NATIONAL HEMOPHILIA FOUNDATION
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MASAC Document #256

MASAC RECOMMENDATION FOR LIVER BIOPSIES IN GENE THERAPY TRIALS FOR HEMOPHILIA

The document was approved by the Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation (NHF) on March 29, 2019 and adopted by the NHF Board of Directors on August 13, 2019.

Currently, adeno-associated virus (AAV) has been identified as the most efficient method to transfer new genes into individuals with a variety of monogenic diseases. Nearly 200 clinical studies have utilized this vector for a number of monogenic diseases. At present multiple clinical trials for Hemophilia A and Hemophilia B are ongoing. This recommendation would also apply to other viral vectors in development for liver-directed gene therapy.

AAV is a small parvovirus, capable of infecting humans but able to replicate only if it has access to the replication machinery of adenoviruses or herpesviruses. It does not cause clinical disease but does induce an immune response including antibody generation. For gene transfer, the 2 AAV genes, *rep* and *cap*, are removed, and the cassette containing the transgene with liver specific promoters/enhancers confers hepatocyte specificity of transgene expression. Many serotypes of AAV capable of infecting numerous mammalian species have been identified, although all of the serotypes, except AAV5, used in clinical trials are human or non-human primate viruses or are derived from them. The serotype confers tissue tropism due to specific interactions with cell surface receptors. Much work is ongoing to derive synthetic AAV capsids in efforts to increase tissue tropism and avoid pre-existing immunity compared to the native AAV capsid serotypes. Once AAV enters a hepatocyte, the DNA is directed into the nucleus, while the viral capsid is targeted for degradation in the proteasome. Much is not understood about this intracellular process, but the DNA becomes double stranded, and assumes a stable episomal configuration capable of RNA transcription and subsequent protein translation. An unknown but small amount of the cassette integrates, although AAV is considered a primarily nonintegrating vector. This raises theoretical concern for potential genotoxicity at insertion sites. Nonclinical studies have revealed some AAV biology, but other species have not fully predicted efficacy and safety in clinical studies.

As gene therapy for hemophilia progresses through Phase 3 licensure studies, many important questions continue unanswered.

- What is the hepatic distribution of individual AAV serotypes?
- How long does it take for the capsid to be catabolized within hepatocytes?

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- Is there evidence of a liver specific innate or cellular immune response not detectable systemically?
- Are there unique transgene effects (ie, Factor VIII) on hepatocyte health and metabolic functions?
- What is/are the cause(s) of transaminase elevations seen periodically?
- What level of integration occurs?
- Where in the genome does integration occur?
- Why are Factor VIII and IX levels so variable within each study?

Many, if not all of these questions can be addressed by serial liver biopsies in at least a cohort of clinical trial participants, providing for predictors of response and toxicities. Liver biopsies are established as the best way to monitor patient status pre- and post-liver transplant, in non-alcoholic fatty liver disease (NAFLD) or nonalcoholic steatohepatitis (NASH) diagnoses and clinical trial endpoints, and in a multitude of rarer genetic and acquired hepatic diseases. While liver biopsies are at risk for producing false negative results due to sampling errors and heterogeneity of disease within the organ, they remain the gold standard for evaluating what is happening within the liver. Safety of liver biopsy is well established in hemophilic, former hemophilic post- liver transplant, and individuals with multiple other liver diseases.

Careful, systematic evaluation within the liver of participants treated in AAV gene therapy trials with both the Factor IX and Factor VIII transgenes is needed. It is incumbent upon sponsors of such studies to work diligently toward answering questions regarding AAV biology. The answers will not only illuminate questions of durability and safety, but likely will offer new research directions to further improve efficiencies and safety of this promising therapeutic modality going forward.

Therefore, MASAC encourages gene therapy study sponsors and investigators to incorporate, at a minimum, liver biopsy sub-studies to investigate, in at least a cohort of patients: Longitudinal assessment of liver histology, viral capsid protein presence, inflammation and immune markers, and distribution of transgene and therapeutic protein across cell types and regions in the liver. Assessment of patients with high expression, lower than expected protein expression, and patients who have progressive diminution of expression is particularly important in understanding the natural history of transduction events in clinical trials. History of HCV infection and residual hepatic disease, as well as other risks for liver toxicity (e.g., NAFLD, alcoholic liver disease) should be considered when enrolling participants into cohorts since all patients are not alike at the time of enrollment.

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