ImmTOR combined with B cell-targeted therapies provides synergistic activity in mitigating anti-AAV capsid antibody responses and enables repeated vector dosing

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Abstract

ImmTOR tolerogenic nanoparticles encapsulating rapamycin have been demonstrated to mitigate immunogenicity of AAV vector, elevate transgene expression and enable vector redosing in several animal models including mouse model of methylmalonic acidemia, an inborn metabolic disease[1-3]. While ImmTOR has been shown to directly inhibit germinal center plasmablasts, the primary mechanism of action is thought to be the induction of tolerogenic antigen-presenting cells that induce antigen-specific regulatory T cells[4]. However, in the mouse model ImmTOR only partially inhibits the initial T cell-independent B cell IgM antibody response and blocks subsequent class-switching to IgG. The residual anti-capsid IgM response can have neutralizing activity and affect the efficiency of vector re-administration. Here we evaluated the combination of ImmTOR with currently available B cell targeting agents to mitigate the IgM response and increase the efficiency of re-dosing. ImmTOR combined with a monoclonal antibody (mAb) directed against B cell activation factor (BAFF), a B cell survival factor, synergistically reduced anti-AAV IgM antibodies, provided more durable suppression of anti-AAV IgG antibodies, and enabled multiple re-administrations of an AAV8 vector. Similar, but a weaker effect was observed when ImmTOR was combined with ibrutinib, a Bruton's tyrosine kinase inhibitor. Most advantageous regimens of ImmTOR (monthly) and aBAFF (bi-weekly) led to complete absence of IgG response and minimal IgM response to AAV. This was seen even after four successive AAV administrations over several months at doses up to 5E12 vg/kg and after two AAV administrations at a high 5E13 vg/kg dose, which is similar to therapeutic doses of AAV used in multiple clinical trials. While ImmTOR alone had little or no effect on total splenic B cells or immature pre-B cells, anti-BAFF mAb reduced total B cells by ~50% and increased pre-B cells by ~2-3 fold. The combination of ImmTOR and anti-BAFF mAb showed a synergistic effect in increasing splenic pre-B cells and reducing B cell plasmablasts. These results suggest that ImmTOR could be combined with belimumab, an anti-BAFF mAb to further mitigate anti-AAV antibody responses and enable repeated AAV administration at sufficiently high, but not excessively elevated AAV doses. This approach may lead to stable expression of therapeutic transgene using AAV doses that have been shown to be well-tolerated in humans and thus provide an immense clinical benefit.

Suboptimal doses of ImmTOR do not always enable successful AAV redosing even if IgG is fully suppressed

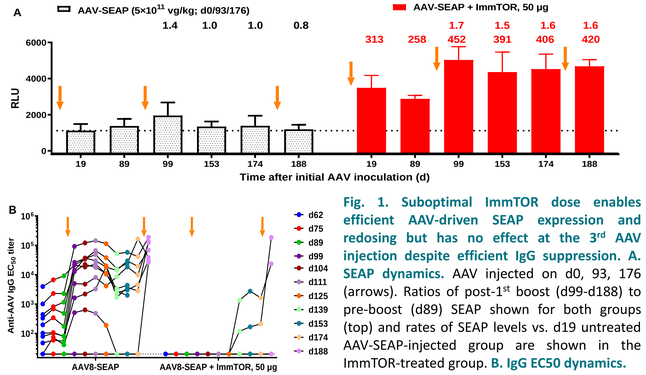


Fig. 1. Suboptimal ImmTOR dose enables efficient AAV-driven SEAP expression and redosing but has no effect at the 3rd AAV injection despite efficient IgG suppression. A. SEAP dynamics. AAV injected on d0, 93, 176 (arrows). Ratios of post-1st boost (d99-d188) to pre-boost (d89) SEAP shown for both groups (top) and rates of SEAP levels vs. d19 untreated AAV-SEAP-injected group are shown in the ImmTOR-treated group. **B. IgG EC50 dynamics.**

IgM: the likely AAV neutralization culprit

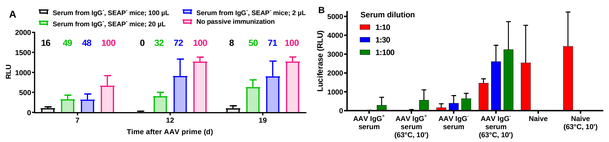
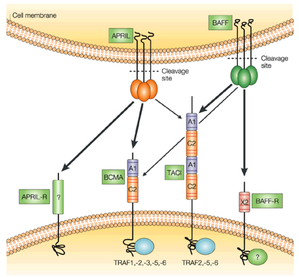


Fig. 2. AAV IgG-negative serum from mice treated with ImmTOR possesses neutralizing activity due to IgM. A. Passive immunization with AAV8 IgG-negative serum blocks AAV8-SEAP transduction. Pooled sera from the mice treated with AAV8-SEAP and ImmTOR as in Fig. 1 was used to passively immunize mice prior to AAV8-SEAP injection (5E11 vg/kg). All individual serum samples used for the pool had an OD <0.04 in AAV8 IgG ELISA and no SEAP activity (meaning no effective vector transduction w/o detectable IgG). SEAP dynamics is shown. **B. In vitro AAV neutralization by IgG-negative serum is inhibited by IgM inactivation.** Pooled sera from the studies in Figs. 1 and 2A either with high levels of IgG against AAV8 or those determined to be IgG-negative were incubated with AAV8-Luc either neat or after IgM heat-inactivation and AAV8-Luc was used for in vitro transduction. This inactivation regimen did not affect AAV8 neutralization by IgG⁺ serum, but efficiently restored transgene expression if IgG-negative serum was used.

Anti-BAFF combined with ImmTOR blocks B cell to plasma cell transition, suppresses IgM and allows more efficient AAV redosing even at very high AAV doses



B-Cell Activating Factor (BAFF)

- TNF family member
- Critical B cell survival cytokine
- Binds three receptors, BAFF-R, TACI, and BCMA

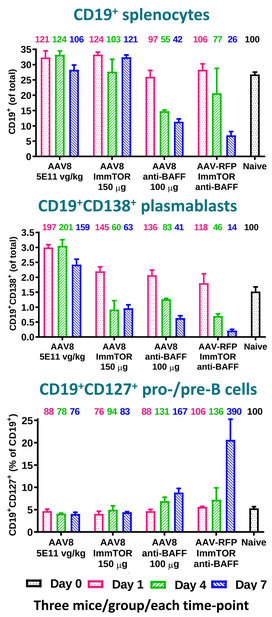
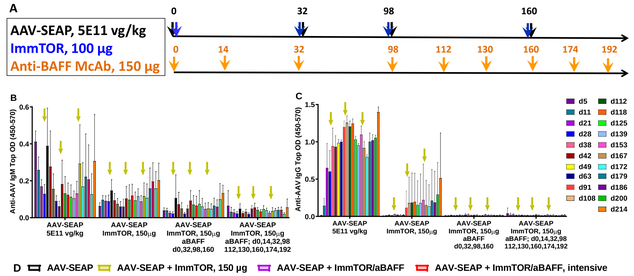


Fig. 3. ImmTOR and aBAFF synergize to decrease splenic B cells, plasmablasts and elevate pro-/pre-B cells counteracting the activation by AAV. The size of each cell fraction vs. that of naïve mice at 100 is shown. ImmTOR decreases splenic plasmablasts, but not mature splenic B cells, while aBAFF affects both populations and synergizes with ImmTOR; their combination leads to accumulation of immature B cells early after AAV challenge.

ImmTOR and anti-BAFF combination for AAV redosing



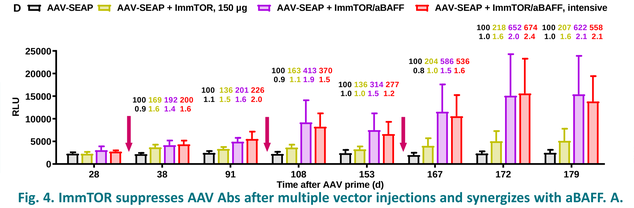


Fig. 4. ImmTOR suppresses AAV Abs after multiple vector injections and synergizes with aBAFF. A. Experimental scheme. B. IgM dynamics. C. IgG dynamics. Times of AAV redosing are shown by arrows. **D. Dynamics of serum SEAP levels.** Relative transgene expression is shown (top) with AAV8-SEAP alone assigned a number of '100'; bottom line: ratio of expression increase after redosing vs. the last pre-redosing time-point. Redosing using ImmTOR and aBAFF lead to ~6× elevation of SEAP.

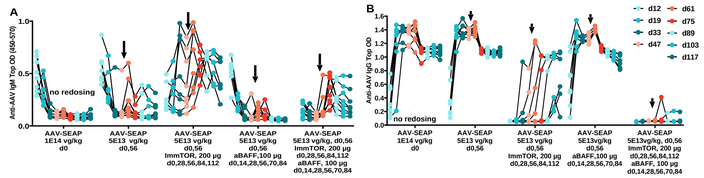
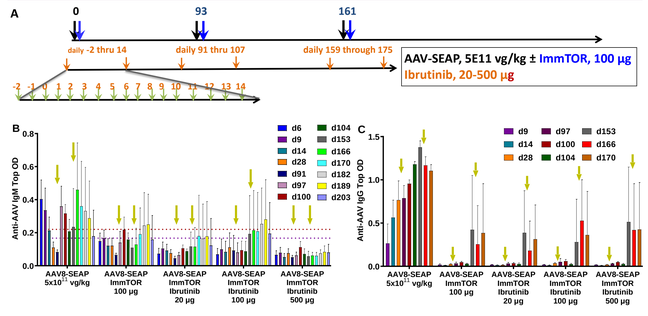


Fig. 5. ImmTOR and aBAFF synergize to prevent AAV IgM and IgG induction (A, B) and enable redosing with 5E13 vg/kg. C. SEAP dynamics. Dotted lines – d19 SEAP in 1E14 and 5E13 vg/kg groups (no ImmTOR)

ImmTOR and ibrutinib for IgM inhibition AAV redosing



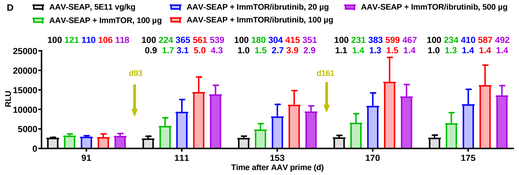


Fig. 6. Ibrutinib combined with ImmTOR leads to efficient AAV IgM control in a dose-dependent fashion and enables vector redosing

A. Experimental scheme. B, C. AAV IgM and IgG dynamics. D. SEAP expression dynamics. Relative transgene expression and ratio of expression increase after redosing is shown (top and bottom lines).

Conclusions

- Even a full suppression of IgG by ImmTOR co-administered with AAV vector does not always enable successful AAV redosing in C57BL/6 mice.
- This is caused by incomplete IgM suppression by ImmTOR followed by effective IgM-driven AAV neutralization.
- B cell-targeting agents, such as a monoclonal antibody against BAFF (aBAFF) or ibrutinib, acting on B cell receptor-activated Bruton tyrosine kinase, provide much better control of AAV IgM when used in combination with ImmTOR and enable effective AAV redosing.
- ImmTOR and aBAFF synergize to inhibit B cell transition to plasma cells and therefore ImmTOR and aBAFF combination is capable of controlling antibody development and enabling efficient vector redosing even if high and clinically relevant AAV doses of 5E13 vg/kg are used.

References

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