Foundation Fighting Blindness

Workshop on Inflammation in Viral Gene Therapy of the Retina

September 14 – 15, 2020

FOUNDATION FIGHTING BLINDNESS

Pre-Meeting Survey Results

Survey circulated to all invited presenters – 16 of 18 responded (89%)

Split into two sections

- Preclinical observations 14 questions
- Clinical observations 13 questions

On average:

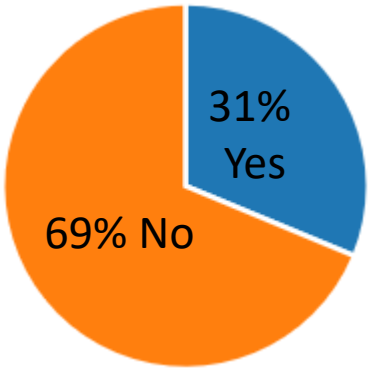
- 15 respondents answered preclinical questions
- 9 respondents answered clinical questions

Not all respondents answered all questions in a section

Average time to complete 15 minutes

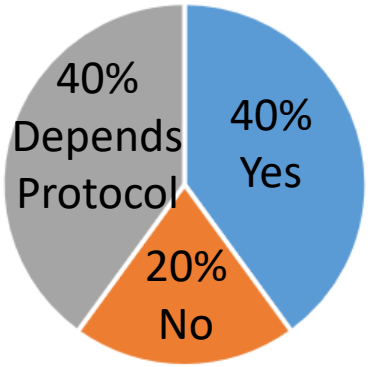
Preclinical

Do you test for pre-existing anti-capsid antibodies prior to intraocular injections?



If measured, do you limit participation if anti-capsid antibodies are found?

"Among *macaca fascicularis* antibody prevalence is very high (>90%)"
"We only use NHPs with low antibodies"
"Yes, if NABs are to AAV"

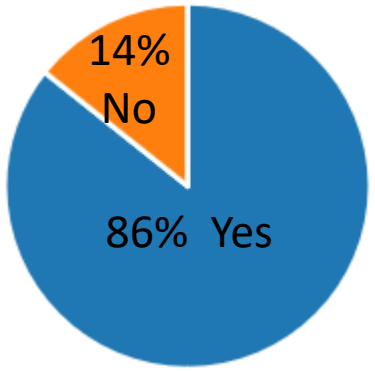


What is your cut-off level for participation if anti-capsid antibodies are found?

"1:10 titers"
">10"

Preclinical

Have you observed antibody or cell-mediated immune responses to intraocular gene therapy injections to any of the capsid isotypes/pseudotypes?



How high have the anti-capsid titers gone?

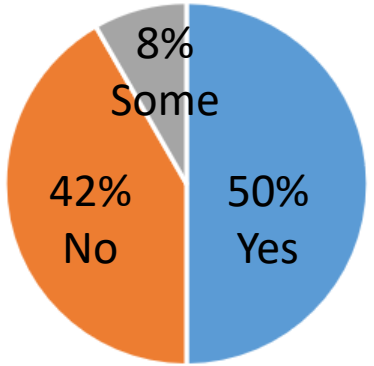
How robust have the cell-mediated responses been?

Ab titer	Cell-mediated Response
1:576	Poor - only a subset of animals and mostly very low and diminished by wk 26
1:1280	
1:2000	Depends on vector, RoA, etc
1:2560 (2)	Dose dependent; Not tested
1:6000	Modest

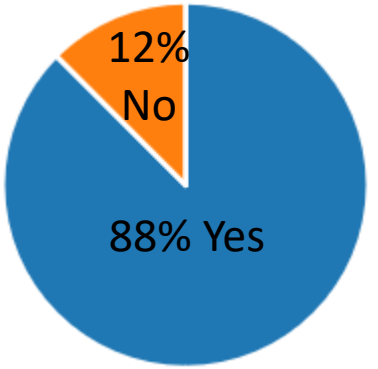
Preclinical

Do you test for changes in anti-capsid or gene product antibodies after therapy?

One commented *"only for capsid, not cargo"*



Have you observed intraocular inflammatory responses after gene therapy injections?



How do you monitor for inflammatory responses?

Most used at least 2 of: clinical examinations, slit lamp exams, OCT, fundus image, histology

1 included flow cytometry

1 included angiography

1 included RNAs for inflammatory genes, microglia number and location

Preclinical

Please describe severity, duration, and treatment approach for the inflammation, if possible.

- Usually modest. Triamcinolone if severe.
- Topical, subconjunctival and oral steroids. Duration and intensity depending on severity
- Tailored accordingly and range from topical steroids, to systemic steroids, intravitreal steroids, and systemic methotrexate
- Increased or prolonged medication with oral Prednisolone
- Steroids (sub conj, topical, oral)
- Moderate clinical ocular inflammation to 5e11vg vitreal. Oral Prednisone 60mg, 6 weeks

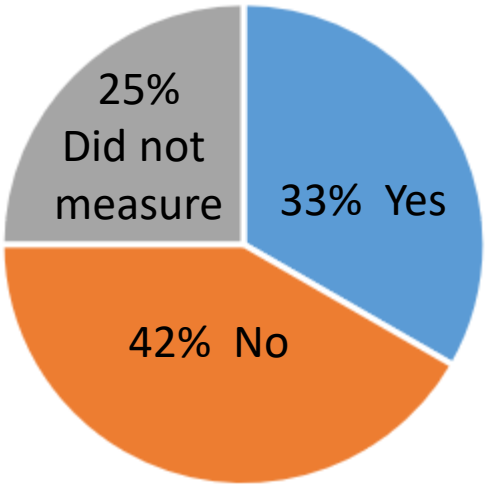
- 5 pigs with 4E11vg AAV8, 1 pig developed severe vitritis starting 2wpi, and slowly self-resolved over time, with complete resolution at 6wpi. Did not treat with steroids as the goal of the study was to understand the inflammation
- Mild inflammation resolving by clinical measures by 1 month. Flow changes persist at 1 month
- None – but researcher reports seeing intraocular inflammatory responses after gene therapy injections

Preclinical

Did intraocular inflammation correlate with efficacy or functional measures (if any)?

Two of the “Yes” noted only if severe inflammation occurred

One “No” indicated insufficient data to reach conclusion



Do you use any form of immune suppression prior to injection?

Compared to those who saw an impact on efficacy:

40% who saw no impact on efficacy did not use immunosuppression

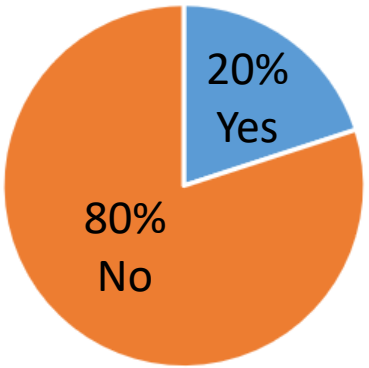
Of the 2 “Yes, only when severe”, 1 used immunosuppression, 1 did not

Of the remainder, 37.5% who used immunosuppression saw an impact on efficacy

Preclinical

What drugs, schedules, and duration of treatment do you use to control inflammation?

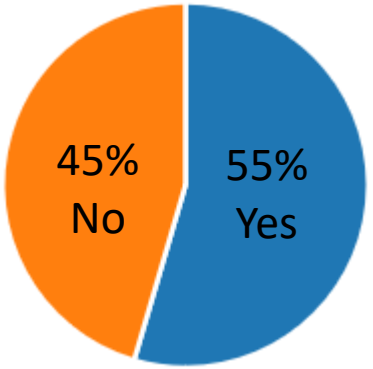
- Pred forte topical hourly and oral prednisone 1 mg/kg
- Topical Pred and oral Prednisone after surgery and a single subconjunctival injection of triamcinolone immediately after surgery
- Prednisone (Oral) start on morning of surgery tappered down over 4 weeks post-injection; Prednisolone (topical) for 4 wks); Triamcinolone Acetonide (Subconjunctival) on day of surgery and 4 wks
- Moderate clinical ocular inflammation to 5e11vg vitreal. Oral Prednisone 60mg. 6 weeks
- Cyclosporine A, 6 mg/kg to reach 150 -200 ng /ml
- Steroids systemic prior to injection/ in some protocols also for a few weeks post-op
- Not in mouse models.

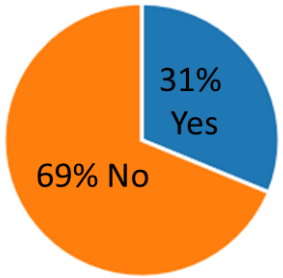


Have you re-treated animals or humans with product after an anti-capsid response has been noted?

Clinical

Do you test for pre-existing anti-capsid antibodies prior to intraocular injections?





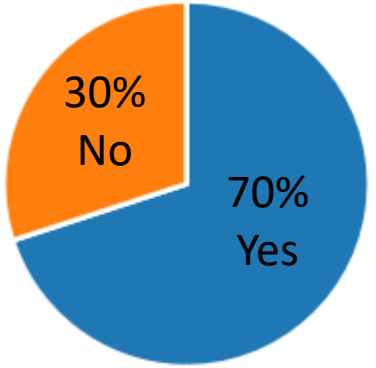
Preclinical

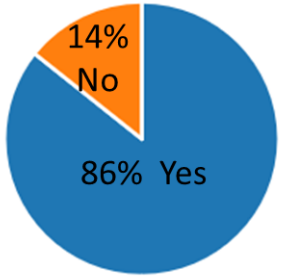
What is your cut-off level for participation if anti-capsid antibodies are found?

- Our clinical studies for AAV8 mediated gene therapy have shown that a humoral immune responses is not elicited (and titer changes after treatment are independent from pre-treatment antibody titers) – group does measure titers however.
- 1:10
- 1:50
- Study protocol defines
- None (50% of responders who do titer)

Clinical

Have you observed antibody or cell-mediated immune responses to intraocular gene therapy injections to any of the capsid isotypes/pseudotypes?





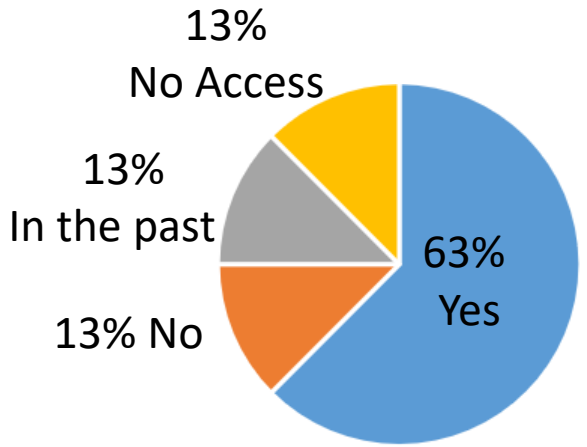
Preclinical

How high have the anti-capsid titers gone?

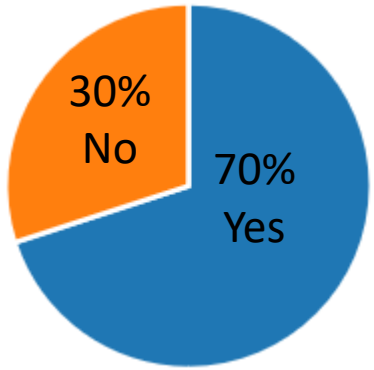
Titer	Cell-mediated Response
1:2000	Mild
1:2560	
1:6000	Modest
Data held by company	vitritis in one subject, retinitis in one subject
Mild/moderate elevation in NAb titer detected in small proportion of cases, and only transiently.	Dose-dependent, and somewhat predictable at high doses, but timing of onset vary between individuals.
1:6620	13 of 15 treated with AAV2/2. Mild anterior chamber inflammation and vitritis were reported at all doses, and all cases were responsive to treatment. A maximum OIS of 9.5 was observed in a patient with history of idiopathic uveitis. Neither ocular inflammation nor immune response could be determined based on the viral dose administered or the patient's immune status at baseline

Clinical

Do you test for changes in anti-capsid or gene product antibodies after therapy?



Have you observed intraocular inflammatory responses after gene therapy injections?



How do you monitor for inflammatory responses?

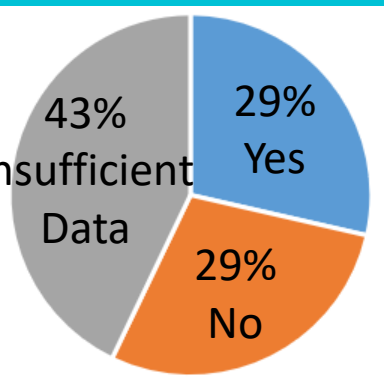
- Most mention 1 or more of: clinical examination, OCT, angiography, fundus image
- 1 includes NAB assay with clinical
- 1 only uses slit lamp
- 2 use OCT only
- 1 mentioned grading according to the international uveitis classification
- 1 mentioned Ophthalmic exam (including IOP measurement) at days 3, 7, wks 4, 6, 9, 11, 13 and 26, serum collected throughout and tested for anti-drug and anti-transgene antibody generation, PBMC collected throughout for T cell activation assay (ELISPOT) using peptide pools to both vector capsid and transgene

Clinical

Please describe severity, duration, and treatment approach for the inflammation, if possible.

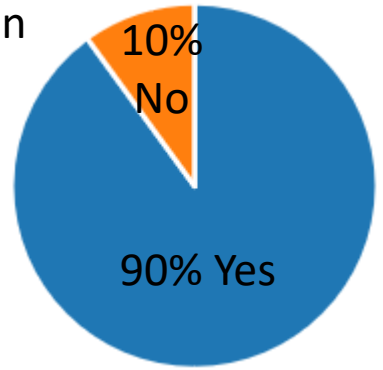
- Mild persistent anterior vitritis that did not respond to oral Prednisone and hourly Prednisolone acetate
- Vitritis was significant, resolved without treatment; subject refused extension of oral steriod. Retinitis required second dosing of steroid. Patient lost vision
- Moderate in many cases, Triamcinolone intravitreal if needed
- Severity dose-dependent, occasionally severe requiring prolonged systemic corticosteroid treatment. Mild cases may settle with local steroid therapy.
- Depends on problem
- A total of 22 AEs of ocular inflammation were treated with topical corticosteroids: Rimexolone (used 9 times), Dexamethasone (used 4 times), Dexamethasone (used twice), and Fluorometholone (used once) (in some cases, concomitant anterior and intermediate inflammation were treated with the same agent).
- Pretreat with oral Prednisone, tapered over 3 months. Topicals as medically indicated.

Clinical



Did intraocular inflammation correlate with efficacy or functional measures (if any)?

Groups saying yes noted mainly in prolonged or severe cases of inflammation



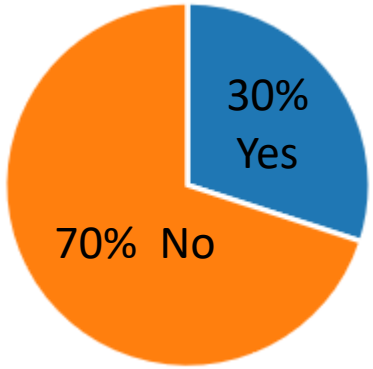
Do you use any form of immune suppression prior to injection?

What drugs, schedules, and duration of treatment do you use to control inflammation?

- Oral Prednisone 1 mg/kg and hourly prednisolone acetate with taper
- Prednisone 1mg/kg/day (2 responses)
- Cyclosporine A, 6 mg/kg SC, to target blood level of 150 to 200 ng/ml
- Oral Prednisolone 1mg/kg starting 3 days prior to gene therapy, then slow taper over 21 days
- Oral Prednisolone for 21 days, starting at 1mg/kg body weight; Prednisolone Acetat Eye Drops 4x daily; Moxifloxacin Eye Drops 4x daily
- For prevention: steroids before injection, in some cases a few weeks after treatment. For inflammatory events steroids, systemic and topical as needed
- Prednisone starting 3 days prior to injection and extending 4 days post injection
- pretreatment with 60 mg oral Prednisone, tapered over 3 months.

Clinical

Have you re-treated animals or humans with product after an anti-capsid response has been noted?



Overall takeaways from survey results

Takeaways	Implications
Intraocular inflammation: • **Inflammation is not rare**; it was actually quite prevalent (70-88% observed it) among the different groups for both preclinical and clinical studies • The severity of inflammation varied among studies • In **more severe or prolonged cases, it was associated with reduced efficacy**	• Preclinical models may be predictive of inflammation observed in clinical studies? • With correct methodologies, intraocular inflammation can be detected, tracked and studied • Prevention and/or management of inflammation key to both safety and efficacy of the gene therapy program
Treatment of inflammation: • There was no consensus regime • Prednisone was most common agent used to treat the inflammation, though different forms (e.g. oral, topical, injected) and lengths of treatment (days to months) were used. • Other immunosuppresants like cyclosporine also being used • Effectiveness of these suppressants varied	• Prophylatic immunosuppression should be considered for most/all therapeutic studies? • Need to better understand optimal immunosuppression protocols