PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	**Germany**
(b)	Notification number	**B/DE/17/PEI2969**
(c)	Date of acknowledgement of notification	**22/12/2016**
(d)	Title of the project	**Double blinded, randomized, Priorix®- and placebo-controlled, trial to evaluate the optimal dose of MV-CHIK vaccine (against chikungunya virus) in regard to immunogenicity, safety and tolerability in healthy volunteers**
(e)	Proposed period of release	**April 2017 – December 2017**

2. Notifier

Name of institution or company: **Themis Bioscience GmbH**
Muthgasse 11/2
1190 Vienna
Austria

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	**(X)**
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class ***Paramyxoviridae***

(b) Identity of the GMO (genus and species)

The MV-CHIK vaccine is a recombinant live attenuated virus vaccine that is based on the backbone of the Schwarz strain measles virus (MV) vaccine (derived from the Edmonston isolate). The heterologous antigens are derived from the Chikungunya (CHIK) virus (CHIKV).

(c) Genetic stability – according to Annex IIIa, II, A(10)

During sequencing analysis of MV-CHIK vaccine six small nucleotide polymorphisms (SNPs) and one insertions was detected.
The risk of re-conversion of the attenuation sites was assessed and the conclusion was, that it is probable that the observed mutations occurred in response to passaging the MV-CHIK in the Vero cell line and that these changes signal an interphase of likely or necessary evolutionary steps needed in order to ultimately adapt the vaccine virus construct to the Vero cell substrate. This means that nucleotide changes as observed can be explained by adaptive processes relevant for the Vero cell line which ultimately might result in a fully stabilized and Vero cell adapted MV-CHIK construct very likely without any measurable change regarding the level of attenuation.
It is also feasible that these nucleotide changes occurred spontaneously, i.e. outside of an adaptation process in only a subset of viruses. This may explain why these changes were not observed previously. This latter interpretation is supported by the fact that the nucleotide insertion results in a frame shift of the P Protein coding sequence and subsequently in a truncated P Protein probably resulting in a non-viable virus particle. Concerns, that the mutations are related to a possible reversion to virulence of the vectored vaccine can be ruled out with great certainty.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (**X**) No (.)

If yes, insert the country code(s) **AT**

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (.) No (**X**)

If yes:
- Member State of notification …
- Notification number B/../../…

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (.) No (**X**)

If yes:
- Member State of notification …
- Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.

The GMO will be closely monitored during utilization in the clinical study and will be accounted for at all times. Residual GMOs will be destroyed at the study end at the

latest. Further, no case of person to person transmission is reported for the backbone measles vaccine and consequently is also not expected for MV-CHIK.
An environmental impact of the release is therefore highly unlikely.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterization:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid	(.)
RNA virus	(**X**)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
(specify phylum, class)	…

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	**Mononegavirales (family: Paramyxoviridae) (subfamily: Paramyxovirinae)**
(ii)	genus	**Morbillivirus**
(iii)	species	-
(iv)	subspecies	-
(v)	strain	**Schwarz strain**
(vi)	pathovar (biotype, ecotype, race, etc.)	-
(vii)	common name	**attenuated measles virus**

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (**X***) No (.) Not known (.)
the wild type form is indigenous to the country where the notification is made; the attenuated form is widely used as measles vaccine, but no cases of natural propagation have been reported.

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (**X***)

the wild type form is indigenous globally; the attenuated form is not able to propagate

If yes, indicate the type of ecosystem in which it is found:

Atlantic	**X**
Mediteranean	**X**
Boreal	**X**
Alpine	**X**
Continental	**X**
Macaronesian	**X**

(ii) No (.)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (**X**) No (.)

(d) Is it frequently kept in the country where the notification is made?
Yes (**X**) No (.)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)

other, specify
Wild type measles virus occurs only in humans and is transmitted by aerosolized respiratory droplets and by direct contact. The attenuated Schwarz strain is able to infect humans, however the pathogenicity is greatly reduced and no case of person to person transmission of measles vaccine has been reported.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
n.a.

5. (a) Detection techniques
Antibody detection; viral culture; viral genome detection

(b) Identification techniques
Polymer chain reaction (PCR) amplification of unique RNA sequences

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (**X**) No (.)
If yes, specify
Classification 1 for the Schwarz strain of measles virus.
(wild type measles virus has classification 2 according Directive 93/88/EEC; however, this classification does not apply for the attenuated form)

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No (**X**) Not known (.)

If yes:

(a) to which of the following organisms:

humans	(.)
animals	(.)
plants	(.)
other	(.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

…

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
"Generation time" of viruses can practically be given as the time of infection until the time of onset of shedding of virions and is approximately 10 to 12 days for the wild type measles virus.
The recipient strain (Schwarz strain) is an attenuated strain and no person to person transmission has been reported. Measles virus vaccine shows the same time course of infection; however, no infectious measles viruses will be shed from the nasopharynx of an infected individual.

(b) Generation time in the ecosystem where the release will take place:
Same as 8. (a)

(c) Way of reproduction: Sexual (.) Asexual **(X)**

(c) Factors affecting reproduction:
n.a.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	…

Measles viruses do not form survival structures.

(b) relevant factors affecting survivability:
Measles virus replication is dependent on human cells and the virus is not stable in the environment. In stability studies Themis showed that MV-CHIK vaccine lost almost complete potency when incubated at ambient temperature for 1 hour (loss of 3 logs; half-life ~6 min).

10. (a) Ways of dissemination
Schwarz strain measles virus is unable to disseminate.

(b) Factors affecting dissemination
Person to person transmission has never been reported.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
…, B/../../…

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(**X**)
(ii)	deletion of genetic material	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
MV-CHIK is a live recombinant attenuated vaccine designed to prevent Chikungunya virus infection and disease. The MV-CHIK vaccine is based on the expression of Chikungunya Virus (CHIKV) antigens on the backbone of the measles virus vaccine strain Schwarz. Nucleotide sequences encoding Chikungunya virus structural proteins were inserted into the Schwarz vaccine strain of MV. The insert was generated by gene synthesis. Donor virus strains were not used during generation of the vaccine.

3. (a) Has a vector been used in the process of modification?
Yes (**X**) No (.)
The plasmid pTM-MVSchw is containing the whole antigenome of the measles virus strain Schwarz. pTM-MVSchw is used to insert the Chikungunya sequences into the measles virus antigenome.
If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (.) No (**X**)
The plasmid pTM-MVSchw is used to insert foreign genetic material (i.e. CHIKV genes) into the MV backbone. Infectious viral particles (MV-CHIK live attenuated vaccine) are generated using a helper cell based rescue system. The antigenome of the modified measles virus is flanked by a T7-RNA-polymerase-promoter upstream and the respective terminator downstream. The rescue cells (HEK239) stably express the T7 polymerase to generate infectious RNA and auxiliary MV proteins

that can form infectious viral particles. The recued viral particles are amplified on Vero cells. Thus the GMO MV-CHIK is composed of live replicating viral particles, which do not contain the pTM-MVSchw cloning vector or sequences thereof.

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid	(**X**)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	…

(b) Identity of the vector
pTM-MVSchw (containing the antigenome of the Schwarz MV vaccine strain)

(c) Host range of the vector
E. coli; eukaryotic cell culture cells

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes (**X**) No (.)

antibiotic resistance	(**X**)
other, specify	…

Indication of which antibiotic resistance gene is inserted
Ampicillin

(e) Constituent fragments of the vector
Besides the ampicillin resistance gene, the vector carries an f1 origin of replication and the measles back bone (measles anti genome).
The measles backbone is flanked by ribozyme sequences promoting self-cleavage of transcribed viral RNA. Flanking further outside a T7-RNA-polymerase promotor (upstream) and a T7-RNA-polymerase terminator (downstream) facilitate transcription of viral RNA in cells expressing T7-RNA-polymerase.
3529 bps inside the measles backbone the cloning site for inserting heterologous sequences is located.

(e) Method for introducing the vector into the recipient organism
Comment: the vector carries the whole antigenome of the Schwarz strain measles virus which is the "recipient organism" according to the wording of this document. Described below are the methods of vector transfer into organisms used for propagation of the vector sequences (E.coli) and virus rescue (helper cells constitutively expressing T7-RNA-polymerase; Human embryonic kidney 293 cells – HEK 293)

(i)	transformation	(**X**) (E.coli)
(ii)	electroporation	(**X**) (E.coli)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	**Calcium phosphate co-precipitation (HEK 293)**

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i)	transformation	(.)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify …	

6. Composition of the insert

(a) Composition of the insert
The insert contains the coding sequence for a polyprotein which, after cleavage, releases the envelope proteins E1, E2, E3, the capsid protein C and the structural protein 6K.

(b) Source of each constituent part of the insert
The donor sequence was derived from the CHIKV strain 06-49, a clinical isolate from La Reunion Island from the 2005/2006 epidemics (Gene Bank Accession No: AM258994.1). The cDNA encoding for the polyprotein including the structural proteins C-E3-E2-6K-E1 from CHIKV strain 06-49 was chemically synthesized. The sequence was inserted in BsiWI/BssHII-digested pTM-MVSchw.

(c) Intended function of each constituent part of the insert in the GMO
Upon vaccination, the heterologous antigens are produced and secreted. These antigens can then induce a specific humoral and/or cellular immune response. The Chikungunya virus antigens include well described epitopes in the E1 and E2 proteins that can induce a neutralizing immune response. In addition, the expression of the entire open reading frame encoding for the CHIKV structural genes ensures a structural integrity of the B cell epitopes.

(d) Location of the insert in the host organism

-	on a free plasmid	(.)
-	integrated in the chromosome	(.)
-	other, specify	**integrated in the viral genomic RNA**

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (**X**)
If yes, specify …

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid	(.)
RNA virus	(**X**)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
(specify phylum, class)	…
other, specify	…

2. Complete name

(i)	order and/or higher taxon (for animals)	n.a.
(ii)	family name for plants	**Togaviridae**
(iii)	genus	**Alphavirus**
(iv)	species	**Chikungunya virus**
(v)	subspecies	n.a.
(vi)	strain	**06-49**
(vii)	cultivar/breeding line	n.a.
(viii)	pathovar	n.a.
(ix)	common name	**Chikungunya virus**

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (**X**) No (.) Not known (.)

If yes, specify the following:

(b) to which of the following organisms:

humans	(**X**)
animals	(**X**)
plants	(.)
other	..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

Yes (.) No (.) Not known (**X**)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

…

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (**X**) No (.)

If yes, specify **Classification 3 according Directive 93/88/EEC**

5. Do the donor and recipient organism exchange genetic material naturally?

Yes (.) No (**X**) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?

Yes (.) No (**X**) Not known (.)

Specify **Survivability of the GMO is expected to be comparable to the recipient (i.e. the GMO is not able to survive outside of a human subject).**

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (**X**) No (.) Unknown (.)

Specify **Growth kinetics are slightly reduced in comparison to the empty measles virus due to the 3.7 kbp insert.**

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (.) No (**X**) Not known (.)

Specify **MV Schwarz strain is unlikely to naturally disseminate; the same characteristic is expected for MV-CHIK.**

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (.) No (**X**) Not known (.)

Specify **The same pathogenicity is expected.**

2. Genetic stability of the genetically modified organism

MV-CHIK vaccine is expected to be stable regarding immunogenicity and level of attenuation.

The original vaccine strain was isolated in the 1950s and it still protects fully against circulating measles virus strains. In addition, the reversion to wild type measles virus has never been observed.

During development and production of the GMO the full length of the vector and the inserted sequences alone were sequenced several times.

In a first approach the MV-CHIK pre-seed after 11 passages on Vero cells was analyzed by deep sequencing. In this project no variants were detected. No viral nucleic acids were found that showed point mutations, deletions or reversions in the insert sequence, when compared to the original sequence.
In a further deep sequencing study, at passage levels 13 and 14, 6 point mutations and one insertion were identified and the risk of reversion to virulence was assessed (for details please refer to heading A.3.c "Genetic stability – according to Annex IIIa, II, A(10)").
Taken together, the sequencing data and the risk assessment support the view, that a possible reversion to virulence can be ruled out with great certainty and the identified mutations either signal an interphase of evolutionary steps of adaption of the viral vector to the Vero cell line substrate or have occurred spontaneously.
Overall, it is expected that the recombinant MV-CHIK-virus is stable in the level of attenuation.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (**X**) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Antibody detection; viral culture; viral genome detection; Antibody specific ELISA from human serum samples (CHIKV and MV specific IgG)

(b) Techniques used to identify the GMO
PCR

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Clinical study on vaccination against Chikungunya virus.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (.) No (**X**)
If yes, specify …

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
n.a.

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
n.a.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
n.a.

4. Method and amount of release

(a) Quantities of GMOs to be released:
Overall 1,36.10^8 $TCID_{50}$ (50% tissue culture infective dose) will be used (all sites, Germany AND Austria).
160 subjects will be treated twice with 5.10^4 $TCID_{50}$ MV-CHIK
120 subjects will be treated twice with 5.10^5 $TCID_{50}$ MV-CHIK

(b) Duration of the operation:
Estimated from Q2 2016 to Q4 2017

(c) Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of the release
Investigational vaccine will be strictly controlled and will be accounted for at all times during the clinical trial. No dissemination through person to person transfer is expected. At the end of the trial all residual vaccine will be destroyed.

5. Short description of average environmental conditions (weather, temperature, etc.)
n.a.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.
No environmental impact is expected.
Human health is affected by vaccination of treated subjects. An earlier phase I study showed that the MV-CHIK vaccine was safe and immunogenic. Neutralizing antibodies were elicited in all treatment cohorts of the phase I trial.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)
(i) order and/or higher taxon (for animals) **Primates / Mammalia**

(ii)	family name for plants	**n.a.**
(iii)	genus	**Homo**
(iv)	species	**H. sapiens**
(v)	subspecies	**H. s. sapiens**
(vi)	strain	**n.a.**
(vii)	cultivar/breeding line	**n.a.**
(viii)	pathovar	**n.a.**
(ix)	common name	**Human**

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Induction of production of neutralizing antibodies directed and specific against Chikungunya-virus like particles assembled from structural proteins expressed by MV-CHIK vaccine. Neutralizing antibodies are expected to convey immunity also against wild type Chikungunya virus.

3. Any other potentially significant interactions with other organisms in the environment
n.a.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (**X**) Not known (.)
Give details
GMO will not propagate post-release; no selection will occur.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
None.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO
n.a.

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

7. Likelihood of genetic exchange in vivo

   (a) from the GMO to other organisms in the release ecosystem:
   Unlikely

   (b) from other organisms to the GMO:

Unlikely

(c) likely consequences of gene transfer:
n.a.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
n.a.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
n.a.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
GMOs will be used restrictively within the clinical study. All doses of vaccine will be accounted for on an ongoing basis; residual vaccine (used, partially used or unused) will be inactivated/destroyed after the end of the trial at the latest.

2. Methods for monitoring ecosystem effects
n.a.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
n.a.

4. Size of the monitoring area (m^2)
… m^2

5. Duration of the monitoring
During the conduct of the clinical trial (currently planned from Q2 2016 until Q4 2017)

6. Frequency of the monitoring
Study sites will monitor the GMOs on an ongoing basis. The clinical sites accountability will be controlled and reconciled by the clinical monitors responsible for the clinical study sites.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
n.a.

2. Post-release treatment of the GMOs
Destruction of GMOs at study end.

3. (a) Type and amount of waste generated
We expect the waste generated throughout the study will be less than 100ml of aqueous GMO solution and less than 100 unused vials of lyophilized GMO.

Rationale: 320 subjects will be enrolled in the study at four clinical study sites; 280 subjects will be treated twice with the GMO in different treatment cohorts. Per site 154 vials of lyophilized product will be necessary (140 for actual use and 14 (10%) overstock).
Per protocol lyophilized vaccine will be reconstituted in 0.4ml of water for injection and 0.3ml of reconstituted drug product will be injected. Per treatment 0.1ml of reconstituted vaccine (GMO in aqueous solution) will remain on site and will be returned to the sponsor and will be destroyed latest at study end. Calculating with this numbers, in the end 14ml GMO solution per site (overall 56 ml) and 14 unused vials per site (overall 56 vials) will remain and will be destroyed after the end of the clinical trial.

3. (b) Treatment of waste
Autoclaving / Chemical inactivation

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Unexpected spread of GMOs is unlikely to happen and can possibly only happen through accidentally dropping of vials or syringes with reconstituted vaccine.
Vaccine that may be spilled during the vaccination procedures will be cleaned using absorbent material and by disinfectants usually used at the clinical study site. All materials used during the cleaning procedures will be disposed of according to standard procedures for the destruction of infectious waste.

2. Methods for removal of the GMO(s) of the areas potentially affected
The GMOs will only be used in a well-controlled way; no areas should be affected or potentially affected (besides accidental and unexpected spread as described above).

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
n.a.

4. Plans for protecting human health and the environment in the event of an undesirable effect
No effect (desirable or undesirable) is expected for the environment.
The safety and wellbeing of the human study subjects will be closely monitored throughout the study; the study subjects will be extensively informed about expected adverse events and will be instructed to return to the study physicians whenever they need assistance.