ImmTOR nanoparticles enhance the level and durability of AAV transgene expression after initial dosing and mitigate the formation of neutralizing antibodies in nonhuman primates

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Abstract

Achieving durable therapeutic activity is a major challenge for systemic AAV gene therapies. Transgene expression can be adversely affected by immune responses to the capsid or transgene product. Moreover, transgene expression may wane over time due to dilution of the nonreplicating AAV vector during cell turnover in response to injury or inflammation of the target organ or cell proliferation in a growing pediatric patient. The loss of transgene expression is exacerbated by the inability to re-treat patients due to the formation of neutralizing anti-AAV antibodies. We have previously demonstrated that tolerogenic ImmTOR nanoparticles encapsulating rapamycin selectively mitigate anti-AAV T and B cell responses and enable vector redosing in mice and in nonhuman primates (NHP) (Meliani et al., Nature Commun, 2018). Here we extend those findings in a larger NHP study and evaluate the different regimens of ImmTOR treatment on the level and durability of transgene expression after a single dose of AAV vector. Five cohorts of NHP (n=3 per cohort) each received 2e12 vector genomes (vg)/kilogram (kg) of AAV8 expressing human secreted embryonic alkaline phosphatase (SEAP) either alone or in combination with ImmTOR. Cohort 1 received a single dose of AAV vector alone. Cohort 2 was treated the AAV vector in combination with a single dose of ImmTOR, administered as sequential IV infusions. Cohort 3 was the same as Cohort 2, except the two components were admixed together prior to infusion. Cohort 4 received a single dose of AAV vector on day 0 and three monthly doses of ImmTOR on days 0, 28, and 56. Cohort 5 was the same as Cohort 4 with the addition of a low dose (0.2e12 vg/kg) of vector administered on days 28 and 56. Expression of the SEAP transgene product increased over time and peaked at day 28 in Cohort 1 animals receiving vector alone. All of the ImmTOR treated groups showed higher levels of transgene expression compared to Cohort 1, with an average of 60% higher levels of SEAP expression at day 28. Strikingly, SEAP expression declined precipitously after day 28 in Cohort 1, indicating an anti-SEAP immune response. In contrast, all of the cohorts treated with ImmTOR showed stable SEAP expression through day 84, with the exception of Cohort 5 which showed increasing expression of transgene from day 28 to day 84, attributable to the additional low doses of AAV vector administered on days 28 and 56. As expected, all three Cohort 1 animals treated with the AAV vector alone developed high levels of anti-AAV IgG antibodies with neutralizing antibody (NAb) titers greater than 1:4700 at day 84. Cohorts 2 and 3 showed suppression of anti-AAV IgG antibodies through day 56, although some animals exhibited a late antibody response at day 84. In contrast, Cohorts 4 and 5 animals treated with three monthly doses of ImmTOR showed suppression of anti-AAV IgG antibodies through day 84. Five of 6 animals in Cohorts 4 and 5 showed NAb titers <1:5 and the sixth animal showed a low titer of 1:8. Taken together, these results indicate that ImmTOR may enhance the level and durability of transgene expression after initial treatment of AAV vector, while inhibiting the formation of neutralizing antibodies that would enable re-administration of AAV therapy if needed.

Methods

Five cohorts of NHP each received 2e12 vector genomes (vg)/kilogram (kg) of AAV8-SEAP either alone or in combination with 3 or 6 mg/kg of ImmTOR. Cohort 1 (n=3) received vector alone. Cohorts 2 and 3 received a single dose of 6 mg/kg ImmTOR. In Cohort 2, ImmTOR and AAV8-SEAP were administered as sequentially IV infusions, while in Cohort 3, the two components were admixed prior to infusion. Cohorts 4 and 5 received 3 monthly doses of 3 mg/kg ImmTOR. In addition, Cohort 5 received two additional low doses (0.2e12 vg/kg) of AAV8-SEAP on days 28 and 56. All cohorts consisted of three animals, except for Cohort 2 in which two animals were inadvertently received 1.4x and 1.7x of the planned vector dose. These two animals were kept on study, but two additional monkeys were added to Cohort 2 and treated with the correct vector dose.

Serum SEAP activity and anti-AAV8 IgG antibodies were assessed at Days 0, 7, 14, 28, 56, and 84. Neutralizing anti-AAV8 antibody titers were assessed at Day 84.

Cohort	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

*Two animals were inadvertently received 1.4x and 1.7x of the planned vector dose. The animals were kept on study, but two additional animals were added and treated with the correct vector dose

Results

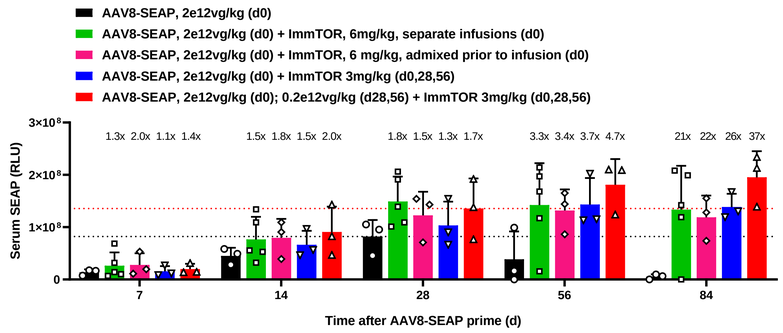
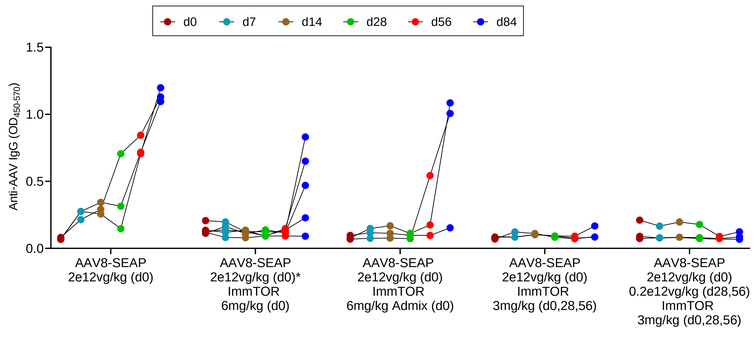


Figure 1 ImmTOR enhances levels and durability of SEAP transgene expression Cohorts of NHP received a single dose of 2e12 vg/kg AAV8-SEAP either alone or together with single dose of ImmTOR or with three monthly doses of ImmTOR. Serum SEAP activity was assessed at Days 0, 7, 14, 28, 56 and 84. SEAP activity increased with time through Day 28. All groups treated with ImmTOR showed increased transgene expression compared to the vector alone treated control, ranging from 1.3x-1.8x increased SEAP activity at Day 28. Strikingly, SEAP activity declined precipitously between Days 28 and 84, consistent with the development of an anti-SEAP antibody response, as previously reported (Majowicz et al., 2017). Unlike for the mouse (Ilyinskii et al, 2021), admixing of ImmTOR with AAV prior to infusion was not required to observe the increase in transgene expression. In contrast, all groups treated with ImmTOR showed stable or increasing transgene expression through Day 84. At Day 84, Cohorts treated with ImmTOR plus a single dose of AAV vector showed 21-26x SEAP activity than the vector alone group at Day 84. Group 5, which received additional low doses of vector on Days 28 and 56, showed a further trend to increased SEAP expression by Day 84.



*Two monkeys in group 2 were inadvertantly dosed with 1.4x and 1.7x AAV8-SEAP and were replaced with two additional animals. Data for all 5 animals shown

Figure 2. Effect of ImmTOR on the formation of anti-AAV IgG antibodies Anti-AAV8 IgG antibodies were assessed at Days 0, 7, 14, 28, 56 and 84. In the control animals treated with AAV8-SEAP alone, antibodies were detected in all three animals as early as 7 days after treatment and antibody levels continue to climb through Day 84. In Cohorts 2 and 3, which received a single dose of ImmTOR at Day 0, anti-AAV8 antibodies were inhibited in all but one animal through Day 56. However, at Day 84 three of the five animals in Cohort 2 and two of the three animals in group 3 developed significant levels of anti-AAV8 IgG antibodies. In contrast, all of the animals in Cohorts 4 and 5, which were treated with 3 monthly doses of ImmTOR showed low or no anti-AAV8 IgG antibodies through Day 84, including one animal in Group5 that showed low levels of pre-existing antibodies at baseline which declined by Day 84.

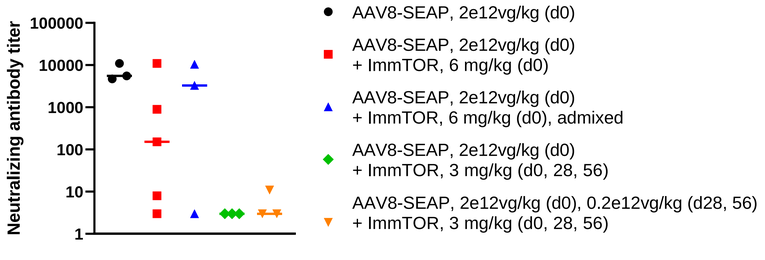


Figure 3. Three monthly doses of ImmTOR inhibits the formation of neutralizing antibodies at Day 84. Anti-AAV8 neutralizing antibodies were assessed at Day 84. Control animals treated with AAV8-SEAP alone nAb titers ranging from 1:4709 to >1:10,395. Cohorts 2 and 3, which received a single dose of ImmTOR at Day 0, showed a wide range of nAb titers. Three animals had no (<1:5) or low (1:8) nAB titers, two animals had intermediate titers (1:153, 1:894), and three animals show high titers (1:3309 to >1:10,935). In contrast, five of the six animals in Cohorts 4 and 5, which were treated with 3 monthly doses of ImmTOR, showed nAb titers <1:5 and the sixth animal showed a low titer of 1:11.

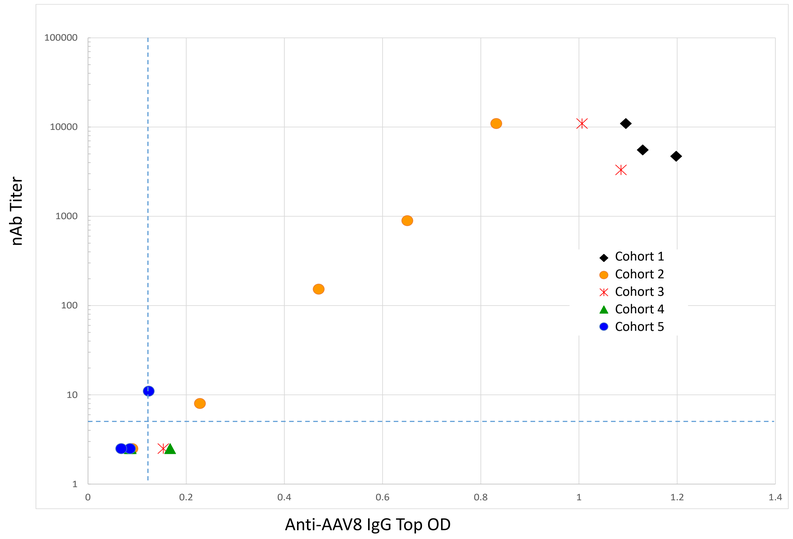


Figure 4. Correlation of anti-AAV IgG antibodies and neutralizing antibodies Individual data for anti-AAV8 IgG and anti-AAV8 nAB titers were plotted for each animal. A high correlation of anti-AAV8 IgG and nAb titers was observed.

Conclusions

- Administration of ImmTOR with an AAV8-SEAP vector induced higher transgene expression after a single dose than vector alone
- A single dose of ImmTOR administered at the time of vector dosing also provided durable SEAP activity compared to vector alone
- A single dose of AAV vector administered with three monthly doses of ImmTOR provided durable inhibition of both anti-AAV8 IgG and nAb antibodies through Day 84
- Five of the six animals treated with three monthly doses of ImmTOR had anti-AAV8 nAb titers <1:5 and the sixth animal had a low titer of 1:11
- These results suggest that ImmTOR has the potential to enhance the levels and durability of transgene expression and enable vector re-dosing
- ImmTOR has been dosed in over 270 patients in combination with a uricase enzyme for the treatment of gout and is currently in Phase 3 clinical testing
- The ability of ImmTOR to inhibit the formation of anti-AAV8 antibodies is currently being investigated in a healthy volunteer study dosed with an AAV8 empty capsid with or without ImmTOR

References

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