Enhanced level and durability of AAV transgene expression and mitigation of anti-capsid neutralizing antibodies by ImmTOR tolerogenic nanoparticles in nonhuman primates

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Abstract

Immune responses to the capsid or transgene product can lead to the loss of transgene product and the formation of neutralizing anti-AAV8 antibodies (NAb), which prevent the ability to re-dose patients. ImmTOR nanoparticles encapsulating rapamycin have been shown to selectively mitigate AAV immunogenicity and enable vector redosing. Here we explored the impact of different dosing regimens of AAV8 encoding human secreted embryonic alkaline phosphatase (AAV8-SEAP) and ImmTOR nanoparticles on NAb formation and SEAP activity in nonhuman primates. As expected, the control group had an early anti-AAV8 IgM response that transitioned to an anti-AAV IgG response and strong NAb titers by day 84. SEAP activity peaked at day 28 and rapidly declined by day 84, suggestive of an anti-SEAP antibody response. In contrast, the addition of a single dose of ImmTOR delayed anti-AAV8 IgG antibody formation until at least day 56 and reduced NAbs on day 84 in some animals. ImmTOR treatment led to increased and sustained SEAP activity in comparison to the control group. The impact of ImmTOR was most striking in groups investigating 3 monthly doses of ImmTOR, in which anti-AAV8 IgM, IgG and neutralizing antibodies were mitigated. Five of 6 animals had NAb titers <1:5 and the sixth animal had a weak titer of 1:11. Combined with the enhanced and sustained expression of SEAP in these animals, these results indicate that 3 monthly doses of ImmTOR may enhance the level and durability of transgene expression, while inhibiting the formation of NAbs and enabling the possibility of vector re-administration.

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Materials and Methods

- Five groups of NHPs were administered AAV8-SEAP and/or ImmTOR intraveneously. When dosed with both AAV8-SEAP and ImmTOR, animals received the treatments sequentially except for group 3, in which AAV8-SEAP and ImmTOR were admixed together and dosed via a single infusion.
- Animals were bled on days 0, 7, 14, 28, 56 and 84 to assess serum SEAP activity, anti-AAV8 IgG and anti-AAV8 IgM levels. Neutralizing antibody levels were assessed on day 84.
- Serum SEAP expression was determined using the Phospha-Light™ SEAP Reporter Gene Assay System (Invitrogen, Carlsbad, CA)
- Anti-AAV8 IgG and IgM were determined using direct bind ELISAs
- NAb antibodies were assessed using a HEK-293 AAV8-Luc cell-based assay.

Study Design

Group	N	Day 0	Day 28	Day 56
1	3	AAV8-SEAP 2e12vg/kg		
2	5*	AAV8-SEAP 2e12vg/kg* ImmTOR 6 mg/kg		
3	3	AAV8-SEAP 2e12vg/kg ImmTOR admix 6 mg/kg		
4	3	AAV8-SEAP 2e12vg/kg ImmTOR 3 mg/kg	ImmTOR 3 mg/kg	ImmTOR 3 mg/kg
5	3	AAV8-SEAP 2e12vg/kg ImmTOR 3 mg/kg	AAV8-SEAP 0.2e12vg/kg ImmTOR 3 mg/kg	AAV8-SEAP 0.2e12vg/kg ImmTOR 3 mg/kg

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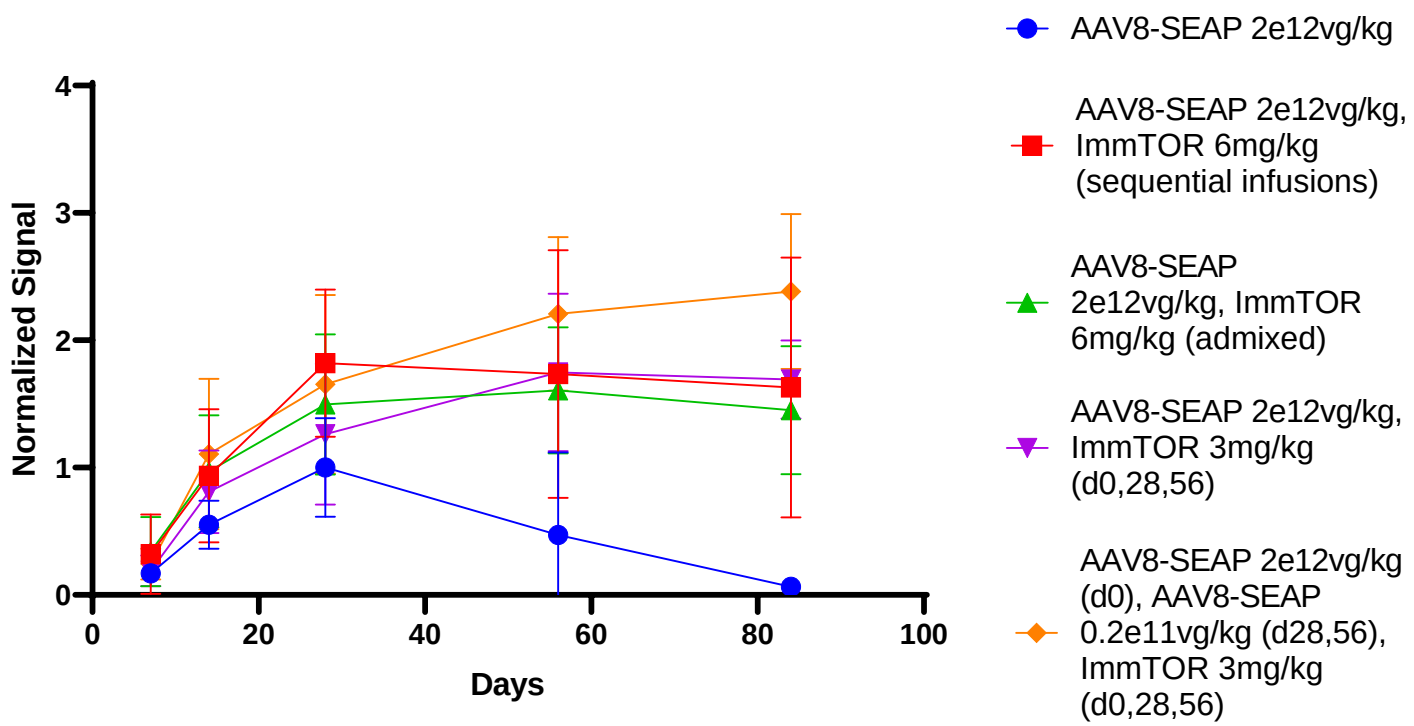
Increased and sustained SEAP expression in combination with ImmTOR



Normalized Signal = Sample SEAP Activity/Control Group Mean SEAP Activity day 7

Mean SEAP activity for the control group (AAV8-SEAP alone) peaks on day 28 and rapidly decreases below day 7 levels. All groups treated with ImmTOR show higher mean SEAP activity than the control on day 28 and remain ~1.5 fold higher than the control of day 28 at the end of the study (day 84). Interestingly, group 5, which received 3 monthly doses of ImmTOR and AAV8-SEAP, (2e12vg/kg d0 and 0.2e12 vg/kg d28, 56) saw a continual rise in mean SEAP activity, indicating the 2 additional doses of AAV8-SEAP were able to supplement the initial AAV8-SEAP transduction.

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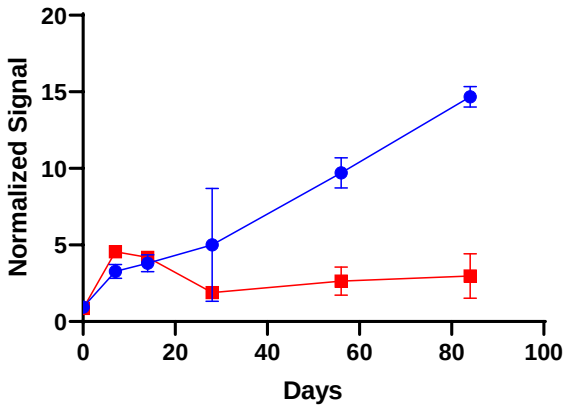
Three Monthly Doses of ImmTOR Mitigates Anti-AAV8 IgG and IgM Formation

A



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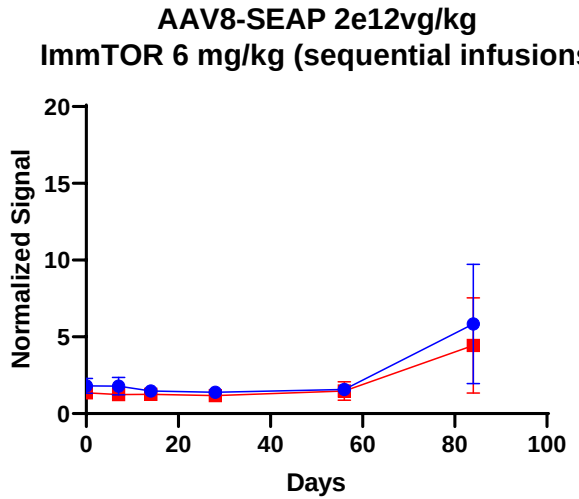

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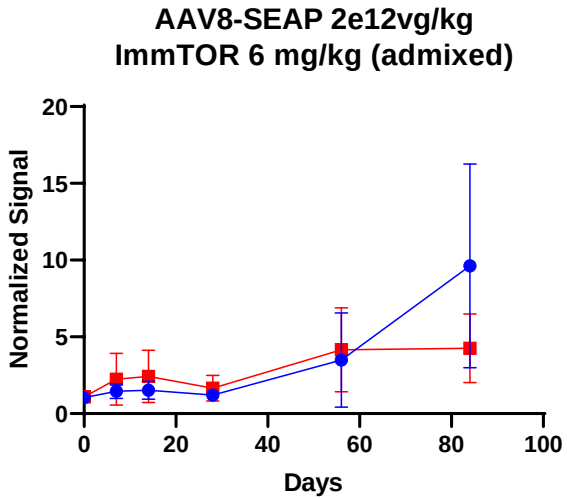
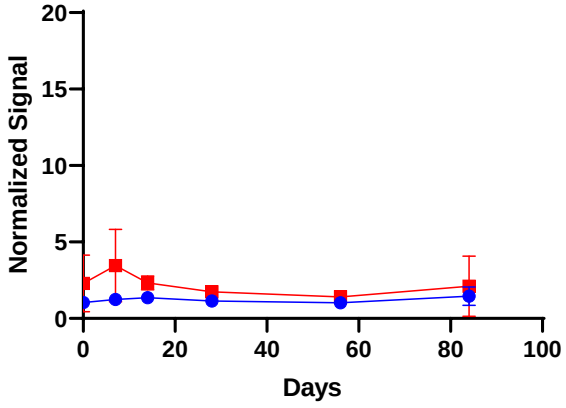


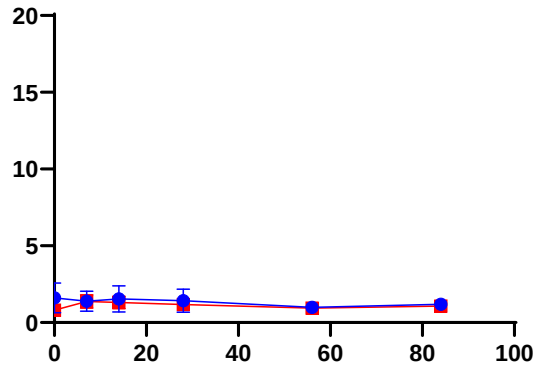
anti-AAV8 IgG

anti-AAV8 IgM

D



E



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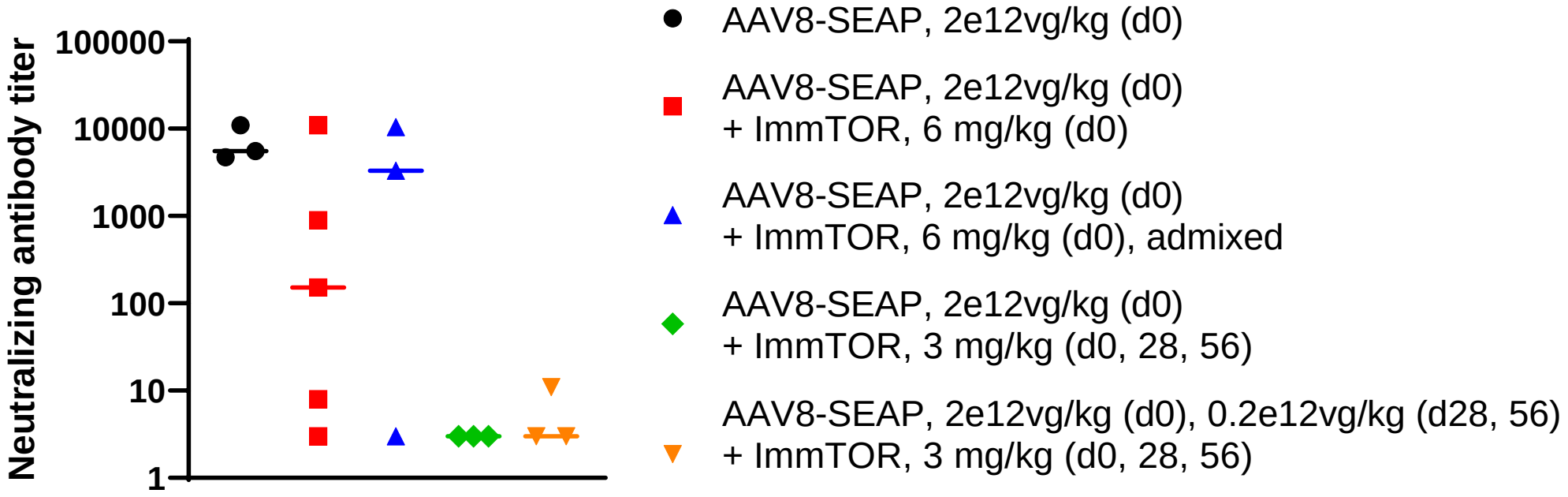
Normalized Signal = Sample OD/Negative Control OD

Three Monthly Doses of ImmTOR Mitigates Anti-AAV8 IgG and IgM Formation

- While a single 6 mg/kg dose of ImmTOR (B, C) delays induction of anti-AAV8 IgG and IgM antibodies compared to the control group (A), by day 84 both groups show an increasing level of anti-AAV8 IgG antibodies. In contrast, in groups that received 3 monthly doses of ImmTOR (D,E), one animal that had pre-existing anti-AAV8 IgM was only transiently positive on day 7 and one animal was positive on day 84 for anti-AAV8 IgM. While anti-AAV8 IgG antibodies were observed transiently, with signals just above the assay cut point, only 1 animal had anti-AAV8 IgG antibodies on day 84 when treated with 3 monthly doses of ImmTOR.

Three monthly doses of ImmTOR are able to mitigate AAV8 NAb development



In the control group which received AAV8-SEAP alone, strong NAb titers were observed in all three animals. A single dose of ImmTOR (groups 2 and 3) resulted in a range of titers, with 2 out of 5 and 1 out of 3 animals with low NAb titers, respectively. Strikingly, 3 monthly doses of ImmTOR (groups 4 and 5) resulted in all 3 animals in group 4 having NAb titers less than 1:5 and 2 out of 3 animals in group 5 having Nab titers less than 1:5. The other animal in group 5 had a low titer of 1:11.

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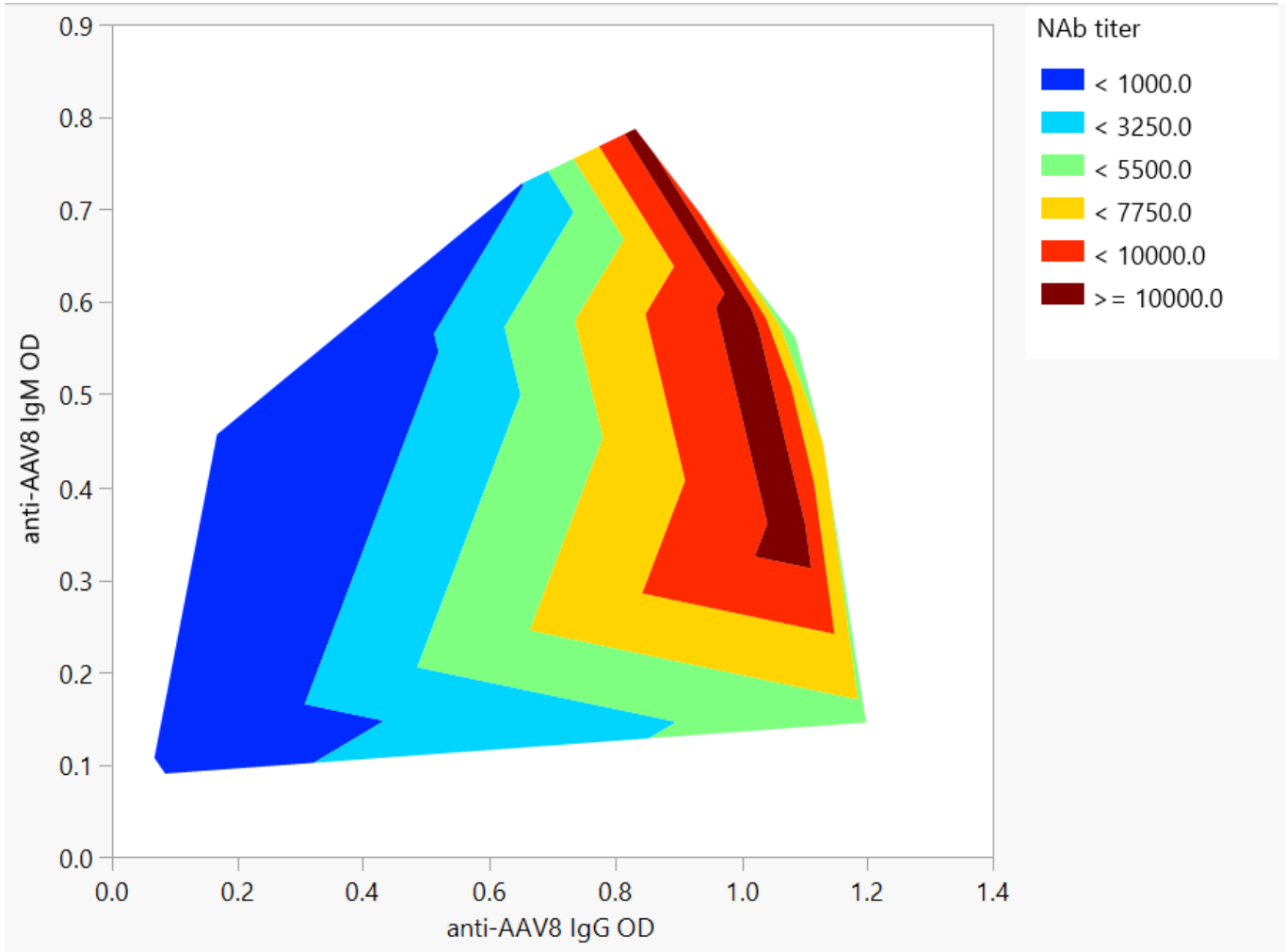
Anti-AAV8 IgG OD Correlates With Anti-AAV8 NAb Titers

A contour plot of anti-AAV8 IgG, IgM and NAb titer, demonstrates that anti-AAV8 IgG are the dominant source of neutralizing activity on day 84, over anti-AAV8 IgM.



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Conclusions

- Administration of ImmTOR with AAV8-SEAP led to enhanced SEAP activity that was durable until the end of the study on day 84
- While a single dose of ImmTOR was able to delay the formation of anti-AAV8 antibodies, 3 monthly doses of ImmTOR was optimal in mitigating the development anti-AAV8 IgG antibody and NAbs.
- Three monthly doses of ImmTOR can enhance the level and durability of transgene expression, while inhibiting the formation of NAbs and enabling the possibility of vector re-administration.

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