



Efficient suppression of IgG antibody responses to high doses of AAV8 capsids by single and multiple administrations of ImmTOR nanoparticles

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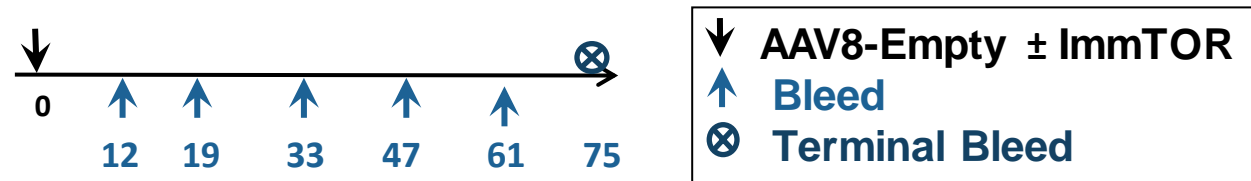
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Efficient suppression of IgG antibody responses to high doses of AAV8 capsids by single and multiple administrations of ImmTOR nanoparticles

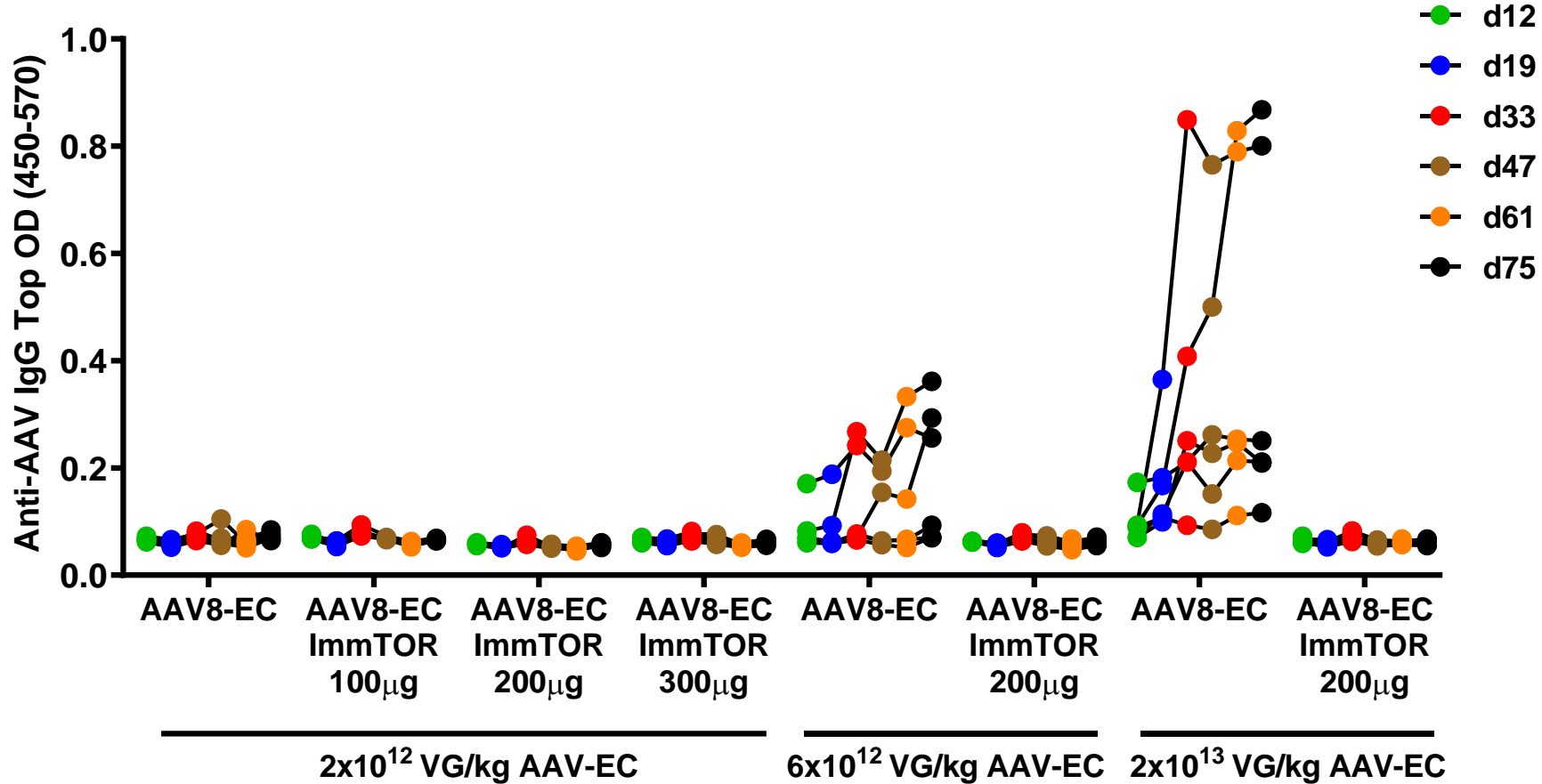
Achieving durable systemic AAV gene therapy may require repeat AAV dosing. Currently, re-dosing is prevented by the formation of neutralizing antibodies. We have previously demonstrated that tolerogenic ImmTOR nanoparticles encapsulating rapamycin mitigate AAV immunogenicity and enable vector redosing in mice and nonhuman primates at moderate vector doses of $\sim 2 \times 10^{12}$ vg/kg. Here we evaluated the ability of ImmTOR to block IgG formation using higher doses of AAV8 empty capsids (AAV8-EC). A single dose of 100 μ g ImmTOR completely abrogated IgG responses to 2×10^{13} vector particles (vp)/kg AAV8-EC through day 62 in Balb/C mice and in the majority (10/12) of C57BL/6 mice at $2-6 \times 10^{12}$ vp/kg AAV8-EC. However, ImmTOR was less efficient at 2×10^{13} vp/kg in C57BL/6 mice, with delayed breakthrough of antibodies observed in most animals at 200 μ g. Higher doses of ImmTOR (300 μ g) inhibited IgG formation in the majority of mice (9/12) at 2×10^{13} vp/kg. We next evaluated administration of two additional monthly doses of ImmTOR. Mice treated with 2×10^{13} vp/kg capsid and three 200-300 μ g monthly ImmTOR doses developed little or no IgG through Day 84. Thus, repeated administration of ImmTOR provides more durable suppression of antibodies against higher viral capsid doses. Monthly dosing of ImmTOR has been shown to be well tolerated and effective in mitigating immunogenicity of a fungal-derived uricase therapy in gout patients and is currently in Phase III clinical trials.

AAV8-EC in BALB/c and C57BL/6 mice (6/group): layout 1

Gr. #	Prime, RO	AAV8-EC Dose	ImmTOR
1	AAV8-EC	2×10^{12} VG/kg	None
2	AAV8-EC + ImmTOR	2×10^{12} VG/kg	100 μ g
3	AAV8-EC+ ImmTOR	2×10^{12} VG/kg	200 μ g
4	AAV8-EC + ImmTOR	2×10^{12} VG/kg	300 μ g
5	AAV8-EC	6×10^{12} VG/kg	None
6	AAV8-EC + ImmTOR	6×10^{12} VG/kg	200 μ g
7	AAV8-EC	2×10^{13} VG/kg	None
8	AAV8-EC + ImmTOR	2×10^{13} VG/kg	200 μ g

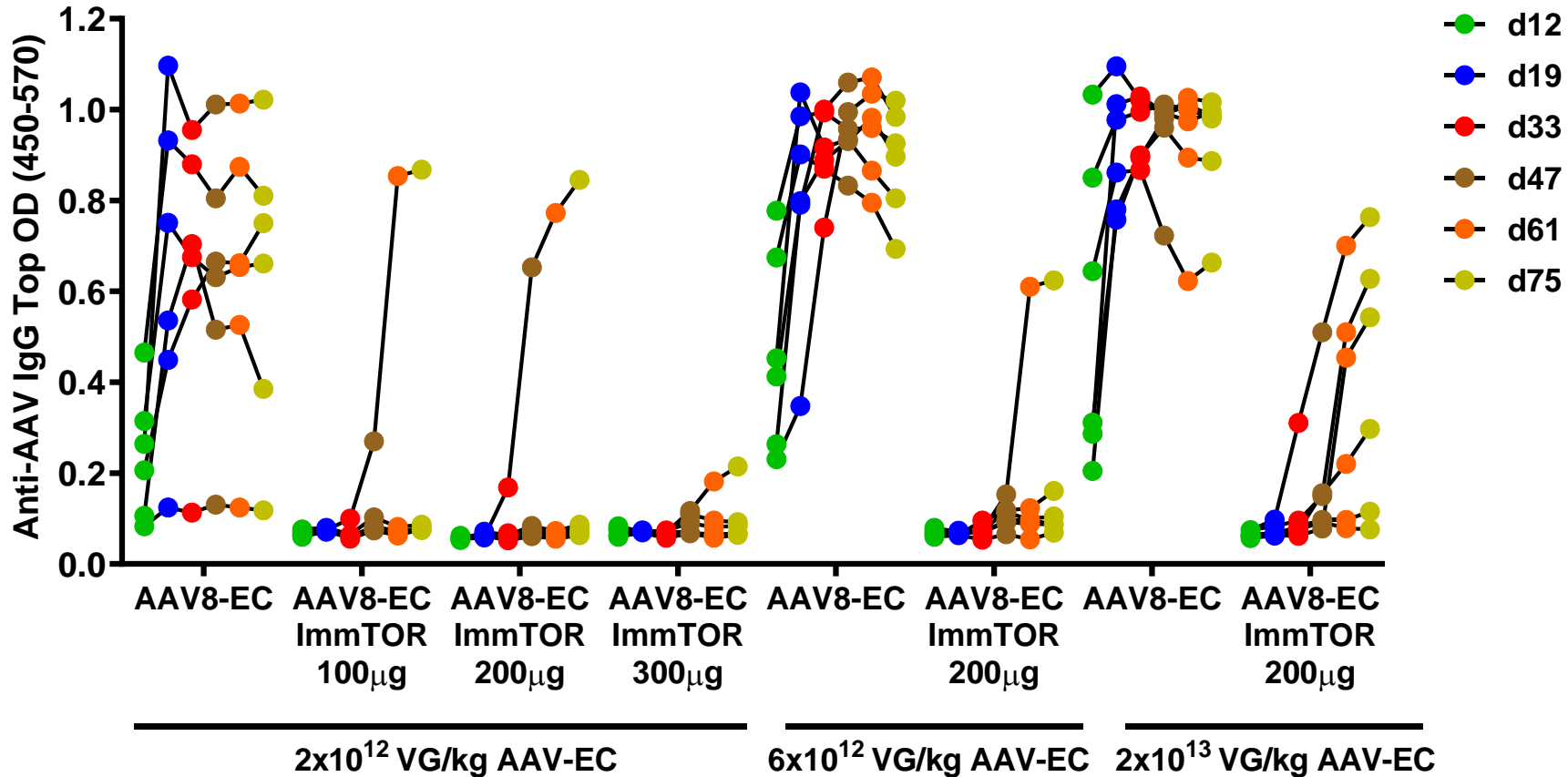


AAV8-EC in BALB/c: IgG dynamics



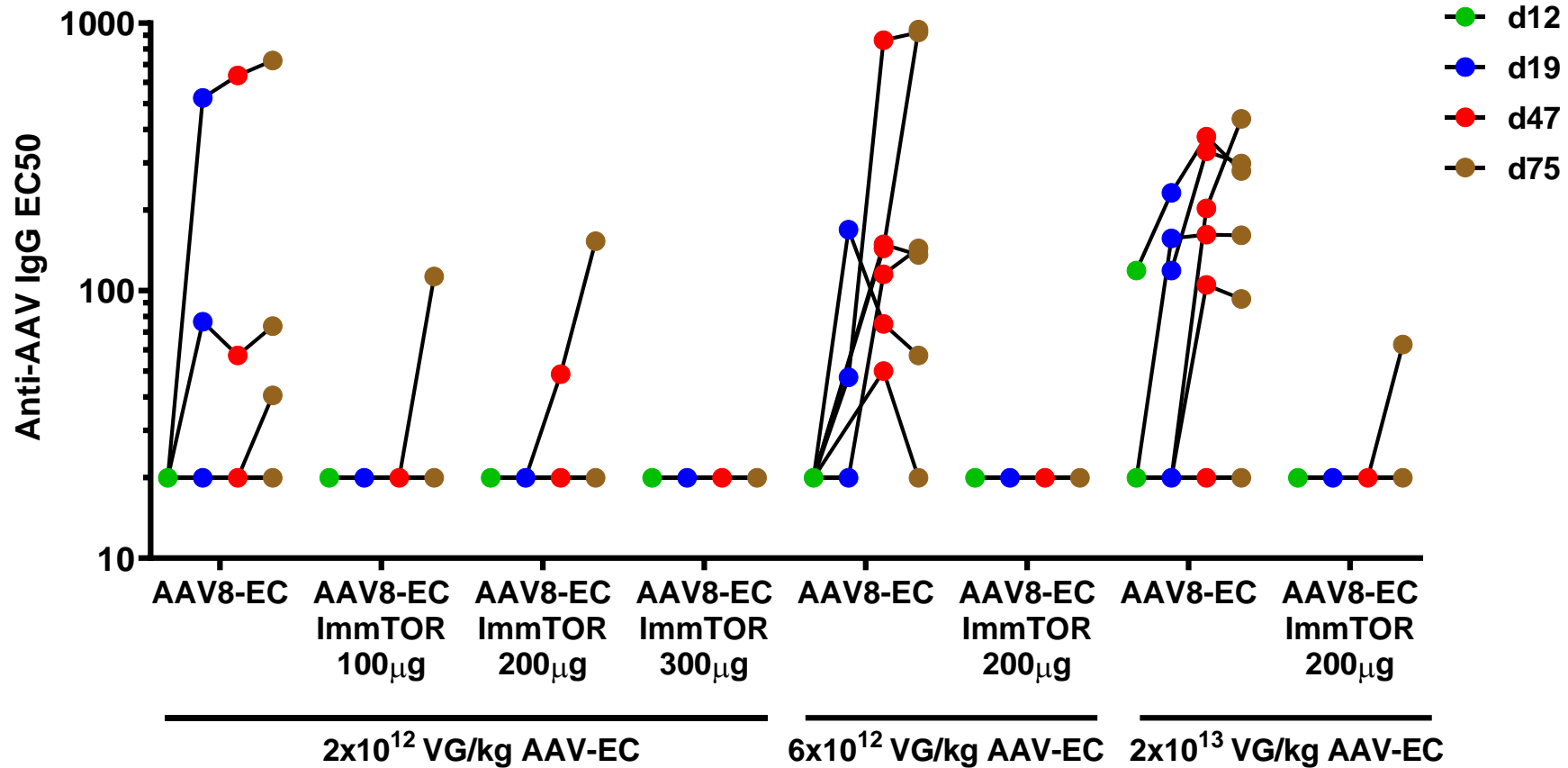
Only high 2×10^{13} vg/kg dose of AAV8-EC is sufficiently immunogenic in BALB/c mice
ImmTOR completely suppresses IgG response to AAV8-EC

AAV8-EC in C57BL/6: IgG dynamics



AAV-EC is strongly immunogenic in C57BL/6 mice, dose-dependent IgG induction; no AAV IgG in all ImmTOR-treated groups by d33, late isolated breakthroughs in low-/mid-dose groups, more by d75 in high EC dose/ImmTOR

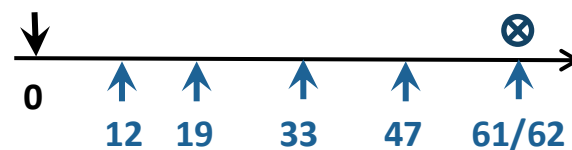
AAV8-EC in C57BL/6: IgG dynamics (EC50)



Dose-dependent IgG induction by AAV-EC; 5/6 mice in mid-/high-dose groups have detectable titers by the end of the study, only isolated mice have detectable titers if ImmTOR is co-administered

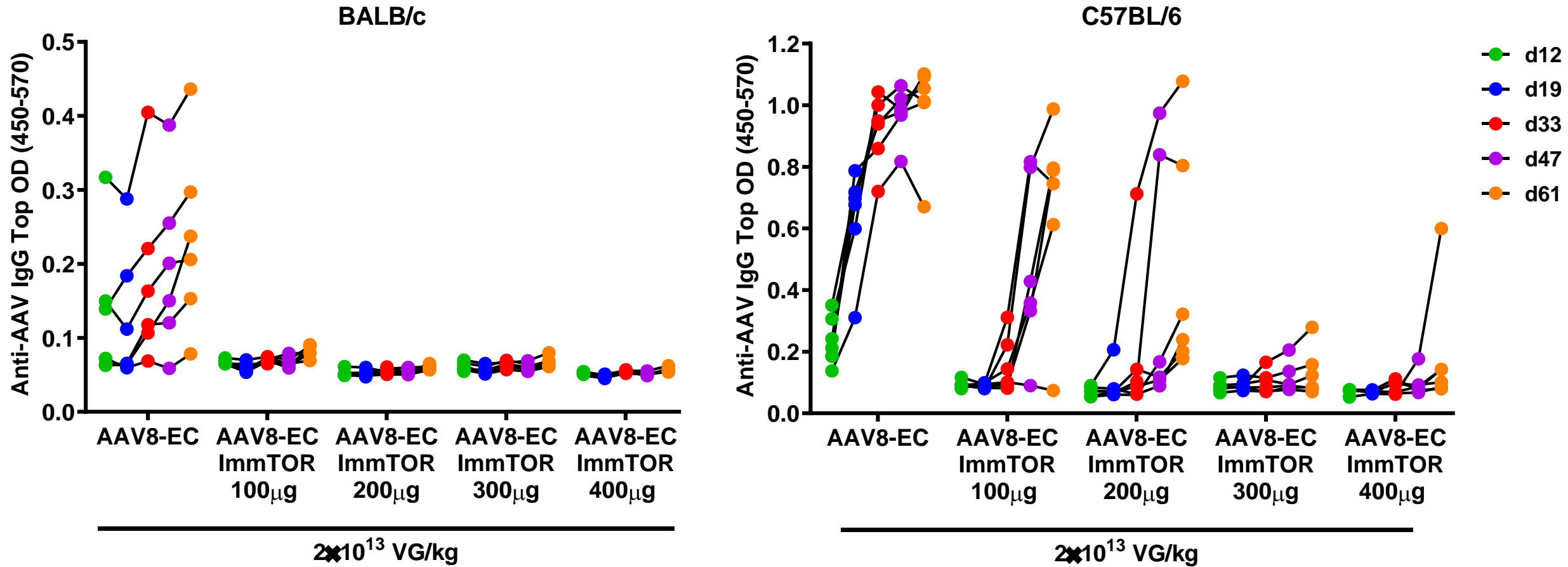
AAV8-EC in BALB/c and C57BL/6 mice: layout 2 (high EC dose)

Gr. #	Prime, RO	AAV8-EC Dose	ImmTOR
1	AAV8-EC	2×10^{13} VG/kg	None
2	AAV8-EC + ImmTOR	2×10^{13} VG/kg	100 μ g
3	AAV8-EC + ImmTOR	2×10^{13} VG/kg	200 μ g
4	AAV8-EC + ImmTOR	2×10^{13} VG/kg	300 μ g
5	AAV8-EC + ImmTOR	2×10^{13} VG/kg	400 μ g



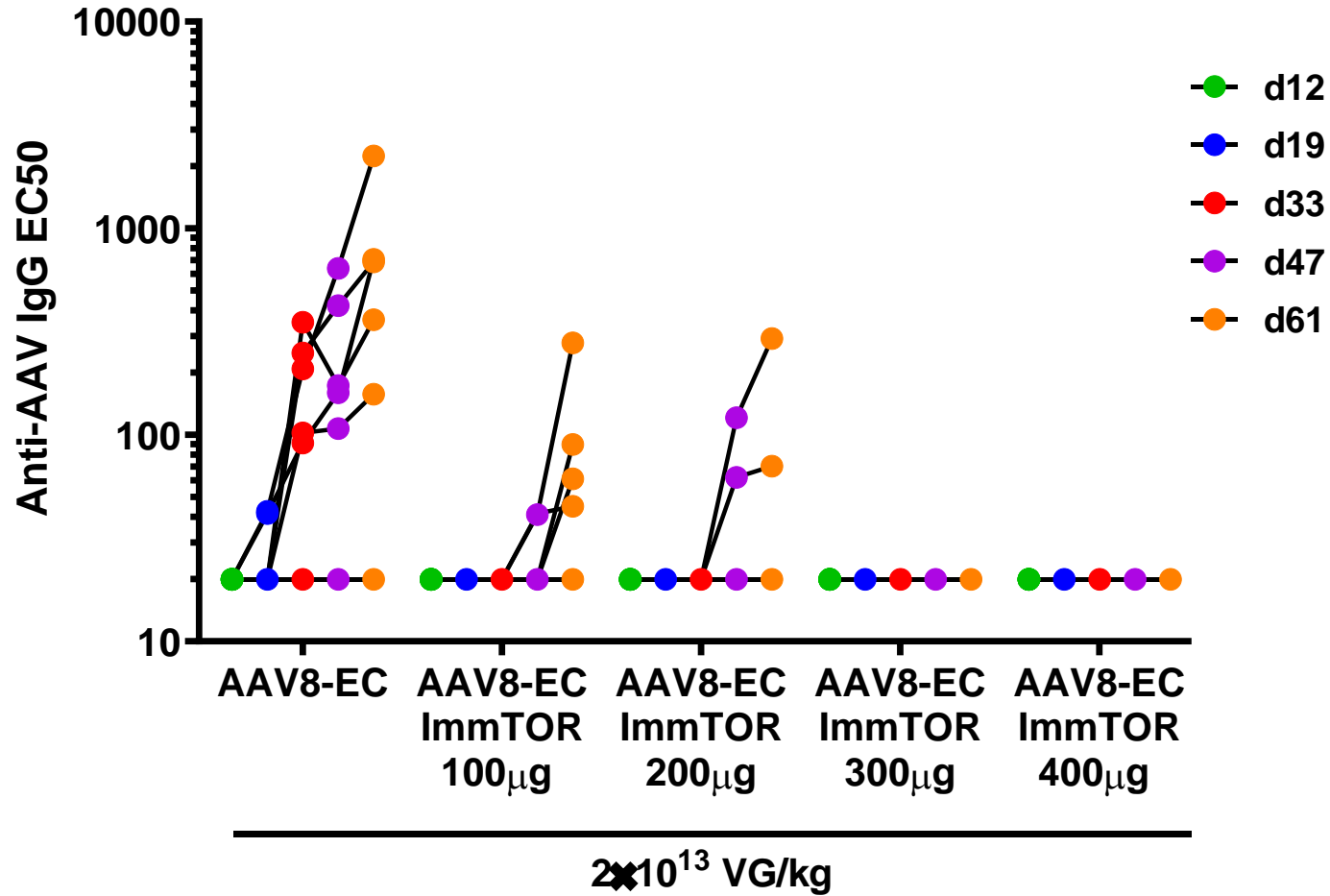
↓ AAV8-Empty ± ImmTOR
↑ Bleed
⊗ Terminal Bleed

AAV8-EC in BALB/c and C57BL/6 mice: layout 2 (high EC dose)



Suppression of AAV8 IgG by a single-dose ImmTOR in BALB/c, $\geq 300 \mu\text{g}$ dose needed for full suppression in C57BL/6 mice

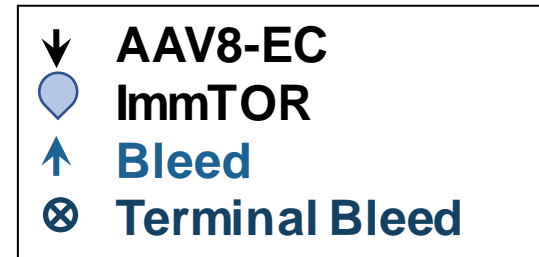
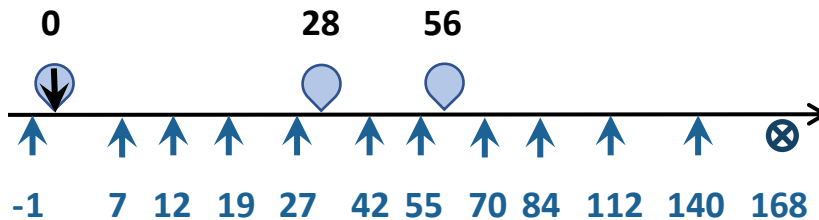
AAV8-EC in C57BL/6 mice: IgG EC50 (high EC dose)



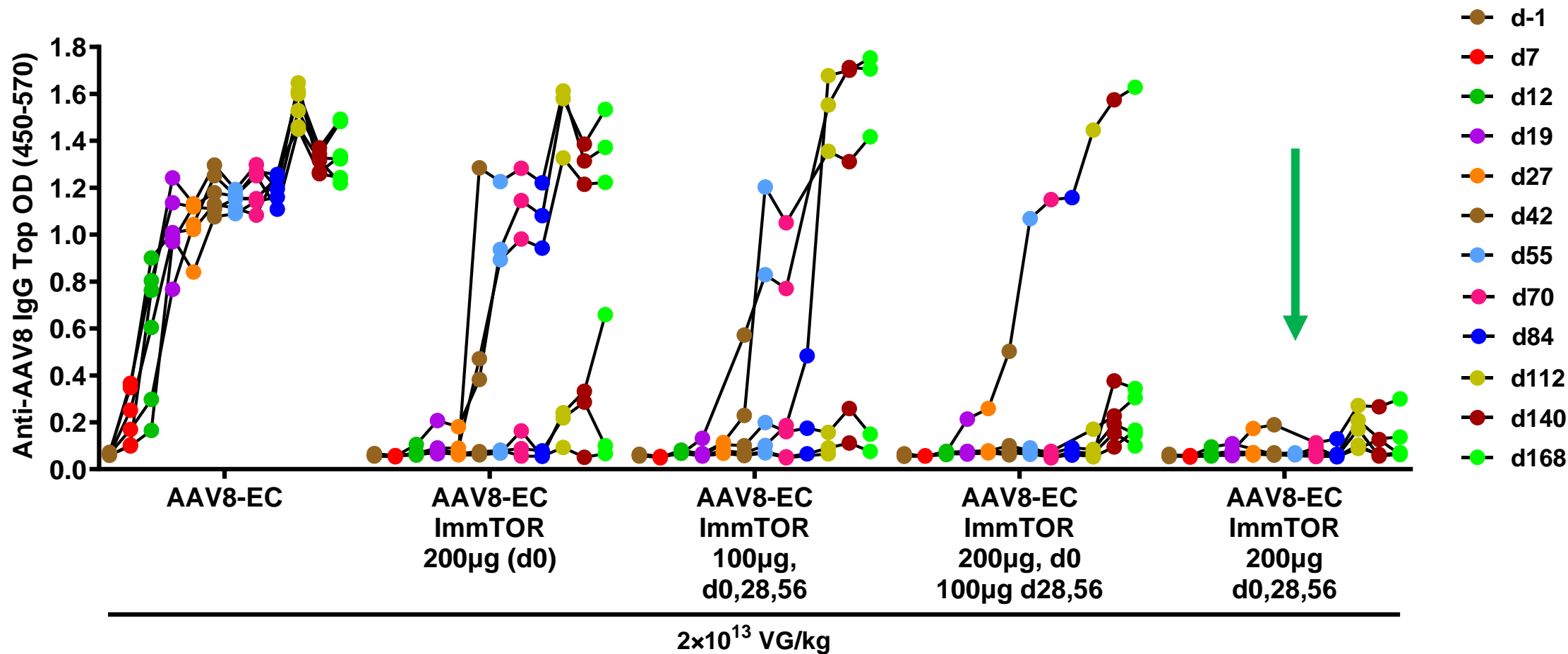
No detectable IgG EC50 titers at a single-dose ImmTOR of $\geq 300 \mu\text{g}$

AAV8-EC in C57BL/6 mice: layout 3 (high EC dose; monthly ImmTOR)

Gr. #	Prime, RO	AAV8-EC Dose	ImmTOR
1	AAV8-EC	2×10^{13} VG/kg	None
2	AAV8-EC+ ImmTOR	2×10^{13} VG/kg	200 μ g, d0
3	AAV8-EC + ImmTOR	2×10^{13} VG/kg	100 μ g; d0, 28, 56
4	AAV8-EC + ImmTOR	2×10^{13} VG/kg	200 μ g, d0 100 μ g, d28 & 56
5	AAV8-EC + ImmTOR	2×10^{13} VG/kg	200 μ g; d0, 28, 56



AAV8-EC in C57BL/6: layout 3 (high EC dose; monthly ImmTOR)



Single mid-dose ImmTOR not superior to 3 monthly low doses
 Full IgG control with 3 monthly injections of mid-dose ImmTOR

Conclusions

- AAV8-EC empty capsids are moderately immunogenic in BALB/c and strongly immunogenic in C57BL/6 mice over the dose range tested (2×10^{12} - 2×10^{13} vg/kg)
- Complete IgG suppression by 100 μ g single-dose ImmTOR to 2×10^{12} - 2×10^{13} vg/kg AAV8-EC in BALB/c mice
- Strong IgG suppression by 200 μ g single-dose ImmTOR to 2 - 6×10^{12} vg/kg AAV8-EC in C57BL/6 mice
- Complete or near-complete long-term IgG suppression with three-monthly doses of ImmTOR even to a high dose of 2×10^{13} vg/kg AAV8-EC in C57BL/6 mice