Molecular Therapy
Editorial

Topics in AAV integration come front and center at ASGCT AAV Integration Roundtable

Adeno-associated viral (AAV) vectors are in preclinical and clinical development to treat numerous genetic diseases with >100 ongoing clinical studies. In contrast to retroviral vectors with specific mechanisms for chromosomal integration, recombinant AAV (rAAV) primarily remains in an episomal form but can integrate at low frequency. These integration events occur by recombination at pre-existing chromosomal double-strand breaks (DSBs), and the distribution of these breaks largely explains the observed quasi-random integration profile. The episomal form provides long-term transgene expression in post-mitotic cells, including muscle, liver, and CNS. While it has been understood for decades that rAAV can integrate into the host genome, there remain many unanswered questions about the frequency of integration, profile within the genome, and potential risks associated with these integrations. Given the number of vector particles typically administered, even low integration rates can result in many integration events. The American Society of Gene and Cell Therapy recently held an AAV Integration Roundtable meeting (https://asgct.org/events/aav-roundtable) to discuss the current understanding of AAV integration and the challenges of assessing the potential risks associated with integration.

The initial studies to evaluate rAAV integration focused on mouse models and were challenging due to the low frequency of events and the limited technology available at the time. The first study to raise concerns about the oncogenic potential of AAV was in an MPS VII mouse model treated with rAAV that resulted in increased incidence of hepatocellular carcinoma (HCC) associated with AAV integrations within a specific locus.[1] This was likely due to a high rate of DSBs and a sensitivity to genotoxic stresses at that locus. Further studies in mice suggested that strain, disease model, and stage of development at AAV administration can influence the risk of HCC.[2] Since mice appear to be more sensitive to development of HCC than large animals or humans, these models may not provide an accurate assessment of risk in humans. Large animal models, such as canines or non-human primates, may better predict oncogenic risk due to longer lifespan and a shared general requirement for multiple genotoxic events to elicit oncogenic development. However, long-term studies in these models are not usually performed, and meaningful oncogenic outcome measures would likely take many years and many subjects to develop. In canine studies there was evidence for clonal expansion of AAV integration events in genes associated with human cancer in several dogs after 6–10 years of follow-up.[3] Development of *in vitro* models for evaluating risk would need to reflect patterns of AAV integration *in vivo* to be informative and must provide outcome measures reflecting rare clonal expansion events. Thus, there are challenges in identifying a model that most likely predicts what the risk may be in humans.

Vector designs that include a strong, ubiquitous promoter and enhancer elements likely increase the risk of oncogenesis due to greater probability of insertional activation.[4] However, any vector sequence can disrupt a tumor suppressor gene, and the relative risks of these events is not known. The fate of the AAV genome and the factors that influence integration are not completely understood. While vector features may drive specific interactions with proto-oncogenes, there is no evidence yet that they influence patterns of integration. While integration predominantly occurs early after vector delivery, it is not known what changes may occur to the AAV genome over time, hence the need for long-term studies. Furthermore, while the discussion has centered around the risk of HCC after liver-targeted gene delivery, this should be a consideration for any systemic AAV administration that may result in vector delivery to the liver. Although AAV-associated tumors in tissues other than liver have not been observed in any animal model, the risk for integration and genotoxicity in other tissues may be dependent on vector load and specific tissue pathology.

Sequencing technologies to investigate AAV integration have greatly improved to provide the ability to sequence large numbers of integration sites, although with some limitations. PCR efficiencies are influenced by the difficulty of sequencing across inverted terminal repeats (ITRs), which can impact recovery of events, and complex rearrangements of vector genomes further complicate these analyses. In addition, large numbers of episomes present challenges to characterizing integrated forms. Target enrichment and long read sequencing may provide insights into the structures of the integrated forms, which are often rearranged or truncated. Another challenge is that tissue sampling only represents a small portion of cells in a tissue, and it is not possible to perform serial sampling of cells in a solid tissue to understand if integration events in those cells will lead to transformation. Estimation of integration frequency is challenging since samples only represent a small subsample of the full population. Standardization of vector genome recovery, amplification, and sequencing methods may improve the ability to compare across studies of AAV integration.

Importantly, there have not been any adverse events related to AAV integration in clinical studies. An additional consideration in human subjects is understanding the background risk in underlying conditions, such as non-alcoholic fatty liver disease and hepatitis, where there is already an increased risk for HCC. While liver biopsies may provide an opportunity to learn more about integration rates, it would only provide a snapshot in time of the liver and the invasiveness of the procedure precludes routine liver biopsies in patients. The use of non-invasive methods for monitoring as well as identification of biomarkers that may allow early detection of expanding clones would be valuable. Long-term monitoring will be important in these trials. Overall, the field is eager to identify more accurate risk-benefit assessments for subjects in clinical studies.

While the AAV integration roundtable initiated the discussion in the field, it will be critical to continue the dialogue to shape the knowledge that can be gained in both preclinical and clinical studies around this important topic so that we can provide better risk-benefit analysis for patients as well as guidance on follow-up in clinical studies.

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