参考資料 3

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

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REFUSAL ASSESSMENT REPORT FOR MYLOTARG

International Nonproprietary Name:
gemtuzumab ozogamicin

Procedure No. EMEA/H/C/000705

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:	Mylotarg
Applicant:	Wyeth Europa Ltd Huntercombe Lane South Taplow Maidenhead, Berks SL6 0PH United Kingdom
Active substance:	gemtuzumab ozogamicin
International Nonproprietary Name/Common Name:	gemtuzumab ozogamicin
Pharmaco-therapeutic group (ATC Code):	Monoclonal antibodies (L01XC05)
Therapeutic indication(s):	re-induction treatment of CD33-positive AML adult patients in first relapse who are not candidates for other intensive re-induction chemotherapy regimens (e.g. high-dose Ara-C) and meet at least one of the following criteria: duration of first remission <12 months, or age >60 years.
Pharmaceutical form(s):	Powder for solution for infusion
Strength(s):	5 mg
Route(s) of administration:	Intravenous use
Packaging:	vial (glass)
Package size(s):	1 vial

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1. BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant Wyeth Europa Ltd submitted on 07 December 2005 an application for Marketing Authorisation to the European Medicines Agency (EMEA) through the centralised procedure for Mylotarg, which was designated as an orphan medicinal product EU/3/00/005 on 18 October 2000. Mylotarg was designated as an orphan medicinal product in the following indication: treatment of acute myeloid leukaemia. The calculated prevalence of this condition was 0.66 per 10,000 EU.

The applicant applied for the following indication: treatment of CD33-positive acute myeloid leukaemia patients in first relapse who are not candidates for other cytotoxic chemotherapy, such as those who are 60 years of age or older.

Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the application contained a critical report addressing the possible similarity with authorised orphan medicinal product Trisenox.

Protocol Assistance:
The applicant received Protocol Assistance from the CHMP on 25 March 1999 and 1 March 2001. The Protocol Assistance pertained to clinical aspects of the dossier.

Licensing status:
Mylotarg has been given a Marketing Authorisation in the following countries:
Cyprus on 23 May 2001, Argentina on 29 December 2000, Brazil on 8 March 2001, Chile on 1 August 2001, Colombia on 24 January 2002, India on 15 September 2002, Israel on 30 June 2003, Japan 25 July 2005, Korea on 10 September 2004, Mexico on 11 September 2001, Singapore on 28 September 2001, south Africa on 11 December 2003, Thailand on 27 August 2002, USA on 17 May 2000, Venezuela on 19 August 2002.

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: **Jens Ersbøll** Co-Rapporteur: **Pasqualino Rossi**

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 7 December 2005.
- The procedure started on 28 December 2005.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 14 March 2006. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 17 March 2006.
- On 20 April 2006, the Biologics Working Party (BWP) adopted a recommendation to the CHMP for the list of questions related to quality aspects.
- During the meeting on 24-27 April 2006, the CHMP agreed on the consolidated List of Questions to be sent to the applicant. The final consolidated List of Questions was sent to the applicant on 27 April 2006.
- A clarification meeting with the Rapporteurs on the List of Questions was held at the EMEA on 31 May 2006.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 10 July 2006.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 7 September 2006.

- On 13 September 2006, the BWP adopted a recommendation to the CHMP for the list of outstanding issues related to quality aspects.
- During the CHMP meeting on 18-21 September 2006, the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- A Scientific Advisory Group in Oncology meeting was convened at the EMEA on 30 November 2006 to address questions raised by the CHMP.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the list of outstanding issues related to quality aspects on 16 January 2007 identifying issues to be addressed at an oral explanation before the BWP.
- During the CHMP meeting on 22-25 January 2007 the Committee agreed to the request from the applicant to postpone the Oral explanation to February 2007.
- During the CHMP meeting on 19-22 February 2007 the Committee agreed to the request from the applicant to postpone the Oral explanation at the CHMP and BWP to April 2007.
- The applicant submitted the responses to the CHMP consolidated list of outstanding issues on 27 March 2007.
- The Rapporteurs circulated the updated Joint Assessment Report to all CHMP members on 3 April 2007.
- During the BWP meeting on 17 April 2007, outstanding quality issues were addressed by the applicant during an oral clarification before the BWP and the BWP adopted a recommendation to the CHMP on quality aspects and a list of post-marketing commitments on 18 April 2007 (Annex 11).
- During the CHMP meeting on 23-26 April 2007 the Committee agreed to an extension of timeframe to allow the assessment of the submitted quality and clinical data.
- The Rapporteurs circulated a further updated Joint Assessment Report to all CHMP members on 7 May 2007.
- During the CHMP meeting on 21-24 May 2007, the CHMP agreed on a second list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- The Rapporteurs circulated an updated assessment report in preparation for the oral explanation to all CHMP members on 15 June 2007.
- During the CHMP meeting on 18-21 June 2007, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the CHMP meeting on 16-19 July 2007, the CHMP agreed on a third list of outstanding issues to be addressed in an oral explanation by the applicant and adopted a report on similarity of Mylotarg and Trisenox.
- The applicant submitted responses to the CHMP consolidated list of outstanding issues on 10 August 2007.
- The Rapporteurs circulated an updated assessment report on the responses to the CHMP third list of outstanding issues on 11 and 14 September 2007.
- During the CHMP meeting on 17-20 September 2007, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 17-20 September 2007 the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a negative opinion for granting a Marketing Authorisation to Mylotarg on 20 September 2007.
- The CHMP opinion was received by the applicant on 28 September 2007.

1.3 Steps taken for the re-examination procedure

- The applicant submitted written notice to the EMEA on 17 October 2007 to request a re-examination of the Mylotarg CHMP Opinion of 20 September 2007.
- During its meeting on 15-18 October 2007, the CHMP appointed Tomas Salmonson as Rapporteur and David Lyons as Co-Rapporteur for the re-examination procedure.
- The detailed grounds for the re-examination request were submitted by the applicant on 27 November 2007.
- The re-examination procedure started on 28 November 2007.

- The Rapporteur's Assessment Report on the detailed grounds for the re-examination was circulated on 21 December 2007. The Co-Rapporteur's Assessment Report was circulated on 18 December 2007.
- During a meeting of the Scientific Advisory Group in Oncology on 11 January 2008, experts were convened to consider the grounds for re-examination.
- During the CHMP meeting on 21-24 January 2008, the grounds for refusal were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 21-24 January 2008, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a final negative Opinion recommending the refusal of granting a Marketing Authorisation for Mylotarg.

2. SCIENTIFIC DISCUSSION

2.1 Introduction

Acute myeloid leukemia

Acute myeloid leukemia (AML) is the most common type of acute leukemia in adults and accounts for ~80% of all cases of acute leukaemia [1]. Worldwide, there are approximately 260,000 new cases of AML each year [2]. With an average incidence of 1 to 3 cases per 100,000 individuals [2], roughly 7000 to 21,000 cases can be estimated per year in Europe. Incidence increases with age and peaks in adults 65 years of age or older [1]. Slightly more than half of all patients are ≥60 years of age [3]. Despite recent developments in understanding the scientific basis of AML and its treatment, there has been little progress in increasing the long-term survival rates in AML patients. If left untreated most patients with AML will die from their disease. If patients are treated, the 5-year survival rate from time of diagnosis is ~20% [4]. With standard treatments, there is a high rate of first remission for patients with de novo (previously untreated) AML, however, ~75% of these patients will ultimately suffer a relapse, in most cases within 2 years [5].

The basic therapeutic approach to AML treatment has changed little over the last 20 years. The approach classically involves 2 separate treatment phases:

(1) induction chemotherapy, with a standard regimen of 3 days of an anthracycline and 7 days of cytarabine, aims at inducing a temporary bone marrow hypoplasia and the regeneration of the normal haematopoietic clone.

(2) consolidation therapy, which consist of multiple courses of intensive consolidation therapy which can be haematopoietic stem cell transplantation (HSCT) or high or intermediate does of cytarabine, aims at reducing the undetectable burden of leukemic cells to a level low enough that long-term disease- free survival (ie, a cure) might be possible.

With standard induction chemotherapy, complete remission is obtained in nearly 70% of the patients [5-7]. For consolidation therapy, the most effective antileukemic approach is allogeneic stem cell transplantation (SCT). However, this technique carries a high risk of initial mortality and a significant risk of long-term morbidity associated with chronic graft-versus-host disease, which tends to offset the therapeutic benefits of a low likelihood of relapse. Chemotherapeutic-based approaches with or without autologous stem cell rescue can be performed relatively safely, but the risk of disease recurrence remains high. Patients with relapsed AML have a particularly poor prognosis. A number of cytotoxic agents and combination regimens have been used for salvage chemotherapy and remission rates from 20 – 70 % have been described. However, the period of remission lasts only typically between 4-6 months.

Currently, authorised drugs for the treatment of AML in the EU include daunorubicin, doxorubicin, cytararabine, mitoxantrone, all-trans-retinoic acid and arsenic trioxide.

About the product

Gemtuzumab ozogamicin (CMA-676) is a humanized (CDR-grafted) IgG4 isotype monoclonal antibody against the CD33 antigen (hP67.6) that is conjugated to a cytotoxic agent N-acetyl gamma calicheamicin dimethyl hydrazide (NAc-gamma calicheamicin DMH) via the bifunctional AcBut linker. The hP67.6 antibody (non-cytotoxic by itself) binds to the CD33 antigen, is internalised and, hence, delivers the calicheamicin derivative to the inside of the leukaemic cell [16-21]. The CD33 antigen is expressed on the external surface of normal and leukaemic myeloid cells, and leukaemic blasts in more than 80% of patients with acute myeloid leukaemia (AML), but not on normal precursor haematopoietic cells [10, 11]. The antibody linked to the conjugate is internalized into lysosomes, where acidification releases the NAc-gamma calicheamicin DMH moiety. The latter undergoes spontaneous reaction with reduced glutathione (GSH) within the cell, where it is activated and the anti-tumour effect can occur [12]. The calicheamicins are potent anti-tumour antibiotics that were initially identified by their ability to damage DNA in screening tests [13]. Their anti-tumour mechanism is thought to occur by binding to the minor groove in the DNA and producing site-specific

double-strand breaks by forming p-benzene diradical [14, 15]. This results in the death of the leukaemic cell.

Mylotarg was approved by the FDA, in the USA in May 2000 for the treatment of AML patients who suffer from a first relapse, are ≥60 years old and are not candidates for other cytotoxic chemotherapy. As a post-approval commitment, the applicant committed to carrying out a randomised consolidation study in de novo AML, which is still ongoing. Mylotarg was also approved in Japan in July 2005 for the indication of relapsed or refractory CD33 positive AML.

An Orphan Drug Designation based on significant benefit was issued 18 October 2000 for the treatment of Acute Myeloid Leukaemia (EU/3/00/005). The current proposed indication is within the designation.

2.2 Quality aspects

Introduction
Mylotarg (gemtuzumab ozogamicin) is a chemotherapy medicinal product containing a recombinant humanised IgG4 kappa antibody linked to calicheamicin, a cytotoxic anti-tumour antibiotic isolated from fermentation of a bacterium. The antibody portion of Mylotarg binds specifically to the CD33 antigen. This antigen is expressed on the surface of leukaemic blasts in more than 80% of patients with acute myeloid leukaemia (AML). Upon binding to the CD33 antigen, the calicheamicin-CD33 complex is internalised within the leukaemia cell and calicheamicin is released from the conjugate via hydrolysis in the lysosome. The released calicheamicin then binds to the minor groove of the DNA causing site-specific double-strand breaks via the formation of a p-benzene di-radical. This ultimately leads to cell death by apoptosis.

Active Substance

The active substance, gemtuzumab ozogamicin, is an antibody-targeted chemotherapeutic agent comprised of three different parts:
A. a humanised IgG4 monoclonal antibody (hereafter termed 'hP67.6') conjugated to
B. the cytotoxic agent N-acetyl gamma calicheamicin dimethyl hydrazide (NAc-gamma calicheamicin DMH) via
C. a bifunctional linker.
The hP67.6 antibody, which is non-cytotoxic by itself, is directed against the CD33 antigen.

1. hP67.6 monoclonal antibody drug substance intermediate

Manufacture
The monoclonal antibody ('hP67.6') is manufactured by an approved manufacturer in compliance with Good Manufacturing Practice (GMP).

The hP67.6 antibody is produced by humanisation of the anti-CD33 murine antibody hP67.6 by complementarity determining region grafting (CDR grafting). The resulting hP67.6 antibody is a genetically engineered human IgG4 kappa antibody, which contains sequences derived from the murine antibody in the antigen-binding region only to minimise human anti-murine antibody response. The IgG4 isotype was chosen because it has the longest circulating half-life of all isotypes and is least likely to participate in immune-mediated mechanisms such as complement fixation and antibody-dependent cellular toxicity.

The established cell banking system is adequate for the reliable manufacturing of a monoclonal antibody. Criteria for the establishment of new WCB were provided. The cell bank testing is adequate. The development of the cell line, the creation of the cell bank and its testing follow a scientifically sound scheme and were sufficiently described. Generally the requirements of the relevant guidelines are met.

Overall the control of source and starting materials has been adequately documented. Materials of animal/human origin used in the manufacturing process of hP67.6 are described below (in the section 'adventitious agents'). Overall, materials used in the manufacturing process are adequately controlled.

The transfected recombinant murine plasmacytoma cell line (designated as KD1) is grown as a suspension culture in a fed-batch process. The fermentation and harvest of hP67.6 antibody is straightforward and has been adequately described. The hP67.6 antibody purification process includes two chromatographic columns including a Protein A chromatography step). Viral inactivation is obtained by low pH treatment and viral DV50 nanofiltration. In-process controls, including control limits, are in place during fermentation, harvest and purification. The manufacturing process and in-process controls have been sufficiently described. Establishment of controls for critical steps and intermediates are described in detail.

Process validation studies were presented demonstrating that the full-scale hP67.6 fermentation and purification process can achieve lot-to-lot consistency and reproducibility. The contribution of each step of the purification process to the removal of impurities derived from the hybridoma cell line and the culture media has been demonstrated, showing a high and consistent removal of the impurities. Further, it has been shown, by a variety of methods, that the hP67.6 antibody integrity is not affected by the purification process.

A detailed and adequate rationale for the changes introduced to the manufacturing process during development has been provided. It has been shown that changes (change in cell line, introduction of viral filtration step and introduction of recombinant protein A resin) were without influence on the quality of the hP67.6 antibody.

The applicant presented a comprehensive structural and functional profile of the antibody, primarily obtained using a combination of chromatographic, electrophoretic and mass spectrometry (MS) techniques. Overall the hP67.6 antibody has bee carefully characterised using a wide range of methods.

Specifications

The analytical methods used in the release control of the hP67.6 antibody intermediate are to a great extent based on non- or semi-quantitative electrophoresis methods. More quantitative methods, such as chromatographic methods are only used to a limited extent. This is acceptable in view of the tests performed on the active substance, i.e. the conjugated antibody. The hP67.6 antibody characterisation study clearly revealed that several varying forms of the antibody exist as well as the presence of aggregated forms and charge variants (determined by isoelectric focusing). This variability is controlled to some extent at the level of the unconjugated hP67.7 antibody and further at the level of the conjugated antibody.

Non-compendial analytical methods used for release testing have been specifically validated for use with the hP67.6 antibody. Batch results from a total of 54 lots have been provided. The data from the batches have met the acceptance criteria of the product specification in place at the time the batches were manufactured.

The level of host cell proteins (HCP) is measured using a semi-quantitative western blot assay which has been sufficiently validated. The Applicant also performed a study with a quantitative ELISA method which confirmed the low levels of HCP found using the western blot assay. The Applicant provided a post-marketing commitment to implement the ELISA method for release of the antibody.

The analytical methods used for release testing of the hP67.6 antibody have been satisfactorily described and validated. The chosen test parameters and limits have been adequately justified with reference to development data, batch analysis and/or stability data.

Stability

The requested shelf life for the hP67.6 antibody is adequately supported by stability data.

2. Activated calicheamicin drug substance intermediate

Manufacture, specifications and stability
The activated calicheamicin intermediate is manufactured by an approved manufacturer.
The nomenclature, structure and general properties of the activated calicheamicin derivative have been described satisfactorily. Calicheamicin is obtained by bacterial fermentation and the manufacturers and the fermentation process with subsequent synthetic steps have been described satisfactorily. The producer microorganism and the raw materials used in the fermentation process and the subsequent synthetic steps are adequately controlled and adequate in-process controls have been established and control ranges are supported by satisfactory validation studies.

The molecular structures of the activated calicheamicin-derivative and related substances have been adequately characterised. The analytical methods used for release testing of activated calicheamicin have been satisfactorily described and validated and the presented batch analysis results indicate that the process is under control. The chosen test parameters and limits have been adequately justified on the basis of batch data and are acceptable. The in-house primary reference material has been adequately characterised and the chosen container closure system provides adequate protection for this substance. Satisfactory stability evaluation has been performed. The proposed re-test period and storage condition are acceptable, when the activated calicheamicin-derivative is stored using the proposed container closure system.

3. Gemtuzumab ozogamicin drug substance

The drug substance is manufactured and controlled by an approved manufacturer in compliance with GMP.

Manufacture
The drug substance gemtuzumab ozogamicin is formed when activated calicheamicin is added to a solution of hP67.6 antibody. The reaction, results in the covalent attachment of activated calicheamicin to the antibody. The resulting active substance consists of a mixture of conjugated and unconjugated hP67.6 antibody. The conjugated molecules differ in the number of calicheamicin moieties attached to the hP67.6 antibody.

Conjugation invariably leads to the formation of some undesired aggregated protein. Size exclusion chromatography is used to separate monomeric antibody conjugate from the aggregated material formed. Process derived impurities are separated from gemtuzumab ozogamicin during the purification process. Impurities are reduced to a low level during purification and are controlled by adequate release specifications.

Two materials of biological origin are used during the manufacture of gemtuzumab ozogamicin. These substances are produced from bovine milk of US origin and are adequately controlled (see also '*adventitious agents*').

Critical in-process controls as well as non-critical controls are specified for the conjugation and purification process to assure a consistent production of the drug substance.

Three consecutive production batches were manufactured to demonstrate that the process met validation acceptance criteria. The gemtuzumab ozogamicin drug substance process validation exercise was successfully completed, documenting the ability to consistently produce drug substance with predictable and acceptable product quality. The properties and consistency of the validation batches of drug substance upon manufacture were evaluated using a battery of release tests.

Gemtuzumab ozogamicin has been characterised structurally by spectroscopic, electrophoretic and chromatographic assays, and characterised functionally by ELISA and immunoassays. All characterisation testing was conducted on reconstituted Mylotarg drug product because of the limited stability of the drug substance. Data shows that the conjugation reaction does not change the antibody

structure, including the glycosylation. Likewise, it has been shown that binding of the monoclonal antibody to CD33 cells is not affected by the calicheamicin conjugation.

Comparability studies have been conducted to evaluate whether changes introduced to the manufacturing process had any impact on the quality of the drug substance. These changes were introduced in particular to optimise the conjugation process, to minimise aggregate formation, and to obtain higher yields. The conjugation reaction is influenced by several factors. The comparability studies cover the very first material produced up to commercial material and consist solely of release test results. Additional characterisation studies were not performed.

The drug substance consists of a mixture of unconjugated antibody and antibodies to which a varying number of calicheamicin moieties are linked. The number of conjugated calicheamicin moieties can vary. The presence of significant amounts of unconjugated antibody in the drug substance was recognised late in the development program after the pivotal clinical trials had been completed and the manufacturing process had been validated, i.e. after 2001. As such, the unconjugated fraction was not tracked in the clinical batches nor was it considered during process development. Therefore the data available from the currently manufactured batches cannot be linked to the material used in clinical trials performed prior to 2001 with regard to distribution of conjugated and un-conjugated hP67.6 antibodies. Full comparison of batches (n=78) produced after 2001, when the test for low conjugation fraction was introduced, showed good consistency with respect to the low conjugation fraction in the commercial product and it is considered that the manufacturing process guarantees consistency in this respect.

As a test for low conjugated antibodies was not available at time of production of material for the clinical trials it can not be assured that the level of low conjugated antibody was the same in the material used in non-clinical and clinical studies as it is in the product to be placed on the market. However, in general, the comparability study covering the very first non-clinical and early clinical batches, clinical batches and the first production batches up to the current production batches shows reasonably consistent release test results among the batches despite differences in the production process. SDS-PAGE analysis shows a higher purity and a lower level of aggregates in the commercial material compared to the very first batches used in non-clinical and early clinical material. On the other hand, different concentrations of calicheamicin have been used in the conjugation reaction in the past compared to the commercial production process. At least in theory, the difference in concentrations of calicheamicin could have an impact on the calicheamicin conjugation level. In this respect it is noted that the proposed specification for the low conjugated fraction has been in place for several years for commercial lots placed on the market in the USA Since the assessment of the clinical efficacy and safety data did not reveal any signals related to the level of unconjugated antibody, there is sufficient reassurance over the clinical efficacy and safety with respect to the level of unconjugated antibody.

Specifications

The analytical procedures used for release testing include state-of-the-art methods such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF), high pressure liquid chromatography (HPLC) and enzyme linked immunosorbant assay (ELISA). These methods have been appropriately validated and are adequate for the assessment of the active substance. The set of specification and limits were adequately justified on the basis of data obtained from numerous batches. All results were within specifications and the results are consistent, except for the very first non-clinical and clinical batches, which had a lower level of purity as shown by SDS-PAGE and a higher level of aggregates and unconjugated calicheamicin.

The specifications include aggregate and unconjugated calicheamicin ozogamicin as well as microbiological (endotoxins by LAL and bioburden) and general tests such as protein content. The non-reducing SDS-PAGE and IEF methods used for identification of the drug substance are only qualitative and were considered insufficient. Therefore, following the request from CHMP the applicant have introduced tests for peptide mapping and oligosaccharide profiling to assure consistency of antibody variants. Potency is tested using a cytotoxicity assay and an immunoaffinity antigen binding ELISA.

The specifications proposed for gemtuzumab ozogamicin are considered appropriate to ensure sufficient quality with respect to purity and level of impurities.

Stability
Gemtuzumab ozogamicin drug substance has limited stability and it is therefore shipped to the lyophilisation site immediately after manufacture prior to the completion of all testing and formal release. Release of the resulting drug product batches is contingent upon acceptable results for drug substance release testing.

Medicinal Product
Mylotarg is a sterile, white, preservative-free lyophilised powder containing 5 mg of gemtuzumab ozogamicin in a 20 ml amber vial. The inactive ingredients are Dextran 40, sucrose, sodium chloride, monobasic and dibasic sodium phosphate. The finished product should be reconstituted with 5 ml of sterile water for injections which is not provided with the product. The reconstituted product is administered as an infusion following dilution in a solution of 0.9% sodium chloride.

Pharmaceutical Development
The development of the lyophilised formulation has been thoroughly described and the rationale for the selection of the formulation and container configuration has been adequately addressed and justified.

Manufacture

The finished product is manufactured by an approved manufacturer (fill and lyophilisation by Ben Venue Laboratories, Inc., USA and packaging by Wyeth Farma SA, Spain) in compliance with GMP. The manufacturing process together with in-process controls has been adequately described. Following the request from CHMP during evaluation, an in-process control test on residual moisture on the lyophilised powder was introduced and the limits for the in-process controls performed during lyophilisation (i.e. temperature, time and pressure) have been stated. The critical steps are defined and controlled and validation data of the critical steps, i.e. sterile filtration, aseptic filling, holding time from start of sterile filtration to start of lyophilisation and container closure system have been provided and are acceptable.

The results from the analysis of production batches show that the manufacturing process used is capable of consistently producing drug product of the required quality.
No new impurities are formed during manufacture and the purity/impurity profile of the drug product is comparable to that of the drug substance.

Product specification

Specifications and release criteria for Mylotarg have been established to ensure identity, purity, potency, quality, and safety. Many of the analytical procedures used for testing the drug product are the same as the drug substance. Additional testing on drug product includes moisture content, uniformity of content, and sterility. Following the request from CHMP during evaluation, the tests for extractable volume, opalescence and osmolality were added. Overall, all methods for release testing of the drug product have been adequately described, justified and validated. The proposed limits are considered acceptable.

Stability
The stability of the drug product has been extensively investigated on clinical and pilot batches. The data show that the drug product is stable for the proposed shelf-life of 36 months at 2-8°C. The data from clinical and pilot batches are comparable and indicate that the commercial batches (manufactured at twice the scale) have a comparable stability. Photostability data shows that the drug product should be protected from light. Simulated clinical admixture infusion studies support a maximum allowed time of 20 hours for product reconstitution, dilution, and administration (through the completion of infusion).

Microbiological aspects were not investigated in the simulated clinical admixture infusion studies therefore, from a microbiological perspective, the product should be used immediately after reconstitution and dilution. If not used immediately, in-use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 20 hours for product reconstitution, dilution, and administration (through the completion of infusion)

Adventitious agents
Materials of animal/human origin used in the manufacturing process of the hP67.6 antibody and gemtuzumab ozogamicin have been properly documented.

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.

It is noteworthy that the hP67.6 antibody was originally manufactured using one material of animal origin obtained from plasma pools not tested in compliance with EU requirements. The selection and screening of donors used for the production of this material was not clearly specified: epidemiological data was not presented, the overall safety strategy was not satisfactorily detailed to ensure traceability, test kits were not described nor validated, etc. The Applicant committed to not place Mylotarg produced with this material onto the EU market. Only Mylotarg produced with a material derived from plasma which complies with EU requirements and for which a plasma master file is available would be put on the EU market.

The viral validation studies have demonstrated efficient and consistent removal of viruses The combined reduction capacity of the chromatographic columns demonstrated an overall virus reduction of at least 4 $\log_{10}$ .for small non-enveloped viruses. Based on the overall data presented; i.e. control of starting materials, control during the process and virus validation, the risk of virus transmission to patients receiving Mylotarg is remote.

2.3 Non-clinical aspects

Introduction

The pharmacology of gemtuzumab ozogamicin was investigated *in vitro* and *in vivo*. Pharmacodynamic and pharmacokinetic studies were not conducted according to GLP standards.

The toxicity studies in rats and cynomolgus monkeys and the reproductive and developmental toxicity studies in rats were conducted according to GLP standards, as claimed by the applicant.

Pharmacology

In vitro studies evaluated the cytotoxicity of gemtuzumab ozogamicin on CD33+ and CD33- cells in tissue culture assays, and comparisons were made with control conjugates with an antibody that does not recognize the CD33+ cells. Ex vivo, colony-forming assays were conducted with bone marrow samples from AML patients and with control conjugates. The behaviour of the antibody-calicheamicin conjugate in the body, the specificity of its anti-tumour activity and the extent to which it may affect normal cells was assessed in vivo. The potential for gemtuzumab ozogamicin to affect vital body systems (ie, cardiovascular, central nervous system [CNS], gastrointestinal, hepatic, and renal) has been investigated.

- Primary pharmacodynamics

The hP67.6 antibody component of gemtuzumab ozogamicin was found to bind only to $CD33^+$ cells and was non-cytotoxic in tissue culture. As a human monoclonal antibody of the IgG4 isotype class, neither complement-binding nor antibody-dependent cellular cytotoxicity (ADCC) was expected or observed in test animals.

In vitro, antigen binding specificity of gemtuzumab ozogamicin was indistinguishable from that of the uncoupled hP67.6 antibody. The relative affinity of four GMP batches of gemtuzumab ozogamicin was determined by competitive binding of gemtuzumab ozogamicin *versus* radio-labelled mP67.6 antibody to $CD33^+$ HEL 92.1.7 cells. Competitive binding was in the order of 10% of that of the hP67.6 antibody, which is within the limit of experimental error for this method. In terms of tissue specificity, neither gemtuzumab ozogamicin nor hP67.6 antibody exhibited any unexpected or unspecific binding to normal human tissues tested. In addition to HEL92.1.7 cells, hP67.6 antibody also stained microglia cells in the cerebellum, which are considered to be of mesodermal rather than neuroectodermal origin. The staining patterns of the microglia cells obtained with hP67.6 antibody were similar to that of the anti-CD68 antibody, confirming that they were indeed microglia cells, which are considered to be macrophages and thus are of mesodermal rather than neuroectodermal origin. The binding specificity of gemtuzumab ozogamicin and the hP67.6 antibody to human normal peripheral blood leukocytes and bone marrow cells was identical.
In vitro cytotoxicity of gemtuzumab ozogamicin in $CD33^+$ and $CD33^-$ cells was investigated in tissue culture assays. The IC_{50} of gemtuzumab ozogamicin for the $CD33^+$ HL-60 cell line was less than 1 pg/ml, while the IC_{50} for the $CD33^-$ Raji cell line was almost [insert value], 100 000-folds higher. Control assays were executed with control antibody conjugates that do not recognise the $CD33^+$ antigen.

The cytotoxic effects of gemtuzumab ozogamicin on HL-60, NOMO-l, NB4, NKM-l cell lines (all $CD33^+$), K562 cells (weakly $CD33^+$), and Daudi cells ($CD33^-$) was investigated. Concentration-dependent and specific cytotoxicity against $CD33^+$ cell lines was observed using flow cytometry to measure cell viability [LC_{50} approximately 0.125 ng/ml of calicheamicin equivalents (cal eq)]. Cells sensitive to gemtuzumab ozogamicin were temporally arrested at the G2/M phase of the cell cycle, except for NKM-1 cells. For the HL-60 cell line, morphological examination and DNA-fragmentation assays indicated that the primary cytotoxic effect of gemtuzumab ozogamicin may be necrotic rather than apoptotic.

The *in vivo* anti-tumour effects of the gemtuzumab ozogamicin were studied in a HL-60 xenograft tumour mouse model. HL-60 xenograft tumours were established by subcutaneous implantation of 8 X 10^6 cells, which had been harvested from ascites. There were 5 nude mice per test group and 10 nude mice in the control group. Tumour mass was determined on a weekly basis. Tumours were staged on average at 8 days post-implantation or when the tumour reached 150 mg. Gemtuzumab ozogamicin was administered as a single dose given intraperitoneally. The data demonstrated a dose-response with an anti-tumour activity at doses between 0.8 and 3.2 mg/m^2 of cal eq. Significant lethality was observed at 3.2 mg/m^2. At the lowest dose of 0.8 mg/m^2, 2 out of 5 mice were tumour free at day 37 post-administration of treatment. Using doses ranging from 0.48 to 2.88 mg/m^2 of calicheamicin equivalents per nude mouse (given on days 7, 11, and 15 post-implantation of tumour?), 5 out of 5 animals were tumour free on day 35. There was >80% inhibition of tumour growth for every group treated with gemtuzumab ozogamicin. Additional experiments with this xenograft mouse model examined the effectiveness of gemtuzumab ozogamicin in comparison with control conjugate non-targeting MOPC-21 antibodies that do not target $CD33^+$ cells specifically but still contain the same calicheamicin derivative. With the corresponding MOPC-21 conjugate, a 20% inhibition of tumour growth was observed.

The effects of gemtuzumab ozogamicin on the colony forming ability of human leukaemia bone marrow samples were investigated. Freshly thawed cell suspensions of bone marrow samples which were known to be capable of forming colonies *in vitro* were exposed to gemtuzumab ozogamicin or control treatments. At 2 ng/mL cal eq, 15% of adult AML patient (n=27) samples had >60% inhibition of colony growth. At 10 ng/mL cal eq the number of samples with >60% inhibition increased to 44%. AML patient samples (n=21) tested at 100 ng/mL cal eq showed high levels of nonspecific inhibition with the non-targeting control and 13 showed a 50% or greater drop in colony growth after incubation with the control conjugate compared to growth with controls at 10 ng/mL cal eq. Results obtained from similar studies using normal bone marrow suggest that normal samples are resistant to gemtuzumab ozogamicin at doses 4-fold higher than those used in the patients.

- Secondary pharmacodynamics

Cytotoxicity studies in normal human megakaryocyte from 6 independent bone marrow donors showed variable results from little or no effect to progressive, cumulative cytotoxicity over 24 hours. Gene expression studies indicated that the observed variability could not be explained by differences in the glutathione pathway or multi-drug resistance genes.

- Safety pharmacology programme

The potential for gemtuzumab ozogamicin to affect vital and other body systems has been investigated.

Cardiovascular system: Three studies were conducted in beagle dogs. In the first study, gemtuzumab ozogamicin administered up to 40 mg protein/m^2 as a single bolus i.v. injection reduced mean arterial blood pressure for 20 minutes (gradually returning toward control values), slightly increased the heart rate but decreased cardiac output. Both P-wave and T-wave amplitudes had a high degree of variability. Vehicle-treated and gemtuzumab ozogamicin-treated animals did not show physiologically significant differences in P-wave and T-wave amplitudes.
In the second study, 13 mg protein/$m^{2\ of}$ gemtuzumab ozogamicin was administered as a 30-minute i.v. infusion, causing no statistical changes in mean arterial blood pressure and cardiac output. However heart rate was significantly increased at all time points starting 15 minutes after infusion. P-wave amplitude was significantly increased at 20 minutes until the end of gemtuzumab ozogamicin infusion (30 minutes) through 90 minutes. T-wave amplitudes were significantly increased at all times starting 15 minutes after infusion.
In the third study, a single dose of 4 mg protein/m^2 of gemtuzumab ozogamicin was administered as a 30-minute i.v. infusion, not causing any change in mean arterial blood pressure, heart rate, and cardiac output, in comparison to vehicle-treated group. P-wave and T-wave amplitudes exhibited considerable variability and were generally not different between vehicle-treated and gemtuzumab ozogamicin-treated dogs. All monitored electrocardiographic parameters were unchanged. Gemtuzumab ozogamicin was without significant physiological effect on overall hemodynamic, cardiac function, or electrocardiographic parameters.

Hepatic function: In rats, i.v. infusion of either vehicle or gemtuzumab ozogamicin at doses of 7.5, 25 and 75 µg calicheamicin eq/kg had no influence on excretion of bromsulphalein sodium compared to the untreated group.

Gastrointestinal (GI) effects: Intestinal motility (mouse *in vivo* study): no significant changes have been observed in urine of levels of protein, sodium, potassium and chloride excretion 5 hours after single i.v. administration of up to 250 µg calicheamicin eq/kg.

Autonomic Nervous System (pig ileum test): gemtuzumab ozogamicin at a concentration up to 0.75 µg calicheamicin eq/ml did not influence spontaneous motility and contractile responses to acetylcholine, histamine, serotonin and barium chloride in isolated guinea pig ileums, suggesting that CMA-676 demonstrates no anti-acetylcholine, anti-histamine, anti-serotonin and Ca^{2+} antagonistic effects.

Effects of vehicle: by using the clinical gemtuzumab ozogamicin sample which contained dextran 40, the effect of gemtuzumab ozogamicin on the volume of urine and urinary electrolytes in rats was not evaluated because of severe hypouresis induced by dextran 40. Hypouresis induced by dextran 40 was not observed in mice.

- Pharmacodynamic drug interactions

Studies on pharmacodynamic drug interactions were not conducted.

Pharmacokinetics

A limited number of pharmacokinetic (PK) studies in animals were performed with gemtuzumab ozogamicin. In light of the absence of cross-reactivity of gemtuzumab ozogamicin (hP67.6 antibody) with the CD33 antigen from other animal species, kinetic studies in animals can only be of limited value, as there will be no binding, specific intracellular uptake, or metabolism of the conjugate that underlies its therapeutic action in humans. This enzyme-linked immunosorbent assay (ELISA) utilizes specific hP67.6 antibody binding to the CD33 antigen. The calibration standard for this ELISA was