A Path to Improving Gene Therapies for Liver-Related Diseases and Cancers through Selectable CRISPR-Cas9 Vectors

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Clinical trials of liver-directed gene therapies are rapidly increasing. Though exciting, there are obstacles to overcome. Significant viral injection is necessary to achieve therapeutic levels of transgene expression. This may lead to life-threatening inflammatory responses such as that which resulted in the death of 18 yr. old Jesse Gelsinger during an OTD gene therapy clinical trial. Additionally, transgene expression through current gene therapies is rapidly lost during cell division. The goal of this project was to use the rapid post-injury rate of hepatocyte regeneration to induce gene-modified hepatocyte selection in vivo. High levels of bile acids are hepatotoxic. However, hepatocytes can be rescued from this hepatotoxic environment through the inactivation of NTCP, a major bile acid transporter. Therefore, in a high bile acid environment, NTCP-deficient hepatocytes have a selective advantage over wild-type hepatocytes. Through transgene integration by AAV and the repopulation of transgenic hepatocytes through selection, therapeutically effective viral transduction no longer mandates high levels of viral particles. NTCP-targeting sgRNA's were designed, synthesized, and verified for cleavage in vitro. An NTCP KO mouse was created while streamlining KO mouse methodology for cost and time-efficiency. Then, pX330 CRISPR constructs were modified to inactivate NTCP and hydrodynamically injected into mice. After three weeks of selection with cholic acid, livers were harvested and immunostained for NTCP. Selection of NTCP-deficient hepatocytes was shown to occur in vivo. Lastly, an AAV was designed to include a transgene and an NTCP CRISPR locus. This system of selection will greatly reduce the risks and increase the effectiveness of liver-directed gene therapies.