European Medicines Agency
Evaluation of Medicines for Human Use

London, 26 April 2007
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WITHDRAWAL ASSESSMENT REPORT FOR CEREPRO

International Nonproprietary Name:
adenovirus-mediated Herpes Simplex Virus-thymidine kinase gene

Procedure No. EMEA/H/C/694

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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1 BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Ark Therapeutics Ltd. submitted on 4 October 2005 an application for Marketing Authorisation to the European Medicines Agency (EMEA) through the centralised procedure for Cerepro, which was designated as an orphan medicinal product EU/3/01/083 on 6 February 2002. Cerepro was designated as an orphan medicinal product in the following indication: treatment of high-grade glioma with subsequent use of ganciclovir sodium. The calculated prevalence of this condition was 0.7 in 10,000 EU population.

The applicant applied for the following indication: Cerepro is indicated for use in conjunction with ganciclovir sodium for the treatment of patients with operable high-grade glioma.

The legal basis for this application refers to:
Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application.

Protocol Assistance:
The applicant received Protocol Assistance from the CHMP on 24 July 2003. The Protocol Assistance pertained to quality, non-clinical and clinical aspects of the dossier.

Licensing status:
The product was not licensed in any country at the time of submission of the application.

The Rapporteur and Co-Rapporteur appointed by the CHMP were:
Rapporteur: Gonzalo Calvo Rojas Co-Rapporteur: Manfred Haase

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 4 October 2005.
- The procedure started on 26 October 2005.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 5 January 2006 . The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 5 January 2006.
- On 14 February 2006, the Biologics Working Party (BWP) adopted a recommendation to the CHMP for the list of questions related to quality aspects.
- During the meeting on 23 February 2006, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated list of questions was sent to the applicant on 23 February 2006.
- The CHMP requested a GCP inspection of the pivotal study 903. The inspection carried out at the following site: Kuopio University Hospital (inspection dates: 10 to 12 May 2006). The final inspection report was issued on 1 June 2006.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 1 September 2006.
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 24 October 2006
- On 8 November 2006, the BWP adopted a recommendation to the CHMP for the list of outstanding issues related to quality aspects.
- During the CHMP meeting on 14 November 2006, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- The applicant submitted the written responses to the CHMP list of outstanding issues on 12 January 2007.
- During the BWP meeting on 13 March 2007, outstanding quality issues were addressed by the applicant during an oral clarification before the BWP and the BWP adopted a recommendation to the CHMP on quality aspects

- During the CHMP meeting on 19-22 March 2007, outstanding issues were addressed by the applicant during an oral explanation before the CHMP on 20 March 2007.
- During the meeting on 24-26 April 2007, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a Marketing Authorisation to Cerepro on 26 April 2007.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

The incidence of brain tumours ranges from 1-10 cases/100,000 of which primary malignant gliomas comprise over 60%. The age-specific incidence of malignant glioma increases from 5/100,000 for those aged under 30, to a peak of 20 cases/100,000 at the age of 75. Anaplastic astrocytoma (WHO grade III), also known as malignant astrocytoma and high-grade astrocytoma, may arise from a diffuse astrocytoma or may arise de novo without indication of a less malignant precursor. Glioblastoma (WHO grade IV), also known as glioblastoma multiforme (GBM), may develop from a diffuse astrocytoma or an anaplastic astrocytoma but more commonly presents de novo without evidence of a less malignant precursor. Using standard multimodality treatment, malignant or high-grade gliomas have a median survival approximately in the range 1 – 2 years from initial diagnosis. The primary therapy of GBM includes surgery, radiotherapy and chemotherapy. In GBM, surgery is always an incomplete debulking, since it is a highly infiltrating tumour and cannot be resected completely. The extent of surgical resection depends on location and eloquence of the brain areas. Whether surgery prolongs survival is debatable, but several studies suggest that survival correlates more closely with the amount of residual tumour observed on postoperative MRI scans. After surgery, radiation therapy remains the most effective adjuvant therapy for the treatment of patients with High Grade Astrocytomas/GBM. Radiotherapy prolongs the median survival by 14-36 weeks. Different methods of administering radiation therapy are available. Chemotherapy probably has a significant effect in prolonging survival when administered with concurrent radiation therapy after surgery.

Cerepro is a gene therapy medicinal product presented as a concentrate for solution for injection containing 1×10^{12} viral particles (vp)/ml. The active substance, adenovirus-mediated *Herpes Simplex* Virus-thymidine kinase gene, is a viral particle consisting of an adenoviral vector containing the *Herpes simplex* virus-thymidine kinase gene (HSV-tk). It is a first generation adenovirus (Ad5 serotype) with an E1 deletion and a partial E3 deletion. When diluted as recommended, the solution for injection contains 1×10^{12}vp in a final volume of 10ml.

Cerepro has been developed for the treatment of patients with operable high-grade gliomas and is used with subsequent intravenous ganciclovir therapy. Cerepro is used as an adjunct to standard care, which at present is surgery followed by radiotherapy and/or chemotherapy. Cerepro is administered by injection into non-tumour tissue at the site of surgical resection of the glioma. The adenovirus transfects dividing and non-dividing cells and the transgene thymidine kinase is subsequently expressed. The thymidine kinase enzyme phosphorylates ganciclovir, injected subsequently, to ganciclovir monophosphate. This monophosphate undergoes further phosphorylation by several cellular kinases, resulting in the production of a cytotoxic nucleotide analogue that kills dividing cells, such as tumour cells, by inducing apoptosis. Apoptosis is induced when the ganciclovir triphosphate competitively inhibits deoxyguanosine triphosphate incorporation into DNA causing chain termination. As apoptosis is predominantly induced in dividing cells, Cerepro treatment results in the selective killing of tumour cells rather than quiescent normal cells.

The claimed therapeutic indication for Cerepro is “Cerepro is indicated for use in conjunction with ganciclovir sodium for the treatment of patients with operable high-grade glioma”. Cerepro is intended to be administered following the neurosurgical procedure that is undertaken to resect the high-grade glioma. Administration is by intracerebral injections into non-tumour tissue at the site of tumour resection only. Cerepro (total dose 1×10^{12}vp in 10 ml volume) is injected into non-tumour tissue at the site of tumour resection under an operating microscope using 30-70 injections to a depth of approximately 10 mm, using a sterile 1 ml tuberculin syringe and the sterile G22 blunt needle. The

total number of injections is dependant upon the surface area of the exposed cavity. Treatment with ganciclovir sodium (5mg/kg twice daily) starts on day 5 after tumour resection and gene transfer. Ganciclovir sodium is administered intravenously according to the relevant Summary of Product Characteristics, twice a day for 14 days.

Cerepro was designated as an Orphan Medicinal Product (COMP opinion dated 06/02/2002). About 16,000 patients will suffer from operable glioma per year in the EU. Protocol Assistance was given with respect to the product development of this gene therapy medicinal product.

Cerepro contains a genetically modified organism (GMO) which requires the evaluation of the potential risk to the environment.

2.2 Quality aspects

Introduction
The active substance, adenovirus-mediated *Herpes Simplex* Virus-thymidine kinase gene (also called Adv.HSV-*tk*), is a viral particle consisting of an adenoviral vector containing the *Herpes simplex* virus-thymidine kinase gene. Cerepro, together with subsequent intravenous administration of ganciclovir, is intended for the treatment of patients with operable high-grade glioma.

Active substance
The active substance is a first generation adenovirus (Ad5 serotype) with deletions of sequences required for replication (E1 and partial E3 deletions), and an expression cassette for the herpes simplex virus-thymidine kinase (HSV-*tk*) gene. The expression cassette contains human cytomegalovirus (CMV) enhancer and promoter elements, and an SV40 polyadenylation signal. Adenovirus is a non-enveloped, icosahedral virus, 70-90 nm in diameter, containing a double stranded, linear DNA genome.

- Manufacture

The active substance is manufactured and routinely controlled at Ark Therapeutics Oy (Kuopio, Finland) in compliance with Good Manufacturing Practice (GMP). The Master Cell Bank (MCB) and Master Virus Seed Stock (MVSS) are manufactured and controlled at a site in the United Kingdom in compliance with GMP.

The HEK293 cell line (human embryonic kidney, adenovirus type 5 transformed) is used as the cell substrate for production of active substance. This cell line is a continuous line of primary human embryonal kidney cells transformed by sheared human adenovirus type 5 (Ad5) diploid DNA and contains and expresses the gene E1a required for packaging of the adenovirus.

The source material for the gene of interest was HSV1-tk cDNA. The adenovirus source material is a first generation adenovirus (Ad5 serotype) with E1 deletion and partial E3 deletion. The expression cassette contains human cytomegalovirus (CMV) enhancer and promoter elements, and an SV40 polyadenylation signal. Overall the genetic characterisation and stability was considered to be sufficiently documented and acceptable.

The applicant has not established a two tiered system (i.e. Master and Working Cell Banks) for either the cell substrate or the virus seed. Although this is not a strict requirement, such a system based on master and working cell banks (MCB and WCB) and virus seeds is generally considered a mainstay to assure durable and consistent active substance production. Nevertheless, the established cell bank is considered acceptable for production of the active substance. Overall it is considered that the Master Cell Bank and Master Viral Seed Stocks (MVSS) have been adequately documented and characterised.

Several different batches of cell banks were prepared during development of the product. The toxicological and clinical trials (studies 902 and 903), as well as the process development studies, were carried out with cell banks different from the MCB to be used for production. Also, several Master Viral Seed Stocks (MVSS) were prepared and used in clinical trials, but the MVSS to be used

in routine manufacture has never been used for production of batches used in clinical study 903. This issue of comparability is discussed below.

Cells are expanded from one vial of Master Cell Bank (MCB) in flasks and cells are infected from one vial of Master Viral Seed Stock (MVSS). After replication of the vector in the infected cells, cells are harvested, sampled for testing and split into sublots which are stored frozen The vector is then released by multiple freeze-thaw cycles and clarified by low speed centrifugation and purified by two successive ultracentrifugations. Collected virus bands are pooled, the buffer is exchanged and the purified sublots concentrated. Each sublot is sterile filtered, sampled for testing and stored frozen.
Sublots that pass quality control are thawed and pooled. If required, the pool may be diluted with buffer. Finally, this formulated active substance is sterile filtered, sampled for testing and filled immediately.

The process is defined as being aseptic from the point at which the purified sublots are placed in the microbiological safety cabinet (Class A) within the aseptic processing area (Class B) and pooled.

The quality and specifications of raw materials of biological origin, as well as other materials used in the manufacture process, are adequately controlled and acceptable.

The manufacturing process and in-process controls have been sufficiently described. Establishment of controls for critical steps are described in detail and are adequate. Validation studies were presented using several batches derived from the final production process, demonstrating clearance of process related impurities and consistency of the active substance manufacturing process.

The active substance has been characterised sufficiently using a number of state-of-the-art analytical techniques. Two tests are used to demonstrate the identity of the active substance. One test is used to show that the protein profile corresponds to the known protein components of the adenoviral particle, as observed in a commercially available adenoviral standard. Identity was also verified by an appropriate method to verify consistency as predicted from the DNA sequence. The biological activity of the active substance was characterised using an appropriate *in vitro* potency assay.

Potential process-related impurities have been identified, as have critical steps for their removal. All batches of active substance are tested for host cell protein content. Host cell DNA in the active substance is routinely quantified using an appropriate method.

Process reagents that may be present as impurities in the active substance include Foetal Bovine Serum, Caesium Chloride, and other low molecular weight compounds that could derive e.g. from trypsin-EDTA.

Two potential product-related impurities have been identified by the Company: replication competent adenovirus (RCA) and aggregates. RCA will not be removed during purification, as its physicochemical properties will be indistinguishable from the desired replication deficient adenovirus. For that reason, RCA content of the active substance is routinely tested during batch release.

Control of other sources of potential impurities such as non-infectious contaminants in starting materials, leaching of impurities from contact materials, residual preservative/sanitation agents from TFF preparation, and the introduction of adventitious agents via starting materials or during production is considered adequate. Endotoxin is only controlled at the level of finished product but this is acceptable. The control for potential adventitious agents is discussed below.

During development, a number of changes have been introduced to the preparation of the active substance. Several different batches of cell banks were prepared during development of the product, and the toxicological and clinical trials (studies 902 and 903), as well as the process development studies, were carried out with cell banks different from the MCB to be used for production. Several viral stocks were prepared and used in clinical trials, but the MVSS to be used in routine manufacture had never been used for production of batches used in studies 902 and 903. The applicant has performed a comparability exercise to demonstrate that batches produced during development are comparable to the product produced for marketing. On the basis of quality data there is evidence that

the material used in the clinical study 903 and material obtained using the commercial process may be considered comparable, provided that outstanding concerns are addressed as part of the post-approval commitments undertaken by the applicant. Nevertheless, Cerepro is a complex product in terms of quality and for such a product the value of a comparability exercise performed on the basis of quality only has limitations. Complete reassurance over the comparability in terms of safety and efficacy may only be established from clinical data obtained with product manufactured using the commercial process.

- Specification

Critical parameters have been included in the active substance specifications to routinely confirm the quality of the active substance by testing pH, content, identity, restriction enzyme digestion analysis, virus proteins, purity and osmolality. The applicant has committed to add a specification for residual bovine serum albumin.

Content in terms of average total viral particles and infectious titre was evaluated on a number of batches. Testing for replication competent adenovirus (RCA) was performed on a number of batches and confirmed the absence of replication. Overall, the proposed assays are considered acceptable and adequately validated. Except for the specification for residual host cell protein, which should be based on batch data obtained with the validated assay, the specifications are adequate to control the quality and consistency of the active substance and have been adequately justified.

- Stability

The formulated active substance is filled immediately into the finished product container. Therefore, stability is evaluated at the level of the finished product and stability of the active substance has not been studied. This approach is justified in the case of Cerepro.

Medicinal product
The medicinal (finished) product consists of Adv.HSV-*tk* in a buffer.
The product is an opalescent colourless solution. Prior to administration, 1 ml of Cerepro must be diluted in saline to give a final volume of 10 ml and a total delivered dose of 1×10^{12} viral particles (vp). Each vial contains an overfill to ensure that 1 ml can be withdrawn. The product is only available in one strength.

The product is presented in a 2 ml Type I glass vial. The vials are sealed using rubber stoppers with a silicone coating. The stoppers are secured with tamper-evident aluminium crimps. The secondary container is a 15-ml sterile vial. The secondary packaging does not have a specific function that affects product quality, but is simply used to protect the glass primary container and prevent breakage during transportation.

- Pharmaceutical Development

The proposed formulation is a simple buffer for the storage and preservation of adenoviral vectors and is considered acceptable.

- Manufacture of the Product

The finished product is manufactured, routinely controlled and batch released by a manufacturing site in Finland. Operations are in compliance with Good Manufacturing Practice (GMP).

The manufacture of Cerepro finished product only involves the aseptic filling of the already formulated active substance into glass vials, which have been pre-sterilised and depyrogenated. After sealing, vials are sprayed with an anti-viral solution, to remove any potential active substance contamination of the outer surfaces. These operations are completed in sequence in a defined period of time.

A batch of finished product is derived from a single batch of active substance.

Finished product manufacture was performed by different manufacturers during development. Initially, filling of Cerepro batches was performed at a site in Finland which was not in compliance with cGMP. At a later stage in development, filling was performed in the United Kingdom in compliance with GMP. Recently, filling has been relocated to the site in Finland since the GMP status of this site had been certified in the meantime. This relocation involved some changes to the manufacturing process and the applicant has presented data that show consistency of the production process as well as comparability between processes.

- Product Specification

Critical parameters have been included in the finished product specifications to routinely confirm the quality of the finished product by testing appearance, extractable volume, subvisible particles, content, particle / infectivity ratio, potency, endotoxin, sterility and particle size analysis (aggregation). pH, appearance, extractable volume, test for subvisible particles, endotoxin content and sterility are performed in accordance with Ph Eur.

The total virus particle concentration in viral particles (vp)/ml, the particle / infectivity ratio (P:I) and the potency are measured by appropriate methods.

Analytical methods applied for the release of finished product have been validated. The specifications have been appropriately justified on the basis of batch analysis and are acceptable to ensure the consistency of manufacture.

Determining total viral particles, P:I ratio and the in vitro potency assay were considered to not be sufficient to cover all aspects of Cerepro functionality and it was considered that a transgene expression assay should be developed as a release test or to demonstrate a clear correlation between gene expression and the proposed potency test.

Stability of the product

The finished product is filled in glass vials (compliant with Ph. Eur. requirements) and stored at -70°C. Supporting stability studies on batches using the initial manufacturing process were presented as well as preliminary data from batches representing the final manufacturing process and are considered satisfactory to support the claimed shelf-life. The stability of these batches continues to be monitored as described by the study protocol. It was considered that additional data would have to be provided when available and that aggregation studies should be conducted.

- Adventitious Agents

Mycoplasma and sterility testing were performed on the MCB, the pre-MVSS (Master Viral Seed Stock) and MVSS and are also performed on each batch of the bulk harvest, foetal bovine serum (FBS), trypsin-EDTA and the finished product. Bioburden testing is performed on the purified sublots. This is considered adequate and all tests are performed in accordance with Ph.Eur.

The raw materials of biological origin used in the manufacturing process of Cerepro, the production of cell banks, and the production of virus seed stocks are foetal bovine serum (FBS) and porcine trypsin-EDTA. The source of FBS has been issued a Certificate of Suitability according to the Monographs of the European Pharmacopoeia. Compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products has been documented.

The manufacturing process of FBS contains two virus inactivation procedures which have been adequately validated. Adequate testing using validated methods is performed on FBS and trypsin during their routine manufacture. There are no viral or TSE contamination issues associated with other starting materials.

The tests performed on the MCB and on the MVSS to detect the presence of adventitious viruses are adequate. They are well described and the validity criteria established for each of the assays are adequate. Results show that MCB and MVSS are free from adventitious viral contamination. The pre-MVSS was characterised with respect to the presence of RCAs (replication competent adenovirus).

No other assays were performed to detect the presence of adventitious viral agents. This is acceptable considering that the MVSS (which was derived form the pre-MVSS) was well characterised with regard to the possible presence of adventitious viral agents.

Tests carried out to detect adventitious viral agents in the bulk harvest are considered satisfactory.

In summary, the TSE and virus safety of the product has been sufficiently assured.

2.3 Non-clinical aspects

Introduction

Preclinical studies were conducted in association with the A.I. Virtanen Institute at the University of Kuopio in Finland. These studies assessed the safety and efficacy of HSV-*tk* gene therapy using a syngeneic BT4C rat glioma model. The preclinical work was followed by a series of toxicological studies using Cerepro. Several non GLP-compliant studies were performed in academic settings for the non-clinical development of Cerepro. Subsequently, one major GLP-compliant study was conducted to address the pharmacokinetics and the toxicity of Cerepro, and some aspects of the pharmacodynamic properties. The dossier presented results from these studies as well data from scientific literature.

Pharmacology

- Primary pharmacodynamics

Four studies were performed to determine the most suitable type of vector and how the level of transfection correlated with efficacy. Initial work employed a replication deficient retrovirus, produced in a PA317 packaging cell line, as the vector, based on its ability to infect dividing cells such as tumour cells whilst normal brain tissue remained unaffected. Expression of the HSV-*tk* gene was driven by the 5' Moloney murine sarcoma virus LTR. The work was conducted using the syngeneic BT4C rat glioma model. This model mimics the human situation as the tumour is able to grow in the rats without inducing a significant immune response. The studies are summarised below.

Hakumaki *et al* (1998) used the syngeneic BT4C rat glioma model to examine the effectiveness of HSV-*tk* and ganciclovir treatment and brain tissue reactions in BDIX rats. The results showed that ganciclovir-induced apoptosis of these experimental gliomas transfected with the thymidine kinase gene was associated with a substantial accumulation of polyunsaturated fatty acids which was monitored using ^{1}H magnetic resonance spectroscopy *in vivo*.

Poptani *et al* (1998) used nuclear magnetic resonance imaging (MRI) to monitor the progression of tumours in rats injected with BT4C cells that had been transfected *in vitro* with a retrovirus carrying the HSV-*tk* gene. The results of the study show treatment responses in the form of local necrosis as soon as Day 4 after ganciclovir administration. However, rats with wild type BT4C tumours that were given retrovirus/HSV-*tk* packaging cell injections intratumourally, followed seven days later by ganciclovir, had a substantially smaller response without any effect on overall tumour growth and outcome. It was postulated that the lack of any treatment effect following direct intratumoural injection was because the packaging cells were injected when the tumour was too large and that the bystander effect was not sufficient to cope with the large tumour burden.

Sandmair *et al* (1999) reported similar findings in the same model following intra-tumoural injections of retroviral packaging cells, namely small necrotic foci in the tumours, with evidence of apoptosis and astrogliosis but no tumour regression. In addition, the treatment had no impact on survival,

demonstrating the limited efficiency of gene therapy using intra-tumoural injections with retrovirus/HSV-*tk*-producing packaging cells and ganciclovir treatment in the BT4C rat glioma model.

Sandmair *et al* (2000) subsequently utilised the BT4C rat glioma model to study the effect of HSV-*tk* and ganciclovir treatment on tumour growth, tissue reactions and survival time. The results showed that after 14 days of ganciclovir treatment, only tumours implanted with ≥10% BT4C-*tk* cells showed a significant reduction in tumour size ($p<0.05$) and prolonged survival time ($p<0.01$).

The results from these studies confirmed that HSV-*tk* can be delivered into the brain using retroviral vectors but the vectors did not always result in sufficient levels of transfection to produce a therapeutic effect.

The dose-ranging clinical study, 901, allowed a direct comparison between adenoviral and retroviral vectors and demonstrated the superiority of the adenovirus vector in terms of the level of transfection. As a result, a replication deficient adenoviral vector was evaluated in the toxicology work conducted at the A I Virtanen Institute and used in all further clinical studies.

Tyynelä *et al* (2002) subsequently demonstrated that three intratumoural injections of an Adv.HSV-*tk*, manufactured using the same process as that used for the material administered in clinical studies 902 and 903, and treatment with ganciclovir for 14 days had a significant effect on survival of rats implanted with BT4C malignant glioma cells into the brain ($p<0.05$). Furthermore, 20% of animals treated were cured (survival > 6 months).

Expression of the HSV-*tk* transgene *in vivo* was investigated using Reverse Transcriptase-Quantitative Polymerase Chain Reaction (RT-QPCR). This study was performed in conjunction with the GLP-compliant single dose toxicity study and the associated biodistribution study. A summary of the study follows in the pharmacokinetic section.

Male and female Crl:WI(GLX/BRL/Han) rats were assigned to nine groups and dosed with *Cerepro* and ganciclovir at 1.2×10^9 to 1.2×10^{11} viral particles and administered by intravenous, intracerebral, and intracerebral+intraperitoneal administration.

In the information given below, the dosing regimen is described in the same way as in the final study report. The original value for the batch of Cerepro used in the toxicology study (AdTK48) was reported as 0.6×10^{12}vp/ml. Since the production of the final study report qualification of the viral particle assay has resulted in the titre of the batch of Cerepro being re-calculated as 1.0×10^{12}vp/ml.

Animals from Groups 1 to 6 were killed on Days 3, 30, 60 and 90 (High dose only), and animal from Group 8 were killed on Days 30, 60 and 90.

At each time point a variety of tissues were assayed for the presence of Cerepro using Quantitative Polymerase Chain Reaction (QPCR). Of the tissues which were positive for the presence of the vector, those which contained the highest number of copies of Cerepro DNA were also analysed for the presence of HSV-*tk* mRNA using RT-QPCR. These tissues were selected because the RT-QPCR method was not considered sensitive enough to detect HSV-*tk* mRNA in samples which only contained low copy numbers of Cerepro DNA.

As is common in studies which employ PCR technology there was a scatter of samples that showed inhibition of the reaction so that test nucleic acid could not always be detected. This only affected a small number of samples and did not prevent adequate assessment of the results.

HSV-tk was expressed in the 3 tissues analysed (blood, spleen, and lungs) from animals which had been administered Cerepro by the intravenous route. The amount of transgene expressed in the tissues analysed decreased over time, and whilst mRNA could be detected in the spleen at Day 90, it was below the limit of quantification.

Analysis of samples collected from animals dosed intracerebrally with Cerepro demonstrated that the transgene was expressed in the brain and spleen, but not in the blood at Day 3. The level of expression

in the brain and spleen decreased at Day 30, and no expression could be detected in the spleen at Day 60. Minimal levels of expression of the transgene were detected at Day 60 and 90 in the brain.

These data demonstrate that Cerepro has the ability to infect cells and express HSV-*tk* mRNA after IC injection in rats.

Other literature

In addition to the preclinical work described above, a series of studies, using a variety of animal models, have been performed by other researchers. These studies show some evidence that gene transfer of the HSV-*tk* gene followed by ganciclovir treatment is active against malignant brain tumours, and supports its use for treating patients with operable high grade gliomas. The data provided in the literature should be considered with caution since they represent different situations to the clinical setting of Cerepro. Therefore, it can be considered as additional data but not as supportive to pharmacodynamic effect of Cerepro.

- Secondary pharmacodynamics

No studies have been submitted. The Applicant supports this by the absence of any indication of additional actions in the *in vivo* studies of Cerepro and other Adv.HSV-*tk* transfections in the brain and other tissues, or of *tk* expressed in the brain and elsewhere by transfection with retroviral and non-viral vectors (see discussion on non-clinical aspects).

- Safety pharmacology programme

No studies have been submitted (see discussion on non-clinical aspects).

- Pharmacodynamic drug interactions

Specific safety pharmacology studies have not been conducted. The Applicant argues that as Cerepro comprises a replication deficient adenoviral vector that produces transfection and expression of the HSV-*tk* gene in the vicinity of the site of localised injection in the brain, and since the *tk* gene is not associated with particular pharmacological effects, specific safety pharmacology studies have not been done.

Clinical observation of animals during the *in vivo* studies of activity and in the GLP-compliant Biodistribution and Toxicity tests have not shown specific effects attributable to pharmacological actions of Cerepro and GCV treatment. In those studies it is likely that only gross behavioural changes or major effects on the cardiovascular system would have been detected but nothing was seen that could not reasonably be attributed to the stress and trauma of IC and IV injections and tumour implants.
The other major component of the therapy, GCV, is a well known and approved medicine, and it is not known to exert relevant pharmacological actions although the possibility of hepatotoxicity is known.

Pharmacokinetics

Conventional studies of absorption, distribution, metabolism and excretion are inappropriate for Cerepro. This is recognised by the recommendations in ICH S6 and the guidelines of the CPMP and FDA for such biotechnology products (ICH S6, 1997; CPMP 1998, 2001; FDA, 1998). What has been explored is the disposition of the viral vector and transgene at the site of injection and elsewhere in the body and the distribution and duration of expression of the transgene.

Methods of analysis

The methodology used for amplification and detection of PCDNA I HSV1-TK plasmid was deemed to be validated with respect to specificity, accuracy, linearity and range, precision, quantification limit and robustness.

Biodistribution study

In a GLP compliant study rats were given Cerepro intravenously or intracerebrally or they received intracerebral Cerepro and intraperitoneal GCV at doses ranging from 1.2 x 10^9 to 1.2 x 10^{11} virus particles. One group was also given GCV IP on its own. Animals were killed at specified times. PCR (QPCR) and RT-QPCR were applied to samples from the brains and representative tissues to measure the vector and transgene expression using validated viral DNA primers and techniques.

The IC dose was the maximum that could be given in terms of the volume that could be injected by that route and the titre of vector that could be produced by a GMP- process.

Early after IC injection, Adv DNA was found in the brain, blood and spleen, sometimes in the liver and rarely in the lung. None was found in the gonads at any time except for a single instance in the ovary of one animal on Day 3 but below the limit of quantification. The amount of viral DNA found tended to be related to dose and it fell with time. There was still viral DNA in the brain and spleen at Day 90.

Following IV injection there were much higher levels in the viscera, including the spleen, liver, lungs, heart and kidneys, especially on Day 3, and a very low level in the brain. There was a rough correlation between dose and viral DNA in the tissues. With time the levels fell sharply and by Day 90 there were low levels predominantly in the spleen, liver and in some instances also in the heart. The testes and ovaries did show a low level of vector DNA on Day 3 and in some instances on Day 30 but not subsequently except for a further isolated finding in the ovary of one animal on Day 60, but this was below the limit of quantification.

Vector DNA was not found at sites from which environmental dispersion might readily occur apart from a low level in the lung and urine on Day 3 in animals injected IC and low levels transiently in the lung from animals injected IV.

Expression of transgenic *tk*: RT-PCR study

Samples from animals in the GLP Biodistribution study were analysed to study expression of mRNA from the *tk* transgene after IC and IV injection of Cerepro. These samples were selected because they had the highest levels of vector DNA. The transgene mRNA was compared with specific mRNA from BT4C cells infected *in vitro* with a retrovirus carrying the same transgene as Cerepro. The study complied with GLP. Study results allowed to follow the increase and decay over time of target mRNA in the brain, spleen and other viscera.

No specific pharmacokinetic studies have been conducted. The Applicant considers that if patients are treated with Cerepro and GCV therapy, there may be need for caution if they were simultaneously to receive an antiviral nucleoside to treat a herpetic infection elsewhere in the body. No other special pharmacokinetic studies were conducted.

Toxicology

- Single dose toxicity

Two limited non-GLP exploratory studies were done in normal rats and mice and a separate study using intratumoral injection into an IC transplant of a glioma in a rat.

A formal GLP compliant toxicity and biodistribution experiment using Cerepro has been done in the rat and this is considered as pivotal for the assessment. The study design was discussed and agreed with the EMEA as part of the Protocol Assistance in July 2003. This experiment included the biodistribution and transgene expression studies, and was compliant with GLP. The batch of Cerepro used was claimed to be equivalent to the preparation that will be supplied for commercial use, except in a few minor details of the manufacturing process. Rats were given Cerepro 1.2 and 6 x 10^9vp IC, 1.2 x 10^{10}vp or 1.2 x 10^{11}vp IV , or 1.2 and 6 x 10^9 IC vp and GCV IP. 3 males and 3 females from each dose group were killed on Day 3, 30 and 70. GCV 25mg/kg b.i.d. was injected IV from Day 5 – Day 19. Full conventional clinical observations were made as well as comprehensive blood and urine tests, autopsy and histopathological investigations, as well as specialised immunohistochemical examination of the brain of animals injected IC.

Results: Two males in the high dose IC+GCV group died prematurely, one from suppurative meningoencephalitis on Day 6 and one from peritonitis on Day 9. One rat in the low dose IC group also died for unknown reasons on Day 7. No animals died after IV dosing. Injection of the vector by both routes and administration of GCV was well tolerated clinically and so was the combination of vector plus GCV. The results of the blood tests showed only scattered variations. In rats injected IC there was slight-mild local rarefaction and scarring around the needle track and some infiltration by lymphocytes and rare plasma cells there and in the adjacent cerebral, ependymal and meningeal tissues. Polymorphs were rarely seen except in one low dose male in the IC injection group that died on Day 30 with suppurative meningoencephalitis. There was more reaction in animals injected IC with vector than in the controls given the viral vehicle alone. There was no clear association between the changes and the dose of virus injected. Immunocytochemical phenotyping of the inflammatory infiltration in the brain showed a typical response of active lymphocytes, some plasma cells and activated macrophages. No difference in the brain or viscera other than the testis was seen in the groups given GCV but the small numbers of rats limits interpretation of this point. After IV injection a few low dose group rats showed myeloid hyperplasia of the bone marrow, some atrophy of the white pulp of the spleen and hypertrophy of the red pulp at Day 3, and there was an increase in the relative spleen weight on Days 3 and 30. The spleen weight in females then and on Day 70 was appreciably higher than in males; the reason for the difference is not known. By Day 70 the other changes had greatly regressed. Antibody against Cerepro was not detected at any time. In every group treated with GCV the weight of the testes and epididymes was reduced and there was degeneration of the germinal epithelium, which had not disappeared by Day 70. Considerable animal-animal variation in the findings on analysis of semen meant that no effect of GCV treatment on sperm morphology or motility could be determined. The effect of GCV on the testis was claimed to be as expected as consequence of administration of this nucleoside analogue.

- Repeat dose toxicity (with toxicokinetics)

No repeat dose toxicity study of this type has been submitted using Cerepro. The Applicant provides information about published reports which used *Adv.HSV-tk* that was manufactured using the same process as that used for material administered in clinical studies 902 and 903 (Tyynelä *et al*, 2002). Cerepro was injected IC once or on three consecutive days into BT4C glioma transplants in the brain in BDIX rats; GCV was given subsequently intraperitoneally for 14 days. The single injection plus GCV had a limited effect on the growth of the glioma or survival of the animals. Of rats given 3 injections, 20% survived for 6 months and the tumours were not detected by MRI or on subsequent autopsy and histopathological examination of the brain. In other rats in this group growth of the neoplasm was slowed but it eventually killed the rats. Neuropathological examination of the brain and MRI scanning in life revealed acute necrosis and inflammatory infiltration and macrophages early after GCV treatment succeeded by focal gliotic scarring at the site of the implanted glioma cells. There was no evidence of continued degenerative changes in neurons, axons and their processes and

myelin sheaths in white matter, or of other reactive changes in long-term survivors or in rats killed after 30 days. Inflammatory infiltration, including activated macrophages, was seen around residual gliomas. The ventricles were dilated in long term survivors. The inflammatory infiltration was acute in type and was restricted to the region of the injected glioma. Anti-adenovirus antibody appeared in rats about 14 days after the first dose of vector and reached a plateau after 21 days. Conventional haematology and clinical chemistry tests did not show any convincing abnormality associated with the gene therapy and GCV.

- Genotoxicity

No genotoxicity studies have been submitted (see discussion on non-clinical issues).

- Carcinogenicity

No carcinogenicity studies have been submitted (see discussion on non-clinical issues).

- Reproduction Toxicity

No reproductive toxicity studies have been submitted (see discussion on non-clinical issues).

- Local tolerance

The local tolerance has been addressed during single and repeated dose toxicity studies.

- Ecotoxicity/environmental risk assessment

The outcome of the Environmental Risk Assessment (ERA) is that there is a negligible risk for human heath and the environment.

Discussion on the non-clinical aspects

Pharmacodynamics

Little information is available for Cerepro itself. One study (Tyynelä et al., 2002) addresses the non-clinical efficacy of Cerepro. However, the experimental setting differed markedly from the mode of administration intended for Cerepro. Further, as part of the pharmacokinetic study, expression of *tk*-mRNA after administration of Cerepro is analyzed in various organs. These limited set of experimental data for Cerepro is supposed to be completed by bibliographic references. However, the review of literature presented is not adequate at all to substitute for experimental data, since most of the references cited refer to distinct animal/vector/tumour models and do not support the statements made. Taken together, the mode of action and the efficacy of Cerepro are not regarded to be established in an appropriate animal glioma model. Scientifically, there is no doubt that expression of HSV-tk followed by treatment with ganciclovir (GCV) results in cell death. However, it remains unclear how, and if a non-replicative adenoviral vector will reach the single distributed tumor cells that remain in the brain after surgical resection of the tumor body. Similarly, the mechanisms of action and the relevance of the 'bystander effect', possibly contributing to efficacy remains unclear although the Applicant has provided a plausible reasoning that would need further reassurance to be confirmed.

There is evidence that the use of Adv.HsV-tk with standard treatment of GCV could be efficacious in the treatment of glioma but data on the exact mechanism of Adv.HSV-tk/GCV are lacking although this is not a main concern considering the severity of the disease to treat and that final overall assessment could be positive.

Secondary pharmacodynamic studies have not been conducted with Cerepro. The justification provided by the Applicant is considered as acceptable.

The justification provided by the Applicant for not conducting specific safety pharmacology studies was considered acceptable. Safety pharmacology has been monitored during GLP toxicology and biodistribution study in the rat and the human data from the two clinical studies carried out.

The Applicant has not conducted any study on pharmacodynamic drug interactions with drugs that could probably be administered concomitantly. Taking into account that clinical studies have not shown any evidence of interactions with any concomitant drugs that the patients may receive, the lack of such interactions studies in animals is justified.

The recommendations in ICH S6 and the guidelines of the CPMP and FDA for such biotechnology products as Cerepro indicate that the lack of this type of experimentation is justifiable under appropriate circumstances (ICH S6, 1997; CPMP 1998, 2001; FDA, 1998).

The absence of conventional safety pharmacology testing does not, therefore, represent an unexplored risk to patients.

Pharmacokinetics

A comprehensive biodistribution study has been performed in rats, in which QPCR was used to assess the distribution and persistence of Cerepro after intravenous and intracerebral administration. Extensive exposure was seen after high dose intravenous administration. Throughout the course of the study the amount of vector present, and the number of tissues in which the vector was detected, decreased over time. The vector was found mainly in the spleen and liver, with only very low levels of DNA found in the heart, lung, kidney and brain. After a single high dose administration by intracerebral injection, the vector was mainly observed in the brain and spleen, with low amounts detected in the blood, liver, lung, urine, kidneys, ovaries and heart. By Day 90, vector sequences were found only at very low levels in the brain, spleen and liver. No vector sequences were detected in either the testis or ovaries from Day 30 onwards.

No specific pharmacokinetic interaction studies have been conducted. The Applicant considers that if patients are treated with Cerepro and GCV therapy, there may be need for caution if they were simultaneously to receive an antiviral nucleoside to treat a herpetic infection elsewhere in the body. Adequate information on this possibility would have to be included in the SPC.

Toxicology

Single-dose toxicity studies were limited. Only a limited range of doses of Cerepro was investigated but it is considered that the top dose was probably the highest feasible due to the physical limitation on intracerebral dosing in rodents. GCV alone produced degeneration of germinal epithelium and dilatation of testicular tubules. The absence of encephalo-meningitis or hydrocephalus in the brain, and the lack of disseminated cytotoxicity there and in systemic organs is noteworthy as the simultaneous biodistribution study demonstrated wide occurrence of vector DNA in the brain and visceral cells at the relevant times.
No formal repeated-dose toxicity study has been conducted with Cerepro. The Applicant provides information about a published report which used Adv.HSV-tk that was manufactured using the same process as that used for material administered in clinical studies 902 and 903.

Adv.HSV-tk was limited to once or three consecutive days. The justification provided by the Applicant is considered acceptable taking into account mainly the short duration of treatment with Cerepro and the severity of the disease to treat. From a Protocol Assistance given to the Applicant in July 2003, it was concluded that the use of only one animal species for toxicity studies could be sufficient.

There is no accepted method for testing a non-replicating, non-integrating virus for ‘genotoxicity’, nor is there a strong theoretical reason for associating Adv.HSV-tk with such an action. The recommendations in ICH S6 and the guidelines of the CPMP for such biotechnology products indicate that the lack of this type of experimentation is justifiable under appropriate circumstances (ICH S6,

1997; CPMP 1998, 2001). The genotoxicity of GCV is well known and is properly considered in the clinical evaluation of Cerepro and GCV treatment in patients with a short life span.

Cerepro is given as a single dose of a replication deficient viral vector to patients with a life-threatening disease. There is no theoretical reason to associate the vector with a risk of tumour formation because of the two deletions in its genome as adenoviruses do not integrate into host DNA. GCV, as a proven mutagen, may represent a risk but that is already known and has been accepted in the treatment of other serious but non-life-threatening disorders. The recommendations in ICH S6 and the guidelines of the CPMP for such biotechnology products indicate that the lack of this type of experimentation is justifiable under appropriate circumstances (ICH S6, 1997; CPMP 1998, 2001). The lack of these data does not, therefore, present a serious and unknown hazard to patients.

The Applicant argues that considering the nature of the disease to be treated and the proven fetotoxic and teratogenic effects of the GCV treatment, it is considered reasonable not to have done such experiments. The tests would not add to the risk management of patients and so would not be a justifiable use of animals. The recommendations in ICH S6 and the guidelines of the CPMP for such biotechnology products indicate that the lack of this type of experimentation is justifiable under appropriate circumstances (ICH S6, 1997; CPMP 1998, 2001).

2.4 Clinical aspects

Introduction

GCP

The CHMP requested an inspection of Kuopio University Hospital to ascertain whether study 903 has been performed in accordance with Good Clinical Practice (GCP). The GCP inspection took place between 10 and 12 May 2006 (the outcome of the inspection is summarised below).

Pharmacokinetics

No pharmacokinetic evaluation of Cerepro was submitted. Considering the nature of this application a traditional pharmacokinetic evaluation approach is not needed. Very limited biodistribution data has been submitted.

Pharmacodynamics

No formal clinical pharmacology programme has been conducted as part of the clinical development programme, as would be expected for a standard new chemical entity. Supportive non-clinical and clinical data have been provided in order to provide an experimental basis supporting the mechanism of action of HSV-tk+ganciclovir therapy in malignant gliomas.

Clinical efficacy

Study 901 was performed to investigate the effectiveness of different vectors in transferring a β-galactosidase marker gene (lacZ) into human malignant gliomas. Two open-label, single-center studies have been submitted to support the efficacy of Cerepro, one phase I (902) and one phase II (903) study. Both studies were conducted at the same center. Altogether 24 (7 + 17) patients have been treated with the investigational product plus Ganciclovir in these studies.

- Dose response study(ies)

<u>Study 901</u>

The aim of study 901 was to investigate the effectiveness of different vectors in transferring a β-galactosidase marker gene (lacZ) into human malignant gliomas. Only the published reference (Puumalainen et al. 1998) has been made available. Neither the study protocol nor the study report were submitted. Twelve patients with suspected malignant glioma underwent stereotactic biopsy to

confirm the diagnosis. A catheter was then inserted into the tumour using the same stereotactic co-ordinates and left in place until tumour resection. Retrovirus-mediated beta-galactosidase gene transfer was performed in three patients and adenovirus-mediated gene transfer was performed in seven patients. The range of doses within the adenovirus subset was 3×10^8 to 3×10^{10} pfu. Tumour resection was performed four to five days after catheter insertion. Two patients served as controls and received no gene transfer. The procedure was well tolerated with no significant toxicity across the dose range administered. Gene transfer efficiency with retroviruses varied between <0.01 and 4%. With adenoviruses gene transfer efficiency varied between <0.01 and 11% with higher rates of transfer occurring apparently with higher doses of vector. The lowest level of measurable gene transfer efficiency was expressed as <0.01% since this was the lowest level of detection available. In the Applicant's view, the results of this study show that the adenovirus led to more efficient gene transfer than did retrovirus and that >10% gene transfer efficiency is required before a therapeutic effect of the herpes simplex virus thymidine kinase and ganciclovir treatment can be expected, which could be met by the dose proposed by the Applicant, although with the data from Puumalainen's study only one patient showed a mean gene transfer efficiency higher than 10%.

Study 902

This phase I open-labeled study was conducted to evaluate the safety and efficacy of HSV-*tk* gene therapy, mediated through either adenoviral vector or retroviral-packaging cells and given in conjunction with ganciclovir, in patients with operable primary or recurrent malignant gliomas. Patients with both anaplastic astrocytoma and glioblastoma multiforme were considered. All patients with malignant glioma referred to the investigating hospital were evaluated for trial entry, with fourteen consecutive patients giving informed consent. The data from an historical control group of seven patients from Study 901, who received an injection with the *lacZ* marker gene four to five days before tumour resection were used for comparative purposes. After confirmation of histology by frozen section, as much of the tumour as possible was resected and either HSV-*tk* retrovirus packaging cells or Cerepro (3×10^{10} pfu/10ml) were injected, using an operating microscope, into the margins of the entire tumour cavity. (between 30 and 70 injections per patient were given depending upon the surface area of the tumour cavity). Ganciclovir (5mg/kg) was delivered intravenously twice daily for fourteen days. Treatment with ganciclovir began 14 and 5 days after gene transfer in the retrovirus and adenovirus groups, respectively. The difference in the timing of the commencement of ganciclovir was due to the different times of maximal gene expression for retrovirus packaging cells and the adenoviral vector. The groups appear well balanced for age, sex, tumour type, primary/recurrent tumours, Karnofsky score, previous therapy and extent of resection. Thirteen patients had recurrent disease. All patients received steroids and anti-epileptic therapy and patients with primary tumours also received radiotherapy. Mean survival times were similar for the historical control group (8.3 months, n=7) and the retrovirus-mediated group (7.4 months, n=7), whereas, by comparison, mean survival for the Cerepro group approximately doubled (15.0 months, n=7) (Sandmair et al, 2000).

- Main study(ies)

Study 903

Study 903 was an open, single centre, controlled, randomised Phase II study involving patients with operable primary or recurrent malignant glioma.

METHODS

Study Participants

The study population included patients with operable primary or recurrent malignant glioma. The protocol specified that 60 patients would be recruited into the study and randomised to the control or active group at a ratio of 1:1. As the prognosis for patients with recurrent tumours is considerably poorer than for primary patients the randomisation was stratified to ensure equal numbers of primary and recurrent patients were in the active and control group

The main inclusion criteria were:

- Age >18 years and ≤ 75 years.
- Diagnosis of malignant glioma (to be confirmed by histology during the operation).
- Karnofsky score > 70.

The main exclusion criteria were:

- Pregnancy.
- Renal or liver disease that causes significant insufficiency in the organ function (CREA >250 µmol/l; ALT >160 U/1).
- Multilobar or intraventricular tumour or infiltration of corpus callosum.

The withdrawal criteria were

- Diagnosis other than malignant glioma as shown by histology performed at the time of operation.
- Any severe neutropenia (<0.5 x 10^9/1), thrombocytopenia (<25 x 10^9/1) or changes in liver enzymes during the first 7 days of ganciclovir treatment.
- Development of any condition specified in the exclusion criteria.
- A subject wishes to withdraw his/her consent.
- Subject lost to follow-up

Treatments

Cerepro (EG009) was given after surgical resection to the patients in the active group. It was administered at a dose of 3 x 10^{10} pfu, in a 10m1 volume, which was injected into the healthy tissue underlying the site from where the tumour was resected. This was followed by intravenous ganciclovir at a dose of 5 mg/kg twice daily, beginning five days after surgery and continuing for fourteen days. Those patients randomised to the control group underwent surgical resection but did not receive Cerepro or intravenous ganciclovir.

In patients randomised to the active group, EG009 was given as a single dose at the time of surgery. After resection of the tumour EG009 was injected into the healthy tissue underlying the site from where the tumour was resected, using 30-70 injections of 0.1-0.4 m1 to a depth of approximately 10mm. Five days after surgery ganciclovir was given through a central venous cannula at a dose of 5mg/kg twice daily for fourteen days.

Objectives

The purpose of study was to evaluate safety and efficacy of adenovirus-mediated thymidine kinase gene therapy and ganciclovir medication for the treatment of malignant glioma.

The primary objective of this study was to assess survival

The secondary objectives were:

- response to treatment as assessed by magnetic resonance imaging (MRI)
- safety as assessed by clinical chemistry, immunological measurements and PCR

- Quality of life as assessed by neurological testing

Outcomes/endpoints

The primary endpoint of the study was survival from the date of first operation as defined by an endpoint of death or re-operation for recurrence. The definition of the primary endpoint has been changed (the draft SAP stated that the primary endpoint is the patient's lifetime after operation, the final SAP specified that survival time will be calculated either defined as time to death or time to re-operation.

A post-hoc analysis of all-cause mortality and subgroup analysis was also performed.

The secondary endpoints of the study were an assessment of tumour progression using MRI, an assessment of quality of life and assessment of safety. MRI was performed at baseline and at eight weekly intervals after surgery. All scans were read in a blinded manner by two independent radiologists. The eight week scan was taken as the secondary endpoint and compared to the immediate post-operative scan
Surgical resection was be graded as 1) total; if at first postoperative day MRI more than 98% of volume is resected; 2) subtotal; if less than 98% but more than 66% of tumour tissue is removed, and ; 3) partial; if less than 66% of volume is removed. According to follow-up MRI, tumour grading will be 1) progressive; if even a slightest sign of local tumour regrowth was seen (volume increase over 5% in gandolinium enhanced T1 MRI), or there was a distant (over 4 cm) new lesion; 2) stable; if the MRI image was the same and; 3) regressive; if the volume decreased.

Performance was assessed using standardised neuropsychological testing including Weschler's memory scale, quickness and attention, flexibility of behaviour, psychomotor quickness and a symptom and mental agility questionnaire.

Safety was assessed by collecting all serious and non-serious adverse events, relevant laboratory parameters, anti-adenoviral antibody titres and vector biodistribution. An adverse event was defined as any untoward medical occurrence in a patient which does not necessarily have a causal relationship to the treatment. This included events which were not present at baseline or events which were present at baseline but which worsened during the study period. Events were recorded from the first study-related activity after the patient had signed the consent form and included illnesses and pre-existing conditions found as a result of the screening procedures undertaken. Adverse events were rated as mild, moderate or severe and their relationship to study medication was rated as unlikely, possibly, probably or definitely.

Sample size

According to the second version of the protocol 60 patients should have been recruited, 30 patients for treatment group and 30 patients for control group based on the practical knowledge of the investigator.

Randomisation

The randomisation was performed as follows: A computerised program was used to create a block of six random sheets indicating whether or not a patient would receive EG009. The program was designed to assign an equal number of patients to the active and control group in each block. When three randomisation envelopes were left in the first randomisation block, another block of six envelopes was generated and added to the original set of envelopes. Opening of the envelopes was usually performed by the nurse and witnessed by the principal investigators. Separate sets of randomisation envelopes were generated for the primary and recurrent patients.

Statistical methods

The protocol dated 23 November 2001 stated that the data would be analysed by Cox-regression analysis. During the review of the protocol, a statistical analysis plan was compiled which stated that survival would be analysed using either Cox-regression analysis or Log-rank analysis

According to the statistical analysis plan (12.03.2003) submitted by the Company the statistical methods were as follows:

All randomised patients, who have met the eligibility criteria at screening visit and those criteria have been confirmed with histology taken during the operation, are included in the intention-to treat (ITT) analysis (primary analysis). For all analysis the two-way significance level is set to 5%.

A Cox regression analysis was used to look at the effect of different prognostic factors in the model.

An interim analysis was carried out on December 1st 2001. The following variables were included in the interim analysis: age, survival, tumour progression, serious adverse events and Karnofsky score.

According to the description provided by the Applicant the final study protocol was not written after the decision to stop the trial, but during study conduct prior to the interim analysis. The Applicant submitted a draft statistical analysis plan (SAP) dated 29th November 2001. This draft SAP is similar to the final SAP and does not provide clear-cut criteria for stopping the trial following the interim analysis.

RESULTS

Participant flow

All patients received their allocated treatment and no patients were withdrawn due to adverse events. No patients were withdrawn due to non-compliance and no patients were lost to follow up. Two patients received ganciclovir for twelve days. One patient received ganciclovir for eight days before being discontinued due to a serious adverse event and one patient received ganciclovir for twelve days in total (eight days of treatment, two days off treatment, then four days of treatment), ganciclovir being withheld for two days for a serious adverse event. Both these patients fulfilled withdrawal criterion No. 2, but were continued in the study by the investigator.

Recruitment

The study was carried out in a single centre, all patients were entered at the University of Kuopio. Between May 1998 and January 2002, 36 patients were recruited into the randomised study. Seventeen patients received Cerepro and 19 patients were randomised to the control group. A cut-off date of 01 September 2002 was used for the efficacy analysis

Conduct of the study

An interim analysis was done. The cut-off-date was 01.12.2001. The variables included in this analysis were: age, survival, tumour progression, Karnofsky score and serious adverse events. Following the advice of the steering committee who reviewed the data generated from this interim analysis, recruitment into the study was terminated. Due to the premature conclusion of the study, only 17 patients were included in the treated group and 19 patients in the control arm.

The study was open to be audited by the sponsor or persons designated by the sponsor or inspected by regulatory authorities. The study was monitored after December 2001, by means of on-site visits, telephone calls and regular checking of the CRFs. The frequency of these checks was sufficient to ensure subject enrolment, compliance with the protocol, the completeness and accuracy of data entered in the CRFs and the recording of adverse events. The source data verification was performed during the period between December 2001 and February 2002 following receipt of information from the Finnish Ministry of Social Affairs and Health, approval of the ethics committee and permission from the chief medical officer of Kuopio University Hospital. In March 2002 the National Agency for Medicines (NAM) sent a letter to Kuopio University Hospital stating that the University Hospital

could not give permission for source data verification to be performed on patients who did not give consent for their personal information to be reviewed by a third party. Therefore, the necessary information was collected onto CRFs by study staff and transferred to the CRO for data entry. There were no major protocol deviations.

The CHMP requested a GCP inspection for Kuopio University Hospital which took place between 10 and 12 May 2006. For this inspection the key following aspects were checked:

a. The availability of informed consent for each patient and the procedure to obtain the consent.

b. Adherence to inclusion and exclusion criteria, paying special attention to age, type of tumour and surgical procedure.

c. Source data verification on baseline data and endpoints with focus on the primary endpoint data.

d. Verification of the randomisation procedure.

e. Verification of the doses of the study medication administered in the treatment group and follow up.

f. Adverse Event reporting with special focus on the PCR analysis to assess the level of vector distribution and verification of adverse reactions in all patients.

g. GCP training received by the investigator team

h. Monitoring

The results of the GCP inspection of study 903 are summarised as follows:

The project started as an academic trial. A notification to the NAM was made in 1996 and the trial started. In the original protocol, a brief description was given for two phases of the trial. The first phase was conducted and reported to the NAM. The second phase (the present study 903) was started in 1998 without notifying the authorities of the substantial amendments made. This is a violation of the NAM regulation.

In late 2001, the sponsorship of the study was shifted to Ark Therapeutics, who started to update the documentation and procedures to meet the modern regulatory standards. The protocol was rewritten, CRFs were created and the authorities were informed of the new status of the trial.

Since the trial procedures were thoroughly registered into the hospital patient records from the very beginning of the study, filling of the CRFs was possible by using the source data. The collection of the data was verified by the investigators and source data verification was also performed during the monitoring procedures.

Originally, the investigators planned to recruit 60 patients but the steering committee suggested an interim analysis to be made. The analysis consisted of 36 patients, and a recommendation to stop the recruitment was given. Two additional patients were recruited before the investigators were informed. The report does not include results of these additional two patients; one in the active treatment group and the other in the control group The major disadvantage of the study can be found on the handling of formal details. The authorities were not sufficiently informed of the amended details and status of the trial 903. Although the study 903 was systematically performed, a valid amended protocol or other valid amendment document was missing for several years during the conduct of the study.

In conclusion; the essential data reported was inspected against the source data of all 36 patients. The conduct of the study was clarified in detail during the interviews and inspection of the documents.

The NAM regulation and the principles of good clinical practice were not followed in all aspects of the study 903.

Despite the findings of the inspection, the document concluded that:

1. The patients were treated well and their rights were respected.

2. The results reported give an accurate description of the trial and source data.

Although the clinical data collected in this study could be verified, the concerns with respect to data quality remain. CRFs were created retrospectively. Thus, only information available in the patients' records was collected retrospectively. However, there is a large amount of information that is either poorly documented or is lacking in many patients (e.g. QoL, MRI data and laboratory parameter) prohibiting meaningful analyses.

Baseline data

In patients with malignant glioma there are several prognostic factors that can significantly influence survival. The most consistently validated factors are histology, age and performance status. With respect to histology, patients with anaplastic astrocytomas need to be differentiated from those with glioblastoma as the former have a more favourable outlook for survival than the latter (median survival of 36 months and 12 months respectively). With respect to age, after diagnosis younger patients tend to live longer than older patients and this parameter may be even more important for outcome than histology. Performance status as assessed by the Karnofsky scale is also correlated with likely outcome. Patients with a score >70 generally have a better prognosis than those with a score <60. In this study, patients were only recruited if they had a baseline score > 70.

Table: Patient and tumour characteristics.

	Cerepro	**Survival at cut off date**	**Control**	**Survival at cut off date**
Number	17	4	19	1
Gender: Males	12	3	12	0
Females	5	1	7	1
Mean Age (Years, range)	51.9 (39 – 68)		56.6 (35 – 75)	
Tumour Type: Primary	12	3	12	1
Recurrent	5	1	7	0
Number of GBMs	12	2	17	0
Number of AAs	4	2	1	1
Karnofsky Score (Mean)	85.2		84.2	

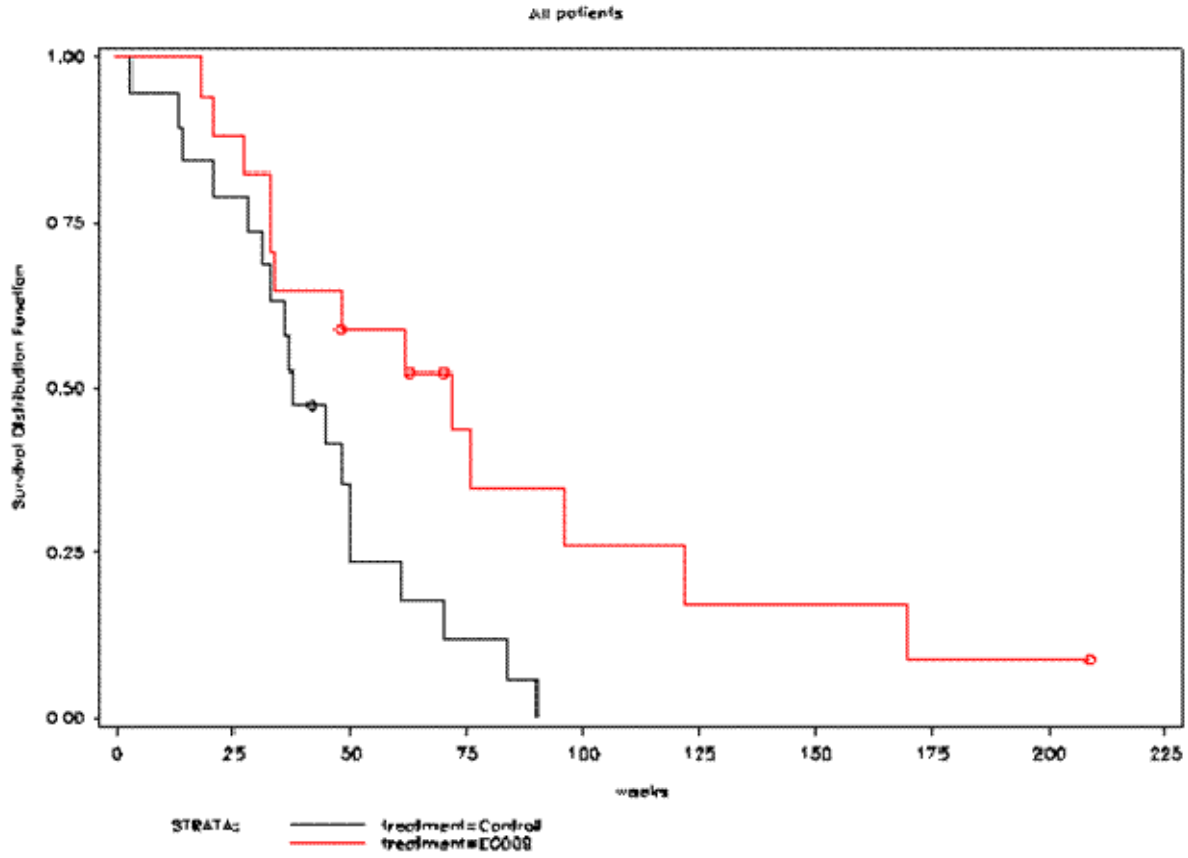
Numbers analysed

Thirty-six patients were included in the intent-to-treat analysis.

Outcomes and estimation

The median survival time for the Cerepro group was 62.4 weeks and 37.7 weeks for the control group. Mean survival time for the Cerepro group was 70.6 weeks (SD=52.9, n=17) and 39.0 weeks (SD=19.7, n=19) for the control group. The difference in the distribution of survival times between the Cerepro and control group was statistically significant (logrank *P*=0.0095). At the efficacy cut-off date, four patients remained alive in the Cerepro group and one remained alive in the control group.

Figure: Survival (death or re-operation) of patients following gene therapy and controls



Primary Endpoint	Mean	Median	Log Rank test
All patients	***Cerepro: 70.6*** ***Control: 39.0***	***Cerepro: 62.4*** ***Control: 37.7***	***P=0.0095***
All primary tumour patients	Cerepro: 74.2 Control: 42.8	Cerepro: 66.4 Control: 40.3	P=0.0322
All GBM patients	Cerepro: 62.4 Control: 38.2	Cerepro: 55.3 Control: 37.0	P=0.0214

Secondary endpoints

a) Tumour progression

MRI was performed at baseline and at eight weekly intervals after surgery. All the scans were read in a blinded manner by two independent radiologists. The eight week scan was taken as the secondary endpoint and compared to the immediate post-operative scan. Progression was seen in six of fifteen patients (40%) in the Cerepro group and in eight of sixteen patients (50%) in the control group.

By twenty-four weeks ten of thirteen patients (77%) in the Cerepro group had shown signs of progression compared to nine of ten patients (90%) in the control group. At thirty-two weeks eight of nine patients (89%) in the Cerepro group had shown signs of progression compared to five of six patients (83%) in the control group.

Table deleted

b) Performance

Performance was assessed using standardised neuropsychological testing including Wechsler's memory scale, quickness and attention, flexibility of behaviour, psychomotor quickness and a

symptom and mental agility questionnaire. Approximately one third of the patients completed at least one questionnaire.

No discernable trends in either an improvement or a reduction in quality of life can be determined using these scales. There is no evidence that those patients who survived longer had any undue deterioration in quality of life compared with those who survived for shorter periods.

There was also no evidence of increased dependency on concomitant drug maintenance during the prolonged survival period further indicating extended quality of life

Ancillary analyses

Post hoc subgroup analysis of the primary endpoint showed an increased median survival in patients with primary tumours (66.4 weeks v 40.3 weeks) and in patients with primary or recurrent gliobastoma multiforme (55.3 weeks v. 37.0 weeks). These differences were statistically significant using Kaplan-Meier survival plots and log rank regression (p=0.0322 and p=0.0214 respectively).

An analysis of all cause mortality on the intent to treat population showed an increased median survival of 17.4 weeks in the active group (62.4 weeks v. 45.0 weeks). Again, the difference in survival between the active and control group was statistically significant using a Kaplan-Meier survival plot and log rank regression (p=0.0256).

- Analysis performed across trials (pooled analyses and meta-analysis)

N/A.

- Clinical studies in special populations

N/A.

- Supportive study(ies)

In addition to the clinical studies performed by Ark Therapeutics to evaluate Cerepro, the results of a number of other clinical studies have been published in which the safety and efficacy of adenovirus-mediated HSV-*tk* has been investigated in patients with malignant glioma (Eck *et al.*,1996 ; Trask *et al.*,2000, Nanda *et al.*, 2001; Smitt *et al.*, 2003; Germano *et al.*, 2003).

- Discussion on clinical efficacy

In the CHMP's view, although some relevant information can be derived from study 901, the contribution of this data towards the "proof of concept" is far from being clear. The extrapolation of the results from study 901 to the intended use of Cerepro in clinical practice remains questionable. Altogether, these uncertainties on the pharmacodynamic data might have limited relevance if clinical data showing undisputable clinical efficacy would have been provided. This is not the case in this application.

Concerning the main study 903, protocol and analysis plan changes in this open label study have been undertaken during the course of the study. This constitutes an important methodological flaw as it cannot be excluded that they were data driven.

Considering the nature of the experimental drug and the complex and strongly investigator-dependent method of administration, the extrapolation of the results from this single-centre study was questioned and further addressed by the applicant in their oral explanation (see below).

The heterogeneity of the studied population and the uneven distribution of relevant prognostic factor make unfeasible a straight forward interpretation of the survival data. In this regard, consistent imbalances favouring the experimental arm can be observed: histology; patients with recurrent tumours, patients in whom total resection of the primary tumour was possible. Importantly, the lack of

summarised information on the total tumour volume makes the data on subtotal resection of limited value in terms of patient prognosis.

The analysis of the overall mortality carried out in patients with primary GBM show no difference in median survival between treatment arms (48.1 vs. 49.6 weeks).

Tumour progression data does not seem to be consistent with the observed survival benefit. The tumour progression in both groups was similar. The quality and consistency of the study results is poor.

Concerning the GCP inspection of study 903, although the clinical data collected in this study could be verified, the concerns with respect to data quality remain. CRFs were created retrospectively. Thus, only information available in the patients' records was collected retrospectively. However, there is a large amount of information that is either poorly documented or is lacking in many patients (e.g. QoL, MRI data and laboratory parameter) prohibiting meaningful analyses.

In conclusion, considering the mentioned methodological flaws and doubts on the level of sponsor and investigator awareness at each step of the decision making process, the results of study 903 cannot be regarded as confirmatory data supporting the safety and efficacy of Cerepro in the claimed indication.

Moreover, the characteristics of study 903 in terms of sample size, baseline imbalances in relevant prognostic factors preclude to draw valid conclusions on the efficacy of Cerepro in the treatment of malignant gliomas.

The applicant, during an oral explanation argued that the significance level associated with the findings of a study carries the same implication whether the study is large or small. The applicant argued that when a small study achieves a significant finding, this is no less or more secure than an individual finding observed in a large study. The applicant also raised a number of other points. Study 903 was claimed to be externally valid based on the demographic characteristics of the recruited population, by the survival of the control group, and by the comparable treatment and survival of patients treated in Finland compared to the rest of Europe. Therefore the findings from Study 903 were claimed to be safe to be extrapolated to a wider European population. The reliability of the key findings of Study 903 depended on GCP compliance, which has been verified by the GCP inspection. The effects seen in the trial could not be put down to any possible bias in the conduct of the trial. Therefore the applicant claimed that the statistical significance of the findings (log rank test) is as reported. Although there was a slight imbalance in prognostic factors the applicant claimed that this does not account for the large difference seen in the primary endpoint and this has been confirmed by an adjusted analysis that allows for imbalances in prognostic factors. According to the applicant, the effect of treatment with Cerepro was clearly significant and robust across many analyses. Lastly, the applicant argued that apoptosis has been demonstrated as the mechanism of cell death *in vitro* and in animals and that it is not ethically possible to conduct appropriate studies in patients with glioma that would demonstrate apoptosis. Therefore, the applicant claimed that whilst the primary pharmacodynamic studies are by necessity limited, the efficacy data from Study 902 and 903 indicate that the use of Cerepro with subsequent ganciclovir is efficacious in the treatment of patients with operable high-grade glioma. Lastly, according to the applicant, additional data provided by the applicant from Study 904 confirm the results of Study 903 and indicate that the level of biodistribution following treatment with Cerepro is negligible and of little, if any, consequence to its safety profile.

The CHMP considered these additional arguments provided by the applicant. However, the CHMP considered that these arguments did not resolve the major concerns with respect to the heterogeneity of the population as to prognosis, the small sample size, and the methodological flaws of the study, as well as the limited size of the safety database.

During the evaluation, the CHMP considered whether the application for Cerepro met the requirements for a conditional marketing authorisation or a marketing authorization under exceptional circumstances. The CHMP concluded that Cerepro did not meet the requirements for a marketing authorisation since the risk-benefit balance of the product is not positive.

Clinical safety

- Patient exposure

The overall safety evaluation of Cerepro is derived from the two clinical efficacy studies, studies 902 and 903. In studies 902 and 903 twenty-four patients received a single dose of Cerepro (3×10^{10} pfu) by intracerebral injection into non-tumour tissue at the site of tumour resection. No patient received more than one dose. All patients who received a single dose were included in this safety analysis. Further preliminary safety data were provided during the evaluation from a further clinical study (study 904).

Table: Demographic profile of patients exposed to Cerepro
(preliminary results from study 904 are not included)

	N(%)
Number	24
Gender :	
Male	17
Female	7
Age (Years):	
Mean	53.0
Range	39 – 68
<18	0 (0)
18-30	0
31-40	2
41-50	7
51-60	11
61-70	4
>70	0 (0)
Tumour Type:	
Primary	15
Recurrent	9
Tumour histology	
AA	5
GBM	18
Other	1

AA = Anaplastic astrocytoma
GBM = Glioblastoma multiforme

- Adverse events

Study 902

Gene transfers were clinically safe with both retrovirus and adenovirus vectors and no severe adverse events were detected. However, epileptic seizures were more frequent in two patients who received adenoviruses, although both of these patients had already had epileptic symptoms before the operation. One patient had partly reversible hemiparesis and aphasia as a complication of bifocal frontal tumour resection. She had retrovirus-packaging cell gene therapy combined with a tumour resection.

Two patients had fever reactions after adenovirus-mediated gene transfer with ventricular openings. The body temperature rose as high as 39°C, but the reactions were short term and reversible without any remaining symptoms.

Study 903

An adverse event was defined as any untoward medical occurrence in a patient which does not necessarily have a causal relationship to the treatment. This included events which were not present at baseline or events which were present at baseline but which worsened during the study period. Events were recorded from the first study-related activity after the patient had signed the consent form and included illness and pre-existing conditions found as a result of the screening procedures undertaken.

Adverse events were rated as mild, moderate and severe and their relationship to study medication was rated as unlikely, possibly, probably or definitely. All patients except one in the control group reported at least 1 AE.

Table: Adverse events by system organ class and preferred terms from study 903 in patients who received Cerepro

System Organ Class	Preferred Terms	Cerepro Events N (%)	Control Events N (%)	EG009 Patients N %)	Control Patients N (%)
Nervous system disorders	Headache	25 (16.7)	14 (19.4)	15 (88.2)	12 (63.2)
	Epilepsy	15 (10)	13 (12.9)	9 (52.9)	8 (42.1)
	Hemiparesis	6 (6.7)	1 (1.6)	6 (35.3)	1 (5.3)
	Dysphasia	5 (3.3)	3 (4.8)	3 (17.6)	3 (15.8)
	Hemianopia	2 (2.2)	-	2 (11.8)	-
	Hypoaesthesia	1 (1.1)	-	1 (5.9)	-
	Monoparesis	1 (1.1)	-	1 (5.9)	-
	Memory Impairment	-	2 (3.2)	-	2 (10.5)
	Ophthalmoplegia	-	1 (1.6)	-	1 (5.3)
Gastrointestinal disorders	Nausea	12 (8.9)	5 (8.1)	8 (47.1)	5 (26.3)
	Abdominal pain	2 (2.2)	-	2 (11.8)	-
	Constipation	1 (1.1)	-	1 (5.9)	-
	Diarrhoea	1 (1.1)	1 (1.6)	1 (5.9)	1 (5.3)
	Dyspepsia	1 (1.1)	1 (1.6)	1 (5.9)	1 (5.3)
	Vomiting	1 (1.1)	-	1 (5.9)	-
	Stomatitis	-	1 (1.6)	-	1 (5.3)
General disorders	Pyrexia	9 (7.9)	5 (8.1)	7 (41.2)	5 (26.3)
	General physical health deterioration	1 (1.1)	2 (3.2)	1 (5.9)	2 (10.5)
	Oedema	1 (1.1)	1 (1.6)	1 (5.9)	1 (5.3)
	Oedema peripheral	1 (1.1)	-	1 (5.9)	-
	Pitting oedema	-	1 (1.6)	-	1 (5.3)
Injury, poisoning and procedural complications	Postoperative complications	6 (6.7)	2 (3.2)	6 (35.3)	2 (10.5)
Investigations	Abnormal liver function tests	3 (3.3)	2 (3.2)	3 (17.6)	2 (10.5)
Psychiatric disorders	Confusion	3 (3.3)	2 (3.2)	3 (17.6)	2 (10.5)
	Depression NOS	2 (2.2)	2 (3.2)	2 (11.8)	2 (10.5)
	Anxiety	-	1 (1.6)	-	1 (5.3)
Infections and infestations	Urinary tract infection	3 (2.2)	1 (1.6)	2 (11.8)	1 (5.3)
	Oral candidiasis	1 (1.1)	-	1 (5.9)	-
	Oral fungal infection	1 (1.1)	-	1 (5.9)	-
	Skin fungal infection	1 (1.1)	-	1 (5.9)	-
	Bronchitis	-	1 (1.6)	-	1 (5.3)
	Pneumonia	-	1 (1.6)	-	1 (5.3)
	Wound infection	-	1 (1.6)	-	1 (5.3)
Respiratory, thoracic and mediastinal disorders	Cough	2 (2.2)	1 (1.6)	2 (11.8)	1 (5.3)
Skin and subcutaneous tissue disorders	Urticaria	2 (2.2)	1 (1.6)	2 (11.8)	1 (5.3)
	Eczema	-	1 (1.6)	-	1 (5.3)
	Eyelid oedema	-	1 (1.6)	-	1 (5.3)

	Localised skin reaction	-	1 (1.6)	-	1 (5.3)
Cardiac disorders	Atrial fibrillation	1 (1.1)	-	1 (5.9)	-
Musculoskeletal and connective tissue disorders	Back pain	1 (1.1)	-	1 (5.9)	-
	Femoral neck fracture	1 (1.1)	-	1 (5.9)	-
Renal and urinary disorders	Urinary retention	1 (1.1)	-	1 (5.9)	-
Vascular disorders	Phlebitis	1 (1.1)	-	1 (5.9)	-
	Deep venous thrombosis	1 (1.1)	-	1 (5.9)	-

- Serious adverse event/deaths/other significant events

Study 902

Only the published paper has been made available on Study 902. No further information on SAR and deaths has been provided.

Study 903

At the efficacy cut-off date, four patients remained alive in the Cerepro group and one remained alive in the control group.

There were no withdrawals due to serious adverse events and all deaths were the result of disease progression. There was no difference in the type of serious adverse events between the two groups with the exception of raised liver enzymes.

All deaths that occurred during the development programme were as the result of disease progression per protocol definitions and were not related to investigational product.
There were no withdrawals due to serious adverse events.

Twenty nine patients reported serious adverse events. There was no difference in the incidence of serious adverse events between the two groups (16 Cerepro, 13 control)

Of the sixteen patients in the Cerepro group who reported serious adverse events, five events in three patients, were considered by the investigator to be possibly (2 events) or probably (3 events) related to treatment.

There were three events (in 3 patients) of raised liver enzymes (compared with none in the control group). One case was considered unrelated and the other two were considered “possibly” related to the study medication by the investigator. No further information is available for the unrelated case. In neither of the other cases was the total bilirubin elevated. In both patients the preoperative liver enzymes were normal and both became elevated to greater than three times the upper limit of normal after 5 to 7 days of ganciclovir treatment. In patient 17 the enzymes returned to normal after a temporary discontinuation of ganciclovir treatment. In patient 7 the enzymes returned to normal four months after discontinuation of ganciclovir treatment. Elevations in liver enzymes have not been reported from patients receiving Cerepro in the five day period before ganciclovir was administered.

There were two events of intracerebral oedema, which were considered by the investigator as probably related to treatment. In another patient it is considered that incomplete resection of the tumour during the initial operation may have resulted in injection of Cerepro into residual tumour and that this may have led to the subsequent intra-cerebral oedema. The second case of intracerebral oedema raises the possibility of an interaction between Cerepro and ganciclovir due to the temporal relationship between the event and the re-introduction of ganciclovir. However by the time that ganciclovir was re-introduced peak expression of the transgene should have passed. Although it is assumed that there would be remaining some thymidine kinase to phosphorylate the ganciclovir, the MAH considers that a direct interaction between Cerepro and ganciclovir is unlikely in this case.

- Laboratory findings

Study 902

There were no major alterations in routine laboratory tests although one patient developed mild, reversible leucopoenia without symptoms during ganciclovir therapy. It is not clear to which group this patient was allocated, but the applicant considers it as largely irrelevant since the adverse event is typical of those seen with ganciclovir irrespective of the vector used.

Study 903
The relevant laboratory parameters were collected at baseline, 1 day after the operation, 19 days and 2 months after the operation and 2 months thereafter during the first year.

A review of laboratory parameters showed no clinically relevant differences between the Cerepro and the control groups, with exception of the raised liver enzymes in the patients as described above.

- Immunological events

Study 902

Anti-adenovirus antibodies increased more than 4-fold in four patients who received adenoviruses; two of them also had fever reactions. The applicant considers that the significant increase in anti-adenovirus antibodies could be related to gene therapy with high-titer adenoviruses and that opening of the ventricle system during surgery seems to correlate with the fever reactions but an increase in antibodies was also seen without ventricle opening.

Study 903

A review of the anti-adenoviral antibody titres showed six patients, all in the active group, with greater than four fold elevations when measured fourteen days after gene transfer. This was not associated with an adverse outcome in any patient.

- Biodistribution

Study 902

No systemic escape of viruses to plasma or urine samples was detected using PCR and wild-type virus assays
An analysis of post-mortem samples from one patient by PCR showed that the tumour and all other analyzed tissues were negative for the transgene 8 months after the gene transfer.

Study 903

Biodistribution of the adenovirus was assessed by measuring the level of adenovirus in the serum and plasma collected at screening and at days 3, 7 and 21 after administration of the adenovirus by quantitative PCR. Two patients were PCR positive in an assay designed to detect Ad.HSV-tk when assayed three and seven days after gene transfer but not thereafter.

Adv.HSV-tk was detected in the serum from one patient at day 3. In addition, the plasma samples collected from this patient at days 1 and 3 were also positive. The samples that were collected at days 7 and 21 from this patient were all negative. In this patient the lateral ventricles had been opened during surgery and the applicant says that this may account for the appearance of the virus in the systemic circulation.

One further patient was PCR positive up to 7 days after surgery in both serum and plasma. The plasma sample collected at Day 21 from this patient was negative.

- Safety in special populations

There were no reports of safety issues in special groups and situations during the clinical development programme.

- Safety related to drug-drug interactions and other interactions

Study 902

The increase in epileptic seizures could be due to the mechanical irritation of the operation but was also reported earlier in relation to retrovirus-packaging cell gene therapy. It is possible that injected virus particles and cell death after GCV medication act as irritative mechanisms together with the tumour resection.

Study 903

In one case, the intracerebral oedema development raises the possibility of an interaction between Cerepro and ganciclovir due to the temporal relationship between the event and the re-introduction of ganciclovir. However by the time that ganciclovir was re-introduced peak expression of the transgene should have passed. Although it is assumed that there would be remaining some thymidine kinase to phosphorylate the ganciclovir, a direct interaction between Cerepro and ganciclovir is unlikely in this case.

- Discontinuation due to adverse events

Study 902

There is no available information

Study 903

All patients received their allocated treatment and no patients were withdrawn due to adverse events. No patients were withdrawn due to non-compliance and no patients were lost to follow up. Two patients received ganciclovir for 12 days. One patient received ganciclovir for eight days before being discontinued due to a serious adverse event and one patient received ganciclovir for twelve days in total, ganciclovir being withheld for two days for a serious adverse event. Both of these patients fulfilled withdrawal criterion No.2 due to an increase in liver enzymes, but were continued in the study by the investigator. The duration of treatment was not considered as major protocol deviation.

- Post marketing experience

N/A

- Discussion on clinical safety

The quantity and quality of the safety information provided is limited. The human safety profile of Cerepro in patients with high grade glioma is mainly based on a small number of patients included in studies 902 and 903 with further preliminary results provided during the procedure from study 904.

Study 901 was also part of the development programme of Cerepro. However, the use of different vectors and different doses, the lack of concomitant treatment with GCV, as well as the different administration of Cerepro in relation to the surgical procedure make difficult the extrapolation of the safety results from study 901 to the intended clinical practice.

Data obtained in the controlled 903 study provided the pivotal evaluation of the Cerepro safety profile in the target population. Only 24 patients received Cerepro at the proposed schedule, and only patients from study 903 (17 in total) received the drug as it is expected to be administered in clinical practice.

All of these issues show that there is an extremely limited patient exposure precluding to reach a sufficiently sound conclusion on the clinical safety of Cerepro.

The most frequently occurring adverse event observed in both groups was headache (15 vs 12 in Cerepro and control groups respectively). Most of them were of mild to moderate intensity and only 1 was considered as related to the active treatment. Importantly, there were 21 (24 according to the listing) epilepsy serious adverse events in the experimental group and 4 (8 according to the listing) in the control group.

Pyrexia, postoperative complication and hemiparesis were reported in a numerically higher frequency of appearance in the Cerepro group. However, due to the limited patient exposure, and the involvement of a surgical procedure which markedly different consequences depending on the tumour location and size, no conclusions can be drawn until more data are available.

Regarding deaths and serious adverse events, all deaths that occurred during the development programme in that study were as the result of disease progression.

Twenty nine patients (16 in the Cerepro and 13 in the control group) reported serious adverse events. Five events in 3 patients in the Cerepro group were considered to be possibly (2 events of raised liver enzymes) or probably (2 events of intracerebral oedema and 1 event of hemiparesis) related to the treatment.

Raised liver enzymes is the most relevantly reported laboratory finding among Cerepro-treated patients, probably related to ganciclovir therapy. No other relevant differences in other laboratoty parameters have been found with the exception of leucopoenia in study 902.

In study 903, adenovirus was found in samples of serum and plasma in 2 patients treated with Cerepro at days 3 and 7 respectively. At day 21 samples were negative for both patients. However, samples were only tested in a minority of patients (*in 7 patients in the Cerepro group and in 4 patients in the control group)*. Of note, even in these few patients sampling was not done at all predefined timepoints. From the report from the company carrying out the PCR analysis it is not entirely clear the number of patients and sample actually analysed. Finally, there were false positive results in the PCR analyses which make impossible to know whether RCAs were present or not in the patients

Since Cerepro is used in conjunction with ganciclovir sodium, it should not be given to those patients in whom the use of ganciclovir sodium is contraindicated. Ganciclovir has the following contraindications:

- patients with hypersensitivity to ganciclovir or valganciclovir or to any of the excipients.
- patients with hypersensitivity to aciclovir and valaciclovir.
- ganciclovir is contra-indicated during pregnancy and lactation

There are no adequate data from the use of Cerepro in pregnant women. Cerepro should not be used during pregnancy unless clearly necessary.

Women of childbearing potential must be advised to use effective contraception during treatment. Male patients should be advised to practise barrier contraception during, and following treatment unless it is certain that the female partner is not at risk of pregnancy

It is unknown if Cerepro is excreted in breast milk, but the possibility of Cerepro being excreted in the breast milk and causing serious adverse reactions in the nursing infant cannot be discounted. Therefore, breastfeeding must be discontinued.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

A detailed description of all routine pharmacovigilance activities and responsibilities were not provided. The CHMP considered that this deficiency would need to be addressed before a marketing authorisation could be granted.

Risk Management Plan

A revised version of the Risk Management Plan was provided by the Applicant during the evaluation. A number of deficiencies were identified and would have to be resolved before a marketing authorisation can be granted. This would include the preparation of an adequate risk minimisation plan. An exposure registry and the long term follow up as well as the educational program for the user/physicians would have to be part of this risk minimisation plan, and would have to adhere to an agreed timeframe.

2.6 Environmental aspects

Introduction

Cerepro is a gene therapy medicinal product based on a genetically modified adenovirus that contains the herpes simplex virus thymidine kinase gene, which is a genetically modified organism (GMO) as defined in directive 2001/18/EC[1]. The scope of this ERA is the environment at large, excluding the patient but including people in the patient's environment. In general the current ERA follows the methodology described in the EU deliberate release Directive 2001/18/EC.

Environmental risk assessment

- Hazard identification

For the purpose of hazard identification, the characteristics of Cerepro that may cause a harmful effect on human health or the environment are identified and the potential consequences of these harmful effects are evaluated. Cerepro is based on an adenoviral vector derived from a human adenovirus serotype 5 (Ad5). Human adenoviral serotypes do not effectively infect non-human species. Harmful effects to non-human species are not expected and are not evaluated in the current ERA.

Worst-case scenario

To identify and evaluate harmful effects that could arise from the use of Cerepro a worst-case scenario is defined. In this scenario a number of parameters are maximised. This scenario does not necessarily correspond to the characteristics and intended use of Cerepro, but is useful as it yields a maximum appraisal of the potential hazards. The actual situation, based on the information provided by the applicant, is taken into account subsequently in the evaluation of the likelihood to determine whether the occurrence of a harmful effect is expected.

- Evaluation of likelihood

The evaluation of likelihood considers the probability that previously identified harmful effects occur. In the worst case scenario described above it was assumed that RCA expressing HSV-tk are present. Furthermore it was assumed that this vector will spread efficiently in the environment. Harmful effects for immune compromised individuals that are treated with ganciclovir at the time of a systemic infection are considered high. Replication, RCA and spreading in the environment were discussed in order to determine the likelihood of events leading to a harmful effect.

Spreading into the environment

[1] • Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC.

RCA may arise during the production in HEK293 cells by homologous recombination. During this process, the HSV-tk expression cassette will be removed from the vector and exchanged for the E1 virus gene. A similar recombination can occur during Cerepro infection of an individual that simultaneously suffers a wild type adenoviral infection.

Based on the vector size and the size of the HSV-tk gene, it is theoretically possible that particles containing both the E1 gene and the HSV-tk gene are formed, as vector particles containing both E1 and HSV-tk sequences do not exceed the critical genome size limit of 105% compared to that of wild type adenoviruses. However, formation of these particles would involve non-homologous recombination. Such particles, if they were to arise at all, would arise much less frequently than RCA resulting from homologous recombination. The chance of an RCA containing the HSV-tk gene being present in a dose of Cerepro is negligible.

Replication of Cerepro may also occur *in vivo* due to complementation of the E1 gene product by a simultaneously present wild type adenovirus. The likelihood of reproduction of Cerepro *in vivo*, followed by enhanced shedding, is negligible. Subsequent infection of other individuals is also negligible.

The likelihood of spreading Cerepro in the environment is also restricted due to the specific administration in the brain. Following administration, Cerepro will in most cases reside in the brain. Spreading into the environment can only occur following the systemic distribution of Cerepro. As long as Cerepro resides in the brain, spreading in the environment is negligible. However, from the 50 patients treated in two clinical studies only a subset of patients was tested for systemic distribution of Cerepro. Two of these demonstrated systemic distribution of Cerepro. Systemic distribution is expected to occur although it will be dependent on the details of surgery.

Nevertheless, it is considered that even if systemic distribution occurs the chance of a release of Cerepro in the environment leading to the exposure and effective infection of other individuals is negligible.

Adenoviral vectors containing the HSV-tk gene have been studied world wide in several clinical studies for treatment of different cancers like head and neck, prostate, ovarian, lung, melanoma, retinoblastoma, colon, bladder and pancreas. It is conceivable that Cerepro will eventually be used for other indications, either off label or after extension of the approved indication. Shedding of Cerepro might be considerably increased i.e. in treatment of prostate cancer.

Nevertheless, the dose that will be released in the environment will be low compared to the dose that is administered to a patient. Also based on the replication deficient character of the vector it can be concluded that the likeliness of an infection of a third party developing a sustainable Cerepro infection followed by a continuous spreading of Cerepro in the environment is negligible.

Ganciclovir

If ganciclovir is present at the start of or shortly after an inadvertent Cerepro infection, the infected cells will be killed before new viral particles are formed, with ganciclovir acting as an antiviral drug. The effects of ganciclovir induce apoptosis, bystander effect, the release of Cerepro and the effect of a Cerepro infection are small. Any harm could increase depending on the scale of the Cerepro infection. The hazard identification assumed that ganciclovir treatment of systemically infected individuals may cause a harmful effect. However, the chance that an individual suffering an accidental severe Cerepro infection is treated with ganciclovir is negligible.

- Estimation of the risk

Risks can only arise in case a harmful effect is identified that is also likely to occur. In the case of Cerepro a harmful effect has been described, however, the evaluation of the likelihood indicates that the necessary events leading to this effect are highly unlikely to occur. Therefore the risk for the environment that is related with the use of Cerepro is negligible.

- Risk management strategies

Based on the estimated risk for the intended intracerebral use of Cerepro there is no necessity for prescription of additional risk management measures with regard to the environment.

- Determination of the overall risk

The overall risk might change in cases where risk management strategies are indicated. Due to the absence of necessary risk management measures the overall risk equals the estimated risk described above.

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

During the evaluation procedure and in accordance with Article 6(3) of Regulation (EC) No 726/2004, Competent Authorities (CAs) established under Directive 2001/18/EC have been consulted and among the consulted CAs, 14 countries have provided comments, which were channelled via the CA from Spain (appointed Lead CA). There was general support of the overall conclusions of the CHMP. Comments have been raised in relation to the monitoring plan, data on biodistribution, the genetically modified organism, production and emergence of RCA, off label uses, PCR assays for detection, worst-case scenario and possible interaction with ganciclovir, other possible target species, waste treatment, standard operating procedures and emergency plan, history of previous clinical approvals, and information to the Public. Comments from CAs specific to environmental aspects have been taken into account. Those concerns related to the quality and safety of the product, rather than the risk to the environment *per se*, were addressed has part of the evaluation of the quality, pre-clinical and clinical aspects.

Conclusion on Environmental aspects

The applicant has proposed a monitoring plan which includes a comprehensive rationale and schedule for testing patients treated with Cerepro. The monitoring plan also includes an overview and a description of the sampling strategy and of the evaluation of the collected data. The applicant has justified why a monitoring plan for systemic distribution would not add further information. In case of an unanticipated effect, the plan outlines which steps should be taken to inform the Authorities. In addition, the monitoring plan identifies who would carry out the various tasks the monitoring plan requires and who would be responsible for ensuring that the monitoring plan is put into place and carried out appropriately.

This plan would be sufficient to monitor unanticipated effects on human health in general.

The applicant has provided documentation that meets the requirements of Directive 2001/18/EC regarding information of the public (namely the Environmental Risk Assessment and the SNIF).

In conclusion, the product contains a genetically modified organism and the outcome of the Environmental Risk Assessment (ERA) is that there is a negligible risk to human health, from an environmental perspective, and to the environment.

2.7 Overall conclusions, risk/benefit assessment and recommendation

Quality

Except for remaining outstanding quality issues, the quality of this product is considered to be acceptable when used in accordance with the conditions defined in the SPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

With respect to the issue of comparability, and except for outstanding concerns that remain to be addressed, it is concluded that on the basis of quality data there is evidence that the material used in the clinical studies 902-903 and material obtained using the commercial process may be considered comparable. Nevertheless, it is noted that Cerepro is a highly complex product in terms of quality and that for such a product the value of a comparability exercise performed on the basis of quality only has inherent limitations. Complete reassurance with respect to the comparability in terms of safety and efficacy can only be established from clinical data obtained with product manufactured using the commercial process.

Non-clinical pharmacology and toxicology

Several non GLP-compliant studies were performed in academic settings for the non-clinical development of Cerepro. One major GLP-compliant study was conducted to address the pharmacokinetics and the toxicity of Cerepro, and some aspects of the pharmacodynamic properties. Non-clinical data reveal no special hazard for humans based on conventional toxicology studies. In preclinical studies the gene transfer adenoviral vector used in Cerepro was detected at low levels in the systemic circulation following intra-cranial administration. The levels declined rapidly with time indicating that the adenovirus was not replicating and had not integrated into the host genome. Adenovirus has been detected in the systemic circulation in two patients up to three days after surgery. No virus was detected after this time. In one of these patients the lateral ventricle had been opened during surgery. This may account for the appearance of virus in the systemic circulation.

Efficacy

The main clinical study 903 was an open, single centre, controlled, randomised Phase II study involving patients with operable primary or recurrent malignant glioma. The purpose of the study was to evaluate safety and efficacy of adenovirus-mediated thymidine kinase gene therapy and ganciclovir medication for the treatment of malignant glioma.

Based on an analysis from this study, a statistically significant difference in overall survival (all cause mortality) has been reported, median survival time being 62.4 weeks for the group receiving Cerepro and 45.0 weeks for the control group.

However, the validity of this finding is questioned since relevant prognostic factors (including histological type of tumour) were unevenly distributed across treatment arms. Moreover, the external validity of the results is also questioned since study 903 is a single centre study with limited sample size. This aspect becomes especially relevant when considering the strong investigator-dependent way of administration of Cerepro. This led the CHMP to consider that the results of Study 904 are necessary to evaluate the added value of Cerepro to the current standard of care in general neurosurgical practice.

In conclusion, the efficacy of Cerepro in the intended target population has not been properly demonstrated.

Safety

The most frequently occurring adverse event observed in study 903 in both groups was headache (15 vs 12 in Cerepro and control groups respectively). Most of them were of mild to moderate intensity and only 1 was considered to be related to the active treatment. It was questioned whether Cerepro caused an increase of the incidence of occurrence of epilepsy but due to the limited number of patient exposure, no conclusions could be drawn.

Pyrexia, postoperative complication and hemiparesis were reported in a numerically higher frequency of appearance in the Cerepro group. However, due to the limited patient exposure, and the involvement of a surgical procedure with markedly different consequences depending on the tumour location and size, no conclusions can be drawn.

Overall, neurological and hepatic reactions appear to be more frequent in the active treatment group. However, the limited size of the database and the insufficient quality of the safety information do not allow to draw firm conclusions on the safety profile of Cerepro.

Risk to the environment

The product contains a genetically modified organism and the outcome of the Environmental Risk Assessment (ERA) is that there is a negligible risk to human health, from an environmental perspective, and to the environment.

Risk-benefit assessment

The validity of the clinical trial results from the pivotal study 903 is questioned since relevant prognostic factors were unevenly distributed across treatment arms, and since study 903 is a single centre study with limited sample size. Due to the heterogeneity of the population as to prognosis, the small sample size, and the methodological flaws, one cannot rule out that the observed study effect might in fact be due to chance and imbalance in important known and unknown prognostic factors. As a result the efficacy of Cerepro in the claimed indication has not been demonstrated.

Concerning safety, the size and the quality of the safety database are considered insufficient. Furthermore, treatment with Cerepro in combination with ganciclovir was associated with an increased neurotoxicity, as derived from a considerably higher frequency of serious epileptic events. Biodistribution data provided were deficient and a new study would need to be submitted before marketing authorisation could be granted. As a result there are concerns with respect to the safety of Cerepro.

There are also remaining doubts with respect to the comparability in terms of safety and efficacy of product used in the clinical trial and product to be marketed.

In conclusion, the CHMP considered that, following review of the data provided, the benefit-risk of Cerepro for use in conjunction with ganciclovir sodium for the treatment of patients with operable high-grade glioma is not positive for the following grounds:

- The pivotal efficacy data submitted, based on an interim analysis of study 903, are insufficient to establish the benefit of Cerepro in the claimed indication;

- There is a need to assess the reproducibility of the results in a multicentre study;

- The size and the quality of the safety database are considered insufficient and there are concerns about the toxicity of Cerepro in combination with ganciclovir;

- The comparability of product used in the clinical trial and product to be marketed was demonstrated only on the basis of quality data. The remaining doubts with respect to the comparability in terms of safety and efficacy can only be addressed with clinical data obtained with product manufactured using the commercial process.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Cerepro in the treatment, in conjunction with ganciclovir sodium, of patients with operable high-grade glioma was unfavourable and therefore did not recommend the granting of the marketing authorisation.