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Supplemental Information

**Ocular Tolerability and Immune Response
to Corneal Intrastromal AAV-*IDUA* Gene
Therapy in New Zealand White Rabbits**

Liujiang Song, Jacquelyn J. Bower, Telmo Llanga, Jacklyn H. Salmon, Matthew L. Hirsch, and Brian C. Gilger

Supplemental Table 1. Injection observations.

Treatment	Rabbit #	Corneal surface leakage (None, Mild, Moderate, Severe)	Anterior chamber perforation (Y/N)	Number of injection attempts
Saline	1	2	N	3
	2	1	N	1
	3	0	N	1
	4	1	N	2
	5	1	N	1
	6	1	N	1
AAV-IDUAe9	1	1	N	1
	2	1	N	1
	3	1	N	1
	4	0	N	1
	6	0	N	1
	7	1	N	1
	8	1	N	1
	AAV-IDUAe10	1	1	N
2		1	N	1
3		0	N	1
4		1	N	1
5		1	N	1
6		1	N	1
7		0	N	1
8		1	N	1
Total Numbers	N=22	15/22 rabbits had none or mild leakage	0/22 perforations	19/22 were single injections

Note: The Right eye was injected with either 50 uL of saline (n=6), AAV-opt-IDUAe9 (n=8), or AAV-opt-IDUAe10 (n=8) using a 31 gauge needle. Most eyes had mild injection site surface leakage but there were no anterior chamber perforations. Most stromal injections could be done with a single needle insertion, but in 3 cases, 2 - 3 needle injections had to be used to ensure a stromal injection.

Supplementary Table 2. Serum Chemistries and Complete Blood Counts

Study month	Rabbit #	Group	Serum chemistries											Complete blood count			
			Glu (mg/dl)	BUN (mg/dl)	Cr (mg/dl)	TP (g/dl)	AP (IU/L)	ALT (IU/L)	AST (IU/L)	Na ⁺ (mmol/L)	K ⁺ (mmol/L)	Cl ⁻ (mmol/L)	Lipase (IU/L)	WBC (x10 ⁹ /UL)	RBC (x10 ⁹ /UL)	Hemo (g/dl)	HCT (%)
<i>Rabbit reference range:</i>																	
0	25	Saline	115	20	1.0	4.7	79	20	14	147	4.6	101	235	5.63	6.72	14.3	41.6
1	25	Saline	112	21	1.1	5.3	59	60	38	146	4.7	102	255	6.41	6.73	14.4	42.3
2	25	Saline	135	21	1.1	5.3	47	43	16	146	4.7	102	251	6.59	6.98	15.1	43.5
3	25	Saline	90	21	1.0	5.5	46	23	13	141	4.4	104	271	6.50	6.64	14.5	42.0
4	25	Saline	116	18	0.8	5.3	29	33	16	144	4.6	109	220	4.54	6.64	14.6	42.3
0	29	Saline	138	17	0.8	4.6	65	25	12	146	4.3	103	162	5.80	6.24	13.3	39.2
1	29	Saline	117	19	1.2	5.6	42	50	28	149	5.0	102	184	5.93	6.69	14.6	43.5
2	29	Saline	155	19	1.0	5.0	27	86	23	143	5.1	106	185	5.34	6.02	13.1	38.0
3	29	Saline	107	19	0.9	5.5	26	48	26	146	4.6	107	167	5.04	6.06	13.4	39.0
4	29	Saline	145	14	0.6	5.6	19	37	12	142	4.7	108	186	X	X	X	X
0	33	Saline	148	14	0.8	4.8	59	52	28	146	4.6	103	229	X	X	X	X
1	33	Saline	128	14	0.8	4.9	45	53	29	145	4.6	103	231	6.82	6.48	13.2	39.4
2	33	Saline	136	16	0.7	5.2	53	52	17	141	5.0	105	230	8.39	6.78	14.1	41.2
3	33	Saline	122	13	0.7	5.4	66	55	41	142	4.2	104	266	8.18	6.67	14.1	41.2
4	33	Saline	140	13	0.7	5.4	30	40	15	142	4.5	109	210	5.88	6.66	14.2	41.3
0	37	Saline	161	18	1.0	4.5	60	27	11	144	4.6	100	225	X	X	X	X
1	37	Saline	237	17	1.1	4.8	53	34	18	144	4.8	103	230	6.98	6.13	13.2	38.4
2	37	Saline	150	24	1.1	5.2	40	38	26	141	4.9	106	276	X	X	X	X
3	37	Saline	114	23	1.0	5.5	52	41	18	139	5.1	106	391	7.58	6.28	14.1	40.9
4	37	Saline	141	18	0.9	5.4	35	36	12	140	4.8	107	287	6.39	6.13	14.0	39.2
0	41	Saline	123	22	1.1	4.9	79	34	20	148	4.7	102	227	8.77	6.78	14.9	42.9
1	41	Saline	117	24	1.1	5.1	36	28	22	147	5.2	101	259	X	X	X	X
2	41	Saline	123	22	0.8	5.0	30	27	13	142	4.6	105	250	X	X	X	X
3	41	Saline	124	23	0.9	5.4	25	27	13	145	4.2	106	357	7.48	6.46	14.5	41.6
4	41	Saline	117	21	0.8	5.2	25	26	13	142	4.9	109	271	8.09	6.56	14.8	42.2
0	45	Saline	150	17	0.8	4.5	90	22	14	144	4.1	102	164	X	X	X	X
1	45	Saline	105	21	0.8	4.8	58	35	22	145	4.6	106	190	X	X	X	X
2	45	Saline	155	22	0.9	4.7	50	46	22	145	4.6	109	208	5.67	6.49	13.2	38.1
3	45	Saline	130	22	0.9	5.2	45	44	36	143	4.5	107	245	4.94	6.82	14.2	40.8
4	45	Saline	137	17	0.8	5.1	33	32	18	141	5.2	110	218	4.78	6.73	14.1	40.2
0	26	Low	124	16	0.9	4.8	126	13	9	148	3.9	101	280	X	X	X	X
1	26	Low	105	17	0.8	5.1	89	21	11	148	4.3	104	276	7.55	6.23	13.2	38.6
2	26	Low	140	18	1.0	5.3	113	18	11	144	4.6	104	393	X	X	X	X
3	26	Low	99	19	0.9	5.5	64	28	18	145	4.2	104	430	7.26	6.22	13.4	39.0
4	26	Low	125	16	0.8	5.5	55	22	11	143	4.0	106	362	5.53	5.87	12.7	36.8
0	30	Low	148	16	0.7	4.7	123	25	13	144	4.2	103	334	5.44	6.64	13.9	39.9

1	30	Low	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
2	30	Low	144	18	0.7	5.5	74	46	17	146	4.5	108	182	X	X	X	X
3	30	Low	139	18	0.7	5.4	64	30	20	144	4.6	104	189	5.37	6.81	14.3	42.0
4	30	Low	142	14	0.6	5.7	44	29	13	143	4.5	107	166	4.63	6.62	14.0	40.8
0	34	Low	123	22	0.9	4.4	31	38	19	146	5.0	104	128	4.99	6.10	12.6	36.8
1	34	Low	90	19	0.9	4.8	28	47	28	148	4.6	106	171	5.16	5.68	11.9	35.3
2	34	Low	134	26	1.0	5.0	26	43	17	146	4.0	106	196	X	X	X	X
3	34	Low	112	21	1.0	5.5	17	36	14	145	4.3	108	173	4.73	6.05	12.8	38.4
4	34	Low	124	20	0.9	5.0	20	31	9	142	4.3	109	193	X	X	X	X
0	38	Low	112	19	1.1	4.8	32	16	11	147	4.4	102	228	6.42	6.40	13.2	39.1
1	38	Low	173	21	1.2	5.0	27	22	17	147	4.4	102	256	5.77	6.36	13.1	38.6
2	38	Low	143	21	1.3	5.1	25	14	16	144	4.1	99	264	7.02	6.17	12.8	37.1
3	38	Low	152	22	1.2	5.0	25	23	21	143	4.3	103	362	6.82	5.93	12.4	36.5
4	38	Low	126	21	1.1	5.2	23	16	15	143	4.5	106	289	6.96	6.18	13.2	38.1
0	42	Low	113	16	0.9	5.0	80	61	23	146	4.5	100	271	6.11	5.93	12.9	38.3
1	42	Low	106	21	1.0	5.2	50	25	17	145	4.9	103	219	6.47	5.96	12.8	38.1
2	42	Low	139	21	0.8	5.0	38	54	13	142	4.4	105	202	5.89	5.65	12.4	36.7
3	42	Low	136	21	1.0	5.5	35	26	11	144	4.1	103	259	6.64	5.75	12.8	37.5
4	42	Low	132	22	1.1	5.7	30	47	20	144	4.5	106	263	6.57	5.66	12.7	37.5
0	46	Low	182	15	0.9	4.7	79	19	14	144	4.2	99	208	6.19	6.62	14.1	40.2
1	46	Low	136	17	0.9	4.9	73	25	17	142	4.9	105	217	7.68	6.67	14.1	41.5
2	46	Low	140	16	0.7	5.1	57	32	41	143	4.5	106	224	7.13	6.74	14.2	41.7
3	46	Low	180	15	0.8	5.2	52	33	34	143	4.5	105	243	6.26	6.56	13.9	41.1
4	46	Low	145	15	0.7	5.3	51	19	33	140	4.9	109	249	X	X	X	X
0	49	Low	149	14	0.9	4.7	42	24	22	147	4.2	101	159	7.88	6.87	14.3	40.9
1	49	Low	122	16	0.9	5.1	41	22	16	142	4.8	105	378	9.07	6.79	13.9	40.5
2	49	Low	139	18	0.8	5.0	33	35	45	141	5.2	106	207	8.20	6.67	13.9	40.2
3	49	Low	182	15	0.9	5.2	32	35	35	144	4.1	104	228	6.96	6.55	13.9	39.9
4	49	Low	140	18	1.0	5.1	27	22	16	141	4.6	107	231	6.28	6.77	14.5	41.5
0	53	Low	198	13	1.0	4.6	61	27	14	145	4.5	99	243	5.41	6.74	14.1	40.7
1	53	Low	140	19	1.1	5.3	47	25	13	144	5.0	104	271	6.97	6.66	14.1	40.8
2	53	Low	146	21	0.9	5.4	37	21	12	143	4.7	102	297	6.38	6.69	14.6	41.9
3	53	Low	136	21	0.8	5.7	41	18	9	142	4.5	105	391	X	X	X	X
4	53	Low	146	24	0.9	5.7	32	19	7	141	4.6	103	424	5.80	6.58	14.5	41.1
0	31	High	132	15	0.9	4.5	51	16	22	145	4.3	101	237	6.15	6.78	14.6	42.4
1	31	High	118	15	0.9	5.0	36	19	16	146	4.5	105	291	7.24	6.39	13.6	39.7
2	31	High	132	16	0.9	4.9	33	19	10	140	4.7	104	272	7.48	6.45	13.9	40.2
3	31	High	107	18	0.9	5.4	26	19	11	143	4.2	106	372	6.40	6.63	14.5	42.3
4	31	High	135	15	0.9	5.1	21	18	12	141	4.2	106	237	X	X	X	X
0	35	High	170	22	1.0	5.2	27	44	29	145	4.5	99	216	5.42	6.07	13.1	37.9
1	35	High	149	18	1.2	5.2	27	67	59	144	4.9	99	227	5.78	5.90	12.7	37.6
2	35	High	134	22	1.0	5.4	32	122	411	141	4.4	103	228	8.01	6.2	13.8	39.3

3	35	High	120	17	1.0	5.5	24	29	23	143	4.4	104	250	X	X	X	X
4	35	High	135	21	1.0	5.4	18	37	17	140	4.4	105	241	X	X	X	X
0	39	High	146	18	0.7	5.0	36	25	17	147	4.1	103	174	5.04	6.51	13.1	38.7
1	39	High	189	18	0.8	5.0	33	43	29	146	4.2	103	170	4.19	6.76	13.8	40.1
2	39	High	130	15	1.2	5.2	45	33	23	143	3.9	104	146	4.16	6.72	13.6	39.3
3	39	High	115	20	0.9	5.2	39	27	19	144	4.2	107	219	4.06	6.61	13.6	39.9
4	39	High	138	15	0.7	5.4	29	22	11	143	4.3	108	171	4.27	6.43	13.6	39.1
0	43	High	161	16	0.8	4.8	33	17	13	147	4.5	103	155	6.92	6.01	13.4	38.9
1	43	High	117	18	0.9	5.1	28	19	14	146	5.5	104	179	8.57	6.06	13.4	39.2
2	43	High	124	20	0.7	5.0	23	18	15	144	4.8	108	171	8.26	5.70	12.8	37.2
3	43	High	120	20	0.7	5.4	21	21	10	144	4.3	109	207	6.53	5.74	13.2	37.9
4	43	High	137	20	0.8	5.6	17	17	11	143	4.6	108	191	6.27	5.78	13.2	37.9
0	47	High	165	18	0.8	5.2	71	26	17	146	4.2	102	169	5.60	6.18	12.5	37.7
1	47	High	136	17	0.7	5.0	47	31	13	144	5.1	108	196	7.27	5.84	11.8	36.0
2	47	High	126	20	0.8	5.0	38	34	11	143	4.7	107	204	7.54	5.82	11.9	36.0
3	47	High	146	20	0.7	5.8	33	22	10	144	4.6	108	187	6.70	5.77	11.8	36.3
4	47	High	136	20	0.8	5.7	24	22	9	142	5.0	107	218	7.11	5.74	12.1	35.7
0	51	High	139	20	0.8	4.5	84	48	32	144	4.6	103	213	7.55	6.09	12.3	36.1
1	51	High	131	24	0.8	4.9	61	40	21	144	5.2	107	238	6.09	6.29	12.6	37.0
2	51	High	135	26	0.9	5.0	46	59	26	142	4.7	107	268	7.61	6.22	12.8	37.0
3	51	High	135	25	0.8	5.4	47	32	17	143	4.7	110	252	X	X	X	X
4	51	High	132	25	0.9	5.4	28	34	23	143	5.2	110	297	6.61	6.35	13.4	38.7
0	54	High	171	17	0.9	5.1	72	23	17	146	4.7	96	170	4.87	6.34	13.0	37.4
1	54	High	134	20	1.0	5.4	56	28	18	142	5.4	103	182	7.18	6.47	13.4	38.8
2	54	High	137	18	0.8	5.5	37	29	14	140	5.2	99	195	X	X	X	X
3	54	High	142	21	0.9	6.2	42	27	18	141	5.4	101	245	6.2	6.43	13.8	40.0
4	54	High	140	20	0.9	5.9	32	22	13	142	5.1	103	238	X	X	X	X

Glu – glucose; BUN – blood urea nitrogen; Cr – Creatinine; TP – Total protein; AP – alkaline phosphatase; ALT - alanine aminotransferase; AST - Aspartate Aminotransferase; Na⁺ - Sodium; K⁺ - potassium; Cl⁻ - Chloride; WBC – white blood cell count; RBC – red blood cell count; Hemo – hemoglobin; HCT - hematocrit

X – data not available

Supplementary Table 3. Serum Neutralizing Antibody at day 176

Rabbit #	Treatment	Titer	Rabbit #	Treatment	Titer
25	Saline	None	46	IDUAe9	None
29	Saline	None	49	IDUAe9	None
33	Saline	None	53	IDUAe9	None
37	Saline	None	27	IDAUE10	None
41	Saline	None	31	IDAUE10	None
45	Saline	None	35	IDAUE10	None
26	IDUAe9	None	39	IDAUE10	None
30	IDUAe9	None	43	IDAUE10	None
34	IDUAe9	None	47	IDAUE10	None
38	IDUAe9	0.043055556	51	IDAUE10	None
42	IDUAe9	None	54	IDAUE10	None

Note: Neutralizing antibodies (NAB) to the viral capsid 176 days following corneal intrastromal injection. There was also no NAB in the aqueous or vitreous humor of eyes injected with saline, IDUAe9, or IDUAe10.

Supplemental Figure 1. Representative optical coherence tomography images of the right and left corneas 176 days after intrastromal injection of AAV-IDUA.

Supplementary Figure 1

