Recommendations for immunogenicity testing requirements for AAV gene therapy

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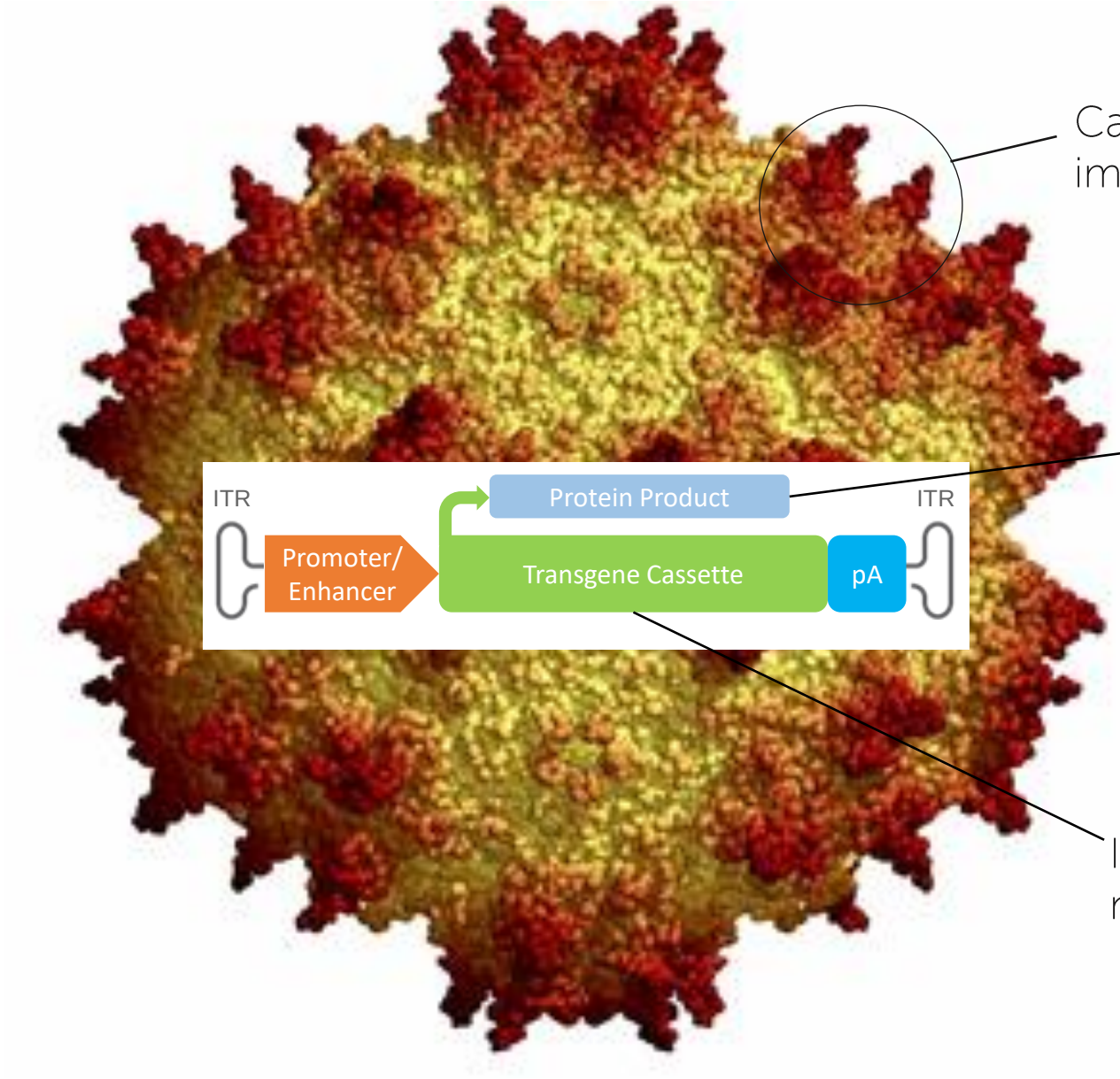
Outline: Immunogenicity Considerations for Gene Therapies

- Complex Drug Design with Multiple Components That May Impact Immunogenicity
- Factors Affecting Immunogenicity for AAV-Mediated Gene Therapy
- Immunogenicity Considerations for Clinical Monitoring
- Patient Screening and Companion Diagnostics
- Repeat Dose Administration and Iterative Dosing
- Streamlined Recommendations for Immune Monitoring

AAV-Gene Therapies Contain Multiple Components That May Impact Immunogenicity



Capsid-specific immunogenicity

Immunogenicity directed toward transgene-expressed protein

Innate sensing of nucleic acids, PAMPs

Patient-Specific Factors That May Impact Immunogenicity: Risk-Based Approach

Host-Specific Factors	Enhanced Immunogenicity Risk	Reduced Immunogenicity Risk
Use of immunomodulators	No use of immunomodulators	Prophylactic/reactive use of immunomodulators
Disease specific changes in target tissue	Pro-inflammatory state, auto-immunity	Healthy
Pre-existing immunity to capsid	Previous exposure to capsid; pre-existing immunity present	Naïve to capsid; no pre-existing immunity
Prior Exposure to Recombinant Protein	Naïve patients or patients with pre-existing Abs against recombinant protein	Patients with prior exposure to recombinant protein with no Ab development; acquired immune tolerance
Underlying Mutation	Null mutations	Missense mutations
Route of Administration	Intravenous, Intramuscular	Immunologically protected sites (Intra-ocular, CNS, parenchymal)

Vector-Specific Factors That May Impact Immunogenicity: Risk-Based Approach

Vector-Specific Factors	Enhanced Immunogenicity Risk	Reduced Immunogenicity Risk
Capsid Serotype	High seroprevalence, evidence of innate immune activation	Low seroprevalence
Dose	High dose	Lower dose
Promoter	Constitutive (Ubiquitous)	Tissue-specific, Liver-specific (tolerance)
Transgene	Large size, secreted	Small size, intracellular
CMC/Manufacturing	(More impurities and variability) Less defined critical quality attributes & critical process parameters	(Less impurities and variability) Well-defined critical quality attributes & critical process parameters
Vector Genome	Self-complementary, CpG-rich, dsRNA intermediates	CpG-low, single-stranded

Immunogenicity Considerations for Clinical Monitoring

Capsid-Related

- Pre-existing immunity to AAV capsid
- Innate and inflammatory activation by AAV capsid (PAMPs)
- Development of adaptive humoral and cellular immune responses to the AAV capsid following dose administration

Transgene and Vector-Related

- Innate immune activation by nucleic acid (TLR, PRR, CpG, dsRNA, etc.)
- Development of adaptive humoral and cellular immune response to the expressed transgene product

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Immunogenicity Monitoring Considerations

Patient Screening

- AAV antibody pre-existing immunity
- Transgene specific immunogenicity
- In vitro diagnostic (may depend on route of administration)

Dose Administration

(Proximal to infusion: Hours-

- Collection of Baseline samples
- Sampling to assess Infusion related clinical events
- Early time points for other markers of innate immune activation (cytokine, complement, etc.)

Short Term Follow Up

(First 2 yrs)

- Monitoring for Humoral and Cellular immune responses against AAV capsid and transgene product
- Markers of innate immune activation and inflammation

Long Term Follow Up

(>2 years)

- Monitor for transgene specific responses and associations with safety and efficacy
- Evaluate clinical value of continued monitoring of capsid-specific immune responses

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Patient Screening – Prior to Dose Administration

Pre-existing immunity against AAV capsid

- May reduce transduction efficiency, alter biodistribution to non-target tissue, and form immune complexes that activate complement and induce adverse events
- Samples should be collected for retrospective analysis to understand the role of pre-existing immunity in clinical studies
- May be less of an issue if gene therapy is administered to an "immune privileged site" (ex. ocular, brain, parenchymal, etc.)

Pre-existing immunity against expressed transgene product

- Pre-existing antibodies to the expressed transgene product may reduce efficacy by neutralizing the activity and/or clearing the therapeutic product
- Safety may be negatively impacted if antibodies bind to nascent gene therapy expressed product or endogenous proteins that resemble the gene therapy product
- Consideration: A determination that patients with pre-existing immunity be excluded from treatment should be based on orthogonal clinical data or non-clinical studies. Impact on safety and efficacy may vary by capsid serotype and transgene product.

Testing for Pre-existing AAV Antibody

The clinical plan should consider studies to assess the effect of pre-existing immunity on safety and efficacy and evaluate a titer threshold

- Needs to be assessed for each GT therapy separately

Pre-existing anti-capsid antibodies can be neutralizing (NAb) on non-neutralizing (binding)

- Both antibody types may negatively impact transduction efficiency and safety
- NAbs are often detected using cell-based transduction inhibition assays
- Total binding antibodies (TAb) are most often detected using ELISA or ECLA-based methods
- The clinical relevance of TAb or NAb results are best determined empirically

In vitro diagnostic should be considered if patients with pre-existing immunity are excluded from clinical trials or known to have an altered safety or efficacy profile

- Zolgensma – Use a laboratory developed test (ELISA); titers above 1:50 were not studied in clinical trials. Patients recommended to have ≤ 1:50 anti-AAV9 Ab titers prior to infusion.
- Luxturna - Pre-existing immunity to AAV2 was assessed during clinical development, but did not appear to affect safety or efficacy, and assessment is not required for administration of Luxturna.

Some consideration should be given for the potential utility of guidance documents addressing more detailed requirements for diagnostics used to test for pre-existing immunity to gene therapies.

The extent of validation necessary should match the stage of product development and that the level of validation should be "fit-for-purpose" or appropriate for the intended purpose of the study

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Human Gene Therapy for Rare Diseases: https://www.fda.gov/media/113807/download

Considerations for Acute Post-Dose Administration – Proximal to Infusion (Hours to Days)

Infusion-Related Reactions: adverse events have been observed within hours of infusion

- Acute hypersensitivity or inflammatory reactions may occur
- Assessment of innate and inflammatory markers should be considered, ex. complement activation, cytokine levels, etc.

Safety events have been observed with days to weeks that may require inflammation monitoring

- Reduced platelets, complement activation (ex. Solid DMD AAV9-GT, Pfizer DMD AAV9-GT)
- Acute kidney injury (Pfizer DMD AAV9-GT)
- Liver inflammation, liver enzyme increases (ex. Zolgensma SMN1 AAV9-GT, Audentes XLMTM AAV8-GT, BioMarin FVIII AAV5-GT)
- Elevated troponin levels (ex. Zolgensma)
- Bacterial infection and sepsis (Audentes XLMTM AAV8-GT)

Considerations for Short Term Follow Up – First 2 years Post-Dose Administration

Adaptive Immunity Monitoring Post-Dose Administration

Monitor for development of anti-capsid humoral and cellular response

- Expected development of anti-capsid antibody response
- Potential for cellular response targeting transduced cells

Monitor for development of **anti-transgene** product humoral and cellular response

- Potential for Ab development against transgene product
- Potential for cellular response against expressed transgene product

- Ongoing monitoring for markers of inflammation – liver enzymes, complement, etc.
- Assess for association between immunogenicity measurements and safety/efficacy parameters

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Considerations for Long Term Follow Up: 2+ years Post-Dose Administration

Adaptive Immunity Monitoring Long-Term

- Monitor for associations with safety or declining efficacy and anti-transgene product humoral and cellular immune responses
- Evaluate clinical value of continuing to monitor anti-capsid humoral and cellular immune responses when capsid antigen no longer detectable
- Some amount of flexibility in decreasing or stopping the monitoring of anti-capsid humoral and cellular immune responses when capsid antigen is no longer detectable may be warranted
- Continued testing may have additional value where repeat dose administration is being considered

Addressing Immunogenicity Considerations

- Sponsors
  - Determine and justify the appropriate mechanism to address considerations through prior experience, literature data, preclinical testing, patient monitoring or patient testing
  - Employ a risk-based approach to respond to treatment emergent immunogenicity concerns
- FDA
  - Share considerations and discuss appropriate approach to justification of various concerns
  - Clarify appropriate testing standards based on level of risk
  - Provide updates on ongoing or emerging immunogenicity concerns

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Repeat Dose Administration

- The Development of high-titer anti-capsid antibody responses following dose administration will likely diminish the efficacy of repeat dosing
- Immune mitigation steps may be appropriate to prevent or reduce immune responses to allow for repeat or iterative dose administration (if needed)

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Summary

- AAV mediated gene therapies represent a complex treatment modality with multiple components that may impact immunogenicity
- ASCGT members request FDA issue immunogenicity recommendations, which are updated regularly to reflect progression and learnings in the field to provide developers with a clear risk-based framework, to facilitate GT development. Recommendations could-
  - Employ a risk-based approach for immune monitoring that accounts for features of the therapy and the patient population (pre-existing immunity, innate/inflammatory immune activation, and adaptive immune responses, etc.)
  - Include the circumstances under which analyses to determine if anti-GT immunogenicity responses are impacting safety and/or efficacy are required
  - Include the circumstances under which immune monitoring in a temporal manner with respect to dose administration is required
- Consider whether future iterations of Human Gene Therapy for Rare Disease guidance documents could provide more detailed information pertaining to the appropriateness and utility of a companion diagnostic for assessing pre-existing immunity and requirements for product approval

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Thank you