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

Lisa Spronck,[1] Martin de Haan,[1] Eileen Sawyer,[1] Liesbeth Heijink,[1] Jaap Twisk[1].
[1]uniQure Biopharma B.V.

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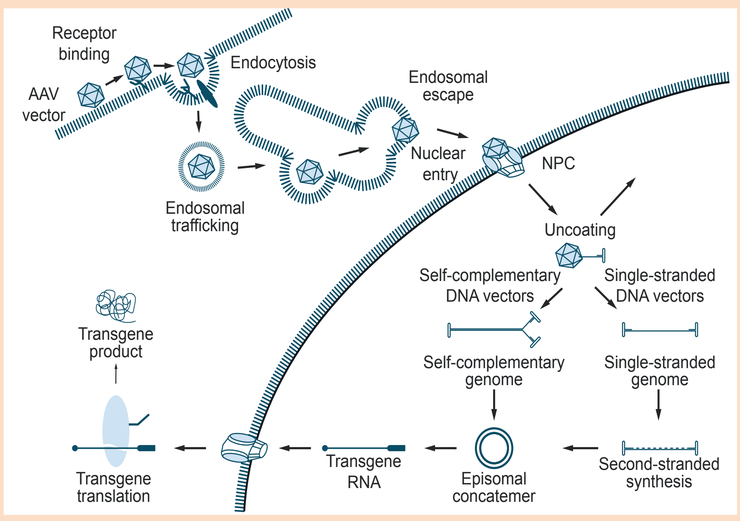
BACKGROUND

- Concerns exist regarding the possibility that gene transfer using viral vectors may lead to vertical germline transmission of the vector DNA to the next generation[1-4]
- Recombinant adeno-associated viral (AAV) vectors are commonly used to deliver genes to human cells
- The risk of germline transmission is limited by the following properties:
  - The AAV vector genome persists in the nucleus as an episome and does not require integration into the host DNA for transcription (**Figure 1**)[5,6]
  - AAVs are not capable of replication

Figure 1. AAV vector genomes remain episomal

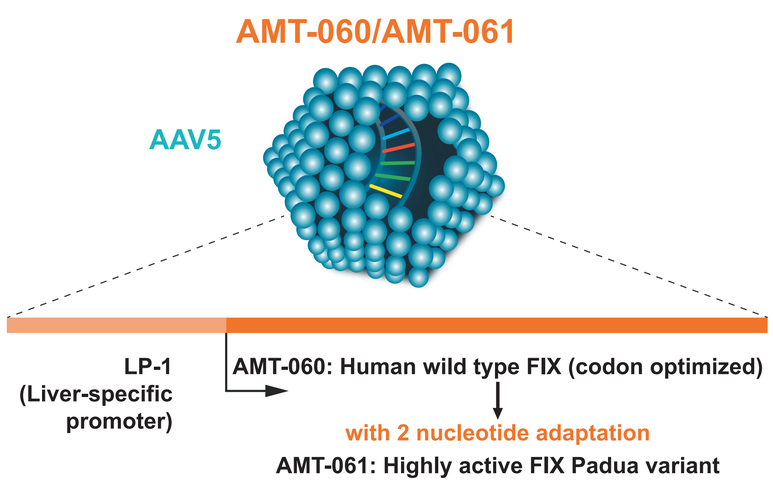


Reproduced from Salganik et al[6]

STUDY AIMS

- To investigate the possibility of germline transmission in mice following intravenous (IV) administration of AMT-060
- AMT-060 and AMT-061 (a modified version of AMT-060) (**Figure 2**) are potential gene therapies for moderate/severe hemophilia B, currently being studied in clinical trials[7-9]
- Since hemophilia B predominantly occurs in male patients, germline transmission through sperm was investigated in mice in a GLP compliant study, according to current gene therapy guidelines (EMEA/273974/2005)

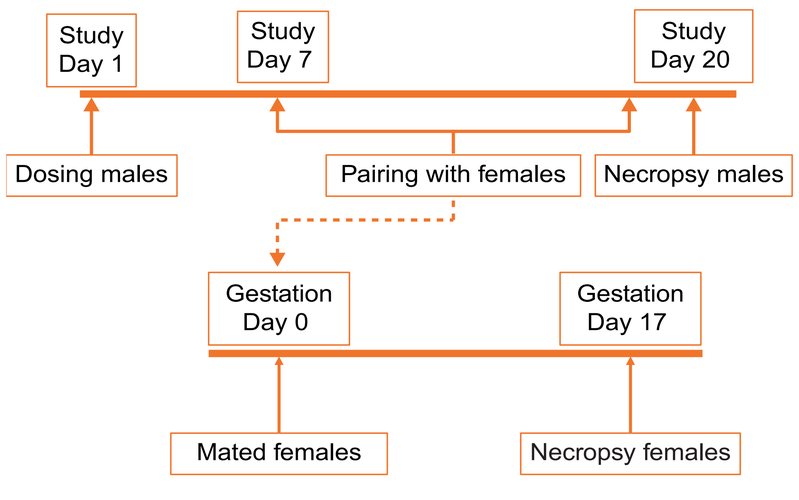
Figure 2. Structure of AMT-060/061



STUDY DESIGN

- Male C57Bl/6 mice each received a single IV infusion of vehicle control (n=5) or 2.3 x 10^{14} gc/kg AMT-060 (n=15) via the tail vein on Day 1 (**Figure 3**)
- After 6 days, each mouse was paired with 2 untreated female mice daily until confirmation of mating by a copulation plug, or for a maximum of 11 days
- A 6-day period between AMT-060 treatment and start of mating was chosen to maximize the chances of vector transmission to offspring
- Males were sacrificed 20 days post-treatment; the seminal vesicle, epididymis, testes and a sperm sample were collected
- Females were necropsied on Day 17 of gestation; the uterus, placenta and fetuses were collected. Each fetus was examined for viability and externally visible abnormalities
- Tissue samples of 5 animals per sex were analyzed for vector DNA by quantitative (Q)PCR

Figure 3. Study design



RESULTS

Pregnancy-related outcomes

- Treatment of male mice with AMT-060 did not affect mating performance, fertility indices or pregnancy performance (**Table 1**)

Table 1. Effects of AMT-060 on fertility and pregnancy outcomes

	Control	AMT-060-treated
Mating performance		
No. males paired	5	15
No. males siring	3	12
Male fertility index (%)	60%	80%
No. females paired	10	30
No. pregnant	5	20
Female fertility index (%)	50%	67%
Pregnancy outcome		
No. pregnant	5	20
Total no. of uterine implants	33	156
Total live implants (%)	29 (88%)	139 (89%)
Total dead implants (%)	4 (12%)	17 (11%)
Mean implants	6.6 ± 2.1	7.8 ± 1.9
Mean live implants	5.8 ± 2.4	7.0 ± 2.0
Mean dead implants	0.8 ± 0.8	0.9 ± 0.9
Mean fetal weight (g)	0.85 ± 0.04	0.89 ± 0.09
No. of fetuses with external abnormalities (%)	0 (0%)	2 (1%)

Observations in male mice

- There were no clinical signs that were considered related to treatment with AMT-060
- Body weight, body weight changes (**Table 2**), and food consumption were comparable between control and treated male mice
- Macroscopic evaluation at necropsy did not indicate any abnormalities in either group

Table 2. Effects of AMT-060 on body weight development in male mice

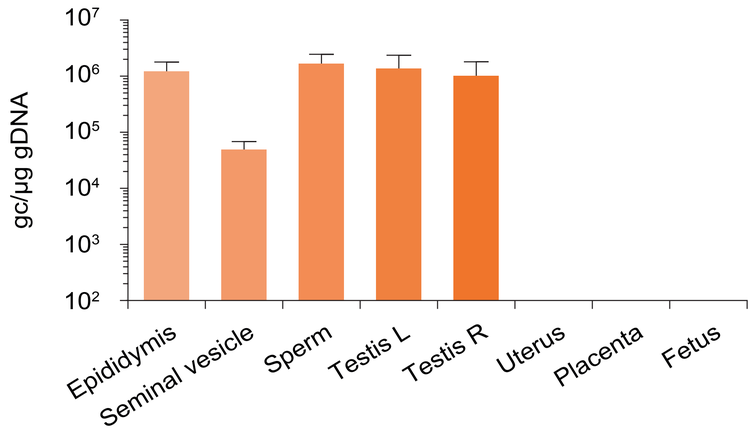
Group		Study day					
		-4	1	4	11	18	Change from Day 1 to 18
Control	mean	22.0	22.6	23.0	23.8	25.0	2.3
	SD	1.6	1.9	1.8	2.3	1.4	0.9
	n	5	5	5	5	5	5
Treated	mean	22.9	23.6	24.4	24.9	26.2	2.6
	SD	1.5	1.4	1.5	1.3	1.4	0.7
	n	15	15	15	15	15	15

SD, standard deviation

Vector DNA germline transfer and biodistribution

- QPCR showed high levels of vector DNA in all male reproductive tissues 20 days after AMT-060 treatment (**Figure 4**)
- In contrast, vector DNA was below the lower limit of quantification (100 gc/µg of genomic DNA) in uterus, placenta and fetuses (**Figure 4**)

Figure 4. Biodistribution of AAV5 vector DNA in mouse tissues



gc, genome copies; gDNA, genomic DNA

CONCLUSION

- Treatment of male mice with AMT-060 was not associated with vertical transmission of AAV5 vector DNA to offspring
- Vector DNA was detected in male reproductive tissues (epididymis, seminal vesicle, sperm, and testes), but not in reproductive tissues (uterus and placenta) from untreated females following mating with treated males
- AMT-060 treatment had no effect on reproductive parameters
- Results lend support to the current view that the (human) risk of germline transmission following gene therapy with AAV vectors is low[1-6]

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DISCLOSURES

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