Presentation Guidance - Preclinical

Each presentation is scheduled for 7 minutes with an additional 3 minutes for participant questions, interspersed with longer general discussion periods.

Rather than a program overview, each presenter is asked to focus specifically on any immunity issues, clinical, or potential subclinical inflammation, they have experienced in retinal gene therapy, including the impact on their studies, and how it was handled or adjusted for.

While no presentation may address all the points below, to promote a productive discussion, and to aid consistency between presentations, we have provided a list of considerations and questions pertinent to inflammation. We hope this will provide some guidance and direction for presentation.

Factors that may impact inflammation in Gene Therapy

Vector Design and CMC

Vector components and manufacturing methods can influence the immunogenicity of the IMP:

1. Type of vector, including serotype/pseudotype (*e.g.*, 'AAV8' or 'HIV-derived lenti pseudotyped with VSV-G'. For AAV if single-stranded or self-complementary)
2. General description of manufacturing process. Details such as potentially immunogenic components of production/storage media, titering method, and purification method
3. General description of quality control (*e.g.*, for AAV: SDS-PAGE, endotoxin levels, empty to full capsid ratio, residual DNA content, etc.)
4. Characteristics of the insert (*e.g.*, CpG content of gene, intron, promoter, enhancer, other transcriptional or translational control sequence)

Study Design:

The choice of non-clinical model can impact outcome:

1. Species and sex of test animal and age at administration
2. Which virus capsids are being used and at what viral load?
    a. Detection of differences in immune response related to viral load or volume injected?
3. Route of administration
    a. For intravitreal, needle gauge and length, position of injections
    b. For subretinal, specific techniques used
4. For AAV, whether pre-existing neutralizing antibody (NAb) titers were measured and what ranges were observed
5. Length of in-life portion of study(ies)
6. Dose(s) administered, including volume and if subretinal, whether single or multiple blebs

7. Any inflammatory events observed (by clinical examinations or other methods such as OCT), including severity, timing, and duration. Did these correlate with functional assays of vision (*e.g.*, ERG) and/or transgene expression, if measured
8. Interventions and outcomes taken for inflammatory events
9. Immunosuppression usage,
    a. Systemic vs. intraocular?
    b. Immunosuppression regimen (agent(s), dose, duration, and timing)
    c. Any measures of intraocular levels of immunosuppression?
10. Any side effects of immunosuppression that were observed.
11. Have there been any correlations betewwn inflammatory events with pre-existing NAb titer, seroconversion, and observed transgene expression?
12. Have there been any inflammation or pathology during post-life analysis
    a. ONL thinning or cellular infiltrate by histology?
    b. Cellular activation of microglia, mueller glia?
    c. Vascular leak or neovascularization?
13. Have antibody or T-cell responses or changes in inflammatory cytokines been measured?
14. Have any immunologic responses (or increased risk of responses) been observed that may have been associated with surgical/drug delivery complications?

Clinical Studies:

For presentations describing human studies, please comment on:

1. What virus capsids are you using and at what viral load?
    a. Have you noted a difference in immune response related to viral load or volume injected?
2. Route of administration of vector:
    a. If intravitreal, please note needle gauge and length, placement of injection
    b. If subretinal, please describe method of injection in detail, including concentration and duration of exposure to agents such as triamcinolone during pars plana vitrectomy
3. Dose(s) and volume and if subretinal, whether single or multiple blebs
4. Age and gender of patient(s), and if null patients
5. If using AAV, do you measure pre-existing neutralizing antibody (NAb) titers?
6. Have you seen intraocular or peri-orbital inflammation after injection?
    a. What kind of reactions?
    b. What is your monitoring schedule? How do you monitor?
    c. When do the reactions start?
    d. How long do they last?
    e. How do you treat?
    f. Did you observe any relationship between inflammation and efficacy or functional outcomes?
7. Do you use immunosuppression?
    a. Please describe the protocol including agent name, dose, time to start immunosuppression, duration, timing, and route of administration

    b. Are all clinical sites using the same protocol?
    c. If not, why not?
8. Have you seen any clinical side effects of immunosuppression (*e.g.*, weight gain, loss of sleep, cataracts, etc.)?
9. What kind of inflammatory events have you seen?
    a. How detected , i.e., clinical examinations, OCT, etc.?
    b. Do you measure functional assays of vision (*e.g.*, BCVA, ERG).
    c. Do you measure changes in systemic or intraocular antibody response? Do they correlate with intraocular inflammation
    d. Do you collect anterior chamber or intravitreal fluid for antibodies or cytokines?
10. If inflammation is observed, how do you treat it?
11. Please describe outcomes of interventions.
12. Have you seen immune responses (or increased risk of an immune response) associated with surgical complications?
    a. How frequently are surgical/drug delivery complications see?