



**ImmTOR combined with B cell-targeted therapies
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AAV capsid antibody responses and enables
repeated vector dosing**

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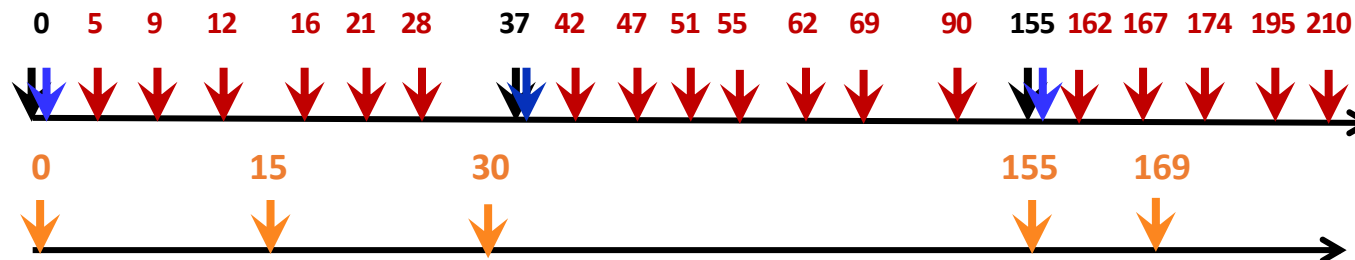
PO Ilyinskii, CJ Roy, AM Michaud, GL Rizzo, T Capela, SS Leung, TK Kishimoto
Selecta Biosciences, 65 Grove Street, Watertown, MA 02472, USA

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

ImmTOR tolerogenic nanoparticles encapsulating rapamycin have been demonstrated to mitigate immunogenicity of AAV vector and enable vector redosing (Meliani et al., Nature Commun 2018; Ilyinskii et al., Science Adv 2021). 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. However, ImmTOR only partially inhibits the initial T cell-independent B cell IgM antibody response and inhibits 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 effects were observed when combined with ibrutinib, a Bruton's tyrosine kinase inhibitor. 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, or ibrutinib to further mitigate anti-AAV antibody responses.

Synergy of ImmTOR and aBAFF for AAV8 antibody mitigation

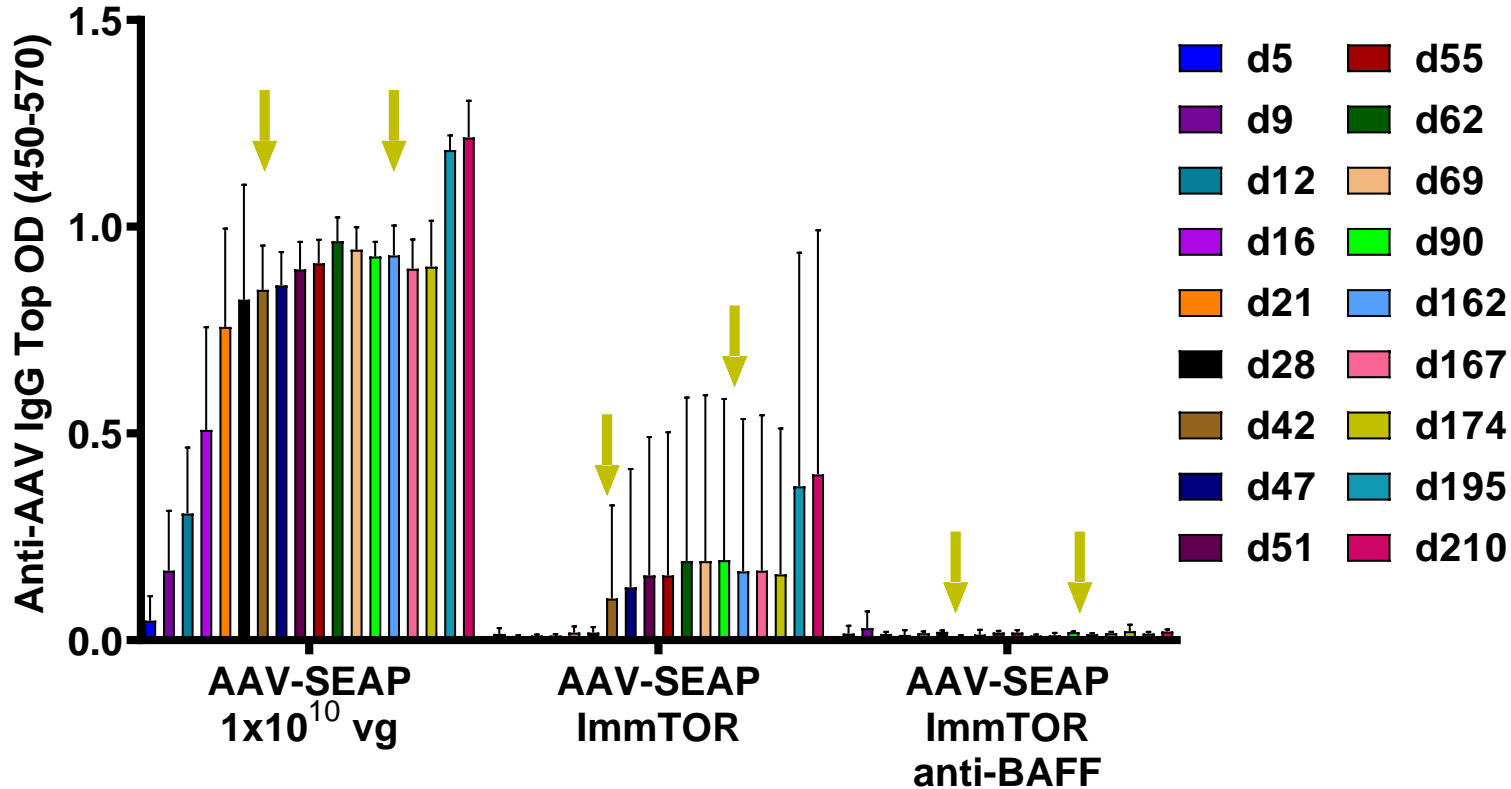
Gr. #	Injection (d0)	ImmTOR d0, 37, 155	aBAFF; d0, 15, 30, 155, 169
1	AAV8-SEAP, 1×10^{10} VG	None	None
2	AAV8-SEAP, 1×10^{10} VG + ImmTOR	150 μ g	None
3	AAV8-SEAP, 1×10^{10} VG + ImmTOR + anti-BAFF	150 μ g	100 μ g, i.p.



AAV-SEAP + ImmTOR
 Anti-BAFF McAb
 Bleed

ImmTOR suppresses AAV IgG after multiple vector injections and synergizes with aBAFF

AAV IgG (d5, 9,12,16, 21, 28, 42, 47, 51, 55, 62, 69, 90, 162, 167, 174,195, 210)

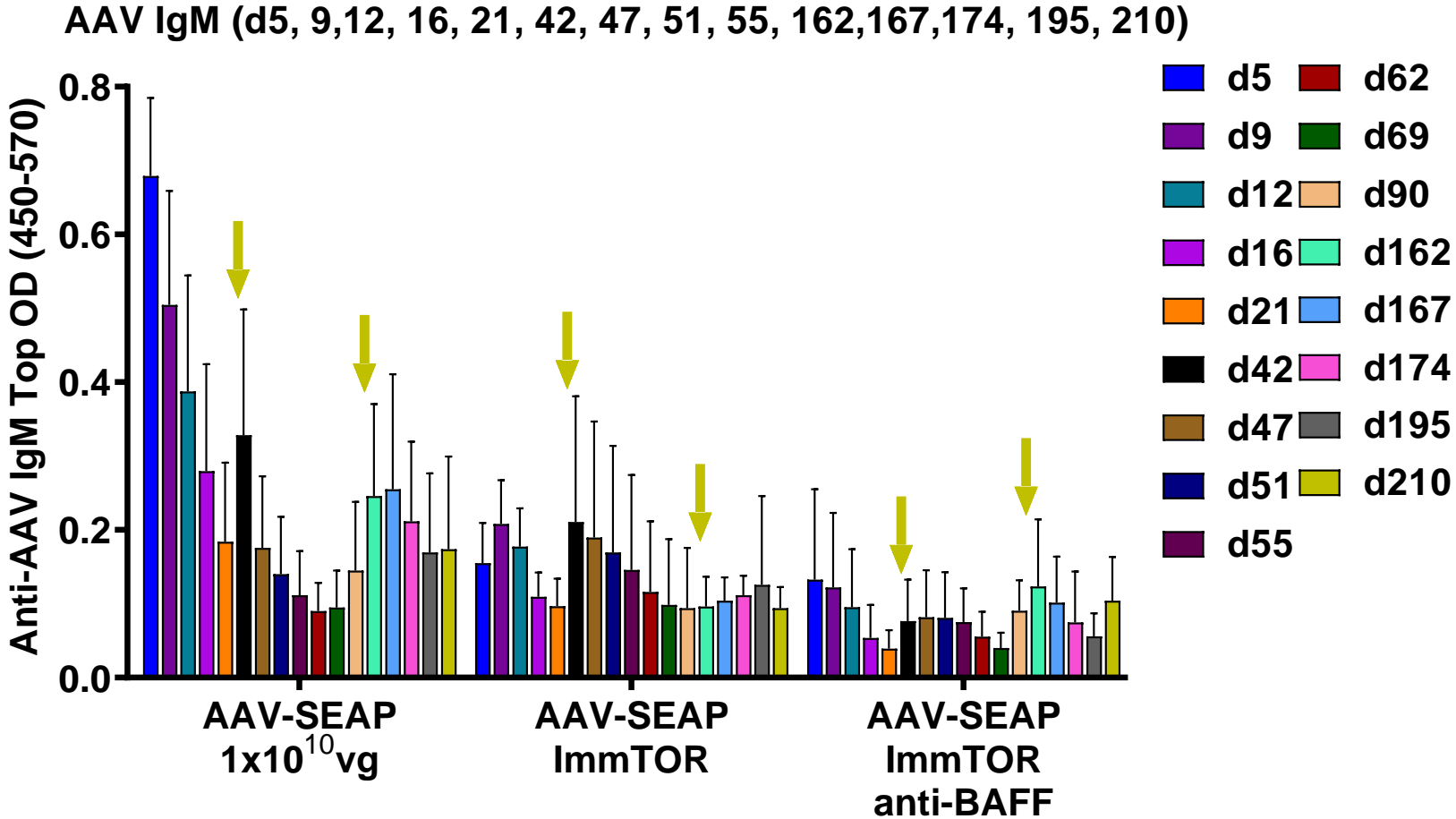


ImmTOR: 1/6 IgG breakthrough after the 2nd dose (d37); 2/6 breakthroughs late after the 3rd dose (d155)

ImmTOR + aBAFF: no breakthroughs after three AAV8 doses up to 210 days

Arrows: times of AAV redosing

ImmTOR suppresses AAV IgM after multiple vector injections and synergizes with aBAFF



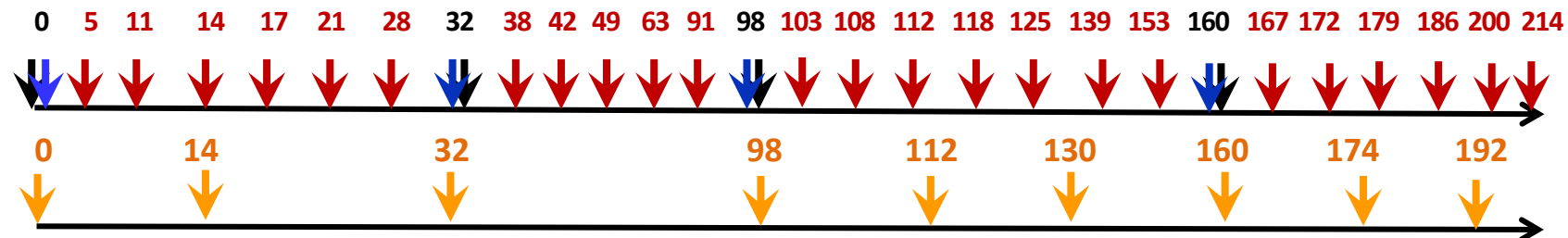
ImmTOR: late and moderate IgM elevation after the 1st dose, another temporary elevation after the 2nd dose
 ImmTOR + aBAFF: low IgM throughout the study with minimal elevation after every AAV redosing

Arrows: times of AAV redosing



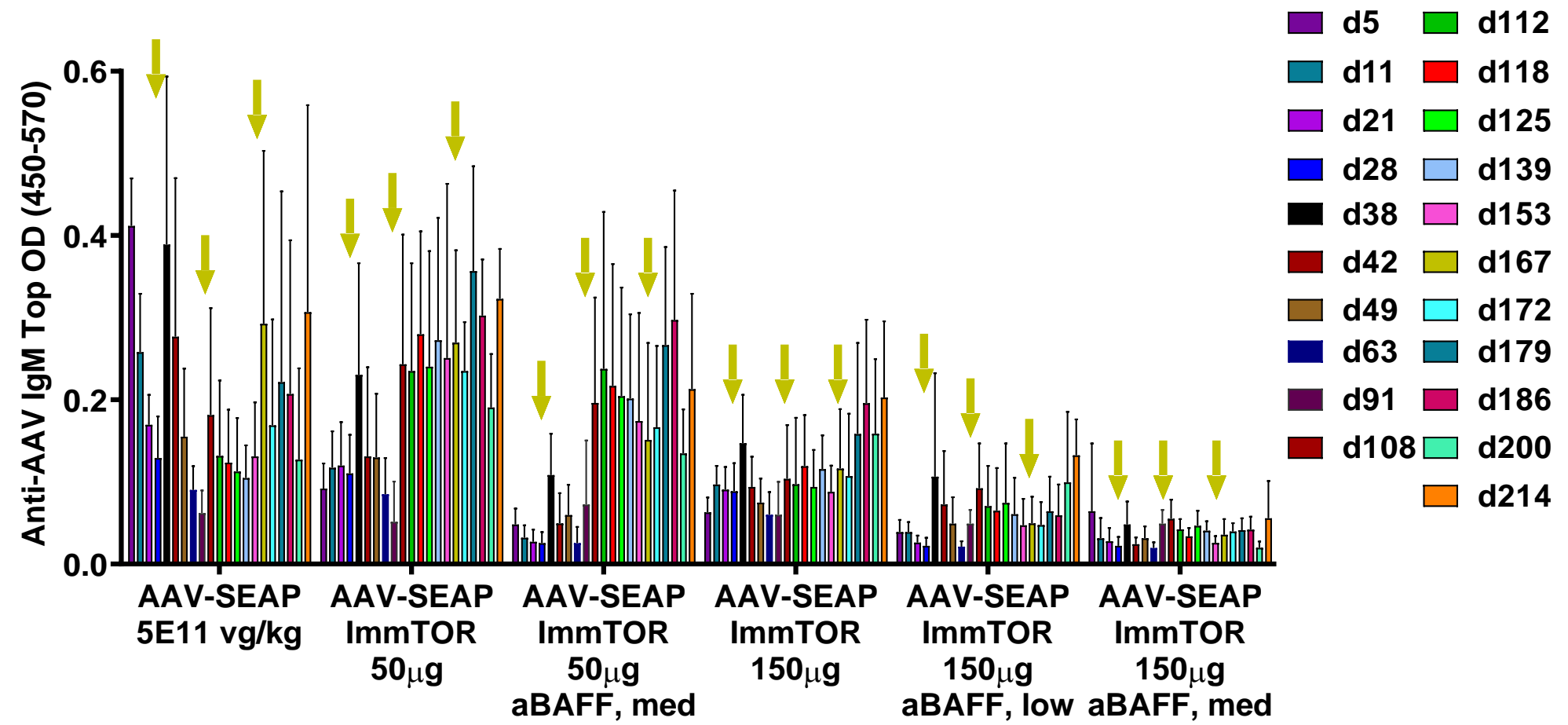
Quadruple AAV redosing in combination with ImmTOR and aBAFF

Gr. #	Injection (d0, 32, 98, 160)	ImmTOR co-injected	aBAFF (i.p.)
1	AAV-SEAP, 1×10^{10} VG	None	None
2	AAV-SEAP + ImmTOR	50 μ g	None
3	AAV-SEAP + ImmTOR + a-BAFF	50 μ g	100 μ g d0,14,32,98,112,130,160,174, 192
4	AAV-SEAP + ImmTOR, high	150 μ g	None
5	AAV-SEAP + ImmTOR + anti-BAFF, low	150 μ g	100 μ g; d0 & 32, 98,160
6	AAV-SEAP + ImmTOR + a-BAFF, med	150 μ g	100 μ g, d0,14,32,98,112,130,160,174, 192



AAV-SEAP
 ImmTOR
 Anti-BAFF McAb
 Bleeds

ImmTOR suppresses AAV Abs after multiple vector injections and synergizes with aBAFF (1): IgM dynamics



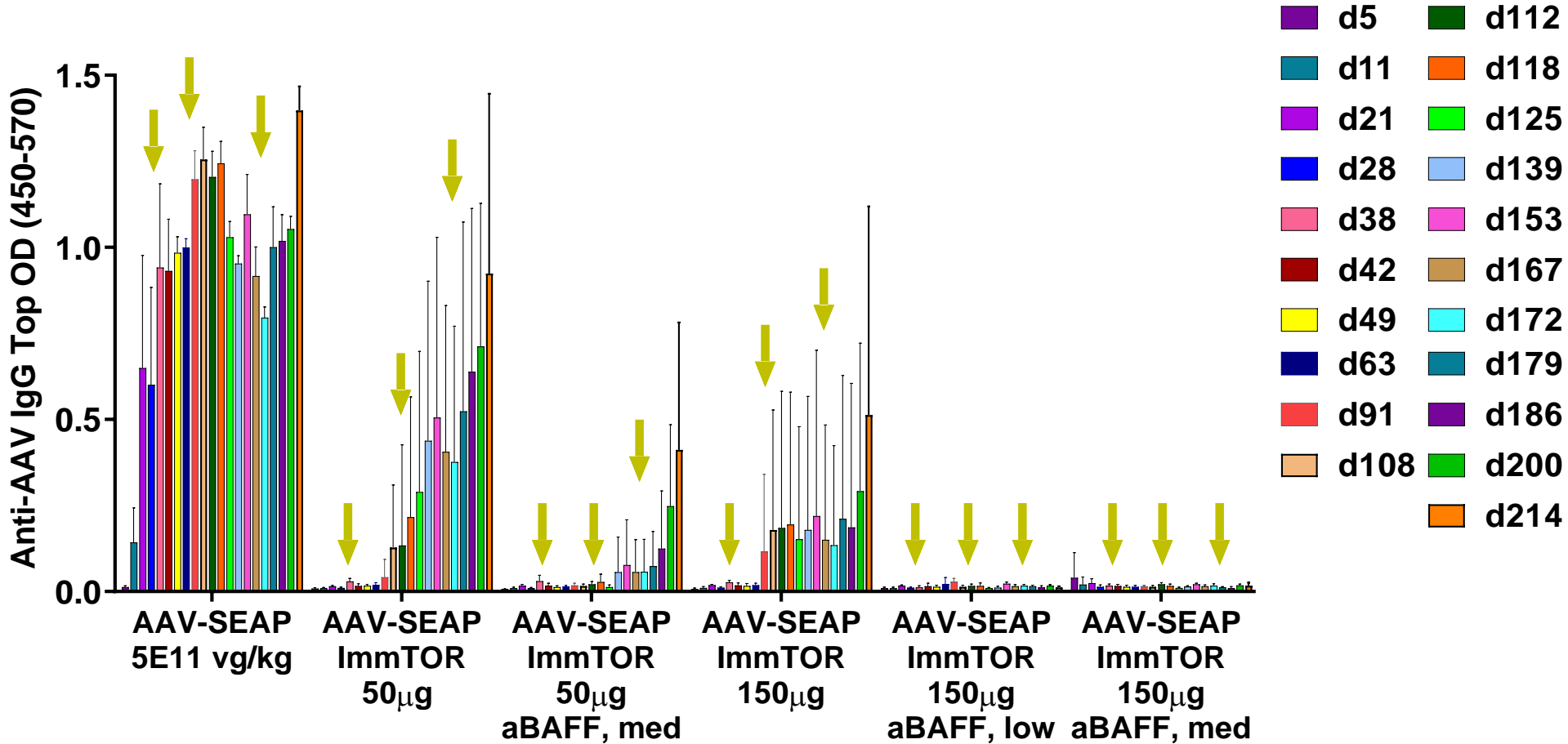
Dose-dependent IgM suppression by ImmTOR is further strengthened by aBAFF

Nearly no detectable IgM after four injections of AAV if ImmTOR is co-administered and combined with three bi-weekly injections of aBAFF after each AAV dose

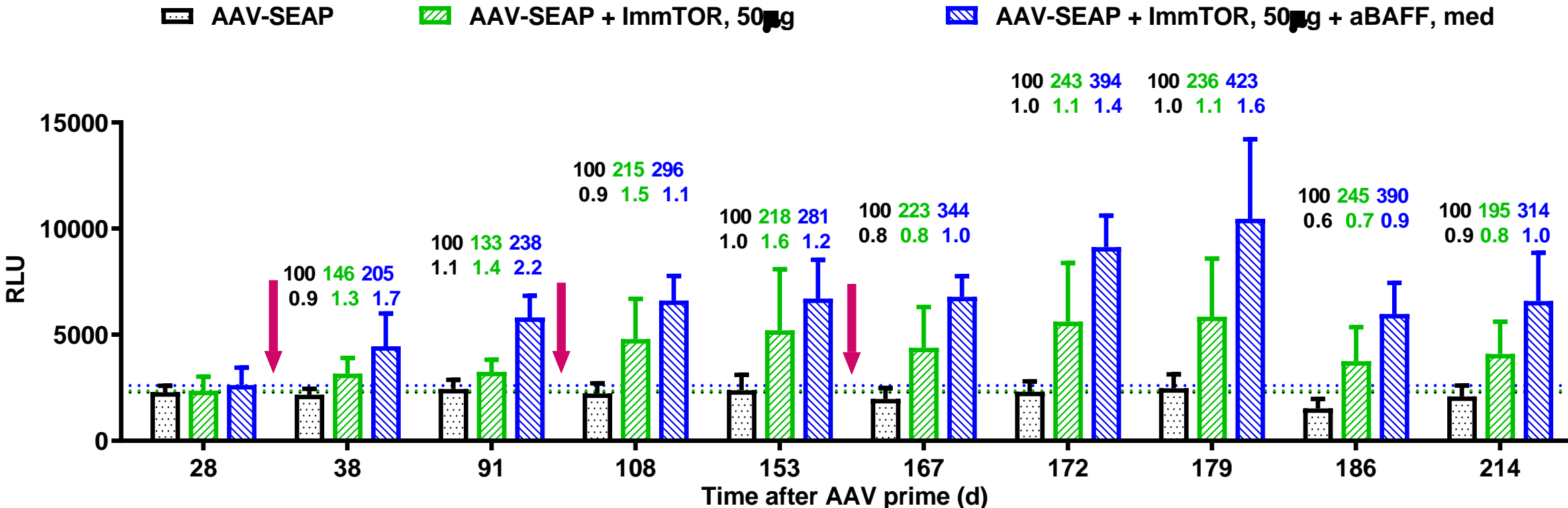
Arrows: times of AAV redosing



ImmTOR suppresses AAV Abs after multiple vector injections and synergizes with aBAFF (2): IgG dynamics



ImmTOR and aBAFF synergize to increase transgene expression (low ImmTOR)



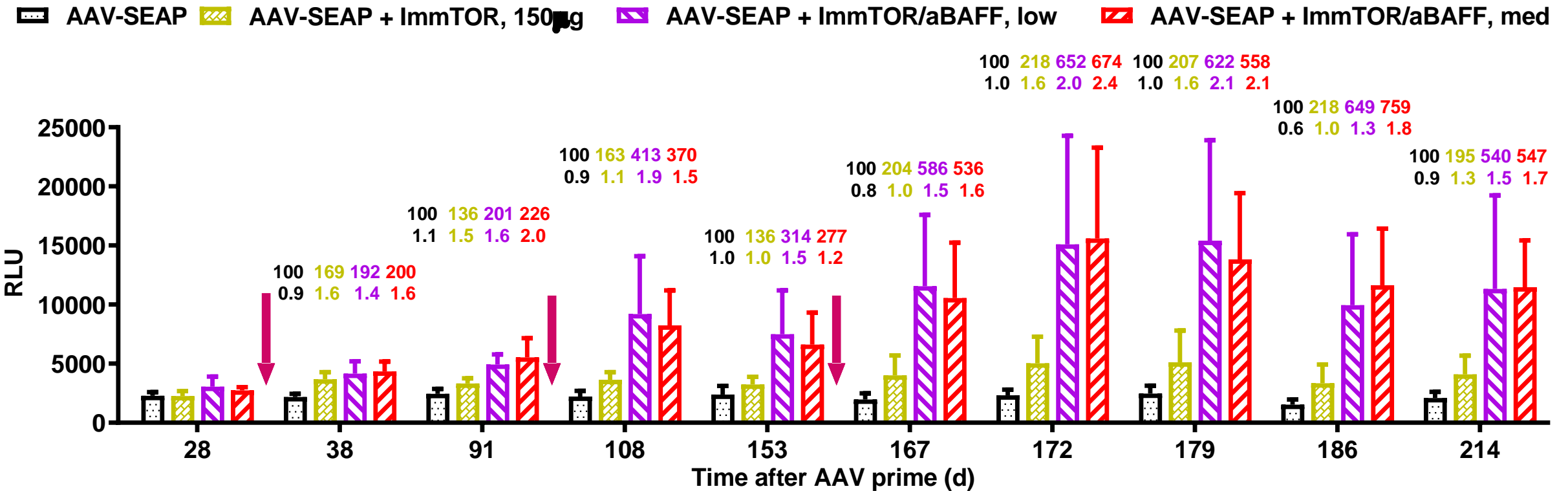
Relative transgene expression is shown for every time-point (mice treated with AAV-SEAP alone assigned a number of '100'); bottom line: ratio of expression increase after redosing vs. the last pre-redosing time-point; dotted lines = d28 expression levels in each group; **arrows: times of AAV redosing**

ImmTOR enables AAV redosing, its efficiency is further elevated by aBAFF

3-4x higher long-term transgene expression if ImmTOR combined with aBAFF



ImmTOR and aBAFF synergize to increase transgene expression (higher ImmTOR)

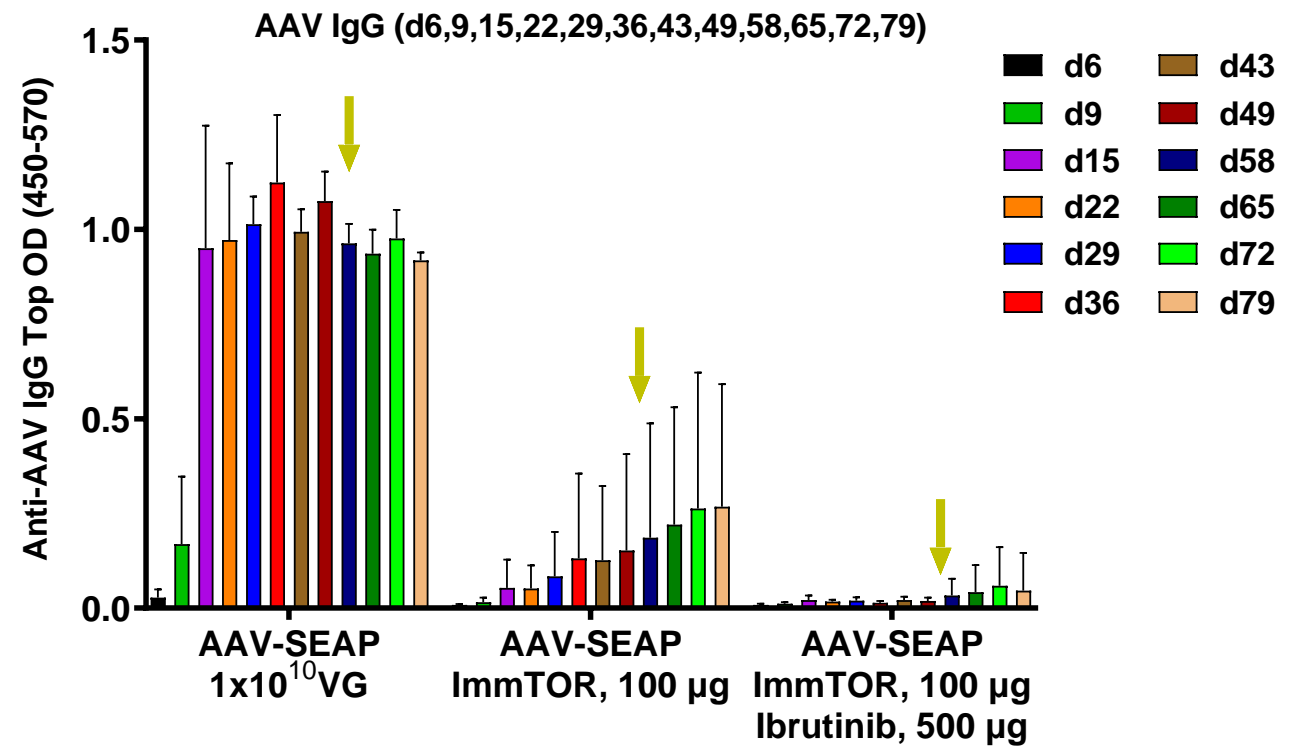
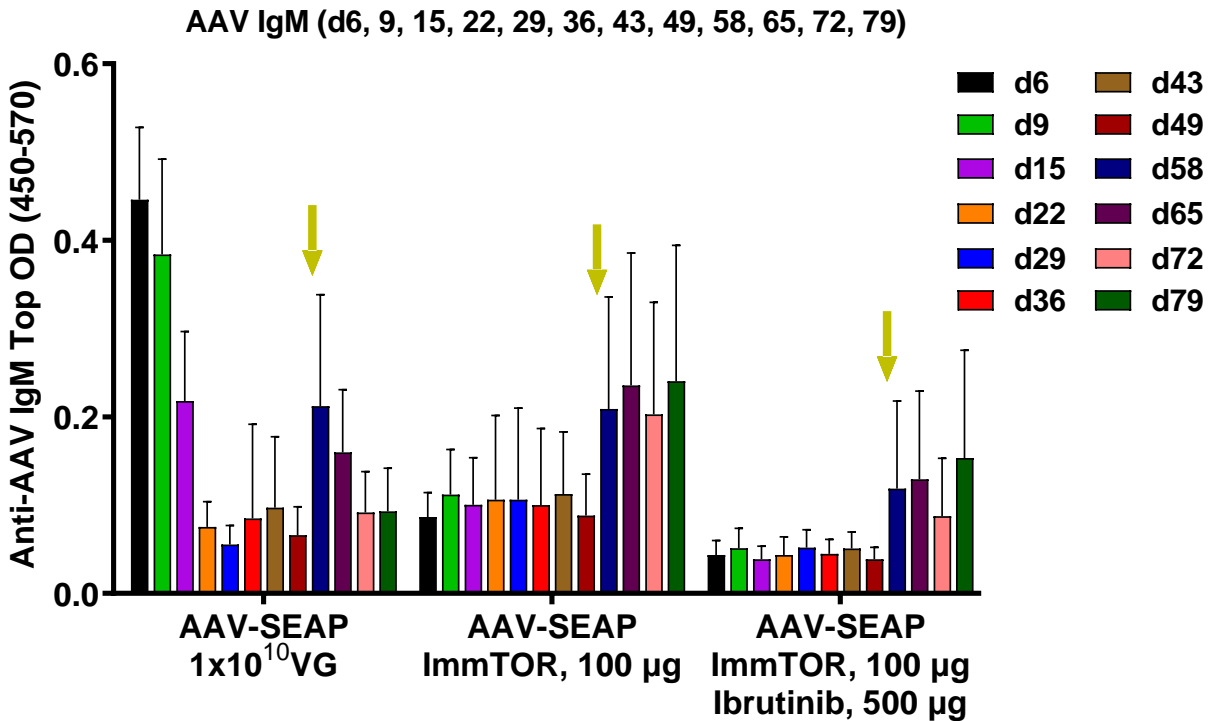


ImmTOR enables AAV redosing, its efficiency is further elevated by aBAFF

Efficient redosing results in long-term 5-7-fold higher transgene expression if ImmTOR combined with aBAFF

Arrows: times of AAV redosing

Relative expression levels and ratios of redosing efficiency are shown as on previous slide

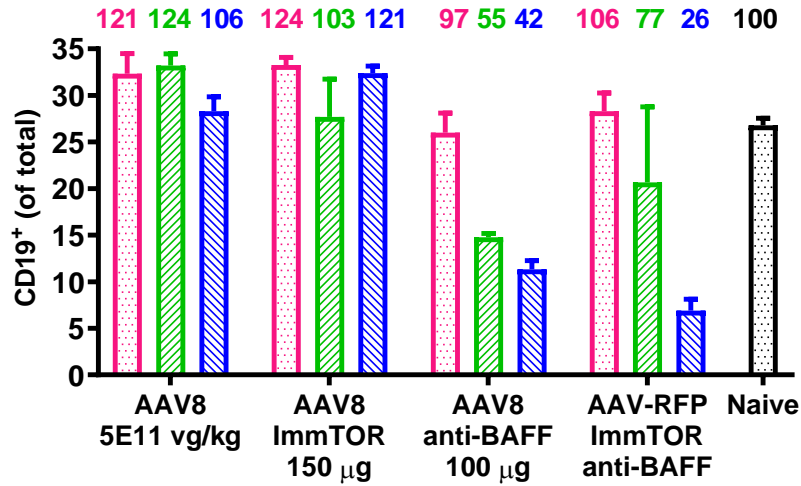


ImmTOR synergizes with ibrutinib to suppress AAV IgM and IgG development after initial and repeat vector administrations

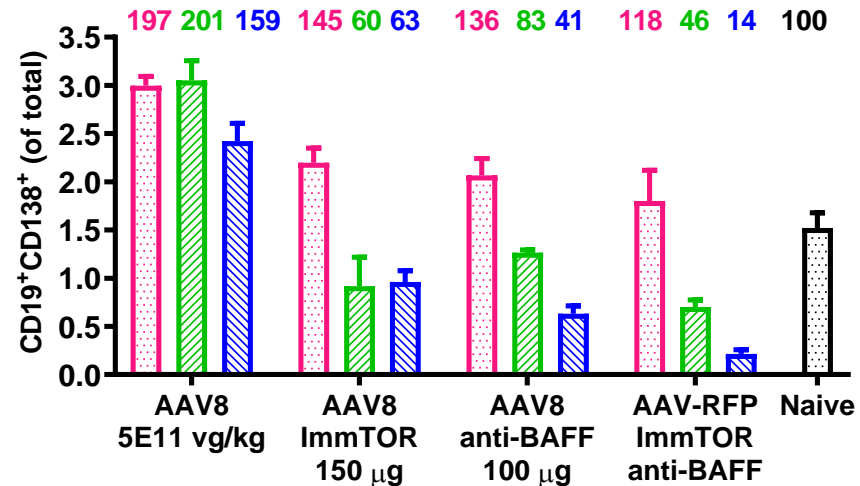
Arrows: times of AAV redosing



CD19⁺ splenocytes



CD19⁺CD138⁺ plasmablasts



CD19⁺CD127⁺ pro-/pre-B cells

Day 0
Day 1

Day 4
Day 7

Three mice/group/each time-point

ImmTOR decreases splenic plasmablasts, but not mature splenic B cells, while aBAFF affects both populations and synergizes with ImmTOR; their combination leads to significant accumulation of immature B cells early after AAV antigen challenge

Conclusions

- **ImmTOR and anti-BAFF monoclonal antibody (aBAFF) synergize to suppress IgM development in response to multiple AAV administrations**
- **This low IgM response maintained by ImmTOR and aBAFF treatments leads to complete suppression of AAV IgG response (as opposed to isolated breakthroughs seen if ImmTOR is used alone; aBAFF used alone is even less effective in IgM/IgG suppression)**
- **Low antibody response against AAV enables multiple AAV re-administrations, which result in significant elevation of AAV-driven transgene expression over more than six months**
- **Another drug directed against Bruton's tyrosine kinase, ibrutinib, also synergizes with ImmTOR to suppress development of IgM and IgG antibodies to AAV**
- **ImmTOR and anti-BAFF synergize to block mature B cell and plasmablast development in spleen early after AAV antigenic stimulation**