# CRA 21

## No evidence of germline transmission of vector DNA following intravenous administration of AAV5-hFIX to male mice

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Clinical Research/Clinical Trials

**Abstract**

Background: Recombinant adeno-associated viruses (rAAV) are replication-deficient, non-integrating viruses commonly used as vectors for gene therapies currently in clinical development. Systemic administration of gene therapy raises the possibility of vertical germline transmission of the vector DNA.AimHere, we investigated the possibility of germline transmission following IV administration of an AAV serotype 5 vector designed for the liver-directed expression of human Factor IX which is being studied in clinical trials for hemophilia B. Methods: Since hemophilia B predominantly occurs in male patients, paternal germline transmission was investigated in mice in a GLP compliant study, according to current gene therapy guidelines (EMEA/273974/2005). Male C57Bl/6 mice (n=15) each received a single intravenous infusion of 2x1014 gc/kg AAV5-hFIX and were mated 6 days later with untreated female mice (n=30). On day 20 post-treatment, males were sacrificed and the seminal vesicle, epididymis, testes and a sperm sample were collected. Successfully mated females were necropsied on day 17 of gestation and the uterus, placenta and fetuses collected for each female. Each fetus was examined for viability and externally visible abnormalities. All samples were analyzed for vector DNA by QPCR. Results: No effect of treatment was observed on male mating performance, fertility indices, maternal body weight, food consumption, pregnancy performance, external fetal abnormalities, or fetal weights. Vector DNA levels of up to 2x106 gc/µg gDNA were detected in male reproductive tissues (epididymis, seminal vesicle, sperm, and testes), but not in female uterus, placenta and offspring. Although vector DNA was detected in the reproductive tissues of males, there was no evidence of transmission of vector DNA to female reproductive tissues or to the fetuses. Conclusion: The risk of paternal germline transmission following AAV5-based vector administration is therefore considered to be low.