European Medicines Agency
Evaluation of Medicines for Human Use

London, 20 March 2007
EMEA/CHMP/273127/2007

REFUSAL CHMP ASSESSMENT REPORT FOR MYCOGRAB

International Nonproprietary Name:
Efungumab

Procedure No. EMEA/H/C/658

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

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PRODUCT INFORMATION

Name of the medicinal product:	Mycograb
Applicant:	*Neu*Tec Pharma plc 2nd Floor, Clinical Sciences Building Central Manchester & Manchester Children's Hospital NHS Trust Oxford Rd Manchester, M13 9WL United Kingdom
Active substance:	Recombinant human monoclonal antibody to hsp90
International Nonproprietary Name:	Efungumab
Pharmaco-therapeutic group **(ATC Code):**	Not yet assigned Not yet assigned.
Applied therapeutic indication:	Treatment of invasive candidiasis in adult patients, in combination with amphotericin B or a lipid formulation of amphotericin B.
Pharmaceutical form:	Powder for solution for injection
Strength:	2 mg/ml
Route of administration:	Intravenous use
Packaging:	Vial (glass)
Package size:	10 vials

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1 BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant *Neu*Tec Pharma plc submitted on 11 March 2005 a full application for Marketing Authorisation referring to Art. 8.3 of Directive 2001/83/EC, as amended, to the European Medicines Agency (EMEA) through the centralised procedure for Mycograb, which was designated as an orphan medicinal product EU/3/01/073 on 5 December 2001. Mycograb was designated as an orphan medicinal product in the following indication: treatment of invasive fungal infections. The calculated prevalence of this condition was approx. 0.3 per 10,000 EU population.

The applicant applied for the following indication: treatment of invasive candidiasis in adult patients, who are receiving co-therapy with amphotericin B or a lipid formulation of amphotericin B.

The legal basis for this application refers to:

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

Protocol Assistance
The applicant received Protocol Assistance from the CHMP on 6 October 2003. The Protocol Assistance pertained to non-clinical and clinical aspects and significant benefit of the dossier.

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

The Rapporteur and Co-Rapporteur appointed by the CHMP were:
Rapporteur: J. Ersbøll
Co-Rapporteur: P. Kurki

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 11 March 2005.
- The procedure started on 28 March 2005.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 13 June 2005. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 8 June 2005.
- The quality list of questions was discussed and adopted at the Biological Working Party (BWP) meeting of 18-20 July 2005.
- During the meeting on 25-27 July 2005, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 28 July 2005.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 23 March 2006.
- Inspections were carried out at the manufacturing sites of the drug substance and drug product contract manufacturers.
  - Biomeva GmbH (prev. BioReliance Manufacturing GmbH) - Czernyring 22 - Heidelberg – Germany – on 10 January 2006 and 11 October 2006 respectively (two inspections);
  - Thymoorgan GmbH Pharmazie & Co. KG - Schiffgraben 23 - Viennenburg – Germany - on 17 February 2006 and 29 September 2006 respectively (two inspections).
- The Rapporteurs circulated the Joint Assessment Report on the applicant’s responses to the List of Questions to all CHMP members on 16 May 2006.
- The quality list of outstanding issues was discussed and adopted at the BWP meeting of 22-24 May 2006.

- During the CHMP meeting on 29 May – 1 June 2006, the CHMP agreed on a List of Outstanding Issues to be addressed in an oral explanation and/or in writing by the applicant.
- The applicant submitted the responses to the CHMP List of outstanding Issues on 21 September 2006.
- The responses to the quality list of outstanding issues were discussed and a recommendation to the CHMP was adopted at the BWP meeting of 9-11 October 2006.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Outstanding Issues to all CHMP members on 11 October 2006.
- During the CHMP meeting on 16 – 19 October 2006, the CHMP agreed on a second List of Outstanding Issues to be addressed in an oral explanation and/or in writing by the applicant.
- The applicant submitted the responses to the second CHMP List of outstanding Issues on 1 November 2006 (clinical aspects) and 6 November 2006 (quality aspects).
- The responses to the quality list of outstanding issues were addressed by the applicant in a hearing in front of the BWP and a recommendation to the CHMP was adopted at the BWP meeting of 7-8 November 2006.
- The response to the clinical question of list of outstanding issues was addressed by the applicant in a hearing in front of the CHMP on 14 November 2006.
- During the CHMP meeting on 13-16 November 2006, in the light of the overall data submitted and the scientific discussion within the Committee, the CHMP issued a negative opinion for granting a Marketing Authorisation to Mycograb on 16 November 2006.

1.3 Steps taken for the re-examination procedure

The Rapporteur and Co-Rapporteur appointed by the CHMP were:
Rapporteur: M. Haase
Co-Rapporteur: D. Lyons

- The applicant submitted written notice to the EMEA on 24 November 2006 to request a re-examination of the Mycograb CHMP opinion of 16 November 2006.
- During its meeting on 11-14 December 2006, the CHMP appointed Dr Manfred Haase as Rapporteur and Dr David Lyons as Co-Rapporteur for the re-examination procedure.
- The detailed grounds for the re-examination request were submitted by the applicant on 19 January 2007. The re-examination procedure started on 20 January 2007.
- The Rapporteur's Assessment Report was circulated to all CHMP and BWP members on 20 February 2007. The Co-Rapporteur's Assessment Report was circulated to all CHMP and BWP members on 21 February 2007.
- The final list of questions for the SAG Anti-infectives and the BWP, respectively, were circulated on 5 March 2007.
- During a meeting of the BWP on 12 March 2007, BWP members and additional experts addressed questions raised by the CHMP. During this meeting the applicant presented an oral explanation. A report of this meeting was forwarded to the CHMP.
- During a meeting of the CHMP Scientific Advisory Group on Anti-infectives (SAG-Anti-infectives) on 13 March 2007, experts were convened to address questions raised by the CHMP. During this meeting the applicant presented an oral explanation. A report of this meeting was forwarded to the CHMP.
- The Rapporteurs' Joint Assessment Report was circulated to all CHMP members on 15 March 2007.
- During the CHMP meeting on 19-22 March 2007, the applicant presented an oral explanation before the CHMP on 20 March 2007.

- During the meeting on 19-22 March 2007, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a final Opinion recommending the refusal of the Marketing Authorisation for Mycograb.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

The spectrum of diseases related to *Candida* species is wide, ranging from superficial candidiasis such as thrush to life threatening infections associated with an overall prognosis comparable with that of septic shock. Invasive candidiasis (also known as systemic or deep-seated candidosis) refers to infection of the visceral organs. It is a life-threatening infection, usually involving multiple organs, the *Candida* having spread via the bloodstream to organs such as the liver, spleen and kidney ("disseminated candidiasis"). Acute disseminated candidiasis is characterised by the rapid onset of fever, sometimes shock, and other signs of sepsis. In immunocompromised patients, a presumptive diagnosis may be made based on multiple positive cultures from non-sterile sites. Because culture-confirmation is relatively slow compared to that achieved with bacterial infections, and may be negative even in the presence of subsequent proven fungal sepsis antifungal therapy is sometimes given on an empiric basis.

The incidence of candidemia has been increasing due to a variety of factors, including iatrogenic immunosuppression, increasingly invasive technologies, and the use of broad-spectrum antibiotics.
Invasive candidiasis accounted for 17% of hospital-acquired infections reported during the European Study on the Prevalence of Nosocomial Infections in Critically Ill patients (EPIC), which included 10 038 patients from 1417 intensive care units (ICUs) in 17 countries in 1992. US data have shown that *Candida* species are the fourth most common cause of bloodstream infection, accounting for 8 to 15% of all nosocomial bloodstream infections. Incidence rate of invasive candidiasis varies considerably and has been estimated to affect 0.2– 1.0 in 10,000 persons per year.
Candida albicans is the commonest species associated with deep infection but other ("non-*albicans*") species of which *Candida glabrata* now dominates are becoming increasingly common.

Antifungal medicinal products currently recommended for the treatment of invasive candidiasis are amphotericin B (lipid-based formulations), fluconazole or caspofungin. In each case, these are given as monotherapy.

Despite the availability of treatments, the mortality and morbidity due to invasive candidiasis is high (mortality varying between 4.5 and 49 %). The frequency of persistent candidaemias in spite of therapy remains 10-17%, while *Candida*-attributable mortality is 10-19%. There is therefore still a need for new antifungal agents.

Mycograb (efungumab) has been developed as a new therapeutic approach. Efungumab is directed to heat shock protein 90 (hsp90) which plays a vital role in fungal cell survival. Efungumab is a single-chain Fv human monoclonal antibody fragment produced in *E. coli* by recombinant DNA technology where the variable domain of the heavy chain (V_H) is connected to the variable domain of the light chain (V_L) with a linker.
The applied indication was for the treatment of invasive candidiasis in adult patients, in combination with amphotericin B or a lipid formulation of amphotericin B.
The proposed dose regimen of Mycograb 2mg/ml powder for solution for injection in adults was 1 mg/kg body weight twice a day (12 hours apart) for 5 days, in combination with amphotericin B therapy.

2.2 Quality aspects

Introduction

The drug substance in Mycograb, efungumab (recombinant human monoclonal antibody to hsp90), is a human-derived single chain variable antibody fragment which binds to the yeast antigen heat shock protein hsp90 (rhMAB-hsp90). It is produced in *Escherichia coli*.
Drug Substance

rhMAB-hsp90 is a non-glycosylated 27 kDa peptide, which contains two disulphide bonds. The protein consists of the variable domains of heavy (VH) and light chains (VL) joined via a flexible linker peptide. For the purposes of purification and detection, a hexahistidine tag has been introduced to the C-terminal end of the protein. The protein does not possess the Fc portion that is responsible for certain biological functions of an antibody.

Manufacture

Manufacturers
The drug substance is manufactured by a contractor, which holds a valid manufacturing license. Following inspection of the site, compliance to Good Manufacturing Practice (GMP) was confirmed although the process validation reports have not been finalised (missing cleaning validation, which according to the applicant will be available beginning 2007).

Genetic development
The description of generation of the production cell genetics is brief but in general acceptable. The rhMAB-hsp90 nucleotide sequence was re-synthesised incorporating codons optimal for expression in *E.coli*, without altering the primary amino acid sequence. The coding sequence was inserted into an over expression vector with an optimised sequence for correct expression. The nucleotide sequence of the optimised gene sequence was determined and shown to result in the correct coding sequence. At the carboxyl terminus of the expression construct, a vector-derived histidine tag is present to facilitate purification. Otherwise, the predicted amino acid sequence of the rhMAB-hsp90 protein from the vector is exactly the same as that found in the parent rhMAB-hsp90 gene.

Cell Banking
Cell banks of an *E.coli* strain were manufactured using animal-free media.
For the preparation of the master cell bank (MCB) in-process controls included determination of OD_{600} and pH, as well as tests for microbial contamination. The MCB is stored under liquid nitrogen in the gas phase. The working cell bank (WCB) is used to directly provide cells for the manufacturing process. For preparation of working cell banks the procedure was essentially the same as that used in construction of the MCB. Testing of the MCB and WCB are identical and adequate.

Genetic stability
Post-production cell bank testing was performed on the first four production batches of rhMAB-hsp90 to establish the genetic stability and robustness of the fermentation process. Testing was performed on cells at the end point of fermentation. Analysis post-fermentation showed that the production cell line continues to produce recombinant protein after the fermentation process and that the nucleotide structure of the coding region remains unchanged. Levels of plasmid retention also remain largely unaltered, further demonstrating genetic stability of the expression strain/construct.

Overall, acceptable information has been provided on genetics development, genetic stability and cell bank stability.

Starting materials
Animal-derived materials used in manufacture of rhMAB-hsp90 are prepared from bovine milk and using bovine bile from cows in countries where cases of BSE have never occurred (Brazil and USA) and where fodder is not prepared from the meat. Serum is not used in the composition of the cell culture medium, including WCB cultivation and cell culture process media. Materials used in the manufacture process are adequately controlled.

Cell culture and purification
Efungumab is produced by fermentation in *E.coli* in the form of inclusion bodies, which are extracted from the cell mass and refolded. Subsequently, the protein is purified by three chromatographic steps under denaturing conditions including an anion exchange chromatography and an affinity chromatography which are used to remove any remaining protein contaminants and endotoxin.

The pH of the resulting eluate is adjusted, concentrated by diafiltration (tangential flow filtration) and the denaturant is removed by buffer exchange to allow the protein to adopt its folded conformation. This is a critical step. Finally, the product is then sterile filtered into sterile bags and kept to await preparation of the finished product. The material that is loaded onto an anion exchange column is defined as a batch.

Overall, the manufacturing process has been well described. Establishment of controls for critical steps are described. In-process controls are in place during fermentation, harvest and purification and limits and ranges are acceptable. Factors impacting on the aggregate size distribution have not been evaluated by the company.
Six consecutive manufactured production scale batches were evaluated to provide evidence that the manufacturing of drug substance is reproducible and leading to suitable product quality as defined in the drug substance specification. The validation program included: demonstration of process consistency, transport validation, validation of critical process steps (removal of process related impurities, stability testing of drug substance and validation on reuse of columns and TFF filters) and a definition of acceptance criteria for the routine manufacturing process. Evaluation and control of the removal of impurities were extended from evaluation of HCP and endotoxin content to include evaluation of DNA and other process related impurities. Some scale-down and full-scale data have been presented.

All in-process controls met the specified IPC acceptance criteria and all six batches showed consistent results and met the drug substance specifications, supporting that the drug substance manufacturing process is sufficiently controlled and operates in a consistent manner. However, control of aggregation was not part of the testing program for validation and therefore consistency with regard to aggregates has not been demonstrated. This issue is connected to the general issue raised on the control of aggregates (see below). Purity of the drug substance is solely evaluated as removal of process-related impurities and none of the in-process controls measures the correct refolding and purity of the protein during the manufacturing process.

Validation of the refolding step has not been performed/finalised. Instead an in-process control at the drug substance level was proposed using a non-validated method and circular dichroism (CD) is proposed in the drug product specification. Since the in-process method was not validated and since the CD data provided have not been convincing, it cannot be concluded that the folding of the protein was properly controlled.

Characterisation
rhMAB-hsp90 and product related impurities have been characterised using a battery of analytical techniques. A high-molecular variant is seen in the SDS-PAGE gel. Measured by two different ELISA assays the antigen-binding activity of the high-molecular variant is shown to be significant and only slightly lower than the parent molecule. This high-molecular variant is therefore considered as a product-related substance. By the introduction of a new SDS-gel method a better separation of the parent molecule and the high-molecular variant is achieved. The method is validated and considered acceptable to control the high-molecular variant in the drug substance and the drug product and a release specification on the basis of this assay was proposed for drug substance and drug product.

The characterisation studies performed under native conditions revealed the presence of an unusually high level of protein aggregates. These aggregates were studied using size exclusion chromatography (SEC). The applicant proposed that the SEC method provides information on the consistency of aggregation between batches. However, consistent results between batches could be shown.

The applicant also provided data using other methods. However, results did neither provide additional assurance of consistent molecular weight distribution between batches, nor explain the root cause for aggregation.
The BWP considered that the control of aggregates is a crucial part of the quality control of this product but the company did not present data which in a convincing way assure that this parameter is well controlled.

In conclusion the lack of a suitable method to control aggregation in the drug substance was considered to be of major concern.

It should be noted that during development, changes have been introduced to the manufacturing process such as a more than 2-fold increase in batch size (with replacement of a 10 L anion exchange column with a 20 L column). In general, using the available methods, comparability has been show throughout manufacturing development. However, with regard to aggregation no acceptable method is available and therefore consistency between batches has not been demonstrated with regard to aggregation.

Specifications

Specifications were proposed on the basis of results from release testing of 10 cGMP manufacturing lots of rhMAB-hsp90. Lots 1-8 were used in pivotal clinical trials. Specifications include:

- protein concentration determined by spectrophotometric measurement at 280 nm
- pH
- activity/potency test by ELISA which measures the specific activity of the active substance
- purity by SDS-PAGE coomassie stained
- endotoxin measured by quantitative kinetic-QCL assay
- DNA measured following phenol extraction
- host cell protein determined by non-quantitative SDS-PAGE and Western Blot method
- degradation products by SDS PAGE densitometry
- high molecular variant by SDS PAGE
- High molecular weight aggregates using a SEC method
- sterility tested in accordance with Ph.Eur using the filtration method

As indicated above (characterisation), the SDS-Page method is acceptable for evaluation of the monomer-, dimer- and high molecular product-related variants. Neither the method nor the specification to control the distribution of high molecular weight aggregates are acceptable.
One host cell protein is co-purifying together with the drug substance in significant amounts. The level is considered high when compared to other parenteral biological medicinal products. The data provided showed that while the proposed HCP assay may be suitable for the detection of a particular HCP and to confirm the level of this particular impurity, it is not suitable for the detection of the overall levels of HCPs.

Stability

Preliminary real-time (96 hours) stability data were presented together with data from storage under accelerated conditions. Under accelerated condition some out of specification results were seen. Under normal storage condition results remained within the specification. Therefore the proposed storage conditions are considered acceptable. However the stability study does not include a suitable test for aggregate size distribution.

Drug Product

The Mycograb product is a sterile, white lyophilised powder for solution for intravenous injection. The lyophilised powder is reconstituted with 5 ml water prior to injection. The water for injection is not provided with Mycograb. The product contains 10 mg efungumab. The reconstituted product contains 2 mg/ml active substance. The excipients are not of human or animal origin.

The product is presented in 5 ml clear, borosilicate glass Type 1 (Ph. Eur.) vials closed by bromobutyl rubber stoppers and aluminium flip caps.

Pharmaceutical Development

Development of the lyophilised formulation of drug product has been described and justified. The reconstituted solution of Mycograb has a high pH. The high pH together with the excipients ensure solubility and prevent precipitation of the protein. Should the pH in solution drop below a certain level, Mycograb precipitates yielding a turbid, white solution, which is unsuitable for therapeutic use. This raises question about protein solubility when the product is entering physiological conditions.
A FS method was developed to evaluate the impact of excipients on the secondary structure of the protein. However, the Applicant has not made a comparison between different batches analysed with this technique and consequently, the consistency of the structural characteristics of Mycograb has not been demonstrated.

A CD spectroscopy method was proposed to confirm the proper folding of the protein however, because of methodological deficiencies, this method is not considered suitable for control of correct refolding of the protein. The issue of refolding is of importance since it is likely that folding may impact on the binding of the antibody to its antigen.

Manufacture
The drug product is manufactured and tested prior to release by a contractor for which compliance to GMP was confirmed.

The manufacturing process for the finished product consists of 0.22µm sterile filtration, aseptic filling, lyophilisation, sealing and packaging. The manufacturing process and in-process controls have been adequately described. Critical steps have been defined and are controlled. The process has been sufficiently validated.

As for the drug substance, the validation of the drug product manufacturing process was still in progress during evaluation and the validation reports have not been finalised. Although preliminary validation results hint towards a consistent and acceptable controlled manufacturing process, the lack a finalised validation makes it impossible to conclude on the adequacy of the drug product manufacturing process. This is a major objection to a positive opinion for this product.

Product specification
Finished product specifications based on the analysis of 10 production batches have been adequately justified and are acceptable. Batch analysis results confirm consistency and uniformity of manufacture and indicate that the process is under control. Some of the tests and specifications for drug product are identical to those applied to drug substance. Tests specifically performed on the finished product are pH after reconstitution, detection of particles and subparticles after reconstitution, residual moisture after drying, endotoxin and sterility.

A specification was introduced to control the level of a *E.coli protein* impurity (present in the drug product. This protein is co-purified with Mycograb and present at a high level for a parenteral medicinal product and which is not acceptable The methods used for detection of total HCP and this E.coli protein give contradictory results and consequently, this specific *E.coli* protein is not adequately controlled. The product should not be used as long as this impurity is not adequately controlled.

Stability
Results for up to 48 months under long term stability conditions at 2-8°C, accelerated conditions for up to 6 months at 25°C, heat stress stability for 24 hours at three elevated temperatures, wet stability (after reconstitution in water) and photostability conditions have been provided.
Long-term stability studies were carried out on 4 batches, accelerated studies were carried out on 3 batches, and stress stability, wet stability and photostability studies were carried out on 1 batch.

When stored at 2-8°C the product was found to remain within the proposed specifications over the 48-month testing period. The reconstituted product remained within the proposed specifications up to 48 hours.
However, as already mentioned for the stability of the drug substance, the stability studies lack an acceptable method to evaluate the aggregate size distribution during storage. Therefore, no firm conclusion can be drawn on the stability of Mycograb.

Adventitious agents
Information on control of mycoplasma, bacteria and fungi, testing of source materials and testing during manufacturing were provided.

The MCB and WCBs, which have been established, are free from TSE-risk substances. Two materials of animal origin are used during drug substance production. Compliance with the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products (EMEA/410/01 Rev. 2) has been demonstrated." The risk of TSE transmission is considered negligible.

Viral validation studies have not been performed and this is considered acceptable since viral contamination is unlikely. Human or animal cell lines are not used in manufacture. The protein is expressed in *E.coli* and the fermentation process occurs in a serum-free medium. The risk of potential contamination with adventitious agents is considered minimal.

In summary, the virus safety of the product has been sufficiently demonstrated.

Discussion on chemical, pharmaceutical and biological aspects
Mycograb is clearly not a conventional monoclonal antibody product and the active substance has a tendency to aggregation. The size of the aggregates has been intensively investigated by the company using several methods. Neither of these methods, however, has provided satisfactory information with respect to the aggregates.
The company have attempted to find suitable methods to control the size distribution of aggregates and proposed SEC for release testing. However, because of methodological deficiencies, the proposed method is not considered adequate for control of aggregation and to demonstrate consistency of the manufacturing process and stability. The control of aggregation is a crucial parameter for the quality control of this product and the company has not been able to demonstrate that the manufacturing process produces a product with consistent aggregation.

In addition, validation of the refolding step has not been performed appropriately and finalised. Instead, an in-process control at the drug substance level is introduced and CD is proposed for the drug product specifications. So far the CD data provided have not been convincing and the method is not considered suitable for controlling refolding of this product. As no validation data have been presented for the in-process method, it cannot be concluded that the applicant has demonstrated that the refolding step of the protein is adequately controlled.

For the determination of *E. coli* host cell protein (HCP) in the drug substance the company has used a commercial ELISA assay. Validation data for the assay have been presented. The level of HCP present in the final product is high when compared to other parental preparations. Such a high level can have an impact on the safety (immunogenicity), which has to be considered with respect to use in patients (repetitive administration) and results from clinical trials.

2.3 Non-clinical aspects

Introduction

The discovery of efungumab stemmed from the observation that patients with invasive candidiasis treated with amphotericin B were more likely to recover from the infection if they produced antibody to the hsp90 antigen. Therefore, the non-clinical testing strategy was designed to determine the protective potential, safety and optimal dosage of Mycograb in combination with amphotericin B in the treatment of invasive candidiasis.
All pivotal non-clinical studies were claimed to be conducted in accordance with principles of Good Laboratory Practices (GLP).

Pharmacology

- Primary pharmacodynamics

Efungumab is a human recombinant monoclonal antibody. It belongs to a new class of antifungal agents which target the immunodominant epitope of *Candida* hsp90.
Its mode of action involves binding to and inactivation of hsp90 present in the fungal cell wall and in extracellular material, particularly around foci of infection. Hsp90 has been suggested playing a key role in cell wall formation and repair, involving the chaperone-mediated folding of cell wall kinases. Inhibition of this process by efungumab would lead to a weakened cell wall. This explains the synergy seen with antifungals active at sites such as the cell membrane (amphotericin B) or cell wall (caspofungin).

For binding to the epitope presented as a synthetic peptide, the Ka value was 2.3×10^4 $M^{-1}s^{-1}$ and the Kd value was 6.47×10^{-4} s^{-1}, showing a high affinity and a very slow rate of dissociation, i.e. once binding is established dissociation has a half-life measured in days rather than hours. The equilibrium dissociation constant (KD; Kd/Ka) was 2.9×10^{-8} M. The dissociation constant for efungumab and *Candida* hsp90 (as a recombinant protein) was 7.2×10^{-7} M. At body temperature (37°C), there was an increase in the association rate constant between efungumab and the peptide epitope, but the dissociation rate constant remained low, consistent with a half-life of several days.

In vitro, efungumab alone has modest antifungal activity with the MIC-0s ranging from 128 to 512 μg/ml. It showed synergistic effect with amphotericin B for a wide range of species of *Candida*.
In in-vitro killing assay of a clinical relevant panel of *Candida* strains, there was no antagonism between efungumab and amphotericin B. All isolates tested produced either synergy or an additive effect, i.e. more than 10-fold decrease in counts in the presence of both agents compared to either compound alone. Efficacy concentrations were clinically appropriate.

In-vivo activity was confirmed in a non-lethal mouse model based on either the production of sterile biopsies or a reduction in colony forming units in the liver, spleen and kidney at 48 hours after treatment with efungumab alone (2 mg/kg) or in combination with amphotericin B (0.6 mg/kg). In a murine model, efungumab alone produced a statistically significant improvement in infections caused by a series of clinically relevant *Candida* strains [*albicans* (strain 7), fluconazole-resistant *albicans*, fluconazole-resistant *krusei* (FA/157), *tropicalis* (NCPF 3242), *parapsilosis* (NCPF 3104), *lusitaniae*, and *glabrata* (NCPF 3240)]. Amphotericin B alone cleared the *Candida tropicalis* infection but failed to fully clear infections caused by *albicans*, *krusei*, *glabrata* or *parapsilosis* strains. When co-administered, efungumab and amphotericin B completely resolved infections with *Candida albicans*, *krusei* and *glabrata*. However, no advantage of combination was observed for *C. parapsilosis* NCPF 3104 and *C. parapsilosis* (clinical isolate). Taken together, these findings support the clinical use of efungumab.

When used in combination with caspofungin, synergy was claimed in mice infected with the outbreak strain of *C. albicans* (spleen at 4mg/kg caspofungin), a fluconazole-resistant strain of *C. albicans*

(liver at 1mg/kg), *C. krusei* (liver at 1mg/kg), *C. tropicalis* (kidney at 4mg/kg), *C. glabrata* (all organs at 4mg/kg) and *C. guilliermondii* (spleen at 4mg/kg). However there are currently no clinical data to support the use of the efungumab in combination with caspofungin.

Synergy, or an additive effect, was demonstrated between efungumab and amphotericin B for 9 isolates of *Cryptococcus neoformans*.

- Secondary pharmacodynamics

The applicant claimed that assessment of complement binding and cytotoxicity are not relevant to efungumab since it does not possess an Fc fragment, which is the component of a whole antibody mediating complement binding and cytotoxicity.
Since efungumab recognises a conserved epitope (LKVIRK) present in both human and yeast hsp90, it would be expected to recognise both human and Candida hsp90. However human hsp90 thought to be essentially cytosolic. The hypothesis of the applicant is that, in normal conditions, hsp90 is not accessible to efungumab *in vivo*. Human hsp90 is only found on the cell surface in disease states such as breast cancer. Induction of antibodies to hsp90, as occurs naturally in patients who recover from invasive *Candida* infections, is not associated with autoimmune phenomena. It remains unclear as whether efungumab can bind to normal cells in healthy individuals or individuals with fungal sepsis.

An immunohistochemical investigation into the cross-reactivity of efungumab with human tissues was conducted according to acceptable standards. These studies demonstrated intracellular staining consistent with the distribution of endogenous hsp90 in human tissues. However, in vitro studies suggest that cells may express hsp90 on their surface in certain conditions, such as malignant transformation.

In vitro, there was no evidence that Mycograb was an inducer of CYP1A2 or CYP3A4 isoenzymes.
Also it did not induce any cytotoxicity (LDH release and decrease in ATP levels) or apoptosis (as measured by Caspase 3/7 activity) when tested up to its limit of solubility (100 µg/ml). Cytotoxicity was seen at highly precipitating concentration ranges (316-1000 µg/ml).

- Safety pharmacology programme

Cardiovascular and respiratory safety parameters were investigated in a predictive animal model administered single dosages of 1, 10 or 20 mg/kg by IV infusion over approximately 15 min. A minor increase in arterial blood pressure was observed in 2 out of 4 animals 30-45 minutes after administration of the 10-mg/kg efungumab. At 20 mg/kg, a significant increase in heart rate and blood pressure, accompanied by a decrease in femoral artery blood flow occurred. These effects, also seen in clinical trials, were likely associated with an increase in femoral artery resistance.
efungumab did not affect any of the respiratory parameters.

- Pharmacodynamic drug interactions

No specific studies have performed besides the above-mentioned studies.

Pharmacokinetics

Initially the pharmacokinetics had not been appropriately assessed (data pooled from a single dose using female animals only, using assays which were not considered validated).

To address this objection, the applicant provided further data to characterise the pharmacokinetics profile of efungumab from repeat dose toxicity studies in mice and in monkeys and a safety pharmacology study in dogs using validated ELISA method.

CD-1 mice of both sexes were dosed for 14-15 days *via* bolus injection twice daily with dose levels of 0, 2, or 10 mg/kg/day.

Results displayed in below table showed that systemic exposure to efungumab increased as dose increased from 2 to 10 mg/kg twice daily. There was evidence of a decrease in systemic exposure to Mycograb between Day 1 and Day 15 of dosing, which could indicate adaptive changes in the kinetics of Mycograb, such as antibody formation to the antibody fragment, following repeat twice daily dosing for 14 days.

Treatment	**Sex**	**AUC (0-tz) (ng.h/mL)**	**AUC (0-∞) (ng.h/ml)**	**AUC %Extrap**	**C_{max} (ng/ml)**	**t_{max} (h)**	**CL/F (ml/min/kg)**	**Vz/F (ml/kg)**	**Vss (ml/kg)**	**$t_{1/2}$ (h)**
4 mg/kg/day of efungumab	Male	20663	NC	NC	9860	0.08	NC	NC	NC	NC
	Female	8483	NC	NC	8775	0.08	NC	NC	NC	NC
20 mg/kg/day of efungumab	Male	88473	NC	NC	25300	0.08	NC	NC	NC	NC
	Female	36572	39407	7.19	53650	0.08	4.23	561.95	382.85	1.53

(NC=not calculated):

Following a single dose of 10 mg/kg and 20 mg/kg given to Beagle dogs, the following toxicokinetic parameters were observed

Treatment	Subject	Sex	AUC (0-tz) (ng.h/ml)	AUC (0-∞) (ng.h/ml)	AUC %Extrap	C_{max} (ng/ml)	t_{max} (h)	CL/F (ml/min/kg)	Vz/F (ml/kg)	Vss (ml/kg)	$t_{1/2}$ (h)
10 mg/kg/day Mycograb	3	Male	12472.23	NC	NC	23800.00	0.25	NC	NC	NC	NC
	4	Male	9690.32	12750.01	24.00	20800.00	0.33	13.07	496.84	481.73	0.44
	7	Female	11881.30	19666.87	39.59	21800.00	0.42	8.47	518.84	494.58	0.71
	8	Female	11819.92	20404.01	42.07	21700.00	0.33	8.17	500.84	494.46	0.71
N			4	3	3	4	4	3	3	3	3
Mean			11465.939	17606.961	35.218	22025.000	0.333	9.905	505.507	490.257	0.618
SD			1219.737	4222.364	9.797	1265.899	0.068	2.747	11.716	7.383	0.155
Min			9690.32	12750.01	24.00	20800.00	0.25	8.17	496.84	481.73	0.44
Median			11850.61	19666.87	39.59	21750.00	0.33	8.47	500.84	494.46	0.71
Max			12472.23	20404.01	42.07	23800.00	0.42	13.07	518.84	494.58	0.71
Geometric Mean			11414.057	17231.392	34.190	21998.300	0.328	9.672	505.417	490.219	0.604
Geometric CV%			11.215	26.603	31.552	5.662	21.101	26.603	2.305	1.513	28.09

Treatment	Subject	Sex	AUC (0-tz) (ng.h/ml)	AUC (0-∞) (ng.h/ml)	AUC %Extrap	Cmax (ng/ml)	tmax (h)	CL/F (ml/min/kg)	Vz/F (ml/kg)	Vss (ml/kg)	$t_{1/2}$ (h)
20 mg/kg/day of Mycograb	3	Male	12913.86	NC	NC	24100.00	0.25	NC	NC	NC	NC
	4	Male	18959.55	22851.74	17.03	38800.00	0.25	14.59	446.46	438.82	0.35
	7	Female	22254.25	31325.61	28.96	39200.00	0.25	10.64	407.86	444.33	0.44
	8	Female	16393.85	24056.66	31.85	29500.00	0.25	13.86	676.29	654.62	0.56
N			4	3	3	4	4	3	3	3	3
Mean			17630.376	26078.006	25.948	32900.000	0.25	13.028	510.203	512.590	0.453
SD			3954.806	4584.321	7.856	7382.412	0.00	2.099	145.123	123.029	0.106
Min			12913.86	22851.74	17.03	24100.00	0.25	10.64	407.86	438.82	0.35
Median			17676.70	24056.66	28.96	34150.00	0.25	13.86	446.46	444.33	0.44
Max			22254.25	31325.61	31.85	39200.00	0.25	14.59	676.29	654.62	0.56
Geometric Mean			17287.988	25823.677	25.046	32246.992	0.25	12.908	497.518	503.494	0.445
Geometric CV%			23.420	17.045	34.714	23.784	0.00	17.045	27.465	23.037	23.65

The impact of antibody formation to the antibody fragment on the toxicokinetics parameters is not fully analysed, therefore the kinetics values should be used with caution.

The toxicokinetics parameters obtained from the repeat dose toxicity study performed in monkeys are displayed in the following tables.

Toxicokinetic parameters following multiple IV doses of Mycograb 20 mg/kg/day (given as two 10 mg/kg doses every 12 hours) to cynomolgus monkeys (Day 5)

Treatment	Method	Sex	AUC (0-tz) (ng.h/ml)	AUC (0-τ) (ng.h/ml)	AUC (0-∞) (ng.h/mL)	AUC %Extrap	Cmax (ng/mL)	tmax (h)	CL/F (mLmin/kg)	Vz/F (ml/kg)	Vss (ml/kg)	t1/2 (h)
20 mg/kg/day Mycograb	1	Male	2855	4443	4443	33.37	13047	0.058	37.51	694.44	676.94	0.214
		Female	2944	3883	3883	23.61	14778	0.058	42.92	586.97	549.59	0.158
	2	Male	3374	3955	3955	8.17	13047	0.058	42.14	556.09	553.44	0.152
		Female	3341	3847	3847	7.83	14778	0.058	43.33	537.66	502.60	0.143
	3	Male	3415	4085	4085	12.76	13047	0.058	40.80	614.04	587.30	0.174
		Female	3384	3979	3979	12.16	14778	0.058	41.89	590.46	542.57	0.163

Toxicokinetic parameters following multiple IV doses of Mycograb 20 mg/kg/day (given as two 10 mg/kg doses every 12 hours) to cynomolgus monkeys (Day 19)

Treatment	Method	Sex	AUC (0-tz) (ng.h/ml)	AUC (0-τ) (ng.h/ml)	Cmax (ng/ml)	tmax (h)	CL/F (ml/min/kg)	Vz/F (ml/kg)	Vss (ml/kg)	t1/2 (h)
20 mg/kg/day Mycograb	1	Male	3115	4067	18221	0.058	40.988	455.75	417.70	0.128
		Female	3136	4124	18406	0.058	40.413	454.92	418.35	0.130
	2	Male	3374	3996	18221	0.058	41.711	388.71	361.18	0.108
		Female	3341	4017	18406	0.058	41.493	380.06	354.82	0.106
	3	Male	3793	4154	18221	0.058	40.129	445.99	399.20	0.128
		Female	3848	4178	18406	0.058	39.890	436.55	391.27	0.126

The small sample size precludes from any firm conclusion on the possible difference between the genders.

There were no animal studies on the metabolism. This is acceptable in view of the biological nature of the product and is in line with the Guideline ICH S6.

Toxicology

- Single dose toxicity

There was no single-dose toxicity study conducted which is acceptable in view of the biological nature of the product.

- Repeat dose toxicity

The applicant presented initially only the results of a 7-day repeat-dose toxicity study conducted in groups of 5 mice given enfugumab twice daily IV (bolus) doses of 0 (control), 2 (low) or 10 (high) mg/kg with a 21 days observation period. This study was not designed to be compliant with any particular regulatory guidelines but is in line with the proposals for toxicity studies for anti-cancer antibodies as outlined in the NIBSC/CRC and MCA guidelines. High-dose mice showed hunched posture, subdued behaviour, piloerection and rolling gait. Clinical chemistry data were not assessable due to insufficient control data. Spleen weights were increased in male mice without obvious histological changes. However, extramedullary haematopoiesis at the high-dose level was marginally greater in both sexes as compared to controls. Haematology was not affected. As the only other finding in this study, treatment-related lymphoid hyperplasia was observed in the 2 and 10 mg/kg groups. Since there was a strong immunogenic response to the humanised antibody, this may well have caused the latter findings. Toxicokinetics and interspecies comparisons were not accounted for.

To address the serious omissions in the repeat dose study in CD-1 mice, the applicant conducted a 2nd study. In a 14-day repeated dose toxicity study, groups of CD-1 mice were given IV dose levels of 0, 2, or 10 mg/kg bid. Local irritation was observed at the tail site of injection. Spleen weights were statistically significant higher in treated mice, and dose-related increases in the number of germinal centres (in the white pulp), clusters of plasma cells and extent of haemopoiesis were observed compared with controls. A few high-dose mice had minor hyperplasia of the bone marrow. Effects on the spleen were considered secondary to an immune response to efungumab. Unexplained mortality occurred in two mice in the high dose group (10 mg/kg bid). The NOAEL was considered to be 2 mg/kg bid, and on a dose-to-dose basis the safety margin to proposed human exposure (1 mg/kg bid) is 2. Qualitative assessment of PAS-stained sections of testes, taking into account the tubular stages of the spermatogenic cycle, did not reveal any treatment related effects.

Considering the immunogenic response to efungumab in mice, a second repeat dose toxicity study was thus performed in Cynomologus monkeys. Escalating doses of efungumab from 5 to 10 mg/kg/day administered intravenously (over 4 days) followed by 20 mg/kg/day (divided in two administrations /12hrs) for 14 days, resulted only in a low level of antibody response. This was higher than the mean levels observed in the patients treated with efungumab but much lower than the levels observed in mice. This antibody response was insufficient to reduce the levels of circulating efungumab in the monkeys, which was in contrast to the situation seen during toxicokinetic analysis of the mice sera. Two of the four monkeys monitored to day 33 had produced a more marked antibody response (predominantly IgM) by day 33, which began to match the levels seen in the mice. There were no mortalities, adverse clinical signs or effects on body weight, or clinical pathology. Two of the treated monkeys had enlarged spleens compared to controls and other treated monkeys, but this correlated poorly with antibody titre and was not associated with histopathological findings. There was no evidence of local irritation due to the injections. There was no evidence of organ related toxicity at doses up to 20 mg/kg/day of efungumab.

- Genotoxicity and Carcinogenicity

Genotoxicity and carcinogenicity studies were not performed which is considered acceptable considering the nature of the compound (human recombinant antibody) and the intended clinical duration of treatment (5 days).

- Reproduction Toxicity

Fertility and pre- and post-natal development studies were not performed.

Toxicity to embryo-foetal development was investigated in groups of 30 pregnant CD-1 mice given efungumab 0, 2 or 10 mg /kg twice daily by IV administration. The maximum dose (10 mg/kg) represented 10x the human therapeutic dose and was the same as in a repeat-dose toxicity study in the same species There were five unexplained deaths in the high dose group. All unscheduled mortalities had enlarged spleens. Local tolerance at site of application was poor as dose-related sores and lesions on the tail were observed. Nonetheless because most of the high dose mice found dead were cannibalised, determination of the cause of death was difficult and the clinical relevance is therefore unknown.
With respect to treatment-related malformations, cleft palate was observed in one 2-mg/kg litter (1 foetus) and in two 10 mg/kg litters (3 foetuses); exencephaly was observed in one foetus in each of the 2 and 10 mg/kg dose groups. The observed dose-related incidences of these 2 specific malformations were higher than historical data for the same strain and testing facility. These findings suggest that efungumab may be teratogenic in CD-1 mice, possibly due to the role of the molecular chaperone, hsp90, in foetal development. There was a concern over the species used since the mice had a significant immunogenic response to efungumab in the repeat-dose toxicity study at identical dose levels.

- Local tolerance

Specific local tolerance studies were not conducted. Local tolerance of Mycograb was poor in the embryo-foetal toxicity study where the majority of clinical observations and necropsy findings were related to the route of administration i.e. sores and lesions of the tail and were dose related in frequency and severity
Mixing blood and Mycograb at relevant concentration did not produce haemolysis, and therefore compatibility with blood was shown.

- Other toxicity studies

Data suggest that the likelihood of significant autoimmune complications is low in spite of the autologous nature of the antigen.

Dependence, metabolite and impurity studies were not performed which was considered acceptable considering the nature of the product.

Ecotoxicity/environmental risk assessment

An assessment of the risk was performed and no significant risk to the environment related to the use of Mycograb is anticipated.

2.4 Clinical aspects

Introduction

The clinical programme consisted of:

- an initial safety and tolerance study performed initially using 0.1 mg/kg and escalating to one and two doses of 1mg/kg/day of Mycograb given i.v. (NTB/Mycograb/001).
- A pilot and confirmatory double-blind placebo-controlled studies of efficacy and safety of 1 mg/kg Mycograb administered iv twice daily over 5 days in the target population (NTB/Mycograb/002).

The applicant subsequently provided results from 2 new pharmacokinetics studies (one in human volunteers and one in patients with advanced carcinoma of the breast) to further characterise the pharmacokinetics profile of Mycograb.

The applicant claimed that clinical trials were performed in accordance with Good Clinical Practices (GCP). A GCP inspection was conducted at 2 sites in the Czech Republic, from which a substantial number of patients were recruited for the confirmatory study 002.

The applicant has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

Pharmacokinetics

Initially the clinical pharmacokinetics of Mycograb was scarcely studied in adult patients with confirmed systemic *Candida* infections already receiving liposomal amphotericin B. Data derived mainly from NTB/Mycograb/001, an initial safety and tolerance study performed in 5 patients initially using 0.1 mg/kg and escalating to one and two doses of 1mg/kg i.v. Mycograb daily.
Two different analytical methods were used to assay efungumab levels in patients, a gel system assay, which was later abandoned, and an ELISA assay respectively. From this 001 study, it appeared that the clearance of the antibody was rapid after the first injection. The concentrations tended to be higher in patients after the second dose; AUC (1-12h) on day 5 was 17.9 + 18.7 µg.h/ml versus 10.6 + 4.9 µg.h/ml on day 1. It remains unclear whether this was due to accumulation or to less efficient clearance (less binding to target antigens), or perhaps to a technical artefact (antigen interference in the assay). These initial data cannot be regarded reliable considering the major technical problems with the efungumab-assay.

Two new PK studies were initiated to further characterise the pharmacokinetics profile. These studies involved a limited number of individuals. Considering the inter-individual variation, the data are not robust:

- A single and repeat dose, open-label, randomised, placebo-controlled, parallel group study to investigate the pharmacokinetics, safety and tolerability of Mycograb in healthy male and female volunteers: (NTP/Mycograb/HuPK/001).
- A Phase 1b, pharmacokinetic, multicentre, open label study evaluating the safety and efficacy of Mycograb administered IV in combination with docetaxel in metastatic or recurrent breast cancer patients (NTP/ONC/001).

- Absorption

After intravenous administration, Mycograb is rapidly distributed into tissues and cleared from the circulation. In healthy volunteers (n = 5) given a single dose of 1 mg/kg Mycograb as an intravenous bolus over 10 minutes, the $t_{1/2}$ was 0.8 ± 1.2 hours (range 0.2 - 2.9 hours), the C_{max} was 2683 ± 318 ng/ml (range 2334 - 3046 ng/ml) and the AUC_{0-tz} was 1336 ± 751 ng.h/ml. The clearance was 13.1 ± 5.6 ml/minute/kg and the volume of distribution was Vz 0.54 ± 0.33 and Vss 0.47 ± 0.22 l/kg.

After single-dose administration to healthy subjects over the range 0.25 to 1 mg/kg infused over 10 minutes, serum Mycograb concentrations quickly declined with time postdose at 0.25 mg/kg but were quantifiable generally for 1 to 4 hours postdose after 0.5 and 1 mg/kg. The peak concentration at the end of the infusion approximately doubled from 0.25 to 0.5 mg/kg but then appeared to plateau at 1 mg/kg. Following administration of the recommended clinical dose of 1mg/kg maximum plasma concentrations of about 2700 ng/ml was reached. Volume of distribution was about 0.5 l/kg and clearance 13 ml/min/kg. Elimination half-life was about 0.8 hours.
Sparse blood sampling during the drug elimination phase was performed in breast cancer patients after a single dose of 2 mg/kg Mycograb infused over 30 or 60 minutes. Half-lives were derivable in 5 patients averaging 1.4 ± 0.4 hours (range, 1.1 to 2.0). These values are in the range measured in healthy subjects.

In healthy volunteers (n = 5, 2 withdrawn) given multiple doses of 0.5 mg/kg Mycograb as an intravenous bolus over 10 minutes twice daily for 9 doses over 5 days, the $t_{1/2}$ was 0.3 ± 0.1 hours (range 0.2 – 0.4 hours), the C_{max} was 1188 ± 190 ng/ml (range 1016 - 1394 ng/ml) and the AUC_{0-tz} was 590 ± 156 ng.h/ml. The clearance was 15.0 ± 4.4 ml/minute/kg and the volume of distribution was Vz 0.33 ± 0.05 and Vss 0.35 ± 0.05 l/kg.

- Distribution

The nature of the Mycograb-binding to plasma proteins remains poorly characterised. Mycograb is highly bound to plasma proteins and several purified serum proteins interfered in the Mycograb-assay, including human serum albumin and gamma globulin. The implications of these interactions may not be restricted to Mycograb assays but may have implications to distribution and elimination as well as to safety.

- Elimination

With respect to metabolism, Mycograb is expected to be degraded into small peptides and individual amino acids. Methodological problems prevented from studying the urinary excretion of Mycograb. Nevertheless, traces of monomer and dimer sized fragment were detected in urine of healthy volunteers. It was suggested that Mycograb is eliminated through renal filtration followed by tubular re-absorption and subsequent metabolic catabolism. However, it is also possible that Mycograb is present larger aggregates in plasma and will not pass the glomerular barrier.

- Dose proportionality and time dependencies

There are no specific studies on dose proportionality. Following repeated dosage, some accumulation with respect to C_{max} is apparent, but the dataset is limited to draw firm conclusion.

A rather large degree of inter-subject PK variability has been shown (AUC steady-state CV% about 100 in the target population and about 40 in healthy volunteers). Data from intra-subject variability are too scarce to draw firm conclusion.

With respect to time-dependency kinetics, repeat dose study in healthy volunteers demonstrated that there was rather an increase than a reduction in Mycograb levels on day 2 when compared to day 1. This is in contrast with results reported for the pilot study in patients in which both C_{max} and AUC were reduced from day 1 and day 5.
Different assays were used in the study of healthy volunteers, on one hand and fungal sepsis patients, on the other hand. The large inter-individual variation and small number of observations hamper the interpretation of the results.

- Special populations

The PK of Mycograb was not specifically studied in subject with renal impairment. Post-hoc analysis stratified according to renal function did not suggest a clinically relevant association, and therefore no dose-adjustment is necessary. Similarly post-hoc analysis stratified by gender and age did not reveal any influence requiring dose adjustment.
There are no data on the pharmacokinetic of efungumab in patients with hepatic impairment nor in data in children.

- Pharmacokinetic interaction studies

No specific interaction studies were performed. Considering that *in vitro* Mycograb was not an inducer of CYP1A2 or CYP3A4 isoenzymes, the potential for pharmacokinetics interaction involving these isoenzymes is unlikely.

Pharmacodynamics

No clinical pharmacodynamic studies were performed. Mycograb is suggested to play a key role in neutralising hsp90 which has entered the extracellular compartment. Hsp90 is intracellular in normal human cells, in the nucleus and cytoplasm, and it is not released as part of cell apoptosis. However, it is released as a consequence of cell necrosis, as occurs when tissues are damaged or diseased. Therefore extracellular hsp90 is associated with disease states. Once in the extracellular compartment, hsp90 can interact with interleukin 6 and nitric oxide synthase. Published data show that interleukin 6 is one of the cytokines released from monocytes when stimulated by amphotericin B and has been identified as a major systemic regulator of C reactive protein during the acute phase of sepsis. The administration of amphotericin B in fungal sepsis is often associated with pyrexia and chills. However, there are no pharmacodynamic studies in humans to support the suggested mechanism of action.

Clinical efficacy

The clinical programme consisted of two multicentre trials involving a total of 133 patients to demonstrate the efficacy and safety of Mycograb in patients with invasive candidiasis: Study 002 Pilot and 002 Confirmatory (table 1). Originally, only a single trial with interim analysis was planned. This was then changed to present the data from two stages with a hypothesis generating pilot study from which sample size would be calculated for the subsequent confirmatory study.

Table 1: Overview of the Clinical Studies

Study ID	Number of Study Centres Location	Study dates Total enrolment /Enrolment goal	Design Control type	Study & Control Drugs	Study Objective	Subjs by arm entered/ completed	Inclusion criteria	Primary endpoint
002 Pilot	7 Belgium Czech UK	June 01- Nov 02 21/20	Double-blind, placebo-controlled, randomised, prospective	Mycograb iv 1 mg/kg bd *versus* iv saline bd 5 days course	Safety, efficacy and kinetics; calculation of sample size & generation of hypothesis to be tested	Mycograb: 11/8 Placebo: 9/8	Culture-confirmed invasive candidiasis, being treated with liposomal Ampho B; active clinical sepsis at time of study entry	Overall response at Day 10
002 Confirmatory	27 Europe* & USA	Dec 02- April 04 139/139	Double-blind, placebo-controlled, randomised, prospective	Mycograb iv 1 mg/kg bd *versus* iv saline bd 5 days course	Efficacy and safety of Mycograb + amphotericin B vs amphotericin B alone. Population kinetics	Mycograb: 68/60 Placebo: 69/63	Culture-confirmed invasive candidiasis, being treated with liposomal Ampho B; active clinical sepsis at time of study entry	Overall response at Day 10

* Belgium, Czech Republic, Denmark, Finland, Iceland, The Netherlands, Slovakia, Spain, Switzerland, UK

- Dose response studies

No conventional dose-response studies were performed. The dose of 1 mg/kg which was selected based on extrapolation from animal studies and AUC and C_{max} exposure findings in the phase 1 studies was considered acceptable.

The proposed duration of therapy of 5 days was supported by the clinical trial results. Prolongation beyond 10 doses was not allowed under the protocol. In order to further characterise the duration of therapy a surrogate marker of infection (level of pro-inflammatory interleukin-6) was measured in a post hoc analysis of sera from patients in the 002 Confirmatory Study. Although the IL-6 data were small and insufficient to provide any clear guide as to whether prolongation or dosage increase could be beneficial, there were to some extent supportive of the 5 days duration chosen. There are currently insufficient data to determine whether prolongation of therapy beyond 5 days could be beneficial.

- Main studies

Study 002 Pilot

The objectives of the study were:

- to obtain preliminary data on the efficacy and safety of a 5 day course of Mycograb (1 mg/kg iv) plus liposomal amphotericin B compared to placebo plus liposomal amphotericin B, in adult, hospitalised patients with deep-seated candidosis;
- to obtain further information on the pharmacokinetics, based on multiple time-points sampling;
- to use these data to optimise the design of the final, confirmatory phase of the trial, particularly with respect to sample size, treatment regimen, selection of endpoints and generation of the hypothesis to be tested.

This study included adult, hospitalised patients with culture-confirmed invasive candidiasis, randomised in seven centres from 3 countries (Belgium, Czech Republic and the UK). Multiple time-points sampling was used with blood samples being taken at 0.5, 1, 4 and 12h post administration on each of Days 1-5, as well as urine samples being collected. Clinical evaluation/disease assessment visits were performed twice a day on days 1-5. Inclusion and exclusion criteria were similar to the following Confirmatory study 002 (see below).

Central randomisation to treatment was stratified according to the germ tube test of the Candida isolated, since *Candida albicans* (germ tube positive) has been associated with a worse prognosis than non-albicans (germ tube negative species).

The test group received a 5 days course of Mycograb (1 mg/kg body weight, every 12h) given as a slow intravenous bolus (over 5-10 minutes). The control group received a 5 days course of 0.9% sodium chloride (for intravenous use) every 12h, given as a slow intravenous bolus (over 5-10 minutes). The volume equated to that which would have been given if the patient had received Mycograb. The study treatment (Mycograb or saline) was started within 12h of the 3rd dose of amphotericin B, and given after completion of the amphotericin B infusion.
All patients (both groups) received a 10 days course of a lipid formulation of amphotericin B –as an intravenous infusion. In this study investigators were allowed to choose between two lipid preparations of either amphotericin B lipid complex (5 mg/kg body weight daily) or amphotericin B liposome for injection (3 mg/kg body weight daily).

Efficacy measure was similar as the ones described in study 002 Confirmatory i.e overall response (clinical and mycological, with the clinical response being regarded as favourable if there were clinical improvement or cure) to treatment (see under study 002 Confirmatory for further details). In the pilot study, a reduction in amphotericin B requirements was also considered as a marker of efficacy, having excluded patients who were withdrawn prematurely due to toxicity. This measure of efficacy was discontinued in the subsequent confirmatory study.

There was no formal statistical analysis plan, patient numbers being too small for statistical analysis. The Mycograb-treated group was compared to the placebo-treated group with respect to efficacy and tolerance. Comparison of the two groups included the investigator`s assessment of the patient`s clinical response to treatment and culture-confirmed resolution of the infection, combined to give a composite overall response to treatment by Day 10.

RESULTS

Patient disposition

Twenty-one patients were randomised, one patient withdrew before receiving the study medication. Eleven patients received Mycograb and nine patients placebo.

Out of the 11 patients who received Mycograb, 8 completed the study. The reasons for withdrawal were: death (1 due to a cardiac arrest in a patient with a recent myocardial infarction and two previous episodes of cardiac asystole before study entry, and 1 due to patient's deteriorating irremediable multiorgan failure due to widespread atheromatosis, secondary to hypercholestrolaemia and hypertension) and withdrawal following complete clinical response and cultures repeatedly negative. The two deaths in the Mycograb group occurred soon after study entry.

In the placebo group, there was one premature termination due to the patient`s failure to respond, and his death 24h later due to multiple organ failure secondary to an aortic graft infected with *Candida*.

Five patients in the Mycograb group and six patients in the placebo group were infected with *Candida albicans*. One patient in each group had a mixed infection. Thus, three patients in the analysis group had other *Candida* strains.

None of the patients had immunosuppression or neutropenia.

Results Pilot Study 002

Table 2: Data used for the calculation of sample size

	Placebo N = 9 (%)	**Mycograb N = 8 (%)**
Overall Response (Day 10)	5 (55.5)	7 (87.5)
Candida-attributable mortality (Day 33)	3 (33.3)	0
Overall mortality (Day 33)	4 (44.4)	2 (25.0)

As displayed in table 3, data were re-analysed using the more stringent definition of overall response as subsequently used in the confirmatory study, in which patients had to have shown a *complete* clinical response to be regarded as favourable i.e absence of pre-clinical symptoms and signs of invasive Candidiasis (one patient excluded from this analysis because the last positive culture was 6 days before study entry).

Table 3: Re-analysis using Definitions applied to Confirmatory Study

	Placebo	Mycograb
Pilot study	N = 8 (%)	N = 8 (%)
Complete Overall Response (Day 10)	3 (37.5%)	5 (62.5%)
Complete Clinical Response (Day 10)	3 (37.5%)	5 (62.5%)
Mycological Response (Day 10)	4 (50.0%)	7 (87.5%)
Candida-attributable mortality (Day 33)	3 (37.5%)	0

A summary of clinical response, categorised into complete, improvement and failure, over time during the study period (table 4) shows that at each time-point, the percentage of patients showing a complete clinical response was higher in the Mycograb-treated group than the placebo group. On Day 5, in the Mycograb-treated group, 3 patients had made a complete response (compared to 0 in the placebo group) and 1 failed (compared to 4 in the placebo group).

Table 4: Summary of Clinical Response Evaluable ITT Population

	Placebo N=8			Mycograb N=8		
Day	Complete	Improvement	Failure	Complete	Improvement	Failure
Day 4 (0 hrs)	0	4 (50%)	4 (50%)	2 (25%)	4 (50%)	2 (25%)
Day 4 (2 hrs)	0	4 (50%)	4 (50%)	1 (12,5%)	6 (75%)	1 (12.5%)
Day 5 (12 hrs)	0	4 (50%)	4 (50%)	3 (37,5%)	4 (50%)	1 (12.5%)
Day 6	1 (12,5%)	4 (50%)	3 (37.5%)	3 (37.5%)	4 (50%)	1 (12.5%)
Day 8	2 (25%)	3 (37,5%)	3 (37.5%)	3 (37.5%)	4 (50%)	1 (12.5%)
Day 10	3 (37.5%)	2 (25%)	3 (37.5%)	5 (62.5%)	2 (25%)	1 (12.5%)

Having excluded patients who died while on amphotericin B, or were withdrawn prematurely due to toxicity, the average duration of amphotericin B therapy in the placebo group was 18 days (range 6-40) with a median of 13 days, while the average duration in the Mycograb group was 13 days (range 2-29) with a median of 9 days.

Study 002 Confirmatory

METHODS

Study Participants

The study included hospitalised male and female patients of 18 years or older with presumed disseminated candidiasis based on: Clinical evidence of active infection (e.g. hyperthermia [>38°C], hypothermia [<36°C], tachycardia [>110/min], hypotension [mean blood pressure <70 mmHg], high white cell count [>11000/mm^3], left shift, need for vasopressor agents or other abnormalities consistent with an ongoing infectious disease process).
-*Plus* documented growth of a Candida species, within 3 days prior to initiation of study treatment from:

- a blood culture
- *and/or* a culture of a specimen from a normally sterile body cavity or tissue
- *and/or* a urine culture, in the absence of an indwelling urinary
- catheter
- *and/or* multiple positive cultures from non sterile sites in a immunocompromised pyrexial patient who is not responding to broad-spectrum antibiotic therapy and is evidently not infected with any other recognisable pathogen.

Patients being treated with a systemic antifungal medicinal product other than amphotericin B lipid complex or amphotericin B liposome for injection were excluded, and the study treatment had to be started within 12h of the third dose of amphotericin B. Patients with candidal endocarditis were also excluded.

Treatments

All patients in both treatment groups received at least a 10 days course of intravenous lipid formulation of amphotericin B (amphotericin B lipid complex, 5 mg/kg body weight daily or amphotericin B liposome for injection, 3 mg/kg body weight daily) combined with Mycograb or placebo on the first 5 days. After the 10 days period, the investigators were able to either stop the antifungal medication, to continue with amphotericin B formulation or to switch to alternative antifungal therapy, when clinically indicated.

Objectives

The primary objective was to compare the efficacy and safety of Mycograb (1 mg/kg iv bid) plus lipid formulation of amphotericin B versus placebo plus lipid formulation of amphotericin B in treatment of deep-seated *Candida* infections. In addition, the study was designed to obtain information on the pharmacokinetics and immunogenicity of Mycograb.
There was a Safety Monitoring Committee for rapid assessment of any serious or unexpected adverse event which may be treatment related.

Outcomes/endpoints

The primary efficacy variable was the overall response (clinical and mycological) to treatment, comparing the test with the control arm at Day 10. This was a composite endpoint comprising both the investigator's evaluation of the clinical response to treatment and culture-confirmed clearance of the infection on Day 10.

A favourable overall response was defined as:
• resolution of pre-treatment signs and symptoms of candidosis
• and culture-confirmed eradication of Candida from clinically significant sites (or presumptive eradication, based on clinical recovery, if repeat sampling inappropriate).

Only patients with a complete clinical response were classified as a success – clinical improvement was considered as a failure.

Failure was defined as:
• lack of change or worsening in pre-treatment signs and symptoms of candidosis
• and/or culture-confirmed persistence of Candida from clinically significant sites.

Secondary efficacy included:
• Clinical response at Day 10
• Mycological response at Day 10 and time taken for cultures to clear
• Candida-attributable mortality and overall mortality (there had to be ongoing signs of sepsis at the time of death to support the investigator's decision that it was a Candida-attributable mortality).

Sample size

The sample size was initially calculated from the unadjusted data (i.e patients required showing both a mycological and clinical response, but the clinical response being either partial or complete) of the pilot study. It was recalculated using adjusted data after applying a more stringent definition of clinical response, in which patients showing partial responses (improvement) were considered as failures.

Randomisation

Central randomisation to treatment (1:1) was stratified according to the germ tube test of the Candida isolated, since *Candida albicans* (germ tube positive) has been associated with a worse prognosis than non-albicans (germ tube negative species).

Blinding (masking)

The investigator and all personnel associated with the study and the patient were blinded to the study medication assignment (Mycograb or placebo) throughout the study.

Statistical methods

The study had a superiority design. The main analysis of efficacy was based on the modified intent-to-treat (MITT) population that included all randomised subjects who had a clinical or mycological assessment 24 hours after starting study treatment with the exception of patients:

- who do not have culture confirmation of disseminated candidiasis within 5 days of starting study treatment
- whose only positive culture for candida is grown from respiratory secretions or throat sites (unless they have a tracheo-oesophageal fistula)
- who do not have clinical or laboratory evidence of on-going sepsis (unless they are immunosuppressed)
- who are found to have candidal endocarditis, characterised by cardiac vegetations and peripheral emboli.

Logistic regression was used to analyse primary and secondary endpoints. Time to clearance of candidemia was assessed by Kaplan-Meier estimation. All tests performed were two-sided and conducted using a significance level of 5%.

A hierarchical test procedure was applied with respect to the secondary endpoints. The effect of this was that no confirmatory claims could be based on variables that had a rank lower than the variable whose null hypothesis was the first that could be rejected.

RESULTS

The disposition of patients is shown in the table 5.

Table 5: disposition of patients

Randomised	139	
Completed	137	
	Mycograb (n= 68)	Placebo (n = 69)
completed	60	63
Withdrawn	8	6
Adverse events	3	2
Death	3	4
other	2	-

2 did not receive treatment due to intolerance to amphotericin B lipid complex

The modified ITT population (MITT) used for efficacy included 56 patients in the Mycograb versus 61 in the placebo one.

Recruitment

Patients were recruited from 24 centres in 10 European countries and 2 centres in the USA. The maximum number of patients in the modified ITT from a single centre was 20.

Conduct of the study

The Good Clinical Practices inspection was conducted at Brno (site 21) and Prague (site 22) in the Czech Republic, from which a substantial number of patients where recruited [total number of patients recruited 51 of the 139 patients randomised (37%)]. Several critical and major findings were detected regarding the ethical, safety and clinical aspects of the trial, including the reporting of the primary efficacy variable, and overall the study was considered not to be reported according to GCP. The finding of the inspection is further discussed under the benefit/risk assessment section.

Baseline data

Main baseline characteristics of patients analysed in the MITT and the demographics are shown in table 6. Overall the majority of patients were Caucasian and elderly, with a fourth being more than 70 years and most baseline characteristics were comparable between the two groups. Compared to previously published studies of disseminated candidiasis, the frequency of polyfungal infections, which have been associated with high mortality, was relatively high. The use of amphotericin B lipid complex (86.5% of the Placebo group and 87.5% of the Mycograb group) and amphotericin B liposome for injection (13.1% of Placebo and 12.5% of Mycograb groups) was matched between the two treatment arms.

Table 6: Summary of Baseline Characteristics of the MITT

CHARACTERISTIC	L-Amphotericin B plus Placebo (N=61)	L-Amphotericin B plus Mycograb (N=56)
Sex – Number (%)		
Male	35 (57)	42 (75)
Female	26 (43)	14 (25)
Age – Median	64	58
Range	19-88	21-76
Caucasian	55 (98.2%)	59 (96.7%)
Underlying conditions and risk factors – Number (%)		
APACHE II score >18	26 (43)	27 (48)
On an intensive care unit	51 (84)	51 (91)
Alcoholic	1 (2)	4 (7)
Smoker *	10 (16)	23 (41)
Chronic Obstructive Pulmonary Disease	7 (11)	10 (18)
Intubated (requiring mechanical ventilation)	31 (51)	34 (61)
Liver failure	15 (25)	14 (25)
Renal failure	24 (39)	23 (41)
Diabetes mellitus	8 (13)	10 (18)
Vasopressor support required	30 (49)	33 (59)
Neutropenia, AIDS[1]	6 (10)	1 (2)
Corticosteroids (prior)	8 (13)[2]	7 (13)
Other immunosuppressant/chemotherapy	3 (5)[2]	1 (2)
Trauma	10 (16)	14 (25)
Surgical	16 (26)	15 (27)
Leukaemia	6 (10)[3]	0
Lymphoma	4 (7)[3]	1 (2)
Cancer (solid tumors)	10 (16)	11 (20)[4]
Antimicrobials (prior)	53 (87)	48 (86)
Total parental nutrition	10 (16)	12 (21)
Urinary catheter	51 (83)	50 (89)
Haemodialysis/Peritoneal Dialysis	9 (15)	9 (16)
Species other than *C. parapsilosis*	58 (95)	55 (98)
Mean APACHE II score (SD;range)	17.5 (6.7;4-32)	18 (7.9;3-39)
Mean Charlson Weighted Index (± SD)	2.92 ± 1.96	3.42 ± 1.88
Prior antifungal therapy for ≥ 4 days		
Number (%)	5 (8)	7 (13)
Average duration in days (range)	9 (4-21)	9 (4-17)
Species of Candida – Number (%)		
C. albicans	39 (65)	35 (63)
C. glabrata	4 (7)	6 (11)
C. tropicalis	3 (5)	2 (4)
C. parapsilosis	3 (5)	1 (2)
C. krusei	2 (3)	0
Unidentified non-*albicans*	0	2 (4)
Multiple species	9 (15)	10 (18)

1. Baseline neutrophil count < $0.5x10^9$/l or CD4 count <$0.2x10^9$/l.; 2. Included one organ transplant recipient,
3. Included the 3 bone marrow transplant recipients; 6 were neutropenic at baseline; 4. Included 4 fatal metastatic cancers
APACHE II: (Acute Physiology and Chronic Health Evaluation 2nd version); *statistically significant

Overall, APACHE scores, which is a validated scoring system devised for patients on intensive care units (ICU) to provide a measure of the severity of their condition and the risk of subsequent death (i.e the higher the APACHE II score, the higher the risk of death) were well matched, with a trend for higher APACHE Scores among Mycograb-treated patients (21-25 and > 25) compared to placebo.
The applicant further detailed description of malignancies and immunosuppression. More patients in the placebo group had malignancies including haematological cancers. In contrast, the rate of solid cancers were comparable, 11 patients in the Mycograb group *versus* 10 patients in the placebo group

had solid tumors, of which 5 were metastatic in the Mycograb group (45%), compared to 2 in the placebo group (20%).

With respect to immunosuppression, 14 (23 %) in the placebo group were severely immunosuppressed compared to 9 (16 %) in Mycograb. No neutropenic patients were included in the Mycograb arm.

Outcomes and estimation

Primary Efficacy

The results on the primary efficacy variable are presented in table 8. In logistic regression analysis, the odds ratio for overall response was clearly in favour of combination therapy with Mycograb.

Table 7: Overall Response at Day 10 - MITT

	Placebo (n = 61)	Mycograb (n = 56)
Complete Overall Response	29 (48%)	47 (84%)
95% CI for % of patients with Complete Overall Response	(35, 60)%	(74, 94)%
Logistic Regression analysis (Mycograb versus Placebo) Odds ratio (se) 95% CI P-value	5.762 (1.561) (2.408, 13.787) < 0.001	

At the request of the CHMP, the main efficacy analysis was redone using the intent-to-treat (ITT) population. In this analysis a conservative approach was taken with missing data classified as failures. There was a statistically significant difference in favour of the Mycograb group with an overall response shown in 79 % (54/68) versus in 51 % (35/69) in the placebo group ($p < 0.001$).

A concern was raised with respect to the potentially low response in the placebo arm. The applicant provided data to show that the MITT complete response rate observed in the placebo arm was within the range of recent response rates reported in other published amphotericin B trials.

Secondary endpoints

Clinical and mycological response at Day 10.

The results for these two secondary endpoints showed a highly statistically significant difference between the two treatment arms ($P < 0.001$).

Table 8: Clinical response and mycological response at Day 10

	Placebo (n = 61)	Mycograb (n = 56)
Complete Clinical Response	32 (52%)	48 (86%)
95% CI for % of patients with Complete Clinical Response	(40, 65)%	(77, 95)%
Logistic Regression analysis (Mycograb versus Placebo) Odds ratio (95% CI) P-value	5.4 (2.2- 13.4) < 0.001	
Culture-confirmed resolution of the infection	33 (54%)	50 (89%)
95% CI for % of patients with Culture-confirmed resolution	(42, 67)%	(81, 97)%
Logistic Regression analysis (Mycograb versus Placebo) Odds ratio (95% CI) P-value	7.1 (2.6-18.9) < 0.001	

Mycological clearance

The rate of mycological clearance over time was determined by recording the time from baseline positive cultures through to the time when the last positive culture was observed. The time to this point was analysed using survival analysis censoring when a last positive culture was not observed because the patient was withdrawn from the study or died. The rate of culture-confirmed clearance was over twice as fast in the Mycograb-treated group compared to the control group (Hazard ratio > 2, P 0.001) (table 10).

Table 9: Statistical Analysis of Time Taken (Days) to Last Positive Culture

	Placebo (n = 61)	Mycograb (n = 56)
Median	23.0	3.0
Number censored (%)	34 (56%)	15 (27%)
Cox proportional hazards regression (Mycograb vs Placebo) Hazard ratio (95% CI) P-value	2.3 (1.4-3.8) 0.001	

Candida-attributable mortality at Day 33

Mycograb treatment was associated with significantly lower Candida-attributable mortality at Day 33 (table 11).

Table 10: Candida-attributable Mortality at Day 33 (MITT population)

	Placebo (n = 61)	Mycograb (n = 56)
Candida-attributable Mortality	11 (18%)	2 (4%)
95% CI for % of patients with Complete Clinical Response	(8, 28)%	(-1, 8)%
Logistic Regression analysis (Mycograb versus Placebo) Odds ratio (95% CI) P-value	0.17 (0.04-0.8) 0.025	

A Kaplan-Meier plot showed that Candida-attributable mortalities were most likely to have occurred by Day 12.

Overall Mortality

The results did not show a statistically significant difference in terms of overall mortality in favour of Mycograb. There was a trend in favour of combination therapy one week after completing the Mycograb (day 12) but this difference had disappeared a month later, as shown in table 11.

Table 11: Statistical analysis of overall mortality at day 33 (MITT population)

	Placebo (n = 61)	Mycograb (n = 56)
Overall day 33 Mortality	20 (33%)	26 (46%)
95% CI for % of patients with Complete Clinical Response	(8, 28)%	(-1, 8)%
Logistic Regression analysis (Mycograb versus Placebo) Odds ratio (95% CI) P-value	1.8 (0.8-3.8) 0.133	

Ancillary analyses

A sensitivity analysis was performed on the primary efficacy variable in which all patients who continued to receive systemic antifungal therapy (of any kind) after Day 10 of the study were classified as failures. This showed a statistically significant difference between the two groups, 52% of the Mycograb group discontinuing systemic antifungals at day 10 have shown a complete clinical and mycological response, compared to 26% of the mono-amphotericin B group (P 0.005).
Two supportive analyses were performed also on the primary efficacy variable.

The first one related to the brand of amphotericin B (amphotericin B lipid complex, or amphotericin B liposome for injection) with which the patient was treated. The use of both medicinal products was well matched: 86.9% (53) of the placebo group and 87.5% (49) of the Mycograb group were on amphotericin B lipid complex, while 13.1% (8) of the placebo group and 12.5% (7) of the Mycograb group received amphotericin B liposome for injection.

The second one related to the result of the germ tube test, a preliminary test for the identification of the species *C. albicans*. If there were discordant results between the germ tube test and subsequent species identification, the latter took priority since this was the definitive test.

Both supportive analyses showed a statistically significant difference between the two treatment arms ($P < 0.001$) in favour of Mycograb group.

Several analyses examining APACHE Scores were done to support the efficacy of Mycograb. Mycograb-treated patients were more likely to have shown complete resolution by Day 10 irrespective of APACHE II score. In patients with APACHE II scores up to 10, the response rate was 100% on Mycograb, and 50-57% on placebo.
The analysis of death showed that in the Mycograb-treated group deaths most frequently occurred in patients with high APACHE II scores (≥25), and the commonest cause of death was bacterial sepsis followed by cancer. In contrast, *Candida* was the commonest cause of death in the group on placebo and, with one exception, these deaths occurred in patients with APACHE II scores below 25. This would suggest that, in terms of overall mortality, Mycograb would likely have greatest benefit in patients with APACHE II scores below 25.

The applicant analysed the primary and secondary endpoints to a subpopulation with a positive candidaemia and/or positive sample from a normally sterile deep site - excluding the 20 patients entered on the basis of positive cultures from urine, abdominal drains or infected burns. This gave similar, statistically significant differences between the two treatments groups as previously observed with the original MITT. For example:

- The overall response at Day 10 doubled from 40% to 80% ($P<0.001$) in patients with candidaemia and/or sterile site culture positive being treated with Mycograb.
- Candida-attributable mortality fell from 21% to 5% in patients with candidaemia and/or sterile site culture positive receiving Mycograb ($P<0.05$).
- The rate of culture-confirmed clearance of the infection remained over twice as fast in the group receiving Mycograb (Hazard Ratio > 2).

Again, all-cause mortality showed only a trend in favour of Mycograb. A subgroup analysis confined to patients with candidaemia showed statistically significant differences in favour of combination therapy with respect to all primary and secondary endpoints.

In addition the applicant performed subgroup analysis examining response according to centre of enrolment (centres 21 and 22 enrolling most patients), immunosuppression, site of infection and prior antifungal treatment (table 12).

Table 12: Comparison of the Efficacy Variables between sub-groups (MITT population)

	Placebo	Mycograb	P-value
Confirmatory study	N = 61 (%)	N = 56 (%)	
Complete Overall Response (Day 10)	29 (48%)	47 (84%)	< 0.001
Complete Clinical Response (Day 10)	32 (52%)	48 (86%)	< 0.001
Mycological Response (Day 10)	33 (54%)	50 (89%)	< 0.001
Median number of days to last positive culture	23	3	0.001
Candida-attributable mortality (Day 33)	11 (18%)	2 (4%)	0.025
Overall Response according to Centre Centre no. 21:Complete Overall Response (Day 10)	n = 6 3 (50%)	n = 14 14 (100%)	
Centre no. 22:Complete Overall Response (Day 10)	n = 11 8 (73%)	n = 9 9 (100%)	
All centres except centres 21 and 22: Complete Overall Response (Day 10)	n = 44 18 (41%)	n = 33 24 (73%)	0.0064
Candida-attributable mortality in immunosuppressed patients (Placebo n=14; Mycograb n= 11)	7 (50%)	0	0.0078
Overall Response according to Site of Infection: Blood (Placebo n = 33; Mycograb n = 35) Abdomen/Thorax (Placebo n =23; Mycograb n = 17) Renal (Placebo n = 25; Mycograb n = 25) Two or more of the above (Placebo n=19; Mycograb n = 21)	 15 (45%) 7 (30%) 14 (56%) 7 (37%)	 31 (89%) 11 (65%) 20 (80%) 15 (71%)	< 0.001
Overall Response in patients who received prior antifungal treatment (Placebo n=7; Mycograb n= 8)	2 (29%)	4 (50%)	

As shown in table 12 a qualitative assessment of these two centres showed a similar trend to that seen in the whole study. Applying the primary efficacy variable to the combined results obtained from the two Czech centres gave a statistically significant difference in overall response at Day 10 between the two treatment groups (Fisher's Exact Test, P = 0.0032). If excluding patients from these two centres from the primary efficacy analysis, a significant difference in the complete overall response rate remained in favour of Mycograb.

With respect to site of infections, patients with abdominal candidiasis were the least likely to have the infection resolved by Day 10.

With respect to prior antifungal treatment, more Mycograb-treated patients had shown a complete clinical and mycological response compared to those on placebo, although the success rates were considerably lower than those obtained from the study overall, but the numbers are limited.

A detailed review of 9 patients who were treated with Mycograb but failed to meet the primary efficacy endpoint showed that 5 patients failed on both clinical and mycological responses.

To examine the relation between response and efungumab doses/concentrations, a subgroup analysis was performed on patients with clinical failure. While these data demonstrate quite large inter patient variations in Mycograb levels, the mean values were not significantly different in clinical failures compared to overall levels in the total population, except at Day 1 when the mean value tended to be lower.

Sera from the confirmatory study were analysed with respect to gender and age. No significant difference between male and female patients was found, although the mean levels were slightly higher in the female patients. This was most pronounced when the Day 3 levels were examined but this trend had disappeared by Day 5. The group of patients who were over 70 years of age was then examined. This demonstrated slightly higher levels on Day 1. This difference had disappeared by Day 3 so that there was no clear difference from the overall levels.
Further, subgroup analysis for respiratory, renal, liver and clinical failure and the presence of antibodies against Mycograb did not demonstrate any group of patients in whom the mean value was more than one standard deviation from base line.

- Clinical studies in special population

The efficacy of Mycograb has not currently been evaluated in paediatric population or in other special population.

Clinical safety

Patient exposure

At the initial submission, the safety database for Mycograb comprised all patients who had received at least one dose of treatment in the open-label and randomised trials.
Safety data were subsequently provided from two additional studies, one in healthy volunteers (n=18 NTP/Mycograb/HuPK/001) and one open label in patients with breast cancer receiving Mycograb+docetaxel (n=20NTP/ONC/001) and from a compassionate use programme in patients with invasive candidiasis (n=17).

Table 13: Demography of Exposure to Mycograb

Indication:	**Invasive Candidiasis**			**Healthy Volunteers**	**Cancer Patients**	**Total**
	Open-label study	Double-blind Studies[1]	Compassionate Use	Randomised open-label	Open-label study	
Dose of Mycograb						
0.1 mg/kg 1 dose	3[2]					3
0.25mg/kg b.d for 5 days				5[3]		5
0.5mg/kg b.d for 5 days				5[3]		5
1 mg/kg 1 dose	3[2]			8	20	31
1mg/kg b.d. for 1 day	3[2]					3
1mg/kg b.d. for 5 days		79[4]	17	2[3]		85
2mg/kg 1-2 doses					20	20
Male	3	51	11	10		66
Female	2	28	6	8	20	60
Age						
Age 0 to 20 years	1		4		0	2
Age 21 to 40 years		11	3	7	0	18
Age 41 to 60 years	4	28	7	11	8	53
Age 61 plus years		40	3		12	53
Concomitant Medication						
Amphotericin B	5	79	10			86
Fluconazole		2[5]	3			2
Caspofungin			5			2
Posaconazole			1			
Docetaxel					20	20
Total	5	79	17	18	20	139

[1]Pilot and Confirmatory, Randomised, Double-blind, Placebo-controlled Trials
[2] In the open-label dose escalating study, a total of 5 patients received 0.1 mg/kg (single dose), 1 mg/kg (single dose) and/or 1 mg/kg bd (for 1 day); 4 received the 1 mg/kg dose o.d. and/or b.d..
[3]Repeat doses of 0.25 mg/kg, 0.5 mg/kg or 1 mg/kg were given as 9 doses each 12 hours apart (10^{th} dose not given so that both first and last doses were given at the same time of day); 2 subjects had the single 1 mg/kg dose and then went on to have the 1 mg/kg repeat dose.
[4]Of whom 71 received at least 9 of the 10 doses.
[5]Two switched to fluconazole because unable to amphotericin B

In the clinical trial, all adverse events were reported by the investigators up to day 33, after which only serious ones were recorded.

Adverse events

Confirmatory trial

In the confirmatory trial 34 (61%) of the Mycograb treated patients were intubated and 38% of patients were given pre-medication to cover amphotericin B. Treatment-emergent adverse events (TEA) was reported by 97 % of Mycograb treated patients compared to 88% of placebo treated patients. However, only 7% of TEA was judged to be possibly directly related to the study drug. Due to the severity of pathologies in the ICU population, the casual relationship between study drug and AE were complicated by co-administration of multiple other drugs including amphotericin B in all patients and vasopressors in half of patients.

Frequencies of AE, which occurred in 2 or more patients receiving Mycograb, are displayed in table 14. The following adverse events were commoner in the Mycograb-treated group: septic shock (17.6% versus 10.1%), urinary tract infections (5.9% versus 1.4%), tachycardia (5.9% versus 0), multi-organ failure (14.7% versus 5.8%), thrombocytopenia (7.4%versus 0), hypertension (7.4% versus 2.9%), decubitus ulcer (4.4% versus 0) and back pain (4.4% versus 0).
AE more common in the placebo group were: Nausea (7.2% versus 0), hypokalaemia (13% versus 5.9%) and drug hypersensitivity (5.8% versus 0), of which 2 cases were caused by hypersensitivity to amphotericin B.

The higher number of septic shock were not considered temporally related to the administration of Mycograb, as the shock occurred days or week after the administration in 11 of 12 patients.

Cardiac disorders were observed in 21% of the Mycograb treated, of which 7% had cardiac arrest. These AE were considered unrelated to Mycograb.

Table 14: All adverse events occurring in 2 or more Mycograb-treated patients in 002 Confirmatory

System Organ Class	Preferred term	Mycograb % (68 patients)	Placebo % (69 patients)
Any adverse event		97.1 (66)	88.4 (61)
Infections and infestations	Septic shock	17.6 (12)	10.1 (7)
	Sepsis	7.4 (5)	5.8 (4)
	Pneumonia	2.9 (2)	4.3 (3)
	Urinary tract infection	5.9 (4)	1.4 (1)
Gastrointestinal disorders	Diarrhoea	5.9 (4)	4.3 (3)
	Vomiting	4.4 (3)	4.3 (3)
	Nausea	0	7.2 (5)
	GI haemorrhage	2.9 (2)	2.9 (2)
	Intestinal fistula	2.9 (2)	0
Investigations	GGT increased	7.4 (5)	5.8 (4)
	Alkaline Phosphatase	4.4 (3)	4.3 (3)
Metabolism/Nutrition	Hypokalaemia	5.9 (4)	13.0 (9)
	Hyperglycaemia	7.4 (5)	4.3 (3)
	Hyperkalaemia	4.4 (3)	2.9 (2)
	Hypomagnesaemia	2.9 (2)	1.4 (1)
Cardiac disorders	Cardiac arrest	7.4 (5)	4.3 (3)
	Atrial fibrillation	4.4 (3)	2.9 (2)
	Tachycardia	5.9 (4)	0
	Cardiac failure	2.9 (2)	1.4 (1)
General disorders and administration site	Multi-organ failure	14.7 (10)	5.8 (4)
	Pyrexia	4.4 (3)	4.3 (3)
	Rigors	2.9 (2)	4.3 (3)
Blood and lymphatic system	Anaemia aggravated	5.9 (4)	5.8 (4)
	Anaemia	4.4 (3)	4.3 (3)
	Thrombocytopenia	7.4 (5)	0
Vascular disorders	Hypertension	7.4 (5)	2.9 (2)

	Hypotension	4.4 (3)	4.3 (3)
Surgical and medical procedures	Laparotomy	2.9 (2)	1.4 (1)
	Abdominal operation	2.9 (2)	0
Respiratory, thoracic and mediastinal	Acute respiratory failure	2.9 (2)	0
	Respiratory failure	2.9 (2)	0
Renal and urinary tract disorders	Acute renal failure	2.9 (2)	4.3 (3)
	Haematuria	2.9 (2)	1.4 (1)
Skin and subcutaneous tissue	Decubitus ulcer	4.4 (3)	0
Injury, poisoning, procedural complications	Postoperative wound complications	2.9 (2)	1.4 (1)
Psychiatric disorders	Agitation	2.9 (2)	0
	Confusional state	2.9 (2)	0
Hepatobiliary disorders	Any event	2.9 (2)	5.8 (4)
Nervous system disorders	Any event	4.4 (3)	2.9 (2)
Musculoskeletal	Back pain	4.4 (3)	0
Immune system disorders	Drug hypersensitivity	0	5.8 (4)
Endocrine disorders	Any event	<2	<2
Neoplasm	Metastases	2.9 (2)	0
Eye disorders	Any event	<2	<2

Serious adverse events and deaths

Among Mycograb-treated, 52% (36) of patients reported SAE, the most commonly observed were sepsis and/or multiorgan failure (21 patients). A similar number of SAE was observed in the placebo-group. There were no SAEs which were thought to be causally related to the study drug by the investigators, the sponsor or the independent expert.

Deaths occurred among 26 Mycograb treated patients up to day 33 compared to 21 in the placebo group. A detailed narrative has been submitted by the applicant, from which it appears that it is improbable that any of the deaths were Mycograb related.

Other Significant Adverse Events and discontinuation

Episodes of hypertension were more common among Mycograb-treated patients and were in most cases directly related to the infusion. In 4 of the 5 affected patients, the hypertensive episode began 0.5 to 1.5h after the first dose of Mycograb and lasted from 20 minutes to 15h. With one exception the hypertension was mild or moderate, and resolved without sequelae and without the need for concomitant treatment. In 4 cases the patients were on vasopressors, because their blood pressure had been too low.

Lumbar back pain was the adverse event most closely associated, in time, with repeated doses of Mycograb. Two patients developed back pain, which was temporally related to the Mycograb infusion. In each case it occurred transiently, recurring in association with several doses of Mycograb, but it did not occur with the last two doses. The severity was categorized as mild in one patient and moderate in the other. In each case it resolved without action being taken. In neither case did the patient or the investigator wish to discontinue study treatment.

A low titre of human anti-drug antibody (HADA) occurred in 11% of patients in the pivotal study, and this was not associated with any loss of efficacy or adverse reactions. The treatment was discontinued in 2 patients in the Mycograb group, due to adverse events compatible with infusion reaction.

With respect to laboratory findings, the data are too small to exclude any role of Mycrograb in the numerically increased incidence of thrombocytopenia (7.4 % versus 0 in placebo).

Safety data in healthy volunteers

In the healthy volunteer study, the tolerability was poor at all dose levels. Infusion-related adverse reactions were much commoner in healthy volunteers than patients with invasive Candidiasis. The commonest adverse reaction after the first dose was nausea and/or vomiting (experienced by 28 out of 30 subjects following the first dose), followed by headaches (26 subjects), rigors (23), pain (19), pyrexia (6), paraesthesia (3) and flushing (1). Most events were mild or moderate and lasted usually a few hours. The adverse events did recur after repeated administration but were usually milder. One out of five individuals with a repeat dose at 0.25mg/kg and three out of five at the 1.0mg/kg dose level withdraw from the study.

All adverse reactions had become mild after the second dose with the exception of headaches (described as moderately severe in two subjects after the third and fourth dose respectively).
The phlebitis observed at the infusion site is probably due to presence of a denaturant, which is used as an excipient. It was not observed in the previous studies in patients with invasive Candidiasis, because the Mycograb was administered as an intravenous bolus injection over 10 minutes using a central venous catheter.

The cytokine data from the healthy volunteers suggest that most observed adverse effect were related to cytokine response. The peak value of the initial TNF-alfa and IL-6 response observed for the proposed clinical dosage of 1 mg/kg were high and comparable to cytokine levels reported in patients who experience severe side effects or tumor lysis from treatment with other antibody based products.

Human anti-drug antibody (HADA) levels in healthy volunteers were similar to those in patients with invasive candidiasis, but occurred more frequently (55% versus 11%). Most healthy volunteers had slightly elevated levels of anti-Mycograb already at day 5. However, there was no link between HADA response and adverse events and HADA was unlikely to be involved in the reactions seen in healthy volunteers during the initial days of treatment.

13 subjects experienced a rise in neutrophil count having peak counts > 8 x109/l.

Safety data from the Breast Cancer Study

In this study Mycograb was given using a dosage schedule significantly different from that used in the treatment of invasive candidiasis or in the healthy volunteer study:

- The dose given was doubled to 2 mg/kg for up to two doses in 8 hours
- The Mycograb was given in cycles, every 3 weeks, which would be likely to maximise any immunogenicity.

Most of the adverse events reported as Mycograb-related by the investigator were as expected, namely (in descending order of frequency): injection site reactions, pain, chills, pyrexia, headaches, nausea and vomiting, flushing, paraesthesia, and, in one patient, hypertension. The interpretation of the observed AE and SAE in this trial remains confounded by the frequent and to some extent overlapping toxicity from the concomitant docetaxel treatment. It is unclear whether all patients had steroids as pre-medication as recommended in the product information of docetaxel.
One patient developed severe hypertension and chills 50 minutes after receiving her first cycle of high dose Mycograb (2 mg/kg) and therefore was withdrawn from the study. This fully resolved within 3 hours, without sequelae. Hypertension occurs with a frequency of 2.4% in patients on docetaxel. Therefore it is likely that the hypertension was causally related to the high dose of Mycograb, but may have been exacerbated by the docetaxel. None of the 20 patients in the cancer study developed hypertension following the 1 mg/kg dose of Mycograb.
There were two further serious adverse events attributed by the investigators to Mycograb, both dyspnoea at rest (which by definition is severe). Both occurred with the second course of 2 mg/kg b.d. Mycograb, administered just after the fifth cycle of docetaxel. Both resolved, without sequelae.
Although most SAE probably were primarily docetaxel related, possible Mycograb related infusion reactions were commonly observed including one hypertensive episode.
Most patients study developed antibodies, mainly of IgG class to Mycograb. IgM class antibodies were also demonstrated along with IgG.

Compassionate Use Programme

Preliminary data on 17 patients with severe invasive fungal infections treated with Mycograb in the compassionate use program during the last year has been provided. Several severe infusion reactions with hypertension were observed.

Safety in special populations

There are currently no safety data in special populations

Safety related to drug-drug interactions and other interactions

So far no interaction has been identified. Adverse interactions with other drugs were not anticipated because Mycograb, being derived from a naturally occurring antibody, is not metabolised by cytochrome P450.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

The CHMP, having considered the data submitted in the application was of the opinion that it was not appropriate to consider the pharmacovigilance system at this time.

Risk Management Plan

The applicant submitted a risk management plan.

The CHMP, having considered the data submitted in the application was of the opinion that it was not appropriate to consider risk minimisation activities at this time.

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

Efungumab, the active substance of Mycograb is a monoclonal antibody fragment. The following quality deficiencies have not been satisfactorily resolved:

- The characterisation studies performed under native conditions clearly indicate that the efungumab protein forms aggregates in solution. The root cause of the aggregation is unknown. The aggregates of Mycograb drug substance are not adequately controlled by the proposed control method.

- Validation of the refolding step has not been performed. Instead, an in-process method is used to control the process at the drug substance level and in addition, CD is proposed for the drug product specifications. In view of the lack of validation data for in-process method, the unconvincing CD data provided and the inappropriateness of this method for controlling refolding, the refolding step of the protein is not adequately controlled.
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- One host cell protein (HCP) appears to co-purify together with Mycograb and can therefore not be removed from the product. This HCP is present at a high level in the final product compared to other parental preparations. Such a high level can have an impact on the safety (immunogenicity), which has to be considered with respect to use in patients (repetitive administration) and results from clinical trials. The proposed HCP assay may be suitable for the detection of this particular HCP and to confirm the level of this particular impurity but it is not suitable for the detection of the overall levels of HCPs.

As a consequence, the quality of the product is not controlled in a satisfactory way and the evidence provided was not sufficient to conclude that the manufacturing process and the methods of control will guarantee the uniform clinical performance of the product. The presence and lack of control of aggregates is of particular concern and needs to be considered from a non-clinical/clinical perspective. The presence of high levels of host cell proteins in the final product can impact on the safety (immunogenicity) of the product. Overall there are remaining issues related to the quality aspects of the product and their potential impact on safety.

Non-clinical pharmacology and toxicology

Efungumab is a humanised monoclonal antibody targeting the immunodominant epitope of *Candida* hsp90. Overall the primary pharmacodynamics of efungumab has been reasonably demonstrated. When used in combination with amphothericin B, Mycograb has greater antifungal activity against a wide range *Candida* species, including *C. albicans*, *parapsilosis*, *C. glabatra*, *C. tropicalis*, *C. Krusei*. In-vivo activity was confirmed in a murine model. Taken together, these findings support the clinical use of efungumab.
Mycograb did not induce cytotoxicity or apoptosis in murine cells cultured in vitro.
The safety and secondary pharmacology has been adequately addressed and a dose-related effect of Mycograb on heart rate and blood pressure as has also been observed in patients given Mycograb was observed in anesthetised dogs. The pharmacokinetic data in animals are limited.
Non-clinical safety data are mainly based on GLP-compliant 14-day repeated dose toxicity studies in the CD-1 mouse and cynomolgus monkeys and an embryo foetal development toxicity study in CD-1 mice. Results did not reveal any special hazard for humans. Increased mortality was observed in mice given repeated doses of Mycograb in the high dose group but the clinical importance of this finding is unknown. Mortality was also reported in the embryo-foetal study.
Mycograb was considered teratogenic in this mouse study (Cleft palate and exencephaly observed). There are no data on the excretion of Mycograb in the milk of lactating women or animals.

Mycograb does not induce human hepatocyte P450 enzymes in vitro, and is unlikely to have any such effect in vivo.

Efficacy

The applicant initially did not provide adequate data to characterise the pharmacokinetics profile of efungumab. The gel assay initially used was abandoned and two new PK studies were initiated one in healthy volunteers and one in patients with advanced state breast cancer. These data gave better insight of the PK although these studies have been performed in too small number of patients to be robust. After intravenous administration, Mycograb is rapidly cleared from the circulation. Overall all PK parameters are associated with significant inter-subject variability.

The demonstration of efficacy rest on the single pivotal trial. This trial 002 was a double-blind, placebo-controlled, randomised, multicenter trial to compare the efficacy and safety of Mycograb plus lipid formulation of amphotericin B versus placebo plus lipid formulation of amphotericin B in treatment of deep-seated *Candida* infections. The majority of 137 patients who completed the trial were Caucasian and elderly, with a fourth being more than 70 years and most baseline characteristics were comparable between the two groups.
Results demonstrated significant superiority for Mycograb treatment in terms of overall response (clinical and mycological), clinical response, *Candida*-attributable mortality and mycological clearance. Results showed that at the recommended dose of 1 mg/kg twice a day for 5 days, clinical and culture-confirmed resolution of the infection was achieved in 47 out of 56 patients (84%) compared to 29 out of 61 (48%) on mono-amphotericin B ($P<0.001$). Prolongation of Mycograb-treatment beyond 10 doses was not allowed under the protocol. The results did not show, however, any benefit in overall mortality, especially at day 33 [20/61 (33%) in placebo versus, 26/56 (46%) in Mycograb]. Submitted sensitivity analysis on baseline characteristics supports the demonstration of efficacy, with overall equivalence in terms of APACHE II scores at baseline and a significant reduction in *Candida*-attributable mortality for Mycograb treated patients, when analyzing deaths in relation to APACHE scores. Further there was overall good agreement between the hard endpoints of

mycological clearance and the softer endpoint of clinical response. In terms of malignancy and immunosuppression treatment arms were not completely comparable, however the applicant demonstrated efficacy across a range of sensitivity analysis taking these measures into account.

Interpretation of this trial is complicated by the high presence of serious underlying disease, the composite primary endpoint, which included the somewhat subjective endpoint of investigator-assigned "resolution of pre-treatment signs and symptoms of candidosis" and by difficulties to exclude the impact of baseline characteristics, related to co-morbidity, such as malignancy and immunosuppression. These issues, led to a GCP inspection at Brno (site 21) and Prague (site 22) in the Czech Republic, from which a substantial number of patients where recruited. According to the GCP inspectors, the protocol was not clear with respect to inclusion and efficacy assessment and assessment of clinical response was subjective at best. In addition, samples for fungal and bacterial cultures were not taken for all patients as dictated by the protocol. A further issue was related to the reporting of data and assessment by the so-called independent expert who was assessing the adverse event reports. The inspection team concluded that the pivotal study had not been conducted and reported in compliance with GCP.
The CHMP considered that, while the GCP inspection has raised several critical findings, the main efficacy findings of the pivotal study were not invalidated. From the two centers, in general data and assessment of most patients included in the analyses seemed to be correct. Further, in most cases, clinical response was supported by mycological response and in general there was overall agreement in the assessment of clinical response between the investigator and independent expert. In the inspectors report all numerical data reported were regarded as valid. Further, no inconsistencies were detected between previous medical records, case report forms and sponsor database.

There is currently insufficient clinical experience of Mycograb in combination with antifungal drugs other than amphothericin B.

The efficacy of Mycograb has not been tested in neutropenic patients.

Safety

The safety database comprised the data from the early studies and the confirmatory trial together with data derived from the studies performed in human volunteers, patients with breast cancer and from compassionate use programme. The total numbers being exposed to Mycograb attained just over 100 patients, which is still a small size database rendering difficult the interpretation of adverse events especially in the critically ill patients with various co-morbidities.

In the confirmatory trial 34 (61%) of the Mycograb treated patients were intubated and 38% of patients were given pre-medication to cover amphotericin B. Treatment-emergent AE was reported by 97 % of Mycograb treated patients compared to 88% of placebo treated patients. Septic shock was most frequently reported followed by multi-organ failure and hypertension occurring after the 1st dose, and thrombocytopenia. However, only 7% of treatment-emergent adverse event was judged to be possibly directly related to efungumab. Due to the severity of pathologies in the studied ICU population, the casual relation ship between study drug and AE were complicated by co-administration of multiple other drugs including amphotericin B in all patients and vasopressors in half of patients.

In the study in healthy volunteers, Mycograb was poorly tolerated with four individuals withdrawing from the study and virtually all patients experiencing adverse event: pain in back or extremities, headache, chills, nausea, vomiting, diarrhoea, syncope, hypotension, dyspnoea, and injection site reactions. Most events were mild or moderate and lasted usually a few hours. The adverse events did recur after repeated administration but were usually milder.
Data on circulating cytokine levels from this study suggest that most of the observed adverse events were related to cytokine response. Although cytokine release and initial infusion reactions such as chills, nausea and headache became less severe with repeated administration it also noted that there was no clear association between the severity of adverse effects and the levels of IL-6, TNF-α or IFN-γ.

In the breast cancer trial, interpretation of observed AE and SAE in this trial remains confounded by the frequent and to some extent overlapping toxicity from the concomitant docetaxel treatment. Although it is acknowledged that most SAE probably were primarily docetaxel related, possible Mycograb related infusion reactions were commonly observed including one hypertensive episode. In addition it is unclear whether all patients had steroids as pre-medication as recommended in the product information of docetaxel.

Data from the compassionate use program has also been submitted. A total of 17 patients have now been treated. Several severe hypertensive episodes were observed in connection with first infusions of Mycograb.

In the healthy volunteer and breast cancer trial the majority of patients developed a HADA response, however there are so far no data to suggest that the development of HADA is linked to specific adverse effects, the cytokine release syndrome or diminished response to Mycograb. So far, limited data from the pivot trial suggest that the development of weak HADA responses will not impair the response to Mycograb. However, it remains possible that HADA could be of consequence in patients who should need re-administration of Mycograb.

The use of Mycograb is commonly associated with a cytokine release syndrome, which may have significant clinical consequences, including severe hypertension. Based on the structure and target of Mycograb, this syndrome was not expected and the pathogenesis of the cytokine release is currently unknown.

During an oral explanation in front of the CHMP, the applicant addressed further the safety issues in relation to the cytokine release, hypertension and the potential impact of the aggregates on the safety profile of the product. The applicant argued that the cytokine release syndrome observed is not linked to the presence of aggregates and Mycograb itself but linked to hsp 90 that may be present on the surface on human white blood cells. This hypothesis raises new safety concerns. The applicant suggested also a mechanism of hypertension and claimed that there were no clinical manifestations of aggregations seen in the safety such as amyloidosis and renal nephropathy.

User consultation

The applicant performed an initial user consultation, followed by a second one which was overall considered adequate.

Risk-benefit assessment

Invasive Candidiasis continues to carry a high mortality despite available anti-fungal agents.
Mycograb (efungumab) has been developed as a new therapeutic approach intended for the treatment of invasive candidiasis in adult patients, in combination with amphotericin B or a lipid formulation of amphotericin B.
Efungumab is directed to heat shock protein 90 (hsp90) which plays a vital role in fungal cell survival.
Efungumab is a single-chain Fv human monoclonal antibody fragment produced in *E. coli* by recombinant DNA technology where the variable domain of the heavy chain (V_H) is connected to the variable domain of the light chain (V_L) with a linker.
Mycograb was designated as an orphan medicinal product.

The CHMP considered that there are remaining issues related to the quality aspects of this product and their potential impact on safety. The evidence provided was not sufficient to conclude that the manufacturing process and the methods of control will guarantee the uniform clinical performance of the product. In particular: the quality of the product is not controlled in a satisfactory way with respect to the distribution of high molecular weight aggregates, the refolding of the molecule and the levels of host cell proteins.

Based on the data provided, the efficacy has been demonstrated with reasonable certainty, with an important reduction of Candida-associated mortality although it did not translate into a reduction of

overall mortality. This discrepancy may be due to imbalance in the co-morbidities but may also be due to unexpected toxicity.

The safety of Mycograb was considered not to be fully established. The causal relationship between efungumab and adverse events was difficult to assess because of the severity of pathologies in the critically ill population in Intensive Care Unit, complicated by co-administration of multiple other drugs including amphotericin B in all patients and vasopressors in half of patients. The other important concern related to a cytokine release syndrome which could manifest itself as clinically significant AEs, including severe hypertension commonly associated with the first dose of Mycograb. The pathogenetic basis of the cytokine release is unclear.
The size of the safety dataset was also too limited to alleviate the concerns related to the quality aspects

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of Mycograb was unfavourable for the applied indication.
The CHMP has recommended therefore the refusal of the granting of the Marketing Authorisation for Mycograb.

3 RE-EXAMINATION OF THE CHMP OPINION OF 15 NOVEMBER 2006

At the November 2006 CHMP meeting following discussion of the Marketing Authorisation Application for Mycograb, the CHMP concluded that the overall benefit/risk for Mycograb in the treatment of invasive candidiasis in adult patients, in combination with amphotericin B or a lipid formulation of amphotericin B was negative.

The grounds for refusal stated in the negative opinion for Mycograb were:

- The evidence provided was not sufficient to conclude that the manufacturing process and the methods of control will guarantee the uniform clinical performance of the product. In particular: the quality of the product is not controlled in a satisfactory way with respect to the presence of aggregates, the refolding of the molecule and the levels of host cell proteins.

- The safety profile of Mycograb is not fully established because of the uncertainties over the pathogenetic basis of the cytokine release syndrome, which is commonly associated with administration of Mycograb and which may manifest itself as clinically significant adverse effects, including severe hypertension.

- The size of the safety dataset is too limited to alleviate the concerns related to the quality aspects.

The applicant submitted written notice requesting a re-examination on 24 November 2006, and the detailed grounds for the re-examination request were submitted on 19 January 2007. A meeting of the BWP including additional experts was held on 12 March 2007 and the CHMP Scientific Advisory Group on Anti-infectives (SAG-Anti-infectives) was convened on 13 March 2007 in preparation of the CHMP meeting on 19-22 March 2007.

The applicant gave oral explanations at the BWP and SAG-Anti-infectives meetings and at the CHMP meeting, respectively on 12, 13 and 20 March 2007.

Grounds for refusal 1:

– The evidence provided was not sufficient to conclude that the manufacturing process and the methods of control will guarantee the uniform clinical performance of the product. In particular: the quality of the product is not controlled in a satisfactory way with respect to the presence of aggregates, the refolding of the molecule and the levels of host cell proteins.

1. Presence of high molecular weight aggregates

Efungumab has "a clear tendency to form aggregates. Aggregation is not uncommon amongst biologically-active proteins, including single-chain antibody fragments (scFvs), and its existence is not considered to be a problem *per se*. Aggregates would be acceptable if they are properly characterised and consistency of production can be demonstrated. The uncontrolled presence of aggregates has potential to influence the *in vivo* activity of Mycograb, and in particular may have an adverse impact on the clinical safety, and therefore it needs to be controlled by means of the application of appropriate specifications, lot to lot consistency needs to be established for it, and the stability studies for the drug substance (DS) and drug product (DP) need to be taken it into account.

During the course of the initial assessment, the applicant described how a variety of techniques for monitoring protein aggregation were investigated, and the applicant proposed size exclusion chromatography (SEC) as a suitable method.

The previous findings with respect to this method were re-examined and the 2006 CHMP opinion is maintained, i.e. that the previously presented SEC method was neither suitable for controlling aggregation in the product nor adequately validated. Instead of providing arguments in support of the previously described SEC method, the company has submitted new data with their grounds for re-examination to introduce a modified SEC system to measure efungumab aggregates. The re-examination procedure may be based only on the scientific data available when the Committee adopted the initial opinion. Even if the modified method were to be considered, outstanding issues would remain that would need to be addressed concerning this method as well as with respect to the control of aggregates.

Therefore it is not possible to conclude that the manufacturing process and the methods of control will guarantee an appropriate control of aggregate size and as a consequence guarantee the uniform clinical performance of the product. On the basis of the assessment of the quality information submitted by the applicant with their grounds for re-examination, the conclusions of the 2006 CHMP opinion are maintained and the major objection remains.

2. High level of host cell protein (HCP)

For control of HCP in Mycograb two assays have been used. A commercially available HCP ELISA measures *E. coli* lysate proteins by using anti-*E.coli* polyclonal antiserum. The ELISA is used for drug product release. As it became clear that this assay does not detect one specific HCP, a second method was developed to measure this HCP.

- Though no general limits for host cell proteins are defined in guidelines or monographs, the proposed limit for one specific HCP is unusually high for a highly purified monoclonal antibody. On the basis of experience with the assessment of recombinant proteins which have been licensed, it is known that there are technical possibilities to reduce these impurities to lower levels. In comparison to these drug products – including recombinant proteins produced in *E. coli* – the total level of HCP in Mycograb is much higher.
- Based on the fact, that a second HCP was necessary to quantify a specific HCP, it is not clear if the currently used ELISA is suitable and able to detect and quantify all other relevant HCPs from *E. coli*. There is concern, that other HCPs, which are not detectable by both assays could increase the total amount of HCP in the product to higher levels.
- Generally the presence of host cell proteins as impurities in drug products of recombinant proteins raises the question of an adverse impact on immunogenicity. This is of particular concern for Mycograb which contains 10 mg DS per vial.

- The HCP impurity was discovered by the inspectors at the occasion of a GMP inspection conducted late during the evaluation process..

Therefore, on the basis of the re-examination of previously submitted data, it remains a fact that efungumab may include a high level of one specific HCP. On the basis of the assessment of the quality information submitted by the applicant with their grounds for re-examination, the conclusions of the 2006 CHMP opinion are maintained and the major objection remains.

3. Control of protein refolding

There are two aspects of the structure of the refolded protein which need to be addressed, namely control of secondary structure and control of the folded structure. The amount of information available on the nature of the structure of efungumab appears to be limited.

Towards the end the initial assessment procedure (end of 2006), the applicant was introducing a proposal to use an in-process method to monitor the development of the secondary structure of efungumab during production, i.e. use it as an in-process control. One of the grounds for the negative opinion on Mycograb at the time was that validation of the in-process method was lacking. The basis of that negative opinion has been re-examined, and is maintained on the basis of the information available at the time of the first CHMP opinion.

With the submission of their grounds for re-examination, the applicant has submitted new data to document that the in-process method has now been validated according to EU/ICH guidelines. The re-examination procedure may be based only on the scientific data available when the Committee adopted the initial opinion. If the validation data were to be considered, the in-process method could be accepted as suitable for use as an in-process control for monitoring secondary structure formation (although this has not been directly demonstrated).

Towards the end the initial assessment procedure (end of 2006), the applicant was proposing to apply a routine CD release test at the DP level. One of the grounds for the negative CHMP opinion in 2006 was the inadequacy of the proposed CD release method, which was not fully validated. The information has been re-examined and the negative opinion on the issue is maintained.
Instead of supporting the previously described CD method as a suitable batch release method, the applicant has submitted new data with the grounds for re-examination to propose a much-modified version of the CD assay. The re-examination procedure may be based only on the scientific data available when the Committee adopted the initial opinion. If this new data were to be considered, this method could be accepted as a method to distinguish between unfolded and folded molecules and considering this method together with the in-process testing, it could be considered that the lack of suitable methods to control protein refolding would no longer constitute a major objection.

Therefore, on the basis of the data available at the time of the CHMP opinion of 16 November 2006, the grounds for re-examination provided by the applicant do not change the initial opinion of the CHMP which was that the quality of the medicinal product is not controlled in a satisfactory way with respect to control of the refolding of the molecule.

Grounds for refusal 2:

– The safety profile of Mycograb is not fully established because of the uncertainties over the pathogenetic basis of the cytokine release syndrome, which is commonly associated with administration of Mycograb and which may manifest itself as clinically significant adverse effects, including severe hypertension.

a) Cytokine Release Syndrome

Regarding the measurement of IL-6 levels in the clinical trial the CHMP concludes that although IL-6 is likely a relevant proinflammatory cytokine the determination of IL-6 alone may not be considered sufficient to characterise a potential cytokine release syndrome in this complex patient population. Despite the arguments from the applicant the CHMP considers the healthy volunteer study as a valid and important pointer to the fact that administration of Mycograb may in some circumstances cause a cytokine release syndrome. With regard to the pathogenetic mechanism for the cytokine release syndrome the CHMP still considered this to be unclear; the basis for the differential secretion of cytokines, as proposed by the applicant, has not been sufficiently addressed and remains unknown.

Cytokine release syndrome is a clinical syndrome caused by a variety of medicinal products. In the confirmatory study with patients suffering from severe candida sepsis, 61% of the patients receiving Mycograb were intubated, therefore in those patients identification of symptoms of cytokine release syndrome is difficult and for some symptoms as chills, headache, and nausea impossible. Therefore the incidence of those symptoms may be underestimated; the true incidence of cytokine release in this patient set is unknown and could only be estimated by direct determination of a comprehensive set of cytokines. Furthermore, sepsis symptoms and symptoms of cytokine release will be very similar; therefore it will be difficult or even impossible to identify newly occurring symptoms of cytokine release syndrome.

The SAG Anti-infectives concluded that it may be difficult to diagnose cytokine release syndrome clinically in the intended population, and that IL-6 levels cannot be used to diagnose a cytokine release syndrome including its severity. The position was however that cytokine release syndrome could be detected if clinically overt and managed with corticosteroids and anti-histamines on a short-term basis; possible long-term consequences cannot be predicted.

The CHMP, taking into consideration the recommendations from the SAG Anti-infectives as well as the assessment report from the Rapporteurs, concluded that that the cytokine release syndrome could be managed in this particular patient population. However, more data from a controlled study would be needed to further explore the cytokine response; the Mycograb and control group should reflect the use of other products for the treatment of candidiasis.

b) Hypertension

The applicant hypothesises that the observed hypertension is a mere reversal of hsp90 induced hypotension via NO synthesis. Overall, this explanation for hypertensive episodes is not considered plausible on the basis of the data provided which appeared to be related to human hsp90 rather than candida hsp90.

The CHMP considers the observed hypertension of relevance due to findings in preclinical studies in a predictive animal model, the breast cancer trial as well as healthy volunteers data, respectively. It is recognized though that cytokine release syndrome is, in its severe form, accompanied by a decrease in blood pressure; therefore it appears possible that this hypertensive reaction is independent from cytokine release.

The SAG Anti-infectives concluded that on the basis of the available data hypertension does not appear to be a major clinical problem and could be dealt with by appropriate safety measures during the administration of the product, e.g. appropriate monitoring and prolonged administration time. However, it was emphasised that the adequacy of these recommendations for administration would need confirmation by actual study data.

The CHMP, taking into consideration the recommendations from the SAG Anti-infectives as well as the assessment report from the Rapporteurs, concluded that hypertension needs to be recognized with the use of Mycograb and that clear instructions with regard to blood pressure monitoring are required; the reduction of the infusion rate might help to mitigate the issue however supporting data needs to be generated.

Grounds for refusal 3:

– The size of the safety dataset is too limited to alleviate the concerns related to the quality aspects.

Significant safety concerns relating to Mycograb were cytokine release syndrome and hypertension, which appear to be manageable.

The applicant's analysis which concentrates on active treatment only and which divides an incomplete population into seven according to the drug batch they received is not helpful in addressing the safety concerns; the apparently huge inter-batch variability is likely to be an artefact of the variable and small numbers exposed to each batch.

The size of the safety database is of concern. The SAG Anti-Infectives expressed the need for a dedicated safety study, that should have a control group to further explore in particular the safety issues cytokine release and hypertension. The control group should reflect the use of other products for the treatment of candidiasis.

The CHMP, taking into consideration the recommendations from the SAG-AI as well as the assessment report from the Rapporteurs, concluded that overall size of the safety database is of concern and hence - also to further explore the described issues - a comparative safety study should be requested to generate an adequate dataset; this study should also compare Mycograb with current treatment options.

Overall conclusions on benefit/risk assessment

Quality aspects

On the basis of the data available at the time of the first CHMP opinion of 16 November 2006, the grounds for re-examination provided by the applicant do not change the initial opinion of the CHMP which was that the quality of the product is not controlled in a satisfactory way with respect to aggregates, the refolding of the molecule and the levels of host cell proteins.

With the submission of the grounds of re-examination, the applicant has presented new data. Even if this new data were to be considered, it would not change the initial opinion with respect to the aggregates and the levels of host cell proteins.

Aggregation is not uncommon amongst biologically-active proteins, including single-chain antibody fragments (scFvs), and its existence is not considered to be a problem *per se*. Aggregates would only be acceptable if properly characterised and if consistency of production can be demonstrated.

As a consequence, the CHMP concludes that in view of the limited characterisation of aggregates and poor control of batch-to-batch consistency with respect to these aggregates, the manufacturing process and the methods of control do not guarantee the uniform clinical performance of the product. Overall it is acknowledged that Mycograb presents an innovative and clinically promising approach, nevertheless there are remaining issues related to the quality aspects of the product, which would need to be resolved due to their potential impact on efficacy and safety.

Clinical safety aspects

The CHMP concludes that the safety issues concerning cytokine release syndrome and hypertension, although not entirely resolved, are manageable in clinical practice. Therefore, the CHMP removed their concern stated in the CHMP opinion in November 2006 regarding the cytokine release syndrome and hypertension. Nevertheless, further studies are warranted to explore the potential impact of the prolonged infusion time in order to mitigate the events related to cytokine release syndrome as well as hypertension.

The size of the clinical database is however considered too small and requires additional data from a comparative safety study.

Benefit / risk assessment

Overall, the data presented support a clinical benefit for Mycograb for the indication i.e. treatment of invasive candidiasis in adult patients, in combination with amphotericin B or a lipid formulation of amphotericin B. As regards the observed safety profile, the safety issues concerning cytokine release syndrome and hypertension are considered clinically manageable. However, there are remaining major outstanding issues with regard to the quality of the product, and in particular in view of the limited characterisation of aggregates and poor control of batch-to-batch consistency with respect to aggregates; based on this it is considered that the manufacturing process and the methods of control do not guarantee the uniform clinical performance of the product. Recognising the orphan designation of Mycograb the CHMP considered that taking into account the limitations of the safety database these quality issues do no allow for a positive risk-benefit balance of Mycograb in the sought indication.

CHMP conclusion on benefit/risk

Having considered the grounds for the re-examination from the applicant, the discussion during the BWP and SAG-Anti-infectives meetings and the CHMP members' discussion during the oral explanation, the CHMP is of the opinion that the benefit/risk for Mycograb in the claimed indication *remains negative.*

Grounds for refusal

- The evidence provided was not sufficient to conclude that the manufacturing process and the methods of control will guarantee the uniform clinical performance of the product. In particular: the quality of the product is not controlled in a satisfactory way with respect to aggregates, the refolding of the molecule and the levels of host cell proteins.
- The size of the safety dataset is too limited to alleviate the concerns related to the quality aspects.