Dose-dependent inflammation signatures following Ixo-vec administration in non-human primates

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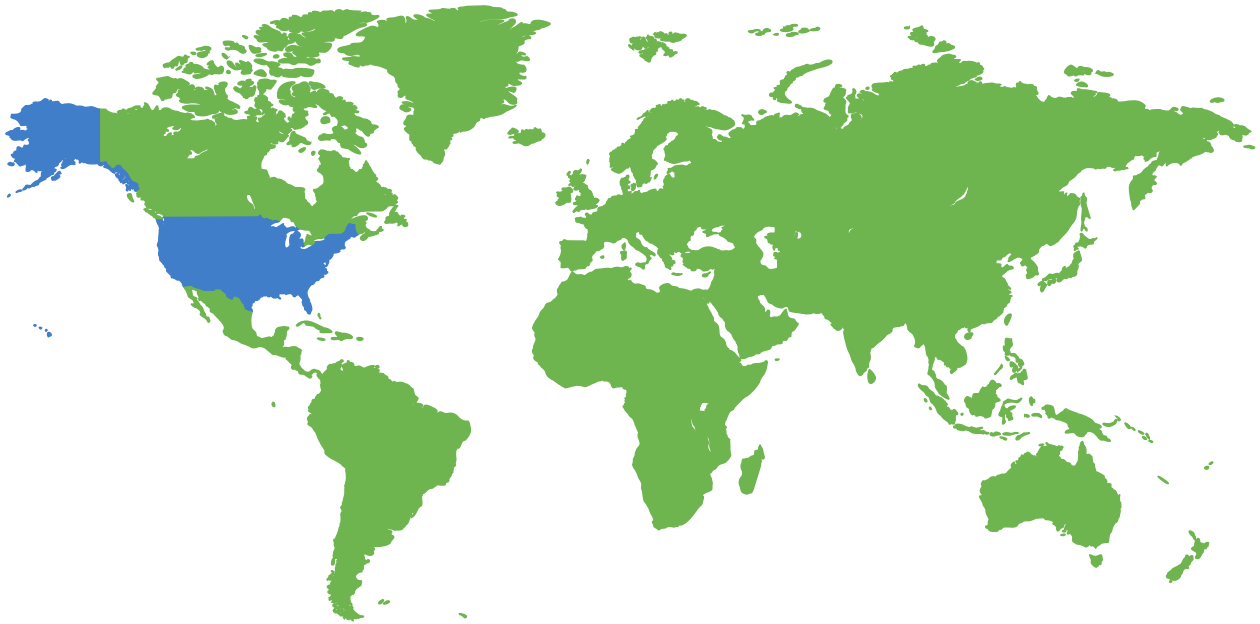
Wet AMD: leading cause of blindness in patients over 65

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

200,000 new cases every year.[1,2]



Up to 42% of patients develop bilateral disease in the first two to three years following diagnosis in the primary eye[6]

Leading Cause of Blindness Over 65

TODAY	TOMORROW
Lifetime injections burden	One and done
Frequent IVT injection Vision Loss due to poor compliance[3,4]	Durable and Disease modifying
$139 billions spend due to vision loss (US)[5]	Readily deployable in outpatient setting

[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*
[6]Gangnon RE et al. (2015) JAMA Ophthalmol; 133 (2): 125–132. Rasmussen A. et al., (2017) *Eye* 31, 978–980 (2017). Wong TY, et al. (2020) *Retina*. 40, 599-611 Zarranz-Ventura J et al. (2014). Ophthalmology; 121 (10): 1966–1975.

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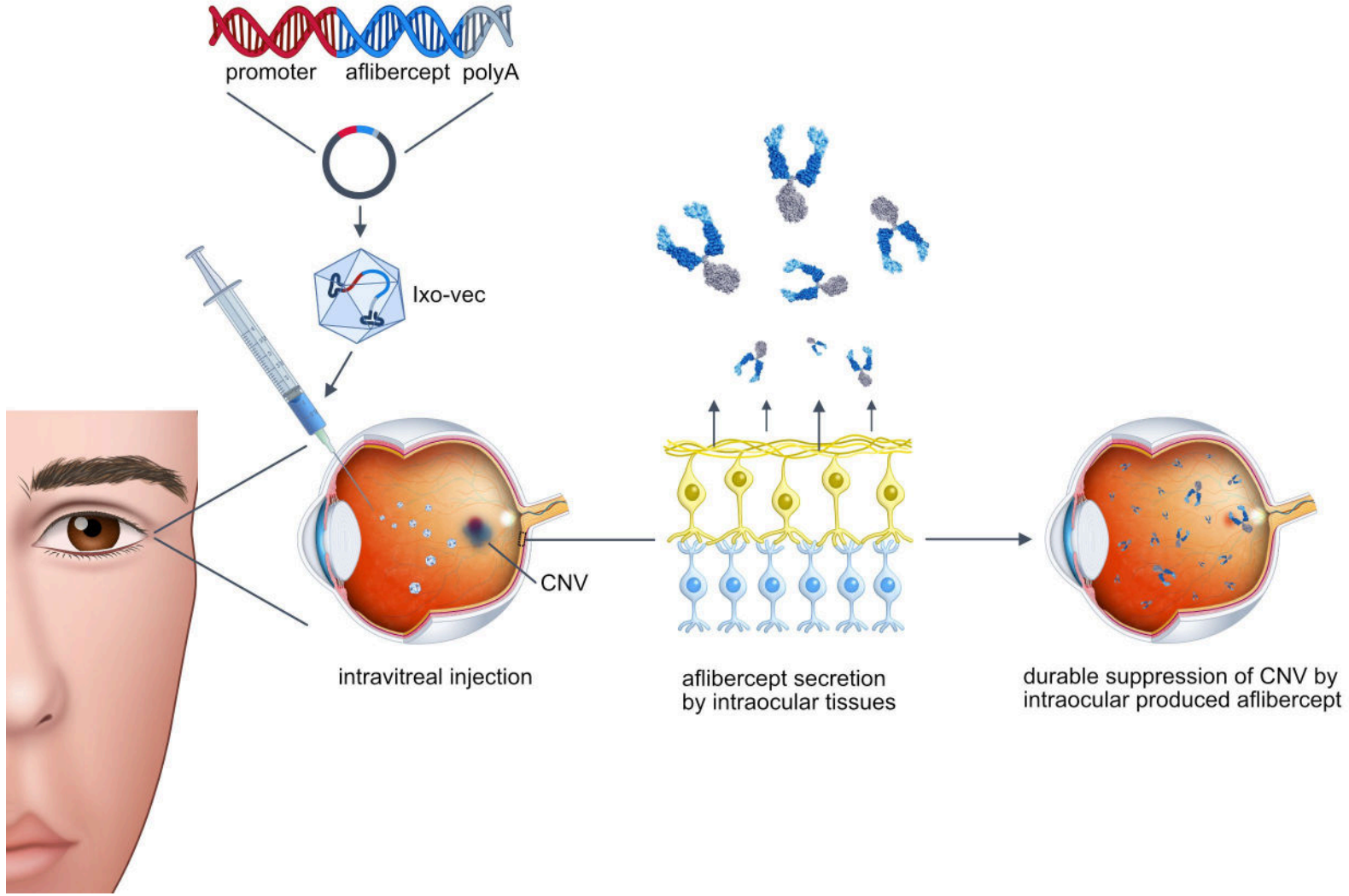
Ixo-vec is a gene therapy biofactory approach designed for continuous delivery of aflibercept (anti-VEGF) by single intravitreal injection



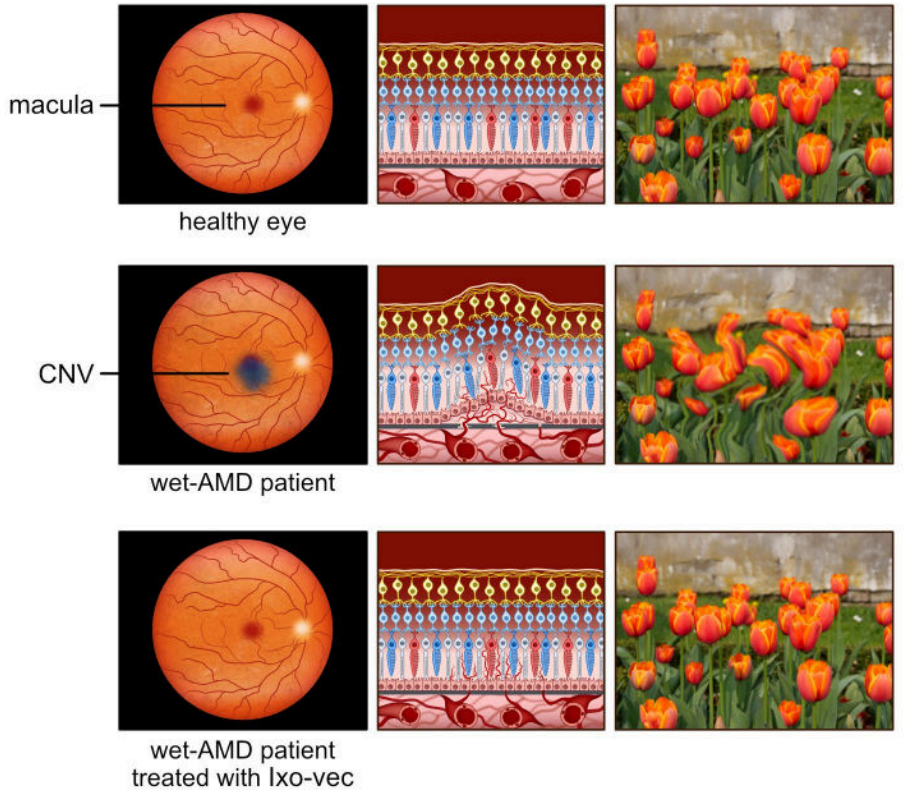


Illustration: Gardenia Gonzalez Gil

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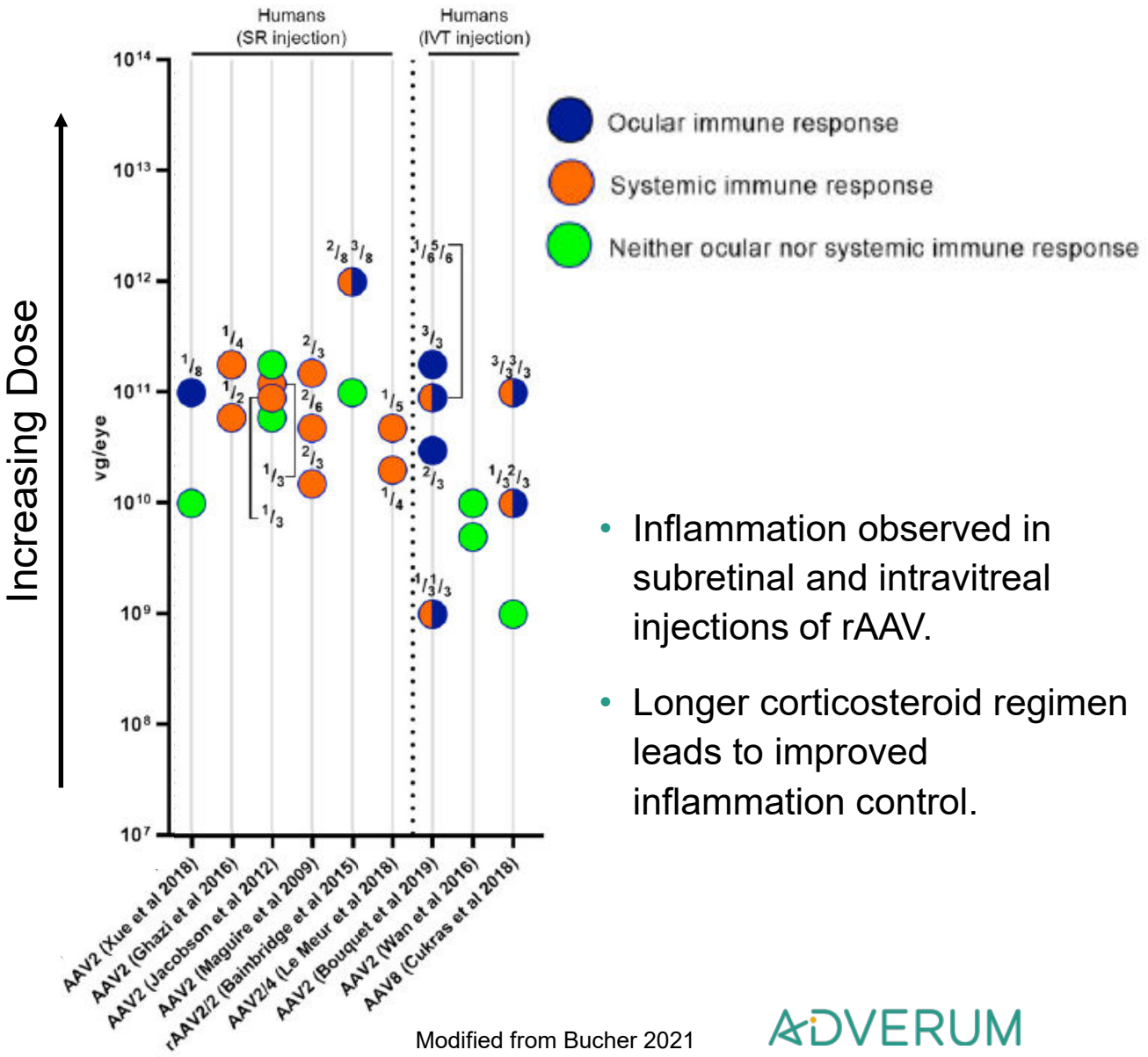
Clinical implementation of gene therapy products is influenced by rAAV immune mediated inflammatory response

- 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.
- Liver gene therapy monitors AST/ALT levels for rAAV immunogenicity. What would be the appropriate markers for non-liver gene therapy?



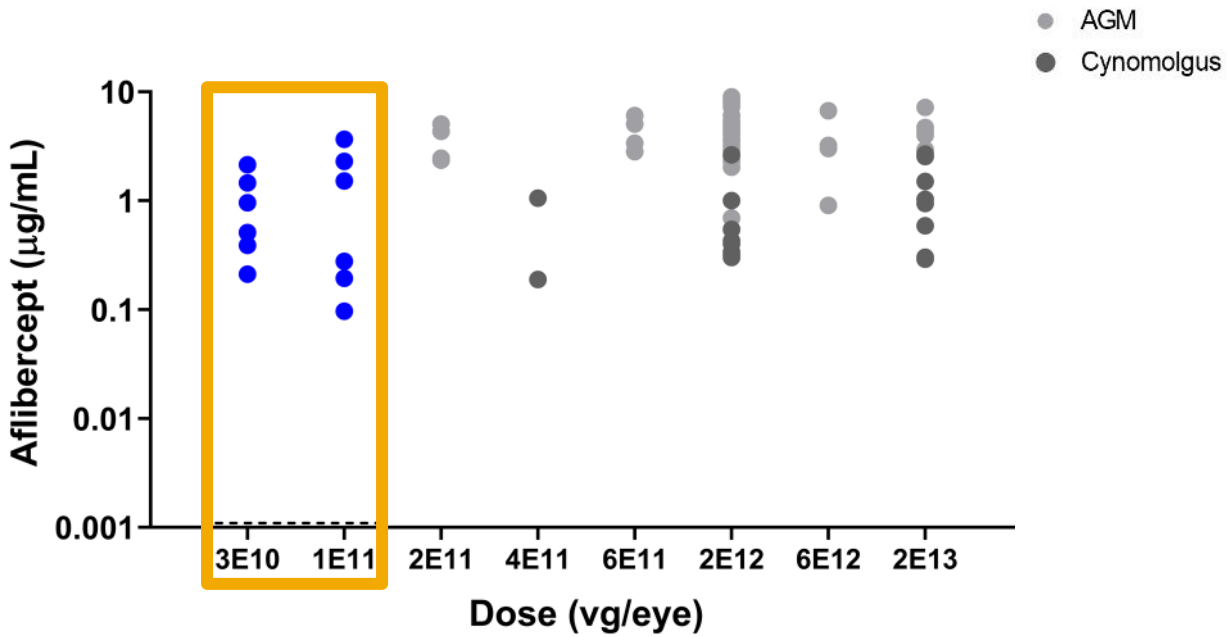
- Inflammation observed in subretinal and intravitreal injections of rAAV.
- Longer corticosteroid regimen leads to improved inflammation control.

Modified from Bucher 2021

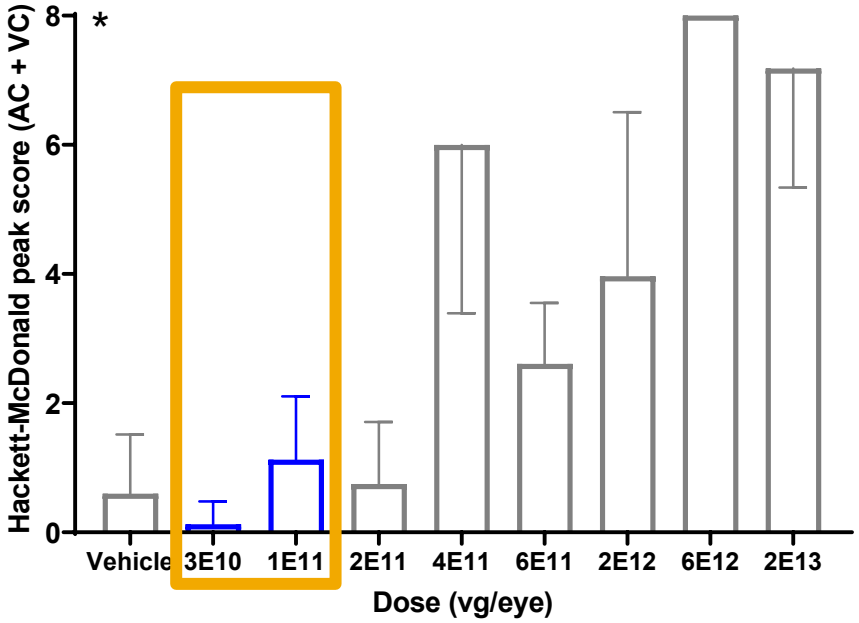
Ixo-vec potency enables ability to dose down to improve inflammation profile

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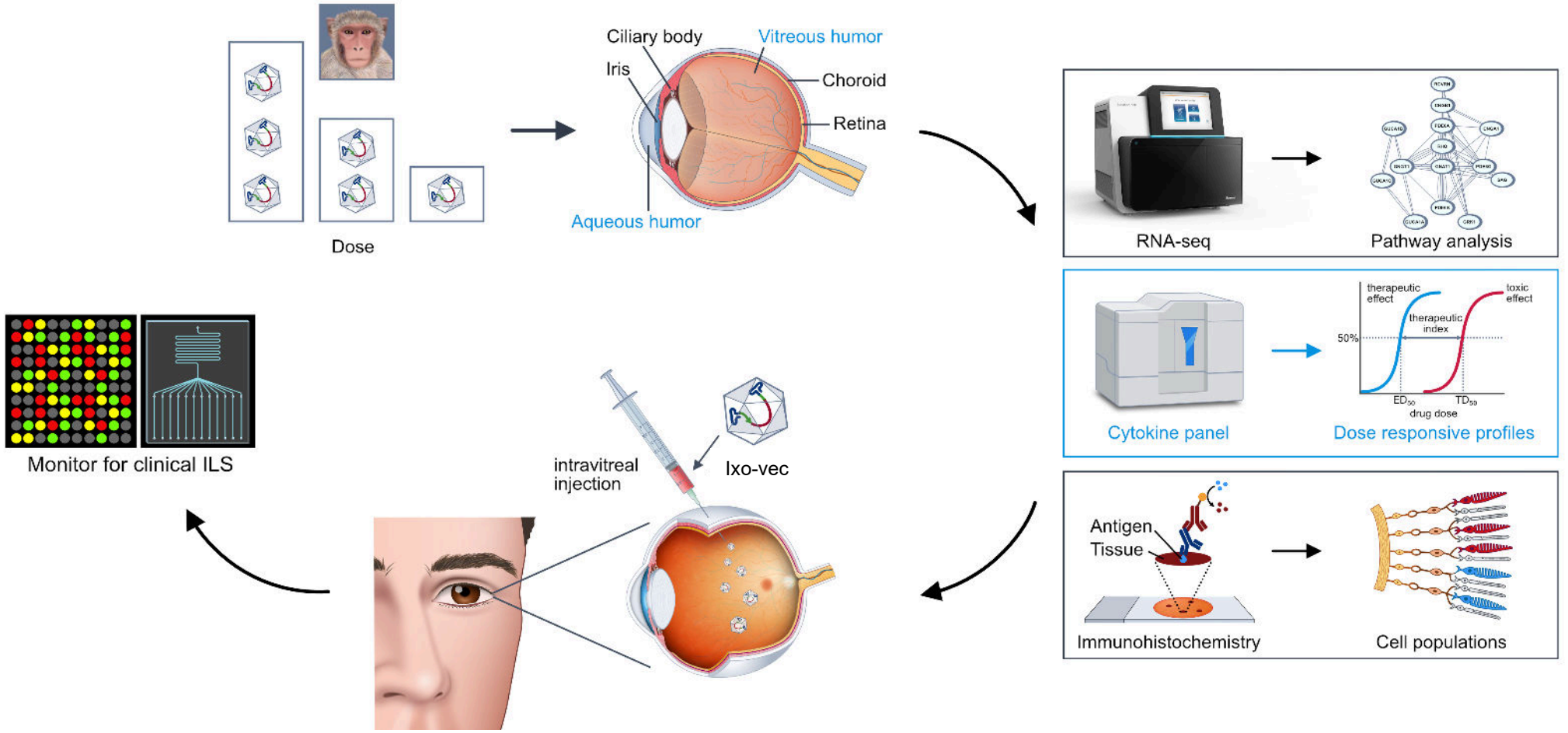
Defining immune landscape signatures of Ixo-vec at supra-clinical doses



- Doses spanning 3-log 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, RT-qPCR of blood, and cytokine panel of serum.

Illustration: Gardenia Gonzalez Gil

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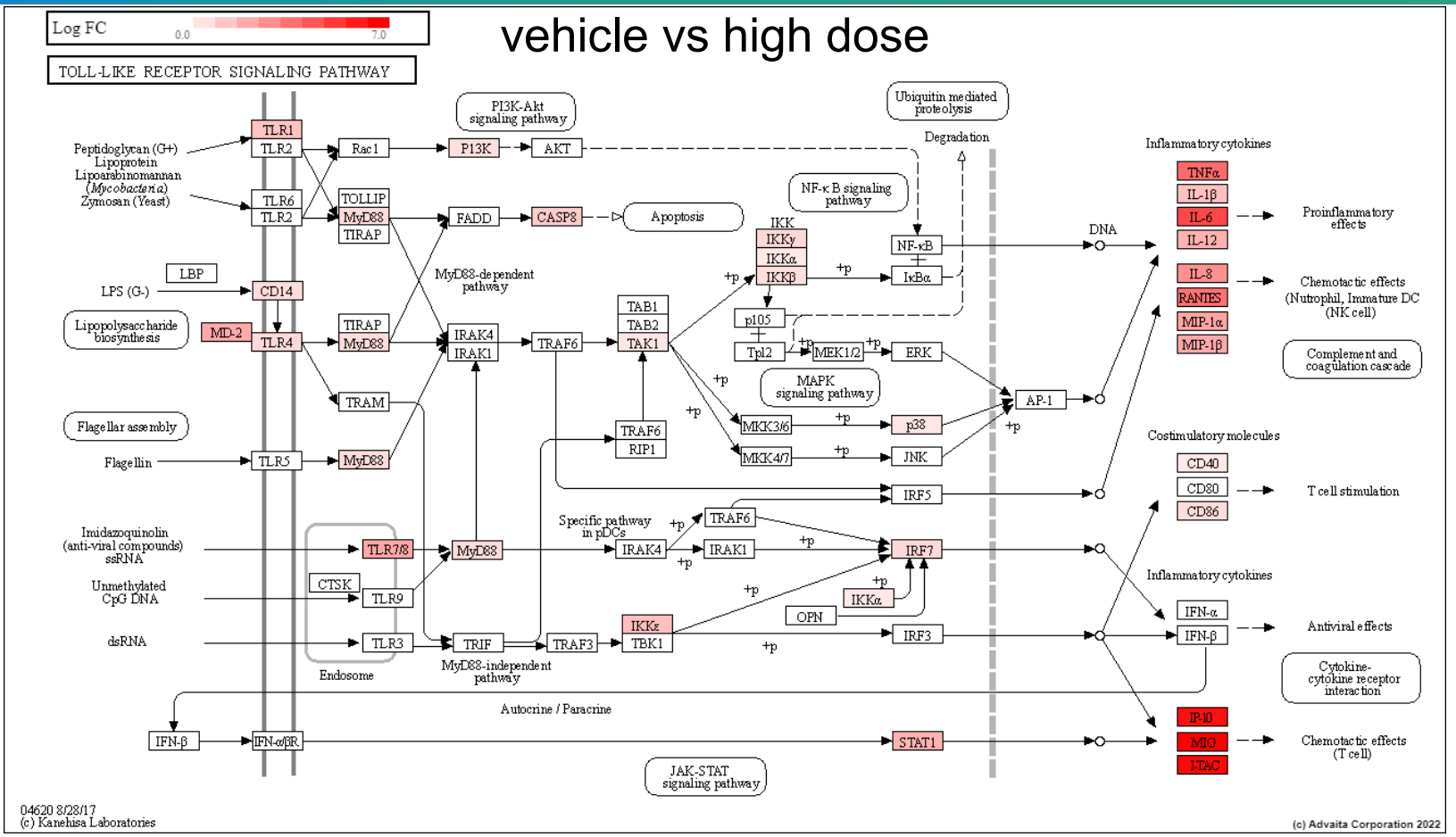
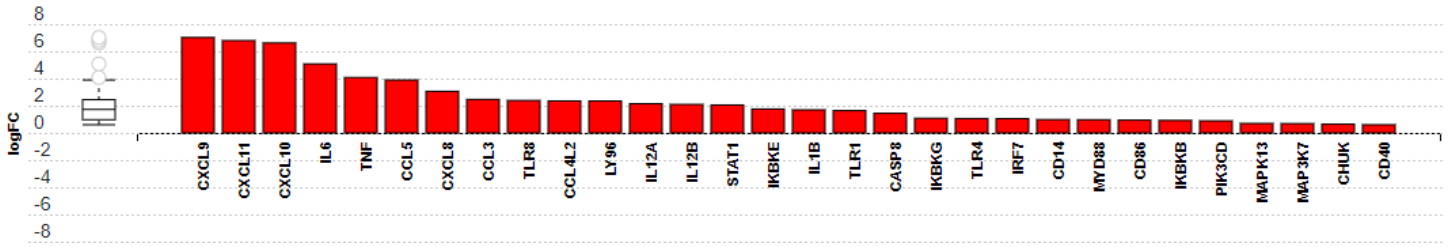
Converging dataset strongly supports dose-dependent inflammatory response

- 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.

Toll-like receptor signaling increased in a dose-responsive manner





- TLR8 suggests response to exogenous mRNA load.[1]
- TLR1/2/4 have reported activation by viral proteins.[2, 3]
- No evidence of unmethylated CpG DNA – TLR9 – MYD88 axis at endpoint.

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

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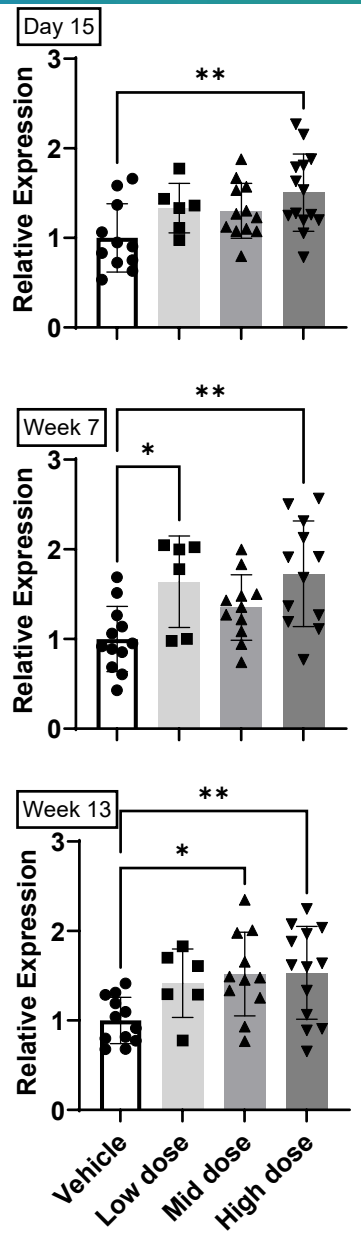
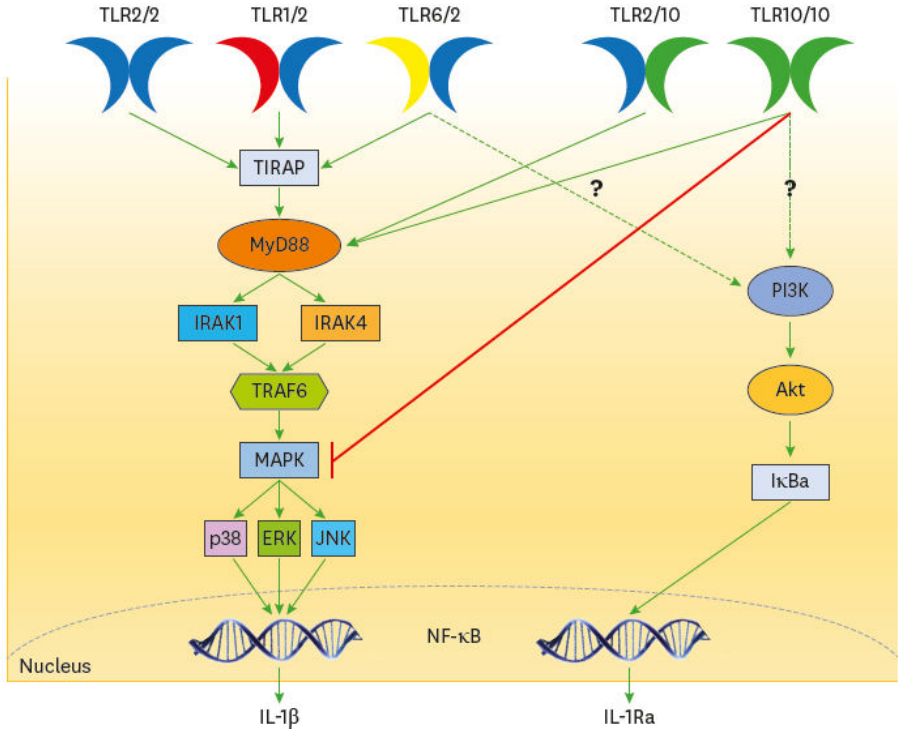
Are ILS biomarkers detectable in non-ocular tissues?

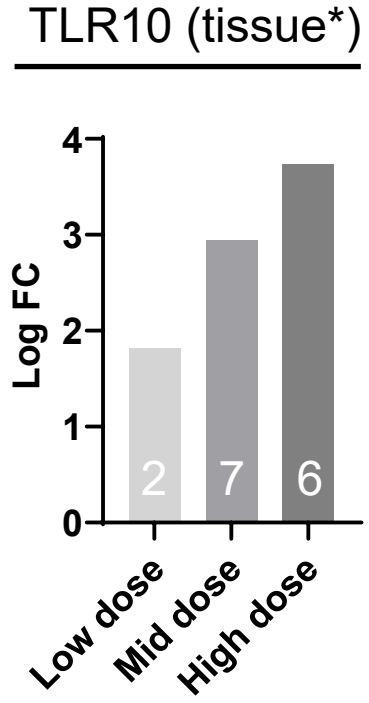
- Initial characterization of ILS focused on ocular tissues and the activation of immune response pathways.
- Peripheral biospecimens such as blood and serum may facilitate monitoring and/or mitigating for immunogenicity to rAAV vectors.
- Cross-referencing ocular NHP RNA-seq data with public RNA-seq AMD datasets to identify candidate ILS biomarkers that would be unique, or enriched, to rAAV-associated inflammation.
- Focus on genes encoding secreted and/or transmembrane proteins.
- Examine at multiple time points after dosing.

TLR10 expression increased in dose-responsive manner





Fore 2020



*N values shown in group columns. Error bars absent from RNA-seq LogFC calculation.

- TLR10 expression increased in blood (left).
  - Expressed in immune cells (T and B lymphocytes, dendritic cells, granulocytes) and non-immune cells (e.g., Muller glial cells).
  - TLR10 has reported activation by viral proteins and additional PAMPs also detected by TLR2.[1, 2]
- Dose-dependent expression also observed by RNA-seq in ocular tissues (top right).
  - Upregulation of both TLR8 and TLR10 reported in viral keratitis.[2]

[1]Su 2021 *Scand J Immun ;* [2]Mohammed 2011 *Cornea*

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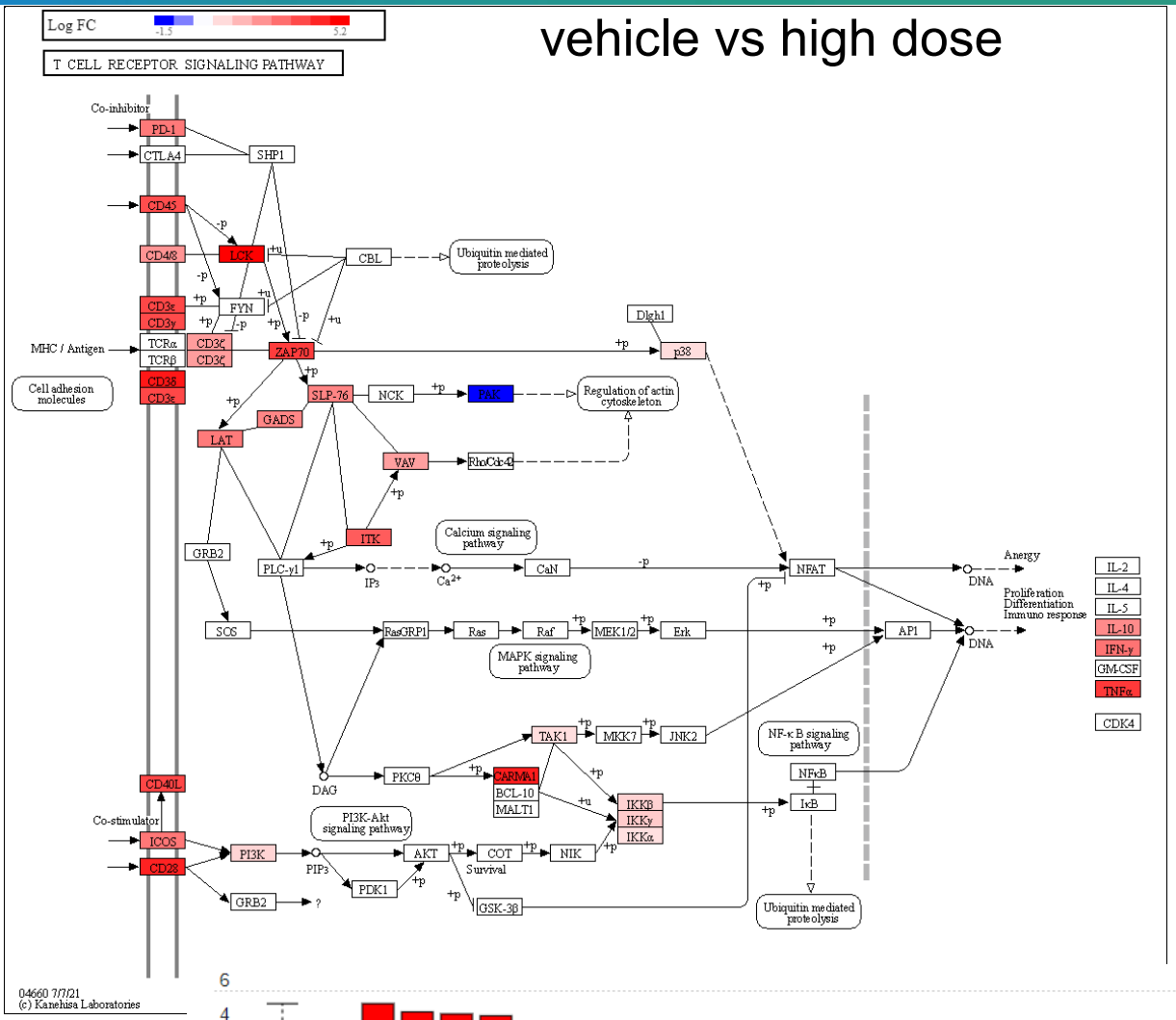
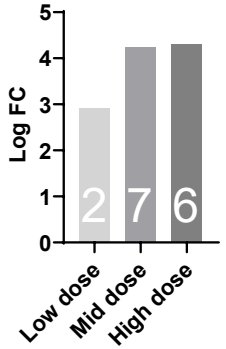
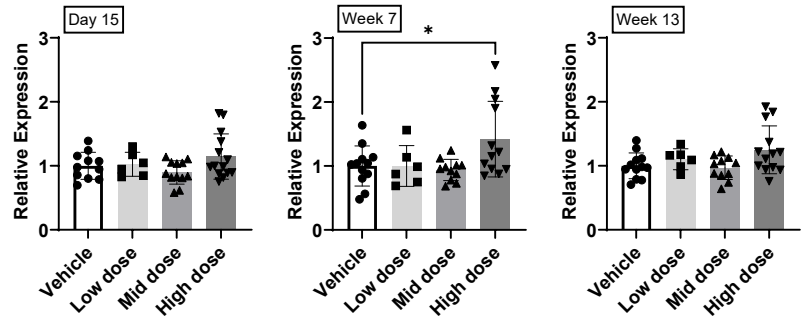
T lymphocyte activation and recruitment increased in dose-responsive manner



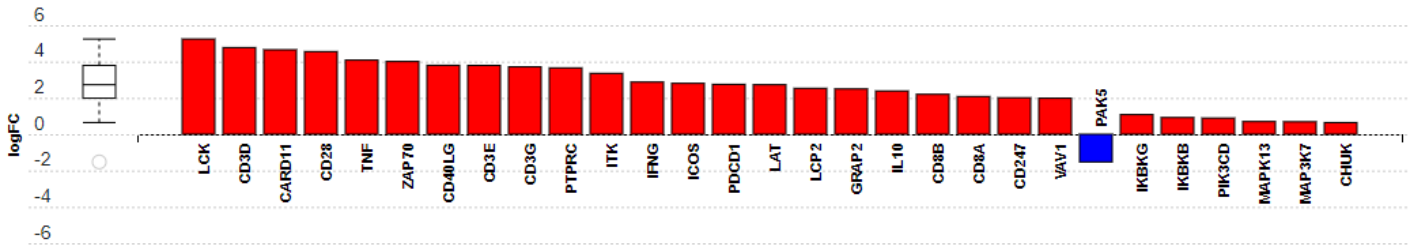
CXCR3 (tissue*)



CXCR3 (blood)



- T lymphocyte recruitment is mediated by chemokine receptor CXCR3. Observed transient increase in blood of high dose group.
- Key chemokine ligands include CXCL9, CXCL10, and CXCL11 (all upregulated in dose-responsive manner) which are induced by INFγ and type I interferons.



*N values shown in group columns. Error bars absent from RNA-seq LogFC calculation.

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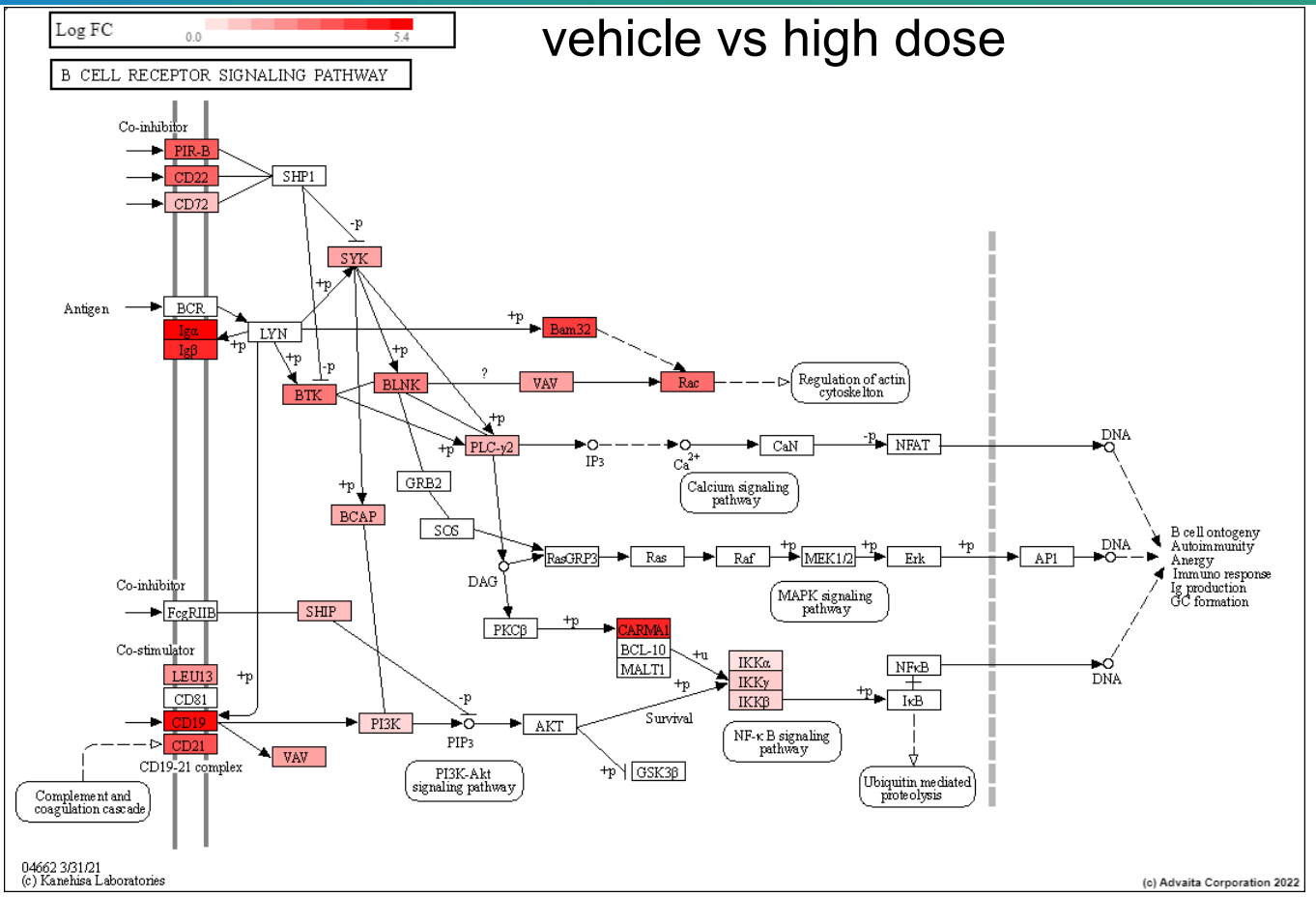
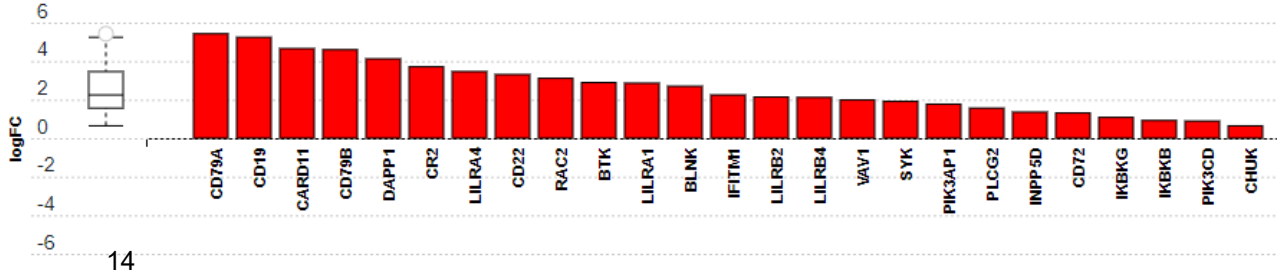
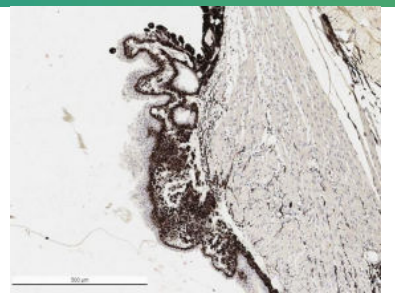
B lymphocyte activation and presence increased in dose-responsive manner

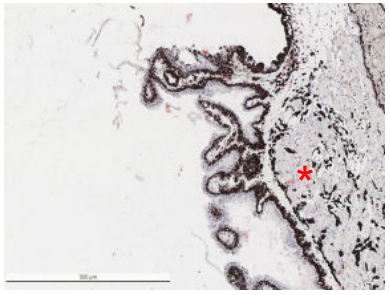




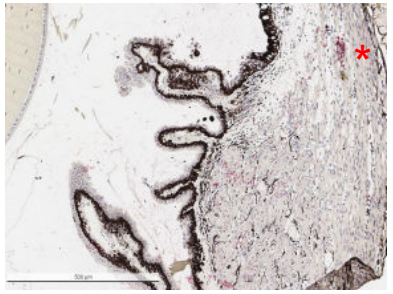
CD20+



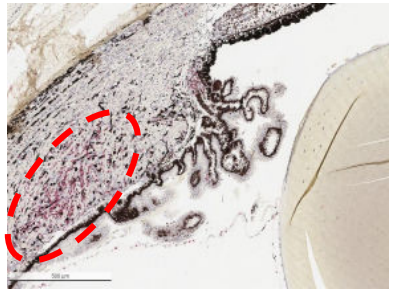
Vehicle



Low dose



Mid dose



High dose

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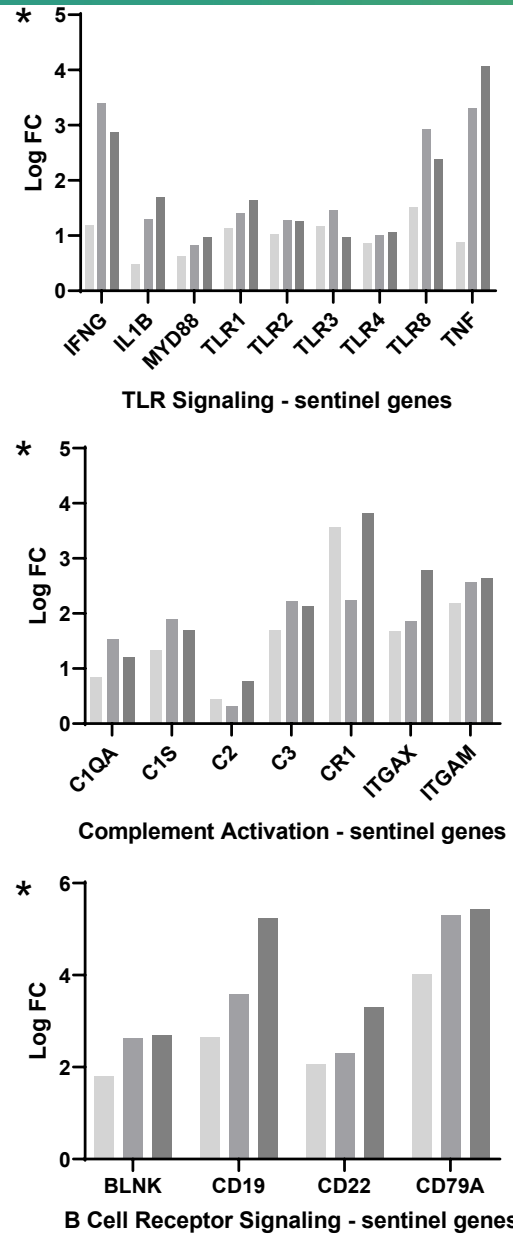
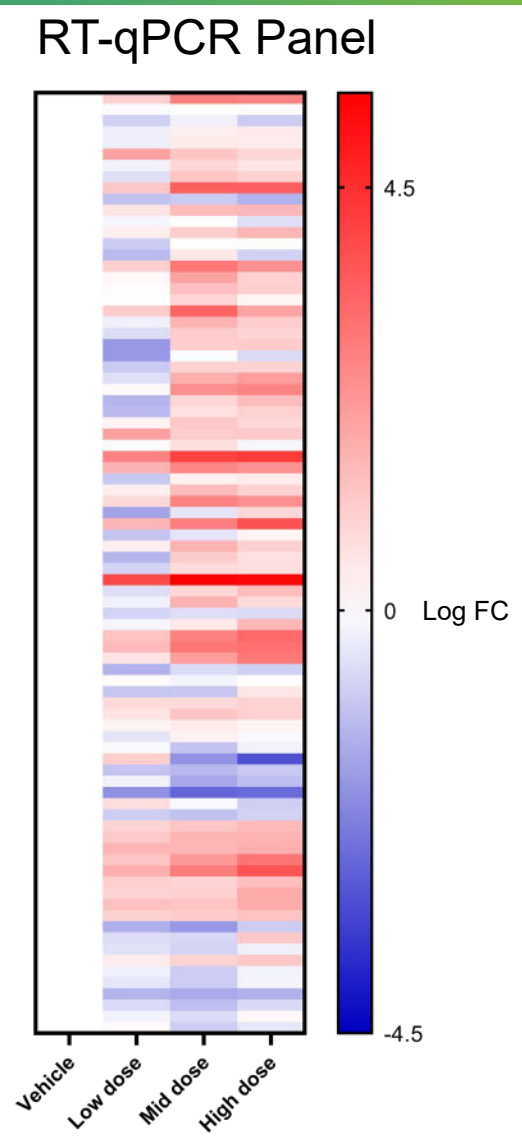
Immune landscape signatures: potential for improved patient management

- 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
  - Management of 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.





*N values shown for each group in figure legend. Error bars absent from RNA-seq LogFC calculation.

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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.
- Identified biomarkers of immune response in peripheral blood.
- 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.

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