Effect of tolerogenic ImmTOR nanoparticles on the formation of anti-AAV8 antibodies in mice, nonhuman primates, and healthy human volunteers

Peter G. Traber[1], Shari Gordon[2], Petr Ilyinskii[1], Sheldon Leung[1], Teresa Capela[1], Alicia Michaud[1], Holly Schroeder[2], Tanika Williamson[2] Gina Rizzo[1], Canwen Jiang[2], David Farve[2], Lloyd Johnston[1], R. Jude Samulski[2,3], Takashi Kei Kishimoto[1]

[1] Selecta Biosciences, 65 Grove Street, Watertown, MA 02472, USA; [2] AskBio HQ, 20 T.W. Alexander Drive, Suite 110, Research Triangle Park, NC 27709, USA;

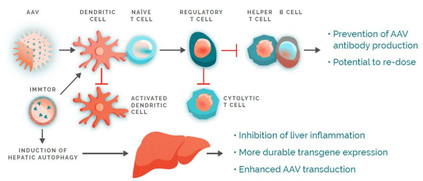
[3] Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Abstract

We previously described the ability of tolerogenic ImmTOR nanoparticles, encapsulating the small molecule immunomodulator rapamycin, to mitigate the formation of anti-AAV antibodies and enable vector re-dosing in mice and nonhuman primates. Here we describe the results of a randomized, placebo controlled, double blind experimental medicine trial in 23 healthy volunteers who were administered a single dose of 2E12 viral particles (vp)/kg of an AAV8-empty capsid formulation (EMC-101), either alone (n=8) or in combination with an immediate pre-treatment with a single dose of 0.15 or 0.30 mg/kg ImmTOR nanoparticles (n=15). Subjects showed baseline anti-AAV8 NAb titers of <1:5 at screening and were followed for 90 days after dosing. The combination treatment was safe and tolerated in healthy volunteers with no serious adverse events. AAV8 empty capsids elicited a strong immune response with peak median anti-AAV8 neutralizing antibody (NAb) titers of 1:6875. ImmTOR showed dose-dependent inhibition of NAb with median Day 30 titers of 1:25 and 1:5 in the 0.15 mg/kg and 0.3 mg/kg ImmTOR cohorts, respectively. All 6 subjects who received 0.3 mg/kg of ImmTOR exhibited an anti-AAV8 neutralizing antibody titer of 1:25 or less, and 4 of 6 had a titer of 1:5 or less at 30 days. At 90 days, 2 of 6 subjects in the 0.3 mg/kg cohort showed sustained control of neutralizing antibodies with titers of 1:5 and 1:25. However, the other four subjects developed neutralizing antibody titers similar to those of control subjects receiving AAV8 capsid alone. Nonclinical studies of C57BL/6 mice dosed with a single dose of ImmTOR combined with a comparable dose of 2e12 vp/kg of the same empty capsid preparation showed breakthrough anti-AAV8 antibodies in only 1 of 6 mice through Day 84; however, mice dosed with a 10-fold higher dose of empty capsid (2E13 vp/kg) showed delayed induction of high titer anti-AAV8 antibodies in 3 of 6 mice. At both empty capsid doses, the administration of two additional doses of ImmTOR on Days 28 and 56 resulted in sustained reduction of anti-AAV8 antibodies in all mice. Similar results were observed in nonhuman primates (NHP), where 2 of 5 animals treated with a single dose of ImmTOR with 2e12 vg/kg AAV8-SEAP showed sustained reduction of anti-AAV8 antibodies through 3 months, while the other three animals showed delayed development of antibodies. Interestingly, a single administration of ImmTOR in NHP and humans resulted in a similar distribution of NAb titers at Day 84 in NHPs, and Day 90 in humans in the 0.3 mg/kg ImmTOR cohort. In the NHP study, 5 of 6 animals treated with three monthly doses of ImmTOR (Days 0, 28, 56) showed neutralizing antibody titers <1:5 at Day 84, while the sixth animal showed a low titer of 1:11. These results show for the first time that empty AAV8 capsids can elicit a robust NAb response in humans and that co-administration of a single dose of ImmTOR can substantially inhibit NAb formation through Day 30. The delayed formation of NAb observed in most subjects suggest that additional monthly doses of ImmTOR may be required to durably inhibit NAb antibody formation.

Introduction

Currently systemic AAV gene therapy cannot be re-dosed due to the formation of neutralizing anti-AAV antibodies. Tolerogenic ImmTOR nanoparticles, encapsulating the small molecule immunomodulator rapamycin, have been shown to induce antigen-selective immune tolerance to co-administered biologic drugs by the formation of antigen-specific Tregs [1]. ImmTOR has demonstrated dose-dependent inhibition of anti-drug antibodies against a fungal derived uricase enzyme[2] and is currently in Phase 3 clinical trials. ImmTOR may have multiple advantageous effects in AAV gene therapy including mitigation of the formation of anti-AAV antibodies and enabling vector re-dosing as demonstrated in mice and nonhuman primates[3,4], inhibition of capsid-specific CD4 T[3] and CD8 T cells (abstract #Tu-213), enhance transgene expression at the initial dose[3], and inhibition of inflammation as shown in a concanavalin A-induced model of liver inflammation[5].



Here we show that ImmTOR inhibits the formation of anti-AAV antibodies after treatment with an empty AAV8 viral particle in mice and humans.

Materials

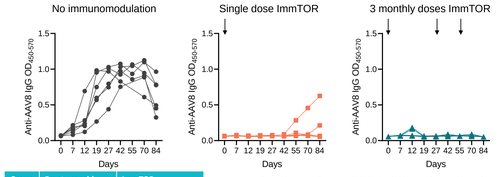
ImmTOR – Biodegradable synthetic nanoparticles comprised of rapamycin, a small molecule inhibitor of the mTOR pathway, embedded in a matrix of poly(lactide) and pegylated poly(lactide).

AAV8 empty capsid – Adeno-associated virus-8 capsid devoid of DNA payload

AAV8-SEAP – Adeno-associated virus-8 encapsulating human secreted alkaline phosphatase transgene

Preclinical results

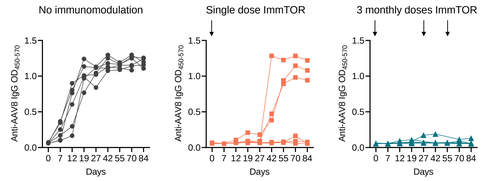
2e12 vp/kg empty AAV8 capsid in C57BL/6 mice



Group	Empty capsid	ImmTOR
1	2e12 vp/kg, day 0	None
2	2e12 vp/kg, day 0	100 µg, day0
3	2e12 vp/kg, day 0	100 µg, day0, 28, 56

A single dose of ImmTOR inhibited antibody formation in all mice out to day 42, but several animals developed antibodies by day 84. Three doses of ImmTOR prevented development of antibodies in all animals at day 84.

2e13 vp/kg empty AAV8 capsid in C57BL/6 mice



Group	Empty capsid	ImmTOR
1	2e13 vp/kg, day 0	None
2	2e13 vp/kg, day 0	200 µg, day0
3	2e13 vp/kg, day 0	200 µg, day0, 28, 56

A single dose of ImmTOR inhibited antibody formation in all mice out to day 27, but half of the animals developed antibodies by day 84. Three doses of ImmTOR prevented development of antibodies in all animals at day 84.

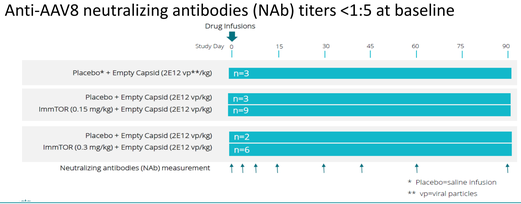
2e13 vg/kg AAV8-SEAP in nonhuman primates

Group	Empty capsid	ImmTOR
1	2e13 vp/kg, day 0	None
2	2e13 vp/kg, day 0	3 mg/kg, day0
3	2e13 vp/kg, day 0	3 mg/kg, day0, 28, 56

A single dose of ImmTOR inhibited antibody formation in all monkeys out to day 56, but several of the animals developed antibodies by day 84. Three doses of ImmTOR prevented development of antibodies in all animals at day 84.

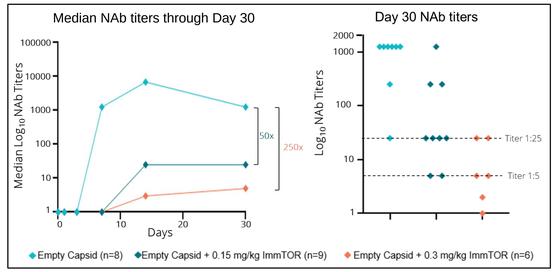
Human Clinical Trial Design

- Randomized, placebo controlled and double-blind study in healthy volunteers; n=23 (14 males and 9 females)
- Anti-AAV8 neutralizing antibodies (NAb) titers <1:5 at baseline

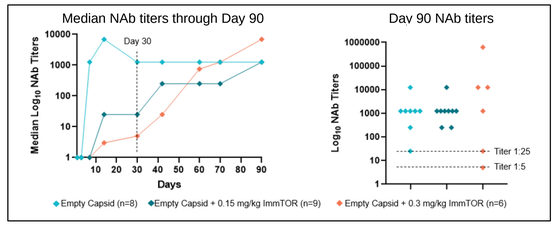


Clinical Trial Results: NAb Titers

A single dose of ImmTOR resulted in a dose-dependent reduction in NAb titers at day 30. ImmTOR 0.3 mg/kg resulted in titers ≤1:25 in all subjects at day 30, including 4 of subjects with titers ≤1:5.



By day 90, NAb titers were similar between groups. In the 0.3 mg/kg ImmTOR group, there remained two subjects with low titers.



Clinical Trial Results: Safety

All treatment-related AEs were expected for ImmTOR, as observed in over 450 subjects treated in other trials, readily monitorable, mild to moderated in grade, and transient.

Overview of Treatment-Emergent Adverse Events (Safety Population)

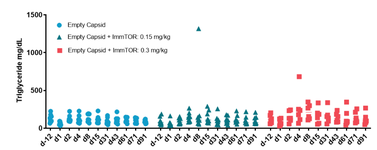
	Control / Normal Saline + EMC-101 (2E12 vg/kg) (N=8) n % m	*SEL-110 (0.15 mg/kg) + EMC-101 (2E12 vg/kg) (N=9) n % m*	*SEL-110 (0.3 mg/kg) + EMC-101 (2E12 vg/kg) (N=6) n % m*	*All SEL-110 + EMC-101 (2E12 vg/kg) (N=15) n % m*
Number of Subjects with any TEAE	5 (62.5) 9	9 (100.0) 40	6 (100.0) 32	15 (100.0) 72
Serious TEAE	0	0	0	0
Non-Serious TEAE	5 (62.5) 9	9 (100.0) 40	6 (100.0) 32	15 (100.0) 72
Severity Grade ≥ 3 TEAE	0	1 (11.1) 1	0	1 (6.7) 1
Fatal TEAE	0	0	0	0
Known ImmTOR/sirolimus AEs				
Stomatitis*		3 (33.3) 4	6 (100.0) 6	9 (60.0) 10
Rash**		3 (33.3) 3	3 (50.0) 3	6 (40.0) 6

TEAE = Treatment-Emergent Adverse Event, n = number of subjects, m = number of events, N = number of subjects in the safety set per treatment

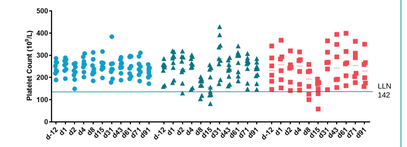
* All grade 1 or 2 (mild or moderate), steroid mouthwash ameliorated symptoms; ** All grade 1 (mild), no treatment required

Results: Key Laboratories

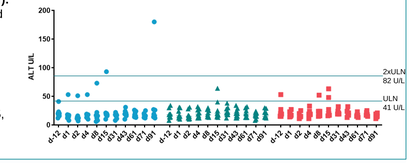
Triglycerides: Two subjects in the ImmTOR groups had transient, asymptomatic increased levels, as observed previously with sirolimus and ImmTOR.



Platelets: Reductions below LLN were seen in 6 subjects in ImmTOR groups at day 8 and 15 after administration with recovery by day 31, as observed previously with sirolimus and ImmTOR.

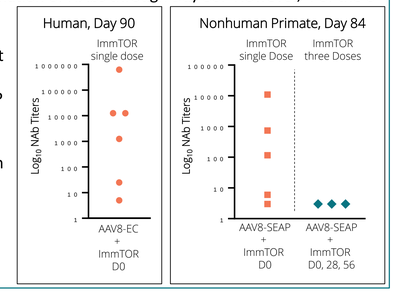


Alanine Aminotransferase (ALT): 2x ULN elevations were observed in 2 subjects receiving Empty Capsid alone, one at day 15 and another at day 91. A small increase of over 1x ULN was observed in one subject in 0.15 mg/kg and two subjects in 0.3 mg/kg ImmTOR groups at day 15, respectively. AST levels mirror these results.



Discussion

We demonstrate here for the first time that empty AAV8 capsids elicits a rapid and robust neutralizing antibody response humans. A single dose of 0.3 mg/kg ImmTOR mitigated the formation of NAbs through Day 30. However, 4 of the 6 subjects developed high NAb titers by day 90. The distribution of NAb titers at 3 months is reminiscent of NAb titers observed in NHP treated with a single dose of ImmTOR. Our results suggest that administration of three-monthly doses of ImmTOR may be required to durably inhibit the formation of NAbs, as observed in NHP.



Conclusions

- AAV8 empty capsids elicited a strong immune response with peak median anti-AAV8 NAb titers of 1:6875
- ImmTOR inhibited the formation of anti-AAV8 NAb in a dose-dependent manner at Day 30.
- After Day 30, 2 of 6 subjects treated with 0.3 mg/kg ImmTOR maintained NAb titers ≤ 1:25, while remaining ImmTOR-treated subjects showed delayed formation of NAb reaching control levels by Day 90
- Animal studies suggest that, if NAb are inhibited at Day 30, administration of two additional monthly doses of ImmTOR may maintain control of NAb beyond 90 days
- Safety findings previously observed with ImmTOR, with no new signals.
- This promising study in healthy volunteers provides support for the potential use of ImmTOR for the inhibition of neutralizing antibodies to AAV8 in gene therapy clinical trials

References

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