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	Belgium
(b)	Notification number	B/BE/09/BVW1
(c)	Date of acknowledgement of notification	05/05/2009
(d)	Title of the project	

Clinical trial NV25025 : "*A randomized, double blind, placebo controlled, parallel group, multicenter study of the safety and response rate of 3 subcutaneously administered doses of 5 x 10^7 pfu RO5217790 in patients with high grade cervical intraepithelial neoplasia grade 2 or 3 associated with High Risk HPV infection.*"

(e)	Proposed period of release	From 01/09/2009 until 31/12/2010

Patients will receive 3 injections of 5x10^7 pfu of RO5217790 or placebo 1 week apart i.e. Days 1, 8 and 15. Release is planned for approximately 12 months after the inclusion of the first patient

2. Notifier

Sponsor

F. Hoffmann-La Roche, Ltd.
Bldg. 74, Grenzacherstrasse 124
4070 Basel
Switzerland

EU Representative

Roche Registration Ltd.
6 Falcon Way-Shire Park
Welwyn Garden City AL7 1 TW
United Kingdom

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class …

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

Non replicative, recombinant vaccinia vector consisting of the modified vaccinia virus of Ankara (MVA) genome containing inserted transgenes that encode three proteins: the modified forms of the E6 and E7 proteins (delE6 and delE7) and the human cytokine IL2 (hIL2).

(c) Genetic stability –

A genetic stability program was designed to assess the genetic stability of RO5217790 at several steps of the production process: Pre Master Virus Seed 1 (PMVS1), Master Virus Seed (MVS), Final Drug Product (DP) and DP + 3 passages

The genetic stability of the RO5217790 genome was assessed in a series of tests. Testing of the expression, functionality, characterization and the nucleotide sequences of the genetic inserts as well as the immunoplaquing assays were performed. RO5217790 still has its expected characteristics 3 passages beyond the passage intended for the production of clinical material.

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) ***FR, DE, ES, FI, NL, BE***

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 (**X**) No ()

If yes: **the previous notifier was Transgene (France**)

- Member State of notification
- Notification number B/../../…

- Member State of notification France…
- Notification number :

Study TG4001.06: Afssaps reference: TG01.07.01 // Biosafety Commission references (CGB) : B/FR/01.07.01
Study TG4001.05: Afssaps reference: TG-01.09.02 // Biosafety Commission references (CGB) : B/FR/01.09.02
Study TG4001.07: Afssaps reference: TG04.02.01 // Biosafety Commission references (CGB): B/FR/04.03.02

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

The likelihood of RO5217790 becoming persistent and invasive in natural habitats is very low for the following reasons:

There is no known human poxvirus able to complement MVA (parent of RO5217790) to generate a replication competent virus

No spontaneous reversion of MVA to replication competent vaccinia virus (VV) has ever been documented.

RO5217790 is unable to produce progeny vector particles in primary human cells, in addition, in human studies, RO5217790 appeared to remain localized at the injection site as vector DNA could not be detected by PCR in the urine or blood of patients (n=18). Based on these observations it is considered unlikely that any significant shedding of infectious particles occurs.

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 (.)

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	*Poxviridae / Chordopoxvirinae*
(ii)	genus	Orthopoxvirus…
(iii)	species	Vaccinia Virus
(iv)	subspecies	…
(v)	strain	Modified Vaccinia Virus Ankara
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	MVA

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**)

RO5217790 will be present at the manufacturing facility at Transgene and the distribution depots for Clinical Trials.

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	

MVA is not found in natural ecosystems. It is severely host cell-restricted. It grows well in avian cells and BHK cells, but is unable to propagate in normal human and most other mammalian cells tested.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques

See 5 (b) Identification

(c) Identification techniques

The identity of MVA can be confirmed by PCR. It is based on the presence of MVA deletion II, characteristics encountered only in the MVA strain of vaccinia virus.

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 Europe and in the USA, the manipulation of recombinant MVA or vectors derived from MVA requires Biosafety Level 1 when used in clinical/research and is dependent on the country and biosafety committees.

Examples of classifications:

French biosafety Commission (Commission de Génie Génétique): Class 2

***US NIH:** Biosafety Level 1 recommended*

US ATCC classification: Risk Group 2

***Swiss classification:** Risk Group 1, Biosafety Level 1 recommended*

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

The parental MVA was safely used in over 120,000 individuals for vaccination against smallpox in Germany in the 1970s; MVA was found to be well tolerated. The most frequently reported adverse events reported in patients receiving MVA administered SC have included injection site reactions, headache, fatigue, malaise, and fever.

MVA does not pose a risk for integration or activation of latent proviruses, as the vector resides exclusively in the cytoplasm.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Not relevant as MVA is not found in natural ecosystems. It is severely host cell-restricted. It grows well in avian cells and BHK cells, but is unable to propagate in normal human and most other mammalian cells tested

(b) Generation time in the ecosystem where the release will take place:
Not relevant

(c) Way of reproduction: Sexual .. Asexual ..
Not relevant

(d) Factors affecting reproduction:
Not relevant

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 …
Not relevant

(b) relevant factors affecting survivability:
It has been demonstrated that MVA vectors can be destroyed with bleach [≥ 1.6° Cl i.e. 5g/l of active chlorine] or autoclaving at 105°C for 10 minutes

10. (a) Ways of dissemination
The GMO as the parental MVA remains localized in the cell cytoplasm until the cell has been destroyed. From clinical studies data, no shedding of the vector was observed, the vector is assumed to be localized in the injection site.

(b) Factors affecting dissemination
Not relevant

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

In this product, a recombinant MVA encoding modified E6 and E7 proteins (delE6 and delE7) from HPV will be delivered in the subcutaneous space. There it can transduce cells including dendritic cells and, in the lymph node draining the injection site, which is away from the tolerogenic local milieu of the lesion itself, express and present E6 and E7 epitopes in a context which should allow development of a targeted cell mediated immune response. It is hypothesized that RO5217790 will transduce specialized antigen presenting cells (APCs) that will present the delE6/delE7 antigen epitopes through the MHC class I pathway to CD8+ effector T-cells. In turn, this will initiate a killer T-cell response against the transformed epithelial cells that express the HPV E6/E7 antigen epitopes and will enable the eradication of the cervical lesion.

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
pTG8042

(c) Host range of the vector
Escherichia Coli

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes *(**X**)* No (.)

antibiotic resistance (.)
other, specify
E. Coli UidA marker gene encoding for beta glucuronidase (used as a selection marker for colorimetric assay)

Indication of which antibiotic resistance gene is inserted
Not relevant

(e) Constituent fragments of the vector

RO5217790 is a recombinant attenuated MVA-based viral vector containing DNA sequences coding for the human Interleukin-2 protein (hIL2) and for mutated forms of E6 and E7 proteins from HPV16.

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

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

Co-transfection (MVA and pTG8042) in chicken embryo fibroblasts

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 three donor genes: delE6, delE7, and hIL2. Other elements (secretory and transmembrane sequences) used in the constructs were derived from the measles and rabies viruses and non-transcribed promoter sequences were derived from vaccinia virus,

(b) Source of each constituent part of the insert

The primary donor sequences are the HPV16 E6 and E7 genes (DONOR 1) and the hIL2 gene (DONOR 2). The E6 and E7 coding sequences were derived from HPV16 viral DNA. The hIL2 gene was derived from human peripheral blood mononuclear cells.

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

RO5217790 is an HPV16 targeted immunotherapy derived from a replication defective strain of vaccinia virus (Modified Vaccinia Ankara, MVA) engineered to express modified forms of the HPV16 early proteins E6 and E7(delE6 and delE7) as well as un-modified human interleukin 2 (hIL2).

The HPV E6 and E7 genes of HPV16 were modified by deletion to eliminate the interaction of their encoded proteins with the respective tumor suppressor proteins pRb and p53, while retaining their immunogenicity. The safety and immunogenicity of these two proteins has potentially been further enhanced by use of heterologous (measles and rabies virus) viral sequences to direct the translated proteins away from the nucleus and anchor them on the cell membrane, thus avoiding nuclear localization of the E6 and E7 gene product and limiting their interaction with pRb and p53.

hIL2 is a cytokine that has been shown to be an essential factor in cell-mediated and humoral immune responses . IL2 therapy has been recognized as effective for patients with metastatic renal adenocarcinoma and in metastatic melanoma. Secretion of this cytokine locally produces very low levels in the circulation while presumably augmenting immune activation.

(d) Location of the insert in the host organism

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

Following transduction, the insert remains in the cytoplasm as part of the viral vector genome

(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

The following information relates to the organisms from which transcribed transgenes were derived. Other minor regulatory sequences are present from the parental plasmid but these are not transcribed.

(1). E6 and E7 modified genes (delE6 and delE7) derived from human papillomavirus genotype 16

1. Indicate whether it is a:

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

2. Complete name

(i)	order and/or higher taxon (for animals)	*Papillomaviridae*
(ii)	family name for plants	…
(iii)	genus	*Alphapapillomavirus*
(iv)	species	*Human papillomavirus 16*
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	*HPV16*

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	(.)
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):

In RO5217790, the E6 and E7 genes have been modified to abolish (E7) or to significantly reduce (E6) interactions with the pRb and p53 tumor suppressor genes, respectively. In addition, an in vitro cell transformation assay was found to be negative, thus providing strong support for the assertion that this product is unlikely to have any oncogenic potential.

(2) Secretory and transmembrane anchoring sequences from rabies virus linked to delE7.

Indicate whether it is a:

viroid (.)
RNA virus (X)
DNA virus *(.)*

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

other, specify …

2. Complete name

(j)	order and/or higher taxon (for animals)	*Mononegavirales*
(ii)	family name for plants	Rhabdoviridae
(iii)	genus	*Lyssavirus*
(iv)	species	*Rabies*
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	*Rabies 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:

(c) 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 (**X**.) Not known (.)

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

(3) Secretory and transmembrane anchoring sequences from measles virus linked to delE6.

Indicate whether it is a:

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

2. Complete name

(k)	order and/or higher taxon (for animals)	*Mononegavirales*
(ii)	family name for plants	Paramyxoviridae
(iii)	genus	*Morbillivirus*
(iv)	species	*Measles virus*
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	*Measles 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:

(d) to which of the following organisms:

humans	*(**X**)*
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
(1) *Classification 2 for Human papillomaviruses*
(2) Classification TBC for Measles virus
(3) Classification TBC for rabies virus

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

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

hIL2

1. Indicate whether it is a:

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

2. Complete name

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

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:

(e) 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 (.) No ***(X)***
If yes, specify …

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 …

(f) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No ***(X)*** Not known (.)
Specify …

(g) 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

A genetic stability program was designed to assess the genetic stability of RO5217790 at several steps of the production process: Pre Master Virus Seed 1 (PMVS1), Master Virus Seed (MVS), Final Drug Product (DP) and DP + 3 passages

The genetic stability of the RO5217790 genome was assessed in a series of tests (Annex III - Table3). Testing of the expression, functionality, characterization and the nucleotide sequences of the genetic inserts as well as the immunoplaquing assays were performed. RO5217790 still has its expected characteristics 3 passages beyond the passage intended for the production of clinical material.

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
quantitative Polymerase Chain Reaction (qPCR).

(b) Techniques used to identify the GMO
Genomic structure by restriction enzyme mapping(using at least two restriction enzymes).

F. Information relating to the release

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

The release in this context will be the administration of the product, in a hospital or clinic setting, by subcutaneous injection to patients as a part of a multinational, multicenter clinical trial protocol. Details of the trial design and its objectives are in the attached protocol synopsis (see annex 1). There are no foreseen problems of this release.

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.)

Note: *The complete immunotherapeutic course will be delivered by 3 sub-cutaneous injections in the thigh in a clinical setting and is thus similar to the use of MVA (smallpox irradication).*

If yes, specify …

3. Information concerning the release and the surrounding area

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

In Belgium RO5217790 will only be administered in the following sites:

Universitair Ziekenhuis Brussel ***Dr Philippe De Sutter*** *Laarbeeklaan 101* *1090 Brussel* *BELGIUM*	*U. Z. Antwerpen* ***Dr Wiebren Tjalma*** *Wilrijkstraat 10* *2650 Edegem* *BELGIUM*
Algemeen Ziekenhuis Heilig-Hart ***Dr Gilbert Donders*** *Kliniekstraat 45* *3300 Tienen* *BELGIUM*	*AZ Middelheim* ***Dr Frans Wesling*** *Lindendreef 1* *2020 Antwerpen* *BELGIUM*
U. Z. Gasthuisberg ***Dr Willy Poppe*** *Herestraat 49* *3000 Leuven* *BELGIUM*	*Universitair Ziekenhuis Gent* ***Dr Steven Weyers*** *De Pintelaan 185* *9000 Gent* *BELGIUM*
ULB Hôpital Erasme ***Dr Philippe Simon*** *Route de Lennik 808* *1070 Bruxelles* *BELGIUM*	

(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

The RO5217790 drug product must be prepared under conditions compliant with injectable preparations. All transfers of the preparation must be done observing universal precautions.

For the administration of the study drug, we propose that the patients will stay in a conventional clinic examination room. Patients will be monitored for half an hour after each study drug injection.

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

Not Relevant

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

Not Relevant

4. Method and amount of release

(a) Quantities of GMOs to be released:

Three subcutaneous injections of $5x10^7$ pfu

(b) Duration of the operation:

3 injections 1 week apart on Days 1, 8 and 15.

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

As with all biological agents, "universal precautions" and sterile technique should be used when dosing patients with RO5217790. All transfers of the preparation must be done using a closed container. As the product is administered subcutaneously, the risk of inadvertent release will be extremely low. Should any inadvertent spill occur in the clinics, a standard disinfectant can be used.

Prior to the administration of the product, the suspension of vectors particles must be prepared under aseptic conditions. In addition, the area used to prepare RO5217790 for injection will be decontaminated before and after manipulation with a standard disinfectant (eg, bleach > 1.6₀Cl; ie, 5 g active chlorine per litre of water) based solution.

There should be negligible effects of RO5217790 on persons working appropriately with this GMO. However, if inadvertent exposure to RO5217790 occurs, for example through needle sticks or inhalation, the same direct and indirect effects of RO5217790 would be expected to occur (ie, immune response to MVA, HPV E6, E7). These are unlikely to cause any adverse effects.

5. Short description of average environmental conditions (weather, temperature, etc.)
Not Relevant

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.

Since 1999 this product has been released in the context of clinical trials on 7 prior occasions, 5 of which involved clinical sites in either France or Switzerland. A total of 111 patients have been treated with at least 1 injection. RO5217790 has been found to be generally safe and well tolerated during these trials with the main adverse event reported being injection site reactions.

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)	Animalia…
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
Local expression of hIL2, modified E6 and modified E7 (delE6 and delE7) gene products.

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

There is minimal potential for gene transfer to other species under the proposed release of the GMO. As mentioned above, the GMO will be released in a conventional clinic examination room and is unlikely to come in contact with other animal species. In order for the viral genes encoded by RO527790, including delE6, delE7, and hIL2, to transfer into the genome of other species of poxviruses, susceptible cells would need to be simultaneously infected with pox virus and transduced by vector which is extremely unlikely.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No ***(X)*** Not known (.)
Give details
No selective advantage or disadvantage has been conferred to RO5217790, and the parental organism (MVA) is not endemic in the human population.

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

RO5217790 is not predicted to interact with non-target organisms because of its highly restricted host range and because of the manner of its proposed release. In the unlikely event of inadvertent administration to non-target organisms further spread would be unlikely as several studies have demonstrated that MVA is non-virulent in immunocompetent and immunodeficient laboratory animals and in primary human cell cultures.

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

Not 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 …

7. Likelihood of genetic exchange in vivo

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

(b) from other organisms to the GMO:
…

(d) likely consequences of gene transfer:
…

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.):
…Not applicable

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
…Not applicable

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Monitoring of the direct and indirect effects of the GMO on patients will be achieved using the following clinical assessments: physical examinations, ECG, vital signs (heart rate and blood pressure), adverse event reporting, assessment of injection site reactions, cervical cytology, histology and colposcopy and immunological assessments. Every effort will be made to follow all patients for 2.5 years after their injections.

2. Methods for monitoring ecosystem effects
… Not applicable

No viral shedding was shown in humans injected with the GMO and no significant dissemination of the GMO outside the injection site was observed in animal studies providing evidence for the non spreading character of the GMO which appears to remain localized to the injection site.

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

Not applicable as RO5217790 is not predicted to interact with non-target organisms because of its highly restricted host range, the manner of its proposed release and the expected transient nature of its gene expression.

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

All treated patients will be monitored as described in section H1 above.

5. Duration of the monitoring

According to the Protocol, i.e. 2,5 years following the first administration. The initial follow-up period following treatment is 6 months with 2 years of follow-up after surgical excision

6. Frequency of the monitoring

Monitoring visits planned on Days 7, 15 and 29 after treatment and 3, 6, 12, 18, 24 and 30 months post treatment (follow-up).

I. Information on post-release and waste treatment

1. Post-release treatment of the site
The place where the product will be prepared for injection will be decontaminated before and after the manipulation with a standard disinfectant based solution.

Following the patient's discharge home, the room and the toilets will be cleaned in a standard way using a standard disinfectant based solution for cleaning the surfaces and floor.

2. Post-release treatment of the GMOs
3. (a) Type and amount of waste generated *See below*
3. (b) Treatment of waste *See below*

Material in contact with recombinant MVA viral vectors such as RO5217790 must be considered as contaminated by infectious material. It must be stored in appropriate biohazard containers and decontaminated prior to disposal. Decontamination and disposal of all contaminated materials and unused or partially used product will occur at a central destruction facility as directed by the sponsor.

J. Information on emergency response plans

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

A detailed procedure for the preparation of the product RO5217790 to be injected will be provided to all personnel involved in handling of the product and posted in the preparation room. A technical sheet detailing the procedure for injection, the conditions of waste disposal and the procedure to follow in case of accidental spillage will also be posted in the room.

All transfers of the preparation must be done using a closed container. Prior to the administration of the product, the product must be prepared under conditions compliant with injectable preparations.

Furthermore, the staff will follow the standard hospital policy recommended for the manipulation of the live virus vaccines.

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

In case of an incident with the use of the RO5217790, it is recommended to treat as indicated below.

Accidental Shedding
In the case of accidental shedding of RO5217790, every contaminated surface area should be treated with a standard disinfectant based solution active on RO5217790 (eg, bleach > 1.6°Cl; i.e., 5 g active chlorine per liter of water). Leave in contact for at least 30 minutes.

Skin Contamination
In case of skin contamination with RO5217790, wash and disinfect the affected area immediately with hydrogen peroxide (3%) and then wash thoroughly with soap and water. Remove contaminated clothes, which must be treated as biohazardous material.

Needle Stick Injury
In case of needle stick injury with RO5217790, wash and disinfect the affected area immediately with hydrogen peroxide (3%), then wash thoroughly with soap and water and cover with a sterile gauze dressing, which should be appropriately discarded when removed. The injured person should be seen by a physician and be closely followed for a period of at least 2 weeks.

Eye Contamination
In case of eye contamination with RO5217790, rinse the affected eye immediately for 15 minutes with tap water, making the water flow laterally into the affected eye. If only one eye is affected, avoid contaminating the other one (the affected eye must be below the other one). Maintain the eyelids open and ask the affected individual to look up, down, and sideways, thus moving the eyeball. If available, instill one drop of a solution of trifluridine 1% or ribavirin. The injured person should undergo an ophthalmological examination and follow-up as soon as possible.

Ingestion
In case of ingestion with RO5217790, do not induce vomiting and consult a doctor immediately. The person should be closely followed by a physician for a period of at least 2 weeks.

Inhalation
This product is an aqueous solution. In case of aerosol inhalation with RO5217790, seek immediate medical attention.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
Not applicable

4. Plans for protecting human health and the environment in the event of an undesirable effect
…
The probability of propagation is very low since, as mentioned above, RO5217790 is replication defective. Thus, any propagation is unexpected. In order to generate a vector capable of propagation, a complementing propagation-competent pox virus would be necessary. This event is unlikely as there is no endemic wild pox virus is present in the

human population. Because MVA and hence RO5217790 differs from smallpox at multiple loci, it is unlikely that several independent mutations could occur, including restoration of the deleted regions of the genome, that would restore this genome to that of the smallpox virus or even a propagation competent variation of MVA. This phenomenon has never been observed during smallpox vaccination in humans, and a mechanism able to cause and select for an event of such a magnitude is hardly conceivable. Furthermore, studies have shown that repair of multiple genes is required to fully restore the ability of MVA to replicate efficiently in human cells. This is consistent with the inability to detect spontaneous revertants and supports the safety of MVA as a gene transfer vector.

ANNEX 1:

SYNOPSIS OF PROTOCOL NUMBER NV25025B

TITLE	A randomized, double blind, placebo controlled, parallel group, multicenter study of the safety and response rate of 3 subcutaneously administered doses of 5 x 10^7 pfu RO5217790 in patients with high grade cervical intraepithelial neoplasia grade 2 or 3 associated with High Risk HPV infection
SPONSOR	Hoffmann-La Roche CLINICAL PHASE 2
INDICATION	Treatment of patients with CIN2/3 associated with High-Risk (HR) HPV infection
OBJECTIVES	*To assess the efficacy of RO5217790 compared to placebo:* *Primary Objective* • Histologic **resolution** at Month 6 Secondary Objectives • Viral clearance by DNA and mRNA at Month 3 and Month 6 • Cellular and humoral immunological response following treatment • Safety and tolerability
TRIAL DESIGN	*A 2.5 year prospective, randomized, multicenter, international, double-blinded, 2-arm, parallel group, placebo-controlled trial*
NUMBER OF SUBJECTS	*200 patients are expected to be randomized 2:1 to RO5217790 or placebo*
TARGET POPULATION	*Principle inclusion criteria:* 1. Are ≥ 18 years old 2. Have a diagnosis within 2 months prior to the first dose of RO5217790 of CIN 2/3 confirmed by colposcopy-directed punch biopsy; patients must have at least 1 quadrant of residual CIN2/3 disease remaining after biopsy. Entry to the trial will be allowed based on the local assessment of this criterion; however, CIN 2/3 diagnosis will have to be confirmed by the central pathologist for the purposes of analyzing the study 3. Have satisfactory colposcopy, i.e. the entire aceto-white or disease area as well as the entire squamocolumnar junction visualized by

	colposcopy 4. Have detection at screening of a single or multiple HR-HPV infection by analysis of liquid based cytology (LBC) material on the Roche Linear Array assay **consistent with any** of the trial strata as specified in section 3.1 *Principle exclusion criteria:* 1. Have colposcopically visible CIN2/3 disease extending over more than 2 quadrants 2. Have any anatomical condition of the cervix, including that resulting from previous cervical surgery, congenital malformation or other condition, that would interfere with a complete evaluation of the transformation zone and surveillance of CIN. If an ECC is performed, and the endocervical curettings reveal CIN, patients are eligible as long as the endocervical lesion is directly extending from the primary lesion and is colposcopically visible in its entirety 3. Have vulvar (VIN) or vaginal (VAIN) intraepithelial neoplasia 4. Have atypical endometrial or glandular cells or evidence of carcinoma on biopsy 5. Have a serious, concomitant disorder, including active systemic infection requiring treatment 6. Have a prior history of or current malignancy other than adequately treated skin cancer (squamous cell cancer or basal cell carcinoma), unless the history of skin cancer is at the site of study treatment administration 7. Have a proven or suspected immunosuppressive disorder or autoimmune disease 8. Have any significant cardiac, hepatic or renal disease 9. Have active viral infections including HIV, HCV, HBV, CMV, and EBV within 30 days of receiving study treatment. Mild viral infections such as HSV-1 or common cold are not excluded.
LENGTH OF STUDY	*Screening period:* 2 months (maximum) *Treatment and follow-up:* 2.5 years
END OF STUDY	*The definition of end of trial for this study is Last Patient Last Visit (LPLV) for the follow-up period, i.e. the last visit is the Month 30 visit.*
INVESTIGATIONAL MEDICAL PRODUCT(S)	RO5217790 is a viral vector containing the MVA genome with genes inserted that code for three

DOSE/ ROUTE/ REGIMEN	proteins: the human cytokine IL-2, and modified forms of the Human Papilloma Virus 16 (HPV-16) proteins E6 and E7 *Dose:* 5 x 10^7 pfu/injection *Route & Regimen:* subcutaneous injection 3 x q1week
COMPARATOR "DRUG" (or STANDARD OF CARE) DOSE/ ROUTE/ REGIMEN	Placebo (same buffer solution to be used in active arm excluding RO5217790) with same injection volume, route and regimen as for active treatment.
ASSESSMENTS OF: - EFFICACY	*Efficacy of RO5217790 will be based on the following measures:* *1. Histological evaluation of cervical tissue excised at Month 6 (see appendices 4, 5 and 6 for details)* *2.* Roche Linear Array (LA-HPV) assay *will be used for HPV genotyping at baseline and to assess viral clearance.* *3.* NucliSENS EasyQ® HPV assay *will be used to assess the presence at baseline and clearance of HPV 16, 18, 31, 33 and 45 mRNA for E6 and E7.*
- SAFETY	*Safety will be assessed by physical examinations, ECG, vital signs, adverse event reporting, assessment of injection site reactions, cytology and colposcopy.*
- IMMUNOLOGY	• Cellular immune responses (e.g. IFN-γ ELISPOT) to RO5217790 • Humoral immune responses to RO5217790 (e.g. anti-E6, E7, hIL-2 and MVA)
- QUALITY OF LIFE (QOL)	• Short form-36 questionnaire • 100mm Visual analog pain scale

PROCEDURES (summary

See schedule of assessments Section 5.0.

STATISTICAL ANALYSES:

An interim analysis will be performed on the data to Month 6 from a subset of the 200 patients randomized.

The primary analysis of efficacy for the study will be primarily exploratory and will be focused on estimating the individual histological response rates at 6 months for RO5217790 and placebo and the relative efficacy of RO5217790 to placebo as measured by the ratio of response rates.

Secondary analyses comparing treatments with respect to viral clearance and time to viral clearance will be based on regressions models, logistic and Cox, with factors as for the primary analysis.

For the evaluation of long-term efficacy beyond 6 months, comparing treatments will be based on regression models as above.

The following null and alternative hypothesis will be tested:

H_0: The treatment response rate is less than 60% OR the treatment response rate is less than double the control response rate.

vs

H_1: The treatment response rate is at least 60% AND the treatment response rate at least double the control response rate.

Rejection of the null hypothesis and acceptance of the alternative hypothesis will support a decision to continue clinical development of RO5217790 in phase III.

All safety parameters will be summarized and presented in tables based on the safety population.

All immunological parameters will be summarized and presented in tables.