European Medicines Agency
Pre-authorisation Evaluation of Medicines for Human Use

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WITHDRAWAL ASSESSMENT REPORT FOR CONTUSUGENE LADENOVEC GENDUX

International Nonproprietary Name:
Contusugene Ladenovec

Procedure No. EMEA/H/C/1041

Day 120 Assessment Report as adopted by the CHMP with all information of a commercially confidential nature deleted.

This should be read in conjunction with the "Question and Answer" document on the withdrawal of the application: the Assessment Report may not include all available information on the product if the CHMP assessment of the latest submitted information was still ongoing at the time of the withdrawal of the application.

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LIST OF ABBREVIATIONS

AE	Adverse event
BSE	Bovine spongiform encephalitis
CHMP	Committee for Medicinal Products for Human Use
CMV	Cytomegalovirus
CPMP	Committee for Proprietary Medicinal Products
DMEM	Dulbecco's modified Eagle medium
DP	Drug product
DS	Drug substance
EMEA	European Medicines Agency
EudraCT	European Clinical Trials Database
FBS	Fetal bovine serum
FDA	Food and Drug Administration (United States)
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation of Technical Requirements
IU	Infectious units
LAL	Limulus amoebocyte lysate
mRNA	Messenger ribonucleic acid
MVB	Master virus bank
Pfu	Plaque forming units
RCA	Replication competent adenovirus
RT-PCR	Reverse transcription polymerase chain reaction
SOP	Standard operating procedure
UK	United Kingdom
US	United States
USA	United States of America
VWB	Virus working bank
WHO	World Health Organization

I. RECOMMENDATION

Based on the review of the data on quality, safety and efficacy, the CHMP considers that the application for Contusugene Ladenovec Gendux, a medicinal product in the treatment of adult patients with recurrent or refractory squamous cell carcinoma of the head and neck as monotherapy, is not approvable since "major objections" have been identified, which preclude a recommendation for marketing authorisation at the present time.

The major objections precluding a recommendation of marketing authorisation pertain to the following principal deficiencies:

- Clinical benefit of Contusugene Ladenovec Gendux was not demonstrated
- Excess mortality was observed in “unfavourable patients” under treatment with Contusugene Ladenovec Gendux.
- Clinical data on biodistribution, shedding and transmission, presented in the dossier, are judged to be not valid. Signals of biodistribution in various organs, body fluids shedding and transmission seen in the studies were not adequately addressed in the further development program of Contusugene Ladenovec Gendux.
- The safety data base does not allow comprehensive evaluation of the safety profile due to its small size and methodological limitations in generating the data
- New uncharacterized open reading frame (ORF) in the vector sequence
- Insufficient analysis of replication competent adenovirus (RCA)
- Unclear role of RCA for the mode of action of Contusugene ladenovec Gendux
- Lack of adequate biodistribution studies
- Possible germ line integration of vector DNA
- Lack of adequate repeat dose toxicity studies
- Insertion of an ORF of unknown impact
- Occurrence of p53-carrying RCA not excluded
- GMP certification of manufacturing site lacking
- Ratio of total particle number to infectious particle number insufficiently specified
- Stability of drug substance and drug product not demonstrated
- Consistency of production not demonstrated

Proposal for Inspection

The manufacturing site for Contusugene Ladenovec is undergoing GMP inspection in relation to procedure EMEA/H/C/914 for Advexin. An inspection of another manufacturer is also required in relation to the evaluation of Advexin. Outcome of these inspections are relevant to this procedure.

The assessment of the pivotal study T-301 revealed some GCP deviations: i.e. missing signature of the protocol, inconsistencies regarding the patient numbers and classification of the individual patients with respect to their p53 profile. A GCP inspection of study T-301 would be necessary but is not recommended at this stage in view of other major objections raised.

II. EXECUTIVE SUMMARY

II.1 Problem statement

The annual incidence of squamous cell carcinoma of the head and neck (SCCHN) is app. 72,000 cases per year in Europe. Risk factors for SCCHN include male gender, age > 50 – 60 years, use of tobacco and alcohol use.

Head and neck cancer can be divided into 3 groups: (1) early disease (Stages I and II), which is characterized by small primary T1 or T2 lesions with no clinically detectable lymph node involvement; (2) loco-regionally advanced disease (stages III and IV), which is characterized by large primary tumours, and possibly significant lymph node involvement, but no detectable distant metastasis; and (3) recurrent or metastatic disease characterized by local or regional recurrence after first-line treatment. Head and neck cancer is generally a local or loco-regional disease when diagnosed.

Most therapeutic interventions are aimed at loco-regional disease treatment and control.

The most important prognostic factor at the time of initial treatment is the stage of disease at presentation. If the patient presents with limited disease, as in 25% of new cases, the standard treatment is surgery, radiation, or the combination of these therapies.

Approximately 60 to 90% of these patients can be expected to be cured. On the contrary, for patients presenting with advanced disease (75% of new diagnoses), the failure rate following first-line therapy reaches 70% and the pattern of failure is primarily local and regional recurrence

II.2 About the product

Contusugene Ladenovec Gendux is a sterile suspension for injection, containing $1.1x10^{12}$ viral particles/ml, and is to be administered intratumourally. Each vial of Contusugene Ladenovec Gendux is formulated to contain $2.2x10^{12}$ viral particles in 2 ml of Dulbecco's phosphate buffered saline with 10% (v/v) glycerine.

The active ingredient is *contusugene ladenovec* (Ad5CMV-p53), an adenoviral vector containing a functional copy of the human p53 gene. The adenoviral vector was derived from adenovirus serotype 5 (Ad 5). The inserted p53 tumour suppressor gene is under control of the cytomegalovirus (CMV) promoter. Structurally, Contusugene Ladenovec Gendux consists of a protein capsid with the Contusugene ladenovec Gendux genome packaged as a nucleoprotein complex. Contusugene Ladenovec Gendux has been genetically engineered to render the vector replication incompetent. However, due to the manufacturing process a low level of infectious adenovirus particles are present in the product.

The indication applied for is as follows: "Contusugene ladenovec Gendux is indicated in adults for the treatment of patients with recurrent or refractory squamous cell carcinoma of the head and neck as monotherapy"

Contusugene Ladenovec Gendux is a gene transfer medicinal product. When introduced into target tissues, Contusugene ladenovec Gendux binds to cells where it becomes internalized and uncoated. Contusugene Ladenovec Gendux genomic DNA is then transported to the nucleus where it causes the production of p53 mRNA (messenger RNA) and p53 protein.

The proposed mechanism of action is that the p53 protein produced from Contusugene ladenovec Gendux triggers changes in the expression of numerous genes which, in cancer cells, activate cellular growth arrest and apoptosis. Additionally, Contusugene ladenovec Gendux may inhibit tumour angiogenesis and stimulate the host's immune response to the tumour. Cellular senescence and tumour clearance was described following transient restoration of p53 activity in a murine liver carcinoma model (Xue et al. 2007).

II.3 The development programme/Compliance with CHMP Guidance/Scientific Advice

Compliance with CHMP Guidance/Scientific Advice

N / A

Clinical development programme

The application contains four clinical studies to support the claimed indication "treatment of patients with recurrent or refractory squamous cell carcinoma of the head and neck as monotherapy".

- In two phase I studies (single arm) and one phase II study (dose controlled) to evaluate the safety and response to Contusugene Ladenovec Gendux treatment, a total of 207 patients with recurrent or refractory squamous cell carcinoma of the head and neck were enrolled.
- One open label randomised phase III study to compare the overall survival and safety of intratumoural Contusugene Ladenovec Gendux administration vs. methotrexate. The study was terminated prematurely after the enrolment of 123 patients with recurrent or refractory SCCHN.

Interim data of a further phase III study to compare the efficacy and safety of Contusugene Ladenovec Gendux treatment in combination with chemotherapy vs. chemotherapy alone in patients with recurrent SCCHN is also included in the submission.

An additional analysis including pooled data from T301 and T201 (a dosing study) is provided.

One phase II study in the same patient population was submitted to support the PK profile of Contusugene Ladenovec Gendux rather than the efficacy.

Additionally the study reports of five phase I / II studies in patients with solid tumours were provided as supportive data.

No development program in other special populations has been performed.

Paediatric development programme

No paediatric program development and no development in other special populations have been performed

II.4 General comments on compliance with GMP, GLP, GCP

GMP

The GMP inspection of the active substance manufacturer is on-going as part of the MAA procedure for Advexin (EMEA/H/C/914). The outcome of that inspection is relevant to this procedure. Currently there is no evidence of GMP compliance of this facility, only page 1 of a 22 page GMP inspection report for this manufacturer was provided in the dossier. Full GMP compliance must be demonstrated prior to approval of this procedure.

The formulation buffer is not manufactured by the finished product manufacturer; it is purchased from another manufacturer as a raw material. GMP compliance of this facility has not been provided. However, it is the assessor's opinion that this is unnecessary as it falls to the applicants QP and their QA procedures to ensure appropriate qualification of this supplier.

GLP

Non-clinical studies were mostly performed not in compliance with GLP. However several toxicological studies were conducted in compliance with GLP. In principle, this approach is regarded as acceptable except for pharmacokinetic studies addressing a possible germ line integration of vector DNA. Additional studies performed with respect to that issue should be conducted as GLP-compliant studies.

GCP

The applicant has stated that the clinical trials carried out outside the European Union meet the ethical requirements of Directive 2001/20/EC.

The study report of the prematurely terminated study T-102 is missing; this is a serious deviation form the GCP principles as laid down in ICH E6.

II.5 Type of application and other comments on the submitted dossier

The applicant Gendux Molecular Ltd submitted an application for Marketing Authorisation for Contusugene Ladenovec Gendux to the European Medicines Agency (EMEA), through the centralised procedure falling within the Article 3(1) and point 1 of Annex of Regulation (EC) No 726/2004.

The legal basis for this application refers to: Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application. The application is a complete dossier composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests.

The clinical part of the application is of poor quality, multiple errors and inconsistencies have been identified throughout the documentation.

III. SCIENTIFIC OVERVIEW AND DISCUSSION

III.1 Quality aspects

Contusugene ladenovec Gendux is a replication-impaired type 5 adenoviral vector, containing a functional copy of the human p53 gene under the control of the cytomegalovirus (CMV) promoter (Ad5-CMV-p53). It is intended for the treatment of patients with recurrent and or refractory squamous cell carcinoma of the head and neck as a monotherapy.

From a quality perspective, Contusugene ladenovec Gendux is the same product as Advexin for which an application for marketing authorisation was withdrawn by the applicant on 19 December 2008.

Drug Substance

Contusugene ladenovec Gendux is an adenoviral vector containing the p53 gene (Ad5-p53) intended for administration into tumours of adult patients with refractory squamous cell carcinoma of the head and neck. The serotype 5 adenoviral vector has been modified to replace the E1 region with a p53 expression cassette consisting of the cytomegalovirus (CMV) promoter, the human wild-type p53 gene, and an SV40 polyadenylation signal. Aside the therapeutic gene, the vector harbours an insertion of 646 base pairs of DNA in the E3 region. The inserted DNA is not identical to any DNA sequence in databases, human or otherwise. Only a small portion of 140 bp of the whole 646 bp insert shows a significant similarity to salmon DNA, which is supposed to originate from salmon sperm DNA historical used as a carrier in transfections during generation of Ad5-p53.

This deletion/insertion affects several open reading frames, which will on one hand impair expression of the viral E3 proteins and generates a modified E3 10.4K protein fused to an unknown peptide. On the other hand, a novel ORF is introduced with the inserted sequence. By RT-PCR, Gingras *et al*. (1996) demonstrated RNA expression from this ORF in cells transduced with Ad5-p53-derived vector particles. This may result in a putative protein of 132 amino acids, extending in the viral genome. The impact of the new protein on pathogenicity, immunogenicity, or allergenicity is not clear and was not sufficiently addressed by the applicant. The lack of any information on this concern is addressed as a major objection.

For production of the Contusugene ladenovec Drug Substance, the viral vector is amplified by infection of an adherent continuous cell line harbouring an Ad5 genome fragment containing the E1

region, flanked on both sides by additional viral sequences. This cell line thus complements for E1 deficiency and allows propagation of replication-defective adenoviral vectors. Due to homology between vector sequences and E1 flanking sequences in the cell line, homologous recombination events can occur and may often result in the formation of replication-competent adenovirus (RCA). Since RCA may lead to adverse events in patients, the occurrence of RCA should be minimised according to Ph.Eur. The specification of infectious RCA per dose seems to be acceptable. However, RCA were regularly detected in batches used in clinical trials and the significance regarding clinical effects should be discussed thoroughly. The RCA present in Contusugene ladenovec was not sufficiently characterized by the applicant. Besides the expected RCA type (Adenovirus containing the E1 but lacking the p53 sequence), RCA carrying a p53 expression cassette might occur. This would exhibit a major concern, since the effect of high level expression of p53 in normal cells as a result of infection may cause significant harmful effects. Moreover, the presence of p53 expressing RCA will influence the results of some of the analytical methods used like potency assay or p53 ELISA assay.

For manufacture of Contusugene ladenovec Drug Substance, cells are thawed from a working cell bank (WCB) and expanded in increasing large numbers and used to seed a bioreactor. After growth inside the bioreactor, cells are infected with the Ad5-p53 viral construct from an established Working Virus Bank. Following propagation of the virus, the infected cells are lysed using a lysis solution and the lysate is harvested, cleared by filtration, and then concentrated and diafiltered into a buffer appropriate for downstream chromatography. Residual host cell RNA and DNA and unpackaged viral DNA are enzyme digested, and the viral suspension is then filtered and purified by anion exchange chromatography. The column eluate material containing the purified Ad5CMV-p53 vector is concentrated by tangential flow filtration and then diafiltered against a Formulation Buffer. Diafiltered material is diluted with Formulation Buffer to obtain a target concentration of 1.1×10^{12} virus particles/ml. The diluted material is filtered into a flexible container to obtain the Contusugene ladenovec Drug Substance. The Drug Substance is stored at $\leq$ -60°C to be released for further manufacture of the Contusugene ladenovec Drug Product.

The analytical analyses performed on Contusugene ladenovec bulk Drug Substance before freezing are: p53 ELISA (identity), RCA (impurity), virus particle enumeration (potency), residual BSA (purity), HPLC-IEC (purity), residual host cell protein (purity), huDNA quantitation (purity). Assays to detect mycoplasma, bioburden, adventitious viruses or endotoxins are performed on unpurified harvest or the prefiltered bulk drug substance, respectively.
Several points regarding assay validation have not been satisfactorily addressed. Validation of the assay to quantify human DNA content or RCA determination in particular is unsatisfactory, with additional issues raised for a number of other assays.

Drug Product

To yield the sterile final bulk Drug Product, bulk Drug Substance is thawed, sterile filtered and filled into borosilicate glass vials. The product is tested for identity (p53 ELISA, pH, osmolality), purity (HPLC, sub-visible particles), potency (bioactivity, virus particle/ IU ratio, virus particle enumeration), safety (bacterial endotoxins, sterility, bulk sterility). In accordance with Regulation 726/2004 release testing of Contusugene ladenovec is conducted in the EU.

In the course of Contusugene ladenovec development, a number of changes have been introduced to the production process. The latest production process (designated as “Commercial process”) differs from previous processes and up to now was not used for production of lots with which clinical data were generated.

The applicant did not sufficiently validate the final manufacturing process. It should be confirmed that the acceptance criteria for the critical/key steps identified for the process are satisfactory and the ranges stated do not impact on product quality. Furthermore the validation exercise should confirm that the company has adequate control of the process and that is operates in a consistent manner which was not demonstrated.

Product comparability through out clinical development (taking into consideration the manufacturing changes made) is not conclusively shown. This is pertinent to product purified using chromatography resins that are not now used for purification purposes.

A major change in the Commercial Process is revealed in the dossier. The formerly used assay to assess the infectious titre is replaced by an infectious unit (IU) assay. Since the sensitivity of this assay to detect infectious particles is higher than the previous assay (about 9-fold), the respective specification (total particle/ infectious particle ratio) may not be maintained but should be adjusted accordingly to guarantee lot-to-lot consistency.

Stability studies for drug substance and drug product manufactured according to the commercial process are initiated, but not completed yet. However, available data on the stability of drug substance and drug product indicate a significant loss of infectivity over 10 month (drug substance) and 3, 6 and 9 months (drug product), which can only be assessed after the specification for total particle/infectious particle ratio is clarified. At present, stability is shown, neither for drug substance nor for drug product.

The Drug Product is administered intratumourally. Depending on the tumour size, the SPC foresees a dilution of Contusugene ladenovec with Dextrose 5% water. The volume to be administered per dose of Contusugene ladenovec ($2.0x10^{12}$ viral particles) is recommended to be 5-30 % of the total estimated volume of the lesion to be injected. Dextrose 5% Water, USP (D5W) is intended to be used as diluent.

Summary on adventitious virus/TSE safety

Contusugene ladenovec Gendux is produced in a human recombinant cell line. Except foetal bovine serum (FBS) no other material from animals with a TSE risk are used at production. Compliance of FBS with "Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products" (EMEA 410/01 rev02) has been demonstrated by a TSE certificate from the EDQM.

Cell banks and virus banks used for production of Contusugene ladenovec Gendux have been extensively screened on virus contamination. Potential adventitious virus contamination during production of Contusugene ladenovec Gendux is controlled by routine testing of virus harvests on extraneous agents following the principles of Ph. Eur 2.6.16 (tests for extraneous agents in viral vaccines for human use).

To summarize, from the quality point of view, a positive opinion cannot be given to this product as a number of major concerns have been identified. The comparability of the final production process with that used in clinical trials, the stability of the product, and absence of p53-carrying RCAs has not been demonstrated. Validation of analytical assays to characterize drug substance and drug product is unsatisfactory. Validation of the manufacturing process for Drug Product is also incomplete. The significance of genome regions of unknown origin is not clarified, and the particle to infectivity ratio has not been satisfactorily justified, as the increased sensitivity of the infectivity assay to be used for release of commercial product over that which has been used to release product used in clinical study has not been confirmed. Furthermore, there are a number of other concerns that need clarification or additional information as described below.

III.2 Non clinical aspects

Pharmacology

The pharmacodynamic properties of Contusugene ladenovec Gendux were addressed by in vitro and in vivo studies. In vitro, various cancer cell lines, mostly characterized by a mutant or deleted p53 gene were analysed with respect to their reaction towards transduction with the p53-encoding adenoviral vector. Cell lines derived from SCCHN tumors as well as other cell lines were investigated. Results from these in vitro studies demonstrate that treatment with Contusugene ladenovec Gendux resulted in expression of p53, increased expression of p53-regulated proteins (i.e. p21), increased apoptosis and decreased cell proliferation and thus support the proposed mechanism of action for Contusugene ladenovec Gendux. However, as stated in the clinical part of the dossier, but not discussed in the non-clinical part, the applicant claims Contusugene ladenovec Gendux to be favourable in tumor patients with non-mutated wild type p53 and unfavourable in tumor patients with

mutated p53, also showing positive immunohistology. This mode of action is not addressed in the non-clinical part of the dossier.

In parallel to the analysis described above, also formation of replication competent adenovirus (RCA) was assessed in some of the described studies. RCA were either not detectable or present at low amounts (1-20 in $3x10^{10}$ vp). However, the control vectors AV1.0CMV and AV1.0CMV.βGal/Luc, expected to be as replication incompetent as the therapeutic vector, have been shown to replicate and to produce infectious progeny virus after transduction of e.g. H1299, HCT116, and ECV304 cells. The scientific reasons and consequences of this finding are not adequately addressed in the present dossier and should be further elaborated by the applicant.

Pharmacodynamic studies were also performed in vivo, mainly applying xenograft tumour mouse models. Various human cancer cell lines, mutated or deficient for p53 or over expressing p53 inhibitory proteins, were transplanted into immunodeficient mice, and were treated with Contusugene ladenovec Gendux either in a monotherapeutic approach or in combination with common chemotherapeutic drugs. Application routes included repeated intra tumoural (IT) and intra peritoneal (IP) injections. Dependent on the respective cancer cell type and the applied dose, the percentage of cells infected with Contusugene ladenovec Gendux was in the range of 2% to 50% of tumour cells, with a maximal rate of 75% after 12 h. Expression of p53 and subsequent apoptosis of cells could be demonstrated in transduced tumours by RT-PCR, immunohistochemistry and TUNEL staining for several days up to one month.

However, the efficacy of a monotherapeutic treatment with Contusugene ladenovec Gendux appeared to be limited, being characterized mostly by a tumour growth delaying activity rather than tumour regression. Particularly, therapeutic effects of Advexin, applied IT as a monotherapy in nude mouse models of cancer were observed at doses of $5x10^{12}$ to $5x10^{13}$ vp/kg, which is by far exceeding the intended clinical dose ($3x10^{10}$ vp/kg). Thus, the proof of concept (for tumours showing mutated or deficient p53; see above) in principle may be achieved, but several findings may raise concerns regarding the efficacy of the product and need to be settled by appropriate clinical data.

In addition to the described monotherapeutic approaches, Contusugene ladenovec Gendux was also investigated for a tumour inhibiting capacity in combination with various cancer chemotherapies (cisplatin, cyclophosphamide, doxorubicin, docetaxel, 5-FU) in nude mouse xenograft models. Depending on the tumour type and virus dose partial and complete tumour responses, i.e. tumour regression, were observed in these studies. In accordance with the *in vitro* data described above, anti-tumoural activity of Contusugene ladenovec Gendux in combination with chemotherapeutic drugs in some studies appeared to be synergistic.

Control vectors (AV1.0CMV and AV1.0CMV.βGal/Luc) in monotherapeutic as well as in combinatorial approaches showed slightly lower but similar tumour inhibiting effects as the applied therapeutic Ad-p53 vector. It is argued in the dossier that this effect may be attributable to the higher extent of replication competent viruses present in the control vector preparations. On the other hand, it was demonstrated that addition of RCA to Contusugene ladenovec Gendux does not influence the therapeutic efficacy of Contusugene ladenovec Gendux. These results appear somehow conflicting, since the first observation hint at a therapeutic effect of RCA, while the second one may argue against such an effect. In conclusion, the anti-tumoural activity observed with the control vector is not sufficiently understood and should be explored further, to elucidate any possible impact on the mode of action of Contusugene ladenovec Gendux. In particular, any contribution of RCA to the therapeutic effect of Contusugene ladenovec Gendux needs to be clarified.

Pharmacokinetics

The pharmacokinetic properties of Contusugene ladenovec Gendux were addressed by investigating the biodistribution, persistence and potential genomic integration of vector DNA and the expression of the encoded protein. Distribution of adenoviral DNA was analyzed in mice and rats which in principle have been justified as adequate animal models. Contusugene ladenovec Gendux in various doses was administered once, mainly by subcutaneous (SQ) injection, but also by intravenous (IV) and intratumoural (IT) injection, in normal animals and in mice with xenograft tumours. These different routes of administration include the intended clinical intratumoural route.

Results obtained from the performed biodistribution analyses show some variability. This, at least partially, may result from methodological deficiencies. The applied assays, i.e. qualitative PCR, immunohistochemistry and luciferase assays were with one exception neither qualified nor validated. Particularly PCR results revealed a wide range of sensitivities, which may be explained by a historically different technical state of the art during the development of the product. None of the relevant studies (SQ, IV, IT) was performed according to GLP, as it is requested for non-clinical safety studies addressing the risk of germline integration.

Dependent on the respective study either local concentration or a more systemic distribution of Contusugene ladenovec Gendux was observed. After SQ administration, vector DNA was always detected at the injection site, and in some animals also in adrenal gland, kidney, heart, liver, lung and spleen. However, after IV administration, systemic distribution of Contusugene ladenovec Gendux was much more prominent and vector DNA additionally was detected in bone, brain and gonads. Intratumoural administration led to a distribution pattern very similar to the one after iv administration. In these short term studies, vector DNA usually persisted in the organs analysed throughout the study duration of 1 month, while it didn't persist in blood samples beyond day 28. Long term studies up to 12 months were performed to further address persistence of vector DNA. As a worst case scenario, vector containing luciferase as a transgene was included in these studies, in order to exclude any apoptosis in transduced cells due to p53. Following iv administration, all organs appeared negative after 1 year; vector DNA was detected in ovaries up to 180 days and in testes up to 90 days. No vector DNA was detected in gonads after sc administration.

Irrespective of this result, no further investigations with regard to inadvertent germ line transfer or genotoxicity were performed. The applicant argues that adenovirus is a non-integrating virus and no signal was detected in gonads after sc administration, which is regarded to best mimic the clinical route of application, i.e. intratumoural. However, this approach appears not to be in line with the study results which indicate that intratumoural administration results in a biodistribution similar to the one after IV administration. It may be argued that xenograft tumours in mice are different from human tumours. Though this may be acknowledged, it may not provide sufficient justification to omit further studies to address the detection of vector DNA in gonads, especially with respect to germline transmission. Lack of such data is not acceptable, unless a scientific sound justification is provided.

With regard to biodistribution, no comprehensive study was provided reflecting the intended clinical application of Contusugene ladenovec Gendux, i.e. intra-tumoural injection with repeated dosing in more than one cycle for a reasonable time span (6 month). Since data indicate a long-term persistence of vector DNA, at least in some organs, the repeated injections may result in accumulative effects, which may also have an impact on the biodistribution. Thus, biodistribution after repeated administration, at least SQ and IT, has to be addressed, maybe in combination with corresponding toxicology studies.

In conclusion, the data presented are not regarded sufficient to adequately address the biodistribution of Contusugene ladenovec Gendux. Further data/studies have to be provided. Particularly, a comprehensive study mimicking the human dosing schedule, with appropriate safety margins, and the route of application is required, applying justified and sensitive detection assays. Since Contusugene ladenovec Gendux DNA can be detected in gonads after IT and IV application for at least 6 months, further investigations (cell fractionation studies, in situ analyses) are required by the respective guidance document in order to exclude transfer/presence of vector DNA in germline cells.

Toxicology

Toxicological studies were performed in normal non-tumour bearing mice and rats, in order to avoid the problem to differentiate between disease progression in the animal and toxic effects of the product. Corresponding to pharmacokinetic studies, the SQ and the IT route were utilized for administration of Contusugene ladenovec Gendux. While the sc route again is regarded as to best reflect the clinical use of Contusugene ladenovec Gendux, the IT route is included as a worst case scenario. Special attention was given to the organs identified in pharmacokinetic studies as possible target organs for Contusugene ladenovec Gendux, i.e. liver, lung, spleen, heart, adrenal gland and kidney.

Standard toxicology study parameters were evaluated in these GLP-compliant toxicology studies, including mortality, clinical signs, body weight, food consumption, haematology, serum chemistry, urinalysis, gross pathology and histopathology. No serious toxic effects were observed, when Contusugene ladenovec Gendux was injected subcutaneously into mice. The NOEL in this case was determined to be $3.7x10^{12}$ vp/kg. Data obtained after intravenous injection indicated the liver to be the most sensitive organ showing elevated serum enzyme levels and hepatocellular cytomegaly. Also the spleen and the blood circulation (decrease in lymphocytes and depletion of platelets) appeared to be affected. A local reaction at the injection site characterized by infiltration of inflammatory lymphocytes was also observed. After systemic application, a NOEL of $3.7x10^{10}$ vp/kg was established in study 01-001-015, being two orders of magnitude lower than the NOEL derived from the above described study after subcutaneous injection.

In summary, single dose toxicity analyses were performed in compliance with GLP and utilising routes of application that appear to be acceptable. The NOEL ranged from 100 fold above the clinical dose, after sc administration to only 1-fold, after intravenous administration. However, since the distribution of Contusugene ladenovec Gendux after intratumoural application rather compares to the IV approach than to SQ application, the lower NOEL may be more relevant.

Repeated dose toxicity was addressed in several pharmacodynamic studies using various administration schedules of Contusugene ladenovec Gendux, including schedules quite closely reflecting the intended clinical use of Contusugene ladenovec Gendux. Various animal models were used including animal tumour models, which were treated with Contusugene ladenovec Gendux as a monotherapy or in combination with various chemotherapeutics. However, toxicological analysis in these studies was partly limited to only a few gross pathology indicators like body weight. Based on these parameters, treatment with Contusugene ladenovec Gendux revealed no major signs of toxicity, especially when compared to the treatment with chemotherapeutics alone. In three studies, where also histopathology was performed, no tissue pathologies were observed in liver, spleen, lung, heart and kidney. In one study, Contusugene ladenovec Gendux alone was administered intraperitoneally in cotton rats and resulted in single cell necrosis in liver cells, increased serum levels of liver enzymes and some changes in spleen. In this study Contusugene ladenovec Gendux was administered only twice and observation period was only 15 days.

In conclusion, repeated dose toxicity is not adequately addressed in the dossier. Limited safety data were collected or the study design does not adequately reflect the intended clinical use of Contusugene ladenovec Gendux i.e. $2.2x10^{12}$ vp injected IT twice weekly, until progression of the tumour. Additional data addressing the toxicology of Contusugene ladenovec Gendux are needed. Such studies should reflect the intended clinical administration schedule and duration of treatment and may also address the issue of germline transmission. Concerning the observed cytotoxic effects, such as single cell necrosis and lymphocyte depletion, these might be due to p53 transgene expression.

III.3 Clinical aspects

Pharmacokinetics

Conventional pharmacokinetic studies to investigate absorption, distribution, metabolism and excretion are not relevant for this type of product.

However, data on biodistribution and transmission pattern of Contusugene ladenovec Gendux were investigated in two phase I / II studies (INT-001 (NSCLC) and INT-002 (SCCHN)) and three phase II studies in recurrent SCCHN (T-207, T-201, and T-202).

Contusugene ladenovec Gendux (DNA and infectious particles) was present in urine, sputum and faeces by one day after administration, and was eliminated in these body fluids in an average of about one week. Urine and sputum seem to be the major routes of elimination of Contusugene Ladenovec Gendux.

Samples were obtained at necropsy from selected patients enrolled in NSCLC study INT-001 (N=6) and SCCHN study INT-002 (N=2). Each of the 8 patients had received repeat doses of Contusugene ladenovec Gendux (2 – 6 doses). Vector DNA was recovered from selected organs, most notably the

site of treatment, for up to 91 days following injection. In the single case examined for which stringent sample collection methods were implemented, vector DNA was recovered from flash-frozen samples from the treatment site, liver, thyroid, a lymph node metastasis, kidney, and a brain metastasis. The presence of vector in liver and other organs is consistent with the analyses of body fluids collected from patients on both these studies; that vector is distributed widely following IT administration.

Regarding the presence of vector DNA in the brain metastasis, it is not uncommon for the blood-brain barrier to be disrupted in patients with CNS disease. Therefore, it is unknown whether vector DNA crossed the blood-brain barrier or was present in the vasculature of the tumour.

Taking the nature of the product into consideration the study program on pharmacokinetics (biodistribution) is adequate. However several flaws were identified in these study reports, which scrutinize the validity of the data. Especially regarding study T-207, the investigator discussed sampling errors in the conduct of the study. E.g. the putatively positive samples from household contacts were declared negative due to numerous procedural errors. This questions the validity of the laboratory data and thus the entire study results

It would have been expected that the signals observed in the early studies such as presence of Contusugene ladenovec Gendux in body fluids for several days would have been taken seriously and adequately followed up; in particular the risk of transmission should be adequately examined in a well conducted study.

Pharmacodynamics

No studies with a primary objective of determining PD effects in humans were conducted.

No prospectively designed studies to elucidate the pharmacodynamic properties were conducted.

The Applicant provided a pooled biomarker analysis of two studies (T-301 and T-201). This analysis was performed in order to identify a population expressing a favourable biomarker profile for the efficacy of Contusugene ladenovec Gendux treatment and it was performed post-hoc using different response definitions from the ones originally applied. Their interpretation is further limited by the small sample size and the questionable validity of the data.

It is suggested that Contusugene ladenovec Gendux treatment results in the induction of biomarkers for cell cycle arrest, apoptosis and senescence leading to tumour response defined as complete response (CR), partial response (PR) or stable disease (SD). Of note, the definition of the tumour response was done post-hoc. The change in the definition of tumour response was driven by a publication (Xue et al. 2007 Nature 445: 656-660), describing cellular senescence and tumour clearance following transient restoration of p53 activity in a murine liver carcinoma model. This modification of the criteria for assessment of the treatment response leads to a higher number of responders than originally reported by the investigators.

Taking also into account that multiple sample errors were reported for some of these studies and the reservations of the investigator, these data have limitations. At best, the data may be seen as hypothesis generating and need to be confirmed in larger well designed GCP compliant clinical studies.

In summary, the Applicant failed to demonstrate convincingly the correlation of p53 expression in tumours and clinical response to Contusugene ladenovec Gendux treatment. The limitations of the analysis are overall validity of the data, post-hoc defined subgroup analyses and small sample size.

In one publication (breast cancer study), local activation of innate immune response and mild inflammatory changes followed by activation of adaptive immunity after administration of Contusugene ladenovec Gendux were reported. Apparently such findings have not been reported in breast tumours treated with conventional chemotherapy. The relative contribution of this immunomodulatory phenomenon to the overall benefit of the treatment is yet unknown. Furthermore, the Applicant failed to evaluate the safety aspect of these findings with regard to the presence of RCA in the final product.

Taking the above into account, the Applicant failed to substantiate the presumed mode of action for Contusugene ladenovec Gendux.

Clinical efficacy

The Applicant submitted four clinical studies in patients with recurrent or refractory squamous cell carcinoma of the head and neck (SCCHN) to support the proposed indication. These studies include phase I / II studies as well as one pivotal phase III study.

Efficacy Studies in Recurrent SCCHN

Study	Number of Patients (ITT)	Phase	Type of Study/ (Mean Dose)
INT-002	34	Phase I	Single arm, uncontrolled (5.6×10^{11} vp)
T-202	61	Phase II	Uncontrolled (3.7×10^{10} vp)
T-201	112	Phase II	Dose controlled, (1.6×10^{12} vp)
T- 301	123 (63 Contusugene ladenovec Gendux)	Phase III	Randomised active controlled vs. methotrexate (2.0×10^{12} vp)

One further study (T-207) was submitted to support the PK rather than the efficacy. However, since secondary objectives were to evaluate the objective response rate (CR or PR), duration of response, time to progression and overall survival, this study is also discussed as supportive study.

One further study in patients with SCCHN (T-102) was terminated prematurely, no data are available.

Study T-301

Study T-301 was an open-label phase III study to demonstrate the clinical benefit of Contusugene ladenovec Gendux in comparison to methotrexate in patients with recurrent, refractory SCCHN.

The primary efficacy endpoint was overall survival time. Loco-regional objective response rate and tumour growth control (TGC = CR + PR + SD) were considered as major secondary endpoints

The study was prematurely stopped having enrolled only about half the patients planned. As median survival was even shorter in patients treated with Contusugene ladenovec Gendux (4.4 months) than in patients treated with methotrexate (6.1 months) the study failed to prove efficacy in the protocol pre-specified patient population. Furthermore, in this population the findings for various efficacy parameters are consistently not providing evidence of any advantage of Contusugene ladenovec Gendux over methotrexate. On the contrary, there are indications that Contusugene ladenovec Gendux might harm patients. A possibly detrimental effect of Contusugene ladenovec Gendux is reported for the so called ‘clinical biomarker’ population. In this population the median survival time was 4.5 months in the Contusugene ladenovec Gendux group compared to 7.3 months in the methotrexate group.

Biomarker analysis

A population expressing a favourable biomarker profile for efficacy of Contusugene ladenovec Gendux treatment was identified.

The current definition favourable and unfavourable contradicts definition given in the previous submission of “Advexin for the treatment of Li-Fraumeni cancer patients”.

Currently patients with “wild type p53” either “negative” or “positive” are classified as “favourable”. Patients with “mutated p53” “positive” are classified as “unfavourable”. According to the definition applied in the previous submission patients with “mutated p53” “positive” would have been expected to benefit from the treatment with Contusugene ladenovec Gendux, whereas patients with the profile “wild type p53” “negative” would have been expected not to benefit from treatment with Contusugene ladenovec Gendux.

Of note the classification is of high importance, since in the recent submission an excess mortality was observed in “unfavourable patients” under treatment with Contusugene ladenovec Gendux.

The applicant is requested to clarify when the final definition “favourable/ unfavourable” as proposed in the current submission was made and clarify convincingly the obvious discrepancies in the definition of the “favourable biomarker profile” between both submissions. The applicant is requested to explain the potential mechanism of this outcome and justify the inclusion of patients with a p53 unfavourable profile in further studies e.g. study T-302.

Supportive study(ies)

- **Study T-302**

Study T-302 is a multicentre open-label randomized phase III study. The Applicant provided treatment related interim information on this ongoing study.

The objective of this study is to compare Contusugene ladenovec Gendux on top of chemotherapy with chemotherapy alone with respect to progression-free survival in patients with SCCHN.

This study started in April of 2001. Information on 89 patients (49 patients Contusugene ladenovec Gendux +chemotherapy, 40 patients chemotherapy) is included into the interim report.

No information on progression free survival is given. However, according to the patient death information with the interim report, 18 of the 89 patients have died so far. The reported death rate is higher in the Contusugene ladenovec Gendux+ chemotherapy group (13/49) than in the chemotherapy alone group (5/40)

The interim information from study T302 does not provide evidence of a beneficial effect of Contusugene ladenovec Gendux when added to chemotherapy in SCCHN patients. On the contrary, a higher mortality in the Contusugene ladenovec Gendux+ chemotherapy group is observed (number of death: secondary efficacy parameter). Thus Contusugene ladenovec Gendux might harm patients when added to chemotherapy.

- **Study T-201**

Study T-201 was an open label multicentre randomized phase II study to evaluate safety and objective response rate (CR + PR) of two dosing schemes of Contusugene ladenovec Gendux in patients with recurrent squamous cell carcinoma of the head and neck (SCCHN)

One hundred seven patients were treated with Contusugene ladenovec Gendux. An overall objective response rate of 7.1% at treated sites was reported. Tumour size was found to have a significant impact on survival.

- **T-207**

Study T-207 was an open label multicentre randomised study to evaluate the biosdistribution transmission effectiveness and safety of two treatment regimes of Contusugene ladenovec Gendux administered by intra-tumoural injections in patients with advanced squamous cell carcinoma of the head and neck (SCCHN).

No patients showed a CR or PR to study treatment.

- **T-202**

Study T-202 was a single-arm, multi-centre study open-label study to estimate the objective response rate of Contusugene ladenovec Gendux in patients with advanced squamous cell carcinoma of the head and neck. One patient (2.0%) showed a partial response to study treatment at all treated sites.

- **Study INT-002 (SCCHN)**

Study INT-002 was a non randomized, single-centre, open-label, parallel group, dose-escalation study in patients with resectable or non resectable SCCHN.

In the non resectable arm 2 patients showed PR, both at the higher dose. At the end of the study 6 patients showed SD, and 9 patients had disease progression. The 15 resectable patients were not evaluated for lesion response because the indicator lesion was removed. Median survival time was greater in the resectable arm (408 days) than the non-resectable arm (127 days).

Based on these data, it was concluded that a Phase II dose of $2x10^{11}$ vp to $2x10^{12}$ vp is indicated.

Studies in other solid tumours

Additionally the study reports of five phase I / II studies in patients with solid tumours were provided as supportive data. One of these studies (T-104) was terminated prematurely; a final study report was issued.

Due to methodological problems or small sample size no conclusion on the efficacy of Contusugene ladenovec Gendux can be made.

Analysis performed across trials

The applicant provided an ISE of studies T-201 and T-301. The pooled analysis of survival confirmed a post-hoc finding from study T-301: median survival for Contusugene ladenovec Gendux treated patients was higher in the p53 Contusugene ladenovec Gendux favourable population (median: 8.5 months) when compared to the p53 Contusugene ladenovec Gendux unfavourable population. However, with this respect the ISE did not provide any additional information of a comparison of Contusugene ladenovec Gendux to methotrexate. Additional analyses dealt with a possible correlation of tumour growth control and survival. In this respect a correlation was found in the Contusugene ladenovec Gendux treated patients but not in patients treated with methotrexate.

Clinical safety

Patient exposure

Although a total of 480 patients were treated with Contusugene Ladenovec Gendux , the safety data base of this submission includes only safety data from 431 patients enrolled in the Phase I / II and III studies.

Of the 431 patients included in this analysis a total of 311 patients were treated for SCCHN, and 120 patients were treated in clinical studies of other solid cancer types.

Adverse events

Of the 311 patients treated with Contusugene ladenovec Gendux for SCCHN, 98.7% reported at least one occurrence of an adverse event. The most frequently reported adverse events for Contusugene ladenovec Gendux patients regardless of causality, included injection site pain (49.8%), fever (43.7%), asthenia (35.4%), pain (32.8%), nausea (27.7%), constipation (22.2%), vomiting (20.6%), chills or dysphagia (19.3% each), headache or anorexia (18.0% each), dyspnea (17.7%), infection (17.0%) and haemorrhage (16.4%). Noted with lesser frequency were face edema (13.5%), rash (12.9%), injection site haemorrhage (12.9%), dehydration (12.5%) and injection site reaction (10.9%).

In study T-301, all patients (100.0%) reported adverse events. More patients on Contusugene ladenovec Gendux than on methotrexate reported chills (37.7% versus 10.9%), fever (54.1% versus 23.6%), injection site haemorrhage (21.3% versus 1.8%) which was mild to moderate (Grade 1-2) in all cases related to the procedure, injection site reaction (9.8% versus 3.6%), general pain (9.8% versus 5.5%) which was most often attributed to the injection, face oedema (18.0% versus 5.5%) which was largely due to swelling at the site of injection), anorexia (36.1% versus 23.6%), dysphagia (34.4% versus 20.0%), dehydration (16.4% versus 7.3%) and weight loss (13.1% versus 7.3%).

Serious adverse events and deaths

A total of 164 of 311 Contusugene ladenovec Gendux-treated SCCHN patients (52.7 %) reported at least one serious adverse event (SAE). The most frequently reported SAEs in over 5% of patients treated with Contusugene ladenovec Gendux in SCCHN studies were aggravation reaction (decreasing performance status, progression of disease, as described above) in 7.7% of patients, dehydration (7.1%), haemorrhage (6.8%) and dyspnoea (5.1%).

For a total of 77/ 311 patients (24.8%) treated in all Contusugene ladenovec Gendux SCCHN studies death was reported as an outcome of the adverse event or serious adverse event. Similar proportions of Contusugene ladenovec Gendux patients and methotrexate patients died in the Contusugene Ladenovec Gendux Phase 3 SCCHN study T301, 15 of 61 treated patients (24.6%) and 14 of 55 methotrexate-treated patients (25.5%), respectively.

Laboratory findings

Changes from baseline to the last visit for which a laboratory value was obtained were assessed for haematology and serum chemistry parameters for all studies and for each of the major tumour types. Laboratory parameters were also compared by therapy type; Contusugene ladenovec Gendux monotherapy, Contusugene ladenovec Gendux plus cisplatin, Contusugene Ladenovec Gendux plus docetaxel/doxorubicin and Contusugene ladenovec Gendux plus radiotherapy.

No clinically significant changes in laboratory parameters were reported.

Fulminant hepatic failure due to disseminated adenovirus infection has been reported in the literature. In order to increase the patient's safety liver enzymes have to be monitored during treatment with Contusugene ladenovec Gendux.

Safety in special populations

No intrinsic factors have been identified which might affect the use of Contusugene Ladenovec Gendux in individual patient populations.

No children and adolescents have been treated with Contusugene ladenovec Gendux.

There is no information on the use of Contusugene ladenovec Gendux in pregnant or lactating females.

The applicant addresses this issue adequately in the SPC.

Immunological events

The majority of patients showed an increase in their plasma anti-adenovirus antibodies during the study with all patients being positive for anti-adenovirus antibodies by cycle 2.

Anti-p53 antibodies were also reported, results were obtained only form one study.

Although the applicant tried to establish a correlation between antibody titre and transgene expression, the evaluation was hampered by low sample size (4 patients) and methodological problems (sampling time). Due to the limited data base no firm conclusion can be drawn.

Evaluation of the immune response and the impact on safety and efficacy (e.g. anti-p53 antibodies) has to be part of the RMP.

Cristofanilli et al (2006) described in the breast cancer study a local immune response in the tumour after Contusugene ladenovec Gendux treatment. The authors proposed that these results indicate that the administration of AdCMV-p53 produces local activation of innate immune response and mild inflammatory changes followed by activation of adaptive immunity. The applicant failed to evaluate the safety aspect of this finding with regard to the presence of RCA in the final product.

Safety related to drug-drug interactions and other interactions

No drug-drug interaction studies have been conducted with Contusugene ladenovec Gendux.

The Applicant submitted a detailed analysis of the use of concomitant medications. However, an analysis of the drug interactions is missing. Due to the limitations of the studies (e.g. methodological flaws, inconsistencies in reporting), the CHMP refrained from requesting a drug interaction analysis

Discontinuation due to AES

Discontinuation due to adverse events was reported for 15.4% of SCCHN patients treated with Contusugene ladenovec Gendux.

In study T-301, slightly higher rates were reported (Contusugene ladenovec Gendux 23.0% and methotrexate 20.0%), however the rates were similar for both treatment arms. Patients discontinuing due to stomatitis or asthenia were reported more frequently in the methotrexate group, and patients discontinuing study due to fever or injection site pain were reported more frequently for the Contusugene ladenovec Gendux group.

Pharmacovigilance system

The CHMP considers that the pharmacovigilance system as described by the applicant has the following deficiencies:

- Missing description of a detailed procedure of signal detection

- Missing flow diagram indicating the flow of safety reports from clinical studies including reports from non-company sponsored trials.
- Missing information on archival activities regarding the objects and the duration of archival storage of safety information

Provided that the deficiencies are rectified prior to the applicant placing the medicinal product on the market, the CHMP may consider that the pharmacovigilance system will fulfil the requirements. The applicant must ensure that the system of pharmacovigilance is in place and functioning before the product is placed on the market.

Risk Management Plan

According to the Guideline on Risk Management Systems for Medicinal Products for Human Use (EMEA/CHMP/96268/2005) the Safety specification should be a summary of the important identified risks and should form the basis of the evaluation of the need for risk minimisation activities. The presented description of non-clinical and clinical data is not detailed enough for evaluation of potential risks and the proposed RMP is therefore not sufficient as a stand alone document.

Pharmacovigilance plan

	Safety Concern	**Action proposed**
1	Risk of shedding adenovirus	Routine pharmacovigilance, literature search
2	Inappropriate administration	Routine pharmacovilance, literature search Specific educational material. Specific administration centres
3	Replication competent adenovirus	No RCA noted in the clinical trial program; no action planed
4	Use in pregnancy	Routine pharmacovigilance, literature search
5	Use in children	Routine pharmacovigilance, literature search
6	Long term exposure	Routine pharmacovigilance, literature search
7	Use in hepatic/renal impaired patients	Routine pharmacovigilance, literature search,

Risk Minimisation Plan

	Safety Concern	**Routine risk minimisation**	**Additional risk minimisation**
1	Risk of shedding adenovirus	T following warning will be inserted in the SPC: “Patients should be instructed to wash hands after urinating or defecating, to use disposable paper tissues in the event of coughing or sneezing and to avoid contact with former tissue or organ transplant recipients and persons known to suffer from severe immunodeficiency disorders (either congenital or acquired), for 40 days following the last Contusugene ladenovec Gendux dose” Isolation of patients is not deemed necessary due to the vector being administered being not	The objective of additional risk minimisation activities for this safety concern is to monitor the possibility of patients shedding Contusugene ladenovec Gendux after treatment. Gendux Molecular proposed to collect samples from patients using molecular and classical virology techniques assay the samples for replication competent adenovirus content

		replication compliment and therefore highly unlikely to be able to replicate and/or transfer infection in a normal human.	
2	Inappropriate administration	The following warning is incorporated into the SPC: "Care should be used when Contusugene ladenovec Gendux is injected into tumours located in the wall of major blood vessels	The objective of additional risk minimisation activities is to reduce the risk of inappropriate administration of Contusugene ladenovec Gendux to patients. Oncology centres will be used for administration of Contusugene ladenovec Gendux, and treating physicians will be trained in the appropriate method for administration of the product
3	Replication competent adenovirus	The SPC and educational material will include the following text…"Contusugene ladenovec Gendux (Contusugene ladenovec) is a replication-impaired adenoviral vector".	None

The pharmacovigilance plan and the risk minimisation plan should be adapted according to all additionally identified risks including those identified from investigations following the clinical and non-clinical questions.

III.4 Environmental aspects

Contusugene ladenovec Gendux is a replication-deficient adenoviral vector containing the human p53 tumour suppressor gene for the treatment of patients with Squamous Cell Carcinoma of the Head and Neck (SCCHN).

Contusugene ladenovec Gendux has been designed using a first-generation vector derived from adenovirus serotype 5 (Ad5). To render the vector deficient for replication, the E1 genes were deleted and replaced with a p53 expression cassette comprising a CMV promoter, wild-type human p53 encoding sequences and a SV40 polyadenylation signal. In addition to deletion of the E1 genes, the vector used for the generation of Contusugene Ladenovec Gendux contains a partial deletion and a concomitant DNA insertion in the E3 region. Importantly, the inserted DNA sequence of 646 bp is of unknown origin and does affect open reading frames (ORFs). The 'regular' E3.10.4K/RIDα viral protein, involved in both blocking TNF-mediated inflammatory responses and prevention of apoptosis induced by a variety of "death" ligands, including TNF, Fas ligand and TRAIL, lacks 18 amino acids at the C-terminus. Instead, it gained 26 additional amino acids encoded by the inserted DNA at its C-terminus. In addition, a novel ORF of 132 amino acids is introduced that originates within the insertion sequences and reads into E3 sequences by 33 amino acids. Predicted protein sequences have no significant similarities to any protein in the databases, except a small sequence of 16 amino acids, which is largely identical to a salmon transposase (result from genebank search perfomed by the assessor). Expression of the novel ORF was detectable by RT-PCR at the mRNA level (Gingras et al., 1996, Cancer Gene Ther. 3, 151-4). It is unknown whether the novel ORF is translated into protein and how this novel protein might affect human health, viral pathogenicity and replication rate.

As the applicant did not provide any information on expression and potential harmful effects of the foreign proteins, this environmental risk assessment is currently only preliminary. A final assessment can only be performed if additional data are provided by the applicant that allows evaluation of any additional risk due to the insertion of foreign DNA in the E3 region.

Replication-competent adenovirus (RCA) was continuously detected in clinical lots of Contusugene ladenovec Gendux at a concentration of 1-2 pfu RCA per $3x10^{10}$ vp Contusugene ladenovec Gendux. In the MAA, RCA for drug substance is specified at 4 pfu RCA per $3x10^{10}$ vp Contusugene Ladenovec Gendux. Thus, up to 267 pfu of RCA may be applied to patients per dose ($2.2x10^{12}$ vp Contusugene ladenovec Gendux). Assuming that patients receive on average 32 doses per year, 8544 pfu of RCA may be applied to a patient per year. No detailed analysis of such RCA providing details of e.g. its genomic organisation or the presence of p53 is included in the applicant's ERA.

Information on shedding presented in the applicant's ERA is regarded not sufficient, particularly in view of the long-lasting shedding of Ad5-p53 vector into patient's body fluids. Shedding of Ad-p53 vector was detected for approximately 4 weeks with highest titre measured shortly after administration. In depth information about shedding, e.g. including levels and type of vector, kinetics of shedding, incidents where increased or prolonged shedding was observed, as well as data on the properties of the Ad5-p53 vector present in samples that turned out positive in CPE assays using complementing cells are required.

Moreover, the applicant states that no clear or direct evidence was seen to support horizontal transmission of administered adenoviral vector. One clinical study (Study T-207, described in Section 5.3.5.3.5) examined horizontal transmission of Ad5-p53 vector from patients to household members by PCR and CPE assay. Though these results in the CHMP's view may indicate transmission of adenoviral vectors but not of RCA, the applicant denies any horizontal transmission of adenoviral vector. The applicant's rationale for this conclusion is not regarded as scientifically sound and acceptable. The findings need to be discussed in much more detail in the applicant's environmental risk assessment. For the moment, it has to be assumed that horizontal transmission of Ad5-p53 vector and potentially RCA might occur upon direct contact with patients that shed Ad5-p53 vector or even RCA. To exclude such a transmission the applicant should provide more rigorous data.

Spreading of Contusugene ladenovec Gendux-derived RCA is considered to be impaired by immunity of the vast majority of adults against Ad5. Additionally, Contusugene Ladenovec Gendux-derived RCA may be more sensitive to the human immune system than wild-type Ad5 due to the partial deletion in E3 that truncates or deletes some of the sequences encoding proteins involved in evading the human immune system. Therefore, the probability that Contusugene Ladenovec Gendux-derived RCA spreads efficiently into the environment is considered low.

Nevertheless, horizontal transmission of Ad5-p53 vector or RCA upon direct contact with Contusugene ladenovec Gendux-treated patients represents a relatively high risk for immune-compromised individuals, which may suffer from a severe disease upon infection. Therefore, risk management strategies should be in place to preclude transmission of Ad5-p53 vector and/or Contusugene Ladenovec derived-RCA from patients to immunocompromised individuals.

The probability for the worst case scenario including formation and spreading of RCA expressing human p53 may be considered negligible as it is unlikely that such a RCA containing E1 genes and the p53 expression cassette arises. First, a non-homologous recombination event would be required and second, the genome size of such a virus would exceed the size limit that allows efficient packaging of the viral genome. However, this theoretical approach should be confirmed by experimental data on the genomic structure of RCA.

In summary, the overall risk of clinical use of Contusugene ladenovec Gendux for immune-competent individuals and the environment is considered low. However, several circumstances render immune-compromised individuals at a high risk, if they come into contact with Contusugene ladenovec Gendux-treated patients. These circumstances include presence of RCA in Contusugene ladenovec Gendux, shedding of Ad5-p53 vector and potentially of RCA for approximately 4 weeks following administration, possible horizontal transmission of Ad5-p53 vector and/or RCA from patients to household members, and a potential additional risk due to the substitution in the E3 region of Contusugene ladenovec Gendux. To lower the risk for immune-compromised individuals, risk management strategies need to be adapted to preclude transmission of Ad5-p53 vector and/or Contusugene ladenovec Gendux-derived RCA from patients to this vulnerable group. For a final risk assessment, it is further necessary to address whether the novel ORF is translated into protein and to evaluate potential consequences on human health and the environment.

Consultation of Competent Authorities established under Directive 2001/18/EC

In accordance with Regulation (EC) No 726/2004, Competent Authorities (CAs) established under Directive 2001/18/EC have been consulted. Of the consulted CAs, 6 countries have provided comments, which were channelled via the CA from Germany (Paul Ehrlich Institute, appointed Lead CA). Overall, there is agreement with the overall conclusions of the assessment report. Where relevant, comments from CAs have been taken into account in the list of questions on environmental aspects.

IV. ORPHAN MEDICINAL PRODUCTS

N / A

V. BENEFIT RISK ASSESSMENT

V.1 Benefits

The applicant failed to demonstrate a benefit of Contusugene ladenovec Gendux therapy in the target patient population.

V.2 Risks

Deficiencies in the quality of the product were identified which did not provide assurance regarding consistency of the manufacturing process and of the potency of the product.

Non-clinical testing was evaluated to have some major deficiencies allowing no final conclusion on the non-clinical safety and efficacy of the medicinal product, i.e. a lack of adequate repeat dose toxicity studies, studies addressing potential non-target toxic effects and lack of convincing data demonstrating preclinical efficacy of Contusugene Ladenovec Gendux when administered as monotherapy. Available data indicate the presence of vector DNA in gonads and possible germ line integration cannot be excluded at the moment. Due to shedding of Ad5-p53 vector into body fluids treated patients may transfer virus to household-members.

The submission is mainly based on findings from the randomised study T-301 designed to compare overall survival in patients receiving Contusugene ladenovec Gendux and methotrexate respectively for the treatment of relapsed or refractory carcinoma of head and neck. In the overall population, median survival was even shorter in patients treated with Contusugene ladenovec Gendux (4.4 months) than in patients treated with methotrexate (6.1 months). Subgroup analyses performed by the applicant generated signals that Contusugene ladenovec Gendux might harm patients in certain conditions. A possibly detrimental effect of Contusugene ladenovec Gendux is reported for the so called ‘clinical biomarker’ population (i.e. patients who received at least 1 dose of study drug, and had a pre-study progression-free survival (PFI) interval $\geq$ 12 months). In this population the median survival time was 4.5 months in the Contusugene ladenovec Gendux group compared to 7.3 months in the methotrexate group. The negative effect of Contusugene ladenovec Gendux was even more pronounced in the population of patients with a ‘p53 unfavourable profile’. In this subgroup of patients median survival for patients treated with Contusugene Ladenovec Gendux was 2.7 months compared to 5.9 months for patients treated with methotrexate.

Further safety evaluation is hampered by the size of the data base, inconsistencies and the methodological flaws. Thus the clinical safety data submitted so far do not allow for a conclusion on the safety profile of Contusugene ladenovec Gendux.

Up to now, several potential risks were identified which may be associated with the use of Contusugene ladenovec Gendux.

V.3 Balance

In conclusion, demonstration of the benefit of a treatment with Contusugene ladenovec Gendux was not accomplished. On the other hand, several potential risks have been identified. Thus, the benefit risk ratio of Contusugene ladenovec Gendux has to be regarded as negative.

V.4 Conclusions

The overall B/R of Contusugene ladenovec Gendux is negative.