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COMMENTARY

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Oracle or false prophet? Can we predict AAV efficacy based on preexisting antibody titers?

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Recombinant adeno-associated viral vectors (AAVs) have emerged as a vector of choice in gene therapy for hemophilia, and over the past few years there has been a proliferation of trials using AAVs introduced through the circulation to target the liver.[1-5] The clinical efficacy of biologics engineered from viruses, however, may be affected by the recipient's prior exposure to the wild-type virus from which the recombinant virion is derived, and both B and T cell responses may present challenges.[6] Data from studies of diverse human populations document that a substantial proportion of adults carry circulating antibodies to AAV,[7,8] and that the proportion of the population that carries these increases with age.[9]

A role for the effects of preexisting antibody titers on clinical efficacy with AAV vectors was surmised early on, and most trials tested these as part of the clinical protocol. The pattern that emerged was that trials that targeted solid organs by direct injection (eg, intramuscular) or that delivered vector to compartments with limited access to circulating antibodies, such as the central nervous system (including the subretinal space), showed effective transduction even in the presence of detectable antibody titers,[10,11] but that delivery of vector through the circulation was sensitive to even low levels of neutralizing antibodies.[1] Subsequent studies in animal models further delineated this observation. In mice, the use of human intravenous immunoglobulin to model preexisting neutralizing antibodies to AAV suggested that this in vivo model may be more sensitive than the in vitro cell-based assays,[12] and studies in non-human primates, which are natural hosts for AAV and thus have naturally occurring antibodies, documented that even low-titer neutralizing antibodies (determined in a cell-based in vitro assay) could fully block liver transduction when vector was infused intravenously.[13] Complicating the straightforward extrapolation of these findings to the clinical arena is the number of different AAV vectors being utilized in clinical studies; conservation of the capsid sequences at the amino acid level varies from as low as 51% up to nearly 100%, and there is some (mostly modest) variation in prevalence of neutralizing antibodies in the population depending on capsid identity.

In the paper by Stanford et al[14] recently published in *Research and Practice in Thrombosis and Haemostasis*, the authors used two different assays to assess preexisting immunity to two different AAV serotypes in 100 hemophilia A patients in the UK. They reported that as many as 30%-40% of these subjects were positive for either antibodies that bind to AAV or an inhibitor of transduction (measured using a cell-based transduction inhibition titer assay) in one or both assays. Beyond the value of understanding seroprevalence against two commonly used capsids in a specific population cohort, the report by Stanford and colleagues highlights two important questions that remain for the most part unanswered thus far.[14] First, which one of the several experimental assays can predict more accurately how the presence of circulating anti-AAV antibodies may impact in vivo transduction? And second, if such a universally accepted assay existed, should the field work together in an effort to standardize it for different capsids?

On the first question, the authors suggest that, while the transduction inhibition assay is considered a standard, a positive signal in either test (binding or neutralizing activity) should trigger exclusion from trials where AAVs are delivered systemically. This notion, perhaps prudent in principle, has been recently challenged by Mingozzi and colleagues on the grounds that binding antibodies may in fact increase capsid internalization and transgene expression and thus NAb assays are better predictors of the outcome of gene transfer.[15] Others have suggested that in vivo neutralization assays, in which Nabs are passively transferred to mice following human serum injection to the animals, are more sensitive than those neutralization assays performed in vitro and

thus better suited for inclusion/exclusion criteria.[16] However, neutralizing assays (both in vivo and in vitro) rely on the ability of a reporter vector to transduce the target cells and mediate quantifiable expression levels that decrease proportionally to the amount of circulating transduction inhibitors. This poses a number of significant limitations to their standardization, as transduction efficiency is highly serotype-dependent and, in general, the sensitivity of the assay decreases as the AAV dose increases, compromising the comparison of NAb titers between serotypes with distinct transduction efficiencies. As an example, the assay used by the authors to measure anti-AAV5 NAbs requires an MOI of 25 000, supplemented with etoposide, an agent that promotes transduction,[17] whereas the anti-AAV8 NAb assay uses an MOI of 200 with no requirement for agents like etoposide.[18] Other characteristics that impact NAb titers when evaluated using in vitro assays include the amount of serum used, the cell number on the plate and the reporter transgene.[16] In this regard, use of assays that do not rely on transduction performance, such as total antibody assays or the assay recently developed by Guo and colleagues, which relies on quantification of AAV binding to the target cells in vitro using a qPCR assay.[19]

Further compounding the intrinsic complexity of each assay are the differences in the AAV investigational products themselves, in terms of infectivity titers and content of empty capsids, both of which influence transduction performance and thus may affect the NAb titer. Empty capsids, which contain the capsid but lack any packaged DNA, are a byproduct of all current manufacturing processes, and have the advantage of functioning to bind and neutralize circulating antibodies to AAV.[20] In in vivo studies in mice and non-human primates (NHP), the presence of empty capsids has been demonstrated to result in more efficient transduction particularly at lower vector doses, by acting as a decoy to bind neutralizing antibodies.[20] Vectors manufactured in insect cells by introducing the DNA sequences using insect cell (baculo) viruses have demonstrated altered capsid composition and lower biological potency,[21] typically owing to reduced content of one of the capsid proteins (VP1), which leads to the formation of defective particles with reduced transduction efficiency. These may function in a manner similar to empty capsids, in that they may bind anti-AAV antibodies without driving transgene expression. These substantial differences in the AAV product from one manufacturer to another further complicate efforts to develop a standardized assay.

As Stanford et al[14] note, the purpose of these assays is to identify accurately those potential trial participants who can be expected to exhibit some level of transduction following intravenous infusion of vector. Thus, it is difficult to judge which assays are of greatest utility without an accompanying clinical dataset. One can debate about best characteristics of the assay, ie, is it better to have a wider definition of eligible (as long as all participants exhibit an adequate level of expression), which may lead to greater variability in clinical outcomes, or is it better to set a tighter range, resulting in fewer eligible participants but greater uniformity of results at a given vector dose? Should we adjust vector doses based on pretreatment antibody titers? Differences among capsids and in final product characteristics make it difficult to extrapolate findings from one product to the next. It is safe to say that we likely have more to learn regarding this critical determinant of clinical success with AAV vectors.

RELATIONSHIP DISCLOSURES

Dr. Anguela reports employment from Spark Therapeutics during the writing of the manuscript. In addition, Dr. Anguela is an inventor in the following patent applications pending to Spark Therapeutics: WO2013158879A1, US20140336245A1, US20150023924A1, US20160375110A1, and WO2017075619A1. Dr. High reports personal fees and other from Spark Therapeutics, outside the submitted work.

AUTHOR CONTRIBUTION

Dr. High and Dr. Anguela jointly outlined the editorial, researched it, drafted it, and revised it.

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