.55	5.7	5.75	NA	NA	NA
RA0773	1C	HD D180	5.65	5.75	5.79	5.98	6.08	6.19	6.45	6.51	6.3	6.5	6.25	5.85	6.05
RA1304			5.25	5.25	5.25	5.18	5.26	5.39	5.14	5.61	5.65	5.7	6.25	6.45	6.65
RA1532			5.39	5.74	5.86	5.8	5.9	6.07	6	5.7	6.05	6.45	6.35	6.05	6.1
RA1534	2	vehicle	5.09	5.15	5.09	5.2	5.4	NA	NA	NA	NA	NA	NA	NA	NA
RA0775			5.84	5.85	5.78	5.72	5.93	6.17	6.1	6.6	7.5	6.4	NA	NA	NA
RA1314			5.45	5.44	5.44	5.52	5.64	5.79	5.79	5.95	6	6.1	NA	NA	NA

**Table S2. Animal weights, study RGX160822p**

Animal ID	Group		BL	D0	D7 (+/- 1)	D14 (+/- 1)	D30	D45 (+/- 1)	D60 (+/- 2)	D90 (+/- 3)
RA1249	1	vehicle	6.60	6.55	6.50	6.60	6.80	6.75	6.90	7.30
RA1329	2	HD	8.85	8.75	8.85	9.00	9.10	8.50	9.00	9.20
RA1219			6.90	6.80	7.00	7.05	7.20	7.10	7.15	7.45
RA1280			7.80	7.90	7.85	8.00	8.30	8.60	8.75	9.25
RA0778	3	LD	4.10	4.10	4.05	4.10	4.10	4.20	4.30	4.25
RA0669			5.80	5.80	5.80	5.90	6.45	6.15	6.15	6.40
RA1231			8.05	7.80	7.75	7.85	7.85	8.10	8.20	8.70
RA1528	4	HD + IS	5.70	5.55	5.40	5.50	5.30	5.60	5.45	5.50
RA0747			5.85	5.75	5.65	5.80	5.75	5.70	5.70	5.85
RA1404			5.60	5.30	5.25	5.30	5.30	5.35	5.25	5.10

**Table S3. CSF WBC counts**

	D0	D3	D7	D14	D21	D30	D45	D60	D90	D120	D150	D180
RA1534 - vehicle	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RA0775 - vehicle	0	0	0	0	0	0	0	0	N/A	N/A	N/A	N/A
RA1314 - vehicle	Blood	1	0	1	0	4	0	0	0	N/A	N/A	N/A
RA1249 - vehicle	0	N/A	0	Blood	Blood	0	1	1	0	N/A	N/A	N/A
RA0778 LD	0	N/A	Blood	0	N/A	1	1	0	0	N/A	N/A	N/A
RA0669 LD	0	N/A	0	0	N/A	0	7	10		N/A	N/A	N/A
RA1231 LD	0	N/A	0	0	N/A	2	1	2	0	N/A	N/A	N/A
RA1492 HD	0	0	0	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RA1292 HD	4	1	2	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RA0502 HD	0	1	0	0	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
RA1470 HD	Blood	2	0	17	12	Blood	28	14	4	N/A	N/A	N/A
RA1514 HD	Blood	3	0	0	12	11	4	1	1	N/A	N/A	N/A
RA1287 HD	0	0	0	0	10	27	2	1	0	N/A	N/A	N/A
RA1329 HD	0	N/A	0	0	N/A	0	0	4	2	N/A	N/A	N/A
RA1219 HD	0	N/A	0	0	N/A	10.0*	1	2	0	N/A	N/A	N/A
RA1280 HD	0	N/A	0	0	N/A	3	2	2	0	N/A	N/A	N/A
RA0773 HD	0	0	0	0	4	4	11	1	0	1	0	0
RA1304 HD	0	1	3	0	1	1	0	2	1	3	0	2
RA1532 HD	0	1	0	6	18	27	11	3	7	16	13	Blood
RA1404 HD + IS	0	N/A	1	1	N/A	0	0	0	0	N/A	N/A	N/A
RA1528 HD + IS		N/A	0	0	N/A	0	0	0	0	N/A	N/A	N/A
RA0747 HD + IS	1	N/A	1	10.00*	N/A	0	0	0	0	N/A	N/A	N/A

\* values excluded: not confirmed by cytosmear analysis

Blood: values excluded when red blood cell count > 500

**Table S4. PBMC IFN- $\gamma$  ELISPOT**

Group	Animal	Study Day	Average SFU per million PBMC							PHA + ctrl	
			Medium	AAV9-A	AAV9-B	AAV9-C	hIDUA-A	hIDUA-B	hIDUA-C		
Vehicle D14	RA1534	0	23	13	23	8	23	0	18	1490	
		14	23	28	10	13	13	10	28	1350	
HD D14	RA1492	0	20	20	18	13	23	15	15	1330	
		14	25	13	23	10	5	20	15	663	
	RA1292	0	23	33	13	18	15	8	15	608	
		14	25	13	8	0	18	8	18	515	
	RA0502	0	15	8	13	10	5	13	3	570	
		14	15	18	8	8	15	13	5	980	
LD D90	RA0778	0	8	28	20	53	40	23	25	TNTC	
		14	18	43	60	60	168	88	53	TNTC	
		30	25	25	23	23	205	NR	NR	TNTC	
		60	80	65	110	105	313	233	358	TNTC	
		90	28	33	50	28	120	93	115	TNTC	
	RA0669	0	0	5	0	5	15	5	0	1235	
		14	5	15	0	0	5	5	10	TNTC	
		30	5	0	3	0	NR	NR	NR	TNTC	
		60	13	18	105	155	150	215	83	TNTC	
	RA1231	0	3	3	5	3	63	3	8	TNTC	
		14	8	0	18	10	85	8	3	TNTC	
		30	13	0	13	3	120	33	20	TNTC	
		60	13	10	8	15	113	128	25	TNTC	
	HD D90	RA1470	0	5	5	5	0	8	5	23	TNTC
			21	5	8	5	15	8	3	3	TNTC
			90	3	15	40	8	73	8	10	TNTC
		RA1514	0	13	18	20	25	40	10	28	TNTC
			21	10	25	28	30	45	45	15	TNTC
90			8	13	15	8	100	20	10	1325	
RA1287		0	5	10	8	5	5	30	10	1185	
		21	5	8	10	25	53	25	8	TNTC	
		90	5	15	13	20	50	85	8	888	
RA1329		0	0	3	13	0	3	8	3	TNTC	
		14	3	35	20	5	63	23	15	TNTC	
		30	3	88	3	25	270	168	48	TNTC	
		60	25	18	35	8	90	53	23	TNTC	
		90	8	20	5	0	43	20	18	TNTC	
RA1219		0	3	5	3	0	5	108	5	TNTC	
		14	15	10	13	20	28	138	10	TNTC	
		30	8	25	10	18	28	73	25	TNTC	
		60	5	0	8	13	20	58	43	TNTC	
	90	3	0	0	0	3	5	3	TNTC		
RA1280	0	13	15	20	10	30	10	13	TNTC		
	14	20	8	20	10	48	5	13	TNTC		
	30	25	10	20	25	78	45	35	TNTC		
	60	20	5	20	18	85	35	60	TNTC		
	90	3	18	10	20	20	55	15	23	TNTC	
HD + IS D90	RA1404	0	5	10	5	5	5	0	35	TNTC	
		30	0	0	0	0	3	3	3	TNTC	
		60	0	0	3	3	0	5	5	TNTC	
		90	35	13	20	40	28	25	20	TNTC	
	RA1528	0	0	0	13	15	13	8	0	TNTC	
		30	8	5	13	10	8	5	0	TNTC	
		60	8	13	28	35	5	18	13	TNTC	
		90	13	38	40	85	28	25	30	TNTC	
	RA0747	0	5	13	18	8	18	15	8	TNTC	
		30	0	13	10	8	20	0	5	TNTC	
		60	20	23	33	38	13	20	0	TNTC	
		90	23	33	68	160	110	95	38	TNTC	

**Table S4.** (cont.) PBMC IFN- $\gamma$  ELISPOT

Group	Animal #	Study Day	Average SFU per million PBMC							
			Medium	AAV9 A	AAV9 B	AAV9 C	hIDUA A	hIDUA B	hIDUA C	PHA + ctrl
Vehicle D90	RA0775	0	20	13	55	35	40	38	38	TNTC
		21	13	13	25	25	10	15	20	TNTC
		90	23	30	30	35	20	30	10	TNTC
	RA1314	0	23	13	13	5	18	18	10	1340
		21	13	3	10	10	0	15	3	1390
		90	3	18	13	13	33	10	20	TNTC
	RA1249	0	15	20	0	5	5	5	0	1425
		14	28	8	13	35	18	25	125	TNTC
		30	28	43	25	NR	25	20	NR	TNTC
		60	53	63	33	20	28	30	35	TNTC
		90	8	8	3	10	10	0	18	TNTC
	HD D180	RA0773	0	5	15	3	10	10	5	15
21			3	8	3	10	13	10	0	1560
90			8	0	8	5	10	15	8	900
180			30	15	20	33	45	45	20	2000
RA1304		0	8	0	0	0	0	0	0	265
		21	5	3	3	0	5	3	5	95
		90	3	3	8	8	0	13	8	253
		180	15	18	18	28	28	20	13	503
RA1532		0	5	3	0	5	8	0	3	TNTC
		21	0	3	0	3	8	8	8	TNTC
		90	23	23	13	30	70	55	20	TNTC
		180	18	68	65	108	95	63	65	TNTC

SFU = Spot Forming Unit. NR = not enough cells

Positive responses, defined as an average value >55 SFU/million PBMCs and at least three times greater than the medium negative control, are highlighted in red. TNTC = too numerous to be counted

**Table S5.** Incidence and severity of the DRG finding

Study	RGX151002p					RGX160822p			
	14		90		180	90			
Group	1A	2A	1B	2B	1C	1	2	3	4
Dose (GC)	10 <sup>13</sup>	Control	10 <sup>13</sup>	Control	10 <sup>13</sup>	Control	10 <sup>13</sup>	10 <sup>12</sup>	10 <sup>13</sup>
Immunosuppression	No	No	No	No	No	No	No	No	Yes
Number of Animals	3	1	3	2	3	1	3	3	3
No. examined	0	0	0	0	0	1	3	3	3
<b>Dorsal root ganglion, Cervical</b>									
Neuronal cell body degeneration with mononuclear cell infiltrate									
No significant abnormalities									
Grade 1									
<b>Dorsal root ganglion, Thoracic</b>									
Neuronal cell body degeneration with mononuclear cell infiltrate									
No significant abnormalities									
Grade 1									
<b>Dorsal root ganglion, Lumbar</b>									
Neuronal cell body degeneration with mononuclear cell infiltrate									
No significant abnormalities									
Grade 1									
Grade 2									

M=missing tissue

The findings in the DRG consisted of neuronal degeneration characterized by central chromatolysis, satellitosis, and mononuclear cell infiltrates that surrounded and invaded neuronal cell bodies (neuronophagia). DRG from the initial study, RGX151002p, were not sampled.

**Table S6.** Incidence and severity of the axonopathy finding

Study	RGX151002p					RGX160822p			
	14		90		180	90			
Group	1A	2A	1B	2B	1C	1	2	3	4
Dose (GC)	10 <sup>13</sup>	Control	10 <sup>13</sup>	Control	10 <sup>13</sup>	Control	10 <sup>13</sup>	10 <sup>12</sup>	10 <sup>13</sup>
Immunosuppression	No	No	No	No	No	No	No	No	Yes
Number of Animals	3	1	3	2	3	1	3	3	3
No. examined	3	1	3	2	3	1	3	3	3
<b>Spinal Cord, Cervical</b>									
Axonopathy, dorsal white matter tracts									
No significant abnormalities	3	1	0	2	0	1	1	0	2
Grade 1	-	-	-	-	2	-	-	2	1
Grade 2	-	-	2	-	1	-	2	1	-
Grade 3	-	-	1	-	-	-	-	-	-
<b>Spinal Cord, Thoracic</b>									
Axonopathy, dorsal white matter tracts									
No significant abnormalities	3	1	0	2	0	1	0	0	1
Grade 1	-	-	2	-	2	-	2	3	2
Grade 2	-	-	1	-	1	-	1	-	-
<b>Spinal Cord, Lumbar</b>									
Axonopathy, dorsal white matter tracts									
No significant abnormalities	3	1	0	2	0	1	0	0	1
Grade 1	-	-	-	-	1	-	2	1	1
Grade 2	-	-	3	-	2	-	1	2	1

The dorsal white matter tracts of the spinal cord had an axonopathy characterized by dilated myelin sheaths with and without myelomacrophages, which was consistent with axonal degeneration.



