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Supplemental information

Use of plasmapheresis to lower anti-AAV antibodies in nonhuman primates with pre-existing immunity to AAVrh74

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SUPPLEMENTAL MATERIAL

SUPPLEMENTAL METHODS

Reverse-Transcription Droplet Digital Polymerase Chain Reaction

RT-ddPCR was used to quantify SRP-9001 transcript expression in collected tissue samples. RNA isolation of tissue samples was performed in AirClean PCR Workstations using the MagMAXTM mirVanaTM Total RNA Isolation Kit on the KingFisher Apex System. RNA concentrations were determined using a Qubit Flex Fluorometer with a broad-range dye. An RNA isolation negative control (INC) containing only DPBS and buffers used in the isolation was present on each isolation plate. Plates were accepted if the RNA INC concentration was below the limit of detection on the Qubit Flex Fluorometer. Primers and probe specific to the SRP-9001 dystrophin transgene [primers (forward, GAAGAGAACCCTGGAGCG and reverse, GTCCTGCAGGGAATCAATCA) and probe (56-FAM/TGAGGCAGG/ZEN/CCGAAGTGATTAAGG/3IABkFQ)] and reference Ap3d1 (PrimePCR Probe Assay: Ap3d1, Rhesus Monkey; Bio-Rad, Hercules, California, USA) were added to the One-Step RT-ddPCR Advanced Kit for Probes (containing Supermix, DTT, and Reverse Transcriptase; Bio-Rad, Hercules, California, USA) to create a reverse transcriptase master mix. A non-reverse transcriptase master mix (containing primers/probe, Supermix, and DTT) was also made to control for DNA contaminants. The master mixes were plated onto a ddPCR-compatible 96-well plate and either diluted RNA sample, the RNA INC, a single control containing a linearized SRP-9001 plasmid and NHP total RNA control, or nuclease-free water (negative control) was added to the respective wells. A QX ONE instrument was used for analyzing each plate. Plates were accepted if the plasmid control yielded a loaded amount of 5 x 104 copies ± 25%, the NHP RNA control yielded ≥ 20 copies/µL, and the no template controls

were below the limit of blank (defined as < 1 copies/µL). All test samples below the limit of quantification (BLOQ; defined as < 5 copies/µL) were considered negative for SRP-9001 dystrophin. Test samples above the limit quantification (defined as > 5 copies/µL) were analyzed by calculating the ratio of SRP-9001 dystrophin to Ap3d1.

SUPPLEMENTAL TABLES

Table S1. Immunosuppression regimens administered throughout Phase 1 to NHPs dosed with delandistrogene moxeparvovec (2.00×10^{14} vg/kg)

Cohort (n=3 each)	Duration
1 (Control)	N/A
2 (Prednisone)	1 day prior to GTT and 30 days postdosing
3 (Prednisone)	1 day prior to GTT and 60 days postdosing
4 (Prednisone)	14 days prior to GTT and 60 days postdosing
5 (Triple IS: rituximab, sirolimus, prednisone)	14 days prior to GTT and 60 days postdosing

IS, immunosuppression; vg, vector genome

Cohort 1 was administered one dose of GTT only.

Table S2. Immunosuppression regimens administered throughout Phase 2 to NHPs redosed with delandistrogene moxeparvovec (2.00×10^{14} vg/kg)

Cohort	Group	Immunosuppression regimens	No. of Animals
1	No redosing	None	3
2-4	Plasmapheresis and redosing	Prednisone	7
2 and 3	Redosing without plasmapheresis	Prednisone	2
5	Redosing without plasmapheresis	Triple IS (rituximab, sirolimus, prednisone)	2

IS, immunosuppression; vg, vector genome

Table S3. Average Blood Chemistry Values for NHPs in cohorts 1-4 (Part 1).

Group	Chemistry Parameter	Unit	Baseline	Post-dosing						Endpoint
				2 Weeks	4 Weeks	6 Weeks	9 Weeks	12 Weeks	24 Weeks	
Cohort 1	ALT	U/L	36.0	36.0	82.5	66.0	62.0†	191.5	87.0	67.5
	AST	U/L	31.0	34.0	63.0	49.5	41.5†	101.5	90.0	50.0
	GGT	U/L	81.0	76.0	76.0	83.0	82.0†	106.0	101.5	94.0
	Platelets	K/mm^3	340.5	326.5	326.5	383.0	326.5†	356.0	345.0	368.0
Cohort 2	ALT	U/L	35.7	71.0	48.3	20.7	26.0‡	25.7	--	--
	AST	U/L	29.0	38.7	33.7	28.3	31.7‡	27.7	--	--
	GGT	U/L	75.3	109.3	95.0	78.3	87.3‡	75.3	--	--
	Platelets	K/mm^3	266.7	236.3	275.0	291.3	264.5‡	264.7	--	--
Cohort 3	ALT	U/L	40.0	47.7	34.3	29.0	31.7‡	57.0	--	--
	AST	U/L	25.7	34.3	27.7	26.0	33.3‡	31.0	--	--
	GGT	U/L	67.3	63.7	53.7	47.3	51.3‡	58.3	--	--
	Platelets	K/mm^3	273.7	278.7	288.7	228.7	317.3‡	263.0	--	--
Cohort 4	ALT	U/L	43.3	39.3	39.7	40.7	--	105.3	--	--
	AST	U/L	27.3	29.7	27.7	27.7	--	47.3	--	--
	GGT	U/L	48.7	51.7	45.0	43.3	--	53.0	--	--
	Platelets	K/mm^3	285.0	318.7	288.0	375.0	--	308.3	--	--

ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase

†9 weeks post-dosing; ‡8 weeks post-dosing

Table S4. Average Blood Chemistry Values for NHPs in cohort 5 (Part 1).

Group	Chemistry Parameter	Unit	Baseline	Post-dosing (weeks)										
				2	3	4	5	6	8	12	15	17	20	24
Cohort 5	ALT	U/L	44.0	121.0	71.0	62.0	58.3	57.7	39.7	143.7	114.0	123.0	102.3	135.3
	AST	U/L	31.3	55.0	50.0	52.3	47.0	50.7	41.7	60.3	61.7	66.0	46.3	65.7
	GGT	U/L	79.7	82.0	80.3	76.7	79.3	73.7	67.0	72.0	74.7	72.7	83.3	130.7
	Platelets	K/mm^3	345.0	419.3	359.0	335.0	317.7	406.7	345.7	352.7	345.3	337.3	319.7	390.7

ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase

Table S5. Average Blood Chemistry Values for NHPs in cohorts 1-4 (Part 2).

Group	Chemistry Parameter	Unit	Number of plasmapheresis exchanges	Pre Redose	Post-redosing							
					1 Day	1 Week	2 Weeks	4 Weeks	6 Weeks	8 Weeks	10 Weeks	Endpoint
Cohort 1	ALT	U/L	0	26	--	--	46	10	10	<6	13	20‡
	AST	U/L		27	--	--	45	29	22	27	28	28‡
	GGT	U/L		58	--	--	68	53	50	55	55	56‡
	Platelets	K/cu3		319	--	--	308	287	--	296	349	339‡
Cohort 2	ALT	U/L	2.25	31†	85	56	34	24	34.5	26	28	35.5§
	AST	U/L		10†	195.5	34	29	30	34	28	29.5	28.5§
	GGT	U/L		<10†	33	61	70.5	67	78	82	82.5	79§
	Platelets	K/cu3		195†	125	288.5	258	229	262.5	198	189	222§
Cohort 3	ALT	U/L	2.2	22.5†	139.5	47.7	25.7	31.3	37.7	27.3	--	32.0‖
	AST	U/L		9.0†	299.5	25.0	22.3	25.7	27.7	26.0	--	23.0‖
	GGT	U/L		<10†	23.5	49.3	49.7	49.7	56.3	62.3	--	57.3‖
	Platelets	K/cu3		217.0†	128.0	343.3	364.0	287.5	322.3	273.0	--	318.3‖
Cohort 4	ALT	U/L	3.0	27.0†	131.5	67.7	38.3	70.0	40.3	51.3	47.0	47.3¶
	AST	U/L		9.7†	195.5	30.7	30.0	52.0	32.0	36.3	30.3	32.7¶

	GGT	**U/L**		**<10[†]**	**30.0**	**45.7**	**49.3**	**50.0**	**59.0**	**62.0**	**62.3**	**57.7[¶]**
	Platelets	**K/cu 3**		**234.0[†]**	**224.5**	**399.0**	**415.0**	**306.7**	**344.7**	**309.0**	**365.3**	**336.7[¶]**

ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase

[†]<10 minutes post-plasmapheresis; [‡]Endpoint, 12 weeks; [§]Endpoint, 11.5-12 weeks; [‖]Endpoint, 9-10 weeks; [¶]Endpoint, 11-12 weeks

Table S6. Average Blood Chemistry Values for NHPs in cohort 5 (Part 2).

Group	Chemistry Parameter	Unit	Pre-redose	Post-redosing					Endpoint (7 Weeks)
				10 Min	1 Week	2 Weeks	4 Weeks	6 Weeks	
Cohort 5	ALT	U/L	66.0	54.0	56.0	116.0	139.0	128.7	126.7
	AST	U/L	32.0	57.0	38.0	57.0	47.0	54.7	50.3
	GGT	U/L	71.0	74.0	56.0	118.7	127.3	140.0	143.3
	Platelets	K/mm^3	444.0	324.0	420.0	337.3	407.3	358.0	368.3

ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transpeptidase

Table S7. Total antibody titers against AAVrh74 in NHPs prior to and following plasmapheresis (before redosing with delandistrogene moxeparvovec)

NHP (Cohort)	Titer after Part 1[†]	Titer after plasmapheresis[‡]	Plasmapheresis cycles, n
RA2292 (2)	1:51,200	1:800	2.5
RA2289 (2)	1:6,400	1:400	3
RA1040 (3)	1:12,800	1:800	3
RA2924 (3)	1:25,600	1:400	3
RA2480 (3)	1:25,600	1:51,200	0.5[‖]
RA2399 (4)	1:12,800	1:1,600	3
RA2279 (4)	1:12,800	1:200	3
RA3000 (4)	1:12,800	1:200	3

NHP, nonhuman primate

[†]12 weeks post-dosing; [‡]Prior to redosing; [‖]Only underwent 0.5 cycles of plasmapheresis due to small size and poor vascular access.

SUPPLEMENTAL FIGURES

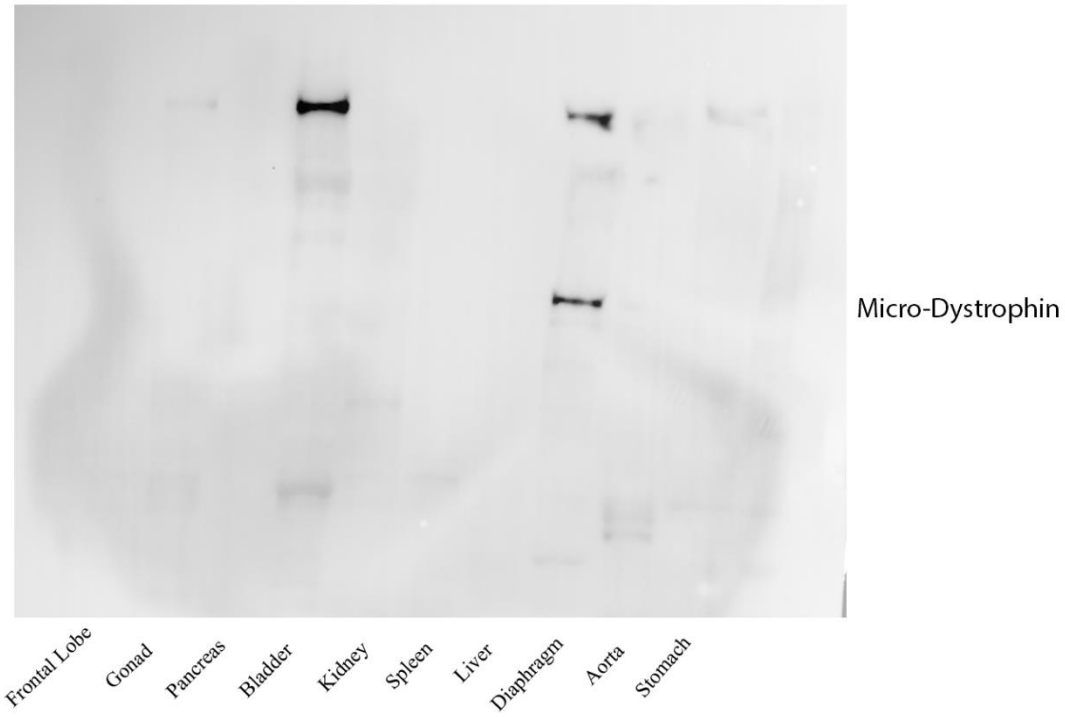

Figure S1. Representative western blots showing SRP-9001 dystrophin expression in off-target tissues from an NHP in cohort 5. Diaphragm is included as a positive control.

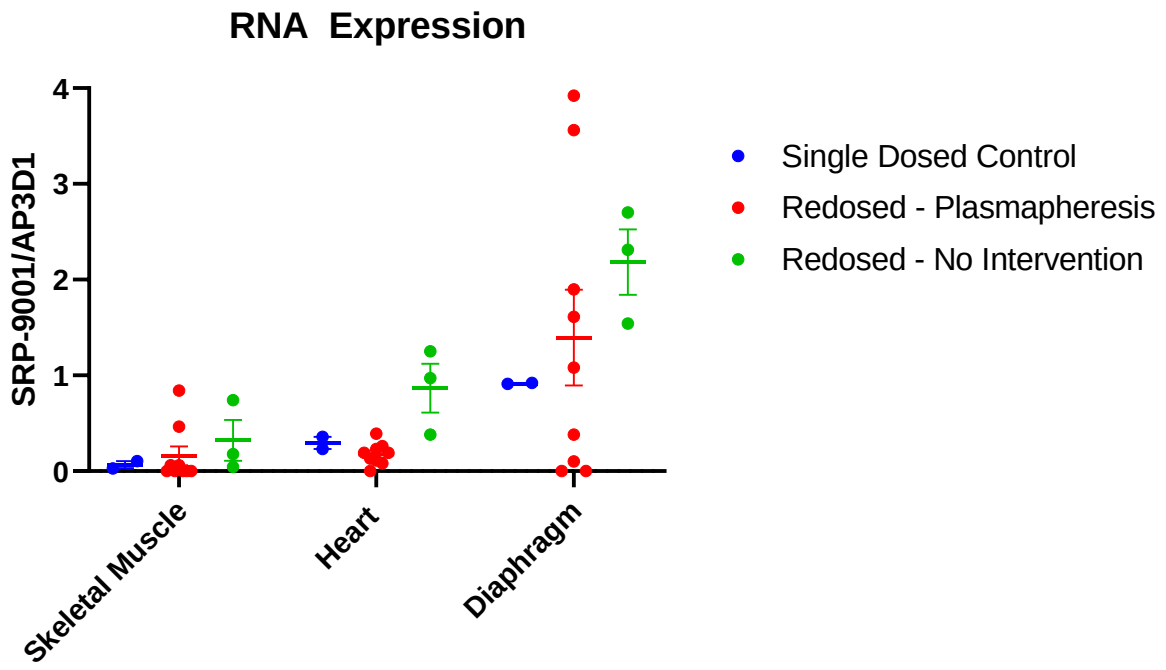

Figure S2. SRP-9001 dystrophin RNA Expression. (A-D) SRP-9001 RNA expression/AP3D1 RNA expression of Endpoint skeletal muscle, heart, and diaphragm samples for single dosed control cohort (cohort 1, blue), redosed with plasmapheresis (cohorts 2-4, red) and redosed without plasmapheresis (cohort 5, green). Bars represent the mean ± standard error mean.