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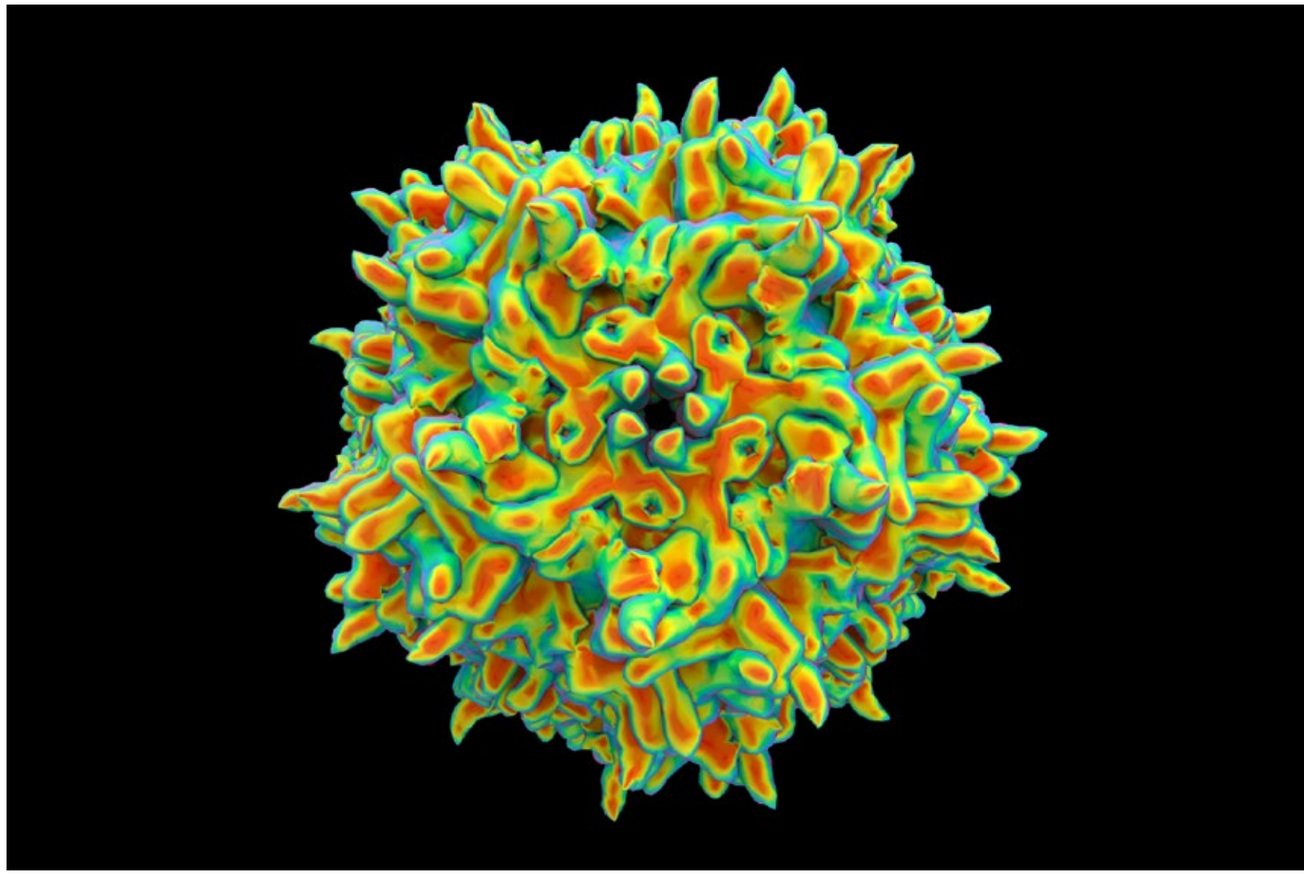

# Immunogenicity and Toxicity of rAAV Gene Therapies in Non-human Primates in Preclinical Safety Evaluation

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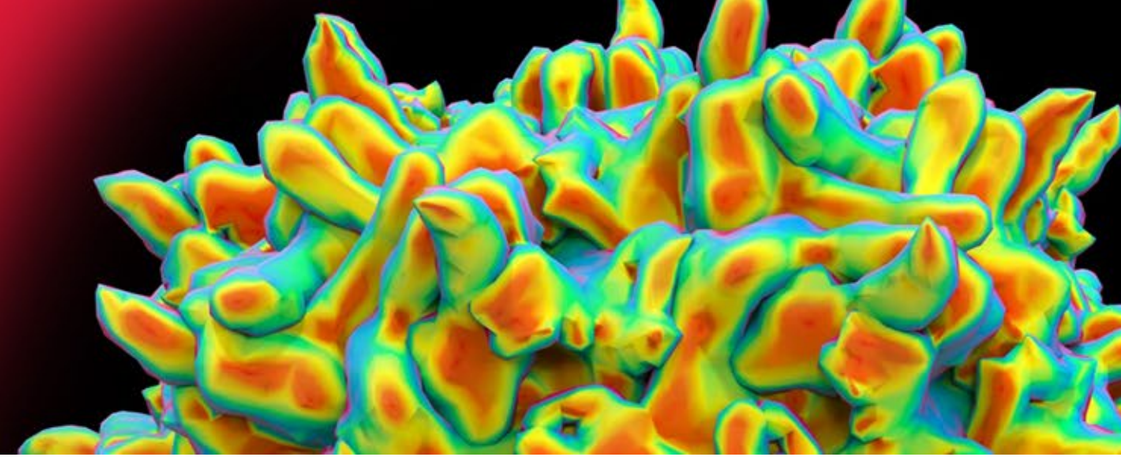


## BACKGROUND & PURPOSE

Recombinant Adeno-associated virus (rAAV) is among the most widely used vectors for gene delivery, yet its clinical applications are challenged by severe immune-mediated toxicities. This study aimed to define a core set of parameters for preclinical assessment of rAAV immunogenicity and toxicity.

## METHODS

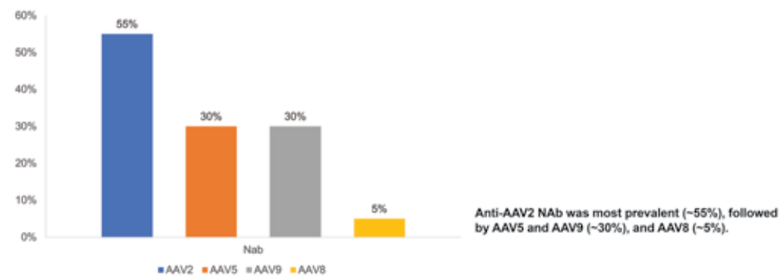
We conducted a pooled analysis of 16 non-human primate (NHP) toxicity studies over the past five years, evaluating a multidimensional dataset: pre-existing immunity, innate immunity (cytokines, complement), adaptive immunity (antibody/T-cell responses), clinical pathology, and histopathology of key organs.

## RESULTS

Pooled analysis across the 16 studies included a total of 538 NHPs administered with rAAV via subretinal, intravitreal, intravenous, or intrathecal injection. By study count, ocular indication was the majority (68.75%), followed by central nervous system (12.5%), hemophilia (12.5%), and cardiac (6.25%) indications. AAV 2, 5, 8, and 9 vectors were selected based on their distinct tissue tropisms. Toxicity profiles were influenced by administration route and vector tropism. As anticipated, ocular delivery was associated with local inflammation, while systemic administration resulted in hepatotoxicity, consistent with the underlying biology of target tissues. To exclude animals with high pre-existing immunity, all studies implemented rigorous serological screening - 87.5% used neutralizing antibody (NAb) assay with titer cut-off ≤1:4 to 1:20 and 12.5% used binding antibody (BAb) assay with an OD value of < 0.5).

## RESULTS

**Figure 1 Pre-existing immunity of different rAAV vectors**




Innate immune activation is a recognized trigger of adverse events in clinical rAAV trials. To investigate this, we monitored early post-dosing biomarkers, including a broad panel of cytokines and complement factors, with sampling within the 24-hour window. Notably, across all studies evaluated, no significant innate immune activation was detected. Adaptive immune response is a pivotal determinant of safety and efficacy profiles. Humoral immunity was assessed by measuring serum and local neutralizing antibody against AAV capsid, as well as binding antibody against transgene product. Cellular immunity was evaluated via IFN-γ ELISpot in response to capsid or transgene product. In these studies, both humoral and cellular immune were evaluated prior to dosing, at 2 to 4 weeks post-dosing, and over prolonged periods (months). After dosing, high-titer serum Nab was detected, while local Nab was low (Table 1) and infrequent anti-transgene antibody (10%-30%) were detected. Critically, cellular immune response was minimal, with positive IFN-γ ELISpot signals detected in only 2 of 13 studies.

**Table 1 The result of Nab after dosing**

| | Animal | Titer |
|---|---|---|
| **Serum** | 70%-100% | 1:256-1:8000 |
| **Local tissue** | 10%-30% | 1:4-1:256 |

Clinical trials of rAAV products have reported hepatotoxicity, thrombotic microangiopathy and dorsal root ganglia toxicity. To evaluate these potential toxicities, key parameters were monitored, including liver enzymes (ALT, AST and GGT) and kidney function (UREA and Cre), histopathology of liver, kidney,

## RESULTS

and dorsal root ganglia. In the present review, most studies showed no evidence of treatment-related liver toxicity. An elevation of ALT without corresponding microscopic findings was observed in only one study, affecting 2 out of 10 animals.

Hepatocellular vacuolation was noted in three studies from the same sponsor but confirmed to be fatty granules via Oil Red O staining. It is noteworthy that the only study to report elevated ALT level was one of the two studies with a positive T-cell response, suggesting a potential association between cellular immunity and hepatocellular injury in that specific context. Kidney function and the histopathology of kidney and dorsal root ganglia were normal across all studies.

The use of immunosuppressants are common in clinical trials to manage AAV immunity but requires cautious application in preclinical studies. If planned for preclinical use, its dosing regimen and potential impact on toxicity must be clearly defined.

## CONCLUSION

This analysis confirms immunogenicity as a critical safety concern for rAAV therapy. While robust anti-capsid antibody responses were prevalent, cellular immunity and transgene-specific responses were minimal. Observed toxicities were mild, including transient ALT elevation and steatosis. A comprehensive panel assessing humoral/cellular immunity, clinical pathology, and histopathology characterized these events, supporting its essential role in the preclinical risk assessment framework to ensure the safe progression of rAAV-based gene therapy.

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