PRESENTATION OF THE FINAL RESULTS OF DELIBERATE RELEASE INTO THE ENVIRONMENT OF GENETICALLY MODIFIED ORGANISMS

IN ACCORDANCE WITH ARTICLE 10 OF DIRECTIVE 2001/18/EC

The final report format shall be completed by the notifier.

- The notifier shall fill in the report format according to the proposed form.
- The notifier shall illustrate as much as possible the reported data by means of diagrams, figures and tables.
- Statistical data could also be provided where relevant.
- In the case of multi-sites and/or multi-events release(s) the notifier shall provide a general overview of the measures taken and effects observed for the full duration of the consent.
- The space provided after each item is not indicative of the depth of the information required for the purposes of this report.
- The information provided in this report is not considered confidential in accordance with Article 25 of Directive 2001/18/EC.

1. General information

1.1. European notification number: *Number can be found on the corresponding SNIF form (i.e. year and number, B/NL/xx/xxx)*	**B/NL/**05/001
1.2. Member State of notification:	**The Netherlands**
1.3. Date of consent: *Date of issue of permit*	July 4, 2005
1.4. Title of the project:	A Study to Determine the Safety and Efficacy in Lipoprotein Lipase-Deficient Subjects after Intramuscular Administration of AMT-010, an Adeno-Associated Viral Vector Expressing Human Lipoprotein Lipase^{S447X} . (AMT protocol nr CT-AMT-010-01)
1.5. Name of institution or company: *i.e. legal entity = notifier*	Academic Medical Center
1.6. Duration of release:	October 3, 2005 – January 31, 2007
1.7. Period of release:	2005 - 2008

2. Characteristics of the release

2.1 Scientific name of the recipient organism:	Homo sapiens
2.2 Transformation event(s) (acronym(s)) or vectors used: *Describe the GMO(s) and/or vector(s) and insert(s) used for the modification*	AAV1-LPL^{S447X}
2.3 Unique identifier, if available: *i.e. product name or GMO name*	AMT-010
2.4 Geographical location(s) (administrative region):	Amsterdam, The Netherlands
2.5 Number of test subjects:	8
2.6 Amount of GMO administered to each test subject:	One dose of 10E11 gc/kg (4 subjects) or One dose of 3x10E11 gc/kg (4 subjects)
2.7 Number of administrations per test subject:	1

3 Risk management measure(s)

Please report the risk-management measures used to avoid or minimize the spread of the GMO(s) outside the site(s) of release, and in particular those measures

- *not originally notified in the application,*
- *applied in addition to the conditions in the consent.*
- *required by the consent only under certain conditions,*
- *that the consent allowed the notifier a choice of measures.*

Answer: No additional measures on top of the application and consent were taken. The environmental risk assessment scored the study as negligible.

4 Post-release monitoring measures

Please describe here any monitoring strategies.

Answer: The shedding of AMT-010 vector was detected by a sensitive quantative PCR. Vector DNA was transiently detected in all body fluids with a rapid clearance. Vector DNA was also transiently detected in the semen of 6 out of 7 subjects.

Table 3. Quantitative PCR analysis of body fluids for vector sequences

Subject	Sample	Days		Weeks								Months (Follow Up)		
		-14	2	1	2	3	4	5	8	10	12	6	9	12
001	Serum	-	$2.0X10^4$	73	35	11	-	6.6	-	-	-	ND	ND	ND
	Saliva	-	$3.0x10^2$	NR	-	-	-	ND	ND	ND	ND	ND	ND	ND
	Urine	-	91	13	-	-	-	ND	ND	ND	ND	ND	ND	ND
002	Serum	-	$1.4x10^6$	$9.0x10^4$	$2.2x10^2$	$1.2x10^2$	56	-	10	12	-	-		
	Saliva	-	$1.2x10^4$	$2.4x10^2$	$3.3x10^2$	38	27	-	-	-	-	ND	ND	
	Urine	-	12	-	-	-	-	ND	ND	ND	ND	ND	ND	
	Sperm	-	3.60E+01	58	38	3.2	6	0.4	-	-	-	-	-	
004	Serum	-	$8.9x10^6$	$3.4x10^3$	77	54	55	35	18	48	-	-		
	Saliva	-	$7.2x10^3$	$3.2x10^3$	$7.1x10^2$	87	-	-	-	-	ND	ND	ND	
	Urine	-	-	9.4	-	-	-	-	ND	ND	ND	ND	ND	
	Sperm	-	ND	21	25	2.7	-	-	-	-	-	-	-	
005	Serum	-	$3.1x10^6$	$4.5x10^3$	22	23	8.4	5.5	-	-	3.5			
	Saliva	-	$1.8x10^6$	$2.1x10^3$	$2.0x10^2$	$1.3x10^2$	14	-	-	11	-			
	Urine	-	-	0.23	-	-	-	ND	ND	ND	ND	ND	ND	
	Sperm [1]	NAV	ND	21	-	5.5	12	-	-	-	-	-		
006	Serum	-	$2.2x10^7$	$5.1x10^3$	$2.3x10^2$	50	26	12	-	-	-	ND	ND	
	Saliva	-	$4.3x10^4$	92	2.2	10	8.1	-	-	-	-	ND	ND	
	Urine	-	-	-	-	1.9	-	-	NAV	-	-	ND	ND	
	Sperm	-	ND	1.2	5.1	-	-	-	ND	ND	-	-		
007	Serum	-	$8.7x10^4$	$1.3x10^3$	$3.0x10^2$	87	$1.3x10^2$	36	8.7	7.6	1.3			
	Saliva	-	$1.2x10^4$	26	-	-	-	-	ND	ND	ND	ND	ND	
	Urine	-	79	115	11	-	-	-	ND	ND	ND	ND	ND	
	Sperm	NAV	NAV	NAV	NAV	NAV	NAV	NAV	NAV	NAV	NAV	NAV	NAV	
008	Serum	-	$3.6x10^5$	$7.4x10^2$	$1.6x10^2$	60	25	2.4	0.04	63	8.9			
	Saliva	-	22	3.7	-	-	-	-	ND	ND	ND	ND	ND	
	Urine	-	$7.2x10^3$	7.7	-	18	-	-						
	Sperm	NAV	NAV	NAV	NAV	NAV	NAV	NAV	NAV	NAV	NAV	NAV	NAV	

Subj 001 is a female, so for this subject sperm was not available. Sperm was not collected at day 2, except for subject 002

Vector DNA sequences are expressed as copies per mL (for serum, saliva and urine) or as copies per microgram DNA (for sperm)

- indicates negative, i.e. below the detection limit of 10-100 copies per sample; ND, not done, NR, no result due to inhibitory sample, NAV, not available

1) As Subject 005 is vasectomized, the DNA in his semen samples was too low to measure. Therefore, for this subject the vector copies are expressed in copies/mL

5 Results of the foreseen and unforeseen release(s)

Consider in the following questions 5.1 through 5.4 all results of the foreseen and unforeseen release(s) in respect of any risk for human health or the environment, without prejudice to whether the results indicate that any risk is increased, reduced or remains unchanged.

5.2 Results of the study

Provide a summary of the study results in respect of any risk for human health or the environment. Include also the results of the monitoring measures (if applicable).

Answer: Administration of AMT-010 was well tolerated by all patients. No laboratory measurements, physical findings or other measurements produced any clinically atypical values in any subject receiving the low and intermediate dose.

5.3 Unexpected effect(s) and adverse effects

'Unexpected effects' refer to effects on human health or the environment, which were not foreseen or identified in the environmental risk assessment of the notification. This part of the report should contain any information with regard to unexpected effects or observations relevant for the initial environmental risk assessment. In case of any observed unexpected effects or observations, this section should be as detailed as possible to allow a proper interpretation of the data.

Answer: Adverse effects were all classified as being mild to moderate, except for two severe adverse events (SAE's) in patient 6. These SAEs were judged to be non-study medication related. No dose-limiting toxicity was observed.

5.4 Unintended release of the GMO

'Unintended releases' refer to any incidents or spills with regard to the GMO that occurred during the study, where possible effect(s) on human health and/or the environment cannot be excluded. Describe these effects, including actions taken to manage the risks.

Answer: no incidents or spills occurred during this study. The risks were managed by specific instructions and training to handle any possible spillage of the GMO during administration.

5.5 Other information

Notifiers are encouraged to supply information, which is outside the scope of the notification but which might be relevant to the trial(s) in question. This may also include observations of beneficial effects.

Answer: Observations are expressed in reports previously sent to Loket Gentherapie:
- Prelimimary safety and efficacy data van 2005 in study IM 05-001, sent to RIVM/Bureau GGO, loket gentherapie, t.a.v. dr. H.P.H. Hermsen, January 4, 2005;
- Year report IM 05-001, December 15, 2006 (confidential), sent to RIVM/bureau GGO, t.a.v. dr. H.P.H. Hermsen, January 8, 2007;
- Verslag van Verrichte Werkzaamheden 2006 - IM 05-001/00/W02, sent to RIVM/Bureau GGO, loket gentherapie, receipt confirmed January 17, 2007 (dr. D.A. Bleijs).

6 Assessment of risk following completion of release

Please provide a reflection of the risk assessment and risk management strategies carried out prior to the release in relation to the obtained results and findings of for instance monitoring and samples taken from the test subjects. Do the results of the study justify the performed environmental risk assessment and conclusion?

Answer: the results justify the performed environmental risk assessment and conclusion.

7 Conclusion

Here the notifier should elaborate on the efficacy and efficiency of all measures taken, and elaborate on the insights gained during this release. Also, specify how the gain in experience can benefit further (future) releases with respect to risk management.

Answer: the results were in accordance with the expectations. The risk of negative environmental side effects occurring was extremely low. The replication deficient vector in AMT-010 was classified as a pathogenicity risk class-1 organism and the environmental risk assessment considered the environmental risk as negligible.