2938 Using a Human Liver Tissue Equivalent (hLTE) Platform to Define the Functional Impact of Liver-Directed AAV Gene Therapy

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Clinical trials employing AAV vectors for hemophilia A have been hindered by unanticipated immunological and/or inflammatory responses in some of the patients. Also, these trials have often yielded lower levels of transgene expression than were expected based upon preclinical studies, highlighting the poor correlation between the transduction efficiency observed in traditional 2D cultures of primary cells *in vitro,* and that observed in those same cell types *in vivo*. It has been also recognized that there are marked species-specific differences in AAV-vector tropism, raising the critical question of the accuracy with which various animal models will likely predict tropism/vector transduction efficiency, and eventual treatment success in humans. Human liver tissue equivalents (hLTEs) are comprised of major cell types in the liver in physiologically relevant frequencies and possess the ability to recapitulate the biology and function of native human liver. Here, we hypothesize that hLTEs can be used as a better model to predict the efficacy and safety of AAV gene therapy in humans. We fabricated hLTEs using 75% hepatocytes, 10% stellate cells, 10% Kupffer cells, and 5% liver sinusoid-derived endothelial cells in 96-well Elplasia plates with 79 microwells per well. hLTEs were transduced at an MOI of 10^5vg/cell, on the day of fabrication, with the clinically relevant serotypes AAV5 (*hLTE-5*) or AAV3b (*hLTE-3b*), both encoding a GFP reporter. After 4 days of self-

aggregation, live/dead assay was performed to confirm viability. Non-transduced hLTEs served as negative controls (*hLTE(-)*), and hLTEs exposed to 20 mM acetaminophen were used as positive controls for liver inflammation/damage. Incucyte® Live-Cell Imaging system was used to track the aggregation and GFP expression of hLTEs. Over the course of the next 5 days, media was collected to determine hepatic functionality, RNA was isolated to assess dysregulation of genes involved in inflammation and fibrosis, DNA was isolated to determine whether AAV vectors integrate into the genome of human hepatocytes and, if so, to define the frequency at which this occurs and the genomic loci of integration, and hLTEs were fixed and processed at appropriate times for histological analyses and transmission electron microscopy (TEM). TEM analysis revealed that all groups exhibited microvilli and bile-canaliculus-like structures, demonstrating the formation of a rudimentary biliary system and, more importantly, proving that hLTEs resemble native liver structure. Incucyte® imaging showed that AAV5 and AAV3b transduction impaired formation of hLTEs (57.57 ± 2.42 and 24.57 ± 4.01 spheroids/well, respectively) in comparison with *hLTE(-)* (74.86 ± 3.8 spheroids/well). Quantification of GFP expression demonstrated that AAV5 yielded the most efficient transduction of hLTEs (fold change in GFP expression compared to control: 2.73 ± 0.09 and 1.19 ± 0.03 for *hLTE-5* and *hLTE-3b*, respectively). Chromogenic assays showed decreased urea production in cell culture supernatants of AAV transduced groups compared to the non-transduced hLTEs on days 6 and 10 of culture, demonstrating decreased hepatocyte functionality. However, ALT and AST levels were similar in all groups. On day 10, *hLTEs* were either used for RNA isolation or fixed in 4% PFA and processed for histology. Masson's Trichrome and Alcian Blue/Sirius Red staining was performed to detect fibrosis, which was then quantified using ImageJ. These analyses showed no significant increase in fibrosis in either *hLTE-5* or *hLTE*-3b compared to *hLTE(-)*. Nevertheless, RT^2 PCR Array for Human Fibrosis detected dysregulation of several genes involved in fibrosis/inflammation in both *hLTE-5* and *hLTE*-3b (16/84 and 26/84, respectively). In conclusion, data collected thus far show successful recapitulation of native liver biology and demonstrate that AAV5 transduces hLTEs more efficiently than AAV3b. However, impaired self-aggregation and decreased hepatocyte functionality was observed in both AAV-transduced groups. Studies to address the incidence and location(s) of AAV integration are ongoing. We have thus shown that the hLTE system can provide critical new knowledge regarding the efficacy and safety of AAV gene therapy in the human liver.

Disclosures: No relevant conflicts of interest to declare.