Identification of Dose-Dependent Immune Landscape Signatures Following Administration of Ixo-vec in Non-Human Primates

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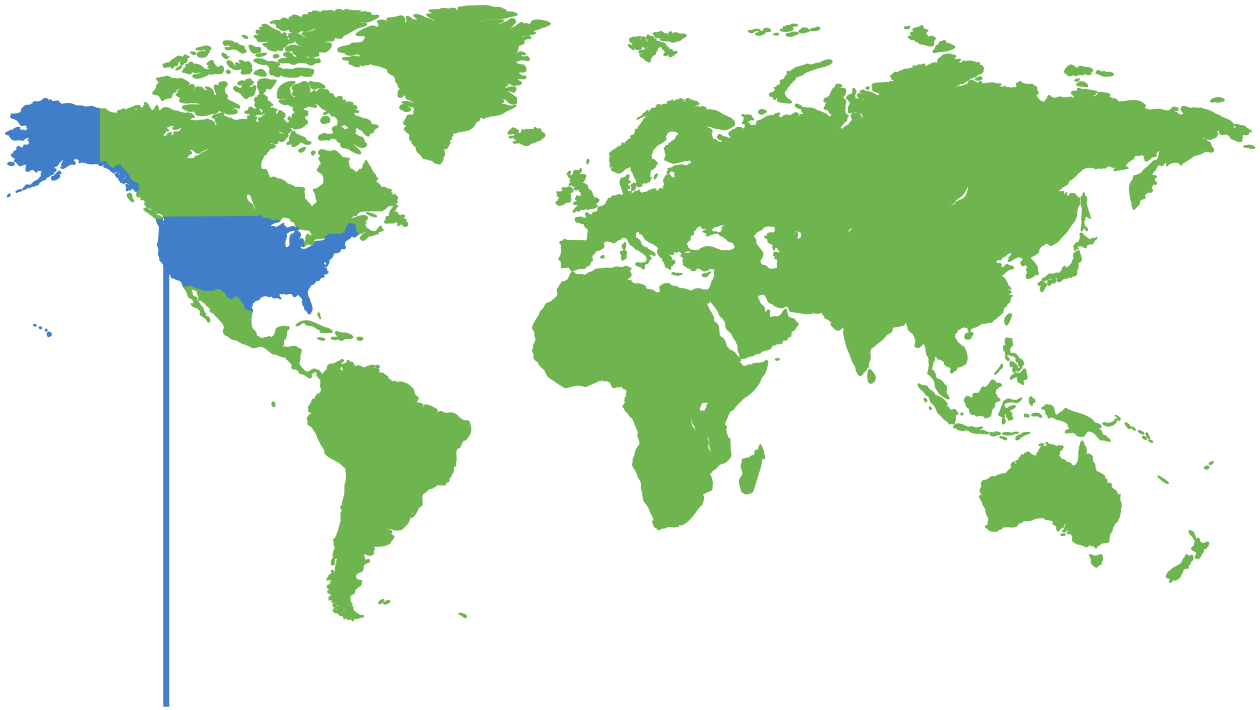
Disclosures

- **Adverum Biotechnologies:** employee, stockholder.

- **Solid Biosciences:** licensing royalties.

Wet AMD: Leading Cause of Blindness in Patients Over 65

20M people living with wet AMD worldwide[1,2]



1.5M people impacted by wet AMD in the U.S.[1,2]

Opportunity for New Wet AMD Treatment

Current Standard of Care Drives Suboptimal Outcomes	Gene Therapy: Overcomes Wet AMD Treatment Burden
Frequent bolus anti-VEGF IVT injections required leading to poor compliance[3,4]	Single, easily administered in-office IVT treatment
Lifetime need for frequent injections overburdens patients, caregivers, and healthcare providers	Durable, disease modifying therapy, potentially “one and done” functional cure
$139 billion in U.S. annual economic burden of vision loss, including falls, cognitive decline and depression[5]	Readily deployable in the U.S. and around the world addressing direct and indirect costs

[1]*Bright Focus Foundation. Age-Related Macular Degeneration: Facts & Figures.*
[2]*Wong WL, et al. Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. Lancet Glob Health. 2014;2:106–16.*
[3]*Holz FG et al. Br J Ophthalmol 2015; 99 (2): 220–226.*
[4]*Khanani A, et al. Ophthal. Retina 2020 Feb; 4(2):122-123.*
[5]*Angiogenesis Foundation: Patient-centered Outcomes in Wet Age-related Macular Degeneration, Boston, MA, October 2017*

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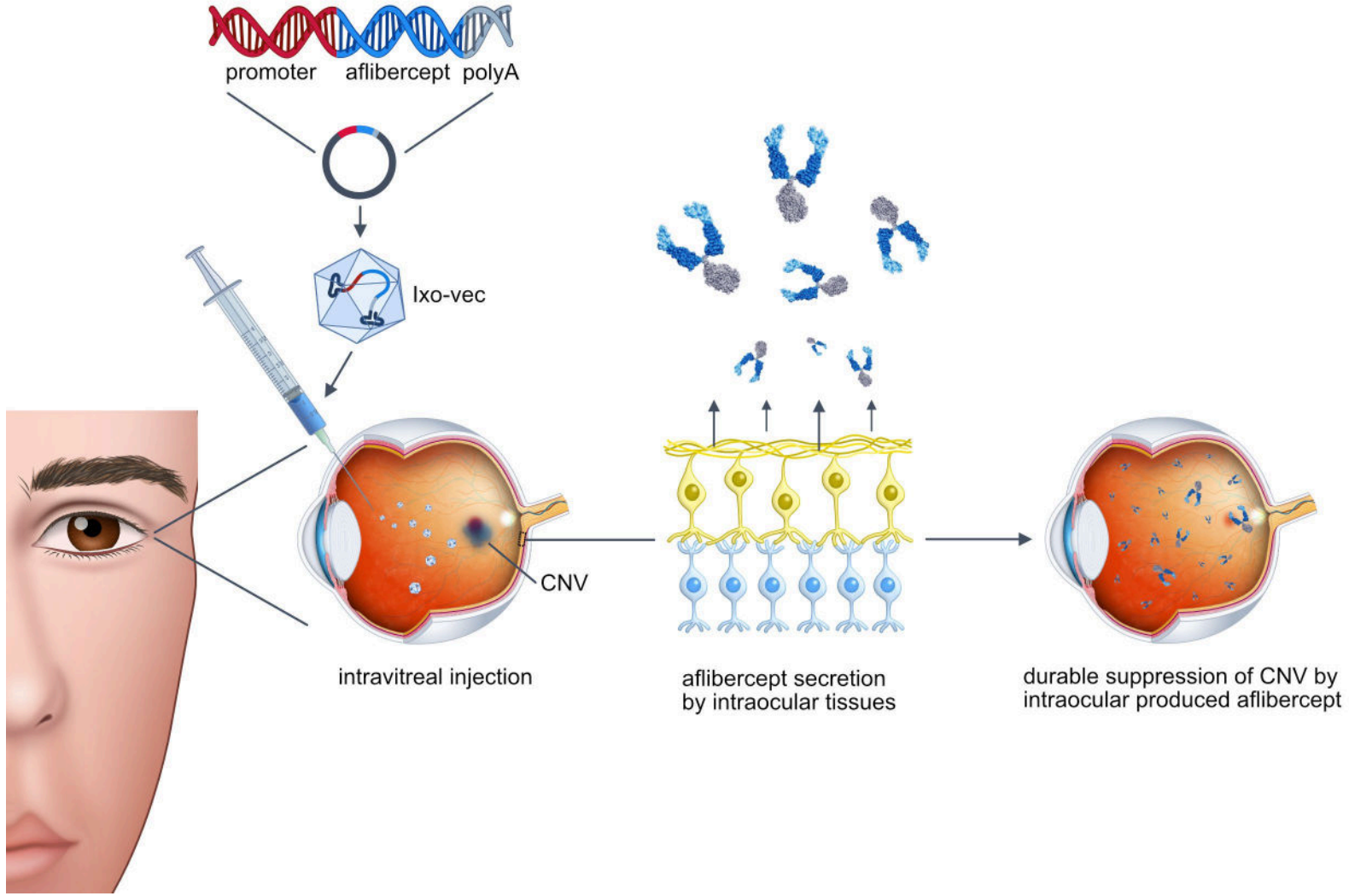
Ixo-vec is a gene therapy biofactory approach designed for continuous delivery of aflibercept (anti-VEGF) by single intravitreal injection



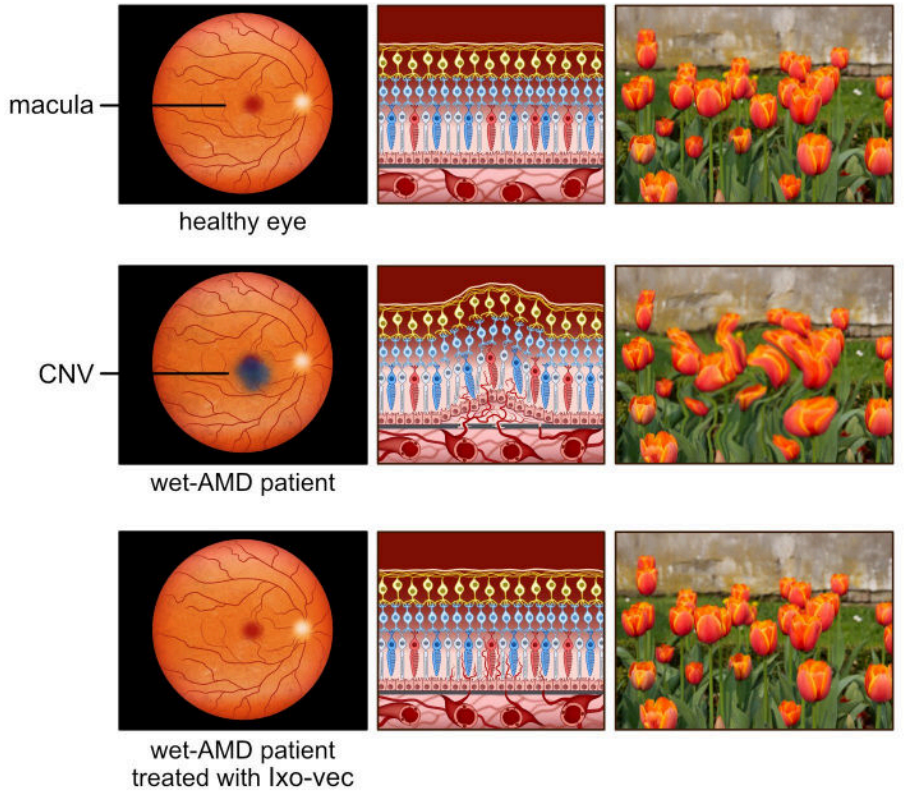


Illustration: Gardenia Gonzalez Gil

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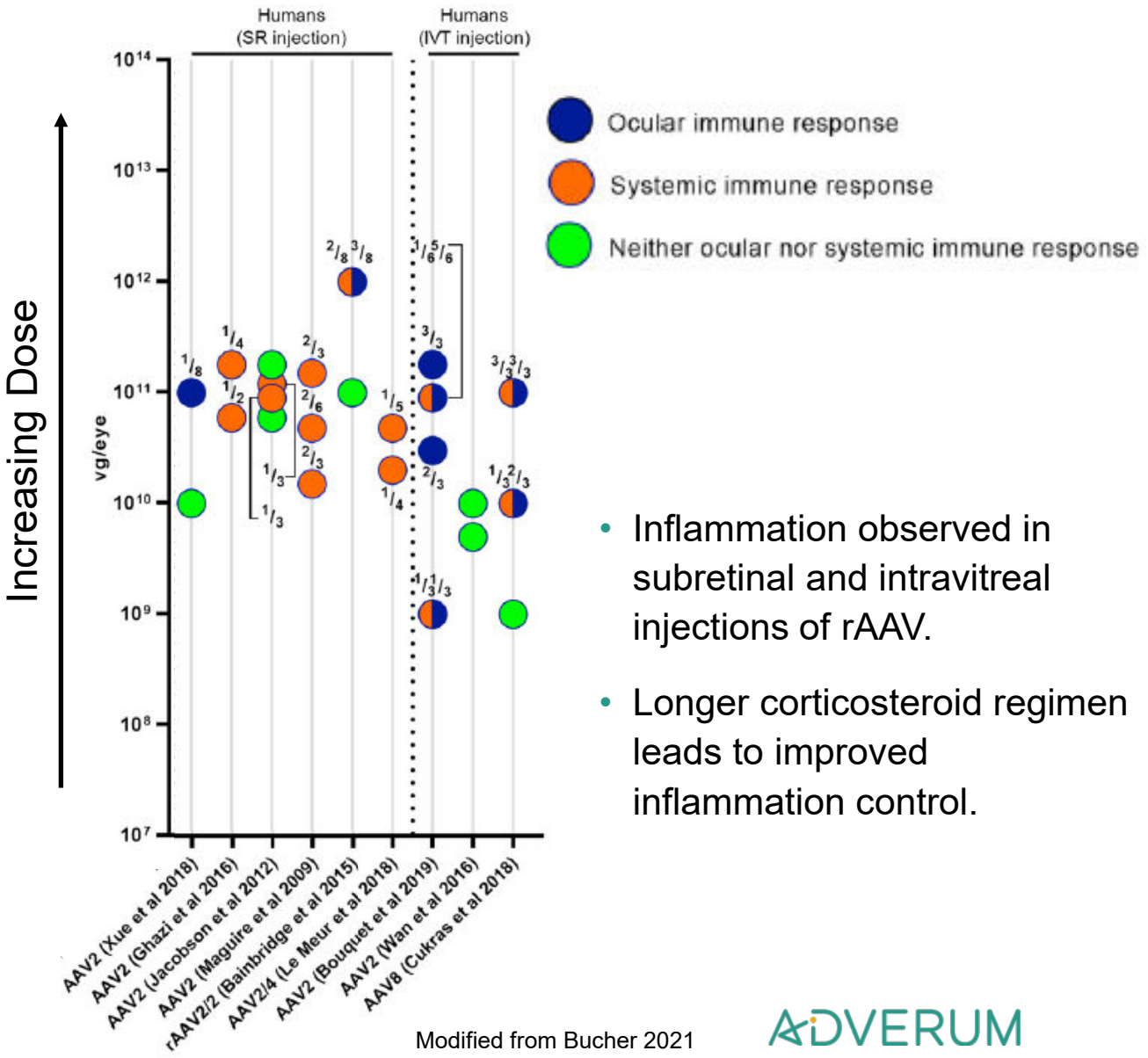
Clinical implementation of gene therapy products is influenced by rAAV immune mediated inflammatory response

- Dose-dependent inflammation is observed across the gene therapy field, irrespective of route of administration and therapeutic modality.
- rAAV immunogenicity can be mitigated by dose reduction and improved corticosteroid regimen.
- Systems-wide analyses of nonclinical models could help identify molecular signatures reflective of the dose-dependent inflammation commonly associated with gene therapy.



- Inflammation observed in subretinal and intravitreal injections of rAAV.
- Longer corticosteroid regimen leads to improved inflammation control.

Modified from Bucher 2021

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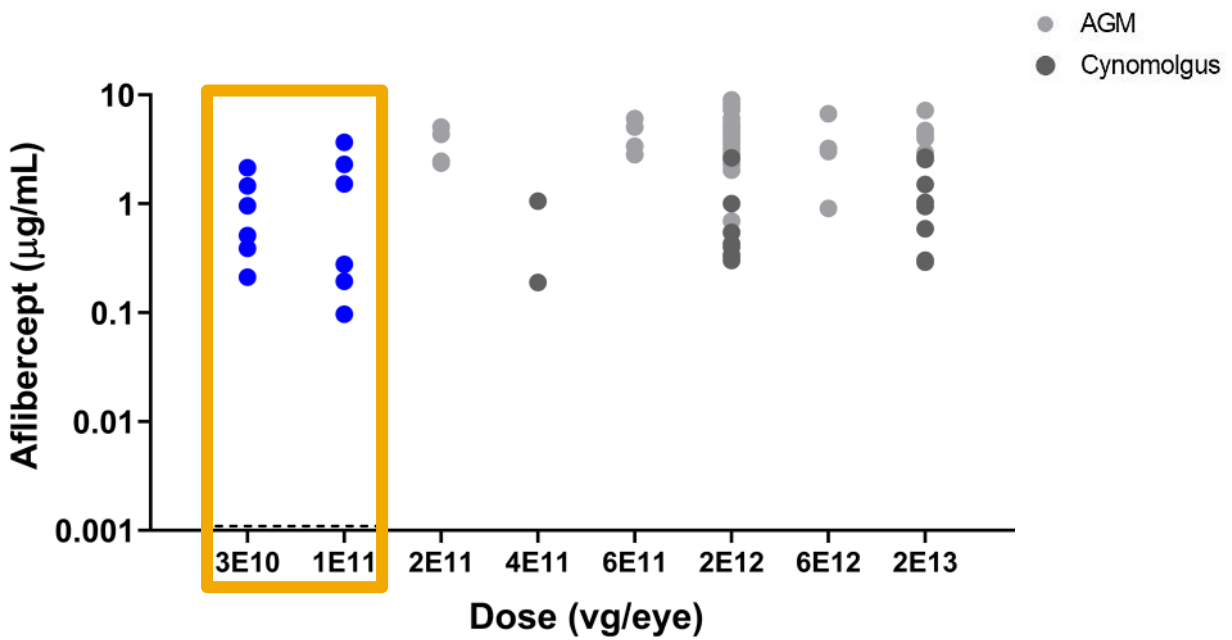
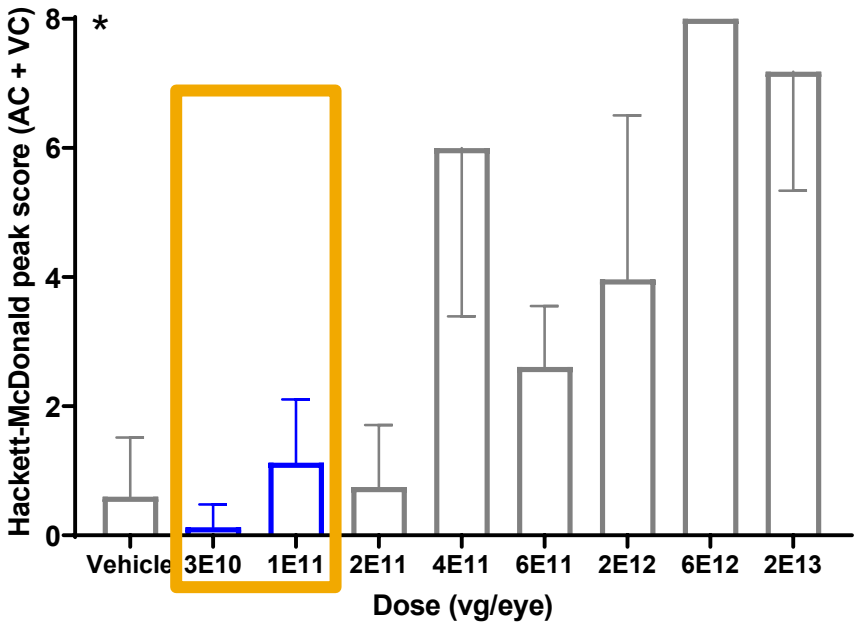
Ixo-vec potency enables ability to dose down to improve inflammation profile

Non-dose proportional aflibercept levels enable dose reduction to improve Ixo-vec inflammation profile while maintaining efficacy.

Vitreous Humor



Inflammation Scores



- NOAEL established at NHP dose of 1E11 vg/eye = 2E11 vg/eye human equivalent dose (HED).
- NHP dose of 3E10 vg/eye = 6E10 vg/eye HED.
- NHP dose of 1E11 vg/eye = 2E11 vg/eye HED.
- Doses in current LUNA clinical study outlined in orange boxes.

*Scale is cumulative of two parameters for maximum score of 8.

Schaefer-Swale K. Non-Clinical Data Support Efficacy and Tolerability of a Human Equivalent Dose of 6E10 vg/eye of ADVM-022 for the Treatment of Neovascular Age-Related Macular Degeneration. Poster presented at: ASGCT; May 16-19, 2022; Washington DC.

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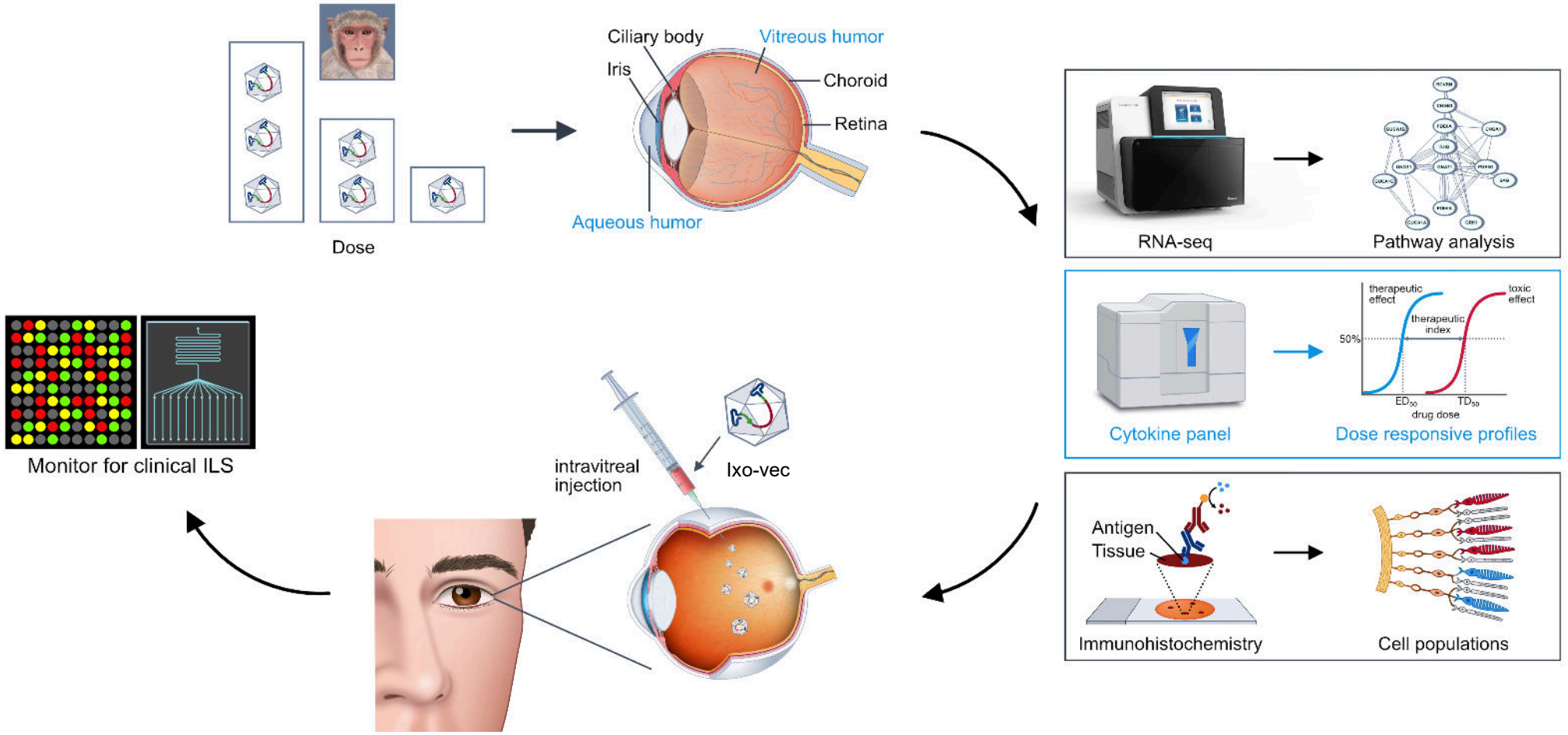
Defining immune landscape signatures of Ixo-vec at supra-clinical doses



- Doses spanning 3-log fold range above NOAEL (1E11 vg/eye):
  - 4E11 vg/eye (low), 2E12 vg/eye (mid), 2E13 vg/eye (high)
- Single eye injected for each animal.
- Endpoint: 3 months post-dose.

- Multiple regions of retinal anatomy submitted for bulk RNA-seq.
- Pathway analysis was performed on RNA-seq samples.
- Parallel effort characterized inflammation by histology.

Illustration: Gardenia Gonzalez Gil

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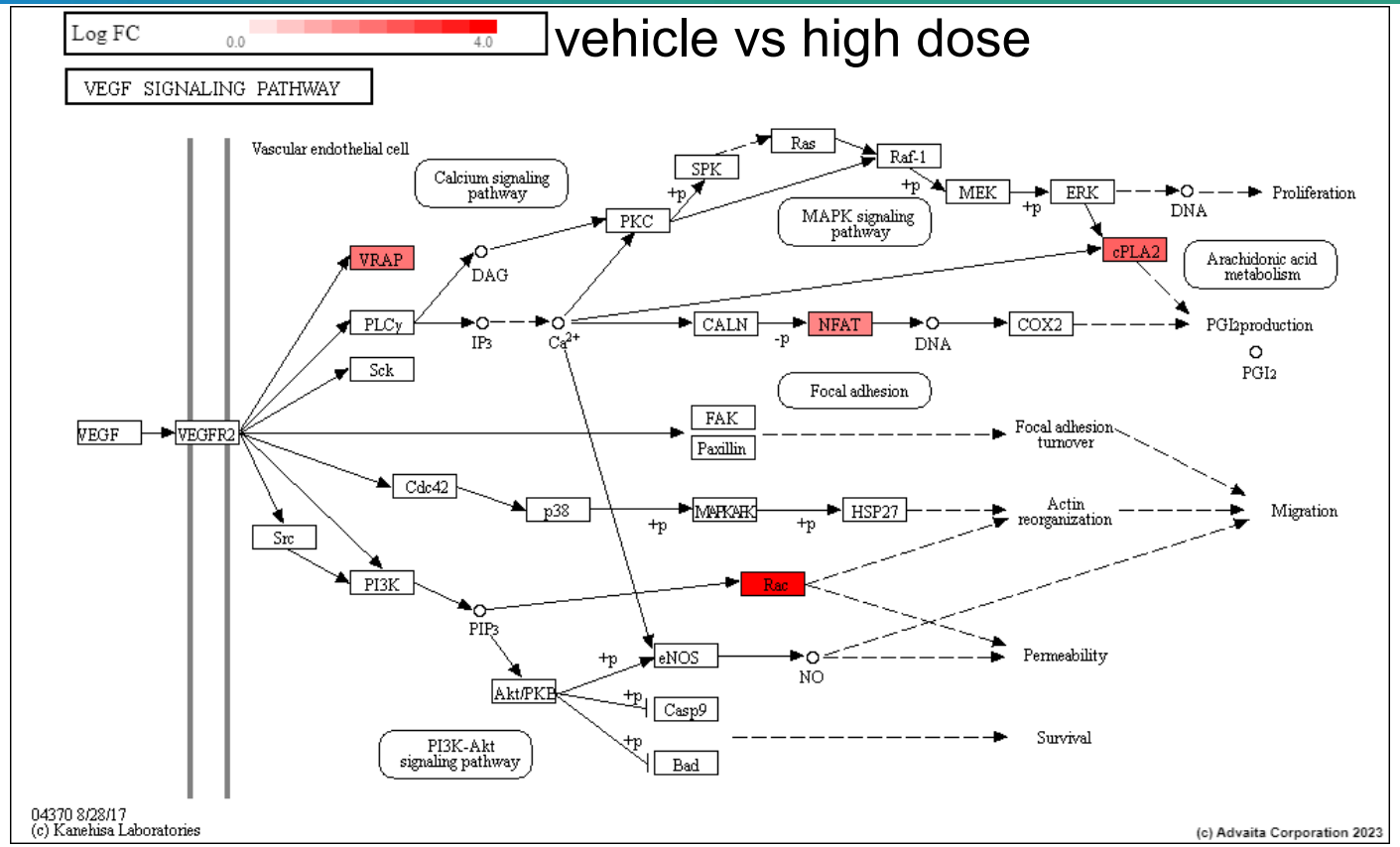
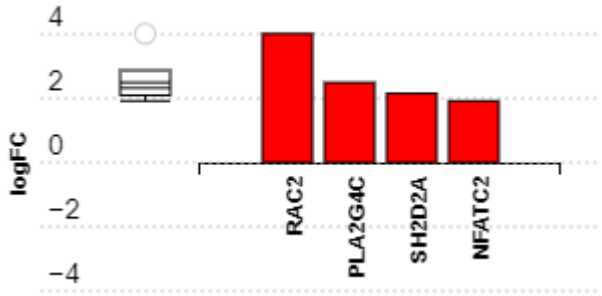
Converging dataset strongly supports dose-dependent inflammatory response

- Pathway analysis was performed using multiple methods (topology and non-topology based).
  - Modulation is determined by the number of differentially expressed genes within a set as well as their position and magnitude of role within a pathway.
- Transcriptomic analysis did not support unfolded protein response (UPR), oxidative or endoplasmic reticulum (ER) stress, neovascularization, nor ciliary body dysfunction.
- Ixo-vec expression detected only in the dosed eye.
- Robust and dose-dependent activation of immune responses, consistent in nature across ocular tissues.
  - Severity in line with anticipated vector biodistribution.
- No evidence that ciliary body architecture was directly affected by Ixo-vec.
  - Only indirectly as dose-dependent inflammation increases.
  - Histology showed no disruption of ciliary body fold formation at any dose.
- RNA-seq and histopathology indicate activation of innate and adaptive immune systems consistent with dose-dependent rAAV-associated inflammation.

VEGF signaling is not activated in ocular tissues





- Observation was similar across doses for all ocular tissues examined.
- Ixo-vec unlikely to exacerbate VEGF-mediated pathology of wet AMD.

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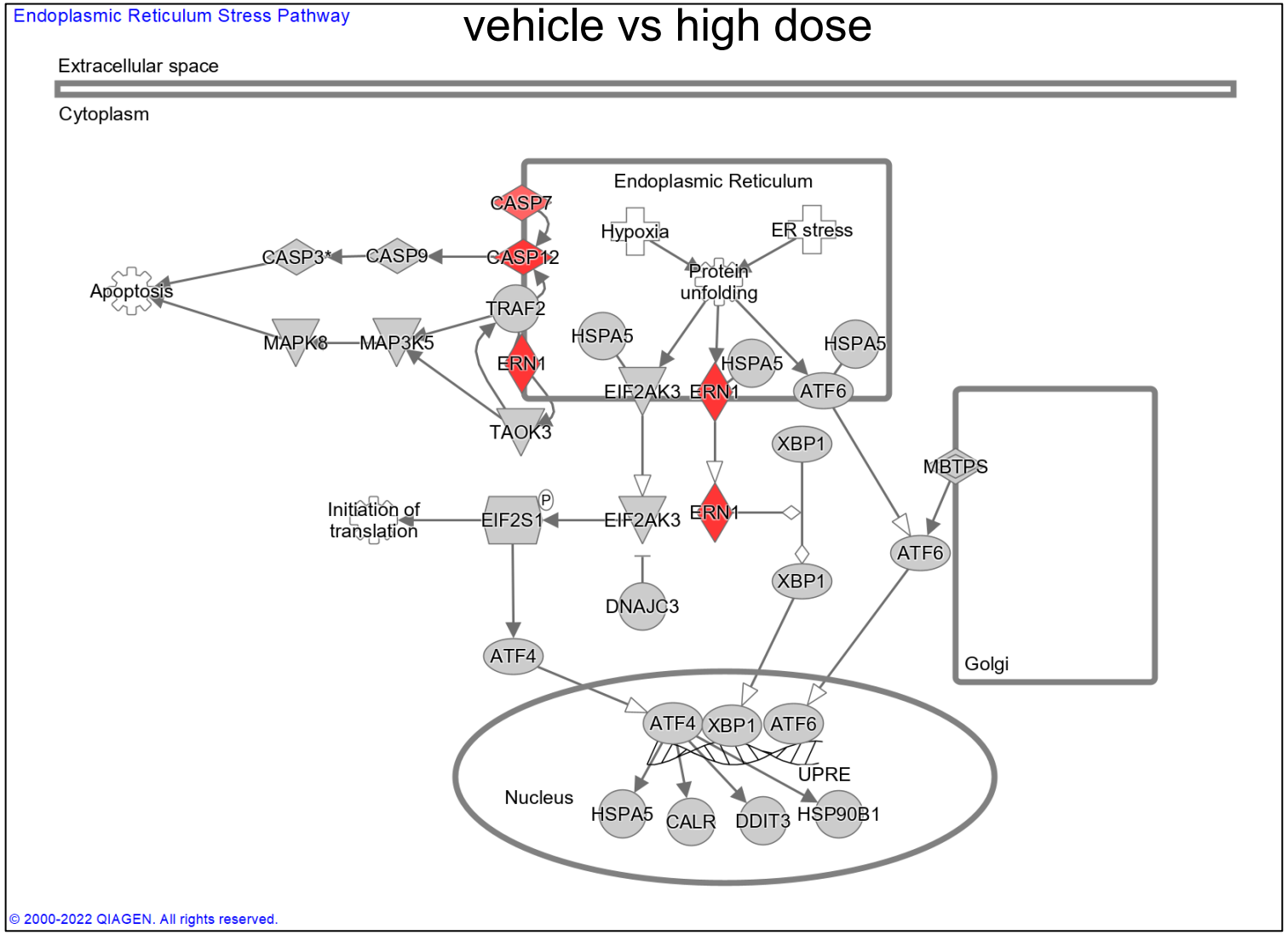
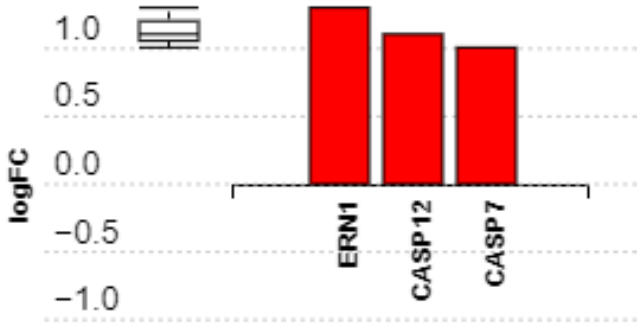
No evidence of UPR or ER stress by Ixo-vec dosing



- Cellular toxicity has been attributed to inefficiencies in transgene translation, folding, and post-transcriptional modification.[1, 2]
  - Ixo-vec expression unlikely induces cellular stress.
- UPR reportedly upregulated by rAAV transduction.[3, 4]
  - Transduction-mediated UPR unlikely to be observed at study endpoint.



- mRNA expression level exhibited little dose-dependence in ocular tissues. No alternative splicing observed.

[1]Malhotra 2008 *PNAS* ; [2]Lange 2016 *Mol Ther Meth Clin Dev* ; [3]Balakrishnan 2013 *PLoS One* ; [4]Cui 2022 *J Virol*

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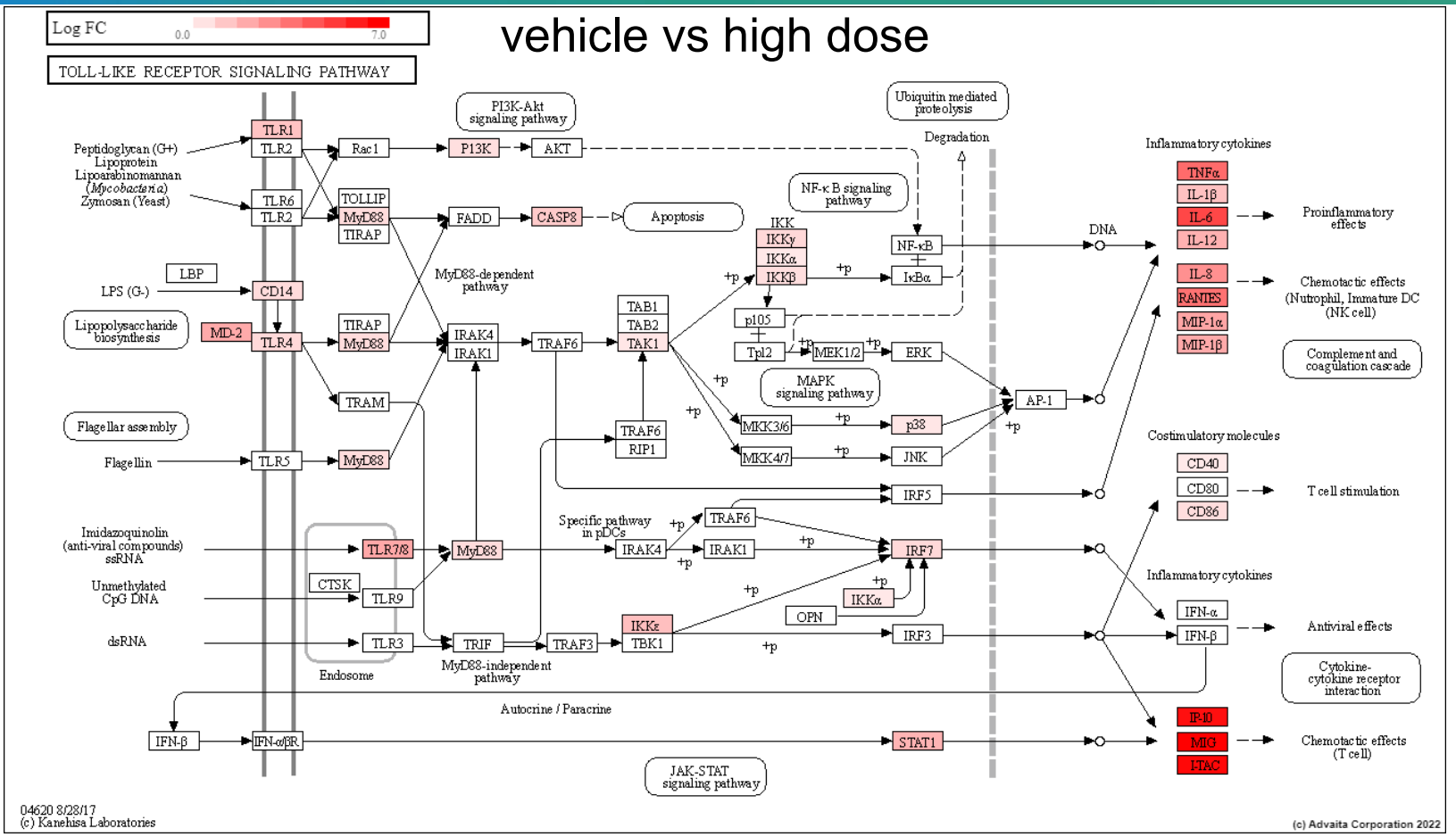
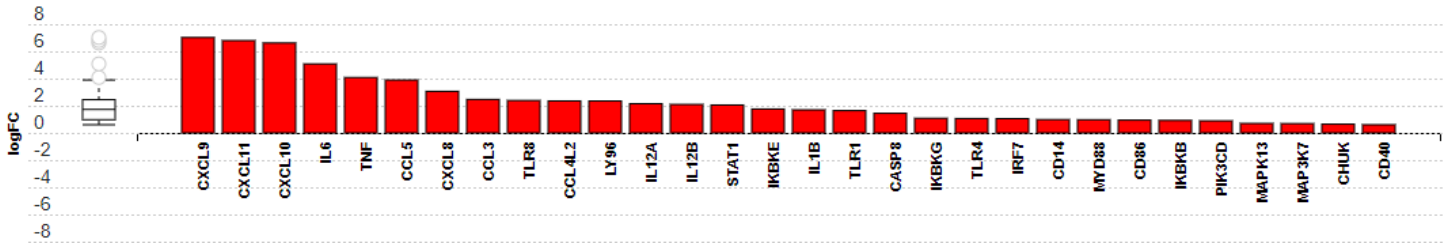
Toll-like receptor signaling





- Unmethylated CpG DNA – TLR9 – MYD88 axis not activated.
- TLR8 suggests response to exogenous mRNA load.[1]
- TLR1/2/4 have reported activation by viral proteins.[2, 3]

[1]Lester 2014 *JMB* ; [2]Hosel 2012 *Hepatology* ; [3]Zhou 2021 *J Med Virol* ;

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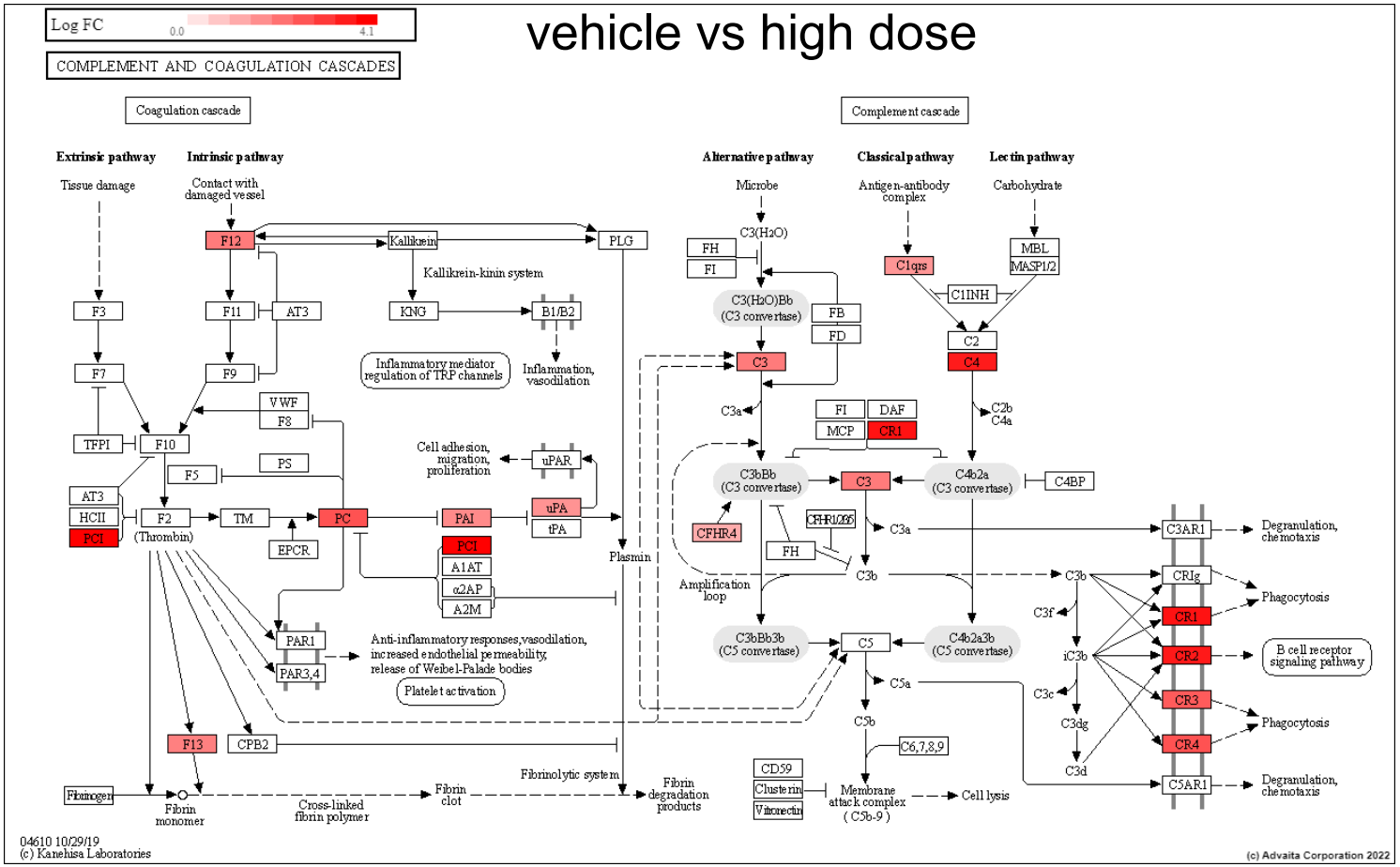
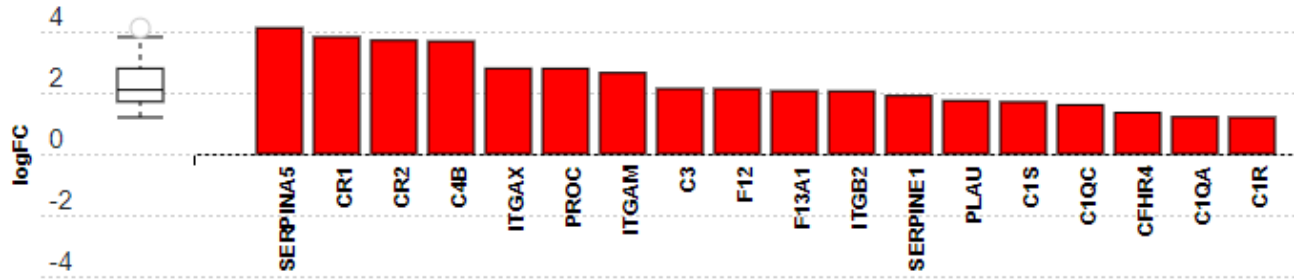
Complement-mediated response observed in dose-responsive manner

vehicle vs high dose





- Could represent antibody complexes bound to rAAV capsid/protein.
- Complement activation previously reported as inflammatory response to high systemic dose of rAAV in clinical trials.[1, 2]
- Complement activation has been known to activate B cells.

[1]Pfizer 2019 *Press release* ; [2]Solid Biosciences 2019 *Press release*

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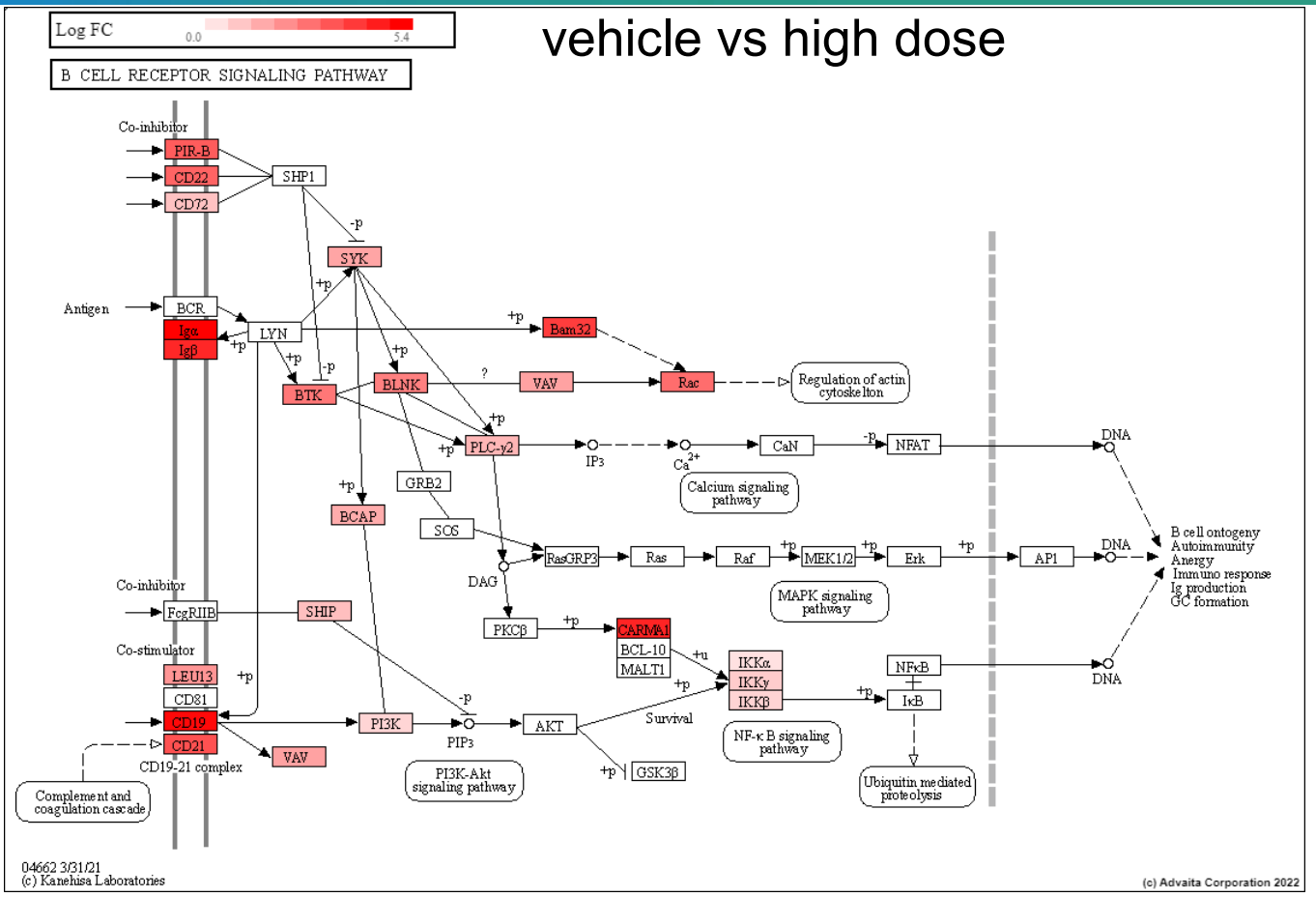
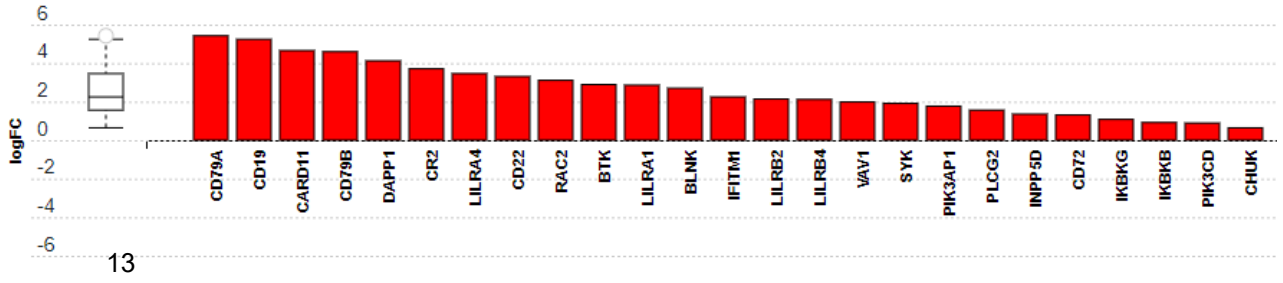
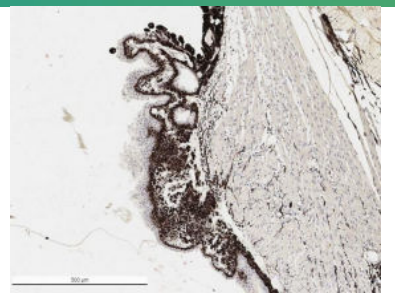
B lymphocyte activation and presence increased in dose-responsive manner

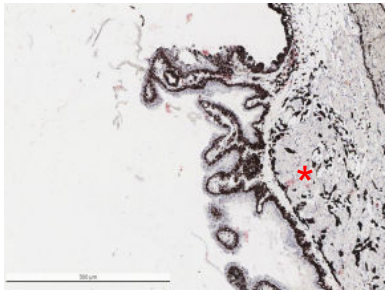




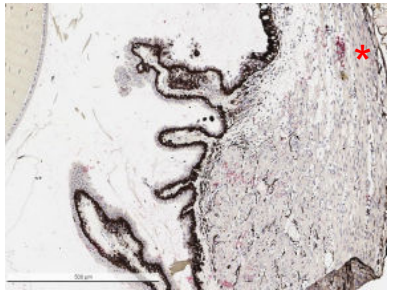
CD20+



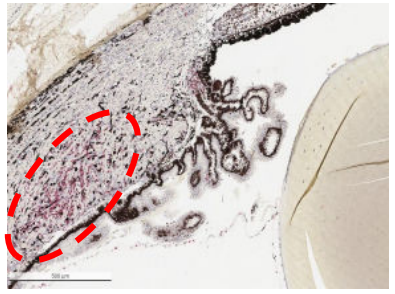
Vehicle



Low dose



Mid dose



High dose

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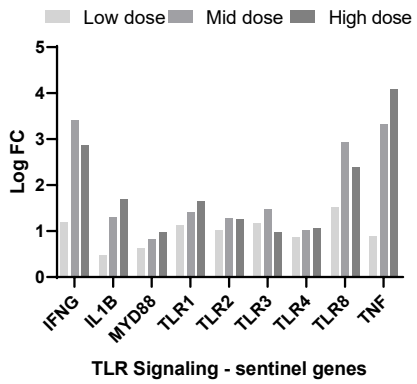
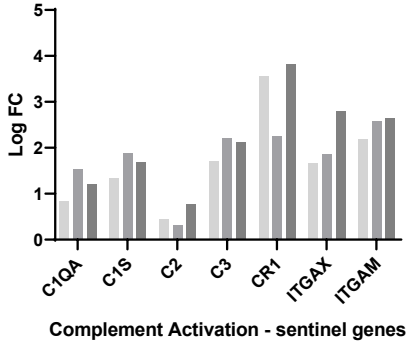
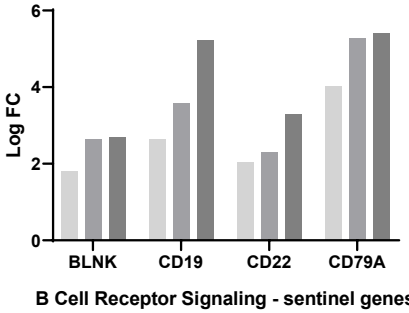
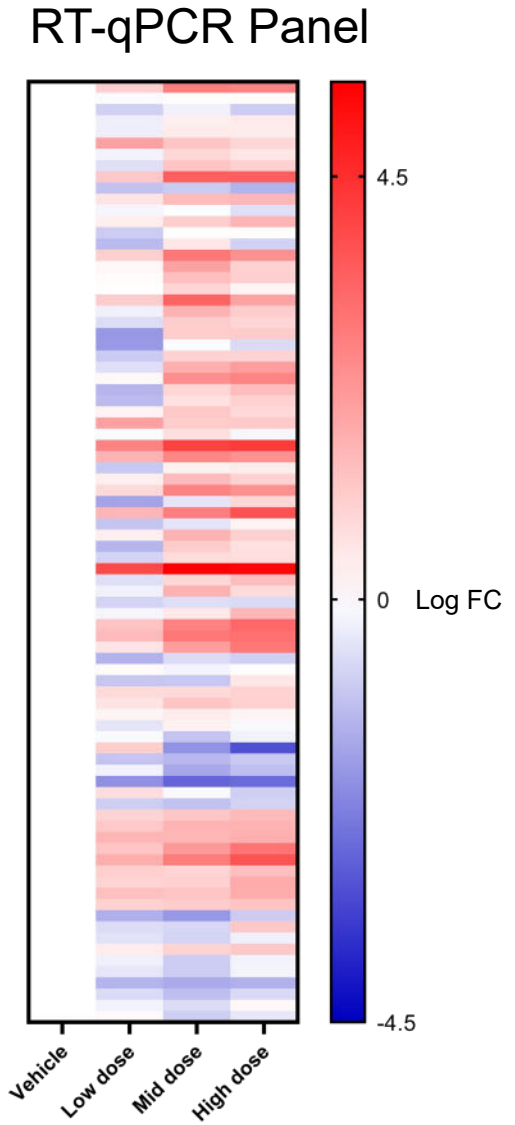
Creation of immune landscape signatures

- Sentinel genes were chosen to represent pathways identified by RNA-seq analysis.
- Concordance found between RNA-seq and RT-qPCR.
- Immune landscape signature (ILS) panel could enable:
  - Nonclinical evaluation to interrogate balance between efficacy/tolerability axis
  - Predict patient outcomes
  - Identify high risk patients (e.g., with microvascular comorbidities)
  - Stratify patients that may not need corticosteroids from those that are more susceptible to rAAV-immunogenicity and require them









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Conclusions

Pathway analysis and IHC converged on dose-dependent activation of innate and adaptive immune response pathways, consistent with rAAV-associated inflammation.

- Aflibercept expression levels plateaued across doses, unlike dose-dependent adaptive immune response.

- Immune landscape signatures of supra-clinical doses are consistent in nature across ocular tissues – predominately in the anterior tissues of the eye and retina.
  - Assessed 3-log fold range above NOAEL.
- No evidence that harnessing ocular cells as biofactories to produce aflibercept leads to expression-associated toxicity/inflammation.
- No evidence that ciliary body architecture was directly affected by Ixo-vec – only indirectly and at supra-clinical doses secondary to dose-dependent increase in inflammation.
- Use of lower doses and an improved steroid prophylaxis, implemented in the LUNA trial, are expected to improve Ixo-vec inflammation profile while preserving therapeutic levels of aflibercept.

Acknowledgements

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- Jenny Vo

Current and **Former* Adverum colleagues

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