Non-Clinical Pharmacodynamic Effects and Immunogenicity Assessment of Prophylactic Immune Modulation Prior to Gene Therapy Dose Administration in C57BL/6 Mice.

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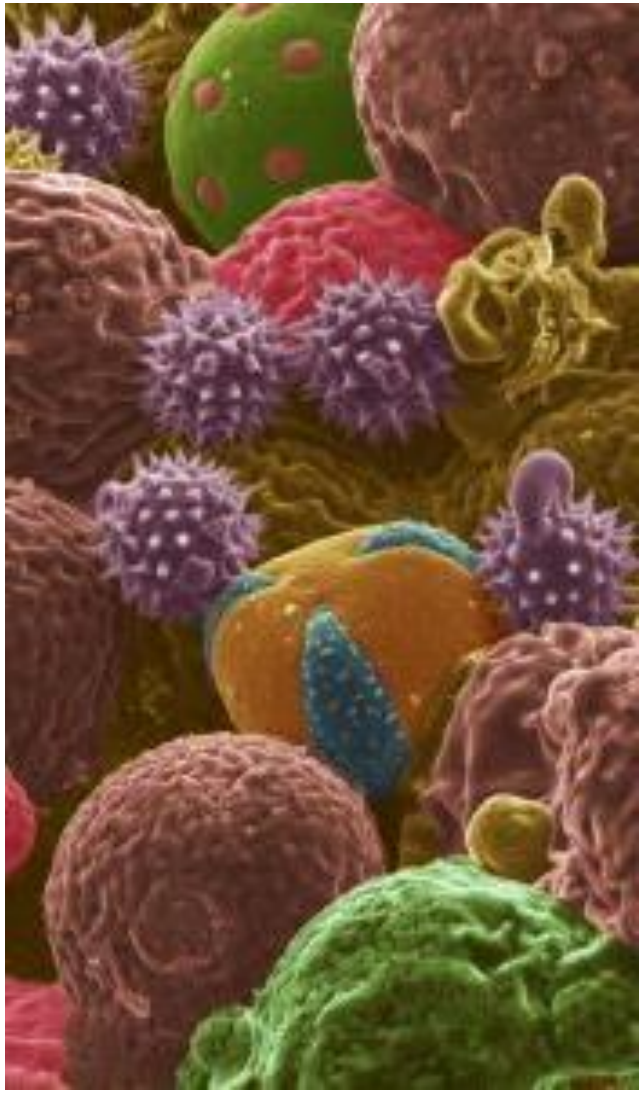
BioMarin Pharmaceutical Inc., Novato, CA, USA

Agenda

- Why use immune suppression in gene therapy?
- Prophylactic Alternative Immune Suppression Mouse Study design
- Key Results: AAV5 TAb, Plasma A1AT, Inflammatory Biomarkers, Vector Genomes in Liver
- Conclusions

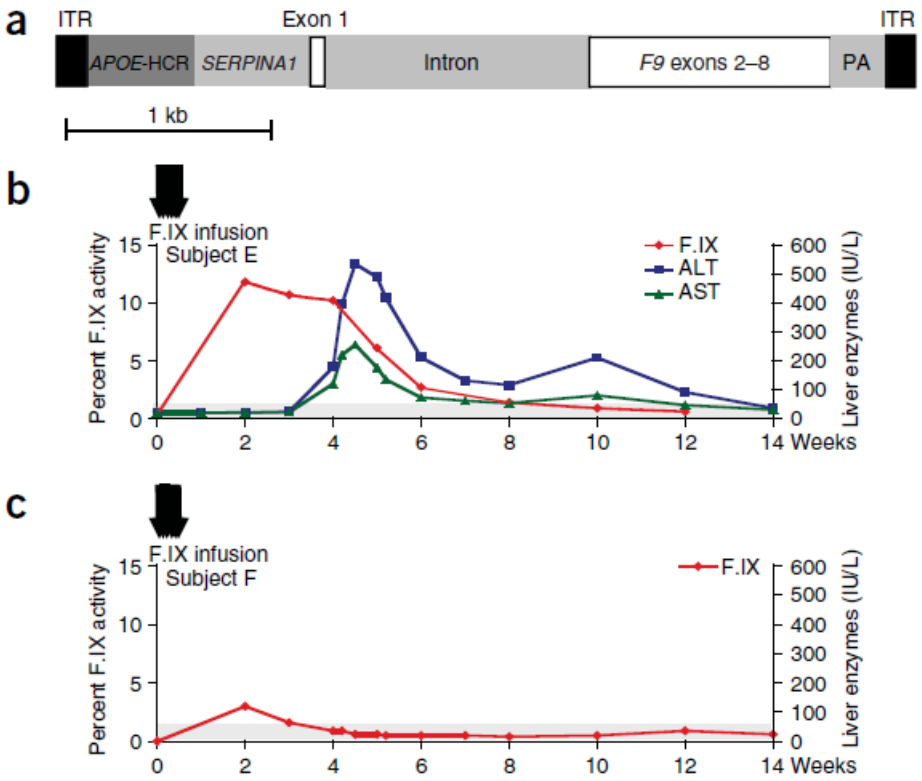


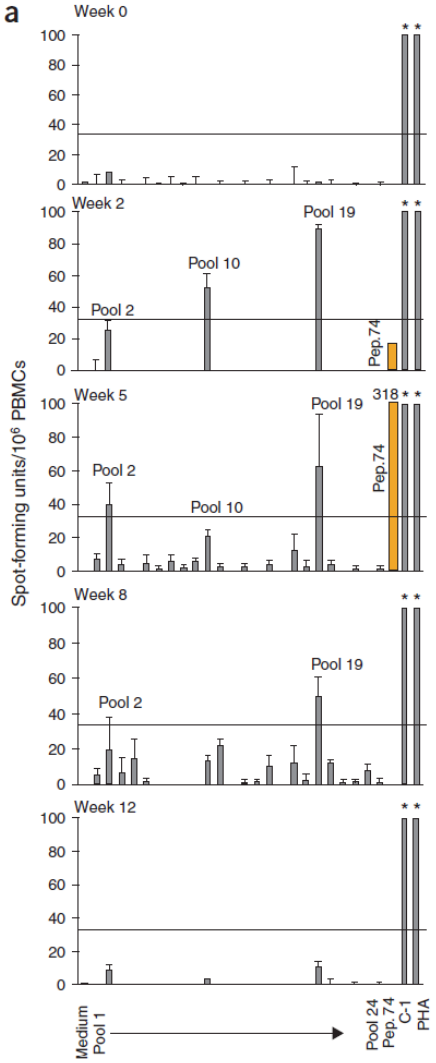
Why Has Immune Suppression Been Used in AAV Mediated Gene Therapies?

Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response

Catherine S Manno[1,2,15], Glenn F Pierce[3,15], Valder R Arruda[1,2,15], Bertil Glader[4,15], Margaret Ragni[5], John J E Rasko[6], Margareth C Ozelo[7], Keith Hoots[8], Philip Blatt[9], Barbara Konkle[10], Michael Dake[4], Robin Kaye[1,2], Mahmood Razavi[4], Albert Zajko[10], James Zehnder[4], Pradip K Rustagi[11], Hiroyuki Nakai[4], Amy Chew[1,3], Debra Leonard[2,12], J Fraser Wright[3], Ruth R Lessard[3], Jürg M Sommer[3], Michael Tigges[3], Denise Sabatino[1], Alvin Luk[3], Haiyan Jiang[3], Federico Mingozzi[1], Linda Couto[3], Hildegund C Ertl[1,13], Katherine A High[1,2,14] & Mark A Kay[4]

nature medicine





Transient, asymptomatic ALT elevations observed at high doses

First to suggest immunomodulation may be required to preserve long-term expression

Temporal relationship between PBMC response to AAV capsid, declining FIX levels and rising transaminases are all consistent with hypothesis that the transaminitis arose from specific destruction of vector-transduced cells by the immune system

Manno et al. Nat Med. 2006 Mar;12(3):342-7.

Why Has Immune Suppression Been Used in AAV Mediated Gene Therapies?

The NEW ENGLAND JOURNAL of MEDICINE

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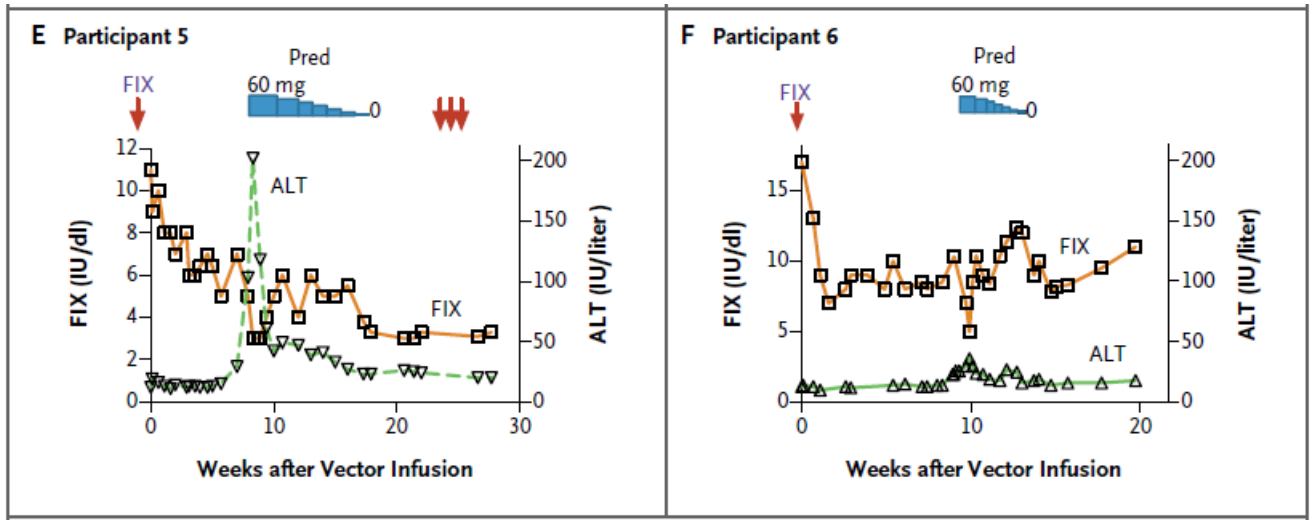
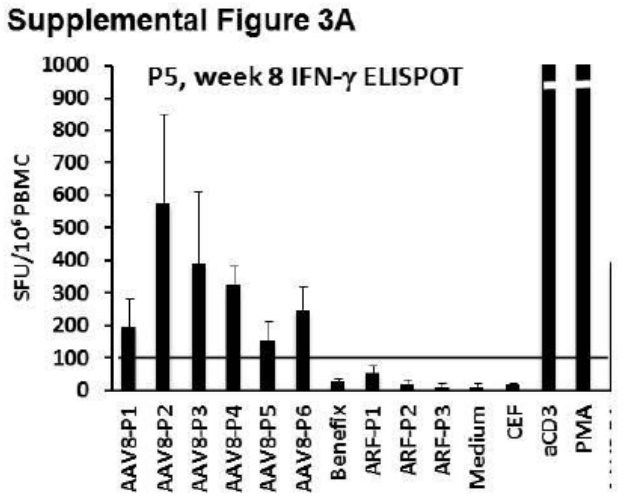
Adenovirus-Associated Virus Vector–Mediated Gene Transfer in Hemophilia B

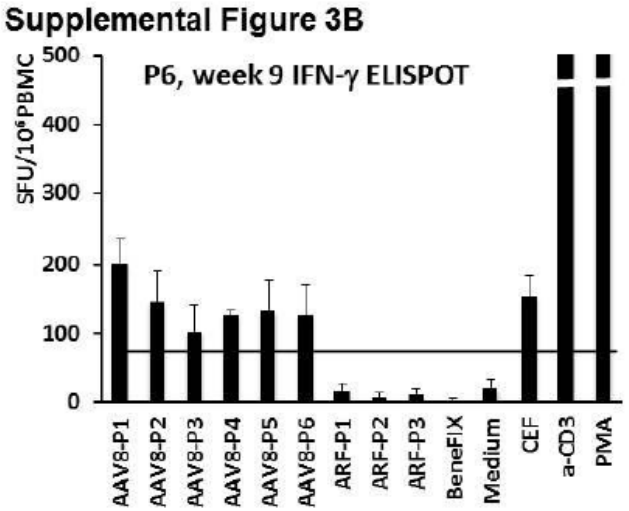
Amit C. Nathwani, M.B., Ch.B., Ph.D., Edward G.D. Tuddenham, M.B., B.S., M.D., Savita Rangarajan, M.B., B.S.,

University College London
AAV8-FIX in Hemophilia B

- Increase in ALT
- Decline in FIX
- Detection of Temporally Related Cellular Immune Response
- Resolved with Prednisolone







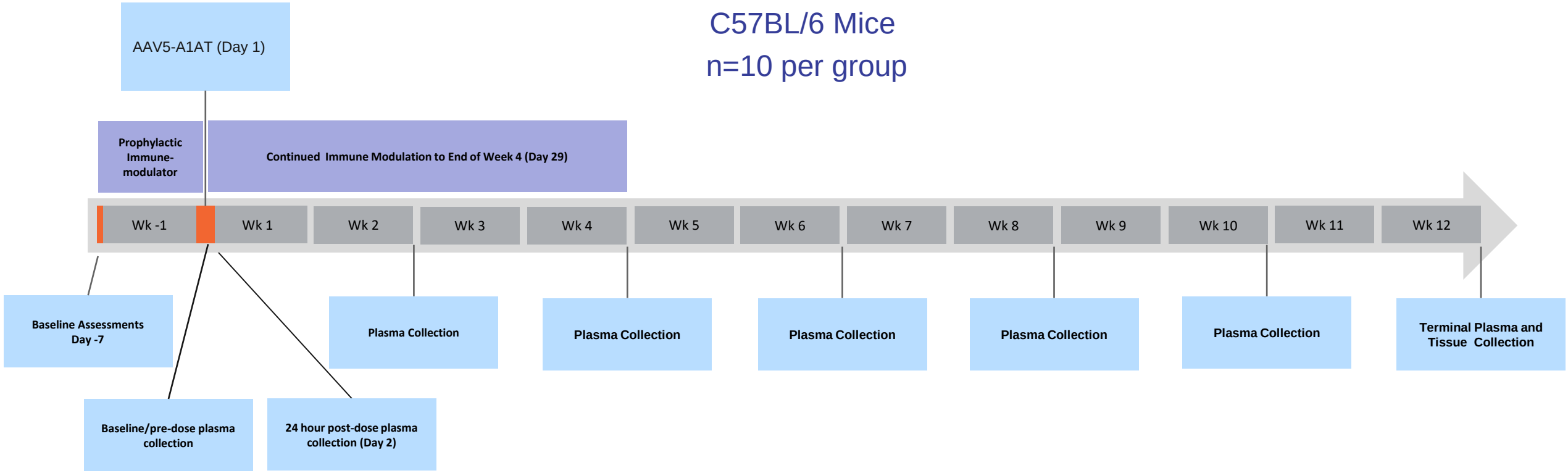
Nathwani et al. N Engl J Med. 2011 Dec 22;365(25):2357-65.

Prophylactic Alternative Immune Suppression Mouse Study design

Tested the following alternative immune suppression approaches:

- Prednisolone, Mycophenolate (MMF), Rapamycin (IP and PO), Tacrolimus, Dimethyl Fumarate (DMF), Fingolimod and anti-IL6R antibody,
- Administered from Day -3 through Day 29 (Day 1 through Day 29 for Rapamycin)

Endpoints: A1AT Plasma protein, Liver DNA/RNA quantification, AAV5 TAb, O-link cytokine expression



Prophylactic Immune Suppression in C57BL/6 Mice

Starting at Day -3 and continuing for 28 days following gene therapy dose administration.

Group No.	Prophylactic Treatment (ROA)	Dose Level (mg/kg/dose)	AAV Vector (6E13 vg/kg)	MOA
1	Saline (PO)	0 (QD)	NA	Saline Negative Control
2	Reference (PO)	0 (QD)	AAV5-A1AT	A1AT Reference Control
3	Prednisolone (PO)	2 (QD)	AAV5-A1AT	Inhibition of gene transcription for COX-2, cytokines, cell adhesion molecules, and inducible NO synthase
4	Mycophenolate (PO)	40 (QD)	AAV5-A1AT	Nucleotide depletion in T and B cells inhibits proliferation and suppresses function.
5	Rapamycin (IP)	4 (QOD)	AAV5-A1AT	mTOR inhibitor blocks T-cell activation and B-cell differentiation by preventing response to IL-2
6	Rapamycin (PO)	10 (QOD)	AAV5-A1AT	
7	Tacrolimus (IP)	1 (QD)	AAV5-A1AT	Inhibits calcineurin to inhibiting both T-lymphocyte signal transduction and IL-2 transcription
8	Dimethyl Fumarate (PO)	100 (QD)	AAV5-A1AT	Interferes with immunometabolism and may inhibit TLR9 signaling
9	TY720 Fingolimod (PO)	0.2 (QD)	AAV5-A1AT	Suppresses the exit of lymphocytes from lymph nodes, leading to a lower level of circulating lymphocytes
10	Anti-IL6R alpha chain (IP)	100 ug/animal*	AAV5-A1AT	Blocks IL-6 receptor activation and cytokine signaling

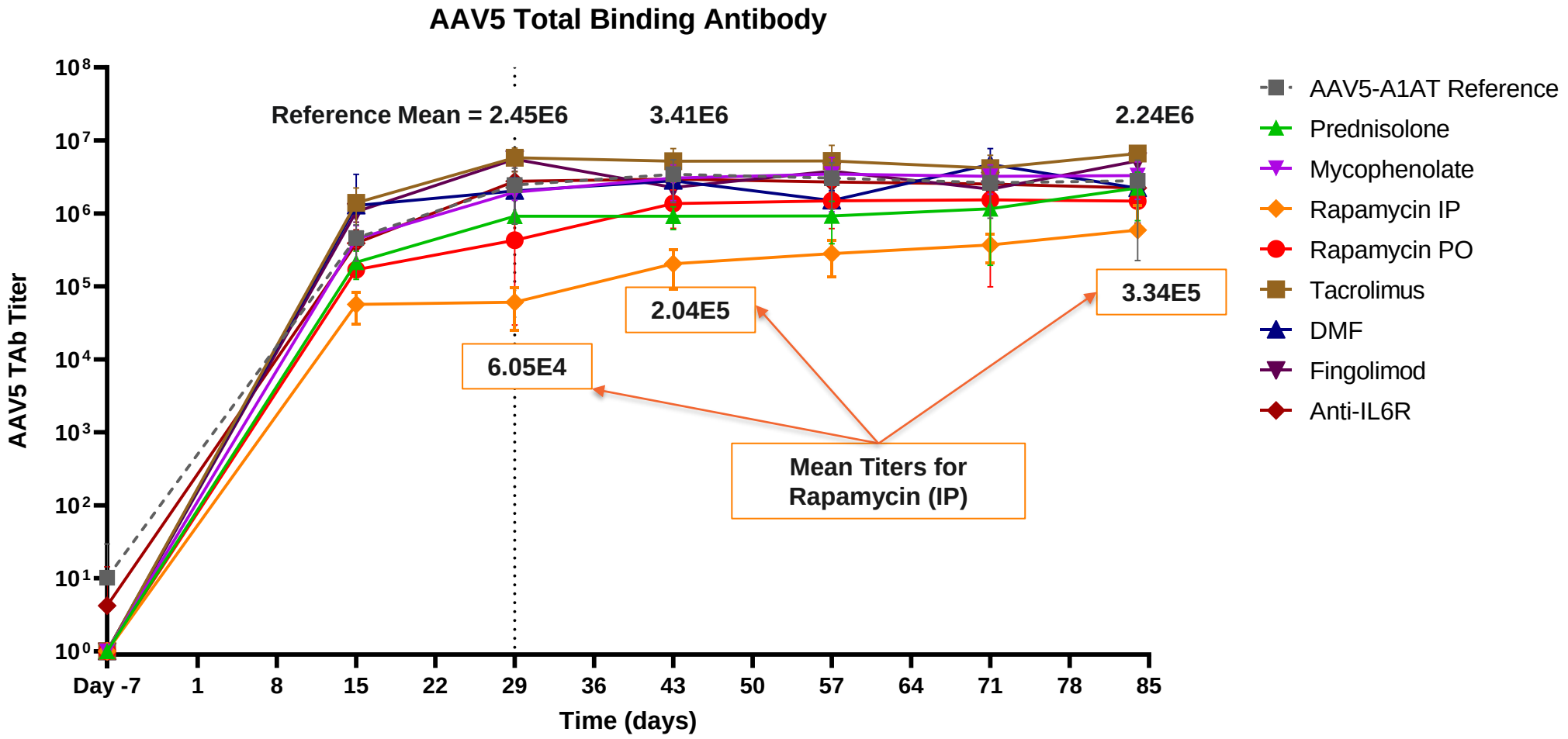
PO – oral gavage, IP - intraperitoneal

*Loading dose of 300ug/mL at Day -3. 100ug/mL on Days 1, 8, 15, 22 and 28

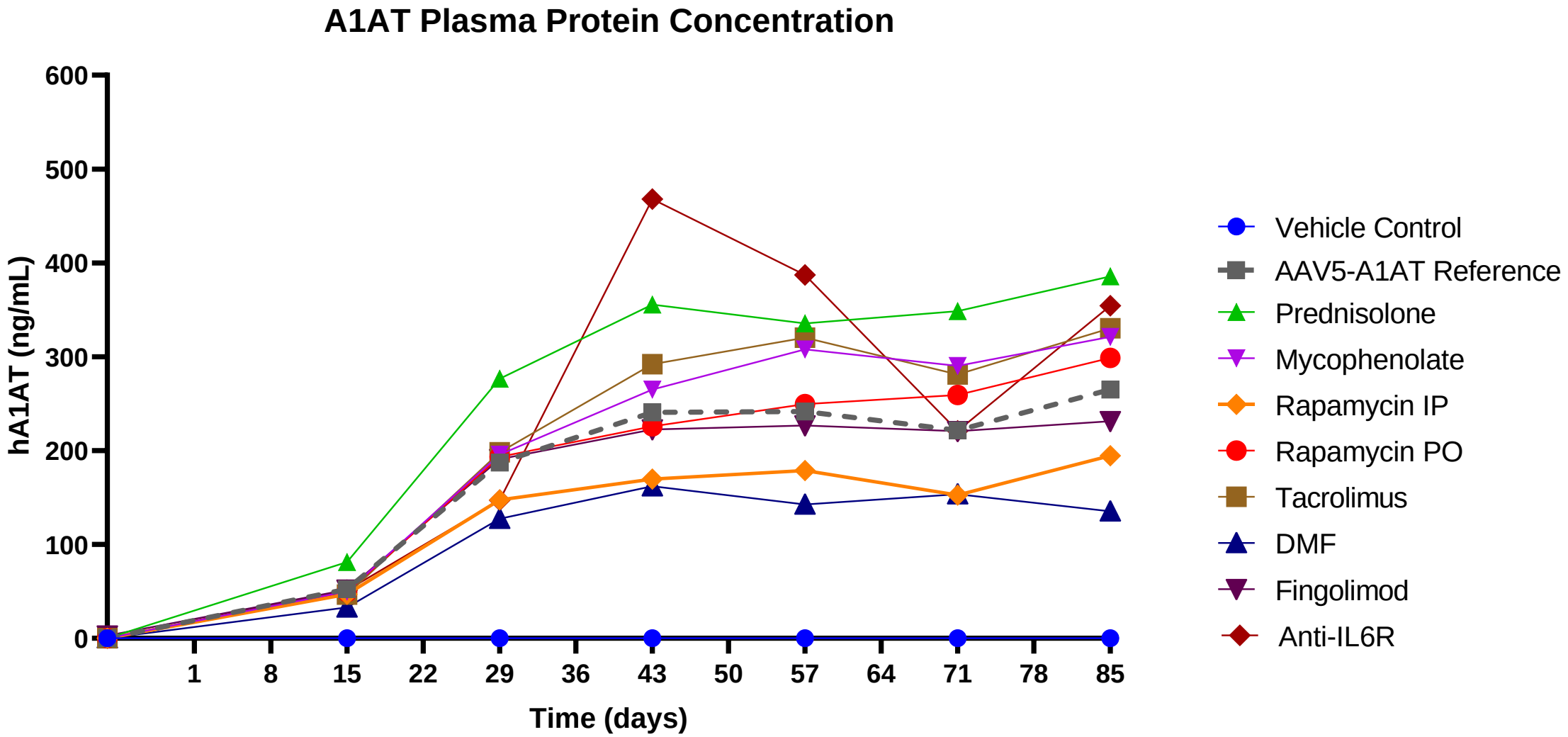
Results Summary

- Rapamycin (IP) administration resulted in a notable (~1 log) reduction in AAV5 TAb titer
- Prophylactic administration of Prednisolone (PO) resulted in a statistically significant increase in hA1AT plasma protein concentrations with associated increase in VG in liver.
- *Trends* of increased transgene expression with Tacrolimus, Mycophenolate, and IL-6R antagonist.
- The rest did not show any benefit over Saline with respect to either hA1AT expression or suppression of AAV5 TAb
- No significant cytokine or inflammatory biomarker increases were observed following gene therapy dose administration in the reference group
- Changes were observed in the cytokine expression profile of mice receiving prophylactic immune modulation.

Prophylactic Rapamycin (IP) Reduced AAV5 TAb Titers



A1AT Plasma Protein Concentration Following GT Dose Administration



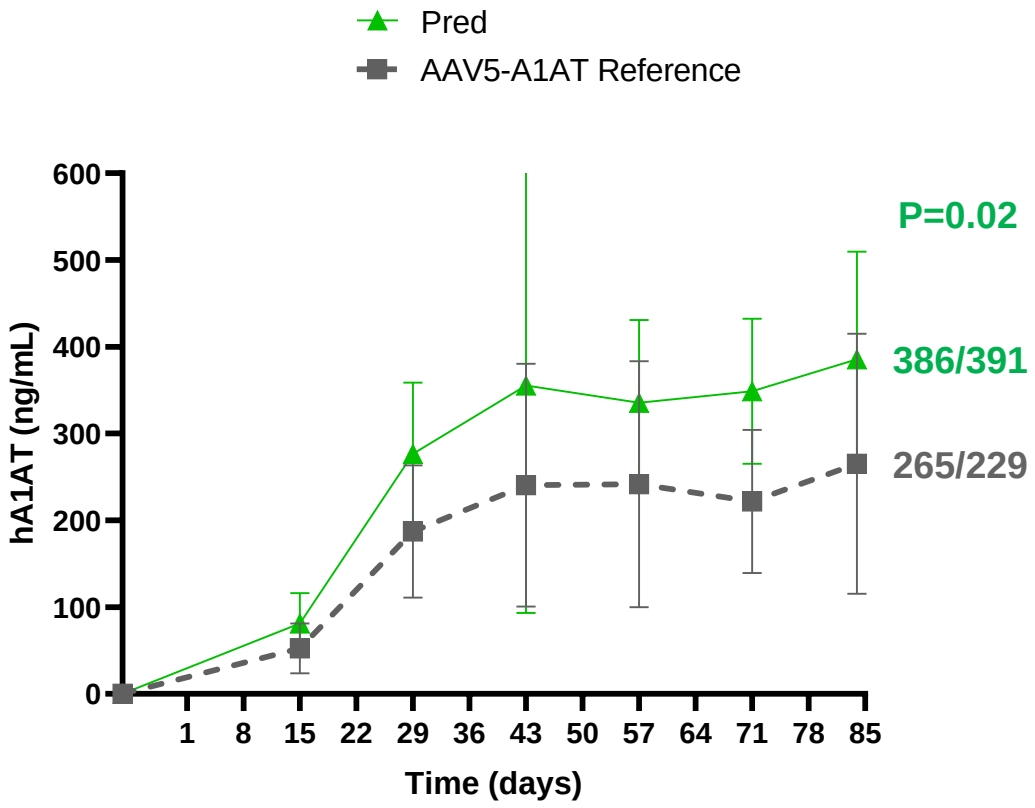
Significant Increase in A1AT Plasma Protein Concentration in the Prednisolone Treatment Group - ANOVA Summary

Group No.	Prophylactic Treatment (ROA)	Mean Plasma A1AT	Median Plasma A1AT	Log Mean	Standard Error	Difference from Reference	P-value
1	Saline (PO)						
2	Reference (PO)	201.4	182.1	5.10	0.123		
3	Prednisolone (PO)	**296.4**	**317.7**	**5.51**	**0.123**	**0.41**	**0.0252**
4	Mycophenolate (PO)	238.9	242.3	4.96	0.381	-0.14	0.7372
5	Rapamycin (IP)	146.5	150.8	4.77	0.125	-0.32	0.0757
6	Rapamycin (PO)	213.1	172.4	4.83	0.381	-0.26	0.5123
7	Tacrolimus (IP)	244.2	265.0	5.12	0.256	0.02	0.9447
8	Dimethyl Fumarate (PO)	124.7	98.0	3.94	0.532	-1.15	0.0606
9	TY720 Fingolimod (PO)	191.2	198.9	4.79	0.381	-0.30	0.4524
10	Anti-IL6R alpha chain (IP)	271.7	180.1	4.83	0.381	-0.26	0.5153

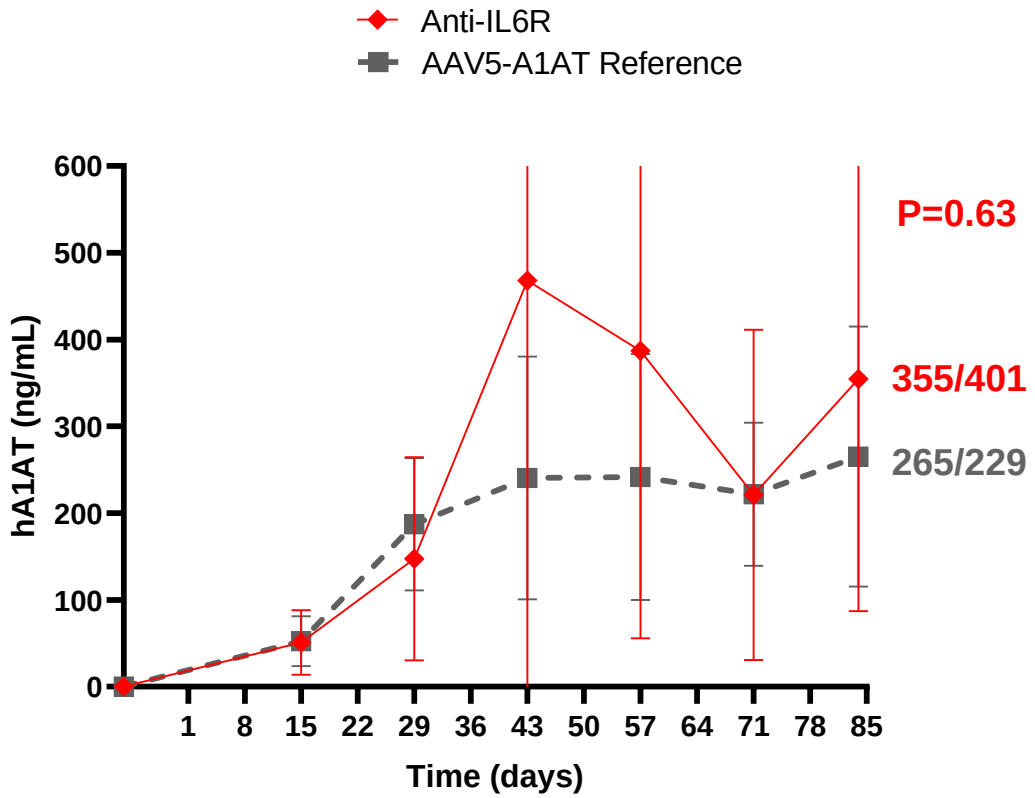
PO – oral gavage, IP - intraperitoneal

Increased A1AT Plasma Protein in Prednisolone Treatment Group

Prednisolone 2mg/kg Daily



Anti-IL6R Antibody 100μg/mL Weekly

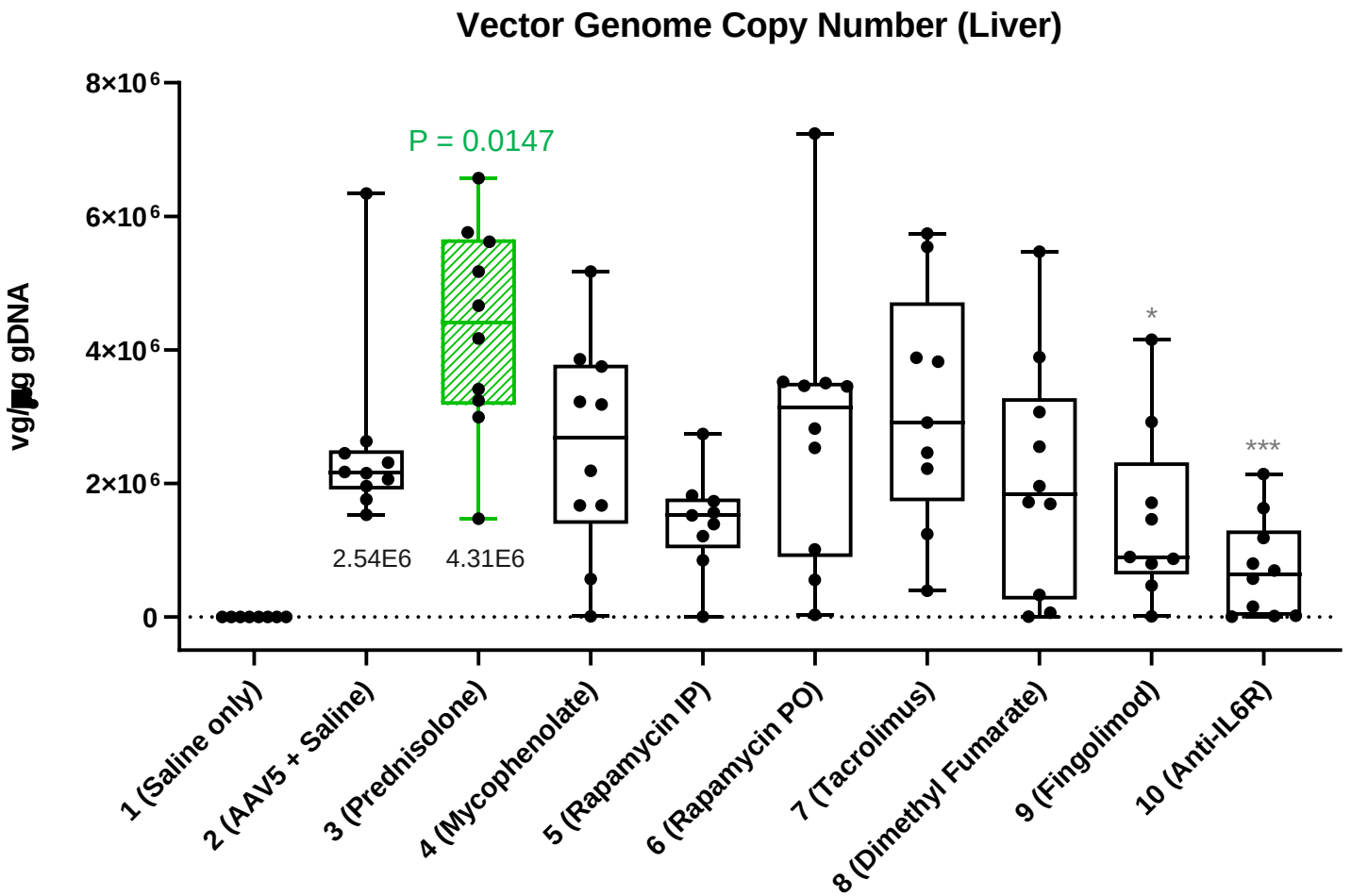


Error Bars are SD

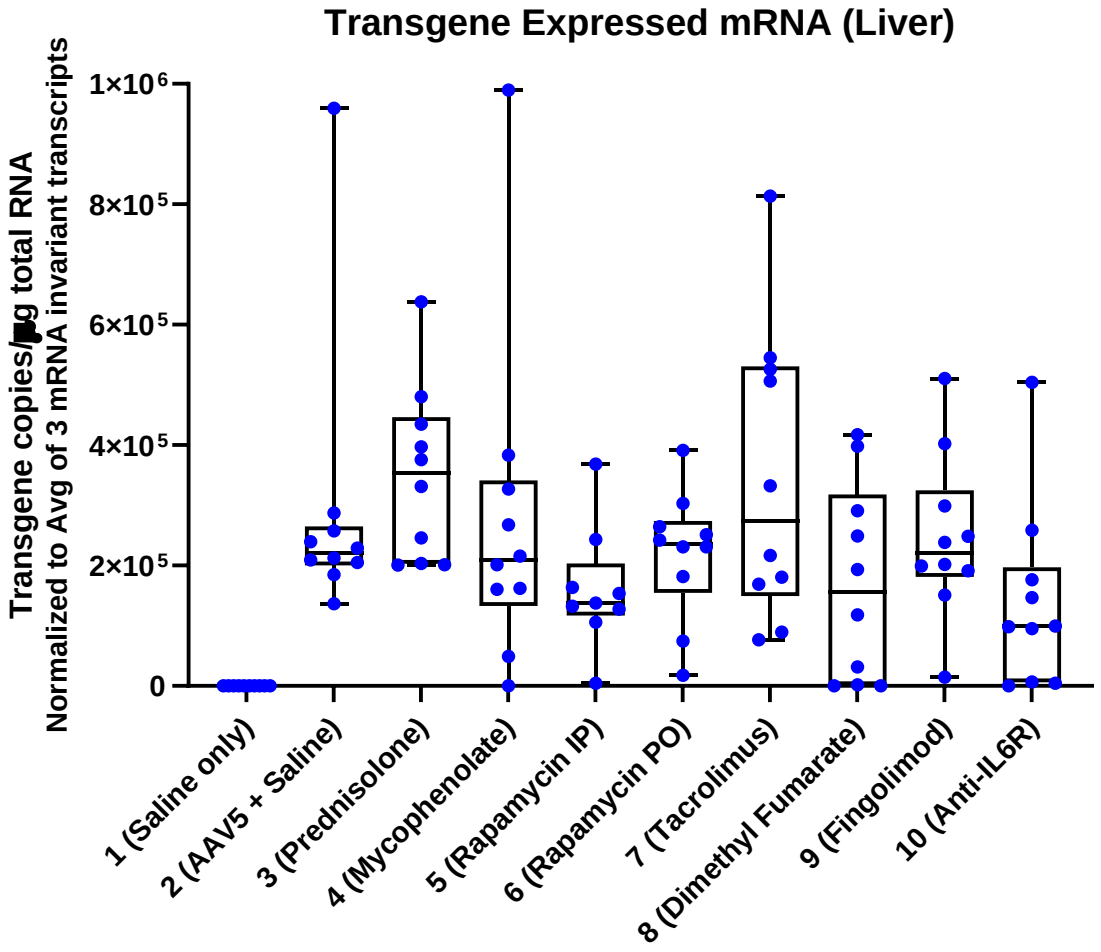
Mann Whitney U test – two tailed at D84 (Mean/Median)

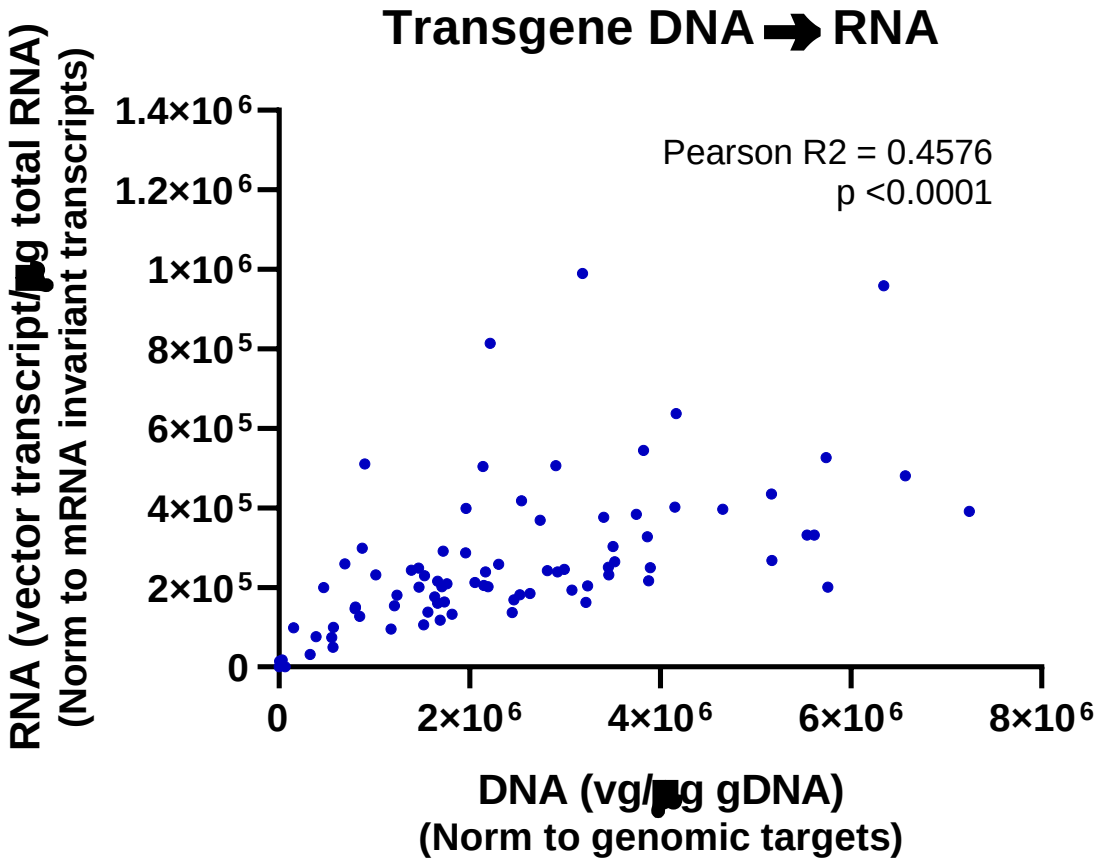
Prophylactic Prednisolone Improved Vector Transduction

- DNA and RNA were each extracted from two frozen pieces of liver tissue.
- DNA was measured and samples were diluted for input into ddPCR of the A1AT transgene and three independent genomic target genes
- Vector DNA normalized to three genomic targets
- Notable amount of inter-animal variability with use of immune suppression



Vector Transgene DNA Correlates with RNA

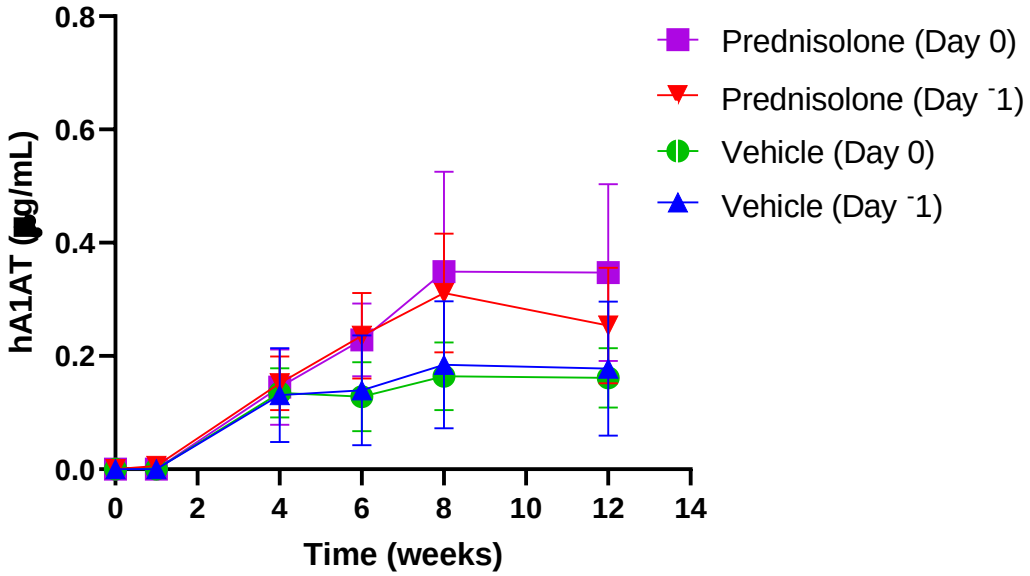


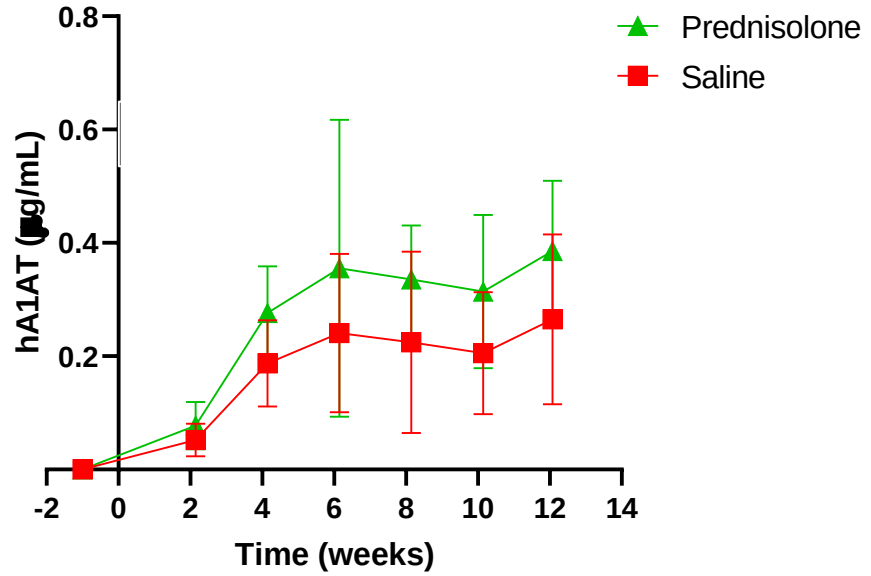


- The use of AIS was not associated with reduced transcription in individual animals.

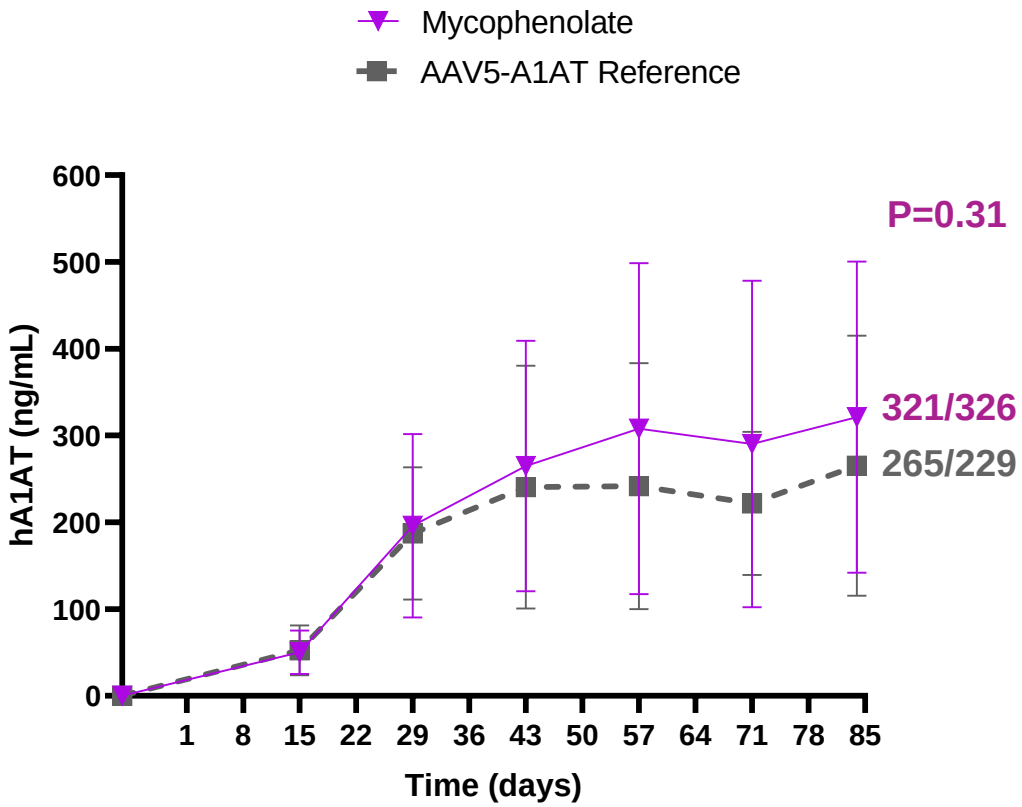
Comparison with Previous Prophylactic Prednisolone Study

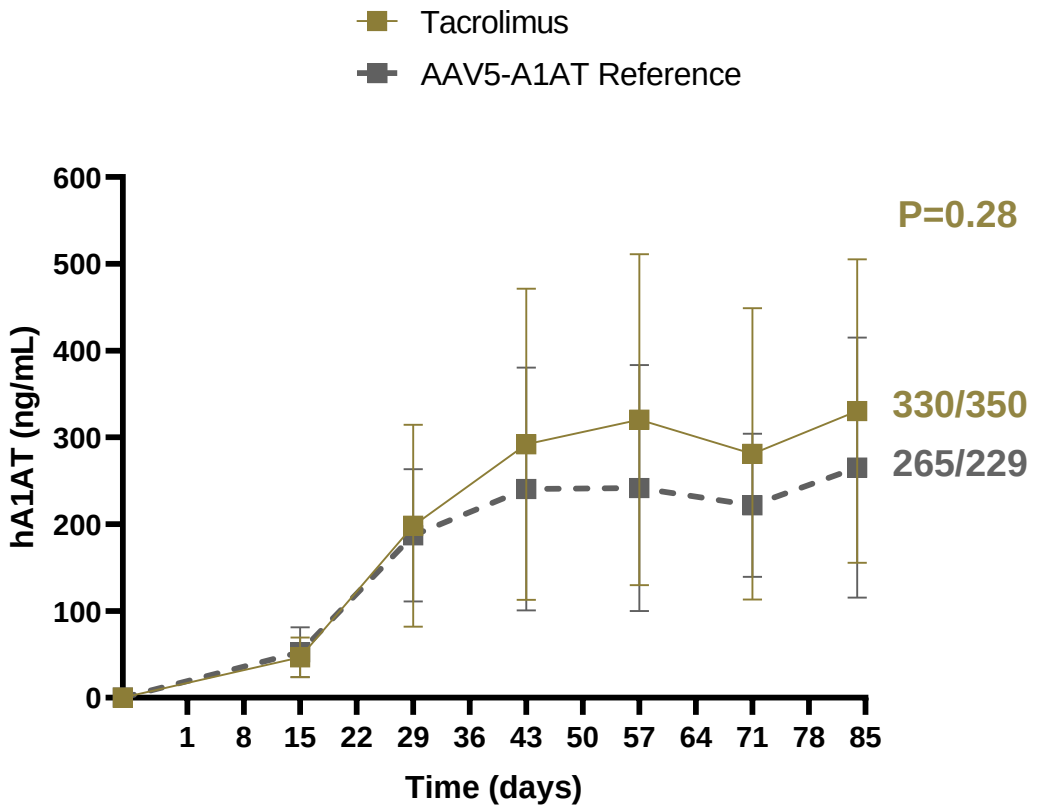
- Consistent with a previous study, levels of secreted hA1AT protein are increased in prophylactic prednisolone treated mice
- The number of hepatocytes transduced with vector DNA and full-length repaired vector genomes was higher than non–prednisolone-treated groups (data not shown).





Minor Increase in A1AT Plasma Protein in Mycophenolate and Tacrolimus Treatment Groups





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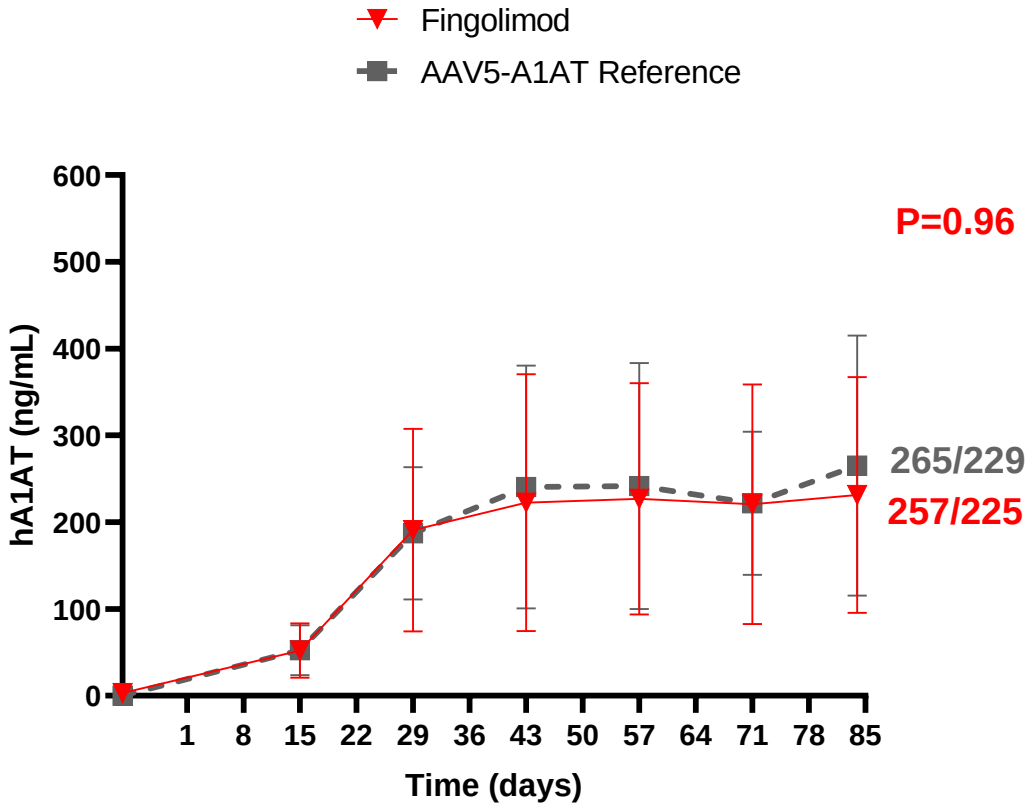
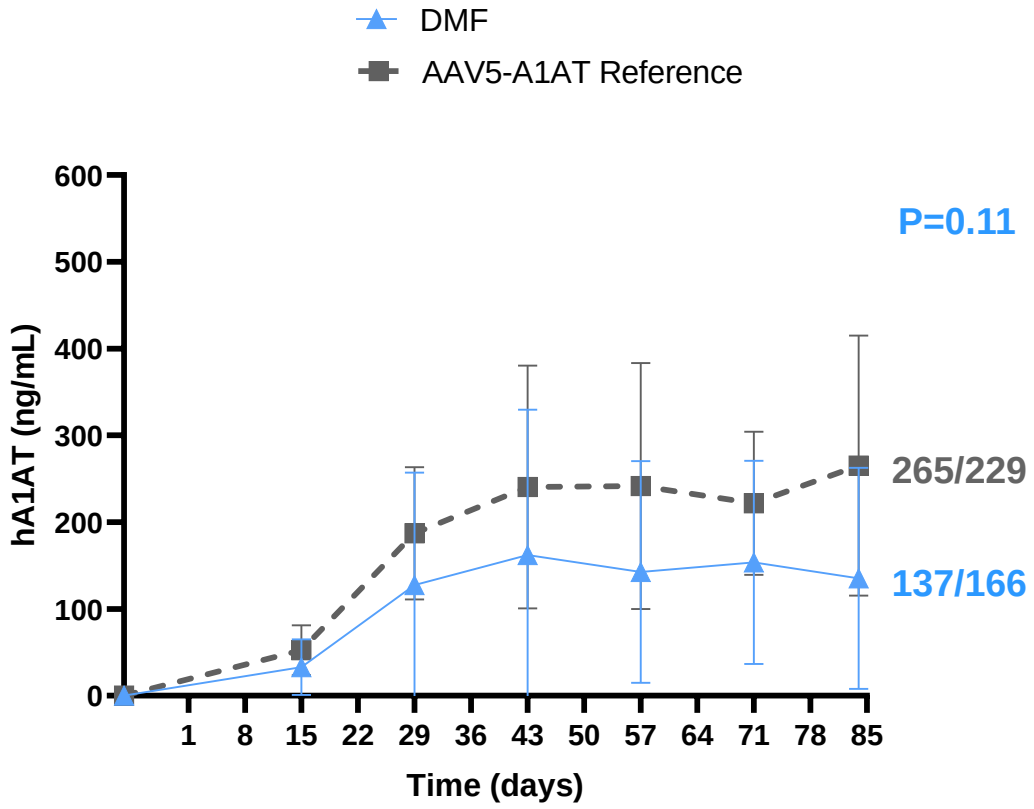
Mann Whitney U test – two tailed at D84 (Mean/Median)

No Benefit to A1AT Plasma Protein in Fingolimod and Dimethyl Fumarate Treatment Groups





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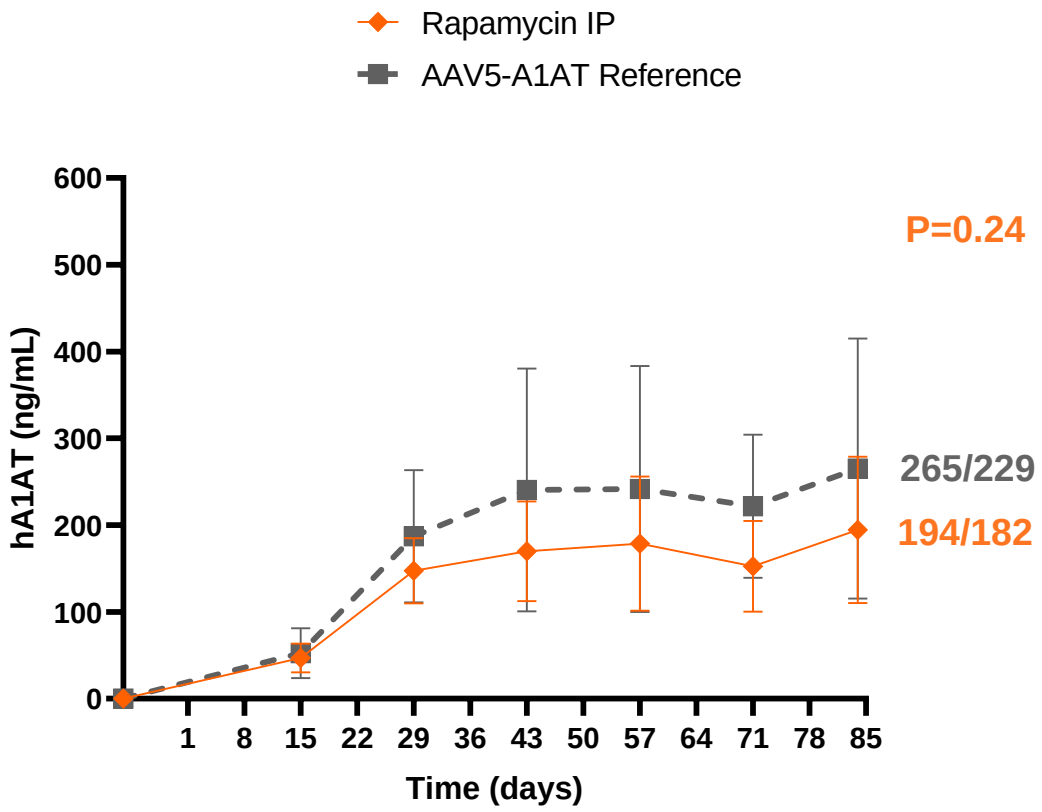
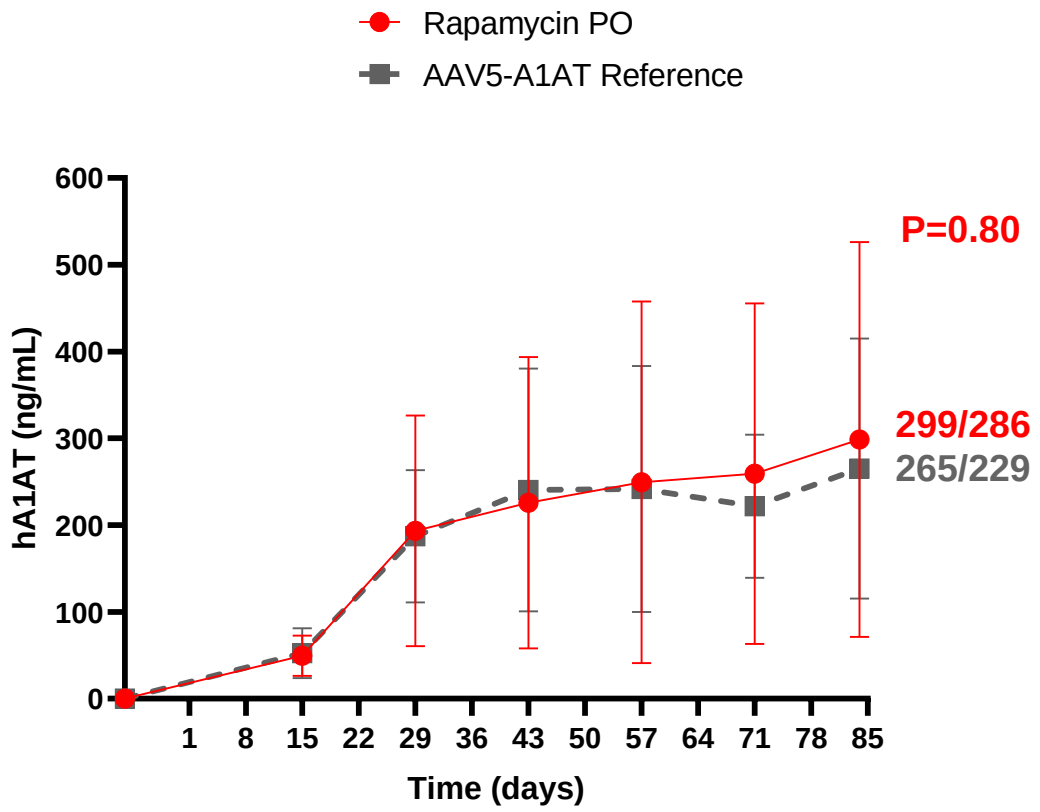
Mann Whitney U test – two tailed at D84 (Mean/Median)

No Benefit to A1AT Plasma Protein in Rapamycin Treatment Groups





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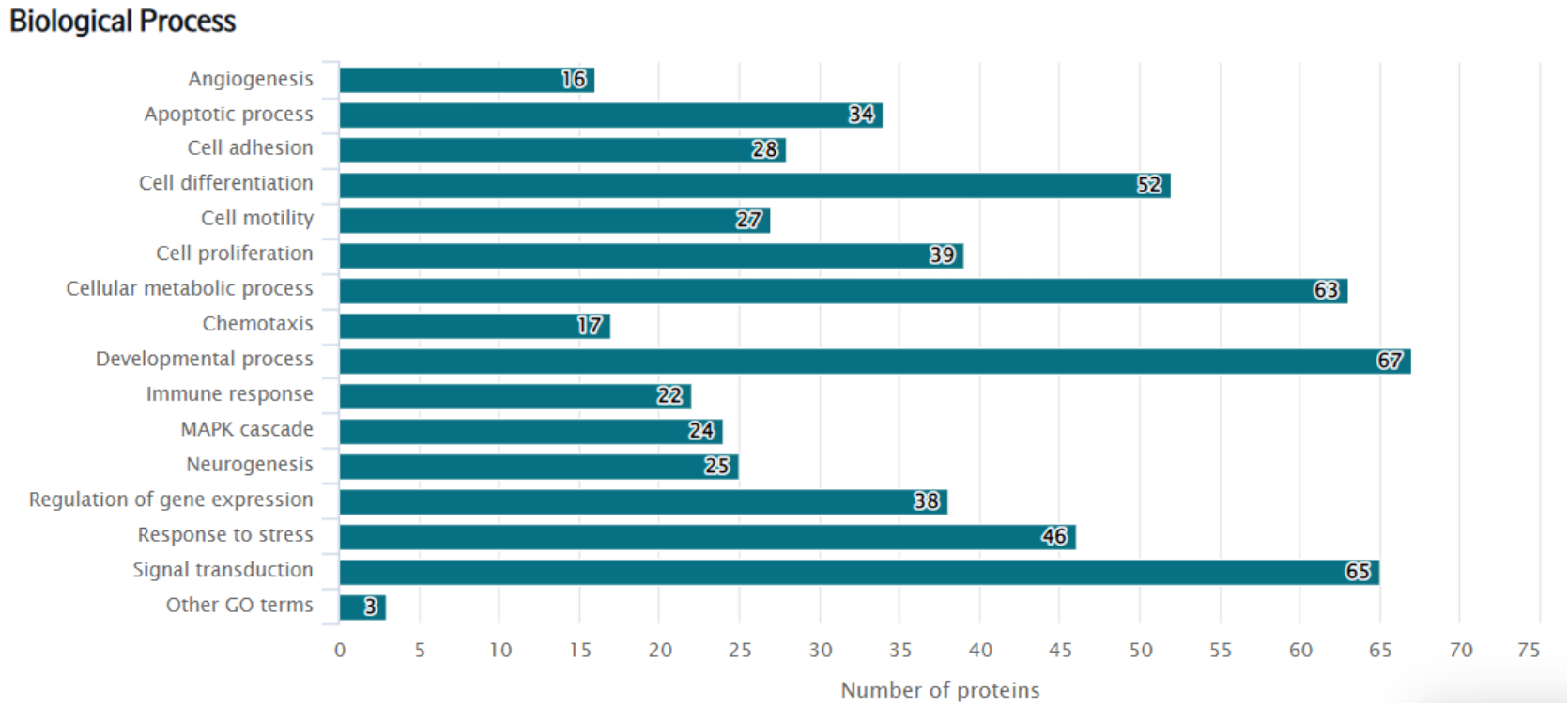
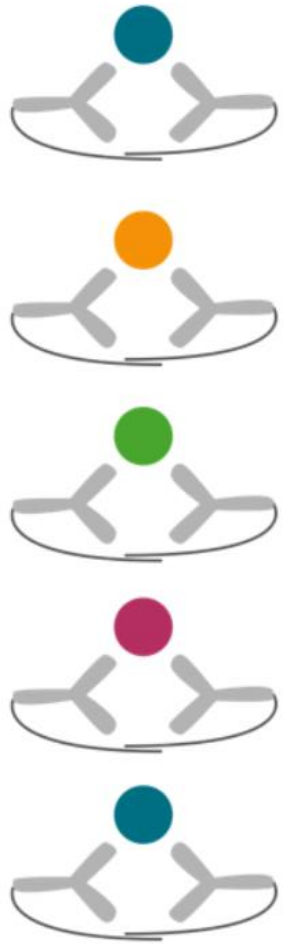
Mann Whitney U test – two tailed at D84 (Mean/Median)

Olink® Target 96 Mouse Exploratory Biomarker Panel

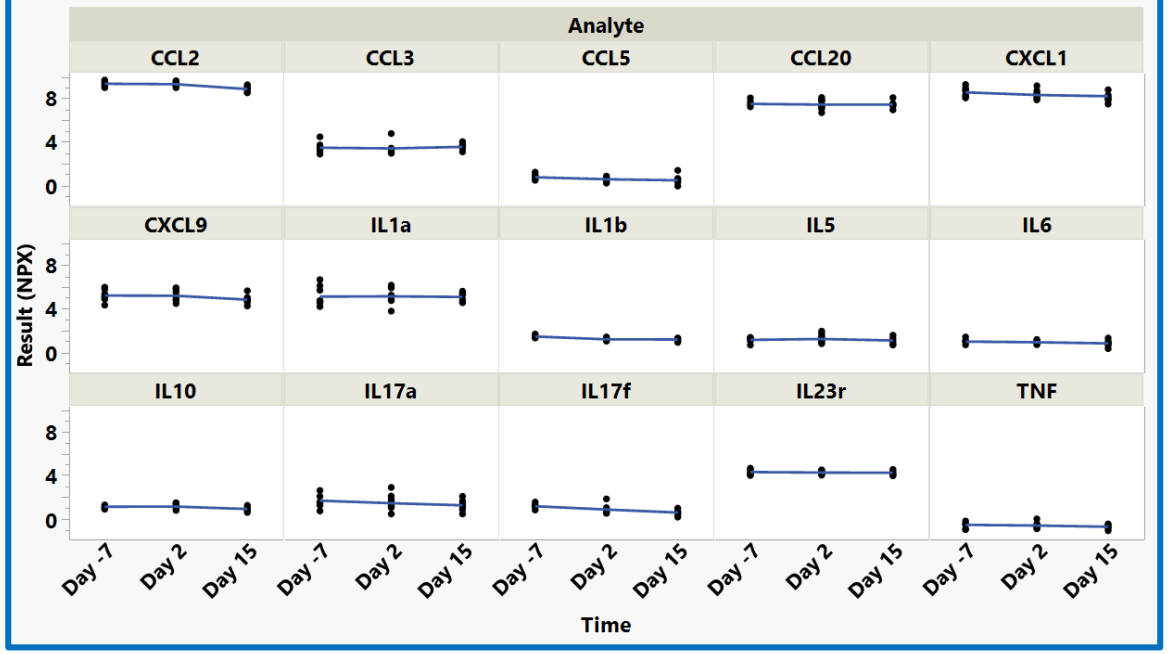
- Simultaneous analysis of 92 protein biomarkers
- Mouse plasma samples from Day -7 (Baseline), Day 2 and Day 15
- Expresses as normalized protein expression (NPX) values



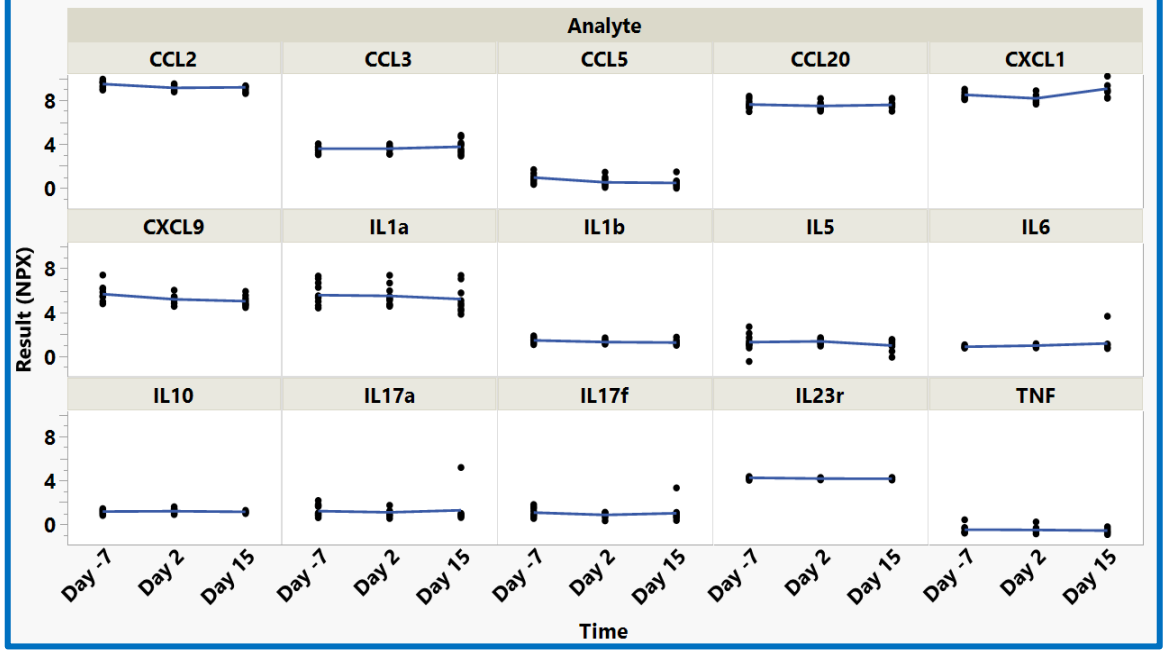


No Notable Increase in Cytokine Expression in Reference Group

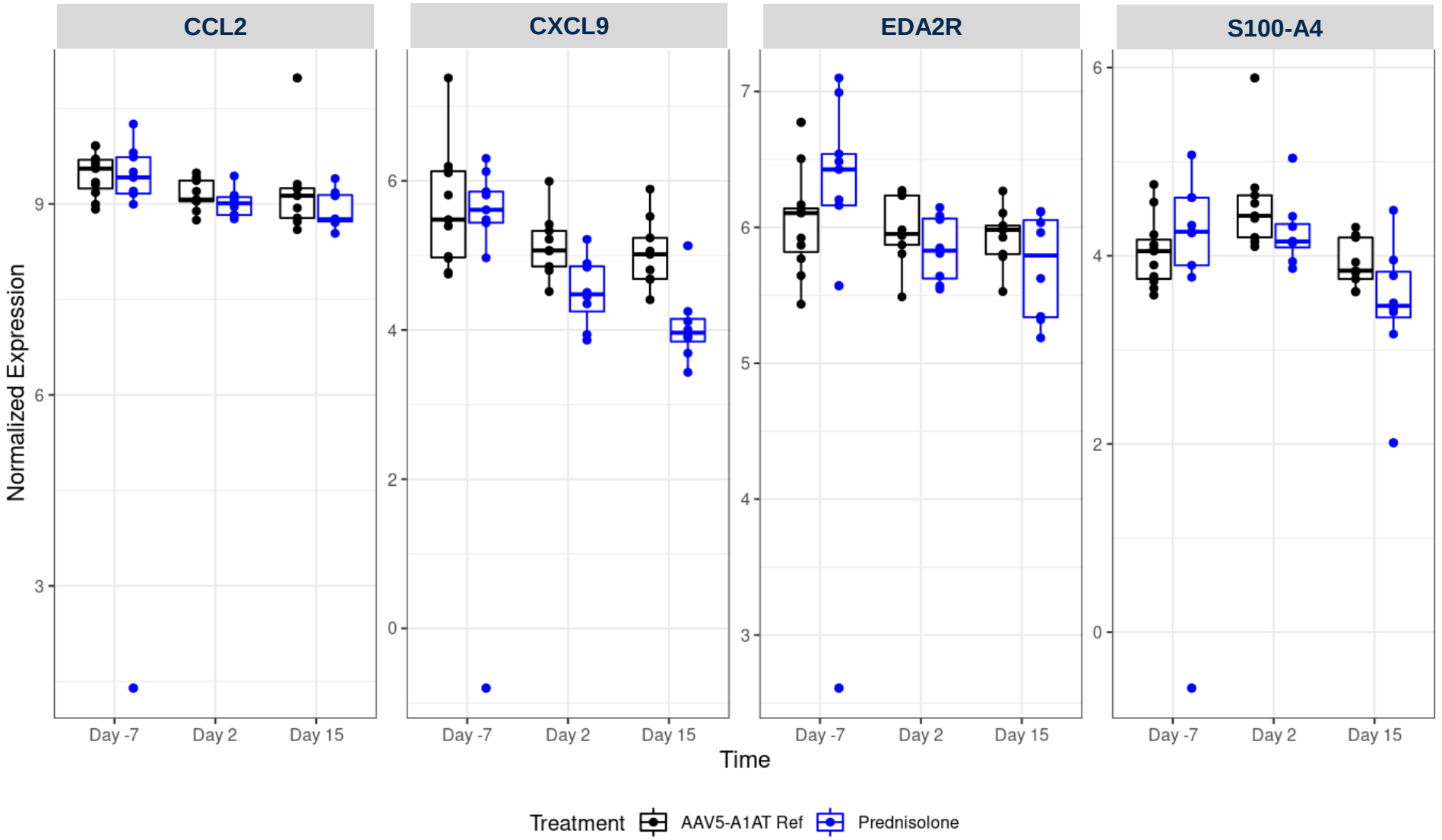
Vehicle Control
No AAV5-A1AT Administered



Reference Control
AAV5-A1AT with no immune suppression



Prednisolone Reduced Expression of CXCL9 Through Day 15



CCL2 and CXCL9 Recruit Cell Populations Involved in Inflammation and Immune Modulation Downstream of Hepatic Injury and IFN-γ Secretion

Hepatocytes, stellate cells, sinusoidal endothelial cells, and activated infiltrating lymphocytes all secrete CXCR3 ligands in response to Type 1 and Type 2 interferons.

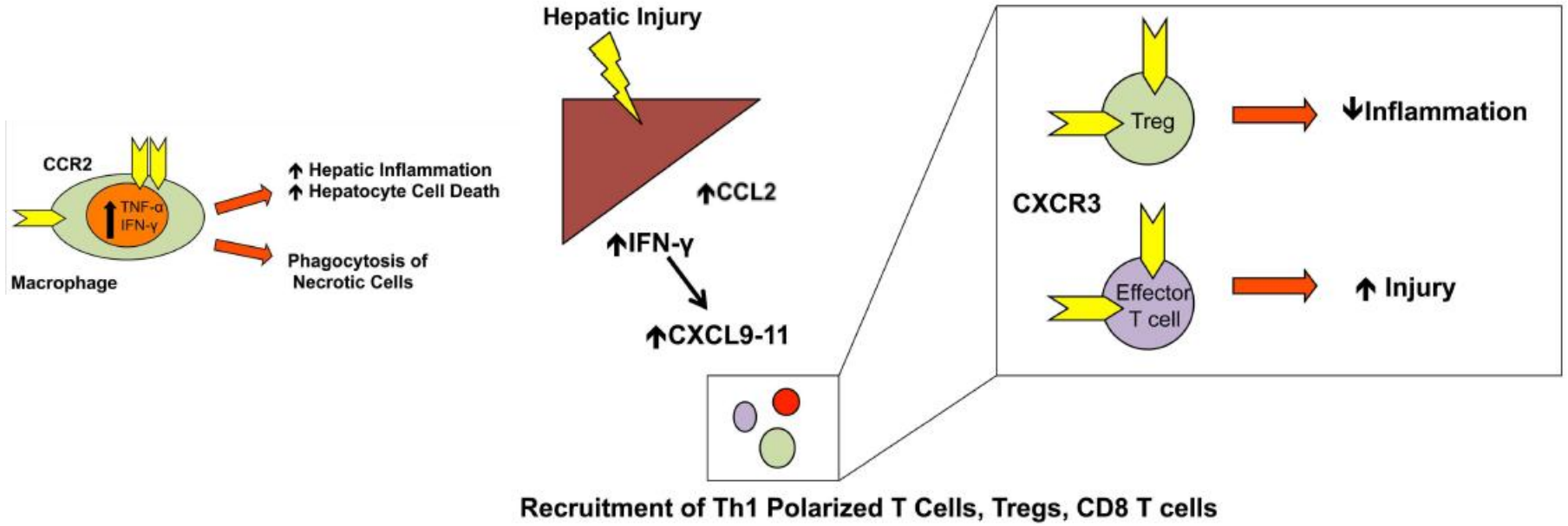


FIGURE 2 | CXCL9–11 T cell recruitment: CXCL9–11 expression is increased in an IFN-γ dependent manner. Numerous T cell populations, including Th1-polarized T cells, Tregs, and effector T cells are recruited to the liver in a CXCR3-dependent manner. The specific type of injury will determine the relative recruitment of Tregs vs. effector T cells and the protective/injurious role of the CXCL9–11/CXCR3 axis.

Saiman Y, Friedman SL. The role of chemokines in acute liver injury. Front Physiol. 2012 Jun 20;3:213.

Conclusions

- Prophylactic administration of 7 different immunosuppressive agents was tested in groups of 10 C57BL/6 mice treated with AAV5-hA1AT
- The prophylactic use of alternative immune suppressive agents did not show any benefit over prophylactic prednisolone
- Rapamycin (IP) administration resulted in a notable (~1 log) reduction in AAV5 TAb titer
- Prophylactic administration of Prednisolone (PO) resulted in a statistically significant increase in hA1AT plasma protein concentrations with associated increase in VG in liver
- Though there were trends of increased transgene expression with Tacrolimus, Mycophenolate, and IL-6R antagonist, the rest of the immune suppressants did not show any statistically significant benefit over Saline with respect to either hA1AT expression or suppression of AAV5 TAb
- No significant cytokine or inflammatory biomarker increases were observed following gene therapy dose administration in the references group
- Prophylactic Prednisolone administration modulated the expression of chemokines involved in immune cell recruitment following acute liver injury.

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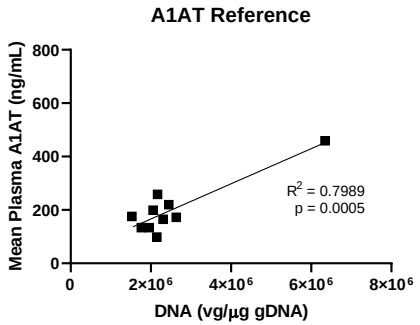
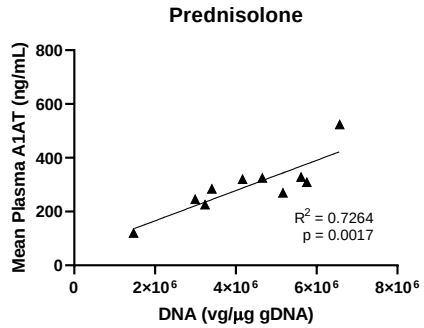
Tara Robinson
Chris Wilson
Cheng Su

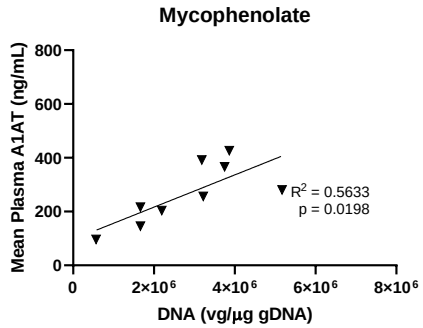
Charles River Laboratories – Ashland, OH

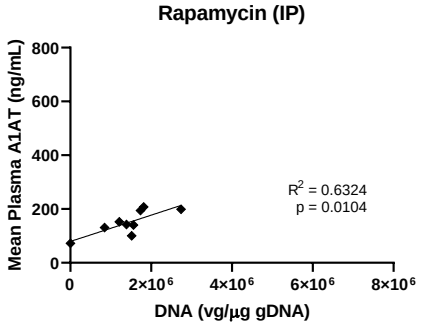
Mindy Tyner

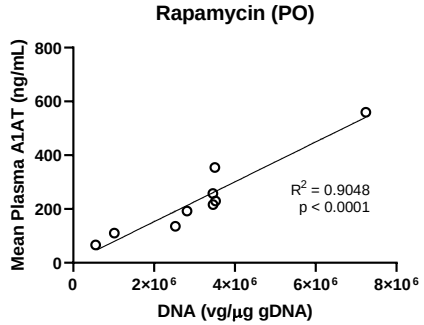
Correlation of hA1AT Plasma Protein with Liver vg Copy Number Individual Treatment Groups

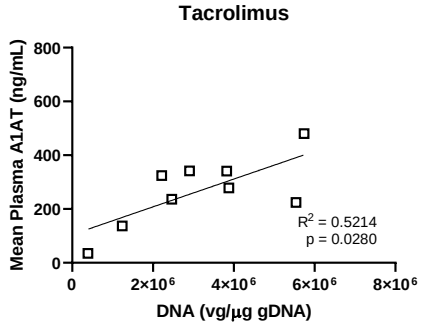


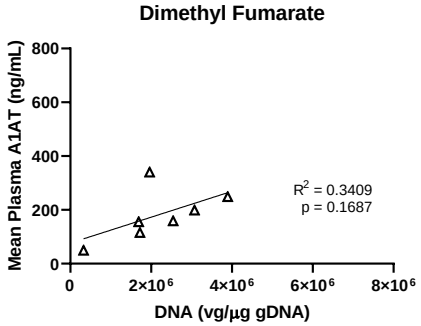


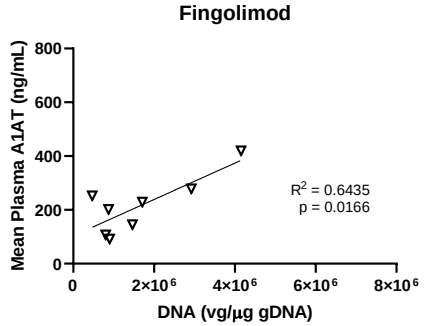


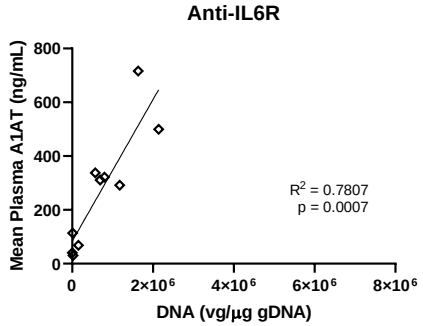












AAV5-A1AT Reference (Saline PO) Control: Biomarker Results Overview