marked decrease in dye extravasation, whereas non-treated *C1E-INH +/-* mice had markedly increased dye extravasation. These results demonstrate that a single treatment with AAVrh.10hC1EI has the potential to provide long term protection from angioedema attacks in the affected population, representing a paradigm shift in current therapeutic approaches.

756. Stable Amelioration of Hemophilia B in Dogs by Intravenous Administration of Lentiviral Vectors Expressing Hyper-Functional Factor IX

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Lentiviral vectors (LVs) are attractive vehicles for liver-directed gene therapy by virtue of their ability to stably integrate in the genome of target cells and the low prevalence of pre-existing immunity against HIV in humans. Over the past years, we have developed a LV platform that can achieve stable transgene expression in the liver, induce transgene-specific immune tolerance and establish correction of hemophilia in mouse models upon systemic administration. This LV is designed to stringently target transgene expression to hepatocytes through transcriptional and microRNA-mediated regulation. We then investigated the efficacy and safety profile of portal vein administration of LVs expressing canine factor IX (FIX) in a canine model of hemophilia B. We produced large-scale batches of LVs qualified for in vivo administration and treated adult hemophilia B dog by portal vein administration. We observed long-term stable reconstitution of canine FIX activity up to 1% of normal and significant amelioration of the clinical phenotype in 3 treated dogs with 6, 3.5 and 2.5 years of follow up. LV infusion was associated with transient signs of inflammatory response and mild hepatotoxicity, which could be abrogated by pretreatment with anti-inflammatory drugs. There was no detectable long-term toxicity or development of FIX inhibitors. In the perspective of clinical translation and to increase therapeutic efficacy, we next treated two 10-kg hemophilia B dogs by peripheral vein administration of LVs expressing a codon-optimized and hyperfunctional canine FIX at a 5-fold higher dose than those previously administered. Intravenous LV administration was well tolerated with mild and self-limiting elevation of aminotransferases in one dog. In the dog that reached more than 1 year of follow up FIX activity ranged between 4-8% of normal. Treatment of two more dogs at a higher dose is underway. Overall, our studies position LV-mediated liver gene therapy for further pre-clinical development and clinical translation. LVs may thus complement other available vectors to address some of the outstanding challenges posed by liver gene therapy of hemophilia and conceivably other diseases.

757. Stable *In Vivo* Transduction of Primitive Hematopoietic Stem Cells After Mobilization and Intravenous Injection of an Integrating Gene Transfer Vector

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Current protocols for hematopoietic stem cell (HSC) gene therapy involve the collection of HSCs from donors/patients, in vitro culture, transduction with retrovirus vectors, and retransplantation into myelo-conditioned patients. Besides its technical complexity, disadvantages of this approach include the necessity for culture in the presence of multiple cytokines which can affect the pluripotency of HSCs and their engraftment potential. Furthermore, the requirement for myeloablative regimens in patients with non-malignant disorders creates additional risks.

We therefore explored the potential for in vivo transduction of HSCs. We developed an approach that involves GCSF/AMD3100-mediated mobilization of HSCs from the bone marrow into the peripheral blood stream, followed by intravenous injection of a Sleeping Beauty transposase-based integrating helper-dependent adenovirus (HD-Ad5/35++) vector system. These vectors target CD46, a receptor that is expressed at higher levels on HSCs than on more differentiated bone marrow and blood cells. We demonstrated in human CD46 transgenic mice and immunodeficient mice with engrafted human CD34+ cells that HSCs transduced in the periphery home back to the bone marrow where they persist and stably express the transgene long-term. In the CD46 transgenic mouse model we showed that our in vivo HSC transduction approach allows for the stable transduction of primitive HSCs, i.e. cells capable of forming multi-lineage progenitor colonies. At 12 weeks after in vivo transduction, we detected GFP marking in bone marrow HSCs in the range of 1 to 2%. Importantly, the proportion of transduced primitive HSCs increased over time. Furthermore, in vivo transduced HSCs were able to repopulate the hematopoietic system of lethally irradiated C57BL/6 mice, showing the functionality of the modified HSCs. Our in vivo HSC transduction approach did not result in innate toxicity or significant transduction of non-hematopoietic tissues. Genome-wide integration site analysis in in vivo transduced HSCs revealed a close-to-random integration pattern without preference for genes and the absence of integration into or near to cancer-associated genes.

In conclusion, our novel in vivo transduction approach allows for stable genetic modification of primitive HSCs without the need of ex vivo culture, myelo-conditioning, and transplantation. Thus, our method is relevant for a broader clinical application of gene therapy of inherited diseases as well as infectious diseases and cancer.

758. A Nonhuman Primate Transplantation Model to Evaluate Gene Editing Strategies Aimed at Inducing Fetal Hemoglobin Production for the Treatment of Hemoglobinopathies

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Fetal hemoglobin (HbF) is the major form of hemoglobin present in newborns but is almost completely replaced by adult hemoglobin after birth, where it constitutes less than 1 percent of total hemoglobin. Hereditary persistence of HbF is linked to mutations at multiple genetic loci that regulate the switch from fetal to adult hemoglobin. The

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