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	United Kingdom
(b)	Notification number	B/GB/12/R45/01
(c)	Date of acknowledgement of notification	18./9/2012.
(d)	Title of the project	A Phase I double-blind, randomized, placebo-controlled, dose-escalation trial to evaluate the safety and immunogenicity of a Sendai HIV vaccine SeV-G(NP) given intra-nasally and Ad35-GRIN administered intramuscularly in prime-boost regimens in HIV-uninfected, healthy adults volunteers.
(e)	Proposed period of release	From December 2012 until December 2015

2. Notifier

International AIDS Vaccine Initiative
125 Broad Street (9th Floor)
New York, NY 10004, USA

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

Sendai virus is a member of a large family of viruses (the paramyxoviridae family) that are common causes of respiratory tract infection [1]. Sendai virus and other members of the paramyxoviridae family are classified in the Mononegavirlaes Order.

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

Sendai virus Z strain [murine parainfluenza virus type 1, HVJ (hemagglutinating virus of Japan)

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

Genetic stability of the GMO has been demonstrated both *in vitro* and *in vivo*. Multiple passaging of the GMO in cell culture has not resulted in genetic instability. In addition, virus isolated from laboratory animals (mice, rabbits and non-human primates) experimentally infected with the GMO revealed no significant differences induced by replication in the animal host.

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

Yes (.) No (X)

If yes, insert the country code(s) …

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 parental Sendai virus does not carry genetic information that can be transferred to other organisms or viruses that provides a growth advantage or resistance to environmental factors. Moreover, Sendai virus does not undergo recombination with other viruses or organisms.

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

1. Recipient or parental organism characterisation:

(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)	Mononegavirlaes
(ii)	genus	paramyxoviridae
(iii)	species	
(iv)	subspecies	…
(v)	strain	Sendai virus Z strain [murine parainfluenza virus type 1, HVJ (hemagglutinating virus of Japan)].
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Sendai

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:

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

(b) Indigenous to, or otherwise established in, other EC countries:

(i) Yes (.)

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

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

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

(c) Is it frequently used in the country where the notification is made?

Yes (.) No (.X)

(d) Is it frequently kept in the country where the notification is made?

Yes (.) No (.X)

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	…	The natural habitat of the parent strain is rodents (mouse, rat). Humans will be the only recipients of the GMO

(b) If the organism is an animal: natural habitat or usual agroecosystem:
…

5. (a) Detection techniques

The parental Sendai virus Z strain and the GMO can be isolated from specimens by propagating virus using standard laboratory procedures for amplifying Sendai virus in cultures of Vero or LLMC-K2 cells [1]. Under these conditions, cultured cells infected with Sendai virus or the GMO will commonly exhibit visible cytopathic effects caused by virus replication. The presence of virus can be confirmed using sensitive hemagglutination-inhibition assays or staining of infected cell cultures with antibodies specific for Sendai virus [1]. The second method of confirmation is used in the assessment of clinical samples with the Cell Infectious Unit (CIU) assay.

Parental Sendai virus Z strain and the GMO can also be detected directly in specimens using sensitive nucleic acid detection methods based on the polymerase chain reaction (PCR) assay [1, 2]. A PCR assay that clearly distinguishes between the parental virus and GMO by the presence of the HIV Gag gene is available. These assays have been use to test samples from laboratory experiments as well as specimens collected from experimentally-infected laboratory animals.

(b) Identification techniques

The Cell Infectious Unit (CIU) assay has been validated with a Limit of Detection of 30 cell infectious units per mL (CIU/mL) and a Limit of Quantitation of approximately 100 CIU/mL.

The PCR assay is capable of detecting the presence or absence of the transgene directly from tissues or specimens to a level of approximately 10^3-10^4 CIU/mL. Virus can be expanded from tissue samples before PCR analysis to increase the sensitivity.

Samples that are positive for Sendai virus can be further analyzed to positively identify the virus in a specimen. Nucleic acid amplified by PCR can be subjected to nucleic acid sequencing. This provides information on the genetic material that can discriminate unambiguously between Sendai virus found in the environment, the parental Sendai virus Z strain, and the GMO.

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

In the United Kingdom, the Advisory Committee on Dangerous Pathogens (ACDP) categorizes Sendai virus ACDP Hazard Group 1 (www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part2.pdf).

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	(X)
plants	(.)
other	(.)

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

The GMO is severely attenuated compared to wild type SeV. Administration of high doses of the GMO to susceptible animals (mice) has resulted in transient non-fatal infection. Administration to non-susceptible animals including directly into the trachea has resulted in limited low level shedding and no clinical pathology.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Not applicable

(b) Generation time in the ecosystem where the release will take place:

Wild type Sendai virus circulating in the environment replicates in rodents and is transmitted in rodent colonies by respiratory secretion [1]. The duration of respiratory illness ranges from 1-2 weeks during which the infectious virus is produced and can be transmitted to other animals in the colony

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

(d) Factors affecting reproduction:

Not Applicable

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 …	

(b) relevant factors affecting survivability:

Like all paramyxovirses, Sendai virus is an enveloped RNA virus [3]. This type of virus is readily inactivated by detergents and disinfecting agents. The virus also does not tolerate dry conditions, thus it does not survive for long periods in the environment [4].

10. (a) Ways of dissemination

There is no indication that the GMO may persist in untreated sewage, river water, seawater or soil [5]. No hazards are anticipated to result from release of the GMO due to the severe attenuation of this strain and the species restrictions.

(b) Factors affecting dissemination

Not Applicable

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)

Not Applicable

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

No genes were removed from the Sendai virus Z strain during construction of the GMO. All six Sendai virus genes (N, P, M F, HN, and L) are essential for propagation of the virus [3]. None of the modifications needed to insert the Gag gene imparted new functional properties on Sendai virus. Insertion of the Gag gene has two consequences: (1.) the GMO can express this HIV protein in infected cells; and (2.) insertion of the extra gene causes some decline in expression of the proteins naturally encoded by Sendai virus [6].

The GMO is less virulent that the parental virus which in turn is substantially less virulent than wild type Sendai virus in experimentally-infected mice and rabbits. The kinetics of the growth of the GMO has been compared to the parental virus in mice, rabbits and non-human primates. The virus replication kinetics were similar although the GMO attained lower peak virus titers compared to the parental virus. No new traits or characteristics were observed. Mild respiratory symptoms were observed in mice but not in rabbits or non-human primates after infection with the GMO.

3. (a) Has a vector been used in the process of modification?

Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Yes (X) No (.)

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

The recombinant SeV-G(NP) vector was generated by Virus Rescue using plasmids encoding three Sendai virus proteins (N, P, and L), a plasmid containing the DNA copy of the GMO genome, and one plasmid encoding T7 RNA polymerase. The virus was generated and amplified to produce a virus seed. A clonal isolate of the GMO was prepared by conducting three rounds of a method called *Limiting Dilution*. A virus clone was selected and amplified to produce the pre-Master virus seed (pMVS). For GMP manufacturing, the pMVS was transferred to a Contract Manufacturing Organization (CMO). Vero cells from a qualified cell bank were infected with the pMVS to produce the Master Virus Seed (MVS). The MVS was used to infect fresh Vero cells to generate the Clinical Trial Material (CTM) that will be used for both pre-clinical safety and clinical studies. Testing on the Clinical Trial Material indicated it meets all the specifications.

(c) Host range of the vector

SeV has a limited host range in animals. These include mouse, rat, guinea pig, rabbit, ferret and marmoset. The pig may be also susceptible. While in enzootic infections, SeV usually produces a subclinical or asymptomatic outcome, epizootic infections as well as experimental infections cause acute pneumonia in mouse and rat. The symptoms are more severe for mouse than for rat. The remaining animals listed above are less susceptible and infections are usually inapparent [7]. It is not naturally harboured by humans and has not been described to be harboured by plants.

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

Yes (X) No (.)

antibiotic resistance	(.)
other, specify	…

Indication of which antibiotic resistance gene is inserted

Sendai virus does not carry any antibiotic resistance genes

(e) Constituent fragments of the vector

The GMO contains unique genetic signatures that can be followed using PCR and nucleic acid sequencing. Application of these methods in the field is illustrated well by influenza virus research programs that routinely monitor the emergence of new virus strains in humans and poultry (ref below). GMO genetic material can be traced if necessary using these methods. The viral genes in the SeV vector, which were derived from the SeV Z strain, have nucleotide sequences that can be distinguished from other Sendai virus strains using PCR and nucleotide sequencing. The *Gag* gene in the GMO is a synthetic gene generated in the laboratory. The nucleotide sequence of this Gag gene is different from the Gag sequence found in HIV, thus it can be uniquely identified in specimens [8].

(f) Method for introducing the vector into the recipient organism

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	Intranasally

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 Sendai virus Z strain RNA genome was cloned into a plasmid DNA where it could be modified using standard molecular cloning techniques. A small synthetic DNA sequence (10 bases) was added to provide a site were the HIV Gag gene could be inserted into the parental Sendai virus Z strain genome. This small DNA insert provides no new functional properties on the virus; it was added to facilitate construction of the GMO in the laboratory. Subsequently, the HIV Gag gene was inserted. Live Sendai virus containing the Gag gene is recovered from a plasmid DNA using established procedures in Vero cells [9-11] qualified for vaccine production.

(b) Source of each constituent part of the insert

The DNA copy of the parental Sendai virus Z strain genome was modified to contains a 10 nucleotide insert 5' to the Nucleocapsid gene. This small insert is the Not I restriction endonuclease cleavage site. The gene encoding HIV Gag was inserted into the Not I site. As a result, the introduced *Gag* sequence is flanked by Not I restriction endonuclease cleavage sites. This same strategy has been used before to generate a Sendai virus Z strain that expressed the firefly luciferase protein [12] or the SIV Gag protein [13].

(c) Intended function of each constituent part of the insert in the GMO

The HIV *gag* gene was synthesized in the laboratory specifically to code a Gag protein that is equivalent to the protein produced by HIV-1 subtype A.

To insert a gene into Sendai virus, the viral RNA genome must be cloned as a DNA copy in a plasmid DNA vector. This genomic DNA clone can then be manipulated by standard molecular cloning procedures, which were used to strategically insert of a small synthetic cloning site (the Not I restriction enzyme cleavage site) that facilitated subsequent addition of the gene encoding the HIV Gag protein. The modified Sendai virus genomic DNA containing the synthetic *gag* gene was then used to recover live virus by a method called *Virus Rescue*. Molecular cloning techniques and Virus Rescue methods used to manipulate the Sendai virus Z strain has been used for more than 10 years [9, 12, 13].

(c) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (X)
- other, specify …

(d) 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 (.)
DNA virus (X)
bacterium (.)
fungus (.)
animal
- mammals (.)

- insect (.)
- fish (.)
- other animal (X)
(specify phylum, class) …

other, specify …

2. Complete name

Sendai virus Z strain [murine parainfluenza virus type 1, HVJ (hemagglutinating virus of Japan)].

Donor: Synthetic DNA sequence generated by chemical synthesis. The donor gene is not directly derived from a virus or an organism. The synthetic gene codes for a protein equivalent to HIV-1 subtype A Gag (Genbank accession number: AY253305).

(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 Sendai

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

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

If yes, specify the following:

(b) to which of the following organisms:

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

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

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

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

In the United Kingdom, the Advisory Committee on Dangerous Pathogens (ACDP) categorizes Sendai virus ACDP Hazard Group 1 (www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part2.pdf).

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 …

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

Yes (.) No (X) Unknown (.)
Specify …

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

Yes (.) No (X) Not known (.)
Specify …

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

Yes (.) No (X) Not known (.)
Specify …

2. Genetic stability of the genetically modified organism

The recombinant SeV-G(NP) vector was generated by Virus Rescue using plasmids encoding three Sendai virus proteins (N, P, and L), a plasmid containing the DNA copy of the GMO genome, and one plasmid encoding T7 RNA polymerase. The virus was generated and amplified to produce a virus seed. A clonal isolate of the GMO was prepared by conducting three rounds of a method called *Limiting Dilution*. A virus clone was selected and amplified to produce the pre-Master virus seed (pMVS). For GMP manufacturing, the pMVS was transferred to a Contract Manufacturing Organization (CMO). Vero cells from a qualified cell bank were infected with the pMVS to produce the Master Virus Seed (MVS). The MVS was used to infect fresh Vero cells to generate the Clinical Trial Material (CTM) that will be used for both pre-clinical safety and clinical studies. Testing on the Clinical Trial Material indicated it meets all the specifications.

Stability of the Gag gene insert in the GMO Pre-MVS has been shown to be stable by PCR for at least 5 generations in cell culture.

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
…

Assay	Specificity for identification
Virus Infectivity Assay	Detection of Infectious Sendai Virus concentration using anti-Sendai antibody.
Western Blot Analysis	Specific Gag protein detection in infected cells using specific anti-Gag antibody.
RT-PCR	Detection of HIV gag insert in context with the Sendai virus back-bone in the NP position.
Virus Sequencing	Detection of HIV Gag insert in context with the Sendai virus back-bone.

(b) Techniques used to identify the GMO

The parental Sendai virus Z strain and the GMO can be isolated from specimens by propagating virus using standard laboratory procedures for amplifying Sendai virus in cultures of Vero or LLMC-K2 cells [1]. Under these conditions, cultured cells infected with Sendai virus or the GMO will commonly exhibit visible cytopathic effects caused by virus replication. The presence of virus can be confirmed using sensitive hemagglutination-inhibition assays or staining of infected cell cultures with antibodies specific for Sendai virus [1]. The second method of confirmation is used in the assessment of clinical samples with the Cell Infectious Unit (CIU) assay.

Parental Sendai virus Z strain and the GMO can also be detected directly in specimens using sensitive nucleic acid detection methods based on the polymerase chain reaction (PCR) assay [1, 2]. A PCR assay that clearly distinguishes between the parental virus and GMO by the presence of the HIV Gag gene is available. These assays have been use to test samples from laboratory experiments as well as specimens collected from experimentally-infected laboratory animals.

The Sendai virus Z strain can be distinguished from Sendai virus naturally circulating in the environment using PCR technology. Viral nucleic acid amplified by PCR can be subjected to nucleotide sequence analysis to clearly show that virus in a specimen is either the parental Sendai virus Z strain, the GMO, or virus naturally found in the environment.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

The purpose of the study is to investigate the safety and immunogenicity of the GMO compared to placebo in healthy HIV-uninfected adult volunteers. In relation to the release of the GMO, the main points are summarised below.

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 (X) No (.)

If yes, specify

The GMO will be administered to healthy volunteers in a randomized, dose-escalation, placebo-controlled Phase I clinical study.

3. Information concerning the release and the surrounding area

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

London, United Kingdom

The UK national (OS) grid reference of the proposed site of release is TQ2677.

(b) Size of the site (m^2):

St. Stephens Centre, HIV Clinical Trials Unit, Chelsea and Westminster Hospital Foundation, 369 Fulham Road, London SW10 9NH.
Clinical Area, SSAT Clinical Trials Unit, St. Stephen's Centre is 20 m^2
Kobler Pharmacy, Ground Floor St. Stephen's Centre is 7.5 m^2
KAVI-KNH: Kenya AIDS Vaccine Initiative, Department of Medical Microbiology, College of Health Sciences, University of Nairobi, Kenya - about 90 m^2
Projet San Francisco, Kigali, Rwanda - about 24 m^2.

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

The site is approximately 1 km from the River Isis (Thames).

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

SeV has a limited host range in animals. These include mouse, rat, guinea pig, rabbit, ferret and marmoset. The pig may be also susceptible. While in enzootic infections, SeV usually produces a subclinical or asymptomatic outcome, epizootic infections as well as experimental infections cause acute pneumonia in mouse and rat. The symptoms are more severe for mouse than for rat. The remaining animals listed above are less susceptible and infections are usually inapparent [7]. It is not naturally harboured by humans and has not been described to be harboured by plants.

4. Method and amount of release

(a) Quantities of GMOs to be released:

The maximum release of the GMO in the study overall will be no more than 2×10^{10} CIU.

(b) Duration of the operation:

December 2012-December 2015

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

Previous clinical experience with a non-recombinant replicating Sendai virus vaccine administered intranasally in humans showed no shedding in nasal secretions. Moreover the GMO is attenuated. It is therefore not anticipated that the GMO will be released outside the human host. However, we cannot rule out the possibility of a transient release of the GMO into the environment after volunteers have been dosed and have left the clinical site.

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

There is no indication that the GMO may persist in untreated sewage, river water, seawater or soil [5]

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.

The GMO SeV-G(NP) has not been evaluated in humans before. Preclinical studies using mice, rabbits, guinea pigs and non-human primates have been conducted.

At very high doses the SeV-G(NP) vector in general caused a self limited respiratory infection in susceptible mice. One mouse died during one of the immunogenicity studies. SeV-G(NP) has been administered to non-human primates, guinea pigs and rabbits with no apparent signs or symptoms of disease.

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)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Target organisms are healthy adult HIV-uninfected human volunteers in the clinical trial.

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

It is anticipated that following intranasal administration of the GMO to human volunteers, the GMO will reach the nasopharyngeal cavities and interact with the local mucosa and spread into the systemic compartment such that a host immune response is generated against the GMO. These immune responses may be protective against Sendai virus, human para-influenza type 1 (cross-reactivity) and HIV-1.

3. Any other potentially significant interactions with other organisms in the environment
None expected

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes (.) No (.) Not known (.)

Give details

The GMO has a disadvantage compared to the parental strain, because it possesses the additional genetic material coding for the HIV-1 Gag protein resulting in a lower ability to replicate. As shown in preclinical studies, the parental strain is severely attenuated compared to wild type SeV strains.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

The GMO may be released in the air. Wild type SeV does circulate in the rodent population. There is no reason to suggest that the GMO may be released into the public sewage treatment system.

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

(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 …

Sendai virus is not known to be pathogenic in humans.

Exclusion criteria have been set for the trial to minimise the risk of transmission of the GMO to potentially vulnerable groups.

Principal exclusion criteria include:

- Subjects with confirmed HIV-1 or HIV-2 infection.
- Pregnant and lactating women.
- Women who do not want to use a contraceptive method from the beginning of the study until 4 months after the last vaccination.
- Subjects with chronic disease, in particular with immuno-compromised condition.
- Subjects with acute or chronic upper or lower respiratory disease.

Contact with rodents or susceptible animals may result in transmission although this is a remote possibility.

7. Likelihood of genetic exchange in vivo

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

The parental Sendai virus does not carry genetic information that can be transferred to other organisms or viruses that provides a growth advantage or resistance to environmental factors. Moreover, Sendai virus does not undergo recombination with other viruses or organisms.

(b) from other organisms to the GMO:

The potential for genetic exchange with any other organisms in the environment is extremely low, given that the GMO consists of single stranded RNA and does not contain any plasmids, will not persist in the subjects and should not persist in the environment.

(c) likely consequences of gene transfer:

The GMO is a single-stranded RNA virus of Paramyxoviridae family, which has been modified to encode the HIV-1 *Gag* gene. Like all paramyxoviruses, the GMO replicates in the cell cytoplasm, without nuclear phase, and no DNA intermediate formed during viral replication. The viral genome integration into the host DNA cannot occur.

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.)

Preclinical studies indicate that there was transient low level shedding for up to 8 days. The level of shed virus was below a level shown to cause disease in susceptible rodents and no disease was apparent in non susceptible rabbits and macaques given much higher doses.

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

No immediate or delayed effects on biogeochemical processes are expected, as there is no known or predicted involvement of the GMO in biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Safety monitoring of the volunteers in the proposed clinical study is a core aspect of monitoring for the GMO. The GMO will be given intranasally in a single dose to volunteers on one or two occasions, who will have regular safety assessments throughout the study. The assessments include close clinical monitoring for any adverse effect of dosing as well as

quantitative assessment of shedding by culture and qualitative assessment of the genetic stability of the virus by PCR.

Data on adverse events and systemic symptoms will be collected after vaccination with the GMO and reviewed at follow-up visits. Any subject reporting significant illness felt to be related to infection with the GMO will be investigated, managed and treated as appropriate.

2. Methods for monitoring ecosystem effects

Participants in the clinical trial will be assessed at selected timepoints for shedding during the first 10 days following administration. Any virus detected by the CIU assay will assessed for identity by PCR to confirm the integrity of the HIV *Gag* gene insert.

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

The GMO contains unique genetic signatures that can be followed using PCR and nucleic acid sequencing. Application of these methods in the field is illustrated well by influenza virus research programs that routinely monitor the emergence of new virus strains in humans and poultry (ref below). GMO genetic material can be traced if necessary using these methods. The viral genes in the SeV vector, which were derived from the SeV Z strain, have nucleotide sequences that can be distinguished from other Sendai virus strains using PCR and nucleotide sequencing. The *Gag* gene in the GMO is a synthetic gene generated in the laboratory. The nucleotide sequence of this Gag gene is different from the Gag sequence found in HIV, thus it can be uniquely identified in specimens [8].

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

St. Stephens Centre, HIV Clinical Trials Unit, Chelsea and Westminster Hospital Foundation, 369 Fulham Road, London SW10 9NH.

Clinical Area, SSAT Clinical Trials Unit, St. Stephen's Centre is 20 m^2
Kobler Pharmacy, Ground Floor St. Stephen's Centre is 7.5 m^2

5. Duration of the monitoring

Monitoring of nasopharyngeal secretion, saliva, urine and blood samples for the presence of the GMO will be performed for 10 days after each administration of the GMO. Stool samples will not be monitored.

Throughout the study subjects will be monitored for clinical and biological signs and symptoms for evaluating the safety and tolerability of the vaccination with the GMO alone or in prime-boost combination with Ad35-GRIN, in particular signs and symptoms evoking an upper and/or lower respiratory tract infection. After vaccination, subjects will visit the clinic for regular assessment. They will also be instructed to contact the study site if they become unwell between scheduled visits and be supplied with an emergency telephone number for a study physician providing out-of-hours contact. In this way the subjects are closely monitored and appropriately assessed and treated should they develop signs or symptoms of clinical evidence of GMO infection.

6. Frequency of the monitoring

Study subjects will be followed up for 12 months after last vaccination as part of the clinical study

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Following each administration of a dose of vaccine, any surface such as table or bench likely to be contact with the vaccine will be wiped clean with a detergent wipe containing 10% bleach. The vaccine will be administered in a separate room within the medical clinic. Only study staff and study participants will not be permitted to be present in the room during clinical procedures.

In clinical areas, routine cleaning of surfaces will be performed using a detergent wipe containing 10% bleach. Larger spillages will be treated as follows: (i) Wear appropriate personal protective clothing (glasses, apron and gloves) and chemically disinfect the area by spraying a disinfectant containing 10% bleach for 10 minutes, (ii) Place absorbent paper towels to absorb the waste and transfer to waste bags, (iii) Wear a new set of protective clothing and clean the area with warm water and discard to waste, (iv) Treat all waste as clinical waste and decontaminate by incineration or autoclaving.

Any equipment used for dosing will be cleaned and decontaminated (e.g. by autoclaving) or disposed of (e.g. by incineration), as appropriate. All disinfection, decontamination and disposal procedures will be performed wearing suitable personal protective equipment in accordance with documented local procedures including those for Infection Control.

2. Post-release treatment of the GMOs

Please refer to previous question.

3. (a) Type and amount of waste generated

- Laboratory waste (plastic ware, liquid reagents, residual GMO).
- Clinical waste (urine, blood samples, mucosal secretions, sharps).
- Miscellaneous waste (disposable clothing, tissues).

3. (b) Treatment of waste

All waste disposal and decontamination procedures will be performed using appropriate personal protective equipment, in accordance with local documented procedures for Infection Control. All waste, including pre-disinfected material, will be placed in biohazard bags and autoclaved prior to removal from the site and final disposal by incineration.

- Laboratory waste: Laboratory waste will be placed in a container and autoclaved, prior to being incinerated on site.
- Clinical waste: Blood, mucosal secretions and urine samples collected into plastic containers for analysis will be autoclaved and then incinerated. Used sharps will be discarded straight into a sharps container at the point of use, prior to disposal.
- Miscellaneous waste: All items that have the potential for contamination, such as disposable clothing, tissues etc will be placed in sealed bags/containers prior to autoclaving and incineration.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

   Following each administration of a dose of vaccine, any surface such as table or bench likely to be contact with the vaccine will be wiped clean with a detergent wipe containing 10% bleach.

   In clinical areas, routine cleaning of surfaces will be performed using a detergent wipe containing 10% bleach. Larger spillages will be treated as follows: (i) Wear appropriate personal protective clothing (glasses, apron and gloves) and chemically disinfect the area by spraying a disinfectant containing 10% bleach for 10 minutes, (ii) Place absorbent paper towels to absorb the waste and transfer to waste bags, (iii) Wear a new set of protective clothing and clean the area with warm water and discard to waste, (iv) Treat all waste as clinical waste and decontaminate by incineration or autoclaving.

   Any equipment used for dosing will be cleaned and decontaminated (e.g. by autoclaving) or disposed of (e.g. by incineration), as appropriate. All disinfection, decontamination and disposal procedures will be performed wearing suitable personal protective equipment in accordance with documented local procedures including those for Infection Control.

2. Methods for removal of the GMO(s) of the areas potentially affected

   Contaminated areas may be decontaminated by the use of standard disinfectants containing 10% bleach.

   The methods for decontamination of areas (surfaces, equipment) affected as a result of unexpected spread will be essentially the same as those described for decontamination of areas following each scheduled release event

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

   Treatments with disinfectants, incineration or autoclaving are all effective means for decontamination of exposed items

4. Plans for protecting human health and the environment in the event of an undesirable effect

The use of the GMO will be strictly controlled according to Good Clinical Practice (GCP) and national regulations restricting its use according to a detailed protocol approved by the MHRA. Each recipient of the GMO will be assessed for undesirable effects regularly by medically qualified personnel according to the approved protocol. Participants in the study also have to ability to contact study staff at any time in the event of an undesirable effect.

Safety monitoring also includes medical oversight by two important teams. A Protocol Safety Review Team which includes a physician from each site as well as the Sponsor's Medical Monitor will review on a weekly basis, reports of all adverse events. The second committee is the Safety Review Board comprising three independent medically qualified people who will assess safety during the trial at predetermined times. Both committees and the Medical Monitor will be available to the local study team to advise on the need to take additional precautions to protect human health.