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 $3x10^8$ 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 $1x10^8$-$1x10^9$ 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.

Poster Session I (cont.)