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Incidence of Neutralizing Adeno-Associated Viral Antibody Subtypes in Cynomolgus Monkeys of Cambodian, Mauritius, and Philippines Origins

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ABSTRACT

Preclinical safety assessment studies utilizing cynomolgus macaques (*Macaca fascicularis*) are an instrumental part of the drug development process. Safety assessment of gene therapy (GT) products, especially those utilizing adeno-associated viral (AAV) vector-based therapeutics, requires prescreening of many animals to obtain adequate numbers for study assignment due to the presence of naturally occurring neutralizing antibodies (nAb) against AAVs. Preexisting antibodies against AAV vectors can impact the effectiveness of gene therapies, with the main challenges being loss of efficacy and loss of GT durability. Due to the increased demand for cynomolgus macaques, attributed in part to unforeseen global factors (e.g., the COVID pandemic and specific border restrictions), continued use of cynos for AAV-based gene therapy has necessitated exploring utilization of animals from other origins such as Mauritius and the Philippines. Genetic and environmental variability between origins can complicate data interpretation and generation of reference data is essential for informed study design of new toxicology programs. Given the unique challenges of working with AAV vector-based test articles, a review of the prescreening nAb data collected from a large number of toxicology studies performed in the past few years was conducted, to identify origin-specific differences in the percentages of nAb negative animals for utilization on AAV studies. AAV neutralizing antibody cell-based assay (ID50 at ≤1:10 serum dilution) was used for confirming negative or low viral titers. A review of the data set indicates no substantial differences in seronegativity rate between origins tested—Cambodian, Mauritian, and Philippines, with the exception of AAV9, where some variability was noted ranging from 40% (Mauritian) to 79% (Philippines). Variations between serotypes were noted, with AAV8 having the lowest seronegativity rate and AAV5 and AAV6 having the highest. In conclusion, before initiating a program utilizing AAVs, it is important to understand the necessity and constraints of screening animals for pre-existing antibodies against specific AAV serotypes. This data compilation serves as an important reference for estimating animal use numbers and selection during the initiation of preclinical safety studies for gene therapies utilizing AAV gene delivery modalities.

INTRODUCTION

An AAV is a small, replication-deficient, virus that can be engineered to deliver DNA to target cells. The aim of gene transfer therapy (GT) is to treat or prevent a disease by adding a functional gene, to compensate for a mutated or absent gene. The intention behind this addition is to allow for the restoration of a functional protein product.

The mechanism of action utilizes the target cell's existing functions by incorporating the AAV capsid through the cell membrane via endocytosis. Following release from the endosome, the vector transits to the nucleus through a nuclear pore. Once the vector DNA transforms into episomal DNA, it is transcribed, and the resultant mRNA is translocated to the cytoplasm, where it is translated, thereby producing the protein of interest. These therapies can potentially have long-lasting treatment effects after only a single administration.

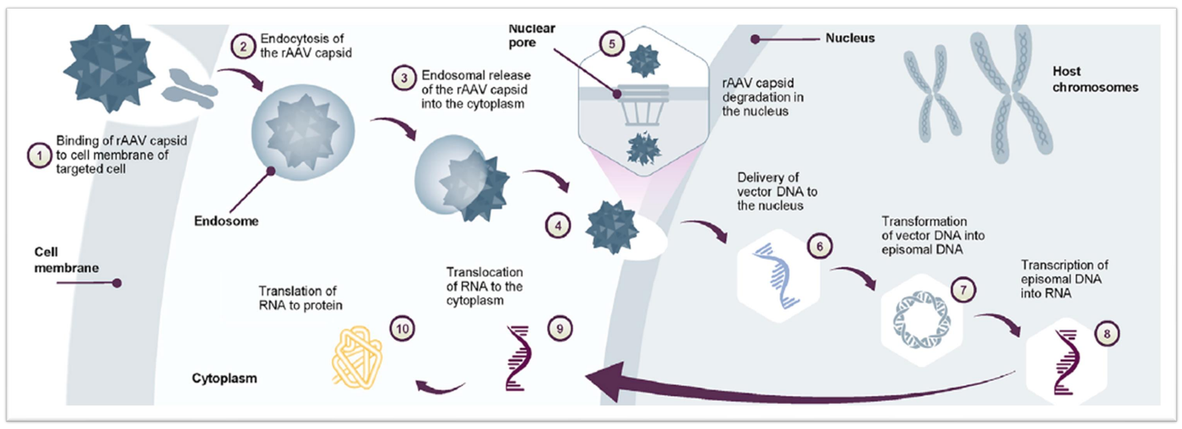

Figure 1. AAV gene transfer therapy mechanism of action

Jerry R. Mendell, et al. Testing preexisting antibodies prior to AAV gene transfer therapy: rationale, lessons and future considerations. Nature Reviews. Molecular Therapy – Methods and Clinical Development. Vol. 25. June 2022.

Neutralizing Antibodies (nAb) Prescreening

Preexisting antibodies against AAV vectors can impact the effectiveness of gene therapies. An effective strategy to circumvent this initial challenge for nonclinical studies is to screen nonhuman primates (NHPs) for preexisting antibodies against the intended capsid, in advance of the study start. The degree of preexisting immunity varies between NHPs; therefore, each animal needs to be screened for antibodies that are specific to its serotypes.

Main Challenges

- **Loss of efficacy:** related to the reduced ability of the AAV to deliver the therapeutic gene inside the target cell.
- **Loss of durability:** due to innate and adaptive immune responses directed against initial products or products generated along the way such as AAV capsid proteins, vector DNA, the transgene product, or impurities during the vector manufacturing

MATERIAL AND METHODS

Criteria for negative or low viral titers were established by AAV neutralizing antibody cell-based assay (ID50 at ≤1:10 serum dilution), for the determination of animal suitability on study.

RESULTS

Table 1. Results of nAb prescreening for 9 serotypes

AAV Serotype/ Origin	% Negative (n = Number of Animals Screened)		
	Cambodian	Mauritian	Philippine
AAV1	81 % (n=152)	-	-
AAV2	77 % (n=30)	-	83 % (n=24)
AAV3	73 % (n=30)	-	-
AAV5	100 % (n=100)	-	97 % (n=34)
AAV6	100 % (n=30)	-	-
AAV7	67 % (n=30)	-	-
AAV8	38 % (n=1119) – 2023 24 % (n=663) – 2024	44 % (n=25)	-
AAV9	61 % (n=160)	40 % (n=25)	79 % (n=34)
AAV10	80 % (n=30)	-	-

There are hundreds of known AAV variants, with about 13 serotypes currently in use for GT. These serotypes differ in their ability to infect certain tissues or cells depending on their target cell surface receptors and their corresponding binding sites. Table 1 shows the results of nAb prescreening in NHPs for 9 serotypes, classified by origin—Cambodian, Mauritian, and Philippines.

AAV8 is one of the more common serotypes used in research; however, it is one of the most challenging serotypes for locating suitable animals for study assignments. Approximately 38% (425/1119) of NHPs of Cambodian origin screened negative for nAb against AAV8 in 2023, and this percentage was slightly lower in 2024, with approximately 24% (159/663) screening negative for this serotype. This could be due to the overall reduction in the Cambodian population available in the United States of America for research due to Cambodian border restrictions still in effect. NHPs of Mauritian origin are a good alternative for locating AAV8 seronegative animals, with approximately 44% negative. Of note, the Mauritian-origin population tested was smaller (N=25) than the Cambodian.

AAV9 seronegativity rate was tested in all three populations with some variability noted, going from 40% in Mauritian, 61% in Cambodian, and 79% in the Philippine population, indicating some differences in pre-existing antibodies for this serotype.

A greater negativity rate was observed for other AAV serotypes with **AAV1**, **AAV2**, **AAV3**, **AAV7,** and **AAV10** ranging from 67 to 81% of the Cambodian population tested. Compared to the Philippe population available for testing, 83% tested negative for **AAV2** which was comparable to the Cambodian population.

Lastly, serotypes with very specialized tissue tropisms such as **AAV5** and **AAV6**, indicated a high seronegativity rate ranging from 97 to 100% for Cambodian and Philippine origins.

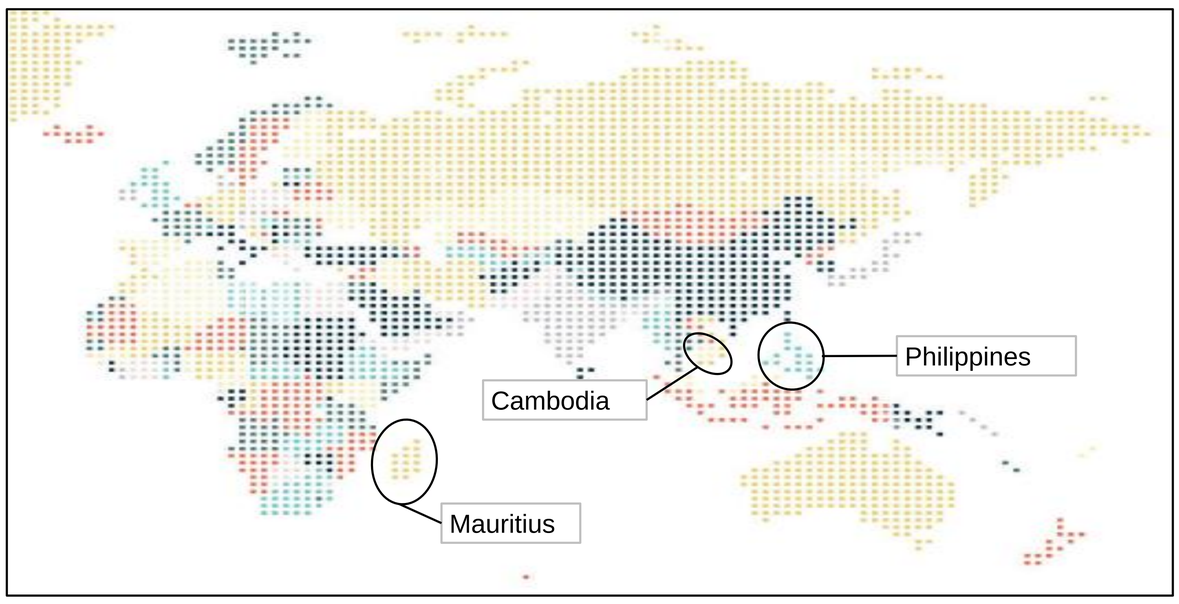

Figure 2. World map indicating country of origin for nonhuman primates tested for pre-existing neutralizing antibodies.

CONCLUSIONS

In conclusion, the diversity of AAV serotypes contributes to the versatility of AAV-based gene therapy, enabling researchers to optimize the delivery of therapeutic genes to achieve desired outcomes. Prior to the initiation of a program utilizing AAVs, it is important to understand the necessity and constraints of screening animals for pre-existing antibodies against the specific AAV serotypes. This data set review serves as a guide for more informed decisions on study designs for AAV vector-based therapeutics and indicates no substantial differences in seronegativity rate between origins tested—Cambodian, Mauritian, and Philippines for specific serotypes tested, except AAV9 where some variability was noted. Variations between serotypes were observed, with AAV8 having the lowest seronegativity rate and AAV5 and AAV6 having the highest. Understanding and managing the impact of preexisting antibodies against AAV vectors is crucial for optimizing the safety and efficacy of AAV-based gene therapies.