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
(b) Notification number B/CZ/16/2
(c) Date of acknowledgement of notification 17/05/2016
(d) Title of the project

A phase I, single-arm clinical trial to evaluate the safety and immune activation of the combination of DCVAC/PCa, an active cellular immunotherapy, and ONCOS-102, an immune-priming adenovirus, in men with advanced metastatic castration-resistant prostate cancer

(e) Proposed period of release

From 3Q 2016 until 3Q 2018

2. Notifier:

Nemocnice na Homolce
Roentgenova 2/37
Praha 5-15030
Czech Republic

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)

Genus Mastadenovirus

Species Adenovirus C, serotype 5

ONCOS-102 (previously known as CGTG-102) is a genetically modified replication competent oncolytic human adenovirus based on serotype 5. It is armed with GMCSF transgene and has a 24 bp deletion restraining the replication exclusively in tumors. The viral capsid has been modified for effective transduction of tumor cells.

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

Properties of the viral genome, such as the size and chemical composition, are identified as major determinants of the mutation rates of viruses. Compared to other viruses, as double stranded DNA viruses with genome size of approximately 36 kb, adenoviruses are considered genetically stable. In addition, adenovirus DNA polymerase acts as a proofreading enzyme and removes mismatched nucleotides during replication.

However, the chance of co-infection enables the natural recombination among adenoviruses. Recombination occurs between strains of the same adenovirus species, predominantly in regions of homology, but not presumable between adenovirus species.

Adenoviruses are also able to recombine with chromosomal DNA, and as a result the vector sequences may become integrated into the host cell genome. However, usually the vector DNA remains episomal and is eliminated when the cell divides or dies. Integration is quite rare event even in an experimental system. In vitro infection of established cell lines with a concentration of 10 virus particles per host cell, result in integration rates between 10E-3 - 10E-5 events per cell (Harui et al. 1999). Furthermore, the integration of viral DNA does not necessarily lead to transformation and most of the integrated viral genomes are defective, containing substantial deletions. Many or most integrations of adenovirus DNA have no recognized biological consequence.

To ensure the genetic stability, A549 host cells are used for ONCOS-102 production. The A549 cell line doesn't possess adenoviral sequences, thus the risk for genomic recombination of the virus during the manufacturing process is negligible. The design of ONCOS-102 construct enhances the genetic stability by restricting the length of inserted sequences and thereby secures the packaging capacity of the virus. Consequently, during virus production the ONCOS-102 genome is efficiently packaged and it is less prone to rearrangements which may lead to unexpected changes in its properties.

From the ONCOS-102 reference batch the complete genome has been sequenced and compared with the NCBI Reference Sequence AC_000008.1. Regions differing from the Ad5 sequence were constructed as follows:

- E1A with 24 base pair deletion: Article (Fueyo et al. 2000) was used as a reference to locate the 24 base pairs deleted from CR2 of E1A, specifically, base pairs 919-943. The base pairs were deleted from the sequence.

- Deletion in E3: As a reference, article (Kanerva et al. 2005) was used to locate the 965 base pair deletion in E3.

- hGM-CSF in E3: A commercial plasmid pORF.hGM-CSF (Invitrogen) was used to clone the hGM-CSF gene to the deletion site on E3. Therefore, hGM-CSF sequence was obtained from Invitrogen. E3 derived restriction enzyme sites for

SunI and MunI were kept at both ends of the gene sequence, as the hGM-CSF gene was amplified with primers containing such sites and sub cloned into the shuttle vector pTHSN.

- Chimeric fiber: The Ad5 knob sequence was replaced by the Ad3 knob, sequence AB361380.1 in NCBI database.

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)
Czech Republic

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

Yes (.) No (X)

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

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

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

Yes (.) No (X)

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

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

ONCOS-102 has been designed with safety as the primary emphasis. ONCOS-102 is an oncolytic virus: it selectively replicates in cancer cells and therefore in theory is unable to replicate in healthy individuals.

In case a healthy person would be exposed to ONCOS-102, it is unlikely that it would cause an infection due to its cancer specific nature. However, even in the case of infection, the symptoms would be mild, mainly flu or mild gastrointestinal symptoms.

The safety data derived from the patients treated in the ONCOS C1-study shows that the side effects of the virus treatment are mild. The most common treatment-related adverse events were pyrexia, chills, fatigue, injection site pain, feeling cold, hyperhidrosis, decreased appetite, and nausea. Keeping in mind that the patients suffered from refractory cancer associated with immunocompromised state, and they were treated with high dose of ONCOS-102, the conclusion is that ONCOS-102 can be considered safe for healthy individuals.

Based on a risk assessment, the major risk for ONCOS-102 is that personnel are exposed to the virus by accidental needle puncture or from surface contaminations. ONCOS-102 handling guideline instructs those who are involved in dose preparation and administration to use universal precautions and appropriate Personal Protective Equipment (PPE). Dose preparation is to be performed in a Biosafety Cabinet (BSC) with a Closed System Transfer

Device to reduce the risks posed by the possibility of generation and inhalation of aerosols. A qualified pharmacist with specific training on the protocol will be responsible for ONCOS-102 material receipt, storage, documentation of traceability of product at the investigational site and reconstitution on the day of administration.
If a person experiences a needle accident, the volume delivered in the body is small, basically one drop that might be at the tip of the needle. Even in the worst case, considering the undiluted virus stock, the estimated maximum delivered accidental dose is less than 5% of the treatment dose. As stated above, the side effects of the treatment with ONCOS-102 are normally only mild. Thus, the accidental dose to a healthy person very unlikely causes any symptoms.
Preventive actions, including standard operating protocols and training of the personnel, have been compiled for minimizing and controlling the risk for surface contaminations. The stability of ONCOS-102 has been studied, and the storability test showed that the infectivity of the virus was lost after 24 hours at room temperature.
The overall risk posed by transmission of ONCOS-102 to the unintended recipient and the environment is considered low. Similarly, the risk posed by secondary exposure from shedding, is considered low.
The risk posed on the unintended recipient by wild type adenovirus, as a contaminant or through recombination, or by viral DNA sequences to the environment is considered low through a combination of the low level consequences of exposure and the low likelihood of this occurring.
Risk management strategies are in place to minimize the risks of exposure to unintended individuals or the environment. Appropriate monitoring strategies are proposed to gather further information on safety, persistence and shedding prior to further wider-scale development.

## 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) Adenoviridae

| | | |
|---|---|---|
| (ii) | genus | Mastadenovirus |
| (iii) | species | Adenovirus C |
| (iv) | subspecies | Serotype 5 |
| (v) | strain | … |
| (vi) | pathovar (biotype, ecotype, race, etc.) | … |
| (vii) | common name | … |

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)
Serotype 5 adenovirus is global and common human pathogen.

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

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

| | |
|---|---|
| Atlantic | .. |
| Mediteranean | .. |
| Boreal | .. |
| Alpine | .. |
| Continental | .. |
| Macaronesian | .. |

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

Adenoviruses are highly stable and resistant to dehydration, temperature and pH. They persist in soil, water or other sites contaminated with human faeces.

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

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

4. Natural habitat of the organism

(a) If the organism is a microorganism

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

Serotype 5 human adenoviruses are specific to humans. Wild type adenoviruses are stable, allowing for prolonged survival outside of the body.

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

…

5. (a) Detection techniques

In vitro cell culture techniques.

(b) Identification techniques

Conventional PCR and sensitive qPCR methods, antibody detection, restriction enzyme analysis, 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

Any adenovirus has been assigned to Risk Group 2 by the National Institutes of Health (NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules, November 2013) and the European Communities (Directive 2000/54/EC).

Biosafety Level 2 containment is required for work with this vector.

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

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

If yes:

(a) to which of the following organisms:

| | |
|---|---|
| humans | (X) |
| animals | (.) |
| plants | (.) |
| other | (.) |

In immune-compromised and immunosuppressed individuals the adenovirus infections tend to be more prolonged, more severe, and sometimes even fatal.

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

Pathogenicity

Wild type adenovirus is a common pathogen of humans. It causes a wide range of illnesses.

Infectivity

Adenovirus is capable of infecting multiple organ systems. It has a worldwide prevalence and it is ubiquitous throughout the year. Serotype 5 is one of the most common serotypes. The infection varies in clinical manifestation and severity; however, most infections are asymptomatic. Group C adenoviruses, types 1, 2, and 5 are associated with respiratory tract infections, but can potentially disseminate in immunocompromised hosts and neonates, causing significant morbidity and even mortality. The mode of adenovirus transmission is through respiratory and fecal-oral routes. Infection can also spread through contaminated fingers, vomits or ophthalmic solutions. Airborne transmission occurs by small-droplet, and to lesser extent, large droplet-aerosols (Robinson 2007).

The alterations in infectivity and pathogenicity of ONCOS-102 are reductions compared to wild type Ad5 and changes due to inserted genes are restricted exclusively in cancer cells where the virus is able to replicate.

Toxigenicity
Various adenoviral factors contribute to the pathogenesis: The pentons are directly cytotoxic and during the process of viral replication and lysis of susceptible cells, the early viral proteins counteract tumor necrosis factor (TNF) and apoptosis, and down regulate the expression of major histocompatibility complex (MCH) Class I molecules thus preventing recognition by cytotoxic T cells.

Virulence
Adenovirus infections are common, have a worldwide distribution, and occur throughout the year. The endemic adenoviruses, which include serotypes Ad1, Ad2, Ad3, Ad5, Ad6 and several others, all together infect more than 80% of the human population early in life, with the peak incidence of infection occurring between 6 months and 5 years of age. In contrast to the endemic serotypes, which infect mainly children, the remaining adenovirus serotypes occur in epidemics and can infect anybody that has not been previously infected with them (Norking 2010).
With broad tropism, adenovirus can infect various cells, both proliferating and quiescent. Thus it is capable of infecting multiple organ systems. The site of entry generally determines the site of infection; respiratory tract infection infections result from droplet inhalation, while gastrointestinal tract involvement results from fecal-oral transmission. Most of the common endemic adenovirus infections are asymptomatic, which enhances the spread of these viruses.

Allergenicity: Adenovirus triggers the immune system to protect the body from harmful effects. It cannot be considered as a hypersensitivity reaction or an overreaction to a harmless substance (an allergen).

Carrier (vector) of pathogen: No

Possible vectors: None

Host range including non-target organism: Humans

Possible activation of latent viruses (proviruses):
Human adenoviruses can exhibit considerable persistence and latency after an acute infection. Some types are capable of establishing persistent asymptomatic infections in the tonsils and adenoids: they can be found in adenoid tissue during routine tonsillectomy.
Adenovirus is resistant to gastric secretions, bile and pancreatic proteases, whereupon it can passage through the stomach and replicate in the intestine.
Adenoviruses can cause latent infection also in mucosal lymphocytes which can result in reactivation of infectious virus production.
Prolonged virus shedding from various body sites aids the transmission.

Ability to colonize other organisms: No colonization to other organisms.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Adenovirus is an obligate human parasite. Virions are metabolically inactive outside the host cell.

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

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

(c) Factors affecting reproduction:
In its host, adenovirus may take advantage of the impaired immunological response: In immune-compromised individuals (the very young or elderly) and those who are immunosuppressed due to a therapy or underlying condition such as immunosuppressive therapy with cytotoxic drugs, use of corticosteroids, radiation therapy, AIDS, malnutrition, or severe burns, the infections tend to be more prolonged, more severe, and sometimes even fatal. Over the last years, adenoviruses have increasingly been recognized as significant viral pathogens, which may be associated with the growth of the immunocompromised population, especially of patients with acquired immunodeficiencies.

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:
Adenoviruses are resistant to lipid disinfectants, but are inactivated by formaldehyde and chlorine (Flomenberg, 2009). Variable inactivation occurs also with iodine and UV light. Viral DNA can be detected long after infectivity is destroyed. The genetic modifications of ONCOS-102 do not affect the sensitivity to physical and chemical inactivation.
Physical inactivation: Wild type adenovirus can be inactivated by heat. Heating to temperatures >56°C for 30 minutes or autoclaving will destroy the infectivity (Robinson & Echavarria 2007). Greater than eight logs of reduction in adenovirus type 5 potency may be obtained upon exposure of the sample to temperatures >70°C and times longer than 20 min (Maheshwari, 2004).
Chemical inactivation: Adenovirus can be inactivated by contact with 1:5 dilution of bleach for 1 minute, or contact with alcohol-based hand gels for 2 minutes (Robinson & Echavarria 2007). Ethyl alcohol, at concentrations of 60%–80%, is a potent virucidal agent inactivating all of the lipophilic viruses and many hydrophilic viruses (e.g. adenovirus).
For adenoviruses, 2.0 % Barrydin solution for 60 minutes or 4.0 % Barrydin solution for 30 minutes is recommended by the manufacturer for short time disinfection.

Virkon®S is a commercially available oxidative disinfectant used against a variety of viruses. 0.9% Virkon®S liquid is proposed for decontamination procedures of adenovirus type 5with contact times greater than five minutes (McCormick & Maheshwari, 2004).
Following reconstitution and administration of ONCOS-102 at a study site, materials used during the procedure should be disposed of according to the appropriate local/regional and institutional requirements for biohazard waste by autoclaving and/or incineration either on or off site. All non-disposable equipment and other materials used during the procedure will be cleaned using a chemical disinfectant capable of virucidal activity for the required duration of contact, or sterilized by autoclaving consistent with local institutional guidelines for handling potentially infectious materials.

10. (a) Ways of dissemination
The mode of adenovirus transmission is through respiratory and fecal-oral routes. Infection can also spread through contaminated fingers, vomits or ophthalmic solutions. Airborne transmission occurs by small-droplet, and to lesser extent, large droplet-aerosols (Robinson 2007).

(b) Factors affecting dissemination
Outbreaks of adenovirus-associated respiratory disease have been more common in the late winter, spring, and early summer. However, adenovirus infections can occur throughout the year. Adenovirus disseminates more efficiently under conditions of crowding.

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

2. Intended outcome of the genetic modification
ONCOS-102 is a serotype 5 adenovirus (Ad5) displaying the following modifications differing from the Ad5 genome:

1. A 24 base pair (bp) deletion in the E1A-gene constant region 2 (CR2). The dysfunctional E1A protein is unable to bind to the cellular retinoblastoma protein (Rb) for the release of the E2F1 transcription factor from Rb, leading to the requirement of free E2F1 for adenovirus gene transcription. Free E2F1 is abundant in cancer cells, where the Rb/p16 pathway is typically disrupted. Thereby viruses with the 24 bp deletion in E1A are able to efficiently replicate in cancer cells but are crippled in normal cells. E2F1 activates other adenovirus promoters eventually leading to replication and lysis.

2. A 965 bp deletion has been introduced in the Early 3 (E3) region coding for 6.7K and gp19K proteins. These proteins are associated with the ability of adenovirus to evade host immune control mechanisms and their functions are expendable for adenoviral replication. In fact, the gp19K deletion may enhance tumor selectivity of the virus. Normally, this protein down-regulates HLA-1 to avoid detection by T-cells. However, since many advanced tumors are HLA-1-negative, this interaction is not needed, while transduced (non-replication permissive) normal cells are cleared faster since they are rapidly recognized by T-cells.

3. A transgene coding for the human granulocyte macrophage colony stimulating factor (GMCSF) protein has been inserted to the E3 region, replacing 6.7K and gp19K. The GMCSF gene transcription into mRNA is being controlled by the endogenous E3 promoter. GMCSF is a potent activator of immune system with established antitumor properties.

4. The serotype 5 fiber knob has been replaced by serotype 3 fiber knob, thereby allowing entry of the virus to cells via the serotype 3 receptor (frequently expressed to high degree in tumor cells) instead of the serotype 5 receptor CAR (frequently down-regulated in advanced tumors).

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 (.) No (X)

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 | (.) |
| bacteriophage | (.) |
| virus | (.) |
| cosmid | (.) |
| transposable element | (.) |
| other, specify | … |

(b) Identity of the vector

…

(c) Host range of the vector

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

Yes (.) No (.)

| | |
|---|---|
| antibiotic resistance | (.) |
| other, specify | … |

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector

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

| | | |
|---|---|---|
| (i) | transformation | (.) |
| (ii) | electroporation | (.) |
| (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 | |

ONCOS-102 was generated and amplified using standard adenovirus preparation techniques. A fiber chimeric plasmid was constructed and recombined with a shuttle vector containing a 24-bp deletion in E1A resulting in plasmid pAd5/3-D24.
An E3-cloning vector pTHSN was created including a 965-bp deletion in the E3 region to insert the human GMCSF gene at the location of the deleted E3 gp19k and 6.7k.
The 432-bp cDNA encoding human GMCSF was amplified and inserted into pTHSN. pAd5/3-D24-GMCSF was generated by homologous recombination between pTHSN-GMCSF and pAd5/3-D24 in Escherichia coli, resulting in the plasmid pAd5/3-D24-GMCSF. pAd5/3-D24-GMCSF incorporated the whole ONCOS-102 genome on a bacterial backbone, which enabled the replication of the viral genome as a part of the circular plasmid in bacterial cells.
The genome of ONCOS-102 was released from the pAd5/3-D24-GMCSF plasmid bacterial backbone by digestion with PacI restriction enzyme and transfected to A549 cells for subsequent amplification and rescue.

6. Composition of the insert

(a) Composition of the insert
Complementary DNA coding for human granulocyte-macrophage colony-stimulating factor (GM-CSF)

(b) Source of each constituent part of the insert
A commercial plasmid (Invitrogen) containing the complementary DNA coding for human GM-CSF.

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

GM-CSF is a potent inducer of antitumor immunity. It recruits antigen presenting cells (APC) and natural killer (NK) cells, and activates and matures APCs at the tumor site, thereby potentiating the ability of ONCOS-102 to induce cellular immunity against the tumor it replicates in.

(d) Location of the insert in the host organism

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

The viral DNA is replicated in the host cell nucleus where the virus utilizes the host cell translation machinery. However, ONCOS-102 is replicated only in Rb-p16 pathway deficient cancer cells that have free E2F transcription factor available.
The vector DNA will remain episomal and will be eliminated when the cell divides or dies.

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

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

1. Indicate whether it is a:

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

(specify phylum, class)
other, specify …

2. Complete name

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

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (X) Not known (.)

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

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (.) No (X)
If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (X) No (.) Not known (.)

Adenoviruses are able to recombine with chromosomal DNA, and as a result, they can exchange genetic material with host cells. However, usually the vector DNA remains episomal and is eliminated when the cell divides or dies. Furthermore, integration is quite rare event and many or most integrations of adenovirus DNA have no recognized biological consequence.

## 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 (X) No (.) Not known (.)
Specify
Wild type adenoviruses are stable. Based on stability studies, infectivity of ONCOS-102 starts to decrease already after 5 hours at room temperature when diluted in 0.9% NaCl. At 24 hours, the infectivity has considerably diminished and at 48 hours no infectious particles are detected. Therefore, it is unlikely that an inadvertently released ONCOS-102 could survive or disseminate in the environment.

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

Yes (X) No (.) Unknown (.)
Specify
ONCOS-102 virus genome has been modified with a 24 base pair deletion in the retinoblastoma binding site of E1A, which allows the virus to replicate only in Rb-p16 pathway deficient cancer cells. Therefore, it is in theory unable to replicate in healthy individuals and in the normal tissues of cancer patients.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X) No (.) Not known (.)
Specify
The infectivity in normal cells has been restricted by a genetic replacement of the knob region of Ad5 with the corresponding domain of Ad3, which allows the virus to bind and entry through the Ad3 receptor, which is expressed to a high degree on tumor cells.
The lack of viral genes 6.7K and gp19K, due to a 965 bp deletion in the E3 region, retains ONCOS-102 unable to evade the host immune system and results in more effective clearing of the GMO.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (X) No () Not known (.)
Specify
Due to the before-mentioned restrictions in replicative abilities of the vector in normal tissues, the replication potential of ONCOS-102 and thereby the pathogenicity of ONCOS-102 is significantly distinct from the wild type parent organism, i.e. ONCOS-102 is significantly less pathogenic.

2. Genetic stability of the genetically modified organism
The genetic stability has been assessed by restriction enzyme assay and sequencing, and ONCOS-102 has remained genetically stable at least for seven passages.
In addition, the expression and functionality of the genetic insert product GMCSF and the specificity of infection vector have been determined by suitable in vitro tests. The results show that the genetic stability is comparable from batch to batch.

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

(a) to which of the following organisms?

| | |
|---|---|
| humans | (X) |
| animals | (.) |
| plants | (.) |
| other | … |

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

Pathogenicity

Compared to wild type adenovirus, ONCOS-102 is less pathogenic due to restrictions in replicative abilities of the vector in normal tissues.

Infectivity
The alterations in infectivity of ONCOS-102 are reductions compared to wild type Ad5 and replication of ONCOS-102 is restricted exclusively in cancer cells.

Toxigenicity
The pentons are not modified and they are as cytotoxic as in parental virus.
The early viral proteins counteract tumor necrosis factor and apoptosis as in wild type.

Virulence
The ability to cause disease has been decreased.

Host range including non-target organism has not changed.

Possible activation of latent viruses (proviruses)
ONCOS-102 replicates only in tumor cells. It is administered intratumorally, which decreases the possibility for persistent asymptomatic infections in the tonsils, adenoids and intestine. It will reach the tissues via circulation, but is unable to replicate in normal cells.

Ability to colonize other organisms has not changed. No colonization.

ONCOS-102 is immunogenic and it induces both an acute, innate inflammatory response and adaptive immune responses resulting in the destruction of transduced cells.

Considerations for human health and animal health as well as plant health:

The potential direct effects on human health are limited to the transmission of ONCOS-102 to an unintended human recipient. The potential adverse effects are expected to be the same as those which may be anticipated in patients receiving the treatment, albeit much lower in intensity.
The potential indirect effects of the release are limited to the consequences of dissemination of ONCOS-102 from the site of injection, shedding, or the release of wild type adenovirus through contamination of the product during manufacture or following recombination in the recipient's cells, which are all highly unlikely to occur.
It is unlikely that ONCOS-102 will be a risk to human health and safety.

Potential effects on the environment could be shedding of ONCOS-102 to the environment or transfer of inserted genetic material into an animal virus. It is anticipated that shed virus or possible recombinants would be non-infectious to other than humans. The consequences of exposure to the environment are therefore minor.
The likelihood of ONCOS-102 constituting a hazard to the environment is very low.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
The presence of infectious virus particles in a sample can be determined by a common, qualitative virus culture method. The presence of virus induces morphological changes in

adenovirus permissive A549 cells (human lung carcinoma), that can be microscopically detected. Changes due to infection are classified as cytopathic effect (CPE) and in case of CPE the sample is considered positive.
The method is not specific for ONCOS-102. Instead, it detects all adenovirus serotypes from the sample and the positive result needs to be verified by identifying ONCOS-102 by qPCR. However, the method is able to detect one infectious particle from a sample, it is scientifically sound and appropriate for the purpose.

Specific and highly sensitive qPCR method can be used for identification of ONCOS-102 specific modifications from swab samples.

(b) Techniques used to identify the GMO
Specific PCR or qPCR methods, antibody detection, restriction enzyme analysis, DNA-sequencing

## F. Information relating to the release

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

The purpose of the release is to evaluate the safety of ONCOS-102 primed with cyclophosphamide (CPO) in combination with DCVAC/PCa, an active cellular immunotherapy, in men with advanced metastatic castration-resistant prostate cancer.

The planned duration of the study is 24 months. Total number of 10-15 patients will be recruited, all with readily accessible soft tissue/nodal tumor lesion (for intratumoral application of ONCOS-102 and biopsy).

The active cellular immunotherapy DCVAC/PCa is an investigational medicinal product containing autologous dendritic cells (DCs) activated by ex-vivo exposure to allogeneic prostate cancer cells. DCVAC/PCa is able to induce immune responses, including cytotoxic CD8+ T lymphocytes, against antigens expressed by prostate cancer cells.

Low dose CPO has been reported to potentiate anti-tumor efficacy by down-regulating immunosuppressive regulatory T lymphocytes, which are key regulators in maintaining immune reactions favourable for the tumor. Low-dose CPO is theoretically beneficial for cancer immunotherapy approaches such as ONCOS-102 or DCVAC/PCa.

Combining of different treatment modalities may help to reduce the tumor mass and enhance the immunogenic response in late stages of prostate cancer. It is hypothesized that ONCOS-102 may enhance the anti-tumor immune responses generated by DCs loaded with tumor antigens and thus assist the immune system to overcome evasive strategies used by tumors.

Justification for the combination of these two investigational medicinal products is supported also by their favourable safety profiles documented in phase I/II clinical trials.

ONCOS-102 is intended for intratumoral administration under ultra sound guidance. Administration will only be performed by trained medical professionals in an approved study site facility.

Guidelines for appropriate handling, personal protective equipment, accidental spills, and waste disposal will be followed during product preparation and administration.
Accidental exposures are reported according to local regulations. In addition, a separate form for reporting the accidents is provided to the clinical trial site.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes (X) No (.)

If yes, specify …
ONCOS-102 will be administered in the clinical site facility with restricted access.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
ONCOS-102 will be administered in the clinical study site located in southern Finland.

Study site address:
Nemocnice na Homolce
Roentgenova 2/37
Praha 5-15030
Czech Republic

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

ONCOS-102 will be handled in the hospital pharmacy in a clean room. Pharmacists are responsible use cleaneroom single-used clothing (overall, booth, gloves). Dose preparation is to be performed in a laminar box in a clean room with a Closed System Transfer Device (PhaSeal) to reduce the risks posed by the possibility of generation and inhalation of aerosols.

The patients will be treated and observed in separate single bed rooms and hospitalized after dosing for 24 hours in these same rooms.

The pharmacy as well the treatment rooms have restricted access meaning a controlled and limited access to authorized hospital staff trained on measures to control infection. The international biohazard symbol will be at each entrance. The biohazard symbol can be taken off the door of the treatment room after discharge of the patient.

Environmental surfaces, hospital rooms, should be routinely cleaned with a virucidal disinfectant based on valid Disinfection plan. All waste should be put to marked container and autoclaved.

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

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

…

4. Method and amount of release

(a) Quantities of GMOs to be released:

The maximum number of patients is 15. Each patient will receive six injections (at weeks 5, 6, 7, 8, 14 and 23) containing 3 x $10^{11}$ virus particles /2.5 mL injection.
ONCOS-102 is formulated as a concentrate at a concentration of 5x$10^{11}$ viral particles (VP) per milliliter (mL). The volume of each vial is 0.8 ml. The product is stored prior to administration in a temperature monitored freezer at -60°C or below. One vial of ONCOS-102 is thawed per dose. The total amount of undiluted ONCOS-102 will be 72 ml in which case the total amount of viral particles for release is 3.6 x $10^{13}$.

(b) Duration of the operation:

From 3Q 2016 until 3Q 2018

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

ONCOS-102 is released for clinical trial use only. The viruses are formulated as a solution, presented in 2 ml glass vials, sealed with a rubber stop and an aluminum cap. A primary label is attached to each vial. The product is stored prior to administration in a temperature monitored freezer at -60°C or below in the pharmacy or other appropriate secure location.

The administration is under the responsibility of the trained medical professionals, according to the clinical protocol and in respect of the Good Clinical Practice. The product must be prepared in aseptic conditions compliant with injectable solutions. Dose preparation is performed in clean room by pharmacist.
The laminar box will be decontaminated before and after manipulation first with virus inactivating agent and then with 70% EtOH.
All staff involved in handling of ONCOS-102 or any potentially contaminated material must wear singlue-used personal protective equipment (PPE). All transfers must be done using a sealed plastic transport box marked with biohazard symbol and a spill kit should follow the transport. The personnel at the site will follow the standard hospital policy recommended for the manipulation of live virus vaccines.
In case of accidental spill, the spill area will be isolated and left empty to allow the aerosols to settle. Personnel that are involved in the clean-up of the spill should wear PPE. Paper towels or wipes are placed carefully over the spill starting from the edges. The spill should be absorbed with paper towels and an active disinfectant capable of virucidal activity should be applied. The contact with the disinfectant will be allowed according to manufacturers' instruction.
All personnel involved in handling the product is informed that in case of:

- Eye splash: the eyes should be rinsed with clean water or physiological saline solution (NaCl 0.9%)
- Intact skin splash: the site should be cleaned with a tissue moistened with virucidal disinfectant, and flushed with clean water for at least 15 minutes. The contaminated tissue should be treated as infectious material.
- Cuts or punctures: the wound should be allowed to bleed before it is flushed

under a running stream of clean, and preferably sterile, water. Then the injured skin area should be covered with a sterile gauze dressing, which should be appropriately discarded according to regular hospital procedure when removed. The individual should be referred to and medically monitor

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

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.

Exploratory open label study of GM-CSF coding oncolytic adenovirus ONCOS-102, with low dose cyclophosphamide in patients with refractory injectable solid tumors, ONCOS C1, from 02 April 2012 to 29 October 2013, EudraCT Number: 2011-001657-82:

This was a classic 3+3 dose escalation study to establish the recommended dose for further development of ONCOS-102 with cyclophosphamide. Five patients out of 12 received the maximum of 9 doses of ONCOS-102 over a period of five months.
All 12 patients received 4 injections of ONCOS-102 within the first month of the study. Therefore, ONCOS-102 was tolerated, as defined in the protocol, at doses of 3 x $10e^{10}$ VP/injection, 1 x $10e^{11}$ VP/injection, and 3 x $10e^{11}$ VP/injection. There were no confirmed dose limiting toxicities (DLTs) nor was there a maximum tolerated dose (MTD) established
All of the patients who participated in the study had at least one adverse event (AE). There was no indication of a relationship between dose of ONCOS-102 and the incidence or intensity of AEs. The most common AE was pyrexia, which was reported in every patient. Other common AEs, reported in 50.0% patients were: chills, fatigue, injection site pain, feeling cold, hyperhidrosis, decreased appetite, nausea, and weight loss. Most of the AEs were Grade 1 or Grade 2. Grade 3 AEs were reported in 6 patients of which AEs in 5 patients were considered treatment-related: pyrexia, increased ALP, increased ASAT, proteinuria, hyponatremia, anemia, fatigue, edema peripheral, and dyspnea.
There were no Grade 4 AEs. Serious adverse events occurred in 5 patients. Five of the 7 SAEs were assessed as either not related or unlikely to be related to study medication: duodenal obstruction, small intestinal hemorrhage, colonic obstruction, muscle rupture, and abdominal pain. One patient had SAEs of Grade 3 peripheral edema and hypoalbuminemia that were assessed by the investigator as possibly related to study medication. However, the patient had advanced metastatic disease. She died of underlying disease more than 1 month after the last administration of ONCOS-102.

Supportive safety evidence on ONCOS-102 in human cancer patients suggests that the applied dose levels have been safe. In an Advanced Therapy Access Program (ATAP), 115 patients with late stage cancer were treated with ONCOS-102 with doses of up to 4 x $10^{11}$ VP. The ATAP was not a trial but an individualized treatment program regulated by the Finnish Medicines Agency (FIMEA) as determined by the Advanced Therapy Regulation, EC/1394/2007.

In the Oncos C1 Phase I study, urine samples and buccal swabs for viral analysis (viral genome copy numbers, the presence of infectious virus) were taken before dosing on Days 1, 4, 8, and 15, and before discharge on Days 2, 5, 9, and 16. Virus was detected before dosing on one occasion (Day 4): in the urine of 1 patient, and in the buccal swabs in 3 patients, but not on subsequent dosing days.

The reason for the positive result before dosing on Day 1 may relate to the fact that 1/5th of the virus dose was administered i.v. on Day 1. On all other occasions the total volume was administered i.t.
I.v. injections will not be given in any future studies.

There is a theoretical risk of spread of ONCOS-102 into the environment from patients who are undergoing treatment. However, in the Oncos C1 Phase I study, no virus was detected in urine or buccal swabs at discharge after dosing in any patient.

## 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 …
   (viii) common name
   Target organisms are humans, cancer patients, men with advanced metastatic castration-resistant prostate cancer.

3. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
   Oncolytic cancer cell killing by ONCOS-102 leads to significant release of tumor epitopes for sampling by antigen presenting cells and represents a potent co-stimulatory danger signal that causes activation of the immune system. It has been demonstrated in vitro that ONCOS 102 induces immunogenic cell death (ICD), as measured by exposure of calreticulin on the cell surface and release of ATP and HMGB1 from dying cancer cells (Liikanen et al, 2013).

   Arming the oncolytic adenoviruses with immunomodulatory transgenes is aimed at enhancing their anti-cancer activity. In a recent Phase I study local administration of ONCOS 102 was shown to induce infiltration of innate immune cells and CD8+ T cells into the tumor area. Simultaneously, induction of tumor-specific CD8+ T cells was detected in the interferon-gamma ELISPOT analysis of peripheral blood mononuclear cells (PBMCs) (Ranki, 2014; Vassilev, 2015).

   Combining of different treatment modalities may help to reduce the tumor mass and enhance the immunogenic response in late stages of prostate cancer. It is hypothesized that ONCOS-102 may enhance the anti-tumor immune responses generated by DCs loaded with tumor antigens and thus assist the immune system to overcome evasive strategies used by tumors.

3. Any other potentially significant interactions with other organisms in the environment
   Human adenoviruses replicates only in human cells. Recombination with other organisms is highly unlikely since this would need simultaneous replication of adenoviruses from different species in a same cell.

In case a healthy person would be exposed to the virus, it is unlikely that it would cause an infection due to the cancer specific nature of ONCOS-102. However, even in the case of infection, the symptoms would be mild, mainly flu or mild gastrointestinal symptoms.

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

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

Give details

Compared to wild type adenoviruses, the pathogenicity, survivability and ability of ONCOS-102 to evade the host immune system of have been decreased.

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

Human adenoviruses replicate only in human cells. ONCOS-102 is anticipated not to interact with other organisms due to the conditions of the proposed release. ONCOS-102 will be confined to the hospital site, including the treatment room, pharmacy, clinical laboratory, and biohazard waste area.

A secondary transmission of ONCOS-102 could potentially originate from shedding. The treated patients may shed ONCOS-102 to sewage water or to their home environment. However, shedding will be unlikely as there were only rare cases of transient secondary transmissions of ONCOS-102 during the C1 study. Furthermore, the stability and survivability of the virus is decreased compared to the wild type, and its infectivity will be shortly lost. It is anticipated that shed virus or possible recombinants would be non-infectious to other than humans.

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 | … |

Medical professionals may get puncture wounds during administration and they may get exposed to spills by accidents. A secondary transmission may occur in patients' family members. Infection would be harmful for instance in immunocompromised hosts and neonates, but patients as well as healthcare personnel belonging in the risk groups will be excluded from study participation.

7. Likelihood of genetic exchange in vivo

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

Highly unlikely.

There is minimal potential for gene transfer to other species under the proposed release of the GMO. The GMO will be released to be administered to patients in hospital operating rooms and is unlikely to come in contact with other animal species.
There is minimal potential for genetic exchange with other human species C adenoviruses as they are endemic in humans. Recombination has been found to shuffle genome fragments within adenovirus species, but not between species.
The opportunity for genetic recombination with animal adenoviruses is probably low since, the recombination event is rare even in vitro settings.

(b) from other organisms to the GMO:
Highly unlikely.

(c) likely consequences of gene transfer:
ONCOS-102 has been designed so that any possible (but unlikely) result of a genetic recombination with a wild type virus is either safer than or as safe as the wild type virus, as an example, the insert (GMCSF) makes ONCOS-102 more visible to the immune system, therefore resulting in efficient clearance of the recombined virus from humans.

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.):
No data are available regarding the behaviour and characteristics of ONCOS-102 in the mentioned environments.

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

## H. Information relating to monitoring

1. Methods for monitoring the GMOs

Monitoring of the direct and indirect effects of the GMO in all patients will be achieved using physical examinations, adverse event reporting, and clinical laboratory assessments throughout the clinical study.
In addition, for the shedding analysis, the presence of infectious virus particles in a sample can be determined by a common, qualitative virus culture method.
The method is not specific for ONCOS-102. Instead, it detects all adenovirus serotypes from the sample and the positive result needs to be verified by identifying ONCOS-102 by qPCR or other methods. However, the method is scientifically sound and appropriate for the purpose.

Specific and highly sensitive qPCR method can be used for identification of ONCOS-102 specific modifications from swab samples taken from the working surfaces.

2. Methods for monitoring ecosystem effects
…

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

It is anticipated, that ONCOS-102 does not integrate into the host genome. The vector DNA will remain episomal and will be eliminated when the cell divides or dies. In addition, ONCOS-102 replicates in human cancer cells but is attenuated in normal non-dividing cells However, the ONCOS-102 genome can be detected with PCR or qPRC methods from other organisms.

4. Size of the monitoring area ($m^2$)
… $m^2$
Not applicable: ONCOS-102 will be reconstituted in hospital pharmacy and administered to patients by intratumoral injections in operating rooms.

5. Duration of the monitoring
Safety assessments will be performed all along the patient's participation in the clinical trial. Safety will be assessed by collection of adverse events (AEs) as well as formal monitoring of pre-specified laboratory values, vital signs and other relevant variables.
Monitoring of the direct and indirect effects of ONCOS-102 in subjects will be achieved by the clinical assessments defined in the clinical trial protocol. Patients will be monitored throughout the treatment by the Study investigators.
An Independent Contract Research Organization (CRO) will be used for study monitoring and data management activities. Any serious adverse event will be reported in the appropriate time-frame to the Sponsor, and as required to each of the national regulatory agencies according to pharmaceutical legislation.

6. Frequency of the monitoring
The Clinical Trial Protocol requires, that the patients will stay for observation in the hospital for 24 hours after each application of ONCOS-102.
After each dose of DCVAC/PCa that is administered alone (i.e., at Weeks 11, 17, 29, 35, 41, 47, and 53), patients will be observed for at least 30 minutes after injection in a supervised out-patient setting.
Vital signs will be evaluated at every visit apart from leukapheresis.
Physical examination and Eastern Cooperative Oncology Group (ECOG) performance status will be evaluated at screening, Week 8, Week 23, and at the EOT visit.
Laboratory evaluation (hematology, biochemistry, and urinalysis) will be done at screening; at the Baseline visit; at Weeks 5, 8, 11, 17, 29, 41, and 53; and at the EOT visit. Coagulation (aPTT, INR) will be evaluated at screening.
Testosterone levels will be determined at screening.
TSH levels are to be measured at screening; at Weeks 11, 29, 53; and at the EOT visit.
Patients will be tested for HIV (HIV1Ab, HIV2Ab), HTLV, HBV (HBsAg, HBcAb), HCV antibodies, and syphilis at screening. National and local guidance and regulations should be followed for all these tests. An additional test before leukapheresis is required due to legislation applicable to DCVAC/PCa manufacturing.
PSA levels will be assessed at screening, at the Baseline visit, at Week 8, Week 11, and then every 6 weeks (+/- 3 days) until PSA progression. After PSA progression, PSA levels will be evaluated every 12 weeks (+/- 3 days) until the EOT visit, ICF withdrawal, or death.
Positron emission tomography (PET) with low-dose computed tomography (CT) will be performed at screening to localize viable tumor tissue.
Disease progression will be evaluated by bone scintigraphy and MRI/CT scanning of the chest, abdomen, and pelvis at screening and then every 4 months, using the same modality throughout the clinical trial.

Blood sampling for research purposes will be collected at the Baseline visit, Week 8, Week 23 (prior to the fifth dose of DCVAC/PCa and the sixth dose of ONCOS-102), Week 53, and at the EOT visit.
Tumor biopsies of one primary or metastatic lesion suitable for repeat biopsy will be performed at the Baseline visit and at Week 8.
Safety tests will include quantitative PCR testing from blood, urine and saliva samples and adenovirus cultures to detect vector shedding in urine and saliva samples (buccal swabs) Shedding samples are taken before and after injection at each ONCOS-102 treatment visit (weeks 5, 6, 7, 8, 14, and 23).

## I. Information on post-release and waste treatment

1. Post-release treatment of the site

ONCOS-102 will be handled primarily in a laboratory facility. The BSC is decontaminated first with Barrydin solution or other virus inactivating agent and then with 70% EtOH, Additionally UV-light can be used. The laboratory surfaces are cleaned by wiping with hospital grade disinfectant.
Following the patient's discharge home, all surfaces of the room and bathroom should be wiped down with hospital grade disinfectant.
If feasible, the patients-care equipment devices can be cleaned with Barrydin solution or other virus inactivating agent. A hospital-grade disinfectant can also be used.
Items such as dishes, utensils, textiles and fabrics will be washed with hot water (>70°C) and detergent. All waste should be autoclaved, incinerated, or treated with virus inactivating agent by personnel who are trained to dispose of biohazard waste.

2. Post-release treatment of the GMOs

All virus, that has been left unused is inside the closed administration assembly and it is disposed of in a manner consistent with the standard practice for biohazard sharps waste at the study site.
During the course of the clinical trial the used and unused extra vials will be destroyed at the site according to hospital practice for Risk Group 2 agents or according to guidance provided.

3. (a) Type and amount of waste generated

Biohazard waste types are: disposable and sharps.

Biohazard waste containers, bags and a puncture-proof sharp item containers are required both in the pharmacy/laboratory and in the treatment room.
The used ONCOS-102 vial and PhaSeal components are placed in a clearly marked biohazard waste container and later disposed. Other disposable waste including plastic and paper waste (covers from the disposables, used wipes and PPEs) are stored in a labeled biohazard bag prior to autoclaving and/or incineration.
Following administration, the complete administration assembly is placed in a puncture-proof sharps container and later disposed of in a manner consistent with the standard practice for biohazard sharps waste at the study site.

By using a closed system transfer device for reconstitution, the amount of waste and waste handling procedures are both minimized if compared to the earlier system. In addition, the risk of generation and inhalation of aerosols is minimized.

The estimated amount of waste per treatment is not much: One reconstitution/administration assembly, used PPEs wipes and covers from the disposables will not fill the waste containers. The waste can be collected from several treatments, if possible. For the study site facility, this will involve temporary containment in sharps bins or clearly marked biohazard bags, prior to autoclaving and/or incineration either on or off site as per local institutional guidelines for handling potentially infectious materials.

All equipment used during the procedure will be cleaned using a chemical disinfectant capable of virucidal activity for the required duration of contact (specified by the manufacturer). Items such as dishes, utensils, textiles and fabrics will be washed with hot water (>70°C) and detergent.

3. (b) Treatment of waste

Universal biosafety practices are followed by medical facilities when handling injectable medicinal products and medical waste. Typically, standard operating procedures for disposal within medical facilities will be consistent with the guidance given in the WHO Laboratory Biosafety Manual, 3rd Ed (2004) as outlined below:

Contaminated (infectious) "sharps"
After use, hypodermic needles should not be recapped, clipped or removed from disposable syringes. The complete assembly should be placed in a sharps disposal container. Disposable syringes, used alone or with needles, should be placed in sharps disposal containers and incinerated. Sharps disposal containers must be puncture-proof/-resistant and must not be filled to capacity. When they are three-quarters full they should be placed in "infectious waste" containers and incinerated.

Contaminated (potentially infectious) materials for autoclaving:
Apart from sharps, which are dealt with above, all contaminated (potentially infectious) materials should be put to marked containers and incinerated.

Other contaminated (potentially infectious) materials:
Biosafety cabinet shall be decontaminated using first a chemical disinfectant capable of virucidal activity then with 70% EtOH following preparation and dosing of ONCOS-102. Work surfaces in the pharmacy shall be decontaminated using a chemical disinfectant capable of virucidal activity following preparation and dosing of ONCOS-102. Precautions outlined above shall be adhered to when administering the product or when dealing with accidental spillages and breakages.

Environmental surfaces, hospital rooms, patients' care areas, patients-care equipment devices should be routinely cleaned with a virucidal disinfectant based on Desinfection plan for the hospital department. All waste should be incinerated, or treated with virus inactivating agent by personnel who are trained to dispose of biohazard waste.

## J. Information on emergency response plans

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

Eye accidents involving ONCOS-102

In case of an accidental occupational exposure through a splash to the eyes, remove the protective gloves that might be contaminated and flush your eyes with eyewash or clean water for at least 15 minutes. See a healthcare provider for signs of systemic (mainly flu-like or mild gastrointestinal symptoms, fever) or local infection (e.g. pain, redness and swelling).

Puncture wounds and cuts
The safe use of needles is shown to prevent puncture wounds and cuts. The standard operating procedures for disposal of contaminated needles within medical facilities will be consistent with the guidance given in the WHO Laboratory Biosafety Manual, 3rd Ed (2004). In case of exposure to needle stick, remove the protective gloves and check if the skin was punctured. Clean the site thoroughly with a virucidal disinfectant such as 2% Barrydin solution. In case of broken skin, wipe the site additionally with antiseptic solution and a sterile cotton pad. See a healthcare provider for signs of systemic (mainly flu-like or mild gastrointestinal symptoms, fever) or local infection (e.g. pain, redness and swelling).

Splatters on the skin or mucous membranes
All personnel handling the virus or material contaminated with ONCOS-102 must observe safety precautions; they have to wear a laboratory coat, sleeve covers and gloves. None of the study staff (e.g., pharmacists, radiologists, nurses) with open skin wounds should come into direct contact with ONCOS-102.
In the event of exposure to healthy skin, clean the site with a tissue moistened with virucidal disinfectant such as 2% Barrydin solution or 1% sodium hypochlorite or Virkon®, and flush the site with clean water for at least 15 minutes. In the event of exposure to mucous membranes, flush the site with clean water for at least 15 minutes. See a healthcare provider for signs of systemic (mainly flu-like or mild gastrointestinal symptoms, fever) or local infection (e.g. pain, redness and swelling).

In case of splatters on clothing, remove the contaminated clothes: spray them with 2% Barrydin solution to inactivate the GMO. Put the single-use clothing and protective gear in the biohazard waste and see to the appropriate washing of the other pieces of clothing.

2. Methods for removal of the GMO(s) of the areas potentially affected
In case of spillage on surfaces, make sure that no outsiders expose themselves to the solution. If necessary, warn other people working in the same space. Always wear the necessary protective clothing (lab coat, protective gloves). Absorb the liquid in paper towels or other disposable towels and put them in an autoclavable biohazard waste bag. Wipe the contaminated area first with adequate amounts of virucidal disinfectant such as 2% Barrydin solution or 1% sodium hypochlorite or Virkon® followed by wiping with 70% ethanol Wash and disinfect your hands carefully first with hand soap and then with hand disinfectant solution. Dispose used PPEs and contaminated waste according to local procedures.

Spreading of GMO over a wide area
In case of spillage on surfaces or breakage of a vial, isolate the space where the accident happened (lock the door and put up a notice outside the space informing of the accident). Make sure that no outsiders expose themselves to the solution. Leave the room for 30 minutes. Always wear the necessary protective clothing (lab coat, protective gloves, glasses, and mask) when going back to clean the spill. Cover the spill with paper towels or other disposable towels. Start from the edge and go toward the center. Pour the virucidal disinfectant such as 2% Barrydin solution or 1% sodium hypochlorite or Virkon® over the

towels starting from the edge toward the center. Let the disinfectant affect for a sufficient contact time. Remove the towels and possible broken vials with gripping tools like forceps. Put the towels in an autoclavable biohazard waste bag and the broken vial into the sharps biohazard waste container, if possible. Wipe the contaminated area with adequate amount virucidal disinfectant followed by 70% ethanol. If the surface cannot be wiped, spray the area evenly with the disinfectant and rinse off the disinfectant by spraying with 70% ethanol. Discard the used PPEs and other contaminated waste in the biohazard waste bag and dispose in compliance with local institutional guidelines.

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
All adverse events occurring during the course of the study will be recorded and assessed by the hospital personnel and the study sponsor, and Health Authorities will be notified when applicable.
The potential direct effects on human health are limited to the transmission of ONCOS-102 to an unintended human recipient. These potential adverse effects are expected to be the same as those which may be anticipated in patients receiving the treatment, albeit much lower in intensity.
The potential indirect effects of the release are limited to the consequences of dissemination of ONCOS-102 from the site of injection, shedding, or the release of wild type adenovirus or genetic variants through contamination of the product during manufacture or following recombination in the recipient's cells, which are all highly unlikely to occur.

If shedding will occur, the exposure would be predicted to be transient and the amount of virus particles low compared to the doses received by patients in the proposed trial. In addition, exposed individuals will likely have been previously immunized with wild type adenovirus. Therefore, public health risks with ONCOS-102 are extremely low.

In the event of an undesirable effect, the treatment decisions should be individualized and done case by case. Because there is no specific treatment for adenovirus infection, the unintendedly exposed human recipient as well as patients suffering from adverse reaction, can be treated with supportive and symptomatic treatment to relieve the symptoms. For instance, pyrexia which is the most common adverse reaction, can be managed successfully with paracetamol (acetaminophen) or ibuprofen. Furthermore, several drugs, such as cidofovir, ribavirin, ganciclovir, and vidarabine, have been used to treat adenovirus infections, especially in immunocompromised patients. In addition, chlorpromazine and apigenin have been shown to prevent adenovirus replication (Kanerva, Raki, Ranki et al. 2001).

Potential effects on the environment could be shedding of ONCOS-102 to the environment or transfer of inserted genetic material into an animal virus. It is anticipated that shed virus or possible recombinants would be non-infectious to other than humans. The consequences of exposure to the environment are therefore minor. The risk of ONCOS-102 constituting an undesirable effect to the environment is very low.

Precautions listed below are taken in order to protect human health and environment:

Design of Viral Construct
Multiple safety features have been incorporated into ONCOS-102. The possibility for creation of stable genetic variants with unintended characteristics is minimized by the design of the ONCOS-102 genetic construct.

Control of Release
ONCOS-102 will only be supplied to approved study sites, where a qualified pharmacist with specific training on the protocol will be responsible for material receipt, storage, documentation of traceability of product at the investigational site, reconstitution on the day of administration and disposal. ONCOS-102 will be administered to subjects by trained medical professionals, in accordance with the clinical trial protocol.
The manufacture, supply and traceability of ONCOS-102 will be controlled and monitored in accordance with pharmaceutical regulations.
The product will be stored prior to administration in the pharmacy or at another appropriate secure location in a temperature monitored freezer at -60°C or below.
The investigational product will not be distributed to any person outside the terms and conditions set forth in the Clinical Trial Protocol. The study medication is to be prescribed by the Investigator or designee and may not be used for any purpose other than that described in the Clinical Trial Protocol.

Transportation precautions
The safe transportation, receipt and storage of ONCOS-102 is instructed in ONCOS-102 Supply Chain Manual. The Material Safety Data Sheet of ONCOS-102 includes instructions for handling spills and it accompanies all shipments.
For transportation of ONCOS-102, the packaging must have a visible label stating "genetically modified organism", or "this product contains a genetically modified organism". This text has to appear also in the accompanying documents during the transport.
Targovax has done a contract with World Courier considering the transport of ONCOS-102 under temperature-controlled conditions and according to Dangerous Goods Regulations of International Air Transport Association (IATA). ONCOS-102 is transported on dry-ice. Concerning the transportation of the reconstituted drug from the pharmacy to the treatment room, the ONCOS-102 Handling Guideline contains instructions for labelling and packaging the prepared dose in a hermetic plastic container, designated for ONCOS-102 administration syringes and marked with a biohazard sign for transport to the administration site. A separate spill kit follows along the transport in case of accidental spills.

Handling and Administration Precautions
Precautions for use are provided in the ONCOS-102 Handling Guideline, issued to the study site by Targovax. Administration will only be performed by trained medical professionals in an approved study site facility. Institutional guidelines for handling, personal protective equipment, accidental spills, and waste disposal should be followed during product preparation and administration.
In the event of accidental exposure, it is recommended to document the course of events as closely as possible (e.g. the amount of ONCOS-102 and persons included, time and place where the accident happened) to enable later reassessment.
Accidental exposures are reported according to local regulations. In addition, a separate form for reporting the accidents is provided to the clinical trial site by Targovax (CLIN-DOC-0680 Reporting instruction for accidental exposures).

Product Labelling

The product labelling and information contains essential information to minimize the risk of exposure to an unintended individual or the environment.
The ONCOS-102 Handling Guideline contains instructions for labelling and packaging the prepared dose in a hermetic plastic container, designated for ONCOS-102 administration syringes and marked with a biohazard sign for transport to the administration site.

Inactivation
Wild type adenoviruses are resistant to lipid disinfectants, but are inactivated by formaldehyde and chlorine. Variable inactivation occurs also with iodine and UV light. Viral DNA can be detected long after infectivity is destroyed. The genetic modifications of ONCOS-102 do not affect the sensitivity to physical and chemical inactivation.
Work surfaces shall be decontaminated using a chemical disinfectant capable of virucidal activity and 70% EtOH before and after preparation and dosing of ONCOS-102.
Physical inactivation: Adenovirus can be inactivated by heat. Heating to temperatures >56°C for 30 minutes or autoclaving will destroy the infectivity.
Chemical inactivation: Adenovirus virus can be inactivated by contact with 1:5 dilution of bleach for 1 minute or contact with alcohol-based hand gels for 2 minutes. Ethyl alcohol, at concentrations of 60%–80%, is a potent virucidal agent inactivating all of the lipophilic viruses and many hydrophilic viruses. 2.0 % Barrydin solution for 60 minutes or 4.0 % Barrydin solution for 30 minutes is recommended by the manufacturer for short time disinfection. 0.9% Virkon®S liquid is proposed for decontamination procedures of adenovirus types 5 and 6 with contact times greater than five minutes.

Communication of Risks and Precautions
Materials provided to the subjects contain essential information to minimize the risk of transmission to an unintended individual.
The ONCOS-102 Handling Guideline contains information including precautions and instructions for handling of genetically modified adenovirus, description of the method and the PPEs to be used, actions to take following accidental exposure, descriptions of the main symptoms of adenovirus infection, with instructions to inform a medical professional, in case of symptoms.

Monitoring Activities
The Clinical Trial Protocol requires, that the six patients enrolled in the safety phase will stay for observation in the hospital for 24 hours (pre-planned hospitalization) after each application of ONCOS-102.
Monitoring of the direct and indirect effects of ONCOS-102 in subjects will be achieved by the clinical assessments defined in the trial protocol. Patients will be monitored throughout the treatment by the Study investigators.
An Independent Contract Research Organization (CRO) will be used for study monitoring and data management activities. Any serious adverse event will be reported in the appropriate time-frame to the Sponsor, and as required to each of the national regulatory agencies according to pharmaceutical legislation.
Vital signs will be evaluated at every visit.
Laboratory evaluation (haematology, biochemistry, and urinalysis) will be done at certain time points.

References

Flomenberg, P. (2009). Adenovirus infections. Medicine, 37(12), 676-678.

Fueyo, J., C. Gomez-Manzano, R. Alemany, P. S. Lee, T. J. McDonnell, P. Mitlianga, Y. X. Shi, V. A. Levin, W. K. Yung and A. P. Kyritsis 2000. "A mutant oncolytic adenovirus targeting the Rb pathway produces anti-glioma effect in vivo." Oncogene. 19: 2-12.

Harui, A., Suzuki, S., Kochanek, S., Mitani, K. (1999). Frequency and stability of chromosomal integration of adenovirus vectors.

Kanerva, A., K. R. Zinn, K. W. Peng, T. Ranki, L. Kangasniemi, T. R. Chaudhuri, R. A. Desmond, M. Wang, K. Takayama, T. Hakkarainen, H. Alfthan, U. H. Stenman, D. T. Curiel and A. Hemminki (2005). Noninvasive dual modality in vivo monitoring of the persistence and potency of a tumor targeted conditionally replicating adenovirus. Gene Ther. 12: 87-94.

Kanerva A, Raki M, Ranki T, Särkioja M, Koponen J, Desmond RA, Helin A, Stenman UH, Isoniemi H, Höckerstedt K, Ristimäki A, Hemminki A. (2007). Chlorpromazine and apigenin reduce adenovirus replication and decrease replication associated toxicity. J Gene Med. 2007 Jan;9(1):3-9.

Liikanen, I., Ahtiainen, L., Hirvinen, M., Bramante, S., Cerullo, V., Nokisalmi, P., Hemminki, O., Diaconu, I., Pesonen, S., Koski, A., Kangasniemi, L., Pesonen SK., Oksanen, M., Laasonen, L., Partanen, K., Joensuu, T., Zhao, F., Kanerva, A., and Hemminki, A. (2013) Oncolytic Adenovirus with Temozolomide Induces Autophagy and Antitumor Immune Responses in Cancer Patients. Molecular Therapy (2013); 21 6, 1212–1223.

Maheshwari, G., Jannat, R., McCormick, L., Hsu, D. (2004) Thermal inactivation of adenovirus type 5. J Virol Methods. Jun 15;118(2):141-6.

McCormick, L., Maheshwari, G. Inactivation of adenovirus types 5 and 6 by Virkon. (2004). Antiviral Research 64: 27–33.

Norking, L.C. (2010). Adenoviruses. In Norking, L.C Virology: Molecular Biology and Pathogenesis. ASM Press

Ranki T., Joensuu, T., Jäger, E., Karbach, J., Wahle, C., Kairemo, K., Alanko, T., Partanen, K., Turkki, R., Linder, N., Lundin, J., Ristimäki, A., Kankainen, M., Hemminki, A., Backman, C., Dienel, K., von Euler, M., Haavisto, E., Hakonen, T., Juhila, M., Jaderberg, M., Priha, P., Vassilev, L., Vuolanto, A., Pesonen, S. (2014) Local treatment of a pleural mesothelioma tumor with ONCOS-102 induces a systemic antitumor CD8+ T-cell response, prominent infiltration of CD8+ lymphocytes and Th1 type polarization. OncoImmunology, 2014 Volume 3, Issue 10.

Robinson, C., & Echavarria, M. (2007). Adenoviruses. In P. R. Murray, E. J. Baron, J. Jorgensen, M. Pfaller & M. L. Landry (Eds.), Manual of Clinical Microbiology (9th ed., pp. 1589) ASM Press.

Robinson, C., & Echavarria, M. (2007). Adenoviruses. In P. R. Murray, E. J. Baron, J. Jorgensen, M. Pfaller & M. L. Landry (Eds.), Manual of Clinical Microbiology (9th ed., pp. 1589) ASM Press.

Vassilev, L., T Ranki, T Joensuu, E Jäger, J Karbach, C Wahle, K Partanen, K Kairemo, T Alanko, R Turkki, N Linder, J Lundin, A Ristimäki, M Kankainen, A Hemminki, C Backman, K Dienel, M von Euler, E Haavisto, T Hakonen, J Juhila, M Jäderberg, P Priha, A Vuolanto & S Pesonen. (2015). Repeated intratumoral administration of ONCOS-102 leads to systemic antitumor CD8+ T-cell response and robust cellular and transcriptional immune activation at tumor site in a patient with ovarian cancer. OncoImmunology Accepted author version posted online: 01 Apr 2015.