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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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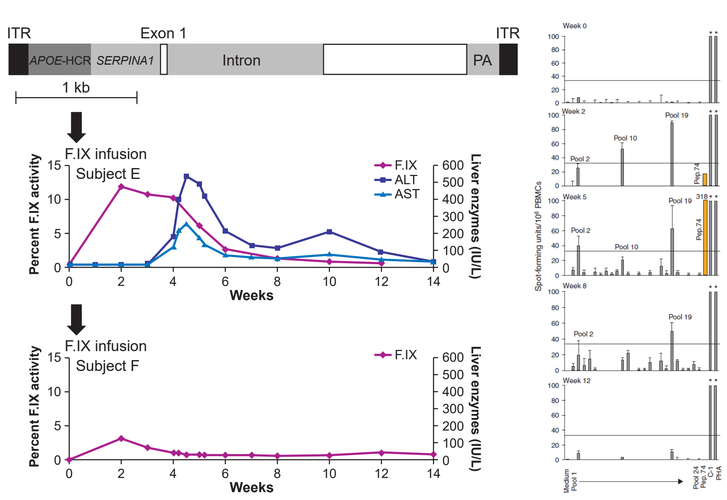
Introduction

The use of immune suppression in AAV-mediated gene therapies

- Transient, asymptomatic ALT elevations have been observed at high doses, suggesting that immunomodulation may be required to preserve long-term expression[1]
- The temporal relationship between PBMC responses to the AAV capsid, declining FIX levels and rising transaminases are all consistent with the hypothesis suggesting that transaminitis arises from specific destruction of vector-transduced cells by the immune system[1]



- A study from the University College London, AAV8-FIX in Hemophilia B, found the following results[2]:
  - Increase in ALT
  - Decline in FIX
  - Detection of Temporally Related Cellular Immune Response
  - Responses resolved with Prednisolone

Methods

Adverse events have been attributed to glucocorticoid treatment

- In the Prophylactic Alternative Immune Suppression Mouse Study design, we tested an alternative immune suppression approach:
  - 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 included: 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 number	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 µg/animal*	AAV5-A1AT	Blocks IL-6 receptor activation and cytokine signaling

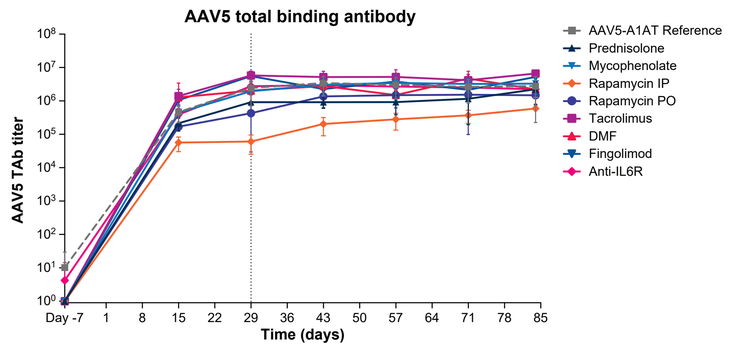
*Loading dose of 300 µg/mL at Day -3. 100 µg/mL on Days 1, 8, 15, 22 and 28. PO, oral gavage; IP, intraperitoneal.

Results

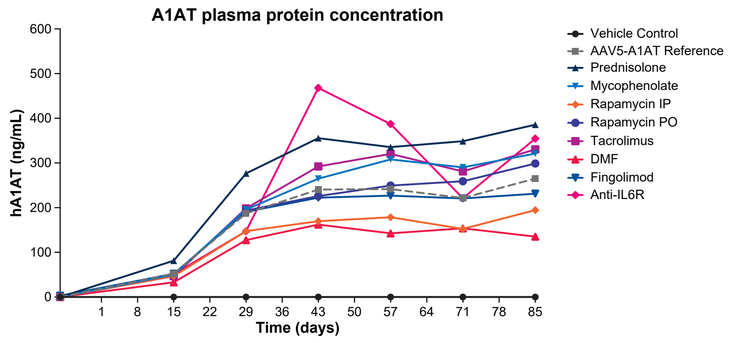
Results summary

- 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

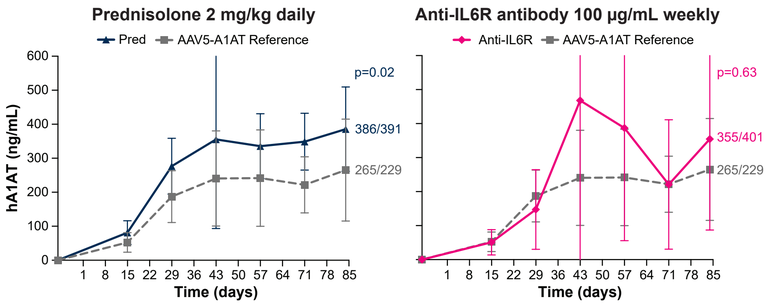


Significant increase in A1AT plasma protein concentration in the prednisolone treatment group – ANOVA summary

Group number	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

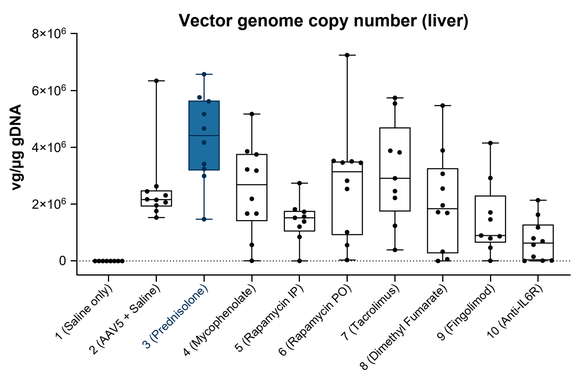
PO,oral gavage; IP, intraperitoneal.

Increased A1AT plasma protein in prednisolone treatment group



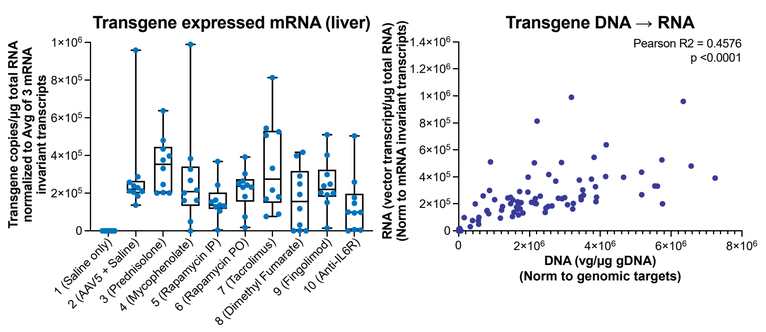
Error Bars are SD. Mann Whitney U test – two tailed at D84 (Mean/Median)

Prophylactic prednisolone improved vector transduction



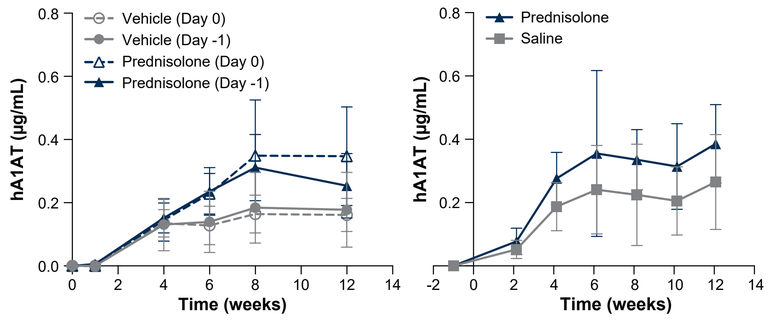
- DNA and RNA were each extracted from two frozen pieces of liver tissue
- DNA was measured and samples were diluted for input into ddPCR of the A1AT transgene and three independent genomic target genes
- Vector DNA normalized to three genomic targets
- Notable amount of inter-animal variability with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

Vector transgene DNA correlates with RNA



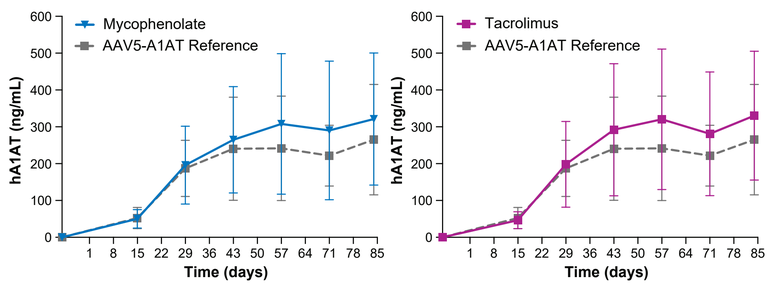
- The use of AIS was not associated with reduced transcription in individual animals

Comparison with previous prophylactic prednisolone study



- Consistent with a previous study, levels of secreted hA1AT protein are increased in prophylactic prednisolone treated mice[3]
- 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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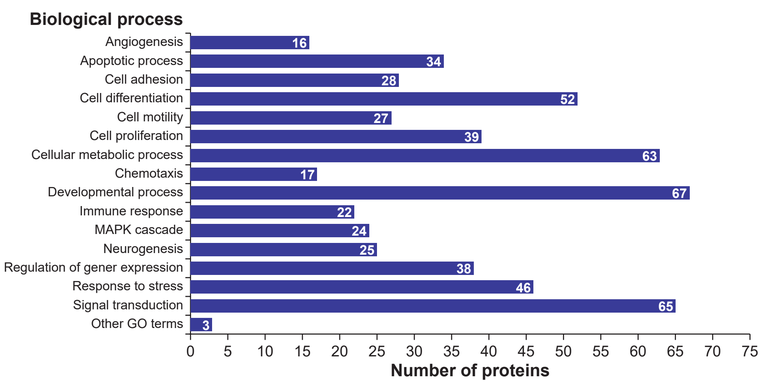
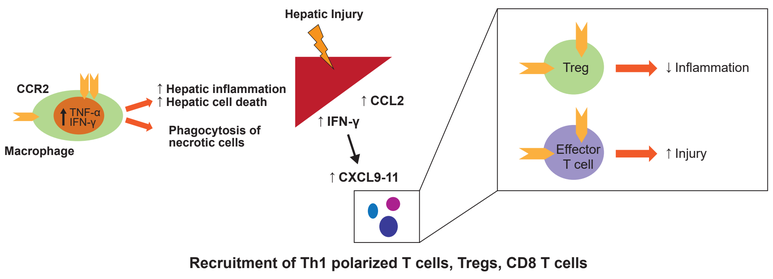
Olink® Target 96 mouse exploratory biomarker panel



- 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

CCL2 and CXCL9 recruit cell populations involved in inflammation and immune modulation downstream of hepatic injury and IFN-γ secretion



Recruitment of Th1 polarized T cells, Tregs, CD8 T cells

- Hepatocytes, stellate cells, sinusoidal endothelial cells, and activated infiltrating lymphocytes all secrete CXCR3 ligands in response to Type 1 and Type 2 interferons[4]

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

References

1. Manno et al. *Nat Med.* 2006 Mar;12(3):342-7. **2.** Nathwani et al. *N Engl J Med.* 2011 Dec 22;365(25):2357-65. **3.** Handyside et al. ASGCT 2022 (Poster 338). The Effect of Prophylactic Corticosteroid Treatment on Adeno-Associated Virus (AAV)-Mediated Gene Expression. **4.** Saiman Y, Friedman SL. The role of chemokines in acute liver injury. *Front Physiol.* 2012 Jun 20;3:213.

Conflict of interest

Brian Long, Britta Handyside, Theresa Seitel, Ryan Boyer, Chris Wilson, Kristin Obrochta Moss, Andrea Van Tuyl, Jeremy Arens, Kelly Lau and Soumi Gupta are employees and stockholders of BioMarin Pharmaceutical Inc.

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