Pig Models for Gene Therapy: Prevalence of Adeno-associated Virus Neutralizing Antibodies in the Yucatan Miniature Swine and the Sinclair Nanopig™

Brocksmith, D.[1], Navratil, N.[2], Cividini, F.[3], Qualls, S.[1], Marsh, T.[1] Bouchard G.[1]

[1]Sinclair Bio Resources, LLC, Auxvasse, MO; [2]Sterling Biomedical Resources, LLC, Sterling, NY; [3]CRI Biotech, Inc., Los Altos, CA

Objective

Gene therapy holds significant promise for treating a wide range of genetic disorders and other diseases, and research in gene therapy continues to expand. Large animal models provide several advantages for translational studies of adeno-associated virus (AAV) vector gene therapy and evaluation of safety and efficacy. Non-human primates are often used due to their genetic and physiological similarities to humans. However, as supply of non-human primates remains unstable, the miniature pig has become another viable large animal model that shares many of the same advantages as NHPs with regards to their biologic similarities to humans. Pigs are the only standard large animal toxicology model that are also well suited for genetic modification. Thus, transgenic disease models can be created in the pig for the long-term evaluation of efficacy. One major limitation of large animal studies is the presence of neutralizing antibodies against wild-type AAVs in the animal model. Both pigs and non-human primates develop neutralizing antibodies against naturally occurring AAVs, and the prevalence and levels of antibodies can vary based on several factors. Therefore, this study evaluated the prevalence of virus neutralizing antibodies (NAb) for many AAV serotypes in cohort of Yucatan Miniature Swine, and two cohorts of Sinclair Nanopigs from Sinclair Bio Resources.

Methods

For the initial study, Serum samples were collected via venipuncture from 50 Yucatan Miniature Swine. The animals were screened for AAV neutralizing antibodies (NAb) at VRL Laboratories using a qualitative neutralization assay for the following serotypes AAV3, AAV5, AAV6, AAV7, AAV9. The results were reported as a positive or negative for three dilutions (1:10, 1:20, 1:40).

Subsequently, serum samples were collected from a cohort of 6 Sinclair Nanopigs from the same production facilities. These samples were also screened using the same methods for neutralizing antibodies for serotypes AAV2, AAV6, AAV8, and AAV9.

A separate cohort of 20 Sinclair Nanopigs were screened for AAV9 by CRI Biotech, Inc. using an ID50 assay, as outlined in Li et al. (2022). AAV9 vectors are attractive given their ability to transduce a wide range of tissues and its lower seroprevalence in the human population compared to other AAV serotypes. Neutralizing antibody titer was designated by the highest dilution factor to exhibit ≥50% inhibition of luciferase activity compared with no serum controls (Li et al., 2022 and Rapti et al., 2012).

Results

Positive results for all serotypes were detected in the cohort of Yucatan Miniature Swine, but at low levels for most of them. The majority of this cohort screened negative for AAV6, AAV7, and AAV9. However, all Yucatan Miniature Swine screened positive for neutralizing antibodies for AAV3, and several screened positive for AAV5. Positive results for all serotypes were detected in the initial Sinclair Nanopig cohort as well, but at low levels. At least 67% or more of the cohort was negative for each serotype evaluated.

For the second cohort of 20 Sinclair Nanopigs, very low levels of neutralizing responses were seen in almost all animals evaluated.

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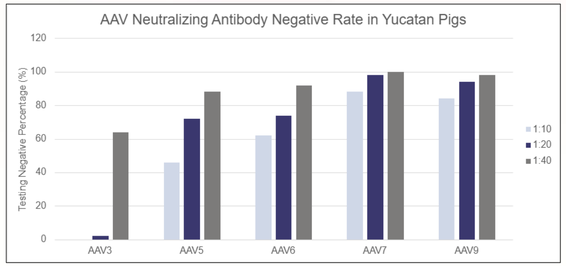
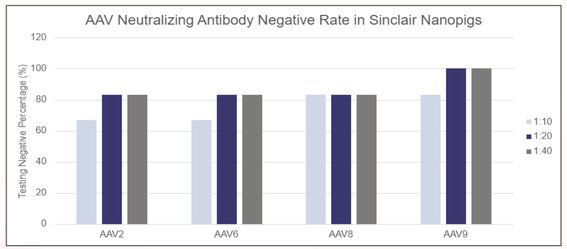
NAb Results for Multiple AAV Serotypes in Yucatan and Sinclair Nanopig™ Lineages

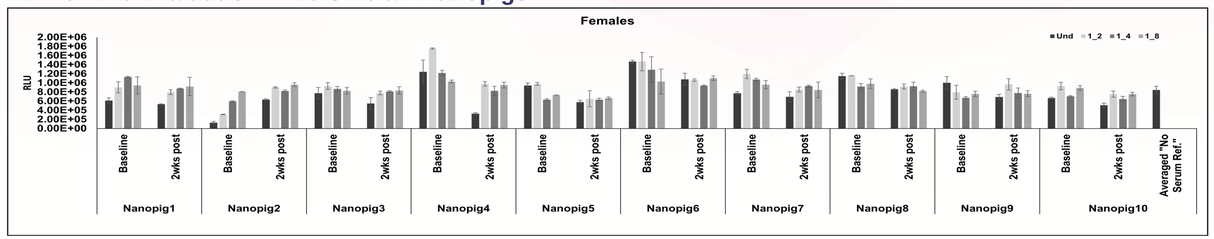
Lineage	Age	n	%(n) negative for AAV3 NAb			%(n) negative for AAV5 NAb			%(n) negative for AAV6 NAb			%(n) negative for AAV7 NAb			%(n) negative for AAV9 NAb		
			1:10	1:20	1:10	1:10	1:10	1:40	1:10	1:20	1:40	1:10	1:20	1:40	1:10	1:20	1:40
Yucatan	2-3 Months	50	0% (0)	2% (1)	84% (42)	46% (23)	72% (36)	88% (44)	62% (31)	74% (37)	92% (46)	88% (44)	98% (49)	100% (50)	84% (42)	84% (42)	98% (49)

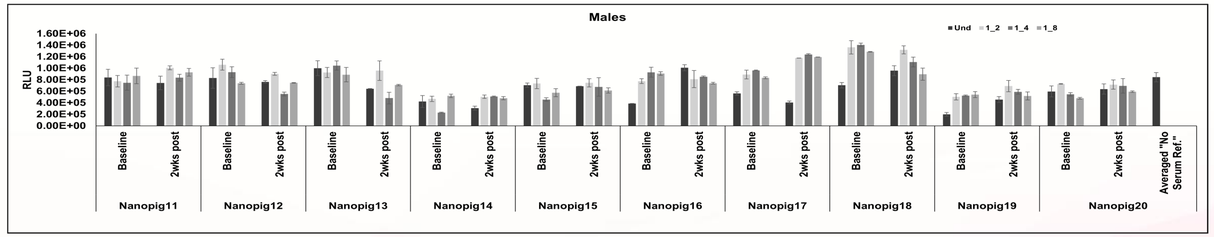
Lineage	Age	n	%(n) negative for AAV2 NAb			%(n) negative for AAV6 NAb			%(n) negative for AAV8 NAb			%(n) negative for AAV9 NAb		
			1:10	1:20	1:10	1:10		1:10	1:40	1:10	1:20	1:40	1:10	1:20	1:40
Sinclair Nanopig	3-6 Months	6	67% (4)	83% (5)	83% (5)	67% (4)		83% (5)	83% (5)	83% (5)	83% (5)	83% (5)	83% (5)	100% (6)	100% (6)





AAV9 NAb Evaluation in 20 Sinclair Nanopigs™





Most RLU values were quite high, across all dilutions, meaning there was little pre-existing neutralizing antibody activity. At 2 weeks post-treatment there was not much drop in RLU for most participants, even at the 1:2 dilution, and several even increased, which is un-expected and could either indicate increased viral activity without any neutralizing antibody activity or assay artifacts. Some samples show a bit of a decrease, but not dramatically. This pattern points to weak or absent neutralizing responses for most of the Sinclair Nanopigs.

Conclusion

This study contributes to the growing body of data demonstrating that swine models can provide significant value for the assessment of AAV-mediated gene therapy efficacy and safety across different therapeutic targets. Previous studies have also evaluated the prevalence of NAbs for serotypes AAV1, AAV2, AAV5, AAV6, AAV8, and AAV9 in Gottingen Minipigs and Yucatan pigs with similar low levels of neutralizing responses, especially for serotypes AAV8 and AAV9 (Jacobsen et al., 2024, and Dai et al., 2022). The AAV9 serotype is known for its ability to cross the blood-brain barrier, and this serotype has been administered intravenously in Bama minipigs. This approach led to widespread and safe transgene expression in the central nervous system, suggesting suitability for neurological disorder treatments (Lin et al., 2023). Thus, additional studies evaluated the NAbs for AAV9 further in Sinclair Nanopigs. The AAV8 serotype has been tested in microminipigs for liver-targeted gene therapy. Intravenous administration of AAV8 vectors encoding luciferase resulted in significant transgene expression in the liver of the pigs, highlighting the potential for hepatic gene therapy applications (Watano et al., 2020). The authors are aware of additional unpublished data demonstrating that levels of NAbs for AAV8 in Sinclair Nanopigs is similar to that for NHPs. This poster summarizes the first studies to include evaluation of Sinclair Nanopigs, and our preliminary results demonstrate that the Sinclair Nanopig appears to have AAV NAbs at a similar prevalence as other miniature swine lineages utilized in drug development. The small size of the Sinclair Nanopigs may also prove advantageous since producing AAV vectors is complex and costly, and using smaller animals minimizes the amount of vector needed

Swine have several advantages that could make them strong alternatives to NHPs for AAV studies. Swine organs, such as the heart, liver, lungs are similar in size and function to humans, making them better models for systemic AAV delivery and whole-organ responses. Advances in CRISPR and other gene-editing tools make it easier to create disease-specific pig models. The existing studies have demonstrated that swine immune systems respond to AAV8 and AAV9 vectors comparable to NHPs, and may even be more comparable to humans. Swine also provide advantages over rodent models, as human-scale delivery method like catheter-based infusions and intrathecal injections can be more easily modeled in swine. Future studies can focus on understanding how different AAV serotypes target swine tissues and the immune responses, how this compares to NHPs and predicts clinical outcomes, and if there are lineage specific differences between miniature swine breeds.

While neutralizing antibodies were detected in both Yucatan and Sinclair Nanopig lineages for the AAV serotypes evaluated in this study, the majority of the samples produced very little neutralizing antibodies, and were consistent with previous studies evaluating other miniature swine lineages. The low prevalence of key AAV neutralizing antibodies in this study provides further support that miniature swine are a beneficial model for the evaluation of AAV vector gene therapy for a variety of diseases.

References

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