WITHDRAWAL ASSESSMENT REPORT

CEREPRO

International Nonproprietary Name: sitimagene ceradenovec

Procedure No. EMEA/H/C/001103

Day 210 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 28 November 2008 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 persons 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:

A - Centralised / Article 8(3) / New active substance.

Article 8.3 of Directive 2001/83/EC, as amended - complete and independent application

The application submitted is a complete dossier: composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain tests or studies.

Information on Paediatric requirements
Pursuant to Article 7, the application included an EMEA Decision P/28/2008 for the following condition(s):

- High-grade glioma

on the granting of a (product-specific) waiver to children aged 0 to less than 1 month for the concentrate for solution for injection for intracerebral use
on the agreement of a deferral for children aged 1 month to less than 18 years.

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 and the evaluation teams were:
Rapporteur: **Jens Ersbøll** Co-Rapporteur: **Sol Ruiz**

As Cerepro is an Advanced Therapy Medicinal Product (ATMP), the advanced therapy regulation was applicable to this procedure. Therefore, during the CHMP meeting of 16 – 19 February 2009, a CAT Rapporteur, a CAT Co-Rapporteur and a CHMP Co-ordinator were appointed.

Rapporteur: **Steffen Thirstrup/Mette Clausen** Co-Rapporteur: **Sol Ruiz**
CHMP Coordinator: Jens Ersboll, Sol Ruiz
CHMP Peer reviewer(s): Christian Schneider

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 28 November 2008.
- The procedure started on 24 December 2008.
- During the CHMP meeting of 16 – 19 February 2009, Dr. Steffen Thirstrup was appointed as CAT Rapporteur and Dr Sol Ruiz was appointed as CAT Co-Rapporteur. In November 2009, as Dr Thirstrup left tits position as CAT member, Dr Mette Clausen (CAT member alternate) was appointed as CAT Rapporteur for Cerepro.

- The Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 18 March 2009. The Co-Rapporteur's first Assessment Report was circulated to all CAT and CHMP members on 18 March 2009 . In accordance with Article 6(3) of Regulation (RC) No 726/2004, the Rapporteur and Co-Rapporteur declared that they had completed their assessment report in less than 80 days.
- On 15 April 2009, the Biologics Working Party (BWP) adopted a recommendation to the CAT for the list of questions related to quality aspects.
- During the meeting on 17 April 2009, the CAT 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 20 April 2009. .
- The applicant submitted the responses to the CAT consolidated List of Questions 24 July 2009.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CAT members on 4 September 2009 .
- On 9 September 2009, the BWP adopted the conclusions relating to the Quality aspects .
- During the CAT meeting on 11 September 2009, the CAT agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant .
- The applicant submitted the responses to the CAT consolidated List of Questions on 19 October 2009.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CAT members on 4 November 2009 .
- During the CAT meeting on 12 November 2009, outstanding issues were addressed by the applicant during an oral explanation.
- During the meeting on 4 December 2009, the CAT, 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 4 December 2009.
- During the meeting on 17 December 2009, the CHMP, in the light of the overall data submitted, based on the CAT opinion and the scientific discussion within the Committee, issued a negative opinion for granting a Marketing Authorisation to Cerepro on 17 December 2009.

2. SCIENTIFIC DISCUSSION

Introduction

High grade glioma is a disease with very poor prognosis. Despite multi-modal therapy involving surgery, radiotherapy and chemotherapy, the average survival of operated patients is a little over a year (Wen & Kesari 2008). Temozolomide is a systemic chemotherapeutic agent employed in the recent years in the treatment of high-grade gliomas. Localized therapies could be effective as well because the disease often presents as a solitary lesion and metastasis does not usually occur outside the Central Nervous System (CNS).
Cerepro is a locally administered first generation adenovirus (serotype 5) with E1 and partial E3 deletions, containing the transgene Herpes-Simplex Virus thymidine kinase (HSV-*tk*). It has an ATC classification of L01XX37.
Ganciclovir (GCV), which is used in the patient after the administration of Cerepro, is a nucleoside analogue, which is metabolised by HSV-*tk* and other cellular kinases to form the cytotoxic nucleotide analogue ganciclovir triphosphate.
The gene therapy system chosen here uses a serotype 5 adenovirus, which is known to transfect glioma and non malignant glial cells (Soudais *et al*, 2001; Thomas *et al*, 2002). The chosen transgene HSV-*tk* is a well-characterised gene, the enzyme product of which metabolises GCV to ganciclovir monophosphate (GCVMP). Cellular kinases then further metabolise the GCVMP to GCVTP, a cytotoxic nucleotide. As well as acting on transduced cells, GCVTP may move to adjacent cells, possibly via gap junctions, and exert its cytotoxic effect on non-transduced neighbouring cells (the 'bystander effect'). Since the toxicity of GCVTP is only manifest in dividing cells (in this case predominantly non-neuronal cells), it is possible to transduce non-dividing cells, hopefully without toxicity, yet exert an effect on other local, dividing (tumour) cells.
The mechanism of action of Cerepro described above is a selective 'cytotoxic' mechanism, which is believed to act through apoptotic pathways. In addition to this, there may be an additional immunological response to the tumour as a result of such treatment.
Historically, a problem of this type of treatment injected directly into the tumour has been the limited extent of transduction of cells around the injection site (Barzon *et al*, 2006). The result is that injection into intact tumours has insufficient effect. As a result of this observation, it was

decided to inject Cerepro into the resection margins of as complete a removal of tumour as possible. The injections into the apparently healthy tissue of the bed may then possibly create a treatment effect through bystander and immunological effects as described above.
The MAA for Cerepro was first submitted in October 2005. The CHMP issued a negative opinion in April 2007 and the Applicant withdrew the application in July 2007. The main grounds for refusal of the MAA were insufficient data on efficacy and safety; this has been addressed by the conduct of a new clinical study (Study 904). The current application which includes study 904 was submitted in accordance with Directive 2001/83/EC, article 8(3): complete independent application for a new active substance.
The proposed therapeutic indication was as follows: 'Cerepro is indicated for use in conjunction with ganciclovir sodium for the treatment of patients with operable high-grade glioma.'
Cerepro was granted Orphan Drug designation in EU for the treatment of high grade glioma (with subsequent use of ganciclovir sodium) on February 6, 2002 (EU/3/01/083).
A paediatric investigation plan was submitted for the condition high-grade glioma in children aged 1 month to less than 18 years for the concentrate for solution for injection for intracerebral use. The conduct of the paediatric studies was deferred. A product specific waiver was granted for children aged 0 to less than 1 month.

2.1 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.

Drug Substance

The drug 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 drug 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. A Working Virus Seed Stock (WVSS) was manufactured at Ark Therapeutics Oy (Kuopio, Finland) in compliance with Good Manufacturing Practice (GMP).

The HEK293 cell line (human embryonic kidney, adenovirus type 5 transformed) is used as the cell substrate for production of drug 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.

A two-tiered system (i.e. Master and Working Viral Seed Stock) has been established for the virus seed. Overall it is considered that the Master Cell Bank and Virus Seed Stocks have been adequately documented and characterised.

Cells are expanded from one vial of Master Cell Bank (MCB) in flasks and infected from one vial of Working Virus Seed Stock (WVSS). After replication of the vector in the infected cells, cells are harvested, sampled for testing and split into sublots which are stored frozen (below -60°C) for a validated holding period). The vector is then released by multiple freeze-thaw cycles, clarified by low speed centrifugation and purified by two successive ultracentrifugation steps on caesium chloride gradients. Collected virus bands are pooled, the buffer is exchanged for final formulation buffer and the purified sublots concentrated by Tangential Flow Filtration (TFF). Each sublot is sterile filtered, sampled for testing and stored frozen (below -60°C for a validated holding period).

Sublots that pass quality control are thawed and pooled. If required , the pool may be diluted to a target concentration of 1.1×10^{12} vp/ml with additional final formulation buffer. Finally, this formulated drug substance is sterile filtered through two consecutive 0.2µm filters, 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 only raw materials of biological origin used in the manufacturing process are foetal bovine serum (FBS) and porcine trypsin-EDTA (ethylenediaminetetraacetic acid). The quality and specifications of the FBS and porcine trypsin, 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 drug substance manufacturing process.

The active substance has been characterised sufficiently using a number of state-of-the-art analytical techniques. Three tests are used to demonstrate the identity of the drug substance. One test demonstrates 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 appropriate methods to demonstrate genomic integrity of the virus and the identity of the HSV1 thymidine kinase transgene.. The biological activity of the drug substance was characterised using an *in vitro* potency assay.

Potential process-related impurities have been identified, as have critical steps for their removal. All batches of drug substance are tested for host cell protein and host cell DNA content..

Process reagents that may be present as impurities in the drug 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 drug 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 drug product but this is acceptable.

Several different batches of cell banks were prepared during development of the product. The pivotal toxicological and clinical trials (study 904) were carried out with the MCB to be used for production. Previous clinical trials (studies 902 and 903) were performed with material derived from different cell banks. Similarly, several Virus Seed Stocks were prepared during development of the product. The Master Viral Seed Stock (MVSS) was used in the preparation of material for the pivotal toxicological and clinical trials (study 904), but the WVSS to be used in routine manufacture has not been used for production of batches used in clinical trials.. The applicant has performed a comparability exercise to demonstrate that batches produced during development are comparable to the product produced for marketing. The results obtained in the pivotal toxicology

study and Phase III clinical study 904 are therefore considered predictive of the stability, safety and efficacy of commercial Cerepro.

- Specification

Critical parameters have been included in the drug substance specifications to routinely confirm the quality of the drug substance by testing pH, osmolality, content, identity by three methods (as above) and purity (host cell protein, residual host cell DNA, caesium chloride and residual bovine serum albumin). A test for replication competent adenovirus(RCA) is also performed on each batch of drug substance.

Batch analysis data were presented confirming consistency, and RCA were not detected in any batch. The specifications for Cerepro-DS are appropriate and adequately justified based on available data, but the applicant should review the release specification levels after 12 months of commercial use.

- Stability

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

Drug Product

The drug product consists of Adv.HSV-*tk* in a formulation 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 of 0.2 ml 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 with a screw-cap closure. 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 manufacture of Cerepro drug product only involves the aseptic filling of the already formulated drug substance into glass vials. The proposed formulation is a simple buffer for the storage and preservation of adenoviral vectors and is considered acceptable. Immediately prior to administration, 1 ml of Cerepro drug product is diluted to 10 ml with sterile physiological saline.

- Manufacture of the Product

The drug product is manufactured, routinely controlled and batch released by Ark Therapeutics Oy (Kuopio, Finland). Operations are in compliance with Good Manufacturing Practice (GMP).

The manufacture of Cerepro drug product only involves the aseptic filling of the already formulated drug 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 drug substance contamination of the outer surfaces. These operations are completed in sequence in a defined period of time.

A batch of drug product is derived from a single batch of drug substance.
Drug product manufacture was performed by different manufacturers during development. Initially, filling of Cerepro batches was performed at Ark Therapeutics (Finland) which was not in compliance with cGMP. At a later stage in development, filling was performed at BioReliance (United Kingdom) to be compliant with GMP. Filling for the pivotal clinical trial (study 904) was performed at Ark Therapeutics Oy (Finland) in compliance with GMP. The applicant has presented data that show consistency of the production process as well as comparability between filling processes.

- Product Specification

Critical parameters have been included in the drug product specifications to routinely confirm the quality of the drug product by testing appearance, pH, extractable volume, subvisible particles, particle size analysis, content, particle / infectivity ratio, potency, endotoxin and sterility. 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 is measured by absorbance at 260 nm after lysis with 0.1% SDS.

The particle / infectivity ratio (P:I) is obtained by dividing the viral particle titre in vp/ml by the infectious titre in NAS IU/ml. The P:I gives an indication of the proportion of viral particles that are actually capable of infecting cells..

The potency is measured by an *in vitro* system using rat (BT4C) glioma-cells.

Analytical methods applied for the release of drug 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.

A transgene expression assay was not adopted as a release test since the potency assay itself relies on expression of functional protein and it was demonstrated that there was a correlation between measured transgene expression and potency in the therapeutic range.

- Stability of the Product

The drug product is filled in glass vials (compliant with Ph. Eur. requirements) and stored below -60°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 of 24 months below -60°C. The stability of these batches continues to be monitored as described by the study protocol. The applicant has sufficiently addressed the issue of potential degradation of the product during the in-use stability study. The proposed in-use shelf-life of 4 hours stored below 25°C is considered justified by the stability data.

- Adventitious Agents

Mycoplasma and sterility testing were performed on the MCB, MVSS and WVSS and are also performed on each batch of foetal bovine serum (FBS) and trypsin-EDTA. Sterility testing is also performed on the drug product. Mycoplasma is also tested on each batch of bulk harvest. Bioburden testing is performed on the bulk harvest and 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 (heat inactivation and gamma irradiation) which have been adequately validated. Adequate testing using validated methods are 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, MVSS and WVSS 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, MVSS and WVSS are free from adventitious viral contamination. The virus seed stocks were characterised with respect to the absence of RCAs.

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

The TSE and virus safety of the product has been sufficiently assured.

The CHMP agrees with the CAT assessment regarding the Quality aspects as described above

2.2 Non-clinical aspects

Introduction

The pharmacology of Cerepro is reported in published literature while the applicant also submitted a combined pivotal pharmacokinetic (biodistribution) and toxicology study. Three study reports were submitted in conjunction with this study. The design of the study performed using Cerepro was discussed with the CHMP during Protocol Assistance in July 2003. It was concluded that the use of only one animal species for toxicity studies could be sufficient. Moreover, the Applicant submitted an interaction study with temozolomide employing the same animal model as in the rest of the non-clinical pharmacology studies and the pharmacokinetic/biodistribution study. In order to model the clinical situation, the BT4C rat glioma model was used and Cerepro was administered intracerebrally but also intravenously to mimic a worst case scenario.

All the pivotal non-clinical studies have been conducted in compliance with GLP.

Pharmacology

- Primary pharmacodynamics

Four studies were submitted which compared different types of vectors and tried to correlate transfection levels with efficacy. These studies employed different types of a replication deficient retrovirus produced in a PA317 packaging cell line, based on their 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 showed the superiority of the adenovirus vector in terms of the level of transfection (see Clinical Aspects). 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 in a study performed in conjunction with the GLP-compliant single dose toxicity study. Male and female rats received different doses of Cerepro via the intravenous, intraperitoneal or intracerebral route. 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. Analysis of samples collected from animals dosed intracerebrally with Cerepro showed that the transgene was expressed in the brain in 3 out of 4 (3/4) animals tested on Day 3, 2/5 on Day 30, 3/5 on Day 60 and 1/3 on Day 90. The Applicant considered that these data demonstrated that Cerepro has the ability to infect cells and express HSV-*tk* mRNA after IC injection in rats.
In the most recent non-clinical study submitted, the effect of Adv.HSV-*tk*/ganciclovir therapy in combination with temozolomide was investigated in the BT4C glioma rat model. The material used in this study was manufactured according to the same process as clinical grade material used in the pivotal clinical trial, 904. Tumour volume and survival was assessed in conjunction with a number of other parameters including limited haematology and clinical chemistry analysis. Treatment with Adv.HSV-*tk*/ganciclovir and Adv.HSV-*tk*/ganciclovir in combination with temozolomide significantly reduced tumour volume. Furthermore, administration of Adv.HSV-*tk*/ganciclovir in conjunction with temozolomide significantly increased survival when compared to temozolomide administration alone (data not shown).
In addition to the preclinical work described above, a number of publications using a variety of animal models and performed by other researchers were submitted (studies not described, see discussion on non-clinical aspects).

- Secondary pharmacodynamics

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

- Safety pharmacology programme

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

- Pharmacodynamic drug interactions

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

Pharmacokinetics

Bio-analytical methods for the detection and quantitation of Adv.HSV-*tk* DNA (qPCR) and RNA (RT-qPCR) were developed and validated. Assays for the analysis of anti-Adv5 antibodies in rat and human serum and for the measurement of C-Reactive Protein (CRP) in rat plasma were also developed. The anti-Adv5 antibody and CRP assays were validated for rat samples (a different assay for neutralising anti-adenovirus antibodies was used in the clinical study)..
No conventional pharmacokinetic and absorption, distribution, metabolism and excretion studies were submitted (see discussion on non-clinical aspects).
A biodistribution study was conducted in conjunction with the single-dose toxicity study. In this study, the presence of DNA originating from Cerepro was examined using the validated qPCR assay in a range of tissues isolated from rats following a single IV or IC administration of Cerepro Groups of Cerepro treated animals and two control groups were investigated for the biodistribution of Cerepro at three or four separate time points . Tissue samples which were strongly positive for the presence of Cerepro (blood, spleen, lungs and brain) were further analysed for the presence of HSV-*tk* mRNA using RT-qPCR following extraction of total RNA (data not shown, see brief description of results in the discussion of non-clinical aspects).

No specific pharmacokinetic studies were submitted. The Applicant considered that in patients treated with Cerepro and GCV there may be need for caution, if they were simultaneously to receive an antiviral nucleoside to treat a herpetic infection elsewhere in the body.

Toxicology

- Single dose toxicity

Summaries of three exploratory academic non-GLP compliant studies were submitted. In these studies, virus corresponding to the originally cloned HSV-*tk* adenoviral construct with similar genetic structure as Cerepro was administered intravenously in healthy mice, in healthy rats by intracerebral injection or in tumour-bearing rats via intra-tumoural injection, respectively.
In a GLP compliant single-dose toxicity study, toxicity and bio-distribution of Cerepro following single-dose IC or IV administration to male and female rats was assessed. Assessment of toxicity was based on mortality, clinical observations, clinical and anatomic pathology evaluations, immunohistochemistry and antibody analysis.
In animals treated intravenously, no deaths and no effect on clinical observations, bodyweights, food consumption, serology or haematology parameters were observed. From the results obtained, no antibody response against Cerepro was detected. Limited systemic changes, such as myeloid hyperplasia in the bone marrow and changes in the red and white pulp in the spleen, probably represent an immune response to the large load of foreign antigens. These changes returned towards normality at the later time points of the study. Dose-dependent increases of spleen weights relative to overall mean necropsy bodyweight were observed, especially in female animals, and persisted throughout the study.
In animals treated via intracerebral Cerepro injection (followed or not by intraperitoneal GCV injection), three deaths occurred. Full histopathological examination was undertaken to determine the cause of death and the potential relation to Cerepro treatment. A male that received $6x10^9$ vp of Cerepro intracerebrally plus intraperitoneal ganciclovir died of suppurative meningoencephalitis, a female that received $1.2x10^9$ vp Cerepro intracerebrally plus intraperitoneal ganciclovir died most likely of peritonitis and a female that received $1.2x10^9$ vp Cerepro intracerebrally without ganciclovir died most likely of multiple emboli. Although no emboli were seen microscopically, bone marrow examination revealed coagulation necrosis, probably due to vascular compromise. The relation to Cerepro injection could not be ruled out. There were no effects on clinical observations, bodyweights, food consumption, serology, clinical chemistry or haematology parameters in any of the animals treated IC with Cerepro. From the results obtained, no antibody response against Cerepro was detected. Mean spleen weights were increased relative to bodyweight only in females receiving the higher Cerepro dose and only at the latest time point (Day 70). Histopathological and brain immunohistochemical examination revealed limited changes along the needle track and both the adjacent subarachnoid (meningeal) space and remotely in the meninges. Occasionally, findings also extended within the corpus callosum or periventricularly and ventricular dilatation and perivascular cuffing was observed.
In the group of animals that only received GCV, there was one death due to peritonitis. There were no other effects on clinical observations, bodyweights, food consumption, clinical chemistry or haematology parameters.
For animals dosed IP with GCV, soft, small dark testes were recorded macroscopically at Day 70 in all animals receiving GCV (with or without Cerepro). Microscopically, these correlated with moderately severe to severe germ cell depletion. In addition, within the testes of all male Day 30 animals treated with GCV (with or without Cerepro), mild to moderate germ cell depletion and tubular cell vacuolation were observed.

- Repeat dose toxicity (with toxicokinetics)

No formal studies of this type were submitted. The Applicant cross-referred to a non-GLP compliant academic study described in the non-clinical pharmacology section (Tyynelä *et al*, 2002) in which Adv.HSV-*tk* was injected intratumourally in rats either once or on three consecutive days. Upon histological examination, necrotic areas were found inside the tumours after treatment with Adv.HSV-*tk*, which were probably related to the effect of the virus and GCV treatment. No glioma cells were found in the brains of rats that had received three consecutive administrations of Adv.HSV-*tk* and survived for six months, instead scar tissue was seen in the place of the tumour, and very strong astrogliosis was seen in the scar area. Weak staining for glial fibrillary acid protein (GFAP, a major component of the cytoplasmic intermediate neurofilaments within astrocytes) was seen inside the tumours near the injection site and weak microglia responses were seen in OX-42 immunostaining in animals treated with a single Adv.HSV-*tk* administration. Analysis of the blood samples showed elevations in aspartyl aminotransferase (AST), alkaline phosphatase (AP), bilirubin and anti-adenovirus antibodies. No change was seen in any of the other parameters.

- Genotoxicity

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

- Carcinogenicity

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

- Reproduction Toxicity

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

- Toxicokinetic data

No toxicokinetic data were submitted (see discussion on non-clinical aspects).

- Local tolerance

The Applicant cross-referred to the submitted toxicology studies, as local effects were assessed in these due to the local nature of the treatment with Cerepro.

- Other toxicity studies

None were submitted.

Discussion on the non-clinical aspects

Four early studies compared different types of vectors and tried to correlate transfection levels with efficacy. These studies employed retroviral vectors instead of the adenoviral vector present in Cerepro and the tumour cells were transfected with the viral HSV-tk gene in vitro before IC implantation. In vitro transfections are expected to yield higher transfection rates than would be expected if the cells were transduced in vivo.
One additional publication (Tyynelä *et al*, 2002), a study conducted in conjunction with the GLP-compliant single dose toxicity study and the most recent study also employing temozolomide, provide some evidence of relevant pharmacodynamic activity for Adv.HSV-*tk* with GCV in glioma. A prerequisite for clinical activity would be to obtain sufficient transfection efficiency following the neurosurgical procedure (through multiple as opposed to single dose injection).
Additional literature studies show some evidence that gene transfer of the HSV-*tk* gene followed by ganciclovir treatment is active against malignant brain tumours, but they should be considered with caution since they represent different situations to the clinical setting of Cerepro use.
Overall, the submitted documentation on primary pharmacology provides some evidence that Adv.HSV-*tk* transfection in conjuction with GCV treatment induces cell death, but the studies are limited and they do not fully mimic the clinical situation. Moreover, the difficulties in translating results obtained in anti-cancer animal models to the clinical setting are acknowledged.
The Applicant justified the absence of secondary pharmacodynamics and safety pharmacology studies by stating that due to the viral nature of the product and the intracerebral administration only localised exposure should occur. They also argued that the GLP-compliant toxicology and biodistribution study in the rat and the human data from the clinical studies confirm that Cerepro has an acceptable safety profile and no novel harmful systemic pharmacodynamic effects have been seen in animals or patients. Moreover, the battery of safety pharmacology studies is generally not conducted in the investigation of live viral agents used for gene therapy and in vaccines, which is consistent with available guidance regarding the development of gene therapy vectors specifically and ICH S6 regarding the development of biotechnology products in general. The CAT considered that secondary and safety pharmacology studies were not necessary.
In terms of drug interactions, the Applicant claimed that there is no systemic exposure to Cerepro. Moreover, the clinical studies have not shown any evidence of interactions with any concomitant medications that the patients may receive. Finally, in a conventional sense, Cerepro is not a drug but an advanced biological therapy, so no pharmacodynamic drug interactions are expected. The CAT considered that animal drug interaction studies were not required. Adequate justification for the potential interaction with antiviral nucleosides to treat herpetic infections would have to be included in the SPC.
Conventional absorption, distribution, metabolism and excretion studies are not applicable for viral products such as Cerepro. However, the distribution, systemic exposure, clearance and transcription of the transgene should be investigated (Note for Guidance on the Quality, Preclinical and Clinical Aspects of Gene Transfer Medicinal Products CPMP/BWP/3088/99).

The applicant has performed a comprehensive single-dose GLP-compliant toxicity and biodistribution study in rats using both IC and IV dosing. The IC route was chosen to mimic the clinical route of administration while the IV route was selected to evaluate potential toxicological consequences of systemic exposure.
Early after IC injection, Adv.HSV-*tk* 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 decreased with time. There was still viral DNA in the brain and spleen at Day 90.
Following IV injection, Adv DNA was found in all tissues tested. As compared to IC administration, the levels were much higher in the viscera (including the spleen, liver, lungs, heart and kidneys) especially on Day 3, while levels were much lower in the brain. The levels decreased with time and by Day 90, Adv.HSV-*tk* DNA was predominantly detected 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.
In terms of toxicity, one animal died most likely due to peritonitis following IP injections of GCV and another one due to meningoencephalitis. Although nosocomial infections following neurosurgery might similarly occur in patients, it is reasonable to assume that the intervention due to the IC inoculation in rats is much more significant than IC injections in patients following neurosurgery, due to the smaller size of the rat brain. Furthermore, it is possible that the meningoencephalitis observed in a single animal could be due to pure housing conditions as compared to the clinical setting.
Several observations were made and reported regarding inflammatory responses after administration of Cerepro, some of them still present at the end of the study. These include increased weights of spleen as well as inflammatory 'non-injection' site reactions. Nevertheless, the evolution of the adverse effects and the temporal course of other parameters like CRP do not suggest persistent inflammatory reactions after administration of Cerepro.
Treatment with a full course of GCV was associated with small foci of necrosis in the brain in some IC group rats but overall the lesions remained small and were confined to the immediate area of the injection track. There was limited ventricular dilatation. Overall, even shortly after commencing GCV treatment, widespread cytotoxicity was not seen.
Even at the highest IV dose of Cerepro there were very limited systemic changes probably representing an immune response to the large load of foreign antigens, such as myeloid hyperplasia in the bone marrow and changes in the red and white pulp in the spleen. All those changes returned towards normality over 30-70 days after injection. However, the toxicological significance of systemic exposure to Cerepro cannot per se be evaluated as subsequent administration of GCV was not included in the toxicity study for the animals dosed with Cerepro IV. It is reasonable to assume that GCV will lead to cytotoxicity and probably apoptosis in the infected cells. On the other hand, GCV produced degeneration of germinal epithelium and dilatation of testicular tubules, when administered either alone or in combination with Cerepro.
No formal repeated-dose toxicity study was submitted. The Applicant referred to a published study in which Adv.HSV-*tk* was administered once or on three consecutive days. In principle, it would have been preferable that a two-week repeated-dose toxicity study in rodents and non-rodents with a similar dosing schedule had been conducted but some limitations should be considered for this biotechnology product, e.g. the possibility of an immune response against the viral vector, hence at the time of Scientific Advice the CHMP considered that studies in one species only could be sufficient for Cerepro. The justification provided by the Applicant was considered acceptable taking into account mainly the short duration of treatment with Cerepro and the severity of the disease to treat.
No carcinogenicity study was submitted. 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 two deletions in the adenoviral genome of the vector, which render the vector replication deficient, and because 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 CAT accepted the justification provided by the Applicant for the lack of carcinogenicity studies.
Regarding genotoxicity, reproductive and developmental toxicity of Cerepro the Applicant considered further studies unnecessary. This was endorsed, given the fact that the vector was not detected in germ line cells. Taking into account the target population to treat, the lack of reproductive and developmental toxicity studies was also at this stage justified.

The CHMP agrees with the CAT assessment regarding the Non clinical aspects as described above

2.3 Clinical aspects

Introduction

Four clinical studies (studies 901, 902, 903 and 904) were submitted in support of the use of Cerepro in the treatment of operable high-grade gliomas.
Study 901 examined the effectiveness of different vectors in transferring a marker gene (*lacZ*) into human malignant gliomas.
Study 902 compared the safety and effectiveness of Cerepro with a HSV-tk retrovirus packaging cell line for the treatment of patients with operable primary or recurrent malignant glioma. The data from a historical control group was used for comparative purposes.
Study 903 evaluated the safety and efficacy of Cerepro in the treatment of patients with operable primary or recurrent high grade glioma in a randomised study.
The pivotal study 904 was an open, multicentre, randomised standard care-controlled study of the efficacy and safety of Cerepro in patients with primary high-grade glioma.
Table 1 summarises the clinical studies comprising the submitted clinical development programme of Cerepro.

Table 1: Overview of Cerepro Clinical Trials

Trial No.	Study Design	Patients enrolled	Endpoint
901	Dose determining study using transfection levels to evaluate the efficacy of gene transfer in patients with operable high-grade glioma. Comparison of an adenoviral vector and a retroviral vector administered directly into the tumour.	12	Transfection levels and distribution Safety
902	Controlled non-randomised study to evaluate safety and efficacy of Cerepro versus HSV-*tk* gene therapy using a retroviral packaging cell line in patients with operable high-grade glioma. The data from historical control patients were also used for comparative purposes.	14	Survival Safety
903	Randomised, controlled safety and efficacy study to evaluate Cerepro for the treatment of patients with operable high-grade glioma.	36	Survival (death or re-operation) Safety
904	Randomised, controlled safety and efficacy study to evaluate Cerepro for the treatment of patients with operable high-grade glioma.	251	Survival (death or re-operation) Safety

Regulatory guidance was received in 2003 regarding the conduct of clinical study 904 (EMEA/CPMP/SAWG/3815/03). Guidance has been followed except with respect to the primary endpoint of the trial, for which the Applicant considered that, based on clinical evidence, time to death or further re-intervention as a composite endpoint is a more reliable endpoint than all cause mortality. Also, several national meetings took place with Denmark (August 2008), Germany (July 2004), United Kingdom (June 2004) and Spain (February 2005).

GCP

Studies 901, 902 and 903 have been previously submitted and assessed. For the new study 904 the Applicant stated that it was conducted in accordance with the principles of the Helsinki

Declaration and in accordance with the principles of Good Clinical Practice. The protocol was approved by the relevant IECs. Patients received oral and written information and participants signed voluntary informed consent before any study related procedures were performed. There has been no GCP inspection of study 904.

Pharmacokinetics

No conventional clinical pharmacokinetic studies were submitted. Some data pertaining to biodistribution were obtained during the pivotal efficacy study, 904, and they are described under Distribution below.

- Absorption

No studies were submitted (see discussion on clinical pharmacology).

- Distribution

Biodistribution of the adenoviral vector containing the thymidine kinase gene in whole blood was assessed by PCR at Screening, Day 1, 2, 5, 19, Months 3 and 6.
PCR analysis of blood samples in study 904 showed that no patient had detectable Cerepro DNA at baseline.
On Day 1 post-procedure, 8 of 112 patients had quantifiable levels of Cerepro DNA in their blood and a further 47 patients had detectable levels but at below the quantifiable sensitivity of the assay. On Day 2, 3 of 110 patients had quantifiable levels of Cerepro DNA and 45 patients showed unquantifiable levels. On Day 5, 1 of 108 patients had quantifiable levels of Cerepro DNA and 24 patients had unquantifiable levels. On Day 19, 1 of 99 patients had quantifiable levels of Cerepro DNA and 15 patients showed unquantifiable levels.
By 3 months post-procedure 3 of 84 patients showed unquantifiable Cerepro DNA levels and after this time point Cerepro DNA was no longer detected.
A small group of patients (up to 16 Cerepro treated patients per time point) underwent assessment for the presence of Cerepro DNA on throat swabs. No virus was detected in the throat swab samples from any patient. The absence of Cerepro DNA in all throat swabs tested indicates a low likelihood of shedding of active virus from patients receiving the treatment.

- Elimination

No studies were submitted (see discussion on clinical pharmacology).

- Dose proportionality and time dependencies

No studies were submitted (see discussion on clinical pharmacology).

- Special populations

No studies were submitted (see discussion on clinical pharmacology).

- Pharmacokinetic interaction studies

No studies were submitted (see discussion on clinical pharmacology).

- Pharmacokinetics using human biomaterials

No studies were submitted (see discussion on clinical pharmacology).

Pharmacodynamics

No conventional clinical pharmacodynamic studies were submitted (see discussion on clinical pharmacology).

- Mechanism of action

No studies to support the mechanism of action were submitted (see discussion on clinical pharmacology).

- Primary and Secondary pharmacology

No studies were submitted (see discussion on clinical pharmacology).

Discussion on clinical pharmacology

No clinical pharmacokinetic studies were submitted. Considering the nature of the medicinal product traditional pharmacokinetic evaluation is not necessary. Limited biodistribution data have been submitted.
No clinical pharmacodynamic studies were submitted, as would have been expected for a standard new chemical entity. Supportive non-clinical and clinical data were submitted (described under non-clinical aspects and clinical efficacy, respectively) in order to provide an experimental basis supporting the mechanism of action of Cerepro/GCV therapy in malignant gliomas.

Clinical efficacy

Four clinical studies comprised the clinical development programme of Cerepro. The first, Study 901, was a dose defining study. Following this, one non-randomised efficacy study (Study 902) and two randomised efficacy studies (Studies 903 and 904) were performed to support the Cerepro programme. Study 904 is the pivotal clinical study, study 901 is described under dose response studies and studies 902 and 903 are described under supportive studies.

- Dose response study

Study 901 was an academic non-GLP compliant trial for which only the published reference (Puumalainen *et al*, 1998) was submitted. The study examined the effectiveness of different vectors in transferring a β-galactosidase marker gene (*lacZ*) into human glioma. Twelve patients (mean age 46 years, range 20 – 70 years) 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 of adenovirus subset was 3 x 10^8 to 3 x 10^{10} pfu. Tumour resection was performed four to five days after catheter insertion. Two patients served as controls and received no gene transfer.
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. The procedure was well tolerated with no significant toxicity across the dose range administered (3 x 10^8 to 3 x 10^{10} pfu).
According to the Applicant, the results of the study showed that an adenoviral vector was more efficient than a retroviral vector in achieving *in vivo* gene transfer and that >10% gene transfer efficiency would be required before a therapeutic effect of HSV-*tk* and ganciclovir treatment could be expected. This percentage was only exceeded by the highest dose of adenovirus administered (3 x 10^{10} pfu). This study formed the basis of the dose selection and posology for further clinical studies and indicated that multiple injections (at approximately 0.5- 1cm intervals) would be required to achieve the necessary transfection rate.

- Main study

Study 904
This was a Phase III, multicentre, controlled, randomised, parallel group, open label study of the efficacy and safety of Cerepro with subsequent GCV for the treatment of patients with operable primary glioblastoma.

METHODS

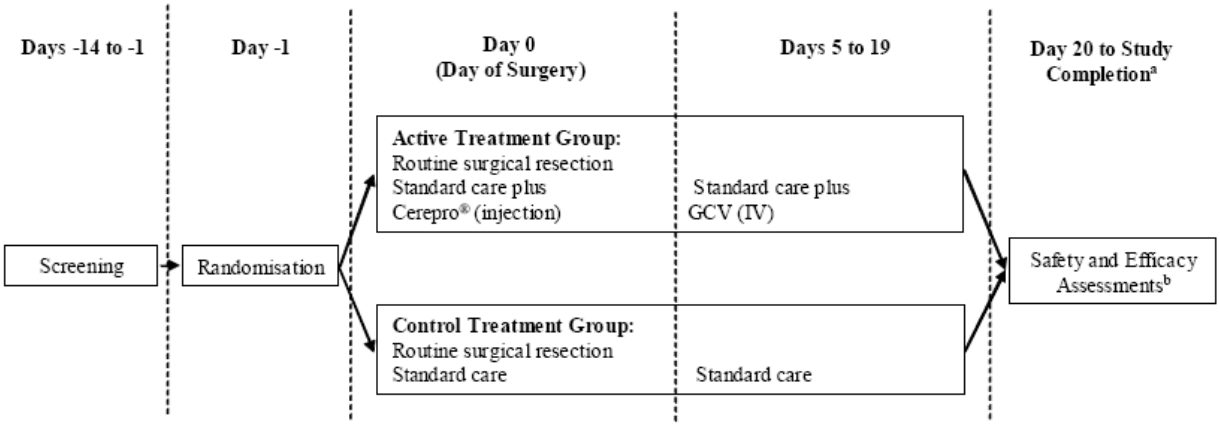
Study Participants
The patient population included adult patients with an operable primary glioblastoma. Overall, 256 patients were screened for the study at 38 sites.
Male or female patients between the ages of 18 and 70 and with a Karnofsky score ≥70 at Screening were included if they had a supratentorial primary GBM (diagnosis based on radiological and clinical grounds). Patients with bihemispheric or multifocal tumours, recurrent glioma, other significant concomitant disease (including renal or liver disease), hypersensitivity to GCV or patients who had received chemotherapy within 6 weeks prior to randomisation were excluded

from the study. Patients were withdrawn from the study if they violated the inclusion criteria (i.e., diagnosis had been changed following the histological assessment).
Patients who withdrew their consent between randomisation and surgery were to be replaced. If a patient withdrew from the study before completion, every effort was made to complete the assessments scheduled for the final evaluation.

Treatments
Patients were randomised (using a 1:1 allocation) to one of the following treatment groups:
- Standard care plus injection of Cerepro during surgery followed by administration of GCV on Days 5 to 19.
- Standard care.

Patients in the active group received a single 1 mL dose of 1 x 10^{12} viral particles (3 x10^{10} pfu) of Cerepro diluted with 9 mL of saline during surgery (Day 0). Administration required 30-70 injections of 0.1-0.4 ml to a depth of approximately 10mm in the tissue surrounding the resection. This was followed by GCV at a dose of 5 mg/kg by intravenous infusion twice daily from Day 5 to Day 19 post-surgery, based on the expected kinetics of HSV-*tk* transgene expression.
The study design is depicted in the following figure 1:

Figure 1: Study Design, Study 904



a: Patients were followed until death or until study completion.
b: Safety assessments included SAEs, AEs, clinical laboratory testing, physical examinations, vital signs, anti-adenovirus antibodies, biodistribution of the vector in whole blood and biodistribution of the vector in oro-pharyngeal mucosa. Efficacy was determined by assessing patient survival, clinical progression confirmed by MRI and Quality of Life.

Objectives
The objective of the study was to evaluate the efficacy and safety of Cerepro (adenovirus-mediated thymidine kinase gene therapy) and ganciclovir for the treatment of malignant glioma.

Outcomes/endpoints
The primary endpoint was defined as time from randomisation to death or re-intervention. Re-intervention was defined as any type of treatment (including surgery, radiotherapy or chemotherapy) given to prolong survival following reoccurrence of a tumour. All re-interventions were confirmed by a Reintervention Committee (RIC). The RIC carried out its assessments towards the end of the study, so all interim analyses were based on re-interventions as assessed by the investigator alone. However, the formal primary analysis was based on re-interventions incorporating the RIC assessment. Patients who were not known to have died or received re-intervention at the database cut-off for analysis were censored at the date they were last confirmed to be alive without re-intervention. To ensure up-to-date information was available, each patient's survival and re-intervention status were to be actively sought prior to the database cut.
The principal secondary endpoint was time from randomisation to all-cause mortality. This was initially the primary efficacy endpoint. Patients who were still alive at the end of the study were censored at the date they were last confirmed to be alive.
Additional secondary efficacy endpoints were: time from randomisation to tumour progression, Quality of Life, safety.

Sample size
The original protocol specified usual statistical methods and a standard fixed sample size calculation as follows: A total of 250 patients would be randomised to the study, in a 1:1 ratio to each of the treatments. This number of patients was estimated as required to observe 150 deaths, which would provide 80% power to detect a 60% improvement in median survival, assuming a one-sided 2.5% significance level, a median survival of 49.5 weeks in the control group, an 18-month recruitment period and a minimum patient follow-up period of 12 months. An interim analysis would be performed after approximately 40% of the required number of deaths has been observed (i.e. 60), to re-evaluate the adequacy of the sample size.

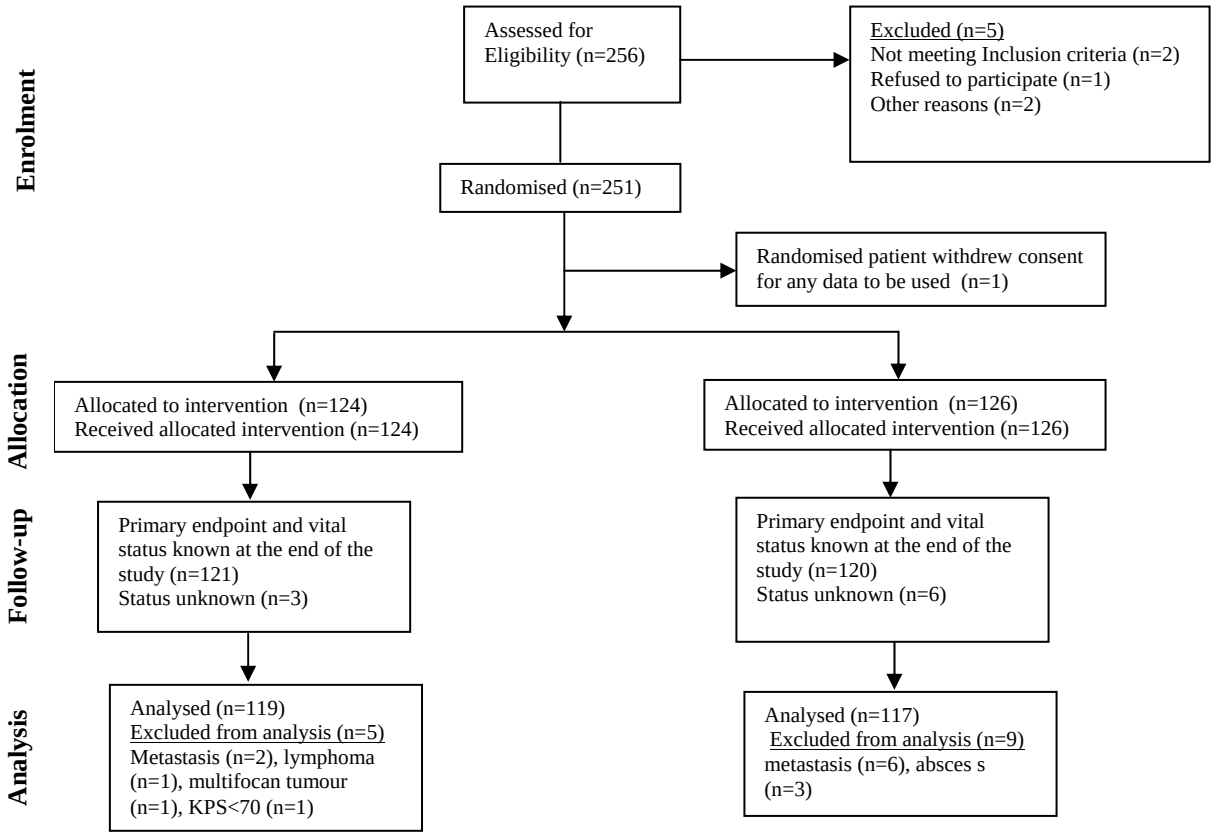
Randomisation
Patients who met the eligibility criteria and provided informed consent were enrolled into the study and randomised (using a 1:1 allocation) to one of the two treatment groups (Cerepro+GCV+standard care vs standard care only). Patients were randomised as close as possible to the time of surgery, but no more than 24 hours before surgery was scheduled.
The use of temozolomide in this study was allowed at the discretion of the Investigator. Temozolomide was not part of the randomized treatment, but its introduction made stratification based on intent-to-treat with temozolomide necessary.
Randomisation was not stratified by centre or country/region due to the relatively large number of sites needed to recruit the patients. Other variables, which may have been associated with survival were investigated in exploratory analyses, but were not used to stratify the randomisation.
Histopathological classification of tumour type could only be determined following surgical resection, and therefore after the patient was randomised. It was possible that some patients were randomised whose initial clinical diagnosis of glioblastoma was not subsequently confirmed either at surgery or by histopathology. The proportion of such patients (e.g., with some types of AA, oligodendroglioma, and oligoastrocytoma) was expected to be small. All histopathologic assessments were reviewed by a blinded, independent expert.

Blinding (masking)
This was an open label study. It was not considered ethical to perform IC injections using carrier alone, nor was it considered ethical to submit patients to 14 days of intravenous injections of saline instead of GCV.

Statistical methods
The statistical methods specified in the original protocol for the pivotal study were fairly standard:
The primary efficacy endpoint was time to death from all causes. Treatment groups would be compared for overall survival using the log-rank test stratified by primary or recurrent tumour status. The hazard ratio and its 95% confidence interval would be estimated from a Cox proportional hazards regression model with terms for treatment and primary or recurrent tumour status. Kaplan-Meier estimates of the survival curves for each treatment would be presented. An interim analysis might be carried out for efficacy at the same time as the sample size re-evaluation. It was not planned to stop the study early to claim efficacy on the basis of this analysis; consequently no statistical adjustment was proposed to the alpha-level (false positive error rate) in the final analysis.
The primary analysis would be performed on the intent-to-treat (ITT) population, comprising all patients randomised. Supportive analyses would be performed on the modified ITT population and the per protocol (PP) population, comprising patients whose clinical diagnosis was confirmed by histopathology and patients who were without major protocol violations respectively.
With the second amendment to the protocol, changes to the statistical methods were implemented as follows: The protocol was revised to allow the data to be analysed at set time points throughout the study, using a continuous sequential design, known as the triangular test. This test comprised a series of interim analyses, the first of which is scheduled for 12 months after the first patient was randomised, the subsequent interim analyses were to take place at 6 monthly intervals. Each interim analysis was to be based on a logrank statistic, Z, stratified for temozolomide use (as predicted at the time of randomisation, see also *Conduct of the Study*). It was also adjusted for age (treated as a continuous covariate) and Karnofsky score at screening (dichotomised into ≤70 or >70) using Cox's proportional hazards regression model. The study was powered to detect a hazard ratio (ψ) of 0.6. If the data conclusively showed that Cerepro was effective then the study was to be stopped early for efficacy, alternatively if Cerepro was not effective the study was to be stopped for futility.

RESULTS

Participant flow



Recruitment

The first patient entered into the study on 30 November 2005 and the last patient completed the study on 10 June 2008.

Conduct of the study

The initial study protocol was dated 07 January 2004. Six amendments to the protocol were made, the second of which was a major amendment. With this amendment dated 15 December 2005, the primary endpoint was changed from 'all-cause mortality' to 'time to death or re-intervention'. The secondary endpoint of the study was also changed from a 'Progression Free Survival' (PFS) to 'Overall Survival' (OS). No PFS as endpoint was included. Under the same amendment, the statistical analysis plan was changed as detailed under *Statistical methods* above and the inclusion criteria were changed to only allow patients with primary high-grade glioma; patients with recurrent tumours or patients with other histologies were excluded from the study. To allow for this the Statistical Analysis Plan was modified such that the primary analysis for the ITT group included patients with a confirmed histology. Finally, amendment 2 also allowed use of chemotherapy within 6 weeks after surgery. Initially, the use of adjuvant chemotherapy was to be excluded under the condition of the protocol. During the course of the study, temozolomide was introduced in the adjuvant treatment of glioma in combination with post-operative radiotherapy. It became clear that it would not be feasible or ethical to deny patients access to temozolomide and the use of temozolomide in this study was allowed at the discretion of the Investigator. Temozolomide was not part of the randomized treatment, but patients were to be stratified based on intended use of temozolomide. Because of the delay between protocol amendment and implementation, for a significant proportion of randomised patients the intention to use temozolomide was not stated; moreover, the intention to use temozolomide was not adhered to and the actual temozolomide use differed substantially from the intended, as also shown in the following Table 2.

Table 2: Temozolomide Use (ITT population)

Parameter	Active group Original stratification for Temozolomide use				Control group Original stratification for Temozolomide use			
	Yes (n=51)	No (n=32)	Unknown (n=36)	Overall (N=119)	Yes (n=51)	No (n=28)	Unknown (n=38)	Overall (N=117)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Actual Temozolomide Use								
Yes	39 (76.5)	7 (21.9)	12 (33.3)	58 (48.7)	39 (76.5)	11 (39.3)	26 (68.4)	76 (65.0)
No	12 (23.5)	25 (78.1)	24 (66.7)	61 (51.3)	12 (23.5)	17 (60.7)	12 (31.6)	41 (35.0)

Baseline data

Demographic and baseline disease characteristics were similar between the two treatment groups.

Table 3: Demographics and Baseline Disease Characteristics (ITT Population)

Characteristic	Active Group (n=119)	Control Group(n=117)
Age (years)		
Mean (SD)	55.8 (10.28)	55.1 (9.90)
Median (Range)	58.0 (20, 70)	57.0 (26, 70)
Age categories (years) n (%)		
≤40	8 (6.7)	12 (10.3)
41 - 50	23 (19.3)	25 (21.4)
51 - 60	46 (38.7)	43 (36.8)
61 - 70	42 (35.3)	37 (31.6)
Gender (%)		
Male/Female	(58.8)/(41.2)	(65.0)/ (35.0)
Histopathology diagnosis[a,b]		
GBM	112 (94.1)	111 (94.9)
Other high grade glioma	4 (3.4)	4 (3.4)
Other	3 (2.5)	2 (1.7)
Location of tumour[c]		
Right	71 (59.7)	60 (51.3)
Frontal	18 (15.1)	11 (9.4)
Parietal	16 (13.4)	13 (11.1)
Temporal	26 (21.8)	27 (23.1)
Other	11 (9.4)	9 (7.7)
Left	48 (40.3)	57 (48.7)
Frontal	13 (10.9)	15 (12.8)
Parietal	10 (8.4)	12 (10.3)
Temporal	14 (11.8)	22 (18.8)
Other	11 (9.2)	8 (6.8)
Ventricular opening (%)[c]		
Yes/No	(22.7)/(77.3)	(15.4)/(84.6)
Time since clinical diagnosis (days)[d], N	108	104
Mean (SD)	9.5 (9.89)	12.5 (13.99)
Median (Range)	7.0 (1, 76)	8.5 (0, 115)
Karnofsky score n (%)		
70	18 (15.1)	11 (9.4)
80	22 (18.5)	23 (19.7)
90	49 (41.2)	47 (40.2)
100	30 (25.2)	36 (30.8)
Estimate of resection during surgery n (%)		
Radical	99 (83.2)	95 (81.2)
Partial	20 (16.8)	22 (18.8)
Estimated extent of tumour resected from postoperative MRI		
<50%	2 (1.7)	3 (2.6)
50-69%	5 (4.2)	8 (6.8)
70-89%	30 (25.2)	22 (18.8)
≥90%	80 (67.2)	80 (68.4)
Not Done	2 (1.7)	4 (3.4)

ITT=intent-to-treat; MRI=magnetic resonance imaging; N=number of patients; SD=standard deviation

a: From Central review (for one patient, 801/143, central histology was not performed, thus the result from the local histology was used).

b: Percentages calculated relative to the number of patients with non-missing data in ITT population within each treatment group. From Central review metastasis and others are not part of the ITT.

Numbers analysed

A total of 251 patients were randomised: one patient formally withdrew consent during the study and requested that all their data be removed from any analysis; thus this patient did not form part of the safety population, and their data were subsequently removed from the clinical database.

Of the 250 patients in the safety population, 124 were randomised to active group and 126 to control group. The primary endpoint and vital status at the end of the study was known for 121 and 120 patients in the active and control groups, respectively.

Two efficacy populations were analysed for this study, the ITT population and the PP population. The ITT population was defined as all randomised patients who had a glioma (low or high grade) as

confirmed by central histology review (or local review if diagnosis from central review was not available). The PP population was defined as all patients in the ITT population who were without major protocol violations and deviations.

Table 4: Efficacy Analysis Populations

Population	Active Gruop n (%)	Control Group n (%)	Total n (%)
Patients Randomised	124	126	250
Analysed for Efficacy			
Patients in ITT population	119 (96.0)	117 (92.9)	236 (94.4)
Patients in PP Population	102 (82.3)	114 (90.5)	216 (86.4)

For the ITT and Safety populations, all patients were analysed according to the randomised treatment.
For the PP Population, patients were analysed as treated. Assuming all other relevant criteria were met, all patients who received no Cerepro and no GCV were analysed as part of the control group and all patients who received any Cerepro or any GCV were analysed as part of the active group.
The reasons for exclusion of FAS (modified ITT) patients from the PP population are detailed in the following Table 5:

Table 5: Protocol Violations and Deviations Leading to Exclusion from the ITT Population

Parameter	Active Group (N=119) n (%)	Control Group (N=117) n (%)
Number of patients with ≥1 major violation/deviation	17 (14.3)	3 (2.6)
Protocol violation/deviation		
Not GBM or histology was not done	7	2
Patient has infratentorial, bihemispheric or multifocal tumors (MRI/surgery)	2	0
Volume of Cerepro® administered was <7mL	7	0
Time between preparation and administration of Cerepro® is >240 minutes[a]	1	0
GCV compliance is <50% across whole GCV administration period (Day 5-Day 19)	1	0
Patient has taken one or more investigational drug for the treatment of glioma before primary endpoint recorded	0	1

GBM= glioblastoma multiforme; GCV=ganciclovir MRI=magnetic resonance imaging.

Note: Patients may have more than one violation/deviation leading to not being included in the PP Population. Percentage of patients with ≥1 violation calculated relative to number of patients in ITT population within each treatment group.

a: Data supporting the stability of Cerepro® allowed an increase from 90 mins to 240 mins (90 mins stated in the Statistical Analysis Plan).

Outcomes and estimation

Primary Efficacy Endpoint
The primary efficacy endpoint was time from randomisation to death or re-intervention. In order to determine the primary endpoint, the type of re-interventions and when these occurred was independently assessed (blinded to treatment) by the Re-Intervention Committee (RIC). Overall, based on the number of patients assessed by the RIC, there was 94.7% agreement in re-intervention as determined by the investigator compared to the RIC. In case of disagreement, the RIC assessment was used.
At the time of the third interim analysis by the DSMB (January 2008), it was recommended that the trial be stopped due to crossing the boundary indication futility. From the final analysis in June 2008, it was concluded that there was no statistically significant difference in efficacy between the active treatment group and the control treatment group in the time to death or re-intervention (p=0.309).

Table 6: Survival Analysis for Time to Death or Re-intervention (ITT Population)

	Active Group (N=119)	Control Group (N=117)	Cox's Proportional Hazards Model		
			Hazard Ratio (Active/Control)	95% CI for Hazard Ratio	P-value
Total No. (%) died or with re-intervention	92 (77.3)	91 (77.8)			
Total No. (%) Censored	27 (22.7)	26 (22.2)			
Quartiles and associated 95% CI for Time to Event (Days)					
25%	201 (154-238)	157 (124-190)			
Median	310 (284-373)	268 (208-313)	0.85	0.60 – 1.16	0.309
75%	485 (430-570)	479 (383-576)			

CI=confidence interval; ITT=intent-to-treat; N=number of patients.

Note: Cox's Proportional Hazards model stratified by intended temozolomide use, and containing terms for continuous age and dichotomous Karnofsky Score at Screening (70, >70)Hazard ratio, 95% confidence interval and corresponding p-value are adjusted for the sequential design Censored refers to patients who did not have an event (i.e., had not died and without re-intervention).

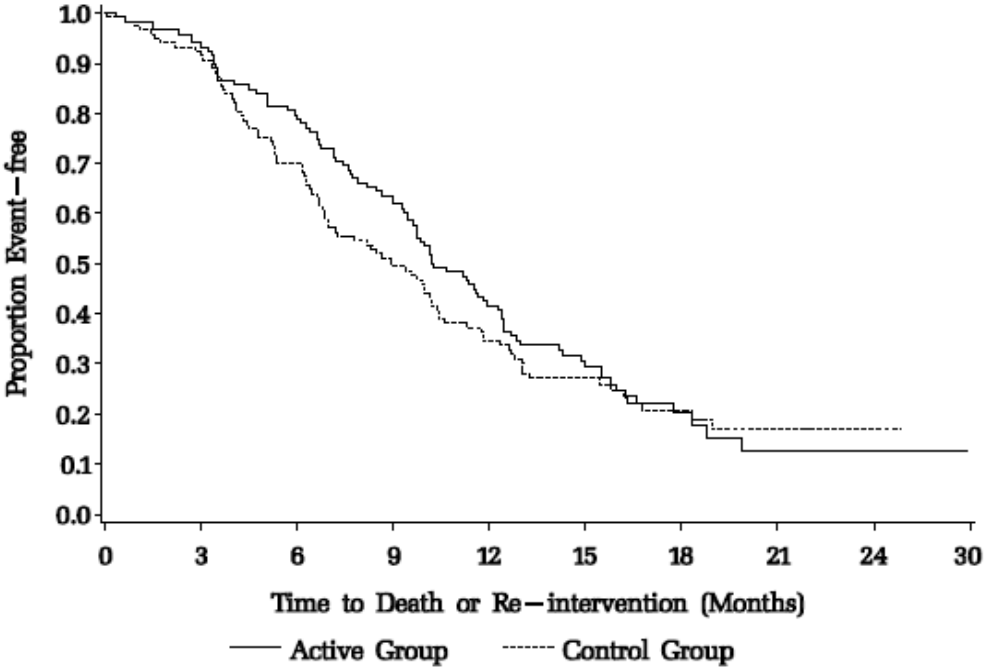
Table 7: Survival Analysis for Time to Death or Re-intervention (PP Population)

	Active Group (N=102)	Control Group (N=114)	Cox's Proportional Hazards Model		
			Hazard Ratio (Active/Control)	95% CI for Hazard Ratio	p-value
Total No. (%) died or with re-intervention	82(80.4)	89(78.1)			
Total No. (%) censored	20(19.6)	25(21.9)			
Quartiles and associated 95% CI for time to event (days)					
25%	200 (153-232)	157 (124-190)			
Median	307 (283-363)	275 (208-315)	0.90	0.66-1.22	0.507
75%	470 (379-539)	491 (383-NK)			

CI=confidence interval; N=number of patients; PP=per-protocol.

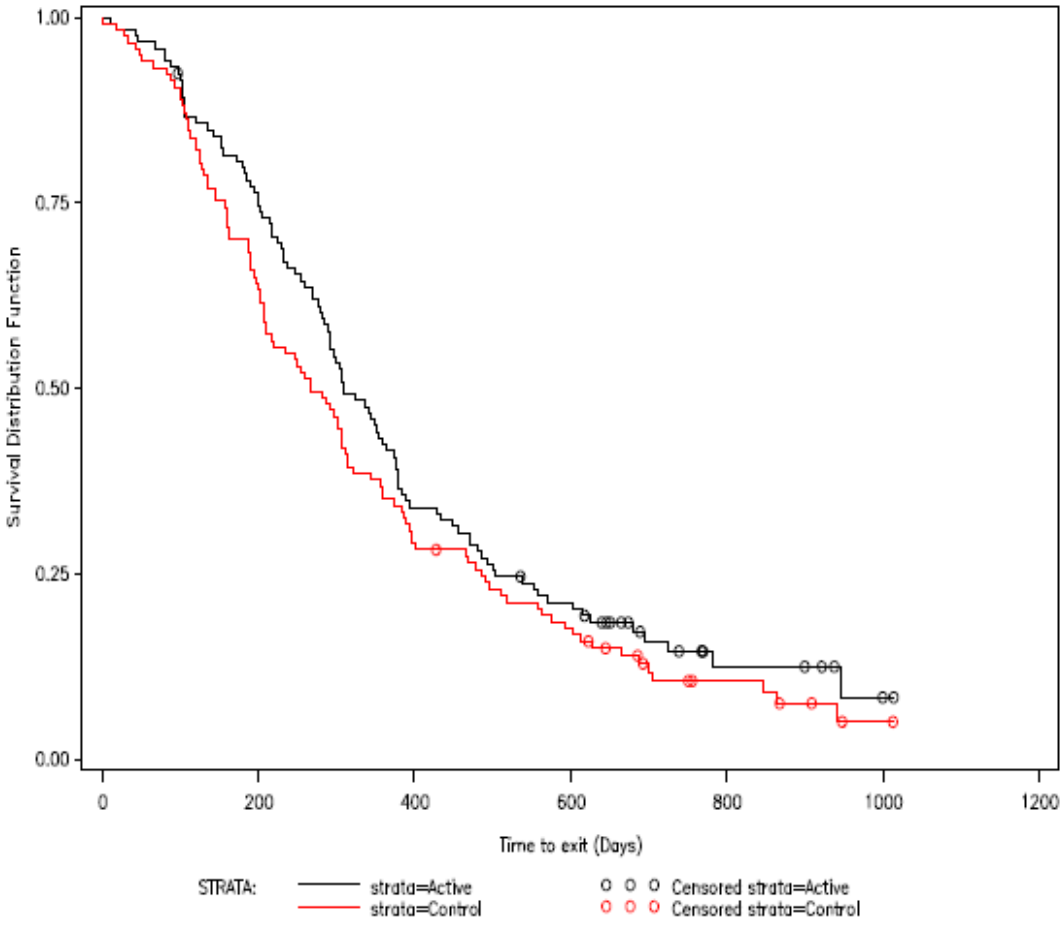
Note: Cox's Proportional Hazards model stratified by intended temozolomide use, and containing terms for continuous age and dichotomous Karnofsky Score at Screening (70,>70). No adjustment was made for the sequential design. Censored refers to patients who did not have an event (i.e., had not died and without re-intervention).

Figure 2: Kaplan-Meier Survival Estimate Curves for Time to Death or Re-intervention (ITT Population, initial submission)



The Applicant submitted an updated analysis of the primary endpoint with a cut-off date of March 2009 when the data had matured further. In a further analysis updated to October 2009, no statistically significant differences were noted (HR=1.26 (95% CI 0.96-1.67), p=0.098 by univariate analysis).

Figure 3: Kaplan-Meier Survival Estimate Curves for Time to Death or Re-intervention (ITT Population, March 2009 updated analysis)



Secondary Efficacy Endpoints

In June 2008, median survival time was 452 days in the active group compared with 492 days in the control group (ITT population). There was an increase in the hazard observed in the active group compared with the control group, although the difference was not statistically significantly different.

Table 8: Survival Analysis for Time to Death from any cause

			Cox's Proportional Hazards model		
	Active Group	Control Group	Hazard Ratio (Active/Control)	95% CI for Hazard Ratio	p-value
ITT Population	N=119	N=117			
Total No. (%) Died	70(58.8)	63(53.8)			
Total No. (%) Censored	49(41.2)	54(46.2)			
Time to Event (Days)					
Median (95% CI)	452 (372-564)	492 (435-600)	1.17	0.83-1.65	0.367
PP Population	N=102	N=114			
Total No. (%) Died	62(60.8)	62(54.4)			
Total No. (%) Censored	40(39.2)	52(45.6)			
Time to Event (Days)					
Median (95% CI)	434 (352-564)	492 (435-600)	1.20	0.84-1.72	0.316

CI=confidence interval; N=number of patients; ITT-intent-to-treat; PP=per-protocol.

Note: Cox's Proportional Hazards model stratified by intended temozolomide use, and containing terms for continuous age and dichotomous Karnofsky Score at Screening (70, >70). No adjustment was made for the sequential design. Censored refers to patients whose last known status was alive.

Although in the original analysis a numerically higher number of deaths were seen in the active treatment arm compared to the control arm. (see also discussion on clinical safety), at the time of the updated analysis (March 2009), the trend had reversed with 88 deaths in the active arm and 92 deaths in the control arm. This is shown in the following table, in which results are stratified by actual (rather than intended) temozolomide use, for reasons discussed under *Ancillary analyses* below.

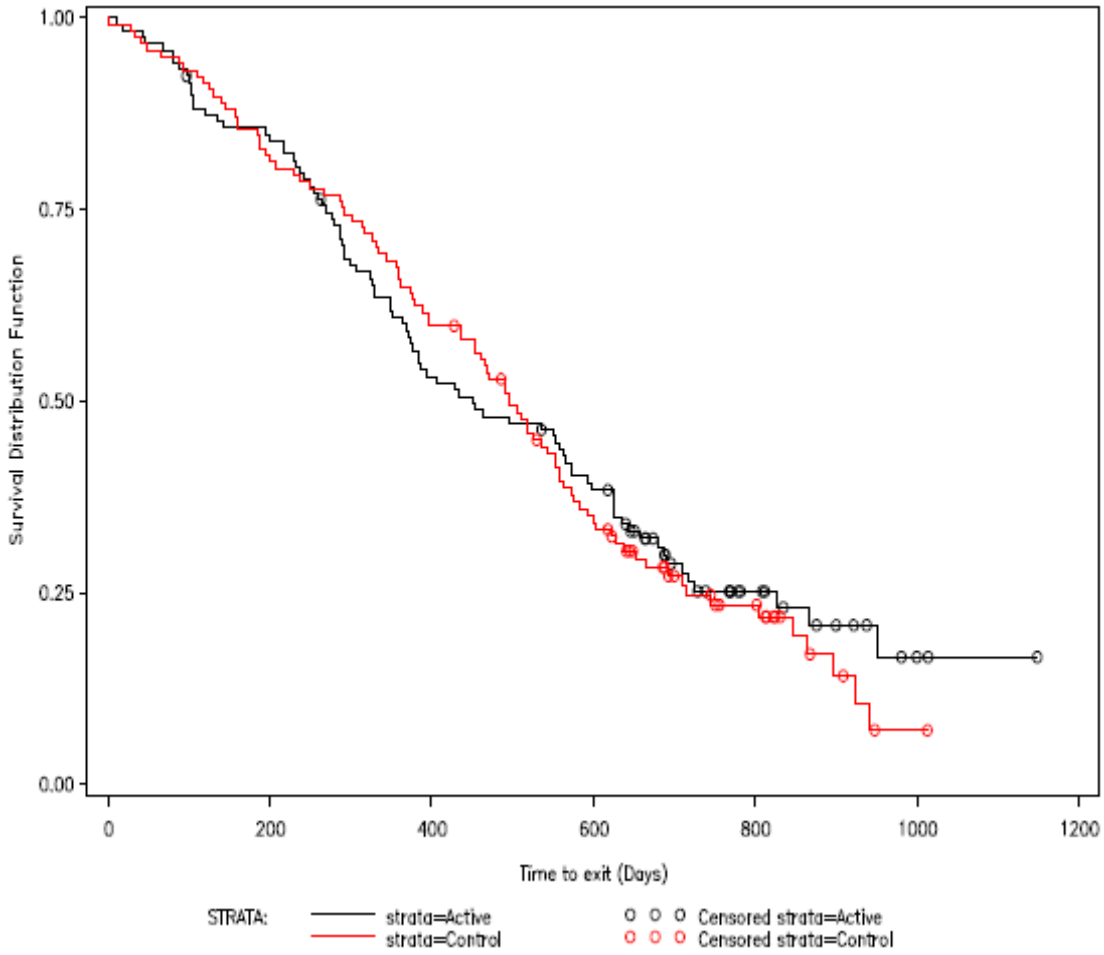
Table 9: Time to All-Cause Mortality by Actual Temozolomide Use (ITT population, March 2009 updated analysis)

	Cerepro		Standard care		
	Used Temozolomide (N=58)	Did not use Temozolomide (N=61)	Used Temozolomide (N=76)	Did not use Temozolomide (N=41)	p-value
Did the patient die?					
Yes	41 (71%)	47 (77%)	58 (76%)	34 (83%)	
No	17 (29%)	14 (23%)	18 (24%)	7 (17%)	
Total	58 (100%)	61 (100%)	76 (100%)	41 (100%)	
Time to All-Cause Mortality					
Median	551.00	385.00	535.00	357.00	
95% CI	(372.0, 626.0)	(300.0, 564.0)	(492.0, 602.0)	(250.0, 467.0)	
Cox-proportional Hazard Modelling*					
Treatment	Hazard Ratio				1.16
	P-value				0.3440
Temozolomide use p-value					0.0017
Treatment - Temozolomide use interaction p-value					0.5157

* Calculated from a Cox's proportional hazards model including terms for treatment, Temozolomide use, age and Karnofsky score. The interaction term is calculated from the additional variation explained in the model by including the treatment by Temozolomide interaction.
A hazard ratio greater than one indicates a positive response for the Cerepro treatment group.

Finally, for the secondary endpoint 'time to all-cause mortality' no significant differences were observed between the treatment arms (HR= 1.11 (95% CI 0.83-1.49), p=0.45 by univariate analysis updated to October 2009).
The Kaplan-Meier depiction of the March 2009 Overall Survival analysis is shown in figure 4 below.

Figure 4: Kaplan-Meier Survival Estimate Curves for Time to All-Cause Mortality (ITT Population, March 2009 updated analysis)



With respect to the other secondary endpoints of 'time from randomisation to tumour progression' and 'Quality of Life', no statistically or clinically significant changes were seen (data not shown).

Ancillary analyses

As stated previously under *Conduct of the study*, intended use of temozolomide showed marked discrepancies compared with actual use. The purpose of stratification to intended temozolomide use was to achieve balance between groups in the use of this agent and to take account of its efficacy in the analysis. However, the non-adherence to the intent confounded the results. In order to compensate for this, actual use of temozolomide was included as a baseline covariate in the Cox proportional hazards model. The results were statistically non-significant It was further noted that the timing of the administration of temozolomide in some patients might have affected the outcome of the study. To account for this possibility, it was suggested that a time-dependent Cox model be applied to the data. The time dependent model included the baseline covariates (age and Karnofsky score at screening) considered in the proportional hazards model, but also treated temozolomide as a time dependent covariate. Again, the primary comparison of Cerepro vs standard care did not show statistically significant differences It was also identified that patients with a lower Karnofsky score were less likely to be administered temozolomide and the Applicant claimed that including D19 Karnofsky score as a covariate in the Cox model may account for some of the temozolomide use bias. Including terms for treatment, age as a baseline covariate as well as actual temozolomide use and Karnofsky score as time-dependent covariates in the Cox model yielded a statistically significant difference between Cerepro and standard care for the primary endpoint but not for all-cause mortality Finally, the company considered that the completeness of surgical resection and the methylation status of the MGMT (O^6-methylguanine-DNA-methyltransferase) gene promoter are important prognostic factors in high-grade glioma. The MGMT gene is involved in DNA repair after the type of damage that temozolomide, among other factors, inflicts on the DNA. Methylation of the MGMT gene promoter has been found to render the gene inactive and to predict sensitivity to temozolomide and improved survival. In a Cox analysis using age, Karnofsky score, extent of surgical resection, MGMT status and intended temozolomide use as baseline covariates and actual temozolomide use as time-dependent covariate, a statistically

significant difference between Cerepro and standard care was found for the primary endpoint but not for all-cause mortality (data not shown).
The Applicant also presented subgroup analyses and claimed statistically significant differences in the subgroup of patients with methylated MGMT promoter status, in patients who followed their intended temozolomide use and in patients with no intention to use temozolomide who indeed did not use temozolomide. In all cases, the statistically significant difference was only found for the primary endpoint but not for all-cause mortality.
The main results of these ancillary analyses based on the updated March 2009 data are presented in the following table (results based on the primary analysis June 2008 data are also reported in parentheses).

Analysis	Primary end point Hazard ratio	Primary end point P value	All cause mortality Hazard ratio	All cause mortality P value	Comment
Time dependent analysis.	1.41 (1.37)	0.022 (0.048)	1.29 (1.07)	0.11 (0.73)	Day 19 Karnofsky and temozolomide as time dependent covariates
MGMT analysis	1.64	0.0024	1.29	0.14	Temozolomide as a time dependent covariate
MGMT analysis	1.66	0.0017	1.43	0.043	Day 19 Karnofsky and temozolomide as time dependent covariates
MGMT analysis in patients with non-methylated MGMT promoter	1.54	0.035	1.32	0.19	Temozolomide as a time dependent covariate
MGMT analysis in patients with non-methylated MGMT promoter	1.58	0.027	1.51	0.059	Day 19 Karnofsky and temozolomide as time dependent covariates
Per protocol analysis of patients who followed their intended temozolomide treatment	1.65 (1.59)	0.020 (0.043)	1.22 (1.11)	0.387 (0.69)	Standard Cox analysis
Per protocol analysis where there was no intention to use temozolomide and no temozolomide was used.	3.85 (3.76)	0.0001 (0.0002)	2.93 (2.46)	0.0026 (0.027)	Standard Cox analysis

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

An integrated efficacy analysis of the two randomised studies (903 and 904) was submitted (data not shown, see discussion on clinical efficacy).

- Clinical studies in special populations

No clinical studies in special populations were submitted.

- Supportive studies

Study 902: This was a phase I, single-centre, open-label study conducted with the aim to evaluate the safety and efficacy of HSV-*tk* gene therapy delivered by either an adenoviral vector or by retroviral-packaging cells and given in conjunction with ganciclovir to 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 a 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 in 0.1 to 0.3ml aliquots to a depth of 10mm (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 HSV-*tk* gene expression mediated by retrovirus packaging cells and by the adenoviral vector.
The groups appeared 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-treated 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).

Study 903: This was a phase II, single-centre, randomised, controlled study with the objective to evaluate the efficacy and safety of Cerepro in the treatment of patients with malignant glioma.
Patients should have a diagnosis of malignant glioma confirmed by histology during the operation. Patients with multilobar or intraventricular tumours or tumours infiltrating the corpus callosum were excluded. Since the prognosis of glioma patients is considerably poorer in case of recurrent tumours compared to primary ones, randomisation was stratified by primary/recurrent tumour.
After surgical resection, Cerepro was given to patients in the active group, while patients in the control group were only observed thereafter. Cerepro was given as a single dose 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.
Treatment groups were generally balanced for age, sex, tumour type, primary/recurrent tumours and Karnofsky score. Median survival (defined as time from operation to death or re-operation for recurrence) was 62.4 weeks in the Cerepro group and 37.7 weeks in the control group (logrank p=0.0095). Progression, documented by MRI performed at baseline and at eight-weekly intervals, was 40% (6/15) in the Cerepro group (vs 50% (8/16) in the control group) at eight weeks.
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).

- Discussion on clinical efficacy

Four clinical studies, 901-904, comprise the clinical data for Cerepro. The first was Study 901, a dose defining study. This was followed by the non-randomised Study 902 and the randomised studies 903 and 904. All studies used the same agent, the same dose (3×10^{10} pfu or 1×10^{12}vp) and the same route of administration. Similarly, the same dose and route of administration of ganciclovir (5mg/kg bd iv x 14 days) was used throughout.
In study 903, the validity of the results was questioned since relevant prognostic factors (including histological type of tumour) were unevenly distributed across treatment arms. Moreover, the external validity of the results was also questioned since study 903 was a single centre study with limited sample size. This aspect was considered especially relevant in light of the potential for investigator-dependent mode of administration of Cerepro.
Study 904 is the pivotal efficacy study for this application. It was a phase III, multi centre, controlled, randomised, parallel group, open-label study, in which 251 patients were randomised either to Cerepro and GCV added to standard care, or standard care alone; this design is in accordance with scientific advice received in 2003. The study was conducted as an unblinded study, because it was not considered ethical to perform IC injections using carrier alone, nor was it considered ethical to submit patients to 14 days of intravenous placebo injections instead of GCV. The design of the study does, however, allow for bias to be introduced given the choice of the primary endpoint of the trial. Contrary to scientific advice which preferred the use of overall survival as primary endpoint for this pivotal trial, with progression-free survival as secondary

endpoint, the Applicant changed the primary endpoint to 'time to death or reintervention' early during the course of the study. Survival was measured as secondary endpoint, while progression-free survival was not assessed. The Applicant claimed that the use of an independent Re-Intervention Committee (IRC) sufficed to exclude introduction of any bias in the decision for re-intervention and hence the primary endpoint. They also pointed out the high concordance rate between the investigator and the RIC results at the time of the primary efficacy analysis.
The CAT considered that the decision to re-intervene is subjective and depends in practice on multiple factors such as the patient's subjective symptoms and complaints, the clinical findings at neurological examination, imaging if carried out and finally the attitude and the demand for active intervention on the part of the patient and his/her relatives. All these factors are subject to bias in the context of an open-label study in which both patient and physician are aware of the type of treatment (active vs control) that the patient has received. The use of the RIC could not compensate for this potential bias, as the committee could only review the CRFs which had been completed by the treating physician when the decision to re-intervene was taken. Thus, the RIC was unable to evaluate whether the information entered into the CRF was objectively correct or biased in some way. In effect, the RIC was only able to check that the treating physician judged re-intervention to be necessary and this was a fact already known. The Applicant provided additional analyses to show that no bias could be demonstrated in the timing of the decision to re-intervene and hence in the adjudication of the primary endpoint (data not shown) but the CAT considered that these analyses did not suffice to consider the primary endpoint acceptable.

The Applicant argued that, based on clinical evidence, All Cause Mortality or Overall Survival is not the preferable endpoint in high grade glioma trials, as it is confounded by subsequent therapies that patients most often receive. On the contrary, the CAT considered Overall Survival as a very relevant endpoint. Overall Survival of high grade glioma patients is short and the risk of confounding the results of overall survival by the effect of sequential therapies was considered minor since few effective treatments are available for 2nd and 3rd line treatments of relapsed GBM and these treatments would be equally available for patients in both treatment arms. Moreover, in study 904, cross-over to Cerepro upon progression was not possible for patients in the control arm. Therefore, the standard univariate analysis of the secondary endpoint (all cause mortality) is considered of most relevance for this pivotal trial.

The pivotal trial 904 did not demonstrate the efficacy of Cerepro and GCV in treating high-grade glioma. There were no statistically significant differences between the Cerepro/GCV arm and the control arm of the study in terms of both the primary endpoint and the more reliable 'All Cause Mortality' in the primary efficacy analysis.
The Applicant made claims on efficacy based on post hoc ancillary analyses, which were conducted without any adjustment for multiplicity. These were multivariate analyses of the Cox proportional hazards regression model adjusting for a number of factors including temozolomide use and Karnofsky Performance Status, MGMT gene promoter status and extent of resection. The submitted post hoc subgroup analyses can only be considered as hypothesis generating at best.
With regard to the pooled efficacy analysis of studies 903 and 904, the CAT considered that the efficacy had not been demonstrated in the individual studies and that consistent with the regulatory prerequisites in current guidance (CPMP/EWP/2330/99) the value of the pooled data was limited and refrained from making any conclusion from this pooled data analysis.

Clinical safety

The overall safety evaluation of Cerepro is based on data from a small proof-of-concept study (study 902) and two clinical trials (studies 903 and 904). Study 902 was not conducted with the rigour of current clinical trials and the safety data are presented in narrative form. The data from the Phase IIb (903) and the Phase III (904) studies have been pooled to allow an overview of the safety profile for Cerepro to be obtained.
All studies evaluated safety by recording adverse events (AEs) and changes in laboratory parameters to identify potential safety issues. Study 904 assessed vital sign parameters and physical examination findings in detail.

- Patient exposure

The Cerepro dose administered was considered in terms of either the number of plaque-forming units (pfu) or the number of viral particles. Plaque forming units is a functional measurement of the number of infective viral units and is logically related to infectivity and therefore efficacy. The term viral particle represents a physical count of the number of virus units, some of which may be non-infective. The number of viral particles is normally considered most important from the safety perspective, representing the quantity of viral material that the patient is exposed to. In addition

the methodology for measuring viral particles is more consistent and reproducible. In the light of these considerations, the dose of Cerepro has been stated in terms of viral particles, as preferred by the regulatory agencies, in the large Phase III clinical trial. For the Cerepro batches used in studies 902 and 903, the dose (3 x 1010 pfu) was calculated to be equivalent to the 1 x 1012 viral particles used in study 904.

Table 10: Cerepro Doses used in Studies Included in Safety Analyses

Trial	Patients Treated With Cerepro	Dose
902	7	A single Cerepro dose of 3×10^{10} pfu administered post-resection. Intravenous ganciclovir (5mg/kg/day) twice daily for 14 days, starting 14-15 days post-resection.
	1	In addition to the patients described above, one further patient received Cerepro for the treatment of operable high-grade glioma at the University of Kuopio. The patient was a 71 year old male with a history of significant cardiovascular disease, who was referred with primary glioblastoma multiforme in the right parieto-occipital lobe. He received a single Cerepro dose of 3×10^{10} pfu administered post-resection and intravenous ganciclovir (5mg/kg/day) twice daily for 14 days, starting 5 days post-resection. Before leaving hospital the patient developed atrial fibrillation and pneumonia and died from a pulmonary embolus, which in the opinion of the treating physician were unrelated to Cerepro therapy. As not formally included in a clinical study the data for this patient are not considered further.
903	17	A single Cerepro dose of 3×10^{10} pfu administered post-resection. Intravenous ganciclovir (5mg/kg/day) twice daily for 14 days, starting 5 days post-resection.
904	119[1]	A single Cerepro dose of 1×10^{12} viral particles administered post-resection. An additional 5 patients were randomised to the Cerepro group but were not administered the study treatment. Intravenous ganciclovir (5mg/kg/day) twice daily for 14 days, starting 5 days post-resection.

[1] In addition to these 119 patients one patient with lymphoma and one with tumour metastasis to the brain also received Cerepro

The demographic characteristics of patients included in the safety analysis are summarised in the following Table 11.

Table 11: Demographic Profile of Patients Randomised to Cerepro

	Study 902	Study 903	Study 904[1]	Studies 903/904[1]
Number N (%)	7 (100)	17 (100)	124 (100)	136 (100)
Gender n (%)				
Male	5 (71)	12 (71)	72 (58)	82 (60)
Female	2 (29)	5 (29)	52 (42)	54 (40)
Ethnicity n (%)				
Caucasian	7 (100)	17 (100)	123 (>99)	135 (>99)
Black	0	0	1 (<1)	1 (<1)
Age (years)				
Mean	55.6	51.9	55.7	55.2
Range	39-65	39-68	20-70	20-70
Age n (%)				
<40	1 (14)	1 (6)	8 (6)	9 (7)
40-60	4 (57)	14 (82)	72 (58)	83 (61)
>60	2 (29)	2 (12)	44 (35)	44 (32)
Tumour type n (%)				
Primary	3 (43)	12 (71)	124 (100)	131 (96)
Recurrent	4 (57)	5 (29)	0	5 (4)
Tumour histology n (%)				
Anaplastic astrocytoma	1 (14)	4 (24)	4 (3)	8 (6)
Glioblastoma multiforme	6 (86)	12 (70)	112 (92)	123 (90)
Other	0	1 (6)	8 (6)	5 (4)
Symptom profile at baseline n (%)				
Seizures[2]	-	-	-	7 (5)
Hemiparesis[3]	-	-	-	34 (25)
Both seizures and hemiparesis				3 (2)
Neither seizures nor hemiparesis	-	-	-	92 (68)
Cardiovascular disease at baseline	-	-	-	9 (7)

[1] A total of 124 patients were randomised to the Cerepro treatment group in study 904, however of these 5 patients did not receive the study treatment. Only the 119 patients who underwent Cerepro administration are included in the pooled analysis of data from studies 903 and 904.

[2] Seizures comprised events of: epilepsy, complex partial seizure, grand mal seizure/convulsions, partial seizure, simple partial seizures, partial seizures with secondary generalisation, uncinate fits, status epilepticus, convulsions and epileptic aura.

[3] Hemiparesis comprised events of: hemiparesis, hemiplegia, transient hemiplegia, monoparesis, paresis, paraparesis and pyramidal tract syndrome.

Exposure to Cerepro and GCV in the pivotal study 904 are summarised in the following Table 12.

Table 12: Cerepro and GCV Administration and Compliance in 904 (Safety Population)

Parameter	Active Group (N=124) n	(%)
Amount Cerepro® administered (mL)		
N	119	
Mean	9.68	
SD	0.961	
Median	10.00	
Range	4.5, 10.0	
0	0	
>0 and <5	1	(0.8)
≥5 and <10	18	(15.1)
10	100	(84.0)
Unknown	5	
Number of injections		
<30	0	
30-50	36	(30.3)
51-70	73	(61.3)
>70	10	(8.4)
Unknown	5	
Estimated surface area covered by injections (cm^2)		
>0-5	1	(0.8)
>5-25	36	(30.3)
>25-100	76	(63.9)
>100	6	(5.0)
Unknown	5	
Injection or leakage of Cerepro® into ventricle		
Yes	18	(15.1)
No	101	(84.9)
Unknown	5	
Compliance (%)		
<100	19	(16.0)
100	100	(84.0)
Unknown	5	
Total amount GCV administered (mg/kg)		
N	118	
Mean	137.31	
SD	18.733	
Median	140.00	
Range	25.0, 160.0	
Number of days dosed		
N	118	
Mean	14.00	
SD	1.783	
Median	14.00	
Range	3.0, 17.0	
Number of doses		
N	118	
Mean	27.47	
SD	3.811	
Median	28.00	
Range	5.0, 32.0	
Compliance (%)		
0	0	
>0-50	2	(1.7)
>50-100	79	(66.9)
>100	37	(31.4)
Unknown	6	

N=number of patients; SD=standard deviation.

- Adverse events

The profile of all treatment-emergent AEs which occurred during studies 903 and 904 is presented in the table below.

Table 13: Summary of Treatment-Emergent Adverse Events (Pooled safety analysis)

Patients Experiencing Treatment-Emergent AEs	Cerepro N (%) (N=136)	Standard care N (%) (N=145)
Patients experiencing AEs	134 (99)	140 (97)
Number of AEs	1581	1333
Patients experiencing:		
Mild AEs	123 (90)	128 (88)
Moderate AEs	124 (91)	125 (86)
Severe AEs	91 (67)	82 (57)
Patients experiencing treatment-related AEs	91 (67)	52 (36)
AE relationship to study treatment		
Unrelated	131 (96)	137 (94)
Possibly	74 (54)	29 (20)
Probably	37 (27)	16 (11)
Definitely	18 (13)	17 (12)
Patients experiencing SAEs	88 (65)	68 (47)
Patients experiencing non-disease associated SAEs	85 (63)	61 (42)
Patients experiencing treatment-related SAEs	34 (25)	2 (1)
AEs leading to study discontinuation	0	0
AEs leading to death	72 (53)	64 (44)
Non-disease associated AEs leading to death	31 (23)	27 (19)

Common AEs (occurring in 10% or more of patients) reported in studies 903 and 904 are presented in the table below.

Table 14: Incidence of Common (in ≥10% of Patients) Adverse Events (Pooled safety analysis)

System Organ Class/ Preferred Term	Cerepro (N=136)		Standard care (N=145)	
	Patients (%)	Events	Patients (%)	Events
Any event	**134 (99)**	**1581**	**140 (97)**	**1333**
Nervous system disorders	**123 (90)**	**473**	**127 (88)**	**418**
Headache	62 (46)	83	68 (47)	93
Hemiparesis[1]	50 (37)	58	39 (27)	47
Seizures[2]	49 (36)	110	54 (37)	84
Aphasia[3]	23 (17)	31	27 (19)	41
Brain and cerebral oedema	15 (11)	18	17 (12)	17
Headache NOS	15 (11)	26	12 (8)	18
Memory impairment	13 (10)	13	9 (6)	9
General disorders and administration site conditions	**90 (66)**	**187**	**73 (50)**	**129**
General physical health	14 (10)	16	9 (6)	11
Pyrexia	54 (40)	72	30 (21)	38
Asthenia	22 (16)	30	19 (13)	23
Pain	15 (11)	21	11 (8)	15
Fatigue	14 (10)	16	17 (12)	19
Neoplasms benign, malignant and unspecified (including cysts and polyps)	**76 (56)**	**92**	**82 (57)**	**112**
Neoplasm progression	68 (50)	84	75 (52)	98
Gastrointestinal disorders	**78 (57)**	**150**	**63 (43)**	**116**
Nausea	29 (21)	39	38 (26)	49
Constipation	24 (18)	27	21 (15)	23
Vomiting	24 (18)	29	14 (10)	17
Diarrhoea	14 (10)	17	5 (3)	5
Infections and infestations	**57 (42)**	**106**	**50 (35)**	**80**
Urinary tract infection	15 (11)	17	8 (6)	9
Psychiatric disorders	**50 (37)**	**77**	**51 (35)**	**86**
Depression	13 (10)	14	11 (8)	12
Metabolism and nutrition disorders	**47 (35)**	**76**	**36 (25)**	**59**
Hypokalaemia	15 (11)	17	15 (10)	15
Hyponatraemia	13 (10)	14	10 (7)	13
Skin and subcutaneous tissue disorders	**37 (27)**	**57**	**39 (27)**	**52**
Alopecia	14 (10)	14	16 (11)	16
Vascular disorders	**36 (26)**	**53**	**31 (21)**	**41**
Hypertension	18 (13)	21	8 (6)	10
Injury, poisoning and procedural complications	**34 (25)**	**46**	**27 (19)**	**34**
Blood and lymphatic system disorders	**36 (26)**	**56**	**23 (16)**	**46**
Thrombocytopenia	17 (13)	19	10 (7)	14
Investigations	**32 (24)**	**51**	**27 (19)**	**36**
Musculoskeletal and connective tissue disorders	**31 (23)**	**45**	**23 (16)**	**28**
Respiratory, thoracic and mediastinal disorders	**27 (20)**	**35**	**16 (11)**	**21**
Eye disorders	**18 (13)**	**20**	**17 (12)**	**19**
Renal and urinary disorders	**16 (12)**	**16**	**10 (7)**	**12**
Ear and labyrinth disorders	**13 (10)**	**14**	**9 (6)**	**10**

[1] Hemiparesis comprised events of: hemiparesis, hemiplegia, transient hemiplegia, monoparesis, paresis, paraparesis and pyramidal tract syndrome.
[2] Seizures comprised events of: epilepsy, complex partial seizure, grand mal seizure/convulsions, partial seizure, simple partial seizures, partial seizures with secondary generalisation, uncinate fits, status epilepticus, convulsions and epileptic aura.
[3] Aphasia comprised events of aphasia, dysphasia, Gerstmann's syndrome, agraphia, acalculia and dysgraphia.

The Applicant submitted more in-depth analyses of AEs, particularly with regard to the temporal occurrence of the events. Moreover, analyses were submitted which focused on focal brain injury as a result of the injection procedure, localised brain inflammation subsequent to the injection and systemic anti-viral response from exposure of the immune system to the viral vector (data not shown, see discussion on clinical safety).

Common treatment-related (possibly, probably or definitely related to treatment) AEs reported during studies 903 and 904 are presented in the table below.

Table 15: Incidence of Common (in ≥10% of Patients) Treatment-Related Adverse Events (Pooled safety analysis)

System Organ Class/Preferred Term	Cerepro (N=136)		Standard care (N=145)	
	Patients (%)	Events	Patients (%)	Events
Any event	**91 (67)**	**369**	**52 (36)**	**130**
Nervous system disorders	**62 (46)**	**142**	**37 (26)**	**59**
Headache	25 (18)	26	25 (17)	28
Hemiparesis[1]	19 (14)	21	4 (3)	4
Seizures[2]	16 (12)	28	4 (3)	4
General disorders and administration site conditions	**36 (26)**	**54**	**14 (10)**	**16**
Pyrexia	25 (18)	27	3 (2)	3
Gastrointestinal system disorders	**19 (14)**	**31**	**12 (8)**	**15**
Investigations	**17 (13)**	**25**	**5 (3)**	**5**
Infections and infestations	**13 (10)**	**17**	**1 (1)**	**1**
Psychiatric disorders	**13 (10)**	**16**	**3 (2)**	**5**

[1] Hemiparesis comprised events of: hemiparesis, hemiplegia, transient hemiplegia, monoparesis, paresis, paraparesis and pyramidal tract syndrome.
[2] Seizures comprised events of: epilepsy, complex partial seizure, grand mal seizure/convulsions, partial seizure, simple partial seizures, partial seizures with secondary generalisation, uncinate fits, status epilepticus, convulsions and epileptic aura.

Overall the most common treatment-related AEs in the Cerepro group were headache (18%), pyrexia (18%), hemiparesis (14%) and seizures (11%). The incidence of common treatment related AEs of hemiparesis, seizures and pyrexia was higher in the Cerepro group than in the 'standard of care' group. Of these events, hemiparesis and seizures are often associated with the disease under study; differentiating true treatment-related AEs from ongoing disease symptoms was therefore problematic. On the other hand, pyrexia could occur as a result of an immune response against the adenoviral vector.
The following AEs (not including events of neoplasm progression/recurrence) were also considered treatment-related for patients in the Cerepro treatment group (occurring in 1-10% of patients):

Blood and lymphatic system disorders	anaemia, lymphopenia, neutropenia, thrombocytopenia
Ear and labyrinth disorders	vertigo
Eye disorders	eye pain
Gastrointestinal system disorders	constipation, diarrhoea, nausea, vomiting
General disorders and administration site conditions	asthenia, fatigue, general physical health deterioration, pain
Infections and infestations	brain abscess, herpes simplex infection, herpes zoster infection, urinary tract infection, wound and post-operative wound infection
Injury, poisoning and procedural complications	cerebrospinal fluid leakage
Investigations	elevated alanine aminotransferase, elevated aspartate aminotransferase, abnormal liver function tests, abnormal nuclear magnetic resonance imaging findings, weight decrease
Metabolic and nutritional disorders	electrolyte imbalance, hyperglycaemia, hyponatraemia
Musculoskeletal and connective tissue disorders	arthralgia
Nervous system disorders	amnesia, aphasia, balance disorder, brain/cerebral oedema, cerebral haematoma and haemorrhage, cognitive disorder, abnormal coordination, dizziness, memory impairment, neurological symptoms, paraesthesia, somnolence, speech disorder, tremor
Psychiatric disorders	anxiety, confusional state, depression, insomnia
Renal and urinary disorders	urinary incontinence
Skin and subcutaneous tissue disorders	alopecia, pruritis, rash
Vascular disorders	hypertension

None of these events occurred with a particularly higher incidence in the Cerepro group than was seen in the standard care treatment group (all <5% difference between groups). The following AEs (not including events of neoplasm progression/recurrence) were also considered treatment-related for patients in the Cerepro treatment group (occurring in <1% of patients):

Cardiac disorders	angina pectoris
Ear and labyrinth disorders	auricular swelling, impaired hearing, Meniere's disease, tinnitus
Endocrine disorders	adrenal insufficiency

Eye disorders	photophobia, reduced visual acuity
Gastrointestinal system disorders	abdominal discomfort, dysphagia, faecal incontinence, gastrooesophageal reflux disease
General disorders and administration site conditions	chest pain, difficulty walking, gait disturbance, hyperthermia, oedema, worsening performance status
Immune system disorders	drug hypersensitivity
Infections and infestations	ophthalmic herpes zoster infection, influenza, oral fungal infection, respiratory moniliasis, upper respiratory tract infection
Injury, poisoning and procedural complications	operative haemorrhage, post-procedural haematoma, post-operative fever, procedural pain, skin laceration, traumatic brain injury
Investigations	elevated alkaline phosphatase, elevated hepatic enzymes, elevated C-reactive protein, decreased blood sodium, decreased blood potassium, abnormal laboratory tests, abnormal renal function
Metabolic and nutritional disorders	diabetes mellitus, hypocalcaemia, hypophosphataemia, vitamin D deficiency
Musculoskeletal and connective tissue disorders	muscle atrophy, osteonecrosis, extremity pain, shoulder pain
Nervous system disorders	apraxia, cerebellar syndrome, reduced consciousness, diabetic hyperosmolar coma, dysarthria, dysgeusia, facial palsy, headache NOS, hemianopia homonymous, hemianopia NOS, hydrocephalus, increased intracranial pressure, neuropathy, peripheral sensory neuropathy, polyneuropathy, subdural hygroma, visual field defect
Psychiatric disorders	abnormal behaviour, affective disorder, apathy, bradyphrenia, psychotic disorder
Renal and urinary disorders	leucocyturia, polyuria.
Respiratory, thoracic and mediastinal disorders	dyspnoea, respiratory failure
Skin and subcutaneous tissue disorders	decubitus ulcer, allergic dermatitis
Vascular disorders	haemorrhage, hypotension, superficial phlebitis, thrombophlebitis, venous thrombosis

- Serious adverse event/deaths/other significant events

A higher proportion of serious adverse events (SAEs) was seen in the Cerepro treatment group, with events occurring in 90 patients (64%) versus 68 patients (47%) in the standard care group. In a condition such as high-grade glioma the risk of disease progression is high and associated events are not always considered SAEs; this was formalised in study 904 where it was specified that disease progression events occurring after 3 months were not to be considered as SAEs. In addition, where SAEs could be clearly associated with disease progression/recurrence they were excluded in an attempt to identify non-disease associated SAEs. It should be noted that the SAEs recorded as non-disease associated could still have been due to disease symptoms or to other aspects of the treatment regimen such as sequelae of surgery, ganciclovir, radiotherapy, steroids or temozolomide.

Table 16: Number of Patients Experiencing Common (in ≥10% of Patients) Severe Adverse Events (Pooled safety analysis)

System Organ Class/Preferred Term	Cerepro N (%) (N=1361)	Standard care N (%) (N=145)
Number of Patients Experiencing SAEs	**91 (67)**	**82 (57)**
Nervous system disorders	**42 (31)**	**33 (23)**
Hemiparesis[1]	15 (11)	15 (10)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	**30 (22)**	**34 (23)**
Neoplasm progression	26 (19)	30 (21)
General disorders and administration site conditions	**18 (13)**	**16 (11)**

[1] Hemiparesis comprised events of: hemiparesis, hemiplegia, transient hemiplegia, monoparesis, paresis, paraparesis and pyramidal tract syndrome.

Overall, the most common severe AEs in both treatment groups were neoplasm progression and hemiparesis. The incidence of specific common severe events was comparable with that seen in the standard care group. In addition, severe seizures occurred in 6% of patients and both severe thrombocytopenia and severe aphasia occurred in 5% of patients following Cerepro treatment; however this was not notably increased above the incidence of 3% reported for such events in the standard care group. Other severe AEs occurred in less than 5% of patients.
The table below summarises non-disease progression associated SAEs that occurred in more than 1 patient per treatment group in the pooled safety analysis of studies 903 and 904. Other SAEs occurred in 87 patients (62%) in the Cerepro group and 61 patients (42%) in the standard care group.

Table 17: Incidence of Non-Disease Progression Associated Serious Adverse Events Occurring in >1 Patient (Pooled safety analysis)

System Organ Class/ Preferred Term	Cerepro (N=136)		Standard care (N=145)	
	Patients (%)	Events	Patients (%)	Events
Any event	**88 (65)**	**218**	**68 (47)**	**150**
Non-disease progression/recurrence-associated events	**85 (63)**	**213**	**61 (42)**	**140**
Nervous system disorders	**55 (40)**	**102**	**34 (23)**	**57**
Seizures[1]	23 (17)	48	16 (11)	23
Hemiparesis[2]	14 (10)	15	9 (6)	11
Hydrocephalus	6 (4)	6	3 (2)	3
Aphasia[3]	7 (5)	8	1 (<1)	1
Brain and cerebral oedema	7 (5)	7	3 (2)	3
Headache NOS	2 (1)	2	5 (3)	5
Neurological symptoms	3 (2)	3	0	0
Cerebral infarction	2 (1)	2	1 (<1)	1
Cerebral haematoma and haemorrhage[4]	1 (<1)	1	2 (1)	2
Subdural hygroma	2 (1)	2	0	0
Memory impairment	0	0	2 (1)	2
General disorders and administration site conditions	**12 (9)**	**14**	**11 (8)**	**13**
General physical health deterioration	7 (5)	7	3 (2)	4
Pyrexia	4 (3)	4	7 (5)	7
Fatigue	1 (<1)	1	2 (1)	2
Infections and infestations	**25 (18)**	**31**	**17 (12)**	**21**
Sepsis (including Escherichia and Staphylococcal sepsis)	4 (3)	4	4 (3)	5
Pneumonia, pneumonia NOS and lung infection	8 (6)	9	5 (3)	5
Wound and post-operative infection	5 (4)	6	0	0
Meningitis and meningitis NOS	1 (<1)	2	4 (3)	5
Brain abscess	2 (1)	2	0	0
Vascular disorders	**8 (6)**	**8**	**10 (7)**	**10**
Thrombotic events[5]	5 (4)	5	9 (6)	9
Injury poisoning and procedural complications	**9 (7)**	**12**	**6 (4)**	**6**
Cerebrospinal fluid leakage	3 (2)	4	2 (1)	2
Extradural haematoma	3 (2)	3	1 (<1)	1
Respiratory, thoracic and mediastinal disorders	**8 (6)**	**8**	**6 (4)**	**7**
Pulmonary embolism	7 (5)	7	3 (2)	3
Gastrointestinal disorders	**7 (5)**	**7**	**4 (3)**	**6**
Nausea	3 (2)	3	2 (1)	2
Vomiting and vomiting NOS	1 (<1)	1	2 (1)	3

System Organ Class/ Preferred Term	Cerepro (N=136)		Standard care (N=145)	
	Patients (%)	Events	Patients (%)	Events
Investigations	**5 (4)**	**5**	**0**	**0**
Abnormal liver function test	3 (2)	3	0	0
Blood and lymphatic system disorders	**3 (2)**	**3**	**5 (3)**	**5**
Bone marrow depression	0	0	2 (1)	2
Thrombocytopenia	2 (1)	2	2 (1)	2
Psychiatric disorders	**4 (3)**	**5**	**1 (<1)**	**1**
Depression	2 (1)	2	0	0
Metabolism and nutrition disorders	**4 (3)**	**4**	**3 (2)**	**4**
Hyponatraemia	2 (1)	2	3 (2)	4
Cardiac disorders	**3 (2)**	**3**	**1 (<1)**	**1**
Myocardial infarction	2 (1)	2	0	0
Surgical and medical procedures	**1 (<1)**	**1**	**3 (2)**	**4**
Tumour/malignant tumour excision	0	0	3 (2)	4
Musculoskeletal and connective tissue disorders	**3 (2)**	**3**	**1 (<1)**	**1**
Skin and subcutaneous tissue disorders	**2 (1)**	**2**	**2 (1)**	**2**

[1] Seizures comprised events of: epilepsy, complex partial seizure, grand mal seizure/convulsions, partial seizure, simple partial seizures, partial seizures with secondary generalisation, uncinate fits, status epilepticus, convulsions and epileptic aura.

[2] Hemiparesis comprised events of: hemiparesis, hemiplegia, transient hemiplegia, monoparesis, paresis, paraparesis and pyramidal tract syndrome.

[3] Aphasia comprised events of aphasia, dysphasia, Gerstmann's syndrome, agraphia, acalculia and dysgraphia.

[4] Cerebral haematoma and haemorrhage comprised events of: cerebral haematoma and cerebral haemorrhage.

[5] Thrombotic events comprised events of: deep vein thrombosis, thrombosis, venous thrombosis, venous thrombosis deep limb.

A higher proportion of patients experienced SAEs in the Cerepro group (64% vs. 47%). Overall, the most common SAEs in the Cerepro group were seizures (17%) and hemiparesis (10%). SAEs that occurred more with a slightly higher frequency in the Cerepro treatment group comprised: seizures (17% of patients vs. 11% in the standard care group), hemiparesis (10% vs. 6%), aphasia (5% vs. <1%), pneumonia and lung infection (6% vs. 3%), pulmonary embolism (5% vs. 2%), general physical health deterioration (5% vs. 2%), brain oedema (4% vs. 1%) and wound infection (3% vs. 0%)

The incidence of AEs leading to death was higher in the Cerepro group. A total of 73 patients (52%) in the Cerepro group and 64 patients (44%) in the standard care group experienced AEs recorded as leading to death. Death was associated with disease progression or disease recurrence for 40 patients in the Cerepro group and 37 patients in the standard care group. For 3 patients in the Cerepro group, death due to disease progression/recurrence was recorded as being treatment-related.

The table below summarises the number of patients experiencing non-disease progression AEs recorded as leading to death that occurred in more than 1 patient per treatment group in studies 903 and 904. Non-disease progression AEs recorded as leading to death occurred in 32 patients (23%) in the Cerepro group and 27 patients (19%) in the standard care group. However, a proportion of neurological AEs (seizures, hemiplegia, aphasia, neurological deterioration) could also have been indicative of disease progression.

Table 18: Number of Patients Experiencing Non-Disease Progression Associated Adverse Events (Occurring in >1 Patient) that Led to Death (Pooled safety analysis)

System Organ Class/ Preferred Term	Cerepro N (%) (N=136)	Standard care N (%) (N=145)
Any event	**72 (53)**	**64 (44)**
Non-disease progression/recurrence-associated events	**31 (23)**	**27 (19)**
Nervous system disorders	**10 (7)**	**13 (9)**
Hemiparesis[1]	2 (1)	3 (2)
Seizures[2]	2 (1)	2 (1)
Neurological symptoms	1 (<1)	3 (2)
Headache NOS	2 (1)	1 (<1)
Brain and cerebral oedema	1 (<1)	2 (1)
Aphasia[3]	0	2 (1)
General disorders and administration site conditions	**5 (4)**	**7 (5)**
General physical health deterioration	3 (2)	5 (3)
Pyrexia	1 (<1)	2 (1)
Infections and infestations	**6 (4)**	**4 (3)**
Pneumonia and pneumonia NOS	2 (1)	2 (1)
Respiratory and thoracic disorders	**6 (4)**	**0**
Pulmonary embolism	4 (3)	0
Vascular disorders	**2 (1)**	**2 (1)**
Cardiac disorders	**2 (1)**	**1 (<1)**
Psychiatric disorders	**0**	**2 (1)**

[1]Hemiparesis comprised events of: hemiparesis, hemiplegia, transient hemiplegia, monoparesis, paresis, paraparesis and pyramidal tract syndrome.
[2]Seizures comprised events of: epilepsy, complex partial seizure, grand mal seizure/convulsions, partial seizure, simple partial seizures, partial seizures with secondary generalisation, uncinate fits, status epilepticus, convulsions and epileptic aura.
[3]Aphasia comprised events of aphasia, dysphasia, Gerstmann's syndrome, agraphia, acalculia and dysgraphia.

The only AE leading to death that occurred with a slightly higher frequency in the Cerepro treatment group comprised pulmonary embolism, which occurred in 3% of patients (vs. 0% in the standard care group).
AEs leading to death were recorded as possibly or probably related to the study treatment for 7 patients in the Cerepro group, with 1 patient in the standard care group experiencing an AE that led to death that was considered definitely related to the study treatment. Treatment-related deaths comprised: pneumonia, respiratory failure, brain abscess, general physical health deterioration, diabetic hyperosmolar coma, status epilepticus, hyponatraemia, with one event recorded as death of unknown aetiology.
Overall, the most common causes of death (either related to treatment or not) were classified as: thromboembolic conditions (6 patients in the Cerepro group vs. 1 in the standard care group), cardiac/cardiovascular conditions (4 patients in each group), neurological symptoms (4 patients in the Cerepro group vs. 7 patients in the standard care group) and sepsis (4 patients in each group).

- Laboratory findings

In the pooled population of studies 903 and 904, a slightly higher incidence of abnormal liver tests was apparent in patients treated with Cerepro over the first 19 days of the study. Five patients (11%) who received Cerepro experienced an AE of abnormal liver function; for 3 patients (2%) the event was reported as serious. In comparison, 10 patients (7%) in the standard care group experienced an AE of abnormal liver function, with none being reported as SAEs. As the incidence of such reactions was highest on Days 6-19 it is probable that ganciclovir administration is aggravating hepatic toxicity during this period; abnormal hepatic function and events such as increased aspartate aminotransferase and alkaline phosphatase levels are common following this chemotherapy. The use of Cerepro does not appear to exacerbate the occurrence of liver function abnormalities above that reported in the ganciclovir SPC (anticipated incidence 1-10%).
Haematological side effects are common events seen during treatment with many chemotherapeutic agents, and in this study they could be attributed to both ganciclovir and

temozolomide. The ganciclovir SPC describes: neutropenia and anaemia as very common undesirable effects; thrombocytopenia, leukopenia and pancytopenia as common; and bone marrow depression as uncommon. When used to treat glioma, the temozolomide SPC describes neutropenia, lymphopenia and thrombocytopenia as very common undesirable effects; and pancytopenia, anaemia and leukopenia as uncommon.
When considering haematological events over the entire study period, only the incidences of anaemia and decreased haemoglobin levels were notably higher in patients who had received Cerepro. As haematological events are likely to be associated with ganciclovir (from Day 6) and temozolomide (after Day 56) administration, the temporal incidence of such events was considered and it indicated that there was a tendency for patients who had received Cerepro to experience a slightly higher incidence of anaemia/decreased haemoglobin levels up to Day 56. The occurrence in Days 0-5 is not clearly Cerepro related. However, over Days 6-56 this event is more common in the Cerepro group and may be related to ganciclovir.
All other haematological AEs were primarily associated with temozolomide treatment and this is consistent with the known propensity for the agent to cause neutropenia, lymphopenia and thrombocytopenia (very common). However, the tendency for patients receiving Cerepro and temozolomide to experience a slightly higher incidence of haematological events (particularly thrombocytopenia- and lymphopenia-related AEs) than those receiving standard care and temozolomide, may suggest a potential for exacerbation of such events with Cerepro/temozolomide combination regimens.
The presence of increased titres of adenoviral antibodies following treatment with Cerepro may indicate systemic exposure to the viral vector. Levels of anti-adenoviral antibodies were routinely monitored during the Cerepro development programme.
In study 903 anti-adenoviral antibodies were increased more than 4-fold in 6 patients in the Cerepro group when measured 19 days after treatment. This was not associated with any adverse outcome.
The changes in adenoviral-antibody levels in the Cerepro and standard care patient groups during study 904 are summarised in the table below. Changes in antibody levels were usually only measured for a patient after Day 19 if the previous test result had shown detectable antibody.

Table 19: Anti-Adenovirus Antibody Levels (Study 904)

Treatment	Baseline		Day 19		Month 3		Month 6		Month 12	
	N	Mean[1]	N	Mean[1]	N	Mean[1]	N	Mean[1]	N	Mean[1]
Cerepro (N=124)	46	161.8	84	3705.5	57	1291.1	49	743.8	5	578.9
Standard care (N=126)	37	168.6	30	134.2	5	156.6	6	221.8	0	-

[1]Geometric mean of dilutional titres based on patients with antibody quantifiable levels.

Prior to treatment all patients had their antibody status assessed but a total of 46 patients (39%) in the Cerepro group and 37 patients (30%) in the standard care group had quantifiable antiadenoviral antibodies, presumably following environmental exposure. An additional 8 patients (7%) in the Cerepro group and 10 patients (8%) in the standard care group had detectable but nonquantifiable levels. By Day 19 there was a notable increase in the number of patients with detectable anti-adenoviral antibody levels following Cerepro treatment (84 patients [75%] with quantifiable and 14 patients [13%] with non-quantifiable levels) and in mean antibody titre (from 162-3706). Both the proportion of patients with anti-adenoviral antibodies and the mean antibody titre gradually declined over the subsequent 12 months. In comparison the mean antibody titre remained relatively constant in patients undergoing standard care. A total of 18 patients experienced injection or leakage of Cerepro into the ventricle during administration but this was not associated with different antibody production profile when compared with other patients receiving the treatment.

- Safety in special populations

A considerable number of analyses were submitted which evaluated the impact of several intrinsic factors on the safety profile, including ethnicity, age, gender, patients with different baseline disease characteristics and different symptom profiles and subjects with cardiovascular diseases and MGMT methylation status.
A higher incidence of a range of AEs in patients aged over 60 was consistent with a poorer overall health status and higher likelihood of concomitant comorbidities in the elderly population; in particular, there was a higher incidence of SAEs associated with underlying cardiovascular disease. For ethnicity no conclusion could be made, as the patient population was almost exclusively Caucasian (>99%). For the rest of the factors analysed, no notable differences were identified. However, the conclusions of these analyses should be taken with caution, due mainly to the low number of patients within each group.
Finally, safety analyses of AEs according to temozolomide administration (extrinsic factor) were submitted (data not shown).

- Safety related to drug-drug interactions and other interactions

Separate analysis of common AEs occurring on or before Day 31 and those occurring after Day 31 was undertaken as this was the median temozolomide treatment start day. This analysis aimed to differentiate between events occurring in patients who did/did not go on to receive temozolomide treatment. The results did not give rise to any concern about a potential interaction between Cerepro/GCV and temozolomide. No data on the investigation of interactions with other drugs were submitted.

- Discontinuation due to adverse events

Patients were considered to have completed the study if they did not withdraw prior to the study being stopped or if they died or required re-intervention before the end of the study. Patient withdrawal was recorded for 8 patients (6%) who received Cerepro and for 25 patients (17%) who received standard care. No patients withdrew from the study as a result of an AE; the primary reasons for withdrawal comprised protocol violation (5 patients receiving Cerepro vs. 12 patients receiving standard care), loss to follow up (1 patient vs. 2 patients), withdrawal of consent (1 patient vs. 10 patients) and other reasons (1 patients vs. 1 patients).

- Post marketing experience

Not applicable.

- Discussion on clinical safety

The applicant has provided detailed information and analyses of safety data from the two randomized trials 903 and 904. However, the analysis of safety data is hampered by the fact that it is difficult to distinguish some of the more serious adverse events from the symptoms of disease progression. Nevertheless, it is clear that treatment with Cerepro is associated with an increased incidence of adverse events and of serious adverse events. Some of these, such as an increased incidence of hemiparesis and seizures are very serious, whereas others, e.g. pyrexia and headache are less serious.
A higher proportion of patients experienced AEs that were considered treatment-related in the Cerepro group compared with the standard care group; assessment of a treatment relationship is problematic in open-label studies and this is reflected by the higher proportion of possibly- and probably-related events in the Cerepro group. Moreover, events of general physical health deterioration and status epilepticus could have been associated with disease progression; a number of other events could also have been associated with other treatment components such as the surgical procedure (brain abscess) and steroid administration (diabetic hyperosmolar coma and hyponatraemia).
The applicant made more in-depth analyses of the adverse events, particularly with regard to the temporal occurrence of the events. Moreover, analyses were submitted which focused on focal brain injury as a result of the injection procedure, localised brain inflammation subsequent to the injection and systemic anti-viral response from exposure of the immune system to the viral vector.
These analyses showed:

- Both hemiparesis and aphasia occur more frequently in the 5 days immediately following surgery with Cerepro administration; as such Cerepro may slightly exacerbate focal brain injury at the tumour site but that this usually resolves after Day 5.

- Though there was no indication that Cerepro increases the likelihood of post-surgical brain inflammation in the days shortly after treatment, the incidence of hydrocephalus was slightly higher in the active treatment group after Day 56 particularly in patients who went on to receive temozolomide.
- The number of patients experiencing seizures was similar in the two groups but there was a higher frequency of seizure events in the Cerepro group during the time of treatment with ganciclovir.
- A transient increase in the frequency of hyponatraemia/low blood sodium was apparent between Days 6-19 post-surgery.
- An increased incidence of fever AEs occurred in the 19 days post-Cerepro treatment, presumably as a result of an anti-adenovirus response. Assessment of vital signs parameters identified an increase in mean temperature on Days 2 and 19 for patients in the Cerepro group that was consistent with the temporal profile of fever AEs.
- Concerns over liver toxicity have been noted in other studies using high doses of adenoviral vector. As a result, the Cerepro doses selected for use in patients with high-grade glioma aimed to minimise the occurrence of adverse hepatic effects. A higher rate of abnormal liver function was noted in the 19 days post-Cerepro treatment, which may be a combination of the effects of both Cerepro and ganciclovir. However, no major safety concerns were raised by laboratory data generated during the Cerepro clinical development programme.
- The development of an anti-adenoviral immune response was confirmed by an increase in the titre and incidence of detectable anti-adenovirus antibodies in patients treated with Cerepro.
- Cerepro/ganciclovir administration may also be associated with an increased incidence of anaemia, and may exacerbate thrombocytopenia and lymphopenia following temozolomide therapy.
- Cerepro may be associated with a slightly higher incidence in the occurrence of thrombotic events and pulmonary embolism/microemboli.
- A slightly higher incidence of urinary tract, oral, viral and wound infections was noted in patients receiving Cerepro, though oral and viral infections appeared to worsen when used in combination with temozolomide.
- The combination regimen of Cerepro with temozolomide may increase the risk of events of thrombocytosis, diarrhoea and memory impairment.

Finally, there was no indication of active adenovirus infection or shedding following Cerepro treatment, or of the systemic persistence of viral vector.

The CHMP agrees with the CAT assessment regarding the Clinical aspect as described above.

2.4 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CAT considered that the Pharmacovigilance system as described by the applicant fulfils the legislative requirements.

Risk Management Plan

The MAA submitted a risk management plan, which included a risk minimisation plan.

The CAT, having considered the data submitted in the application, was of the opinion that the efficacy of the medicinal product had not been established based on the data submitted. In the absence of established efficacy, it cannot be assessed if the proposed risk minimisation activities would be adequate in reducing the risks to a sufficient level to make the benefit risk balance positive. Therefore the CAT was of the opinion that risk minimisation activities cannot be evaluated at this stage.

The CHMP agrees with the CAT assessment regarding the Pharmacovigilance aspects as described above.

2.5 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 environmental risk assessment (ERA) is mostly based on information present in module 1.6.2. of the application for marketing authorisation. 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.

The worst-case scenario for Cerepro assumes a replication competent adenoviral vector (RCA) containing the HSV-tk gene that is able to spread in the environment efficiently, and in that way may also infect immune compromised individuals with a dose that is able to result in a systemic infection.

- 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

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.

[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.

Replication of Cerepro may also occur *in vivo* due to complementation of the E1 gene product by a simultaneously present wild type adenovirus. This might produce enhanced levels of Cerepro and lead to enhanced shedding. Complementation does not give rise to replication competent vectors. The data provided by the applicant does not indicate substantial occurrence of complementation. 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, 6 countries have provided comments, which were channelled via the CA from Denmark (appointed Lead CA). From the ERA performed during the first

submission of Cerepro it has already been concluded that there is a negligible risk for human health and the environment. At that time several questions were asked. The company provided sufficient information to deal with these questions adequately. With the current (second) submission of Cerepro the company has submitted a similar dossier which for some issues includes additional data to support the previous conclusions. As a whole it is concluded that there is a negligible risk for human heath and the environment and no additional scientific questions remain to be solved.

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. The samples selected, nasal swabs, are adequate to address shedding of the virus and emergence of RCAs. The outlined monitoring plan will be sufficient to trace possible effects of Cerepro on human health and the environment. 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 will carry out the various tasks the monitoring plan requires and who is responsible for ensuring that the monitoring plan is put into place and carried out appropriately.

General surveillance is incorporated by the applicant in the proposed monitoring plan. From the launch of the product on the market, all hospitals which handle Cerepro will be advised to keep a registry of individuals who handle Cerepro. These include pharmacy staff, operating theatre staff and staff who care for the patients, and staff involved in cleaning up large spills of Cerepro. The gathered information is retained in the hospital for fifteen years. This plan is 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.

The CHMP agrees with the CAT assessment regarding the Environmental Risk aspects as described above.

2.6 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 to be addressed, the applicant has performed a comparability exercise to demonstrate that batches produced during development are comparable to the product produced for marketing. The results obtained in the pivotal toxicology study and Phase III clinical study 904 are therefore considered predictive of the stability, safety and efficacy of commercial Cerepro.

Non-clinical pharmacology and toxicology

The submitted non-clinical pharmacology studies showed that Adv.HSV-*tk* transfection in conjuction with GCV treatment induces cell death. However, the efficacy of Cerepro treatment in conjuction with GCV depends upon whether sufficient transfection efficiency can be achieved clinically following the neurosurgical procedure.
Conventional toxicology studies were not conducted for sitimagene ceradenovec. A combined single-dose biodistribution and toxicity study showed viral infection and expression of the thymidine kinase gene in various tissues following systemic exposure. While sitimagene ceradenovec in general was well-tolerated, the conclusion of the non-clinical study was complicated

by the lack of administration of ganciclovir following significant systemic exposure to sitimagene ceradenovec.

Efficacy

The efficacy of Cerepro in terms of unbiased clinical endpoints (e.g. overall survival) has not been demonstrated. Although regulatory guidance had preferred 'all-cause mortality' as primary endpoint for the pivotal trial, the Applicant chose to change the statistical method to a continuous sequential design and to change the primary endpoint to 'time to death or re-intervention' during the early course of the study. The revised primary endpoint is considered invalid, since it may be subject to bias by the treating physician in this unblinded trial. In the treatment of GBM, overall survival is a more robust and clinically relevant efficacy endpoint. The risk of confounding the results of overall survival by the effect of sequential therapies is considered minor since cross-over to the experimental therapy was not possible for patients in the control arm upon progression and knowing that few effective treatments are available for 2nd and 3rd line treatments of relapsed GBM; these treatments would be equally available for patients in both treatment arms.
In the pre-specified standard univariate analysis of overall survival in the pivotal trial 904 using the true ITT population and stratifying for intended temozolomide use, no statistically or clinically significant difference between Cerepro and standard of care was found. Additional submitted exploratory analyses suffered from a number of methodological issues including lack of adjustment for multiplicity and lack of pre-specification. The results of subgroup analyses could only be considered as hypothesis generating.

Safety

The safety data presented document that administration of Cerepro is associated with an increased incidence of adverse events and of serious adverse events (e.g. hemiparesis, seizures).

- User consultation

It is noted that User Testing of the Cerepro package leaflet was not conducted since User Testing had been performed for the Cerepro MAA submitted in October 2005. User Testing might have to be performed again to demonstrate that the label and package leaflet can be understood by users.

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

A prerequisite to a positive benefit-risk balance is to establish efficacy of Cerepro in the claimed indication. In the single pivotal clinical trial submitted, no significant treatment effect was observed in the main analysis of the primary endpoint time to re-intervention. Notwithstanding the lack of a treatment effect concerning this endpoint, there were concerns that time to re-intervention may be prone to important investigator bias due to the open design of the study. Concerning the more objective and clinically relevant secondary endpoint of overall survival, no significant differences were observed in the primary analysis population. The efficacy claims made by the applicant are based on mainly non-prespecified exploratory analyses using various statistical techniques and adjustments for imbalances in patient and disease characteristics. Due to the lack of pre-specification of these analyses it cannot be ensured that important biases can be avoided and it is difficult to draw any conclusions to establish the efficacy of Cerepro in the claimed indication based on their results. In conclusion, the efficacy of Cerepro in combination with ganciclovir in the treatment of high-grade glioma has not been established based on the data submitted. Concerning the risks, the use of Cerepro was associated with non-negligible toxicity. In the absence of an established benefit, the benfit-risk balance is considered negative.

Recommendation

Based on the CAT and CHMP review of data on quality, safety and efficacy, the CAT and CHMP considered by majority 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.

Grounds for refusal

In conclusion, the CHMP considered that based on the CAT opinion and 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 efficacy data submitted do not demonstrate the benefit of Cerepro in the claimed indication. The primary efficacy analysis did not show any statistically and clinically significant difference between the active treatment and the control arm; in this failed trial any post-hoc subgroup analyses can only be considered as exploratory.
- In addition, during this open label trial, the company changed the primary endpoint in a sequential design from 'overall survival' to "time to death or re-intervention", which is prone to bias by treating physicians.
- The administration of Cerepro is associated with an increased incidence of adverse events and of serious adverse events (e.g. hemiparesis, seizures). In view of the lack of proven efficacy of Cerepro and the risk management submitted, the documented side effects result in a negative benefit/risk ratio.
- Due to the aforementioned concerns a satisfactory summary of product characteristics, risk management plan, environmental risk assessment and follow-up measures to address other concerns as outlined in the list of outstanding issues cannot be agreed at this stage.

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