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

Doc. Ref: EMEA/692328/2008

WITHDRAWAL ASSESSMENT REPORT FOR ADVEXIN

INN: contusugene ladenovec

Procedure No. EMEA/H/C/919

This report is based on the D120 List of Questions adopted by the CHMP with all information of 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.

7 Westferry Circus, Canary Wharf, London, E14 4HB, UK
Tel. (44-20) 74 18 84 00 Fax (44-20) 74 18 85 45
E-mail: mail@emea.europa.eu. http://www.emea.europa.eu

TABLE OF CONTENTS

I. RECOMMENDATION 4
II. EXECUTIVE SUMMARY 5
II.1 Problem statement 5
II.2 About the product 5
II.3 The development programme/Compliance with CHMP Guidance/Scientific Advice 6
II.4 General comments on compliance with GMP, GLP, GCP 7
II.5 Type of application and other comments on the submitted dossier 7
III. SCIENTIFIC OVERVIEW AND DISCUSSION 8
III.1 Quality aspects 8
III.2 Non clinical aspects 10
III.3 Clinical aspects 13
III.4 Environmental aspects 18
IV. Orphan Medicinal Products 21
V. BENEFIT RISK ASSESSMENT 21
V.1 Benefits 21
V.2 Risks 21
V.3 Balance 21
V.4 Conclusions 21

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 Agency for the Evaluation of Medicinal Products
EudraCT	European Clinical Trials Database
FBS	Foetal 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 Organisation

I. RECOMMENDATION

Based on the review of the data on quality, safety and efficacy, the CHMP considers that the application for Advexin, an orphan medicinal product in the treatment of Li-Fraumeni cancer patients, 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 Advexin was not demonstrated
- Correlation of p53 expression in tumours and clinical response to Advexin treatment was not convincingly demonstrated.
- 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 Advexin.
- The data do not conclusively allow further recommendations regarding the posology such as the duration of therapy, monotherapy vs. combination therapy, type of combination therapy.
- 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)
- Lack of GMP certification and import licence
- Lack of validation data on the release tests of the drug product
- Lack of demonstrated consistency of lots with respect to the ratio of infectious particles to total particles and manufacturing changes during product development
- DP manufacturing process is not fully validated
- Lack of sufficient stability data
- Unclear role of RCA in the mode of action of Advexin
- Lack of adequate biodistribution analysis
- Possible germ line integration of vector DNA
- Lack of adequate repeat dose toxicity analysis
- Several deficiencies in the data and evaluation for assessment of the environmental risk

The applicant requested granting the marketing authorisation under exceptional circumstances according to Article 14 (8) of Regulation (EC) No 726/2004. Due to the major objections precluding a marketing authorisation, no decision can be made on the conditions for marketing authorisation at the present time.

Proposal for Questions to be posed to additional Experts
None

Proposal for Inspection
The EMEA Inspections Sector has reviewed the manufacturer information contained in the application form (Module 1) and determined that all relevant sites underwent GMP inspections by EEA/MRA authorities with a satisfactory outcome within the last 3 years, with the exception of the manufacturer of active substance, finished product and quality control and manufacturers responsible for irradiation sterilisation of vial/stopper assemblies and MCB/WCB storage, for which an inspection is required.

A GCP inspection is not proposed at this time.

II. EXECUTIVE SUMMARY

II.1 Problem statement

Li-Fraumeni syndrome (LFS) is an autosomal-dominant inherited predisposition to develop cancer. In about 70% of LFS patients a mutation in the tumour suppressor gene p53 can be found. p53 is a transcription factor involved in the control of cell cycle and cell growth. Mutations of p53 are regarded to be causally related to the disposition to develop multiple tumours at younger ages.

LFS is associated with a variety of tumours. A database has been created to collect information on families carrying a germ-line mutation in the p53 gene and on families affected by Li-Fraumeni syndromes. So far analysis of 265 families/individuals is included in this database. The most frequent cancer is breast cancer (30.6%), followed by soft tissue sarcoma (17.8%), brain tumour (14%), bone sarcoma (13.4%), and adrenocortical carcinoma (6.5%). Less frequent tumour sites include lung, haematopoietic system, stomach, colorectal, skin, and ovary. The gender distribution for these tumours shows an excess of males for brain tumour, haematopoietic cancers, and stomach cancer, whereas an excess of females was observed for adrenocortical carcinoma and skin cancer. All of the breast cancers were in females. Males and females were equally affected by soft tissue and bone sarcoma, lung cancer, and colorectal cancer. The age at onset of tumours in p53 mutation carriers varies with tumour site; however, all of the inherited tumours show an earlier age at onset compared with their sporadic cancer counterparts (Olivier et al (2003): Cancer Res 63, 6643-50; the database is available online at http://www-p53.iarc.fr/germline.html).

No specific treatment for Li-Fraumeni syndrome exists. Treatment is adapted from the protocols for sporadic cancer therapy. The standard treatments for these tumours include medication with doxorubicin, cisplatin, paclitaxel, docetaxel, 5-fluorouracil (5-FU), etoposide, irinotecan, cyclophosphamide, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and melphalan. Occurrence of second malignancy after successful radiotherapy is discussed due to abnormal sensitivity of LFS patients to radiogenic cancerogenesis (Varley et al. (2003) Mutat 21 (3), 213-20, Limacher et al. (2001) Int J Cancer 96, 234-242). The treatment with Advexin is aiming at reconstitution of significant levels of functional p53 within tumour cells, utilising the above mentioned physiological functions of p53 as an anti-tumourigenic agent.

The p53 tumour antigen is found in increased amounts in a wide variety of transformed cells. The protein is also detectable in many actively proliferating, non-transformed cells, but it is undetectable or present at low levels in resting cells. The p53 protein induces cell cycle arrest or apoptosis in response to sub-lethal or severe DNA damage, respectively, by differential transcription of target genes and through transcription-independent apoptotic functions. It has been suggested that wild type p53 may play a role in DNA repair and that expression of mutant forms of p53 may alter cellular resistance to the DNA damage caused by gamma-radiation. Furthermore, p53 had been thought to function as a cell cycle checkpoint after irradiation, also suggesting that mutant p53 might change the cellular proliferative response to radiation.

LFS is a rare disease and it is estimated that about 400 patients are concerned worldwide.

Somatic p53 mutations are frequent in most types of sporadic human cancer. The frequency varies from 5 – 70% depending on cancer type and stage (IARC database: www-p53.iarc.fr).

II.2 About the product

Advexin is a sterile suspension for injection, containing $1.1x10^{12}$ viral particles/ml, and is to be administered intratumourally. Each vial of Advexin 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 the control of the cytomegalovirus

(CMV) promoter. Structurally, Advexin consists of a protein capsid with the Advexin genome packaged as a nucleoprotein complex. Advexin has been genetically engineered to render the vector replication incompetent. However, due to the manufacturing process a low level of infectious adenovirus particles is present in the product. The proposed therapeutic indication in the present MAA for Advexin is: "Advexin is indicated for the treatment of Li-Fraumeni cancer patients".

Advexin is a gene transfer medicinal product. When introduced into target tissues, Advexin binds to cells where it becomes internalised and uncoated. Advexin 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 Advexin-mediated p53 protein triggers changes in the expression of numerous genes which, in cancer cells, activate cellular growth arrest and apoptosis. Additionally, Advexin may inhibit tumour angiogenesis and stimulate the host's immune response to the tumour.

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

Compliance with CHMP Guidance/Scientific Advice

The applicant received in March 2007 Protocol Assistance from the CHMP (EMEA/SAWP1/64058/2007). The Protocol Assistance pertained to quality, non-clinical and clinical development of the product. Regarding the clinical development of the product the applicant requested advice on the protocol of a clinical study which is not subject to the current MAA.

Regarding the quality issues the applicant appears not to be in compliance with overall recommendations given by the CHMP protocol assistance. The inconsistencies which have been noted for specifications on e.g. MVB2 (sterility, RCA, titre, LAL), WVB (bioburden, titre, particle enumeration) still exist. In this regard, release tests on p53 expression, bioactivity and particle/pfu ratio are still non-uniform between MVB2, WVB, DS and DP, respectively. The ratio of total to infectious particle number is an essential specification for dosing of Advexin. The change-over of a particle/pfu ratio to particle/IU ratio assay during batch production, without adjustment of the specification is even a major objection. The applicant failed to demonstrate product consistency.

Regarding the non-clinical issues, the application is not fully in line with the protocol assistance given. Firstly, the question of germ line integration is still not sufficiently addressed. Secondly, recommendations given with respect to studies on biodistribution and toxicology were followed only partially. Biodistribution and persistence of vector DNA was not investigated in studies mimicking the human dosing schedule; the choice of subcutaneous administration, instead of intravenous application, as being representative for the intratumoural route is not sufficiently justified.

Regarding the ERA, recommendations given in the protocol assistance were followed by the applicant. However, deficiencies were identified regarding issues which were not addressed in the protocol assistance.

Clinical development programme:
A clinical trial program with Advexin was conducted in the late 1990s and early 2000s. Phase I /II studies in patients with various solid tumours were performed. These studies were mainly aimed to evaluate the safety of Advexin treatment also with regard to biodistribution and horizontal transmission. Further objectives included determination of pharmacodynamic markers and treatment response. In 2007 Senzer et al published the case study of a Li-Fraumeni patient treated for cancer with Advexin.

Paediatric development programme:
Not applicable

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

GMP
GMP certificate for some manufacturers of Advexin are not present in the MAA. Inspection of some of the manufacturing facilities is recommended by EMEA which is endorsed.

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 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
Clinical trials carried out outside the European Union meet the ethical requirements of Directive 2001/20/EC. The clinical trials conducted in Europe were performed before the Directive 2001/20/EC came in force and as stated in the clinical trial reports, these trials were performed according to the requirements at that time. For clinical trial T 102 which was terminated prematurely the GCP status was not reported.

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

The applicant Gendux Molecular Ltd submitted an application for Marketing Authorisation for Advexin to the European Medicines Agency (EMEA), through the centralised procedure falling within the 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 revised paper version of the Advexin Marketing Authorisation Application (MAA) is not consistent with main parts of the respective electronic version. This complicates the cross reference, whereas the present Quality-Assessment always refers to the electronic version of the revised MAA 23.11.07. Furthermore, a list of abbreviations for the quality part is lacking.

The applicant requested granting the marketing authorisation under exceptional circumstances according to Article 14 (8) of Regulation (EC) No 726/2004.

III. SCIENTIFIC OVERVIEW AND DISCUSSION

III.1 Quality aspects

Drug Substance

Advexin is an adenoviral vector containing the p53 gene (Ad5CMV-p53) intended for administration into tumours of Li-Fraumeni syndrome (LFS) patients. The serotype 5 adenoviral vector (Ad5-dl309) 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 historically used as a carrier in transfections during generation of Ad5-dl309.
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-dl309-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 addressed by the applicant. The lack of any information on this concern is addressed as a major objection.

For production of the Advexin Drug Substance, the viral vector is amplified by infection of an adherent continuous cell line (HEK293; human embryonic kidney) 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 HEK-293 cell line, homologous recombination events can occur and may often result in the formation of replication-competent Ad (RCA). Since RCA may lead to adverse events in patients, the occurrence of RCA should be minimised according to Ph.Eur. The specification seems to be acceptable. However, RCA were regularly detected in Advexin batches used in clinical trials and the significance regarding clinical effects should be discussed thoroughly. The RCA present in Advexin was not 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 Advexin Drug Substance, HEK 293 cells are thawed from a working cell bank (WCB) and expanded in increasingly large numbers and used to seed a CellCube bioreactor. After 7 days of growth inside the CellCube bioreactor, HEK 293 cells are infected with the Ad5CMV-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. Benzonase is added to digest residual host cell RNA and DNA and unpackaged viral DNA, 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 Formulation Buffer 4 (Dulbecco's Phosphate Buffered Saline with 10% (v/v) glycerin). Diafiltered material is diluted with Formulation Buffer 4 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 Advexin Drug Substance. The Drug Substance is stored at ≤ -60°C to be released for further manufacture of the Advexin Drug Product.

The analytical analyses performed on Advexin 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.

Drug Product

To yield the sterile final bulk Drug Product, bulk Drug Substance is thawed overnight, 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 Advexin will be conducted in the EU, but transfer of test methods is not yet completed. No information on the validation of methods is presented in the dossier. Completed validation of respective Drug Product release assays should be provided.

In the course of Advexin development, a couple 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.

Comparison of the Commercial Process with the previous processes is complicated by the fact that a drug substance has been defined only for the latest process. A study was conducted to compare the quality of former batches to recent batches derived from the final manufacturing process and intended for marketing ("Comparability study 3") but the data and evaluation of this study are not presented. In view of the given major changes of the manufacturing process during development of the medicinal product, consistency of production has to be adequately addressed by presenting data.

However, a further major change in the Commercial Process is that the formerly used pfu assay to assess the infectious titre is replaced by an infectious unit (IU) assay based on TCID50. Since this assay is 9-fold more sensitive to detect infectious particles than the pfu assay, 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 the drug substance indicate variability in the measurement of infectivity of Advexin over 3 to 5 months. This observation and the fact that the IU assay has not been validated make it difficult to interpret the data in relation to the proposed 6 month shelf-life. At present, no data are available for the drug product, and a conclusion on acceptable shelf life is not possible.

Adventitious virus/TSE safety:

Advexin is produced in a human recombinant cell line. Except foetal bovine serum (FBS) no other material from animals with a TSE risk are used in production. Compliance of FBS with the "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 Advexin have been extensively screened for virus contamination. Potential adventitious virus contamination during production of Advexin 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). Details on the testing procedures for cell banks and the bovine serum which is added at production should be clarified.

To summarise, from the quality point of view, a positive opinion cannot be given for 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 have not been demonstrated. The significance of genome regions of unknown origin is not clarified, and critical test methods have not been validated yet. There are a number of other concerns that need clarification or additional information.

III.2 Non clinical aspects

Pharmacology

The pharmacodynamic properties of Advexin 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 tumours reflecting prominent LFS-associated types of cancer (breast, colon, bladder, lung) as well as cell lines only poorly related to cancer observed in LFS patients (liver, head-and neck, prostate, ovary) were investigated. Results from these *in vitro* studies demonstrate that treatment with Advexin 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 Advexin.

In parallel to the analysis described above, formation of replication competent adenovirus (RCA) was also 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 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 Advexin either in a monotherapeutic approach or in combination with common chemotherapeutic drugs. Application routes included repeated intratumoural (IT) and intra peritoneal (IP) injections. Dependent on the respective cancer cell type and the applied dose, the percentage of cells infected with Advexin 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 Advexin 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 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, Advexin 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 Advexin 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 Advexin does not influence the therapeutic efficacy of Advexin. 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 Advexin. In particular, any contribution of RCA to the therapeutic effect of Advexin needs to be clarified.

In summary, proof of concept for Advexin with respect to its supposed mode of action, i.e. an anti-tumoural activity, is provided *in vitro* and *in vivo* using adequate cell culture and animal models.

Pharmacokinetics

The pharmacokinetic properties of Advexin 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. Advexin in various doses was administered once, mainly by subcutaneous (SC) 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 (SC, IV, IT) was performed according to GLP, as it is requested for non-clinical safety studies addressing the risk of germline integration.

Depending on the respective study either local concentration or a more systemic distribution of Advexin was observed. After SC 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 Advexin was much more prominent and vector DNA was additionally 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 obviate further studies addressing the detection of vector DNA in gonads, especially with respect to germline transmission. Lack of such data is not acceptable, unless a scientifically sound justification is provided.

With regard to biodistribution, no comprehensive study was provided reflecting the intended clinical application of Advexin, 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 cumulative effects, which may also have an impact on the biodistribution. Thus, biodistribution after repeated administration, at least SC 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 Advexin. 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 Advexin 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, according to 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 of differentiating between disease progression in the animal and toxic effects of the product. In agreement with pharmacokinetic studies employing the IT administration route, the SC and the IV route were utilized in these single dose toxicity studies for administration of Advexin. While the SC route again is regarded as to best reflect the clinical use of Advexin, the IV route was included as a worst case scenario. Special attention was given to the organs identified in pharmacokinetic studies as possible target organs for Advexin, 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 Advexin 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 hyperplasia. Also the spleen and blood (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 Advexin after intratumoural application compares to the IV approach rather than to SC application, the lower NOEL may be more relevant.

Repeated dose toxicity was addressed in several pharmacodynamic studies using various administration schedules of Advexin, including schedules quite closely reflecting the intended clinical use of Advexin. Various animal models were used including animal tumour models, which were treated with Advexin 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 Advexin 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, Advexin 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 Advexin was administered only twice and observation period was only 15 days.

In conclusion, repeated dose toxicity is not adequately addressed in the dossier. Either the safety data collected were too limited or the study design doesn't adequately reflect the intended clinical use of Advexin, i.e. $2.2x10^{12}$ vp injected IT twice weekly, with up to 6 cycles of that treatment 28 days apart. Additional data addressing the toxicology of Advexin 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. With regard to the situation in LFS patients, toxicity studies in p53-deficient model systems like the knockout mouse should have been considered, as already requested above (section II.1).

III.3 Clinical aspects

Pharmacokinetics

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

Advexin dissemination and shedding, and household contact infection was investigated in phase I / II studies during the early development program of Advexin. Data on biodistribution/shedding of Ad5-*p53* are reported from three phase II studies in recurrent squamous cell carcinoma of the head and neck (SCCHN) (T201, T202 and T207) and two phase I/II studies in patients with non-small cell lung cancer (NSCLC, study INT-001) and SCCHN (study INT-002)).

When administered intratumourally, wide distribution of Advexin in body fluids (faeces, urine and oral gargle), and peripheral blood lymphocytes (PBLs) was reported for up to 17 days. In a post mortem sample from a patient who died in close timely relation (4 days) to Advexin treatment, Ad5-*p53* DNA was detectable in the liver, distant lymph node, kidney, lung and tumour tissue but was undetectable in the testes. In one case persistence of viral vector in the liver was reported.

A signal of transmission of Ad5-*p53 virus* to family members was observed. However, as stated in the study reports, multiple sampling errors occurred during the conduct of the various studies. The investigators suggest that samples are false positive. The signals of horizontal transmission of Ad5-*p53 virus* to family members were also rated by the investigator as false positive since sampling errors occurred.

Taking the nature of the product into consideration the study program on pharmacokinetics (biodistribution) is adequate. However, the data presented on biodistribution, shedding and transmission are judged to be not valid. The applicant failed to follow up these signals on horizontal transmission by conducting further studies under adequate conditions. Moreover, in one report persistence of viable vector in the liver was observed. The applicant failed to discuss the findings in light of virus tropism and to discuss the potential safety consequences.

Pharmacodynamics

Restoring the p53 pathway in cancer cells is known to result in inhibition of proliferation and induction of apoptotic cell death both *in vitro* and *in vivo* (Ventura et al. 2007). The pathogenesis of tumour formation in LFS patients is mediated through a familial mutation in the p53 gene, resulting in a defect in p53 tumour suppressor protein. It is suggested that Advexin treatment and the resulting expression of normal, wild-type p53 protein inhibits cancer cell proliferation and induces cancer cell apoptosis.

A post hoc analysis to evaluate the correlation between abnormal tumour *p53* pre-treatment levels detected by immunohistochemistry and Advexin treatment in a sub-group of SCCHN patients (n=28) suggested a statistically significant increase in loco-regional disease control following Advexin therapy.

The limitations of the analysis are small sample size, sub-group analysis, post-hoc analysis and overall validity of the data. In addition the criteria for assessment of the treatment response in correlation with p53 expression were changed for the conduct of the post-hoc analysis, leading to a higher number of responders than originally reported by the investigators.

During the clinical development program the downstream markers of p53 function were also evaluated. It is suggested that Advexin treatment resulted in the induction of biomarkers for cell cycle arrest and apoptosis while down-regulating the cellular proliferation biomarker and the apoptosis inhibitor.

The applicant failed to demonstrate convincingly the correlation of p53 expression in tumours and clinical response to Advexin treatment. Also the presumed mode of action was not substantiated by the submitted data.

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 Advexin were reported. The relative contribution of this immunomodulatory phenomenon to the overall benefit of the treatment is yet unknown. Moreover the applicant failed to evaluate the safety aspect of this finding with regard to the presence of RCA in the final product.

Clinical efficacy

No clinical study report was submitted. The applicant presented only a summary of a publication (Senzer et al 2007) describing p53 therapy in a patient with Li-Fraumeni syndrome. At initiation of Advexin treatment the patient suffered from progressive cancer disease (pelvic tumours, bone infiltration and brain tumour). Initially one accessible tumour was treated with Advexin. After 4 injections into this tumour, the patient received weekly 8 additional injections of Advexin over a 2-month period targeting tumours at other sites, including the pelvic extension of the primary vaginal tumour. In total, the patient received 12 injections over an approximate 5-month period.

By FDG-PET/CT scan, complete remission of the treated tumour was observed, with the untreated lesions showing further progression. Immunohistochemistry of the tumour was performed, pre-treatment and 7-day post-treatment, to evaluate expression of molecular markers associated with p53 mechanisms of action. The analysis revealed that the p53 signalling pathway was intact in the tumour. Furthermore a relationship between treatment response, radiographic findings and molecular markers of p53 tumour suppression was reported.

No reports with regard to the other treated lesions were submitted.

Increased expression of the coxsackie adenovirus receptor (CAR) after p53 treatment was reported in the publication. This increased expression could enhance the spreading of RCA.

Supportive study(ies)
Further study reports of 10 phase I / II studies in patients with various solid tumours and bibliographic evidence (6 publications) were included in this submission, in order to support the efficacy of treatment with Advexin in Li-Fraumeni cancer patients.

The correlation between abnormal p53 expression in pre-treatment samples and clinical outcome was evaluated in a post-hoc analysis and the evaluated samples represent a subgroup of enrolled patients. However, the applicant failed to demonstrate convincingly the correlation of p53 expression in tumours and clinical response to Advexin treatment; the limitations are small sample size, sub- group analysis, post-hoc analysis and overall validity of the data (see also “Pharmacodynamics”). The data can be seen as hypothesis generating and need to be confirmed in larger, well designed, GCP compliant clinical studies. This has not been accomplished.

Analysis performed across trials

In addition to the phase I/II study reports the applicant submitted an integrated summary of efficacy (ISE), to assess the dose response across multiple dosing regimens and the adequacy of the intended dose, $2.2x10^{12}$ vp/day.

The main findings from the ISE analyses were:
- Better objective response rate in SCCHN with an average dose (vp/injection) and total dose (vp) of $\geq 2x10^{12}$ vp.
- Increased median duration of response for Advexin treated tumours for average doses and cumulative doses of $\geq 2x10^{12}$ vp compared to doses $< 2x10^{12}$ vp.

- A trend towards improved median overall survival for average as well as cumulative doses $\geq 2x10^{12}$ vp when compared to doses $< 2x10^{12}$ vp.

The results from this post hoc analysis provide an indication that Advexin doses $\geq 2x10^{12}$ vp are more likely to be effective than lower doses. However, a confirmatory proof of this finding is missing. Moreover the data do not conclusively allow further recommendations regarding the posology such as the duration of therapy, monotherapy vs. combination therapy or type of combination therapy.

Clinical safety

Patient exposure
The data base of this submission includes safety data from the 372 patients enrolled in the Phase I and II studies with about 232 patients receiving Advexin at the intended dose of $\geq 2x10^{12}$ vp per unit dose.

Adverse events
Of the 372 patients treated with Advexin 364 patients experienced at least one adverse event. The most frequently reported adverse events were fever (51.1% of patients reporting), injection site pain (37.1%), pain not otherwise specified (36.8%), nausea (32.8%), asthenia (31.2%), constipation (23.1%), vomiting (22.8%), chills (18.8%), headache (18.3%) and dyspnoea (18.3%). Chills, fever, flu-like syndrome and rash were more common in higher dose groups.

Serious adverse events and deaths
For a total of 69 patients (18.5%) death was reported as an outcome of the adverse event. The majority of these were attributed to the disease under study (reported as aggravation reaction / progression of disease), neoplasm, and carcinoma. No deaths were attributed to Advexin therapy.

However, a death due to respiratory failure needs further exploration. It is known that adenoviruses most commonly cause respiratory illness. Symptoms of respiratory illness caused by adenovirus infection range from the common cold syndrome to pneumonia, croup, and bronchitis. Patients with compromised immune systems are especially susceptible to severe complications of adenovirus infection.

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; Advexin monotherapy, Advexin plus cisplatin, Advexin plus docetaxel/doxorubicin and Advexin plus radiotherapy.

No clinically significant changes in laboratory parameters were reported. Slight increase in liver enzymes was attributed to the concomitant standard therapy. In order to increase the patient's safety liver enzymes have to be monitored during treatment with Advexin. The issue has to be addressed in the SPC.

Safety in special populations
No intrinsic factors have been identified which might affect the use of Advexin in individual patient populations.

No children and adolescents have been treated with Advexin.

There is no information on the use of Advexin 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 found in the plasma of six patients during the study. Two patients were positive at baseline and four seroconverted following administration of Advexin.

No correlation between antibody titre (anti-adenovirus or anti-p53) and plasma *p53* level was seen. Due to the limited data base no firm conclusion can be drawn.

Cristofanilli et al (2006) described in the breast cancer study a local immune response in the tumour after Advexin 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
Causality for adverse events in studies, in which Advexin was given in combination with radiotherapy or with cisplatin was assigned on the basis of 'study treatment'.
For the breast cancer study, in which Advexin was given in combination with docetaxel/doxorubicin, causality was assigned for Advexin versus chemotherapy separately. The lowest rate of reporting adverse events were seen under Advexin monotherapy, and for Advexin administered with docetaxel/doxorubicin in the breast cancer trial. In the other combination therapy trials, a higher proportion of patients reported adverse events, but it remains unclear to which extent the adverse events were related to Advexin.

The higher rates of asthenia, chest pain, anorexia, dyspnoea, haemoptysis, lung-related disorders (described as lung fibrosis, pharyngitis, pneumonia, pneumothorax) and rash are more likely to be due to the chemotherapy or radiotherapy regimen given as a component of the combination therapy, or the disease under study, rather than directly the result of Advexin injections.
The small sample size and as stated by the applicant the different methodology used to assess the causality in the various small studies hampers assessment.

Discontinuation due to AES
The applicant provided data on discontinuation due to adverse events affecting ≥1% of the patients. These events included aggravation reaction, asthenia, dyspnoea, and haemorrhage. No clinically significant differences were noted between dose groups, or with time of exposure, for discontinuations due to adverse events.

Pharmacovigilance system

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

- Missing description of the collection and processing of individual case reports from Non-EEA countries (in the context of the reporting responsibilities of the QP)
- 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 and the applicant ensures that the system of pharmacovigilance is in place and functioning, the CHMP may consider that the pharmacovigilance system can fulfil the requirements.

Risk Management Plan

Safety specification

The safety specification is incomplete. The following safety concerns were not discussed and not addressed accordingly in the pharmacovigilance plan and the risk minimisation plan:

- Liver toxicity
- Risk of horizontal transfer of adenovirus/RCA
- Risk for hospital staff who handle Adp53 and all other staff involved in transportation and storage of Adp53 (different legal requirements in the member states have to be considered)
- Transmission of Adp53 to sexual partners
- Implications of (long-term) immunity against p53 and Ad5 (Anti-p53/Ad5-antibodies) in patients and contact persons
- Reproductive toxicity
- Long term persistence of the virus

Pharmacovigilance plan

	Safety Concern	**Action proposed**
1	Risk of shedding adenovirus	Samples from patients will be evaluated using both molecular and classical virology methods
2	Inappropriate administration	Specific educational material. Specific administration centres
3	Replication competent adenovirus	Test the initial 5 patients who receive Adp53
4	Use in pregnancy	Routine pharmacovigilance
5	Use in children under 12 years of age	Routine pharmacovigilance, educational material for physicians.
6	Long term exposure	Routine pharmacovigilance
7	Use in hepatic/renal impaired patients	Routine pharmacovigilance

A more detailed description of the routine pharmacovigilance activities according to the description of the pharmacovigilance system is missing.
Events of special interests (e.g. adenovirus type infection symptoms) and the intervals for signal detection should be defined.
Details of the educational material should be provided. Both adenovirus and replication competent virus should be investigated in parallel. A detailed protocol for the proposed procedure should be provided. Limitation to the initial 5 patients regarding sampling for replication competent virus is not justified.
Routine pharmacovigilance activities are not sufficient to monitor the off-label use in children, the use in pregnancy or hepatic/renal impaired patients. A patient registry is recommended for this purpose. Furthermore, a long term follow up programme should be started as soon as possible.
Measures to ensure that use of this treatment is restricted to patients with Li-Fraumeni syndrome should be included as part of the protocol for the registry.

Risk Minimisation Plan

	Safety Concern	**Routine risk minimisation**	**Additional risk minimisation**
1	Risk of shedding adenovirus	The 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	The objective of additional risk minimisation activities for this safety concern is to monitor the possibility of patients shedding replication competent adenovirus after treatment. Gendux Molecular proposed to collect samples from patients using molecular and

		or organ transplant recipients and persons known to suffer from severe immunodeficiency disorders (either congenital or acquired), for 28 days following the last Adp53 dosing"	classical virology techniques assay the samples for replication competent adenovirus content
2	Inappropriate administration	The following warning is incorporated into the SPC: "Care should be used when Adp53 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 Adp53 to patients. Oncology centres will be used for administration of Adp53, 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…"Adp53 (Contusugene ladenovec) is a replication-impaired adenoviral vector"	Additional risk minimisation activity will include testing the first set of 5 patients using CPE assay (direct culture) and PCR assays. The objective of these tests will be to ascertain that the adenovirus remains replication non-competent after administration. Results showing no replication competent adenoviral vectors present in samples taken from patients will provide verification of success of proposed action. This proposed testing will be reviewed after one year.

Many safety concerns have not been addressed. The proposed risk minimisation measures are insufficient. According to clinical data Adp53 is detectable in urine and respiratory tract for one month after administration. Thus, the safety interval of 28 days appears to be short. It has to be justified why isolation is not required.

A detailed handling description for treating physicians in order to avoid inappropriate administration should be included in the SPC.

Methods for minimising off-label use and measures of success are missing.

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

Advexin is a replication-deficient adenoviral vector containing the human p53 tumour suppressor gene for the treatment of tumours in patients with Li-Fraumeni-Syndrome.
Advexin has been designed using a first-generation vector derived form 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 Advexin 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 viral protein involved in prevention of TNF-mediated cytolysis 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. 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 Advexin at a concentration of 1-2 pfu RCA per $3x10^{10}$ vp Advexin. In the MAA RCA for drug substance is specified at 4 pfu RCA per $3x10^{10}$ vp Advexin. Thus, up to 267 pfu of RCA may be applied to patients per dose ($2.2x10^{12}$ vp Advexin). Assuming that patients receive 4 treatment cycles with administration of 2 doses per cycle, 2136 pfu of RCA may be applied to patients in total. 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. Furthermore, formation of helper dependent E1 positive particles (HDEP) should be considered by the applicant. HDEP are generated by a single crossover event between homologous E1 sequences of vector and producer cells and subsequent deletion of significant portions of the viral backbone (Murakami et al., 2004, J Virol 78, 6200-8). Although such particles are replication deficient, they can induce cytopathic effects in the presence of the recombinant adenoviral vector.

Information on shedding presented in the applicant's ERA is considered 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 at least 3 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 HEK293 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, section 5.3.5.3.) examined horizontal transmission of Ad5-p53 vector from patients to household members by PCR and an assay for CPE. Though these results may indicate transmission of adenoviral vectors but not of RCA, the applicant declines 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 to patients that shed Ad5-p53 vector or even RCA. To exclude such a transmission the applicant has to provide more rigorous data.

Spreading of Advexin-derived RCA is considered to be impaired by immunity of the vast majority of adults against Ad5. Additionally, Advexin-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 Advexin-derived RCA spreads efficiently into the environment is considered low.
Nevertheless, horizontal transmission of Ad5-p53 vector or RCA upon direct contact with Advexin-treated patients represents a relatively high risk for immune compromised individuals, which may suffer from a sever disease upon infection. To minimise direct contact between the two groups, risk management strategies should include isolation of Advexin treated patients as long as Ad5-p53 vector and/or RCA are being shed in significant amounts.

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, as requested in the quality part of the assessment report.

In summary, the overall risk of clinical use of Advexin 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 Advexin treated patients. These circumstances include presence of RCA in Advexin, shedding of Ad5-p53 vector and potentially of RCA for at least 3 weeks after 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 Advexin. To lower the risk for immune-compromised individuals, risk management strategies need to be adapted to preclude transmission of Ad5-p53 vector and/or Advexin-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 and of the consulted CAs, 7 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 AR. Where relevant, comments from CAs have been taken into account in the list of questions on environmental aspects.

IV. ORPHAN MEDICINAL PRODUCTS

According to the conclusion of the COMP (Opinion EMEA/COMP/255901/2006, EMEA/OD034/06 dated 11/06/06) the prevalence of the Li-Fraumeni syndrome is 0.05 per 10,000 individuals in the EU.

V. BENEFIT RISK ASSESSMENT

V.1 Benefits

No specific treatment for Li-Fraumeni cancer patients is available. Current treatment is adapted from the protocols for sporadic cancer therapy. However, due to the p53 defect in patients with Li-Fraumeni syndrome, these therapies may be associated with a high risk of secondary malignancies. Due to its mode of action Advexin is not expected to cause secondary tumours and thus may represent a valuable treatment alternative. However, clinical data were evaluated to not be sufficient to establish the efficacy of Advexin.

V.2 Risks

Up to now, several potential risks were identified which may be associated with the use of Advexin. Most importantly, clinical data do not allow concluding on the safety profile of Advexin. Similarly, 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 Advexin 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. Moreover, deficiencies in the quality of the product were identified which do not allow to consider that a consistent manufacturing of a potent product is ensured.

V.3 Balance

In conclusion, demonstrating the benefit of a treatment with Advexin was not accomplished. On the other hand, several potential risks have been identified. Thus, the potential risks are not balanced by the benefit of the product and the benefit risk ratio has to be regarded as negative.

V.4 Conclusions

The overall Benefit-risk balance of Advexin is negative.