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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 macaque (*Macaca fascicularis*) are an instrumental part of the drug development process. Safety assessment of gene therapy 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. 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, with the aim of identifying origin-specific differences in the percentages of nAb negative animals for utilization on AAV studies.

In conclusion, 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 indicates no substantial differences in seronegativity rate between origins tested—Cambodian, Mauritian, and Philippines, except 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.

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 existing functions of the target cell 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 have the potential for long-lasting treatment effects after only a single administration.

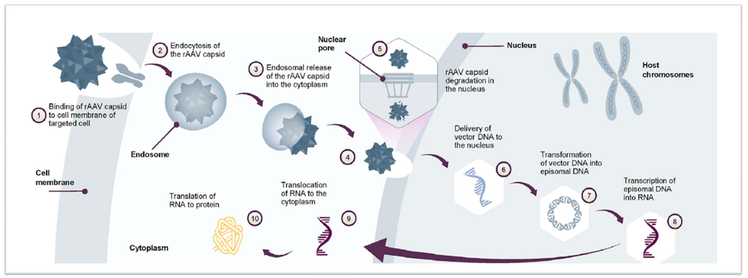

Figure 1. AAV gene transfer therapy mechanism of action

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 the presence of antibodies for specific serotypes.

Many individuals naturally carry antibodies against AAVs due to prior exposure to the wild-type virus in everyday life. NHPs, like humans, can naturally encounter AAVs in their environment, often found in cells that are simultaneously infected with adenovirus. This exposure can lead to the development of antibodies against specific AAV, potentially affecting the success of AAV-mediated gene therapies.

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.

Criteria for negative or low viral titers were established by AAV neutralizing antibody cell-based assay (≤ 5 nAb50 in HEK293 cells), for the determination of animal suitability on study.

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	36 (n=1261)	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 origins - Cambodian, Mauritian, and Philippines.

AAV8 is one of the more common serotypes used in research however, it is one of the most challenging serotypes to locate suitable animals for study assignment. Approximately 36% (454/1261) of NHPs of Cambodian origin screened negative for nAb against AAV8, and 44% for NHPs of Mauritian origin. Of note, the Mauritian-origin population tested was smaller (N=25) than the Cambodian however, a similar proportion of negativity rate was measured.

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, and AAV10 ranging from 73 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.

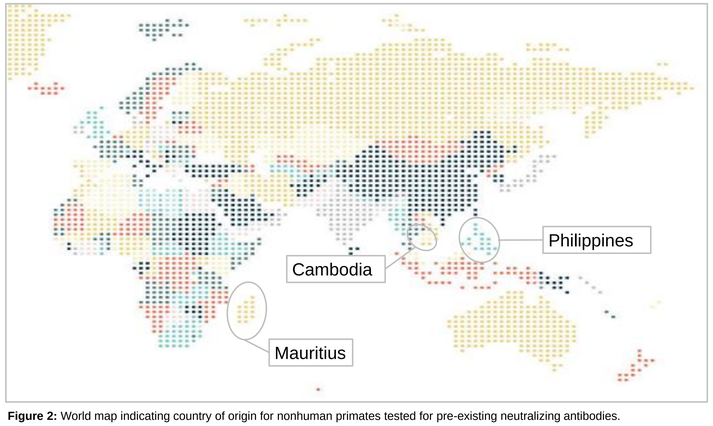

Figure 2: World map indicating country of origin for nonhuman primates tested for pre-existing neutralizing antibodies.

Seroconversion

Seroconversion refers to the development of detectable antibodies in the blood in response to an infection, vaccination, or in the case of AAVs, exposure to a particular antigen. The immune system responds by producing specific antibodies tailored to recognize and neutralize that particular antigen or its components. The process of seroconversion involves the initial absence of detectable antibodies followed by their production and subsequent detection in the bloodstream.

Case Study

For AAV8, rescreening of animals 5 to 7 months following initial viral titer assessment revealed 24% (32/135) were positive (≥10 nAb50 in HEK293 cells) for nAb when they previously had low to negative viral titers.

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 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 decision 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 ranging from 40% (Mauritian) to 79% (Philippines). 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.