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

Clinical study title:

A Phase I/II Open Label Clinical Trial Assessing Safety and Efficacy of Intravesical Instillation of the Recombinant BCG VPM1002BC in Patients with Recurrent Non-Muscle Invasive Bladder Cancer after Standard BCG Therapy

Clinical Study Code:

SAKK 06/14

Version	Date
03	04.08.2017

Investigational Product: VPM1002BC containing rBCGΔureC::Hly^{+}

EUDRACT number: 2014-005330-58

Released by:

Sponsor: Swiss Group for Clinical Cancer Research (SAKK) Effingerstr. 33, 3008 Bern, Switzerland

European Organisation for Research and Treatment of Cancer (EORTC) is the European Sponsor Representative.

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

1. General information

1. Details of notification

(a)	Member State of notification	The Netherlands
(b)	Notification number	B/NL/17/004
(c)	Date of acknowledgement of notification	23/08/2017
(d)	Title of the project	A Phase I/II Open Label Clinical Trial Assessing Safety and Efficacy of Intravesical Instillation of the Recombinant BCG VPM1002BC in Patients with Recurrent Non-Muscle Invasive Bladder Cancer after Standard BCG Therapy
(e)	Proposed period of release	From approval of the trial until 31/03/2022

2. Notifier

Name of institution or company:
Sponsor: Swiss Group for Clinical Cancer Research (SAKK) Effingerstr. 33, 3008 Bern, Switzerland

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

(b) Identity of the GMO (genus and species)
specify phylum, class

Bacteria; Actinobacteria; Actinomycetales; Corynebacterineae; Mycobacteriaceae; Mycobacterium; M. bovis; Strain BCG subtype Prague (recombinant Bacille Calmette Guérin (rBCG)).

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

There is genetic variety in the mycobacteria family. There are no other mycobacterial typical factors known except the general mutational factors like multiple passages and radioactivity and/or chemicals (1). During the scientific development, several passages of the strain VPM1002BC were performed and genetic instability was never observed. To the contrary, it was proven that all strains, starting with parental seed up to the GMP material of VPM1002BC, have a 100% homology in the inserted sequence (see Part A section A3.3). Sequencing data are available for various batch releases of the product, which reveal high stability of the genetic construct.

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)

DE

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

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

If yes:

- Member State of notification DE
- Notification number 2686/01

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:

- Member State of notification CH
- Notification number Swissmedic 2014GT1011

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

The Netherlands Commission on Genetic Modification (COGEM) classified rBCG:ΔureC::hly+ (VPM1002) as an GMO that can be handled at the ML-I / DM-I level without endangering the safety of humans or the environment according to § Dutch law (CGM/070402-05).

In reference to Directive 93/88/EEC (on the protection of workers from risks related to exposure to biological agents at work), *M. bovis* BCG is classified as biosafety level 1, i.e. not pathogenic to humans.

VPM1002BC was originally developed containing an antibiotic resistance against hygromycin for selection purposes. The full name of this original rBCG was live recombinant *M. bovis* BCGΔureC::Hly+::Hyg+ (genetic background "Danish, subtype Prague"). This strain was used as the IMP for the first three clinical trials performed with VPM1002 (Hyg+). In 2011, the hygromycin resistant gene was successfully removed from the original VPM1002 (Hyg+) strain and a VPM1002 (Hyg-) vaccine without hygromycin resistance gene, but otherwise identical genetic modification, was produced.

As of note, during the development several terms were used for identical material. In the context of the present document the term VPM1002BC always relates to the strain rBCGΔureC::Hly^{+}.

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

Bacteria; Actinobacteria; Actinomycetales; Corynebacterineae; Mycobacteriaceae

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	*Mycobacterium*
(iii)	species	*M. bovis*
(iv)	subspecies	…
(v)	strain	BCG subtype Prague
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	BCG

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 (**X**) No (.)
VPM1002BC is stocked at Bilthoven Biologicals, Anthonie van Leeuwenhoeklaan 9-13, 9721MA, The Netherlands

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 parental organism of VPM1002BC, *M. bovis* BCG, has no natural habitat. It is a pure laboratory strain, which is not able to survive in the natural environment.

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

Not applicable

5. (a) Detection techniques
PCR

(b) Identification techniques
PCR, Sequencing

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

BCG is a marketed, authorised medicinal product with a well-known safety profile (e.g. information derives from the Summary of Product Characteristics of BCG-medac, national version: 08/2014).

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

Pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonise other organisms:

Due to the fact that only one truncated gene of *L. monocytogenes* is used, we omit details on the parameters mentioned in headline (d) of the above mentioned document.

M. bovis BCG is a marketed, authorised medicinal product with a well-known safety profile (e.g. information derives from the Summary of Product Characteristics of BCG-medac, national version: 08/2014).

Pathogenicity of *M. bovis* BCG: non pathogenic.

Infectivity: Nonclinical studies with intravesical applied VPM1002BC have demonstrated that VPM1002BC is eliminated within a short time frame, unlike common BCG. This has been substantiated by excretion data from the phase I part of the clinical trial SAKK 06/14. 24 hours after intravesical instillation, the bacterium was no longer detectable in the urine of

the patients (see Part A section A2.16 and A5.1). The reason for this behaviour is believed to be the induction of apoptosis in cells infected with VPM1002BC (2).

Upon intravesical instillation into the bladder the majority of *M. bovis* BCG is voided during the first micturitions following instillation. Only a small portion of the instilled dose of *M. bovis* BCG persists for a long period in the patients.

Toxigenicity: VPM1002BC preclinical proof of concept and safety have been analysed in 19 studies comprising approximately 600 animals for the two strains; VPM1002 (Hyg+) and VPM1002BC. The safety of VPM1002 (Hyg+) was shown in three species (mice, guinea pig, (newborn) rabbits). As supportive data, rhesus monkeys were tested with scientific research material of rBCGΔureC::Hly+::Hyg+. First, IFNγ knockout mice were used. These animals serve as a safety model for estimating the *M. bovis* BCG-infection risk because in humans, *M. bovis* BCG infection is correlated with deficiencies in the IFNγ signalling pathway. Further safety studies with VPM1002 (Hyg+) were performed in SCID-mice which lack an adaptive immune system. None of the animal study findings argues against the use of this drug in humans (see Part A section A5.1).

VPM1002 (Hyg+)-specific human data are available from three clinical trials (3). Two studies in healthy adolescent volunteers and one phase IIa study in healthy newborn infants were performed. Combining the clinical safety data with the preclinical safety data, we conclude that VPM1002 (Hyg+) is better than *M. bovis* BCG in terms of safety.

Further experience having used the same strategy of genetic modification was published by Grode (2;4).

Virulence: *M. bovis* BCG is not virulent. It is tested for virulence according to European Pharmacopeia.

Allergenicity: *M. bovis* BCG is not known for inducing allergic reactions. To the contrary, *M. bovis* BCG is known of preventing immunised individuals of gaining allergies (5;6).

Carrier of pathogen: *M. bovis* BCG does not carry any pathogens.

Possible activation: not applicable.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Being a pure laboratory strain, *M. bovis* BCG, is not able to survive in the natural environment.

(b) Generation time in the ecosystem where the release will take place:
Being a pure laboratory strain, *M. bovis* BCG, is not able to survive in the natural environment.

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

(c) Factors affecting reproduction:
Being a pure laboratory strain, *M. bovis* BCG, is not able to survive in the natural environment. The generation time of VPM1002BC under optimal culture conditions is 8 hours.

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:
M. bovis and *M. bovis* BCG are known to be highly UV sensitive and do not form survival structures such as spores. There is no significant replication of *M. bovis* and *M. bovis* BCG outside its natural hosts, in the case of *M. bovis*, or special culture media, in the case of *M. bovis* BCG.
There is no information that *M. bovis* BCG or VPM1002BC might be more resistant, or might better survive in the natural environment than wild type *M. bovis*. It is unlikely that the introduction of the *L. monocytogenes* listeriolysin into VPM1002BC has changed its non-resistance in the natural environment. Therefore, a similar or shorter survival time of VPM1002BC if it should be released into the natural environment compared to the wild type *M. bovis* can be predicted. Moreover, spread of the vaccine strain *M. bovis* BCG into the environment has never been detected. No natural habitats of *M. bovis* BCG are known. An extensive literature search found no reports.

10. (a) Ways of dissemination

Multiplication or dissemination of *M. bovis* BCG in the environment has neither been a concern nor ever been detected. An extensive literature search found no reports. There is no information that VPM1002BC might be more resistant, or might better survive in the natural environment than *M. bovis* BCG or wild type *M. bovis*. It is unlikely that the introduction of the *L. monocytogenes* listeriolysin into VPM1002BC has changed its non-resistance in the natural environment. Therefore, we presume a short survival time of VPM1002BC if it should be released into the natural environment.

(b) Factors affecting dissemination

Mycobacteria are known to be UV-sensitive. Multiplication or dissemination of *M. bovis* BCG in the environment has neither been a concern nor ever been detected. An extensive literature search found no reports.

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/../../…
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	(X)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
The goal of the genetic modification was to knock out the urease C (*ureC*) gene and site-specifically introduce the listeriolysin gene into *M. bovis* BCG genome.

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

To generate VPM1002BC, an *E. coli* pVEP2003 plasmid was used as a shuttle vector. Such vectors from apathogenic *E. coli* strains are commonly used for cloning.

(c) Host range of the vector
The plasmid is a high copy number plasmid in *E.coli*.

(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
Hygromycin

(e) Constituent fragments of the vector

The plasmid carries an *E. coli* origin of replication but no mycobacterial origin of replication, which makes this plasmid self-limiting in mycobacteria. In addition, the plasmid contains an expression cassette carrying listeriolysin and an expression cassette carrying hygromycin resistance sequence. These sequences are located between UreC-P1-P2 and UreC P3-P4 regions of the plasmid and have been integrated into the BCG genome by homologous recombination. The plasmid carries no transposable elements.

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

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

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 construct inserted into the genome of *M. bovis* BCG contains the mature peptide sequence of listeriolysin (hly) from *L. Monocytogenes* under the regulation of the mycobacterial promoter sequence from the heat shock protein 65 (hsp65) fused to the leading sequences of the Antigen85B (Ag85B).

(b) Source of each constituent part of the insert
Please see (a).

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

Listeriolysin (*hly*) was used as a pore forming protein active in the phagosome after endocytic uptake of a bacterium, enabling the bacterium to move into the cytosol. The leading sequence of the Antigen85B was fused to *hly* sequence to allow the secretion of listeriolysin.

(a) Location of the insert in the host organism

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

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

other, specify …

2. Complete name

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

Bacteria; *Firmicutes; Bacilli; Bacillales; Listeriaceae; Listeria; L. Monocytogenes* renamed by Pirie 1940

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 (.)

Studies suggest that up to 10% of human gastrointestinal tracts may be colonized by *L. monocytogenes. L. monocytogenes* is a significant cause of neonatal sepsis and meningitis. Listeriosis in adults is normally associated with patients living with compromised immune systems, such as individuals taking immunosuppressant drugs and corticosteroids for malignancies or organ transplants, and those with HIV infection. Clinical diseases due to *L. monocytogenes* are most frequently recognized as meningo-encephalitis in ruminants. Soft cheeses made from non-pasteurised milk may be a source for infections with *L. monocytogenes*.

If yes, specify the following:

(a) 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):

Due to the fact that only one truncated gene of *L. monocytogenes* is used we omit details on the parameters mentioned in headline (b).

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

Listeria monocytogenes is classified as risk group 2 organism according to Directive 90/679/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…

(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 …

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

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

Specify…

(c) 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

In VPM1002BC the genetic modification is of genomic and not of exosomal plasmidic origin. Hence, the genetic stability is even higher than that observed by Horwitz (1). As of note, the extraordinary high genetic stability of VPM1002BC is an obstacle for further genetic modification for the scientific development of second generation VPM1002-derivatives.

Furthermore, chromosomal integration of distinct genetic material to VPM1002BC or *M. bovis* BCG is a highly complicated lab-procedure and therefore highly improbable to occur spontaneously.

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)

Annex III A, point II(A)(11)(d)

Pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonise other organisms:

M. bovis BCG is a marketed, authorised medicinal product with a well-known safety profile (e.g. information derives from the Summary of Product Characteristics of BCG-medac, national version: 08/2014).

Pathogenicity of *M. bovis* BCG: non pathogenic.

Infectivity: Nonclinical studies with intravesical applied VPM1002BC have demonstrated that VPM1002BC is eliminated within a short time frame, unlike common BCG. This has been substantiated by excretion data from the phase I part of the clinical trial SAKK 06/14. 24 hours after intravesical instillation, the bacterium was no longer detectable in the urine of the patients (see Part A section A2.16 and A5.1). The reason for this behaviour is believed to be the induction of apoptosis in cells infected with VPM1002BC (2).

Upon intravesical instillation into the bladder the majority of *M. bovis* BCG is voided during the first micturitions following instillation. Only a small portion of the instilled dose of *M. bovis* BCG persists for a long period in the patients.

Toxigenicity: VPM1002BC preclinical proof of concept and safety have been analysed in 19 studies comprising approximately 600 animals for the two strains; VPM1002 (Hyg+) and VPM1002BC. The safety of VPM1002 (Hyg+) was shown in three species (mice, guinea pig, (newborn) rabbits). As supportive data, rhesus monkeys were tested with scientific research material of rBCGΔureC::Hly+::Hyg+. First, IFNγ knockout mice were used. These animals serve as a safety model for estimating the BCG-infection risk because in humans, *M. bovis* BCG infection is correlated with deficiencies in the IFNγ signalling pathway. Further safety studies with VPM1002 (Hyg+) were performed in SCID-mice, which lack an adaptive immune

system. None of the animal study findings argues against the use of this drug in humans (see Part A section A5.1).

VPM1002 (Hyg+)-specific human data are available from three clinical trials (3). Two studies in healthy adolescent volunteers and one phase IIa study in healthy newborn infants were performed. Combining the clinical safety data with the preclinical safety data, we conclude that VPM1002 (Hyg+) is better than *M. bovis* BCG in terms of safety (see Part A section A5.1.

Further experience having used the same strategy of genetic modification was published by Grode (2;7).

Virulence: *M. bovis* BCG is not virulent. It is tested for virulence according to European Pharmacopeia.

Allergenicity: *M. bovis* BCG is not known for inducing allergic reactions. To the contrary, *M. bovis* BCG is known of preventing immunised individuals of gaining allergies (5;6).

Carrier of pathogen: *M. bovis* BCG does not carry any pathogens.
Possible activation: not applicable

Annex III Point II(C)(2)(i) is discussed in detail in section A5.2 of Part A.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Techniques used to detect VPM1002BC in the environment are PCR fingerprint and sequencing. For a brief description, see section A5.12 in Part A.

(b) Techniques used to identify the GMO

Techniques used to identify VPM1002BC are PCR and sequencing, see also section A3.3 of Part A.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
The goal of this study is to develop development of VPM1002BC as a safe, well-tolerated and efficacious immunotherapy against non-muscle invasive bladder cancer. VPM1002BC is to replace the currently used *M. bovis* BCG immunotherapy. The new therapy should be at least as potent as the current strain and should have a better safety profile (2;3;8). VPM1002BC is formulated as live lyophilised bacteria to be resuspended before intravesical instillation. VPM1002 (Hyg+)-specific human data are available from three clinical trials (3). Two studies in healthy adolescent volunteers and one phase II study in healthy newborn infants were performed. Combining the clinical safety data with the preclinical safety data, we conclude that VPM1002BC is better than BCG in terms of safety (For details see section A5.1 in Part A).
In the current trial (SAKK 06/14), VPM1002BC is applied for the first time in the context of non-muscle invasive bladder cancer by intravesical instillation into the bladder. This trial is performed as a combined phase I/ II trial. Primary objective of

the phase I part of the clinical trial: to determine safety, tolerability and the recommended phase II dose (RP2D) of intravesical VPM1002BC. The primary objective of phase II part of the clinical trial: to investigate the efficacy (recurrence-free rate in the bladder at 60 weeks), safety, tolerability and immunogenicity of intravesical VPM1002BC instillations. Secondary objectives: time to recurrence in the bladder, time to recurrence, time to progression, overall survival, adverse events, tolerability, quality of Life. Exploratory endpoints: to investigate immunogenicity of VPM1002BC.

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 (.)

Bein pure laboratory strains, *M. bovis* BCG and VPM1002BC do not have natural habitats and are not able to survive in the natural environment.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
VPM1002BC will be applied to patients at clinical sites in Switzerland (phase I and phase II) as well as in clinical sites(only phase II) in Germany, and one clinical trial site in the Netherlands (Netherlands Cancer Institute, Antoni van Leuwenhoek Hospital). Thereafter patients will be monitored on an outpatient basis in these clinics.

(b) Size of the site (m^2):
Location for release of the GMO is the treatment room at the clinical ward.

(i) actual release site (m^2): Not applicable
(ii) wider release site (m^2): Not applicable

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

VPM1002BC will be instilled into the bladder of the patients. In order to prevent release of VPM1002BC from patients, they will be treated as laid down in chapter I.1. of this document (post-release treatment of the location) and will be monitored on an out-patient basis. They will have usual contacts to other humans or biota. For 1 week after instillation, patients should void while seated to avoid splashing of urine. Urine voided during this time should be disinfected with chlorine tablets (e.g. Javel-Tabs containing Sodium-Dichlorisocyanurate Dihydrate from Steinfels Cleaning Systems) according to the manufacturer's protocol. The patients will be instructed to add two tablets into the toilet bowl before urinating, and after urination to wait 15 min until flushing.

Special precautions will be taken at the first voiding after each VPM1001BC instillation, as most of VPM1002BC is excreted in the urine at this time point. The first voiding will take place in the hospital. Two Javel-Tabs will be added to the toilet bowl 15 min before toilet use, then the toilet will be flushed. Afterwards the toilet can be used as indicated above (after adding again 2 Javel-Tabs). Alternatively, the patient will void in a urinal bottle containing two Javel-Tabs. After 15 min the urine will be disposed.

(b) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Please see previous section.

4. Method and amount of release

(a) Quantities of GMOs to be released:
In detail, each subject will receive a maximum of 15 doses of VPM1002BC, administered by intravesical instillation at dose level 1 , determined as the recommended phase II dose in the phase I part of the clinical trial.

Dosage group	Planned dose levels
Dose level 1	1-19.2 x10E8 CFUs (colony forming units)/50ml
Dose level -1	1-19.2 x10E7 CFUs/46.4ml

(b) Duration of the operation:

2015: first patient in

Q2 2019: end of trial therapy

Q3-Q4 2022: last patient, last visit

In phase I, 6 patients (3+3 dose de-escalation design) were treated with VPM1002BC. In phase II, 39 patients will be treated with VPM1002BC. The patients in phase I who have been treated with the recommended phase II dose (RP2D), will contribute to the number of patients in phase II.

Each patient will receive initiation therapy of 6 instillations of VPM1002BC at weekly intervals plus maintenance therapy of 3 instillations at weekly intervals at months 3, 6, and 12.

(c) Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of the release
Details are described in section A4.2 of Part A: VPM1002BC is re-suspended in 50 ml of diluent and applied into the bladder w/o pressure by holding the instillation bag 15 cm above the bladder level after sterile transurethral insertion of a self-lubricating Charrière 14 Catheter.

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

The study medication is reconstituted by the use of a closed drug transfer system and the instillation must be performed within 3 hours. The BD PhaSeal™ Closed Drug Transfer system will be used (see BD PhaSeal™ System Basic Instructions for details, annex 9 of Part A). The administration site of the GMO is the bladder of the treated patients.

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.

VPM1002 (Hyg+)-specific human data are available from three clinical trials (3). Two studies in healthy adolescent volunteers and one phase IIa study in healthy newborn infants were performed. Combining the clinical safety data with the preclinical safety data, we conclude that VPM1002 (Hyg+) is better than *M. bovis* BCG in terms of safety. (For details see section A5.1 in Part A).

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	…

VPM1002BC will be used in humans, in particular it will be instilled into the bladder of bladder cancer patients. It will target bladder cancer tumour cells.

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

VPM1002BC shall induce immunogenicity directed against the bladder tumour in the patients. For details regarding the mode of action, refer to section A1.3 in Part A.
VPM1002BC is a live recombinant *M. bovis* BCG. It was generated in order to direct mycobacterial antigens to the MHC I pathway. Antigen processing through the MHC I pathway is a prerequisite for improvement of CD8 cytotoxic T cell stimulation (9). The strain secretes listeriolysin (*hly*) of *L. monocytogenes*. It enables VPM1002BC to escape from the phagosome of host cells by perforating the membrane of the phagosome. Inactivation of the urease C gene was necessary to ensure an acidic

phagosomal pH for optimal listeriolysin activity. Perforation promotes antigen translocation into the cytoplasm and facilitates cross-priming through increased apoptosis (2). This process mimics the immune induction of *M. tuberculosis* very effectively (10).
The mode of action is expected to result in an efficacious and well-tolerated immunotherapy against non-muscle invasive bladder cancer, which should be at least as potent as and safer than the currently used classical BCG strain.

3. Any other potentially significant interactions with other organisms in the environment
The target organism for VPM1002BC in the context of the clinical trial is the enrolled patient suffering from recurrent non-muscle invasive bladder cancer, who will be monitored on an outpatient basis after treatment. VPM1002BC has been shown to be non-pathogenic in rodents and non-human primates. It must be noted in this context that there are no natural habitats or reservoirs known for *M. bovis* BCG, and that multiplication or dissemination of *M. bovis* BCG in the environment has neither been a concern nor ever been detected. An extensive literature search found no reports. See also chapter H.1 of this notification.

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

Regarding environmental aspects, VPM1002BC does not have any competitive advantage with the unmodified *M. bovis* BCG or wild-type *M. bovis*. We expect a disadvantage for survival of VPM1002BC in organisms due to our pre-clinical evaluation. Nonclinical studies with intravesical applied VPM1002BC have demonstrated that VPM1002BC is eliminated within a short time frame, unlike common BCG. This has been substantiated by excretion data from the phase I part of the clinical trial SAKK 06/14. 24 hours after intravesical instillation, the bacterium was no longer detectable in the urine of the patients (see Part A section A2.16 and A5.1). The reason for this behaviour is believed to be the induction of apoptosis in cells infected with VPM1002BC (2).

Clinically, VPM1002BC has been designed to replace the currently used *M. bovis* BCG in medicine. VPM1002BC should be at least as potent as the current strain but should have a better safety profile.

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

Treatment will be performed at the clinical sites. Patients will stay at the clinics for at least 4 hours. Patients will be monitored on an out-patient basis. They will have usual contacts to other humans or biota.

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	…

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:

(a) and (b) Brosch et al. (11) analysed the whole genome of several mycobacteria and observed no evidence of any horizontal gene transfer. This was confirmed also for a genetically modified *M. bovis* BCG (1) with a genetical modification of plasmidic origin.

In VPM1002BC the genetic modification is of genomic and not of exosomal plasmidic origin. Hence, the genetic stability is even higher than that observed by Horwitz (1). As of note, the extraordinary high genetic stability of VPM1002BC is an obstacle for further genetic modification for the scientific development of second generation VPM1002-derivatives.

Regarding genetic stability see chapter 1.3.(c) of this document. Furthermore, chromosomal integration of distinct genetic material to VPM1002BC or *M. bovis* BCG is a highly complicated lab-procedure and therefore highly improbable to occur spontaneously.

(a) likely consequences of gene transfer:
Gene transfer is not likely to 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.):

Excessive population increase is not anticipated for VPM1002BC due to the extremely slow cell doubling time and the preclinical *in vivo*-data (see see Part A section 5.1). There are no natural habitats or reservoirs known for *M. bovis* BCG. Multiplication or dissemination of *M. bovis* BCG in the environment has neither been a concern nor ever been detected. An extensive literature search found no reports.

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

VPM1002BC does probably not interact with environmental processes because its environmental life span is too short (please see B.8.(c) of this document).

H. Information relating to monitoring

1. Methods for monitoring the GMOs

We have specific techniques available to discriminate between environmental mycobacteria and VPM1002BC, in case needed.

In case of a known contact to the patient and possible transmission to other persons or objects, the subjects are asked to inform the clinical site about this event. The subjects are asked to report to the site any signs or symptoms reported by a contact person that resemble symptoms of an adverse drug reaction against the immunotherapy. A supervision of the contact persons reporting these symptoms by suitable medical assessments is intended, provided the person in question agrees to this procedure. If the medical supervision confirms the reported symptoms, a further analysis by suitable detection methods (PCR analysis) is intended.

In an earlier clinical trial using VPM1002 (Hyg+) as a tuberculosis vaccine by intradermal injection, samples were collected from 9 subjects treated with three escalating doses of VPM1002 (Hyg+), in order to collect data on possible routes of VPM1002 (Hyg+) transmission into the environment. The blood, urine, stool, and saliva of volunteers were analysed for traces of vaccine using a validated PCR method detecting unique genomic DNA regions of VPM1002 (Hyg+). Samples were collected prior to vaccination at baseline and on day 11 and after 6 months. DNA from VPM1002 (Hyg+) was not found in any sample, at any dose, at any time point. In another arm of the study the volunteers received BCG. Likewise, in these patients receiving BCG, DNA was not detected in any sample at any time point.

To gain data on possible routes of transmission of VPM1002BC into the environment, urine samples were collected before (hour 0) and 2, 4, 8 and 24 hours after the instillation in the phase I part of the SAKK06/14 clinical trial and analysed by CFU counts and PCR. The performed real-time MTB-PCR has been recently published (12). In this publication the used in-house method uses the multi-copy insertion element IS6110 for the sensitive and specific detection of MTB. It was compared to an established method (Abbot RealTime MTB test), and was shown to have 100 % sensitivity, 99.2 % specificity for tested samples, and a detection limit of 10 CFU. Taken together the implemented surveillance tests allowed for collection of data and subsequent evaluation of the potential risks for dispersion of the VPM1002BC into the environment and for human-to-human transmission.

2. Methods for monitoring ecosystem effects

See section H.1

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

The donated genetic material will not be transferred from VPM1002BC to other organisms, see chapter G.7. (a)/(b) of this notification. Even if so, the aforementioned PCR-technique would be applicable.

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

Not applicable. Location for release of the GMO is the treatment room at the clinical ward.

5. Duration of the monitoring

The monitoring will last for 60 weeks per patient. Details regarding the monitoring of the treated patients are described in section A5.12 of Part A.

6. Frequency of the monitoring

The assessment of excretion of VPM1002BC in urine, blood and sputum was performed in phase I during induction (at instillations 1 and 6). In particular:

- Urine (30-50 mL) was collected before instillation and at 2, 4, 8 and 24 hours after instillation.
- Blood 1.5 ml for PCR + 3-5 ml for culture was collected before instillation and at 24 hours after instillation.
- Sputum was collected before instillation and at 24 hours after instillation

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Post-release treatment of the location:

Following the intravesical application of VPM1002BC patients are asked to withhold micturition for at least 1 hour and up to 2 hours. During this time, the patients will be allowed to move freely around the clinics perimeter to encourage optimal contact with the bladder wall.

The patients will be instructed about the following behavioural procedures:

- Special precautions will be taken at the first voiding after each VPM1002BC instillation, as most of VPM1002BC is excreted in the urine at this time point. The first voiding will take place in the hospital. 1 hour after the instillation the patient will void in a urinal bottle containing two Javel-Tabs. After 15 min the urine will be disposed by flushing down the toilette as described in section A.4.3.
- Up to 1 week after instillation patients should void while seated to avoid splashing of urine. Urine voided during this time should be disinfected with chlorine tablets (e.g. Javel-Tabs containing Sodium-Dichlorisocyanurate dihydrate from Steinfels Cleaning Systems). The patients will be instructed to add two tablets into the toilet bowl **before** urinating, and after urination to wait 15 min until flushing down the toilette.

Techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment:

Nonclinical studies with intravesical applied VPM1002BC have demonstrated that VPM1002BC is eliminated within a short time frame, unlike common *M. bovis* BCG (see Part A section A5.1). The reason for this behaviour is believed to be the induction of apoptosis in cells infected with VPM1002 (2).

In the treatment of bladder cancer patients by instillation of VPM1002BC into the bladder, it is expected that the vast majority of the dose will be voided during the first micturition after the instillation. Only a minor portion of the dose will persist in the patient´s bladder.

2. Post-release treatment of the GMOs

See I.1.

3. (a) Type and amount of waste generated

1. Type of waste generated:

VPM1002BC will be supplied as freeze-dried cake containing 1 dose of VPM1002BC, live, 1-19.2 x 10E8 CFU, in an amber glass bottle with bromobutyl stopper and aluminum crimp cap and has to be reconstituted before application. The 50 ml diluent (0.9% sodium chloride, 0.025% Tween 80, in water for injections) for resuspending the cake is supplied in a separate amber glass bottle with bromobutyl stopper and aluminum crimp cap. Reconstitution to the desired dose will be performed in a disposable closed drug transfer system, i.e. glass bottles with rubber stoppers and syringes will be used in this process. Any partially or completely used VPM1002BC vials and all other equipment, packaging and materials exposed to the product are considered as biohazardous materials and will be disposed accordingly. Any material or tools used for handling accidental leakage, spill, break of IMP or cleaning the IMP reconstitution area (e.g. absorbent pads) is considered as biohazardous waste. The type of waste generated during application of the IMP normally would consist of liquid and solid waste (glass bottles containing the reconstituted VPM1002BC, syringes with needles, gloves and absorbent pads used for cleaning the IMP reconstitution area).

In case of urine spill or spread of urine of treated patients due to incontinence, all the materials that get in touch with urine should be treated as biohazardous materials and should be disposed accordingly. In case of surfaces coming in touch with GMO-containing urine, they should be cleaned according to local requirements, using a disinfectant proven to be active on mycobacteria.

Patients will be instructed by the Principal Investigator to follow strict hygiene rules.

2. Expected amount of waste:

The amount of waste expected is rather small comprising of approximately 1200 syringes with needle, ca. 600 small glass bottles (50 ml), ca. 600 small glass bottles (4 ml) containing liquid waste, gloves used by personnel during the IMP handling and material used for cleaning the IMP reconstitution area (e.g. absorbent pads).

3. (b) Treatment of waste

VPM1002BC contains viable attenuated mycobacteria and should be handled as an infectious agent at all times. Any unused VPM1002BC vials must be stored for monitoring drug accountability.

VPM1002BC is classified as a genetically modified organism with Biological and Genetic Safety Level L1/S1. Any partially or completely used VPM1002BC vials and all other equipment, packaging and materials exposed to the product contain viable attenuated mycobacteria and should be handled as an infectious agent at all times. Therefore, they should be immediately placed in a container for biohazardous materials and disposed of as biohazardous waste. Any waste material should be disposed of in accordance to instructions compliant with local requirements. Leakage, spill and break should be disposed of in accordance to instructions compliant with local requirements.
If no instructions are available they should proceed as follows:
For cleaning of contaminated surfaces these should be wiped down the surface with a disinfectant suitable for the inactivation of mycobacteria.

For clean-up of leakage, spill and break, shards or broken containers should be carefully removed; spilled liquids should be soaked up with absorbent material and all waste should be autoclaved.

For decontamination of any tools or equipment used for clean-up and exposed to the product these should be wiped down with the group A disinfectant according to the RKI instructions.

Small heat-, pressure- and steam-resistant objects may also be decontaminated by autoclaving.

Additional cleaning/sanitation activities according to local or on-site requirements not specific for this preparation may be performed after the procedure described here.

Recommended procedure for waste treatment: the waste described in chapter I.3.(a) will be collected in autoclave waste bags positioned in plastic waste containers with a volume of approx. 500 ml. The waste container and instillation bags will be placed in an autoclave located at the sites and GMOs will be inactivated by steam autoclaving. The inactivation process will be monitored using a biological indicator (*Geobacillus stearothermophilus* spores) included in each autoclave run. After inactivation the waste will be collected in hard plastic disposal boxes and will be shipped for professional disposal. The procedures are laid down in the standard operating procedures of the clinical sites.

J. Information on emergency response plans

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

PCR-methodology is available for detection of VPM1002BC in samples. In the highly improbable case of a detected human-to-human transmission leading to an unsustainable

adverse drug reaction a set of suitable rescue medication is available for treatment. Please refer to section A5.12 in Part A.

In case of accidental injection of a partial dose, the same warnings and precautions, liver effects, systemic adverse reactions as for BCG should be expected (see section A5.9 in Part A).

The sites will be instructed about safety precautions and behavioral practices in case of contamination caused by self-injection.

The investigational medicinal product, VPM1002BC, is developed also as vaccine against tuberculosis under the name VPM1002. VPM1002 TB vaccine has been tested in adults in a single dose of up to 2-8x10E5 CFU (colony forming units) given by intradermal injection. Two studies in healthy adult volunteers and one Phase IIa study in healthy newborn infants were performed with VPM1002 TB vaccine. A further phase II study in over 400 newborn infants, including HIV exposed babies, has finished recruitment (Phase II study in South Africa, study code: VPM1002-ZA-2.13TB, ClinicalTrials.gov Identifier: NCT02391415).

Moreover, the parental strain of VPM1002BC, *M. bovis* BCG, is used as active immunization against TB with approximately 4 billion applied doses since licensure and additional doses applied as immunotherapy for the treatment of NMIBC. Due to the similarity of both products, the safety profile of *M. bovis* BCG serves as basis for a conservative assumption of VPM1002BC effects in human. This assumption is supported by the preclinical data, which demonstrates that VPM1002BC has an improved safety profile compared to *M. bovis* BCG. Thus severely immune compromised mice (C.B-17 SCID mice), which do not have an adaptive immune system, survive a subcutaneous dose of VPM1002BC while they die with *M. bovis* BCG. The reason for this is induction of apoptosis in cells infected with VPM1002BC, but not with *M. bovis* BCG self-containing the infection (2). The hitherto existing clinical data contributes to the assumption/proposition of improved safety.

In case of accidental injection of the entire dose of 1-19.2x10E8 CFU, the effects are not generally considered to be more severe than for lower doses, as the immunological response is not proportional to the dose.

<u>Management of accidental self-injections:</u>

Because of the risk of accidental injection into a blood vessel, the affected person should consult a member of the study team immediately via a 24 hour emergency telephone and consult the Principle Investigator (PI) of the Sites or if not present a Sub-investigator (SI) participating in the trial and combination therapy with tuberculostatics should be considered.

Management of systemic infections or persistent local infections following vaccination with BCG tuberculosis vaccine includes treatment with antimycobacterial drugs. The management of accidental self-injections with VPM1002BC should be handled similarly to that of BCG. VPM1002BC is sensitive to all common tuberculostatics (see section A5.9 Part A).

In order to minimize dissemination of the recombinant vaccine into the environment, the injection site should be covered with a suitable dressing immediately after injection. This should absorb any vaccine that may leak out through the needle track. The dressing should

be removed from the injection site at the end of the 30 minute observation period and should be disposed as biohazardous waste, in accordance with the relevant guideline and current local practice. The affected person should receive additional dressings as well as special waste disposal bags for further care of the wound at home.

Clean any blood-contaminated surfaces according to I.3.(b) of this document. Collect any blood-contaminated waste and dispose it as biohazardous waste according to I.3.(b) of this document.

In the event of an accidental self-injection the affected person will receive the following instructions:

- Cover the injection site with a suitable dressing immediately. This will further absorb any vaccine that may leak out through the needle track.
- You may take off the dressing after 30 minutes.
- Inform a physician as well as the PI or if not present a SI of the trial about the incidence.
- Avoid any direct contact with the injection site in order to prevent transmission of the vaccine to other parts of the body, to other persons or objects.
- Use the additional supply of dressings provided to you in the event that the original dressing detaches.
- Discard the detached dressing(s) in the plastic bag you received and return to the clinical site for professional disposal.
- If possible the injection site should not be touched, scraped or rubbed within the first few days, especially if the site has started ulcerating, to prevent an infection of the injection site with ubiquitous agents and spreading of the recombinant mycobacteria into the environment.
- In case of direct contact with the site of injection or with the material that covered it, wash your hands thoroughly using warm water and soap.
- Do not apply any ointments and creams to the injection site until it is completely healed.
- Keep the site of injection dry
- Minimize wetting of the area e.g. during bathing, until the injection site is completely healed.
- If in doubt, follow the SOP of your hospital
- Contact the PI/SI of the trial if any notable local injection site reactions occur. Depending on the progression of the ulceration, subsequent appointments for ambulant visits may be arranged.

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

Disinfectants or disinfectant procedures that are suited for inactivation of vegetative bacteria including mycobacteria as well as fungi and their spores will be used for removal of areas that are potentially affected. Compare also chapter I.3.(b).

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

Not applicable. Surfaces that came into contact with GMO will be disinfected with a disinfectant suitable for the inactivation of mycobacteria

For further measures see also chapter I.3.(b).

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

In the highly improbable case of a detected human-to-human transmission leading to an unsustainable adverse drug reaction, a set of suitable rescue medication is available for treatment.

For further measures see also chapter J.1.

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