Poster Session I (cont.)

P4

CURING DISEASE BEFORE BIRTH: IN UTERO GENE THERAPY FOR THE TREATMENT OF HEREDITARY TYROSINEMIA TYPE 1 IN A SMALL ANIMAL MODEL

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Purpose: In vivo gene therapy can successfully rescue a porcine model of hereditary tyrosinemia type 1(HT1). The aim of this study was to take the in vivo approach one step further: to cure a murine model of HT1 through in utero gene therapy, as well as to evaluate lentiviral vector biodistribution and integration profile in this setting.

Methods: We performed direct fetal intrahepatic injections of a lentiviral vector carrying the luciferase gene under control of the CMV promoter in wild-type mice. Injections were performed at day 15 of gestation and 3x10⁸ TU/fetus. Mothers and fetuses were imaged with the Xenogen IVIS-200 3-5 days after injection. Injections were repeated in Fah⁻/⁻ mice with a lentiviral vector carrying the human FAH gene under control of the hepatocyte-specific alpha1-antitrypsin promoter. Correction of the metabolic disease was followed through histological analysis and through the animals’ ability to thrive off the protective drug NTBC/nitisinone. Direct fetal intrahepatic injections of a lentiviral vector carrying the green fluorescent protein(GFP) gene under control of the SFFV promoter were then performed in a sow at day 63 of gestation and 1x10⁸-1x10⁹ TU/fetus. Here, biodistribution was evaluated through PCR analysis.

Results: Luciferase expression in mothers was limited to the uterus, and in pups luciferase was preferentially expressed in the liver. Three treated Fah⁻/⁻ pups have demonstrated maintenance of healthy NTBC-independent growth curves, suggesting effective treatment of the metabolic disorder, with histological confirmation of FAH-positivity in hepatocytes and complete liver repopulation with corrected cells within three months. Preliminary real-time PCR analysis performed on fetal and maternal pig tissues demonstrated no evidence of lentiviral vector integration in maternal tissues.

Conclusions: In utero gene transfers have the potential to correct the metabolic deficiency in FAH-deficient mice, with preferential lentiviral integration and expression in fetal liver over other fetal and maternal tissues in both mice and pigs.