European Medicines Agency
Evaluation of Medicines for Human Use

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ASSESSMENT REPORT

FOR

Ceplene

International Nonproprietary Name: histamine dihydrochloride

Procedure No. EMEA/H/C/000796

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

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1 BACKGROUND INFORMATION ON THE PROCEDURE

1.1 Submission of the dossier

The applicant EpiCept GmbH submitted on 06 October 2006 an application for Marketing Authorisation to the European Medicines Agency (EMEA) through the centralised procedure for Ceplene, which was designated as an orphan medicinal product EU/3/05/272 on 11 April 2005 Ceplene was designated as an orphan medicinal product in the following indication: treatment of acute myeloid leukaemia. The calculated prevalence of this condition was approximately 0.7 in 10,000 persons in the Community.

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

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

The applicant applied for the following indication: Ceplene in conjunction with interleukin-2 is indicated for maintenance of remission in adult patients with acute myeloid leukaemia in first remission to prolong the duration of leukaemia-free survival.

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 did not seek Protocol Assistance from the CHMP.

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

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

Rapporteur: David Lyons Co-Rapporteur: Bengt Ljungberg

1.2 Steps taken for the assessment of the product

- The request for Accelerated Assessment was rejected on 18-20 July 2006.
- The application was received by the EMEA on 06 October 2006.
- The procedure started on 25 October 2006.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 17 January 2007. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 16 January 2007.
- During the meeting on 20-22 February 2007, 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 22 February 2007.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 06 September 2007.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 19 October 2007.
- During the CHMP meeting on 13-15 November 2007, the CHMP agreed on a list of outstanding issues to be addressed in writing and/or in an oral explanation by the applicant.

- During the CHMP meeting on 20 February 2008, outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During a meeting of the SAG-Oncology on 11 January 2008, experts were convened to address questions raised by the CHMP.
- During the meeting on 17-19 March 2008, 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 Ceplene on 19 March 2008.

1.3 Steps taken for the re-examination procedure

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

Rapporteur: Harald Enzmann Co-Rapporteur: Alar Irs

- The Applicant submitted a letter to the EMEA dated 26 March 2008 requesting the re-examination of Ceplene CHMP Opinion of 19 March 2008.
- During its meeting on 21-24 April 2008, the CHMP appointed Harald Enzmann as Rapporteur and Alar Irs as Co-Rapporteur for the re-examination procedure.
- The detailed grounds for the re-examination request were submitted by the applicant on 27 May 2008. The re-examination procedure started on 28 May 2008.
- The Rapporteur's Assessment Report on the detailed grounds for the re-examination was circulated on 4 July 2008. The Co-Rapporteur's Assessment Report was circulated on 7 July 2008.
- A Scientific Advisory Group (SAG) in Oncology meeting was convened at the EMEA on 10 July 2008 to consider the grounds for re-examination.
- The Rapporteurs' Joint Assessment Report on the detailed grounds for the re-examination was circulated on 18 July 2008.
- During the CHMP meeting on 21-24 July 2008, the grounds for refusal were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 21-24 July 2008, the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a final positive Opinion under exceptional circumstances recommending the granting of a Marketing Authorisation for Ceplene. The applicant provided the letter of undertaking on the specific obligations to be fulfilled post-authorisation on 23 July 2008.
- During the CHMP meeting on 21-24 July 2007, the CHMP adopted a report on similarity of Ceplene and Trisenox.
- The CHMP opinions were forwarded, in all official languages of the European Union, to the European Commission, which adopted the corresponding Decisions on 7 October 2008.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

Acute myeloid leukaemia (AML) is a heterogeneous haematological malignancy characterised by abnormal proliferation of immature haematopoietic progenitor cells of the myeloid lineage. Diagnosis is made by morphologic examination of and immunophenotyping of myeloid progenitor cells from the bone marrow. The complete blood count (CBC) is almost always abnormal and often, leucocytosis, anaemia and thrombocytopenia are present. Current standard treatment includes chemotherapy with or without a bone marrow transplant.

The standard treatment of AML in adults typically is divided into 2 phases: induction and intensification. Induction treatment uses a combination of anthracyclines (most commonly daunomycin) and high dose cytarabine, with or without 6-thioguianine. Treatment is repeated every 4 weeks until complete remission (CR), defined by the National Cancer Institute (NCI) as including all of the following:

- peripheral-blood counts rising toward normal,
- a mildly hypocellular to normal cellular marrow with fewer than 5% blasts,
- no clinical signs or symptoms of the disease, including in the central nervous system (CNS) or at other extramedullary sites.

Complete remission may be achieved in 70-80% of the approximately 30,000 newly diagnosed AML patients worldwide. After induction chemotherapy, intensification (or consolidation) therapy (eradicate any microscopic amounts of leukaemia) can be initiated. Many patients, especially those younger than 60 years at the time of diagnosis and those with favourable cytogenetic patterns, may also receive consolidation therapies (generally, 1 or 2 courses), including high-dose cytarabine (HDAC) and/or with other chemotherapy (daunomycin) and/or allogeneic stem cell transplantation. However, the majority of AML patients (75-80%) in their first CR, who cannot be allotransplanted, will relapse after median remission duration of approximately 1 year. Where allogeneic transplantation is not an option, there is no expert clinical consensus regarding effective therapy for the maintenance of remission beyond the completion of consolidation chemotherapy. As a result, the standard of care for such AML patients in remission is no treatment.

Induction of remission and long-term survival are still major challenges in the care of patients with AML. The survival outcome after a leukaemic relapse is poor; thus, more than 80% of non-transplanted AML patients will die within 2 years after onset of relapse. Overall, about 20-30% of people survive free of disease at 5 years from diagnosis; most relapses occur within 2 years of diagnosis. If a patient survives induction and consolidation chemotherapy then the length of that patient's first remission is considered the primary prognostic indicator for the length of survival.

Novel therapeutic strategies to maintain CR in AML are needed because leukaemic relapse is synonymous with limited long-term survival. Currently, there is no recognised strategy or pharmacological intervention to prevent AML relapse and for this reason the concept of relapse prevention using the combination of subcutaneous interleukin-2 (IL-2) and histamine dihydrochloride (HDC) is of considerable scientific and therapeutic interest.

Histamine dihydrochloride is a synthetic immune modulator. The rationale for its use in combination with IL-2 in maintenance of first remission in adult patients with AML is based on its putative novel mechanism of action in which HDC is thought to improve the effectiveness of the cytokine, IL-2. Activation of IL-2 receptor bearing lymphocytes NK and T-lymphocytes to kill tumour cells is the principal goal of IL-2 and IFN-α therapy.

The therapeutic effect of histamine dihydrochloride is exerted by its effect on monocytes. Histamine dihydrochloride inhibits the function of a key enzyme in oxygen radical formation, NADPH oxidase,

reducing the detrimental effects of oxidative stress on NK and T-cells thereby preserving their cell cytotoxic activity against tumour cells. Histamine is involved in local immune responses, regulation of gastric HCL secretion and the allergic response. Based on its known biological effects, histamine administered subcutaneously is expected to produce vasodilation (manifested as flushing), hypotension and a corresponding increased heart rate, increased gastric acid secretion and local injection site reactions.

Ceplene (histamine dihydrochloride) solution for injection is classified as an immune modulator (ATC code: L03A X14). It is used in conjunction with recombinant IL-2. The proposed indication is 'administered in conjunction with interleukin-2, maintenance of remission in adult patients with acute myeloid leukaemia in first remission to prolong the duration of leukaemia free survival'. In some EU countries, recombinant IL-2 is approved for use in renal cell carcinoma and malignant melanoma.

2.2 Quality aspects

Introduction

Ceplene contains histamine dihydrochloride as the active substance and it is presented as a solution for injection for subcutaneous use. Each vial contains 0.5 ml of the solution of histamine dihydrochloride at a concentration of 1mg/ml.

Other ingredients include sodium chloride, hydrochloric acid or sodium hydroxide and water for injections. All excipients used in the product are of non-animal origin and comply with their corresponding European Pharmacopoeia monographs.

The immediate packaging materials are commonly used for these types of formulations and consist of Type I Ph.Eur. clear, colourless glass vials sealed with bromobutyl rubber stoppers and flip-off aluminium seals

Active Substance

Histamine dihydrochloride ($C_5H_9N_3$. 2HCl) is a well-characterised molecule for which a Ph. Eur. Monograph exists. It is a white to off-white crystalline powder, very water-soluble, but less soluble in ethanol. It has a pKa of 6.12 and 9.85 and is moderately hygroscopic. Histamine dihydrochloride does not contain any optically active centres or isomerable double bonds and exhibits only one polymorphic form.

The proof of chemical structure was achieved by elemental analysis; analysis of the electron ionisation mass spectrum, ^{1}H-NMR and ^{13}C-NMR spectra, IR and UV spectra, while X-Ray diffraction confirmed that histamine dihydrochloride is a highly crystalline powder.

- Manufacture

Histamine dihydrochloride is produced by a proprietary process and purification steps to achieve histamine dihydrochloride of satisfactory quality.

The impurity profile of histamine dihydrochloride has been extensively investigated and meets the ICH requirements. All potential impurities have been identified and characterised. All solvents used in the manufacture of the active substance are controlled in compliance with ICH guidelines.

- Specification

The active substance meets the specification in the current Ph. Eur. monograph for histamine dihydrochloride. The specification includes tests for appearance, identification, assay (HPLC), impurities (HPLC, TLC, GC), water content, loss on drying, solubility. Whilst meeting the Ph.Eur. monograph for histamine dihydrochloride, other physico-chemical methods to characterise and control the quality of the active substance have also been established.

The analytical methods employed are either pharmacopoeial or have been adequately validated.

Batch analysis data have been provided for 10 batches of histamine hydrochloride, the 4 more recent manufactured by the method intended for commercial manufacturing. Earlier batches did not meet the Ph. Eur. requirements for appearance and clarity and as a result the purification process was modified. In all other cases the results comply with the proposed specifications.

- Stability

Stability studies were conducted according to the ICH requirements on three registration batches of histamine dihydrochloride using the commercial manufacturing process. Samples were stored at 40°C°C/75%RH , 25°C/60% RH, and 30°C /60% ..

The parameters tested were appearance, assay, chromatographic impurities, colour and clarity, pH, water content, microbial limit and endotoxins. The analytical methods used were the same as those used for routine testing and have been shown to be stability indicating.

The stability data demonstrate that histamine dihydrochloride is very stable with values of known and unknown substances often below the limit of quantitation.

Stability studies were also carried out at 5°C. In addition, photostability studies under conditions, which were in compliance with the ICH Q1B guidance, were conducted on one batch of histamine dihydrochloride. The studies showed no significant differences between exposed materials and controls, therefore it can be concluded that the bulk histamine dihydrochloride does not require any special precautions to protect from light during storage.

Freeze/thaw testing confirmed that the drug substance did not degrade when exposed to extreme temperature fluctuations as may be encountered during shipping.

Medicinal Product

- Pharmaceutical development

Ceplene is an immunomodulator that is administered subcutaneously as an adjunct to Interleukin-2 therapy and is available in single/unit dose vials. The product is designed for self-subcutaneous administration by the patient. No diluent is required.

Pre-formulation studies were carried out with histamine dihydrochloride dissolved in saline. The content of histamine dihydrochloride is based on findings of pre-clinical work. As the drug product is an aqueous solution of the active ingredient there are no physiochemical or biological properties relevant to the performance of the finished product. The formulation is simple, with sodium chloride to provide an isotonic solution, and water for injections as the solvent. The product is formulated to maintain pH in the range necessary for optimal stability. The formulation has been shown to be stable and to withstand autoclaving. The manufacturing process is standard for this type of formulation.

Originally the product was developed to be filled in vials containing 1.2 ml of the Ceplene solution corresponding to 2 doses, which were to be used within 12 hours of each other. In response to questions raised during the evaluation about the choice of the presentation the applicant reduced the fill volume of the vial to be equivalent to that of a single dose i.e.0.5 ml (with required overfill). Although single dose preparations are typically presented in ampoules, this is acceptable since this product will be self-administered in a home environment, and a vial is obviously more user friendly than an ampoule.

Compatibility studies monitoring the level of extractables from process equipment have been performed and a maximum holding time of the solution prior to filling has been established. The integrity of the container closure system has been evaluated using a challenge study with a microbial bath (*S. Marcescens*). The stoppers used in the finished product have been evaluated for extraction characteristics in accordance with the Ph. Eur. (Type I Elastomeric Closures) and the results indicate compliance with the Ph. Eur. requirements.

The clinical trials were undertaken using the proposed marketing formulation.

- Manufacture of the product

The manufacturing process has been adequately described and all critical process parameters have been identified and suitable in-process controls have been implemented to monitor the active substance dispensing, bulk solution compounding, filtering, filling, sealing, terminal sterilisation and inspection of the filled vials. Batch analysis data demonstrate that the process is reproducible and provides a drug product that complies with the in-process and finished product specifications.

- Product specification

Specifications for the finished product at release and shelf life have been established. The specification includes tests for appearance, identification (HPLC), assay (HPLC), pH, impurities (HPLC), volume in container (Ph. Eur.), pH (Ph. Eur.), sterility (Ph. Eur.), bacterial endotoxins (Ph. Eur.) and particulate matter. All tests included in the specification have been satisfactorily described and validated. Batch analysis data have been presented. All batches met the test limits as defined in the release specification and test methodology valid at the time of batch release.

- Stability of the product

Stability studies were conducted on drug product batches according to ICH guidelines Samples were stored at 5ºC, 25ºC/60% RH and 30ºC/60% RH and six months at 40ºC/75% RH.

Supplementary stability studies were also conducted to assess photostability and syringe stability. A shipping stress study has also been performed. In addition stability studies used to support early clinical development have been conducted on five lots of Ceplene at 25ºC/60% RH and have been provided supporting data.

The analytical methods used to assess stability have been validated as stability indicating or are compendial in nature. In all cases the stability results presented were satisfactory and support the proposed shelf life for the commercially packaged product, as defined in the SPC

Discussion on chemical, pharmaceutical and biological aspects

The quality of Ceplene is adequately established. In general, satisfactory chemical and pharmaceutical documentation has been submitted for marketing authorisation. There are no major deviations from EU and ICH requirements.

The active substance is well characterised and documented. The formulation is based on an aqueous solution. The excipients comply with Ph. Eur. requirements. The packaging material is commonly used and well documented. The manufacturing process of the finished product is a standard process that has been adequately described. Stability tests indicate that the product under ICH guidelines conditions is chemically stable for the proposed shelf life.

2.3 Non-clinical aspects

Introduction

Histamine is a pleiotropic endogenous biologically active amine that is widely distributed in the body and acts on multiple organ systems. Histamine is a mediator of immune and inflammatory reactions. Histamine is formed de novo during decarboxylation of histidine by histidine decarboxylase (histamine forming capacity or HFC) and is stored in the cytoplasmic granules of mast cells and basophils. Histamine synthesis, storage and release from endogenous pools are influenced by a variety of conditions including stress, circadian rhythms, drugs and allergens.

Endogenous histamine acts on a large variety of different cell types including smooth muscle, neurons, endocrine and exocrine cells, blood cells and cells of the immune system. Histamine covers its multiple biological actions *via* one of several receptors including H_1, H_2 and H_3 and most recently, H_4

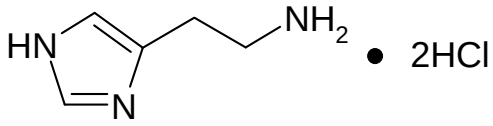
receptors. The receptors have been detected in mammalian brain, respiratory tract, genito-urinary system and vascular system, as well as on several types of leukocytes and haematopoietic cells. The principal receptors throughout the body are the H_1 and H_2 receptors.

- H_1 -- smooth muscle contraction (most muscle other than that of blood vessels), vasodilatation and vascular permeability increase
- H_2 -- leukocyte function, gastric parietal cell and cardiac stimulation
- H_3 -- neurotransmitter in central nervous system (CNS) histaminergic nerve endings (also present in other parts of the body)
- H_4 -- haematopoietic and immunocompetent cells (most recently discovered)

The mechanism whereby HDC exerts an anti-tumour effect is unknown but has been cited in literature since the 1970s. In combination with IL-2, it is thought that histamine acts as an immunomodulatory agent by binding to H_2 receptors on phagocytes, preventing the release of reactive oxygen species (ROS) and protecting natural NK function. This in turn would enhance the activity of immunotherapeutic cytokines like IL-2 by protecting effector immune cells from oxidative damage. The effect is thought to be mediated by both H_1 and H_2 type receptors.

Pharmacology

Structure of the active substance is histamine dihydrochloride.



- Primary pharmacodynamics

<u>*In vitro* effects</u>

In an *in vitro* study blasts recovered from peripheral blood of patients with AML were lysed by heterologous NK cells treated with IL-2, an NK cell activating cytokine (3, 4). The cytokine-induced killing of AML blasts was inhibited by monocytes recovered from peripheral blood. Histamine, at concentrations exceeding 0.1 µM, abolished the monocyte-induced inhibition of NK cells. The effect of histamine was completely blocked by the histamine H_2 receptor antagonist, ranitidine. Catalase, a scavenger of ROS, reversed the monocyte-inducted inhibition of NK cell-mediated killing of blast cells, indicating the inhibitory signal was mediated by products of the respiratory burst of monocytes.

<u>*In vivo* effects</u>

Table 1 presents primary pharmacodynamic studies in various tumour models in the rodents.

Table 1 Effects of histamine monotherapy in animal tumour models

Tumour model	Species	Histamine treatment	Effects	H_1 or H_2 mediated	Ref.
Fibrosarcoma	Mouse	6 mg/day i.p., daily	Reduced tumour growth and improved survival	H_2 blocker (metiamide) no influence on tumour growth but increased survival	(5, 6)
Chemically induced gut tumours	Rat	4 mg/day s.c., daily	Reduced glandular tumour in rats	H_2	(7)
Ascitis sarcoma	Rat	0.005 μg/day i.p.	Improved survival	Not stated	(8)
Chemically induced mammary carcinoma	Rat	0.1 mg/kg/day s.c., daily	Reduced tumour incidence and growth	H_2	(9)
Melanoma	Mouse	2.5–250 mg/kg i.v., single dose	Reduced pulmonary metastasis	H_2 receptor mediated	(6)
YAC-1 lymphoma	Mouse	125 mg/kg i.v., single dose	Reduced YAC-1 lung tumour emboli	H_2 receptor mediated	(10)
YAC-1 lymphoma	Mouse	125 mg/kg i.v., single dose	Improved NK cell clearance of YAC-1 tumour cells	H_2 receptor mediated	(11)
Colorectal carcinomas	Mouse	1 mg/kg/day i.p. daily	Reduced growth of human tumours transplanted to the subrenal capsule	H_2 receptor mediated	(12)
Syngeneic colorectal adenocarcinoma	Rat	0.5 mg/kg/day s.c. daily	Reduced growth rate of transplanted liver tumours in rats	Not stated	(13)
Leydig cell sarcoma	Rat	0.5 mg/kg/day s.c. daily	Reduced growth rate of transplanted liver tumours in rats	H_2 receptor mediated	(14)
Colorectal cancer	Mouse	Local administration via osmotic pump	Enhanced tumour growth	H_2	(15)
Leukaemia	Mouse	5 μg/day; i.p. daily for 5 days	Slightly increased survival in mice inoculated with murine B-cell leukaemia	Not stated	(16)

Note: i.p. = intraperitoneal; i.v. = intravenous; s.c. = subcutaneous

Monotherapy

The effect of histamine on NK cell function was evaluated by Hellstrand (10) using an *in vivo* mouse model assay developed by Hanna and Fidler (17). In this model, mice were injected with histamine, ranitidine or a control solution. Twenty-four hours later they were injected i.v. with ^{51}Cr-labeled YAC-1 lymphoma cells which form tumour emboli in the lungs and which are extremely sensitive to lysis by NK cells. Four hours after the YAC-1 injection the animals were sacrificed and the radioactivity present in their lungs was measured. The levels of radioactivity in lungs has been shown to be inversely proportional to NK cell function in that the greater the lysis by NK cells of the target YAC-1 cells the less radioactivity is shown in the lungs.

Hellstrand et al. showed that histamine treatment reduced the amount of lung radioactivity by up to 2/3 when compared to the control group. On the other hand, treatment with the H_2 antagonist, ranitidine, resulted in a tripling of the level of radioactivity. This suggests that histamine increased the effectiveness of NK cells in killing YAC-1 cell tumour emboli by preventing NK cell suppression by ROS. The inhibition of this effect by blockade of H_2 receptors reduced NK cell lysis of YAC-1 cells by up to two thirds indicating that the effect is mediated through H_2 receptors.

The effect of histamine and H_2 receptor involvement in this effect on NK sensitive cells was replicated by Asea (11) as indicated in the table above. Effects were shown to be most effective when histamine was administered 2-6 hours prior to cell inoculation, whereas administration 5 minutes prior to inoculation made no difference to tumour growth.

Combination therapy with IL-2
The combined treatment with histamine in synergy with IL-2 effectively protected mice against B16 melanoma lung metastases. IL-2 only weakly activates NK cells or T cells in an environment of oxidative stress. By inhibiting ROS production, histamine protects NK cells and T cells from down-regulation and apoptosis and thus markedly improves the IL-2-induced activation of T cells and NK cells in tumours (Table 2).

Table 2 Animal models used to assess anti-tumour activity of histamine in combination therapy with IL-2

Tumour model	Species	Effects	Ref.
B16 melanoma	Mouse	Potentiate reduction of metastatic lung tumours by IL-2	(10)
YAC-1 lymphoma	Mouse	Enhance NK cell mediated clearance of YAC-1 cells from lungs by IL-2	(11)
Dunning prostate adenocarcinoma	Rat	Potentiate anti-tumour properties of IL-2	(18, 19)
Intra-cerebral glioma	Rat	Reduce growth of established intracerebral tumours by 50%	(20)

AML is not able to be fully represented by animal models and therefore support for the use of histamine and IL-2 in this disease comes mainly from *in vitro* studies.

Below is listed the findings regarding cimetidine on tumour growth in animals and humans:

- enhance the growth and metastases of Lewis lung cancer in mice (21)
- slow metastatic development and prolong survival in mice bearing the Lewis lung carcinoma (22)
- in a murine B cell leukaemia model it did not have an effect on BCL1 tumour cell growth (16)
- cimetidine treatment of patients with gastric cancer has resulted in increased survival rates (23)
- mice receiving cimetidine (100 to 200 mg/kg in drinking water) were partially protected against a lethal inoculum of EL-4 lymphoma cells (24)
- cimetidine (100 mg/kg in drinking water) reversed the tumour growth enhancement in mice (25)
- demonstrated that whereas cimetidine suppresses the formation of B16 melanoma metastases in mice at a high dose (100 mg/kg), cimetidine and other H_2 receptor antagonists instead aggravate melanoma metastasis at lower doses (25 to 50 mg/kg) (10)
- significantly increased the incidence of chemically induced intestinal tumours in rats (26)
- long-term exposure (19 mg/mg) increases the frequency of spontaneous lymphoid neoplasms and potentiates chemical carcinogenesis in mice (27)

- Secondary pharmacodynamics

Histamine has proliferative effects on human and murine cells *in vitro*. Tilly and others (1) examined several human and murine cell lines and reported that histamine at a single concentration of 0.1 mM enhanced the *in vitro* growth of 3 cell lines (HeLa carcinoma cells, A431 epidermoid carcinoma cells and A875 melanoma cells) (2). This effect was apparently mediated by H_1 receptors, since the histamine-induced growth was inhibited by the H_1 antagonist, pyrilamine and not the H_2 antagonist, cimetidine. There is no evidence that this is predictive of effects of histamine *in vivo*.

The stimulation of histamine release or the administration of exogenous histamine may produce a multitude of physiological responses including allergic reactions and anaphylaxis, vasodilation and vasoconstriction, gastric acid secretion and neurotransmission. A brief summary of the response of multiple organ systems to histamine exposure in various species, including humans, is provided in Table 3.

Table 3 Summary of pharmacological effect of histamine by organ system

Organ system	Pharmacological effects	Ref.
Central nervous	• Functions as a neurotransmitter in the central nervous system. • Increases wakefulness via H_1 receptors, explaining the sedation by classical anti-histamines. • May inhibit appetite acting through H_3 receptors. • Histamine-containing neurons may participate in the regulation of drinking, body temperature, secretion of anti-diuretic hormone, control of blood pressure and the perception of pain.	(28)
Cardiovascular	• Endogenous histamine causes dilation of small resistance peripheral arterioles resulting in flushing, lowered total peripheral resistance and a fall in systemic blood pressure. • Exogenous histamine has been shown to lower blood pressure via H_1 and H_2 receptors in almost all mammalian species tested except the rabbit. • Tends to increase capillary permeability in certain tissues.	(21)
Respiratory	• Stimulates or more rarely, relaxes various smooth muscles depending on the vascular bed. • In the pulmonary circulation, H_1 receptor responses are associated with vasoconstriction and H_2 receptor responses are associated with vasodilation. • Increases vascular permeability in lungs leading to oedema formation	(22)
Gastrointestinal	• Stimulates gastric secretion and increases gastrointestinal (GI) motility. • An important physiological regulator of gastric acid secretion from parietal cells mediated by H_2 receptors.	(23)
Immune	• Affects the function of various cells including eosinophils, neutrophils, T lymphocytes and suppressor T cells. • Down-regulates several immunological functions through its interactions with H_2 receptors. • Inhibits the further release of histamine from mast cells and basophils. • Shown to modulate the Th_1/Th_2 cytokine balance and may restore the Th_1/Th_2 cytokine network and enhance anti-tumour activity of IL-2. • Triggers the acquisition of CD86 and HLA-DR antigens on human monocytes and thus, may facilitate the development of antigen-presenting dendritic cells from monocyte precursors. • Involved in immediate hypersensitivity and allergic response • Histamine appears to have a synergistic effect with IL-2 activation of NK cells and T cells leading to increased destruction of tumour cells.	(24-26)
Endocrine	• Modulates the release of various anterior pituitary hormones including adenocorticotropic, prolactin, TSH, LH and growth hormone • Participates in the stress-induced release of these hormones.	(27)
Renal	• Appears to be a potent vasodilator of both the systemic and renal circulation. • When infused into the systemic circulation or the renal artery of the dog, histamine causes vasodilation and an increase in renal blood flow. • The complex effects of histamine on the renal circulation appear to be mediated by actions on both H_1 and H_2 receptors in the rat, rabbit and dog.	(28)

Safety pharmacology programme

Histamine acts on multiple organ systems with the cardiovascular response being a major dose-limiting pharmacodynamic effect. For this reason, safety pharmacology studies were performed to evaluate the cardiovascular profile of histamine in the dog (**Study 82-0016**) and an *in vitro* hERG assay (**Study sph03-037**).

In vitro hERG assay (**Study sph03-037**)

The assay evaluated the potential for HDC and its major metabolites, imidazole-4-acetic acid (IAA) and N-methyl histamine (NMH) to inhibit a hERG encoded channel tail current. The effects of 10 µM of HDC, IAA and NMH were investigated after exposure of hERG assay using 100 nM E-4031 (a class III antiarrhythmic agent) as positive control. There was no statistically significant difference between the vehicle- and test article-treated groups, while a substantial tail current reduction was observed with E-4031. Based on these results, there was no evidence that HDC and its major metabolites inhibit hERG current *in vitro*.

Acute cardiovascular response in dog (**Study 82-0016**)
HDC was administered as a 10-minute s.c. infusion of 0.015 or 0.15 mg/kg (0.15 mg/kg approximately 20-fold a clinical single dose) to anaesthetised male dogs (n = 3). Blood sampling was made pre-dose and at 1, 2, 3 and 4 hours following start of infusion. ECG (8 leads) were obtained pre-dose, at 15 and 30 minutes and at 30-minute intervals up to 4 hours after start of infusion. Mortality, clinical signs and gross pathology were also evaluated.

No treatment-related adverse clinical signs or gross pathology findings were observed. The death of one animal at 0.15 mg/kg was judged as related to pre-existing hypoxia (results of pre-dose blood gas analysis) and the animal was replaced. No other treatment-related ECG or blood gas changes were observed. At 0.015 mg/kg, no treatment-related changes were observed. At 0.15 mg/kg, most haemodynamic parameters, including effects on heart rate, systolic and diastolic pressures (systemic, pulmonary), left ventricular systemic pressure, ventricular contraction/relaxation and cardiac output and stroke volume, were minimally to moderately affected. Most of these parameters returned to baseline levels.

- Pharmacodynamic drug interactions

Various functional classes of drugs have the potential to interact with histamine: compounds that inhibit histamine metabolism pathways (e.g., anti-malarial and anti-trypanosomal agents, neuromuscular blocking agents), release endogenous histamine (include a variety of drugs), act as histamine antagonists (e.g., in treatment of gastric peptic ulcers, various anti-depressants) and/or act as cardiotonic agents (e.g., ouabain, digitalis).

The study (**Study 82-0039**) was designed to investigate whether histamine exacerbates the IL-2-induced pulmonary capillary leakage. Male rats (10/sex/group) were administered subcutaneously with IL-2 and HDC in saline twice daily during 4 days and only one dose on the 5th day, as follows:

-	Group #1	Control (saline)	-
-	Group #2	Control (dextrose)	-
-	Group #3	IL-2 (low)	0.25 MIU/kg (to produce minimal to moderate toxic effect)
-	Group #4	IL-2 (high)	2.5 MIU/kg (to produce a moderate to severe toxic effect)
-	Group #5	HDC	100 mg/kg
-	Group #6	IL-2 (low)/HDC	0.25 MIU/kg/100 mg/kg
-	Group #7	IL-2 (high)/HDC	2.5 MIU/kg/100 mg/kg
-	Group #8	HDC/IL-2 (high)	100 mg/kg/2.5 MIU/kg

In each group 5 animals per sex were sacrificed at Day 5 to determine total fluid volume of the organs with no further pathology and the remaining at Day 6 for a complete histopathology performed.

No mortality occurred. Clinical signs of IL-2-induced vascular leak syndrome were observed in a dose-related fashion. Neither IL-2, HDC nor the combination of HDC plus IL-2 had any effects on body weights, haematology, urinalysis or clinical chemistry. Administration of both doses of IL-2 alone produced histological findings (vasculitis of lungs, liver bile duct hyperplasia and inflammation of injection site andiatic nerve). The combination of IL-2 and histamine gave similar histological effects. Female spleen-weight data suggested that the combination treatment might enhance the (high-dose) IL-2 effect of spleen total fluid volume. On the contrary, in males, by adding histamine, a significantly lower high-dose IL-2 effect on lung, spleen and liver total fluid volumes was observed. Thus, IL-2 alone induced a dose-related vascular leakage. The extent of the capillary leakage was not aggravated, but rather tended to decrease by addition of histamine.

Pharmacokinetics

The pharmacokinetic documentation consists of pharmaco- and toxicokinetic studies and published reports on endogenous and exogenous histamine.

- Methods of analysis

In all pharmaco- and toxicokinetic studies performed by the applicant, determination of plasma histamine levels was performed using commercially available ELISA (Immunotech) for rat, dog and rabbit and RIA (Immunotech) for monkey without subtraction of endogenous plasma histamine since none of the assays distinguish between exogenous and endogenous levels.

Rat and rabbit plasma histamine were tested linear in the 250-8.000 nM range, while dog plasma histamine was tested linear between 25-800 nM. Inter-assay precision and intra-assay precision were below 18.5% CV within predefined limits with one exception.

Monkey, but not apparently rat, rabbit and dog plasma histamine was highly unstable in room temperature, but also after frozen storage. The stability was somewhat improved by the addition of aminoguanidine (diamine oxidase inhibitor).

- Absorption

Endogenous levels of histamine were measured in various species with the highest levels found in the rat and the lowest in dogs and humans. The rabbit showed the greatest intra-individual variability in their endogenous plasma levels (Table 4). Higher levels of histamine were found in pregnant rats as also described in the literature.

Table 4 Main pharmacokinetics parameters after single administration

Study	Species	n per dose	Dose (mg/kg)	Route	C_{max} (nmol/l)	T_{max} (h)	AUC_{0-24h} (nmol.h/l)	$T_{½}$ (h)
82-0005TK	Rat male	1	2000	s.c.	563 000	-	313 000[a]	-
82-0010PK	Rabbit female	5	0.05	s.c.	7 200	24	67 100	1.5
			0.5		1 100	4.0	10 900	0.6
			5		5 300	0.5	30 000	1.1
			50		54 700	1.0	273 000[b]	3.7[b]
82-0009PK	Dog female	3	0.05	s.c.	45	0.4	14	-
			0.5		390	0.5	400	0.4
			1.5		1 020	0.5	1 440	1.7
			5		2 600	0.6	5 500	1.8

a AUC(0.83-1h)
b Death of 4/5 rabbits - figures considered as estimates only

Toxicokinetic data from a 28-day study (**Study 82-0008PK**) shows that the C_{max} values were variable but did increase proportionally from between 5 and 100 mg/kg on Day 28. Female animals were generally less exposed than male animals. T_{max} typically occurred within 0.1 to 0.3 hour, while $t_{½}$ was longer at the highest dose (100 mg/kg; about 1 hour). Baseline C_{max} levels were between 400-1,500 nM and AUC values at 0.5 and 5.0 mg/kg increased only slightly with dose; likely due to the high endogenous histamine levels.

Both C_{max} and AUC were dose-proportional between the 5 and 50 mg/kg in rabbits, even though plasma concentrations showed a high variability. This was probably due to the variable and high endogenous plasma levels of histamine in this species (baseline ~ 500 ± 500 nM).

Mean C_{max} at the higher doses were less than dose proportional in dogs. With increased dose, $t_{½}$ increased. AUC values were slightly greater than proportional to dose. Only female dogs were evaluated. However according to results of **Study 82-0105PK**, female dogs were somewhat higher exposed than male animals at the higher dose level (0.75 mg/kg). Clearance (Cl/F) decreased with increasing dose.

No pharmacokinetic parameters could be calculated for 0.05 mg/kg dose since most of the plasma concentration values were below the limit of quantification (25.0 nmol/l).

- Distribution

In mammalian tissues, endogenous histamine is present in all tissues in varying amounts ranging from less than 1 to over 100 μg/g. The distribution of exogenous histamine was determined in male rats in **Study 82-0119** (non-GLP) and in a study performed by Rizell et al (13).

Study 82-0119
Intra-duodenal administration resulted in a rapid systemic absorption of radioactivity (plasma and tissue t_{max} ~1h). Blood-to-plasma radioactivity ratios increased with time from 0.8 to 1.23, suggestive of binding to blood components. Mean tissue-plasma concentration ratios were similar between the 2 doses and remained relatively constant over time. The highest radioactivity concentrations relative to plasma were found in tissues associated with excretion with levels up to 130 μg-Eq./g (i.e., kidney, urinary bladder, liver and colon). The lowest levels were detected in the brain.

Rizell et al. 2002b (13)
In this study, histamine concentrations were measured in interstitial fluid collected from normal and malignant tissues by microdialysis using a RIA assay (^{3}H-histamine) following a single i.v. dose of 0.5 mg/kg. The highest radioactivity (C_{max} and AUC) was found in plasma, liver and in liver tumour while the levels in subcutis and subcutis tumour were lower.

There is no information on the distribution to the foetus.

- Metabolism

Histamine metabolism is well documented in the literature (29). Histamine is mainly metabolised by oxidation by diamine oxidase (DAO) or histaminase and ring methylation by histamine N-methyl transferase (HMT) and there is an extensive first pass metabolism. The metabolising enzymes are conserved between species, but show variations in tissue distribution.

Oxidation by DAO is the preferential pathways of renal histamine catabolism in several species including dogs and humans. In this pathway, the biologically active IAA, which is a chemo-attractant for leucocytes and an inhibitor of histamine release from leukocytes, is formed. At high doses (≥ 50 mg/kg) IAA possesses analgesic and narcotic activities. This oxidative activity seems however low in rabbit and mouse kidney. Elevated DAO activity has been reported in the pregnant mouse, rat and hamster and there are high placental levels of DAO, especially in man and rat.

The kidney is rich also in HMT. In the rat and guinea pig, deamination is the dominant route while in humans, as well as in the mouse, cat and dog, ring methylation appears to be the major pathway. In the rabbit and guinea pig, both routes appear to be important.

One or both of the enzymes have also been found in the intestine, stomach, liver, lungs and heart in various species. In humans, DAO is primarily located in the small intestinal mucosa, placenta, liver, skin, kidney, thymus and granulocytes whereas the HMT activity has been detected in the small intestine, liver, kidney and monocytes.

- Excretion

Histamine and its various metabolites have been identified in the urine of various mammals including man. Only a small fraction of histamine is excreted unchanged (30, 31).

After subcutaneous injection of ^{14}C-histamine to male dogs, urinary N-methyl imidazole acetic (NMIA) acid was predominating, followed by IAA and N^{τ}-methylhistamine (NHM). Only in this species, no unchanged histamine was detected in the urine. In humans, besides a significant proportion of IAA riboside (~20%) and small amounts of unchanged histamine (2-3%), the ratio of histamine and metabolites in the urine in humans after intra-dermal administration was similar in humans as in dogs. After infusion, excretion by the renal route was 85-95% with methyl imidazole acetic acid as the major urinary metabolite. Less than 10% was excreted as free histamine.

Sex differences were prominent in renal histamine metabolism. In the mouse, the kidney (and stomach) of females had a higher histamine-forming capacity (by action of histamine decarboxylase)

compared to males. The females also excrete more histamine than the males, but there is a great individual variation (32).

In **Study 82-0119**, mass balance parameters for a single dose of histamine were determined as shown in Table 5.

Table 5 Study 82-0119 – Mass balance parameter after single administration

Species	n	Dose (mg/kg)	Route	Anal.	Urine (% dose)	Faeces (% dose)	Recovery (% dose)	Time (h)
Rat	3	10	intraduodenal	LSC	48±5	31±15	84±15	24
	3	100	intraduodenal	LSC	54.3±2.6	32.4±0.9	93.1±1.2	24

After 24 hours, the total recovery was 84 and 93% in the 10 and 100 mg/kg dose groups respectively. There were no major differences between the doses.

During pregnancy, urinary excretion of histamine is increased in rodents. During the last third of pregnancy, the rat produces large amounts of histamine as indicated by its secretion in urine (33). The mouse sometimes produces 100 times the pre-pregnant value (34). No such differences were reported regarding the pregnant rabbit. There is no information on whether histamine is excreted in breast milk.

- Pharmacokinetic drug interactions

Two *in vitro* studies showed that histamine and its major metabolites (IAA, NMH) did not show any important effects on induction on (**Study XT033017**) or inhibition (**Study XT035014**) of the CYP450 expression in cultured human hepatocytes.

Toxicology

The applicant has documented the safety profile of HDC alone in studies of single and repeat dose toxicity, reproductive and developmental toxicity, genotoxicity and local tolerance. Subcutaneous administration was generally used. The dog, rat and rabbit were the main species. The dog is similar with human in terms of metabolic pathways as well as having low endogenous histamine levels and is thus considered to be a relevant species for human safety assessment. The rat has substantially higher endogenous histamine levels and it also tolerated much higher doses than the dog.

- Single dose toxicity

Single dose toxicity of HDC was studied in rats (non-GLP studies only), dogs and rabbits. In all species, expected histamine related effects were observed. In rats, one 'acute' maximum tolerated dose (MTD) of 2,000 mg/kg (death within 48 hours, C_{max} 1,000-fold, AUC > 500-fold the respective clinical exposure) and one 'delayed' MTD of 1,000 mg/kg (severe tissue necrosis at the injection site several weeks after administration, 25,000 times a proposed clinical single dose) were established. The highest non-lethal dose was 5 mg/kg in rabbits (C_{max}, AUC about 100-fold the clinical exposure). The NOAEL in this species was 0.5 mg/kg, with C_{max} and AUC 18- and 230-fold the clinical exposure after 1 mg. Dogs were most susceptible to histamine, where a 10-minute s.c. injection of 16.7 µg/kg (100 µg/kg/h) caused an increased heart rate, while < 80 µg/kg/h was without effect. In another dog study (non GLP), clinical signs occurred at 1.5 mg/kg, at C_{max} and AUC 20-30-fold the clinical exposure at 1 mg/day.

- Repeat dose toxicity (with toxicokinetics)

The pivotal repeat dose toxicity studies were undertaken in rats (up to 100 mg/kg bid for 6 months) and dogs (up to 0.5 mg/kg bid for 12 months). Histamine-related clinical signs occurred in both species (in rats: skin discoloration, warm to touch, injection site alopecia, dermal irritation, injection site reactions, in dogs: decreased activity, redness of ear, eyes, muzzle, skin, swelling of head, salivation, lacrimation and blood shot eyes). There were certain treatment-related changes of clinical chemistry and haematology parameters and in organ weights (in rats: increased adrenal, heart, liver and lungs at 200 mg/kg/day, in dogs: increased adrenals, reduced thymus and spleen at 1 mg/kg/day). However, there were no histopathological correlates. These changes were reversed after recovery in

both species. Thus, expected histamine related effects were observed, while there was no unexpected toxicity. In rats, the NOEL was 0.5 mg/kg bid for 6 months, resulted 50-60-fold the C_{max} and AUC the clinical exposure at 1 mg/day. In dogs, the NOEL was 0.05 mg/kg bid for 12 months, resulted in C_{max} and AUC in the clinical dose range while toxicity (mainly clinical signs) was seen at 4-5-fold the exposure.

Table 6 shows comparative systemic plasma exposure to histamine following s.c. administration of HDC in the rats, rabbits, dogs, healthy human volunteers (1 mg s.c.) and patients with metastatic melanoma.

Table 6 Interspecies comparison of systemic plasma exposure to histamine following s.c. administration of HDC

Study number	Species	BW (kg)	Dose (mg/kg)	Mean systemic exposure		Margins animal-to-human ratio of dose/exposure			
				C_{max}[a] (nmol/l)	AUC[a] (nmol·h/l)	BW	BSA	C_{max}	AUC
MP-MA 403	HHV	60	0.017	49	22	-	-	-	-
MP-MA 103	MMP	60	0.017	57	46	1	1	1	1
82-0104TK (6-month)	Rat	0.25	0.5	2 950	2 710	29	5	52	59
			5	19 000	8 530	294	48	333	185
			100	224 000	215 000	5 880	960	3 930	4 674
82-0103TK (12-month)	Dog	10	0.05	48	36	2.9	1.65	<1	<1
			0.25	239	192	15	8	4	4
			0.5	637	546	29	16	11	12
82-0014	Rabbit	3	2.5	NA[b]	NA[b]	147	1110	NA	NA

HHV = Healthy Human Volunteers; MMP = Patients with metastatic melanoma; BW = Body Weight; NA = Not applicable

a total histamine concentration (endogenous and exogenous)

b systemic exposure data were not used for the calculation due to high variability

- Immunotoxicity

Effects of histamine on immune function (35-37) are:

- Affects function of various cells including eosinophils, neutrophils, T lymphocytes and suppressor T cells
- Down-regulates several immunological functions through its interactions with H_2 receptors.
- Inhibits the further release of histamine from mast cells and basophils
- Shown to modulate the Th_1/Th_2 cytokine balance and may restore the Th_1/Th_2 cytokine network and enhance anti-tumour activity of IL-2
- Triggers the acquisition of CD86 and HLA-DR antigens on human monocytes and thus, may facilitate the development of antigen-presenting dendritic cells from monocyte precursors

The most relevant points in this context of this application are outlined below (the well-characterised role of histamine as a mediator in immediate hypersensitivity and allergic response is not further addressed).

Histamine down-regulates several immunological parameters through its interaction with H_1 and H_2 receptors. The suppressive effect on lymphocyte proliferation and cytokine release is a known property of histamine. Histamine inhibits certain functions of phagocytes and granulocytic cells, most notably, their ability to produce ROS and release TNF-α and IL-1-β. The inhibition by histamine on T-cell proliferation and cytokine production was closely related to suppression of autocrine IL-2 synthesis in proliferating or cytokine-producing T cells (38-40). However, the dose and regimen of IL-2 were chosen based on published research indicating that these doses and schedule will have significant biological activities and overcome any suppressive effect of HDC. In support for the notion that histamine does not reduce T-cell activity when administered with IL-2, Hansson et al. (41) showed that the addition of histamine and IL-2 to a mixture of mononuclear phagocytes and T-cells yielded a > 5-fold more efficient activation than that induced by IL-2 alone (as reflected by appearance of the CD69 activation antigen on the T-cell surface).

Consequently, when histamine is administered, a parallel inhibition of T-cell proliferation and phagocyte production of oxygen radicals may occur, which hypothetically could counteract the desired protection of tumour-killing lymphocytes. However, the inhibition of T-cell proliferation by histamine is unlikely to occur during the concomitant administration of histamine with IL-2. Thus, the inhibition is secondary to inhibition of endogenous synthesis of IL-2 in T-cells and the co-administration of IL-2 together with histamine counteracts the histamine-induced inhibition of T-cell function.

In the repeat dose toxicity studies, there was no evidence that HDC causes adverse effects on the immune system. In the rat studies, relevant parameters in terms of immunotoxicity were differential white-cell counts; lymphoid organ weights (thymus, spleen and lymph nodes); and microscopy of lymphoid tissues. Although changes occurred in some haematological parameters at the 6-month timepoint (increased white blood cell count, segmented neutrophils and monocytes in at least one sex), the effects resolved during the 4-week recovery period. No treatment-related changes were noted in lymphoid organ weights and no adverse histopathological effects were detected in lymphoid tissues. The doses tested resulted in several 100-fold higher exposure than the intended clinical use. Moreover, in the dog studies, there were no findings that raise major concerns, although reduced spleen weights were seen. Although not substantially evaluated, there was no indication from the non-clinical primary pharmacology studies that the combination of IL-2 and histamine results in immunosuppression.

- Genotoxicity

Genotoxicity was investigated in accordance with ICH Notes for Guidance (Standard battery for genotoxicity testing of pharmaceuticals [ICHS2B] [CPMP/ICH/174/95] and Specific aspects of regulatory genotoxicity tests for pharmaceuticals [ICHS2A] [CPMP/ICH/141/95]) i.e., testing mutagenic potential in bacteria, a mouse lymphoma assay and a micronucleus assay in the mouse (Table 7).

Table 7 Genotoxicity studies: overview

Study ID **Type of test**	**Test system**	**Concentrations** **Concentration range** **Metabolising system**	**Results** **Positive/negative/equivocal**
82-0025 Gene mutations in bacteria, plate incorporation assay	S. typhimurium (TA1535, TA1537, TA98, TA100) E. coli (WP2 uvrA)	+/- S9 0 to 5000 µg/plate (selected from prel. toxicity test)	No mutagenic response, no precipitate, no toxicity at any concentration. Positive controls as expected. Negative
82-0026 Gene mutations in mammalian cells	Mouse lymphoma assay L5178Y cells	+/- S9 (4 h incub) -S9 (24 h incub) 0 to 5000 µg/ml	No toxicity (≤ 50% reduced total growth) at 4 hours, only at 5000 µg/ml at 24 hours Criteria for valid test fulfilled Negative
61-0006 Chromosomal aberrations in vivo	Mouse, micronuclei in bone marrow. Males only, 10/harvest time	0, 20, 63, 200, 632, 2000 mg/kg	Dose-related (fr LD) clinical signs, necrosis at injection site (≥ 632 mg/kg), 3 HD mice died Dose- and time related PCE reduction (bone marrow toxicity) (signif. fr 632, 2000 mg/kg) No effect MN/ PCE ratio Negative

There was no indication that HDC has a genotoxic potential.

- Carcinogenicity

Neither *in vivo* assay was performed for investigating carcinogenic potential nor formal carcinogenicity study was found in the published literature.

- Reproduction toxicity

Reproductive and developmental toxicity study was conducted in rats with doses up to 100 mg/kg bid. In all studies, the high dose caused toxicity in the parent generation (injection site reactions, reduced

body weight and food intake). Effects on male fertility (decreased weights of prostate and seminal vesicles and increased sperm density) were observed only at 100 mg/kg bid. In females dosed 15 days before mating to gestation Day 7, reduced numbers of implantations and of viable foetuses were found. The incidences were similar at all doses and the reduction was statistically significant *vs.* controls at low and high dose. No systemic exposure data were obtained but in non-pregnant rats, the low dose corresponded to an AUC > 80-fold the clinical AUC after 1 mg HDC. No developmental toxicity was seen after dosing of rats (high dose 100 mg/kg bid) or rabbits (high dose 1.25 mg/kg bid) during the organogenetic period.

In the peri- and post-natal development study, the HDC caused maternal toxicity and the offspring showed toxicity during lactation (fewer live pups at lactation Day 21 compared to lactation Day 4) but not after weaning. The F2 generation was not affected. The maternal and offspring NOAEL was 10 mg/kg/day. There is no information on exposure of maternal rats, of pups or about milk transfer. Based on these data, it is recommended that women treated with Ceplene should not breastfeed.

- Local tolerance

Local tolerance studies have been conducted in the rabbits and guinea pigs with an HDC gel formulation. In the primary ocular irritation test, HDC was classified as a minimal eye irritant. No effects were seen in the delayed contact hypersensitivity test or in the primary dermal irritation test. In addition, injection sites have been investigated in detail in the repeat dose toxicity studies. Injection site reactions were common findings, primarily at high doses in rats (200 mg/kg/day).

- Other toxicity studies

The applicant has conducted one interaction/combination toxicity study (**Study 82-0039**) in rats to study the potential for histamine, known to increase capillary permeability, to aggravate IL-2 induced capillary leakage (see Pharmacodynamic drug interactions section).

Antigenicity
Not applicable

Dependence
Not applicable

Metabolites
No study was conducted

Studies on impurities
All impurities are generated during the drug substance manufacturing process and there are no new impurities in the final product. These impurities have been identified and characterised in accordance with the relevant ICH guidelines. The process impurity D704 has been fully characterised with a specification level of ≤ 0.2% w/w.

One residual solvent (cyclohexanol) does not have a release limit in ICH Q3C (Note for guidance on Impurities: Residual Solvents [CPMP/ICH/283/95]). The applicant has provided calculations of a permitted daily exposure (PDE). The maximal daily intake is < 1% of the estimated PDE raising no concern.

Ecotoxicity/environmental risk assessment
The applicant has provided an environmental risk assessment, taking into account the Guideline (Environmental Risk Assessment of Medicinal Products for Human Use [CPMP/SWP/4447/00]) that came into operation on December 1, 2006. In line with this guideline, the applicant has identified a refined Fpen, which was justified by market penetration data, but also taken a worst case assessment with a higher Fpen. The following is concluded:

- The low market penetration factor of 0.007% is not unexpected for a new treatment in an orphan indication. However, in a worst-case assessment, an Fpen of 0.05% (i.e., the disease prevalence threshold for orphan designation) have been applied as follows: The $PEC_{SURFACE\ WATER}$ is

estimated to be 1.0 x 10^{-4} µg/l, one hundred times less than the action limit of 0.01 µg/l that triggers a Phase II assessment.

- The above low predicted surface water concentration for HDC, combined with the innocuous nature and likely ready biodegradation of histamine and its human metabolites, indicates that the use of HDC in the proposed indication should pose a negligible risk to the environment.
- No precautionary measures are considered necessary regarding environmental release following the use of HDC in patients.
- No special labelling requirements regarding environmental aspects are considered necessary for HDC.

Discussion on the non-clinical aspects

From a non-clinical primary pharmacodynamic point of view, the applicant did not provide any proprietary studies for their proof of concept. The applicant commented that AML has no valid animal model that can simulate the disease correctly and therefore the best way to show its effect on AML is by *in-vitro* work. Although there is a plausible scientific rationale for the efficacy of histamine in combination with IL-2, a definite conclusion cannot be made on the basis of the non-clinical information. There are too many uncontrolled variables in the literature studies such as timing of dosing, type of tumour, doses used, length of treatment, etc. The question of efficacy in AML is deferred to the clinical dossier.

Histamine is a pleiotropic endogenous biologically active amine that is widely distributed in the body and exerts multiple functions. Physiological responses to histamine include anaphylaxis, vasodilation, vasoconstriction, gastric acid secretion and neurotransmission. The cardiovascular response to histamine may be considered a major dose limiting effect and in overdose profound tachycardia, cardiac arrhythmias, hypotension, and shock could be expected. Potential adverse reactions, including effects on the CNS, cardiovascular system, immune/inflammatory response, and respiration could be anticipated from the known pharmacology of histamine in an overdose situation. These effects influenced the selection of the therapeutic dose and the choice of 0.5 mg/day twice daily as likely the maximum, safe, well-tolerated dose in man administered on an outpatient basis.

The applicant conducted *in-vitro* and *in-vivo* studies to show that at doses up to 21 times the clinical dose, effects on cardiac conduction should be unlikely. Other haemodynamic parameters were affected at the highest dose but had returned to baseline by the end of the monitoring period.

Toxicity to IL-2 is related to its effect on increased capillary permeability. A proprietary study was conducted to show that histamine does not exacerbate the effects of IL-2 on capillary permeability as evidenced by histology. No other studies on the combination treatment were performed.

HDC has the potential to interfere with certain drugs. Certain P450 isozymes that modulate gene function by controlling the level of oxygenated lipids represent at least one common intracellular target of growth-regulatory biogenic amines (such as histamine). In addition, exogenous drugs including anti-oestrogens, anti-androgens and certain antidepressants also have the potential to target such isozymes and modulate cell growth. These findings could be viewed as being of potential relevance to the use of HDC as part of a treatment for AML. Critical drug interactions are not anticipated under the current protocol.

The doses and regimen of IL-2 were chosen based on published research indicating that these doses and regimen will have significant biological activities on the target lymphocytes. The dose levels were selected to target primarily high affinity and intermediate affinity IL-2 receptors on NK cells and T cells.

Histamine binding to H_2 receptors on parietal cells has been shown to produce a comparable median effective dose (ED_{50}), which led to the selection of the human dose of 0.5 mg of the histamine dihydrochloride salt as a compromise to allow for maximum gastric acid secretion and minimal adverse side effects. Twice-daily dosing appears to be appropriate to allow safe and effective

protection of NK cells and T cells, and yet, not compromise the innate defence against infectious agents.

Similarities exist in the pharmacokinetic profile of histamine across the species studied (rats, rabbits, dogs, humans). Histamine was measured in rats, rabbits, dogs and humans by a validated enzyme immunoassay. In monkeys, radioimmunoassay was used for histamine measurements. There was no way to differentiate between endogenous and exogenous histamine and therefore total histamine measurements in plasma were reported with the assumption that at low doses the measurement would mainly be of endogenous levels, whereas at high levels, the most likely source for histamine was exogenous. Endogenous circulating levels of histamine in plasma vary widely among species with the highest levels being reported in rat and guinea pig, followed by dog and monkey, and the lowest levels in man.

The proprietary pharmacokinetic studies conducted by the applicant were limited. There were absorption studies conducted in the rat (single high dose), rabbit and dog. These studies showed that histamine is rapidly absorbed with t_{max} reached in approximately one hour. Histamine has a short plasma half-life that is associated with rapid clearance from the body and a large volume of distribution. C_{max} was slightly less than dose proportional but as the dose increased the plasma half-life increased and clearance decreased. Its extensive distribution outside of the plasma compartment is consistent with the pleiotropic effects that histamine has on various organ systems. As mast cell granules are the main storage site for histamine it logically follows that the distribution of mast cells in the body correlates with the areas where histamine levels are highest, such as skin, connective tissue, gastric mucosa and mucosa of the bronchial tree. The liver is the preferential site for histamine uptake.

The metabolism of endogenous or exogenous histamine is qualitatively similar in all mammalian species. Methylation, catalysed by HMT, is the major pathway of metabolism in humans, as well as in the mouse, cat, and dog. In the rat, deamination via the DAO (or histaminase) pathway is the dominant route. Most of the product, NMH, is converted by monoamine oxidase to NMIAA. Each metabolic pathway can be blocked by enzyme inhibition or interfering compounds. Histamine metabolism has been largely elucidated by analysis of urinary metabolites after the administration of radiolabelled histamine. From the applicant's proprietary studies, it is unlikely that histamine or its major metabolites are able to interact with CYP P450 enzyme system in any way.

Histamine is rapidly cleared from the body primarily via the kidneys at higher doses.

The toxicity associated with the use of histamine dihydrochloride is related to exaggerated pharmacological responses in the species studied (rats, dogs, rabbits and monkeys). The dog was the most sensitive species to the effects of histamine whilst the rat was the least sensitive species. This is evident from the wide variation in the dose that was maximally tolerated and the respective NOAEL.

The general lack of cross-species consistency of the various effects, the ready normalisation of the effects during the recovery period and the absence of any histopathological correlates, strongly suggest a pharmacodynamic rather than a toxicological mechanism. In rats: red skin, warm to the touch, effects on adrenals, heart, liver, lungs. In dogs: dermal irritation, hypoactivity, increased respiration, scratching, biting, rubbing dose site, vocalisation during and after dosing, warm to touch, recumbence, excessive salivation, decreased blood pressure (Day 1), selected organ weight changes (adrenals, spleen, prostate and ovaries not correlated with adverse histopathology findings, and thus, not considered to be biologically relevant), minor effects on clinical chemistry and urinalysis parameters (not associated with gross pathology), and lymphoid depletion in the thymus. All findings were resolved following the recovery period. In addition, the effects noted seem most unlikely to be clinically relevant. Although the precise nature of the pharmacological mechanisms involved in the causation of the reversible effects noted in the chronic toxicity studies remains unclear, it seems possible that changes in microvascular permeability may play a role (42-44).

Histamine dihydrochloride is devoid of genotoxic potential as described by the ICH battery of genotoxicity tests performed on the product. No carcinogenicity studies were performed. This was considered acceptable based on criteria such as the conversion of the active ingredient to a

nature-identical substance following administration; the transient increase in plasma histamine in patients leading to a relatively minor increase in the overall burden; the serious nature of the disease being treated and the associated low life expectancy; the non genotoxic nature of the drug substance.

The lack of carcinogenicity studies is acceptable, considering the severity of the disease with a short life expectancy, histamine is an endogenous substance, the use of HDC will lead only a transient increase in plasma histamine in patients leading to a relatively minor increase in the overall burden, the non genotoxic nature of the drug substance and no absence evidence of pre-neoplastic pathology in the repeat dose toxicity studies.

Histamine dihydrochloride is not considered to have any adverse effects on reproduction. The effects noted during the reproduction studies were similar to those noted during the repeat dose studies. Although doses were, in some cases, maternally toxic, this did not have any effects on the embryo and foetus.

Dermal irritation tests performed in New Zealand white rabbits revealed that histamine dihydrochloride would be classified as a minimal irritant, but the study showed no associated microscopic lesions caused by the treatment. HDC does not cause delayed contact hypersensitivity.

Ceplene solution for injection 1 mg/ml does not represent a risk to the environment primarily due to the low market penetration factor of 0.007% and the calculated PEC $_{SURFACEWATER}$ for histamine dihydrochloride is below the 0.01 µg/l guideline threshold concentration.

2.4 Clinical aspects

Introduction

Ceplene has been investigated clinically for use in combination with cytokines such as IL-2 and IFN-α for the treatment of patients with malignant melanoma, renal cell carcinoma and AML.

GCP

The clinical trials were performed in accordance with GCP as claimed by the applicant.

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.

The CHMP requested a GCP inspection of **Study MP-MA-0201** which was conducted in May 2007 and concluded the sites were ICH-GCP compliant.

Pharmacokinetics

Seven clinical studies evaluated the pharmacokinetics and potential PK/PD correlations, following the subcutaneous administration of HDC solution.

No clinical pharmacokinetic studies of HDC were conducted in the AML target patient population.

- Analytical methods

Three bio-analytical methods were used (RIA, HPLC and ELISA). Pharmacokinetic parameters were calculated using non-compartmental methods.

- Absorption

The data from a total of 51 healthy subjects and 29 patients with malignant disease (mainly melanoma) were analysed. Each subject/patient received 1 mg HDC, infusion rates differed between studies (10-25 minutes). The calculated PK parameters for each study are presented in Table 8 for total and baseline corrected histamine.

Table 8 Summary of pharmacokinetic parameters for total histamine in plasma after subcutaneous injection of 1 mg

Population	Study	N	Baseline histamine (nmol/l)	C_{max} (nmol/l)	t_{max} (h)	AUC_{0-t} [d] (nmol•h/l)	$AUC_{0-\infty}$ (nmol•h/l)	$t_{1/2}$ (h)	CL/F (l/h/kg)	V_z/F (l/kg)
Total histamine										
HV	403[a]	21	1.5±0.9 (0-3.0)	49.10 (19.54)	0.28 (0.09)	22.3 (7.8)	24.5 (7.6)	0.27 (0.11)	3.46 (1.68)	1.53 (1.43)
HV	0501/ 0502[b]	6	2.4±2.3 (0-6.8)	42.8 (25.7)	0.389 (0.086)	34.9 (12.3)	44.6 (11.6)	1.51 (0.26)	1.38 (0.46)	2.92 (0.74)
HV	504[b]	24	-	35.0	0.40	25 (10)	31 (15)	0.71 (0.27)	2.9 (0.9)	3.1 (1.5)
MM	0103[b]	14	4.9±6.6 (1.3-25.6)	57.1 (25.0)	0.53 (0.47)	46.4 (25.6)	58.5 (47.8)	0.75 (0.79)	1.97 (0.96)	1.42 (0.41)
MM	0503[b]	8	2.0±2.3 (0-7.4)	33.4 (14.4)	0.531 (0.339)	28.2 (10.4)	33.5 (10.5)	1.69 (1.86)	2.21 (0.90)	6.76 (9.05)
MM	MM2[c]	7	0.7-4.2	21.7-86.9	0.13-1.00	10.8-44.5	16.0-49.2	0.39-2.80	1.7-5.3	1.7-17.5
Baseline-corrected histamine										
HV	403[a]	21		47.6 (19.6)		20.9 (7.8)	22.3 (8.0)	0.23 (0.09)	4.05 (2.4)	1.52 (1.56)
HV	0501/ 0502[b]	6		40.4 (25.4)	0.39 (0.09)	27.3 (11.8)	35.2[d] (7.3)	0.90[d] (0.34)	1.69[d] (0.36)	2.32[d] (1.25)
MM	0103[b]	14		52.2 (23.5)		36.4 (21.4)	36.9 (22.9)	0.43 (0.38)	2.78 (1.22)	1.45 (0.97)
MM	0503[b]	8		31.4 (13.3)	0.53 (0.34)	24.2 (12.1)				
MM	MM2[c]	7		20.9-84.9	0.13-1.00	7.2-30.4	7.4-30.8	0.12-1.62	2.5-12.6	0.8-12.5

HV: healthy volunteers, MM: malignant melanoma patients

a infusion duration 10 minutes

b infusion duration 20 minutes

c infusion duration 10, 15 or 25 minutes, range given

d last sampling time (t) was in study 403: 60 minutes, 0103: 120 minutes, 0501, 0502 and 0503: 240 minutes, MM2: 300 minutes, 504: 24 hours

Inter-individual variability is high. CV% was 40-60% for C_{max} and 35-55% for AUC_{0-t}, with a higher variability in patients than in healthy volunteers.

Pharmacokinetic data from two different occasions is available in one patient. There was a large difference in exposure in this patient (AUC_{0-t} was 27.8 and 10.8 nmol·h/l, although the same dose was given at both occasions), suggesting large intra-individual variability.

The absolute bioavailability has not been determined.

- Distribution

There is no information regarding plasma protein-binding. As there are no data after intravenous administration, information regarding V_{ss} is not available and V_{ss}/F could not be estimated. However, V_z/F (parameter of limited value) was reported (Table 8) and differed considerably between studies.

- Metabolism

No data on metabolism were provided. The pathways of synthesis and catabolism of histamine in man are well known (45).

Under physiological conditions, the metabolites of histamine have very little or no activity and are excreted in the urine.

- Elimination

Histamine is mainly eliminated by metabolism in the liver and other tissues. As there are no data after intravenous administration, information regarding CL is not available. CL/F varied considerably between studies and was on average around 2 l/h/kg. Half-life also varied between studies and was on average between 0.5 and 1 hour for exogenous histamine and between 0.75 and 1.5 hour for total histamine (Table 8).

The renal handling of infused ^{14}C-histamine was studied in healthy male subjects by measurement of ^{14}C-histamine and its metabolites in arterial and renal venous blood and in the urine. In man, the radioactivity of exogenously-administered ^{14}C-histamine is largely excreted in the urine during the first 6 hours. A large part of the histamine removed from the blood is metabolised in the kidney with only a small fraction excreted unchanged in the urine. The major metabolites found in the urine of healthy persons are N-methylhistamine and N-methyl imidazole acetic acid (MIAA).

The renal extraction ratio of histamine from human kidney is 0.7-0.8. This high extraction ratio for whole blood implies that a tubular transport mechanism in the kidney is responsible for renal removal of histamine from the blood.

- Dose proportionality and time dependencies

Dose proportionality and time dependency has not been studied. Pharmacokinetic data are available only after single dose administration of 1 mg.

- Special populations

Impaired renal function

The pharmacokinetics of HDC was evaluated 6 healthy and 17 renally impaired subjects (**Study MP-MA-0501**). HDC was administered at a single dose of 1 mg/ml by s.c. infusion over 20 minutes on Day 1. Renal impairment has no significant influence on the pharmacokinetics of histamine.

Published data show that when renal function is compromised due to renal disease, e.g. in the case of chronic pyelonephritis (inulin clearance was 5 ml/min), the extraction of ^{14}C-histamine from whole blood was reduced, 51% in patients *vs.* 74-86% in healthy volunteers. In addition, in chronic renal failure in 23 patients with a mean serum creatinine concentration of 5.1 mg/dl, the mean plasma histamine concentration was 5.5 ± 2.0 nmol/l, compared to 1.9 ± 0.4 nmol/l in 16 healthy controls.

Vital signs were monitored and it was reported that in the group with severe renal impairment, decreases in systolic and diastolic blood pressure (for which baseline values were higher compared to the other groups) were observed at plasma histamine concentrations, which caused no appreciable decrease in blood pressure in the other groups.

Impaired liver function

The pharmacokinetics of HDC was evaluated 6 healthy and 18 hepatically impaired subjects (**Study MP-MA-0502**). Each subject was allocated to 1 of 3 grades (Grade A, B and C) using Pugh's modification of Child's classification, during a pre-study visit performed within 1 week of Day 1. On Day 1, each subject received a single dose of HDC at 1 mg/ml by s.c. infusion over 20 minutes.

A summary of pharmacokinetic parameters is presented in Table 9.

Table 9 Study MP-MA-0502 - Summary of pharmacokinetics of total histamine

	Normal function n = 6	**Mild impairment n = 6**	**Moderate impairment n = 6**	**Severe impairment n = 6**
Baseline histamine (nmol/l)	2.1±1.1 (0-3.0)	1.9±1.6 (0-3.8)	118±175 (2.0-442)	77±77 (8.6-214)
C_{max} (nmol/l)	31.8±5.2	35.6±21.0	371±438	151±54.7
t_{max} (h)	0.417±0.053	0.386±0.165	0.389±0.174	1.201±1.542
AUC_{0-t} (nmol•h/l)	35.2±5.3	27.6±13.2	527.5±600.7	319.7±197.4
$AUC_{0-\infty}$ (nmol•h/l)	59.8	21.5±20.2	992.8	NA
CL/F (l/h/kg)	1.38±0.46	6.66±5.61	0.04	NA
VZ/F (l/kg)	1.08	3.13±2.30	0.07	NA
$t_{1/2,Z}$ (h)	2.1	0.3±0.1	1.3	NA
C_{max}/Dose (nmol/l/nmol/kg)	0.49±0.12	0.58±0.29	0.45±0.2	1.86±0.77
$AUC_{0\rightarrow\tau}$/Dose (nmol•hr/l/nmol/kg)	0.544±0.129	0.371±0.212	10.206±11.554	4.113±2.952
$AUC_{0\rightarrow\infty}$/Dose (nmol•hr/l/nmol/kg)	0.928	0.233±0.196	27.480	NA

Median plasma histamine concentrations were similar in subjects with normal hepatic function and in subjects with mild hepatic impairment, while median plasma histamine concentrations were higher in subjects with moderate and severe hepatic impairment.

Vital signs were monitored and it was reported transient effects (decreased in supine systolic and diastolic blood pressure more pronounced according to the hepatic impairment) in which returned to baseline value within approximately 1 hour after the start of the injection. No significant differences were observed in histamine, baseline-corrected, N-methylhistamine or baseline-corrected N-methylhistamine pharmacokinetic parameter estimates among the study groups. Therefore, no alteration in dosing is indicated in subjects with hepatic impairment, although higher plasma histamine concentrations may be encountered in subjects with moderate to severe hepatic impairment.

Gender
Potential difference in pharmacokinetics between men and women was evaluated in healthy volunteers and in patients.

No difference is observed in pharmacokinetic parameters using baseline-corrected histamine concentrations between male and female patients with advanced malignant melanoma except a shorter half-life than men (men: 0.53 hour, women: 0.28 hour).

Race
A comparison of pharmacokinetic parameters by ethnic origin was provided by the applicant. Although the study was small, with 5, 33 and 10 Asian, Caucasian and Hispanic subjects, respectively, no clinically significant differences between groups were observed.

Weight
In the overall pharmacokinetic analysis, weight was found to have no significant influence on the AUC of histamine.

Elderly
The pharmacokinetics of histamine was examined across an age continuum via a linear regression analysis. The minimum age was 19 years and the maximum age was 80 years. The mean age was 50 years. Age did not have any significant effect on any of the pharmacokinetic parameters of histamine.

Children
Neither paediatric study was conducted nor patients less than 18 years of age enrolled in the clinical studies.

- Pharmacokinetic interaction studies

In vitro
Two *in vitro* studies (**reports XT035014 and XT033017**) to determine the effect of histamine on a battery of CYP 450 isoenzymes have been conducted. The results indicated that histamine, N-methylhistamine and imidazoleacetic acid had little or no capacity to inhibit CYP 450 isoenzymes neither to induce CYP1A2 or CYP3A4 in primary cultures of human hepatocytes.

In vivo
In **Study MP-MA-0503** the interaction between HDC and IL-2 was evaluated in a total of 12 patients with either malignant melanoma (8) or renal cell carcinoma (4). This was a single-site, crossover study whereby each patient received HDC (1 mg) alone; IL-2 (18 MIU) alone; IL-2 (18 MIU) followed by HDC (1 mg); and HDC (1 mg) followed by IL-2 (18 MIU) during separate clinic admissions. Table 10 summarises the study results.

Table 10 Study MP-MA-0503 - Summary of pharmacokinetics of total histamine and IL-2

	Histamine			IL-2		
	HDC alone	**IL-2 before HDC**	**HDC before IL-2**	**IL-2 alone**	**IL-2 before HDC**	**HDC before IL-2**
C_{max} (nmol/l)	41.7 (18.6)	32.9 (12.8)	33.7 (13.5)	2488 (933)	1739 (745)	1982 (900)
t_{max} (h)	0.503 (0.239)	0.576 (0.365)	0.399 (0.153)	2.95 (1.67)	2.7 (1.7)	2.63 (1.46)
AUC_{0-t} (nmol•h/l)	31.7 (14.6)	26.8 (11.8)	28.7 (10.1)	15866 (4165)	12329 (3850)	13687 (4430)
$AUC_{0-\infty}$ (nmol•h/l)	36.8 (13.3)	29.5 (14.6)	39.9 (27.8)	16885 (5129)	13024 (3985)	14153 (4464)
$t_{1/2}$ (h)	2.53 (1.58)	1.15 (1.27)	2.42 (3.53)	3.02 (0.58)	3.10 (0.91)	2.85 (0.73)

IL-2 appeared having no clinically significant influence on histamine pharmacokinetics. HDC reduced IL-2 C_{max} in malignant melanoma cancer patients but had no or limited effect on histamine pharmacokinetic parameters in patients with renal cell carcinoma and on the other pharmacokinetic parameters than IL-2 C_{max} in patients with malignant melanoma.

- Pharmacokinetics using human biomaterials

In vivo pharmacokinetic interaction studies were conducted on cultures of human hepatocytes.

Pharmacodynamics

No pharmacodynamic studies have been submitted. Donskov et al. (46) have published pharmacodynamic data in renal cell carcinoma.

- Mechanism of action

No clinical studies to elucidate the mechanism of action of HDC in conjunction with low dose IL-2 in AML have been presented.

- Primary and secondary pharmacology

Primary and secondary pharmacology

Study MP-MA-103 comprised a substudy with the objectives to evaluate changes in biomarkers indicative of oxidative stress and immune cell function after HDC + IL-2 administration. The primary endpoints were changes, over time, in biomarkers, including CD3ζ,, neopterin, IL-6 and oxidative burst. Treatment with HDC and IL-2 provided a durable effect leading to an increased expression of CD3ζ, associated with a significantly increased proportion ($p < 0.0001$) of circulating NK cells (CD56+) in responding patients. Oxidative stress parameters at baseline and during therapy were significantly elevated in patients with progressive disease.

Secondary pharmacology

Study MP-MA-403 was a Phase I, dose-escalating, safety and pharmacokinetic study of HDC in healthy human volunteers. Three dose levels were tested 10, 15 and 20 μg/kg by subcutaneous injection BID for 14 consecutive days. Thereafter subjects were engaged in 14 days of outpatient follow-up. On Day 29 they received a 1-mg dose of HDC given repeatedly at least 2 hours apart in 3 successively increased rates of injection (50, 100 and 150 μg/min). For safety and tolerance 25 subjects were analysed.

The study drug was generally well tolerated. No clear dose-response relationship was revealed. Systolic and diastolic blood pressures tended to fall from baseline (range 5-30 mmHg) and heart rate increased post-dose. These effects resolved without treatment in 40 to 60 minutes.

All subjects experienced at least one drug-related adverse event (AE). These were numerous (such as flushing, headache, nausea and injection site reactions), but generally they were expected effects of histamine, transient and resolved without treatment or sequelae. No serious adverse events (SAE) occurred in the study. Two subjects discontinued due to an AE, one with nausea and an ear disorder of moderate intensity and the other with severe asthenia. Apart from transient changes in one subject, no marked changes in haematology and chemistry laboratory parameters were seen.

Clinical efficacy

A total of 15 clinical studies were conducted evaluating the efficacy and safety of HDC, only 2 clinical studies (**Studies AML-1** and **MP-MA-0201**) in the patient population of the proposed indication (Table 11), the other studies in malignant melanoma, renal cell carcinoma, chronic hepatitis C.

The clinical efficacy data arises from a population of 359 AML patients. **Study AML-1** was a single-arm, exploratory dose-finding Phase II single-arm study in 39 patients. Study **MP-MA-0201** in 320 patients was a multi-national, randomised, pivotal, Phase III study designed to assess the efficacy of HDC administered in combination with IL-2 compared to standard of care (defined as no treatment).

Table 11 List of clinical studies in AML patients

Study number	Design	Study objective	Dosage	Number of patients by arm: entered	Diagnosis	Study duration
AML-1	Phase II, investigator-sponsored, single-arm, non-controlled	Assess the clinical safety and effect on QoL of long-term immunotherapy	IL-2: 1 MIU/m^2, s.c., BID, Days 1–21 HDC: 0.3–0.7 mg, s.c., BID, Days 1–21; Other maintenance low-dose chemotherapy (cytarabine and thioguanine) was administered as alternating courses	39	Acute myeloid leukaemia	2 years or until disease relapse
MP-MA-0201	Phase III, multicentre, open-label, non-controlled, randomised	Determine if HDC and IL-2 could prolong duration of leukaemia-free survival compared with no treatment in AML pts in CR	Arm A IL-2: 1 µg/kg, s.c., BID, Days 1-21 HDC: 0.5 mg s.c., BID, Days 1-21 Arm B No treatment Rest period of 3 to 6 weeks between cycles	160/160	Acute myeloid leukaemia	18 months, consisting of ten cycles plus rest periods

- Dose response study

No dose-response study was performed.

Selection and timing of dose for the pivotal study was based on published data as mentioned in the primary and secondary pharmacology section.

Bibliographical data concerning the primary pharmacology were presented regarding IL-2 and HDC. Published data on histamine in normal human volunteers indicated that doses of 0.3-0.7 mg histamine base (corresponds to 0.5–1.0 mg HDC) were maximally effective on H_2-receptors to induce gastric acid secretion, yet minimising intolerable side effects such as intense flushing, hypotension and headache. Histamine binding to H_2-receptors on parietal cells (leading to maximal acid secretion) and leukocytes has been shown to produce comparable ED_{50}-values. This assumption led to the selection of the human dose of 1 mg of HDC, producing maximum gastric acid secretion and tolerable adverse side effects. Twice-daily dosing was judged to be appropriate to allow safe and effective protection of NK- cells and T-cells and yet, not compromise the innate defence against infectious agents (See clinical efficacy supportive study AML-1).

For the evaluation of HDC in conjunction with IL-2 as immunomodulating therapy in patients with AML in remission, a dose of 0.5 mg HDC was chosen. This dose was tested in study AML-1 and found to be acceptable for chronic outpatient administration in clinical practice.

- Main study

Study MP-MA-0201 was conducted as a pivotal, confirmatory, clinical study in patients with AML. This was a multicentre, randomised, open-label study to evaluate the safety and efficacy of subcutaneous histamine dihydrochloride plus IL-2 versus no treatment in patients with acute myeloid leukaemia in first or subsequent complete remission (CR)".

Methods

Study participants

This was a multicentre study conducted at 100 investigational sites in 10 countries.

Main inclusion criteria were:

- cytochemically-verified AML in CR 1 (patients in first remission) or CR 1+ (patients post first remission) and documentation of patient's remission status by bone marrow examination within 28 days of randomisation
- age 18 years or older
- prior induction therapy or consolidation therapy as per standard practice at the institution, including autologous stem cell transplantation
- life expectancy of more than 3 months and able to comply with study requirements
- a WHO Performance Status of 0 – 1 or Karnofsky score of 70 or greater

Main exclusion criteria were:

- prior treatment with allogeneic stem cell transplant
- abnormal cardiac function
- presence of other active malignancies, except carcinoma in situ of the cervix, localised squamous or basal cell carcinoma of the skin
- presence of active peptic or oesophageal ulcer disease or with past peptic ulcer or oesophageal disease with a history of bleeding
- requiring treatment for hypotension
- history of asthma treated in previous 5 years
- currently receiving continuing systemic treatment with clonidine, steroids and/or H_2 receptor blocking agents
- history of hypersensitivity to histamine or histamine products, severe allergies to food or contrast media requiring treatment in the last 5 years

Treatments

The eligible patients were allocated to one of the two treatment arms:

- Combination of IL-2, 16,400 IU/kg (1 µg/kg, twice daily) was first administered subcutaneously and 1-3 minutes after HDC 0.5 mg (twice daily) also subcutaneously over 5-15 minutes during a 21-day cycle. For Cycles 1-3, each cycle is followed by 3 weeks of rest, for Cycles 4-10, each cycle followed by 6 weeks of rest
- Standard of care: no active treatment/observation

The absence of treatment in the control arm is justified, since no treatment is the standard of care and there is no evidence that IL-2 alone improves leukaemia-free survival (LFS).

Treatment with HDC+IL-2 was started as soon as clinically feasible after completion of consolidation treatment and confirmation of complete remission. The protocol allowed 10 cycles of HDC+IL-2, approximately 18 months or until bone marrow relapse or death.

Treatment duration was chosen to maintain a protective level of immuno-stimulation during the high-risk phase that is during the initial 18 months post-consolidation of remission when > 85% of relapses would be expected.

Objectives
The original objective was double:
1. to determine if active treatment could prolong leukaemia-free survival (LFS) compared to standard of care in patients in first remission
2. to determine if active treatment could prolong LFS compared to standard of care in patients in subsequent remission

Due to poor recruitment of patients in subsequent remission the hypothesis was amended to determine if active treatment could prolong LFS compared to standard of care in patients in first or subsequent remission. These groups have different prognosis but the change of primary objective is not problematic since randomisation was stratified based on first or subsequent remission.

The secondary objectives included LFS at 6, 12, 24 and 36 months after randomisation, effects of treatment on LFS of patients in CR 1 and CR 1+, overall survival, safety, toxicity and quality of life.

Outcomes/endpoints
The duration of LFS was the primary endpoint. LFS was defined as the time elapsed from the date of randomisation to the date of relapse of AML or death from any cause. The widely accepted and clinically relevant laboratory definition of remission was < 5% blasts in bone marrow without extramedullary leukaemia, the standard goal in studies of leukaemia treatments.

Secondary efficacy endpoint of this study included duration of overall survival was treated in the same manner as LFS, Kaplan-Meier estimates of proportion of patients alive and relapse-free at specific timepoints and disease-free survival (DFS) in various prognostic groups of AML patients. In addition to these secondary endpoints, quality of life (QoL) was studied.

Sample size
The original statistical hypotheses were:
1. For a hypothesised improvement in median LFS of 50% in CR 1 patients in the combination group compared with control, a sample size of 96 patients with CR 1 in each treatment group reaching an evaluable endpoint within the 36-month follow-up would provide a statistical power of 80% to detect the hypothesised difference between treatment groups with a type I error rate of 0.05.
2. For a hypothesised improvement in median LFS of 75% in CR 1+ patients in the combination group compared with control, a sample size of 51 patients with CR 1+ in each treatment group would provide a statistical power of 80% to detect the hypothesised difference between the treatment groups with a type I error rate of 0.05.

The planned enrolment was 360 patients (240 in CR 1 and 120 in CR 1+), with equal distribution of each subset between the treatment arms.

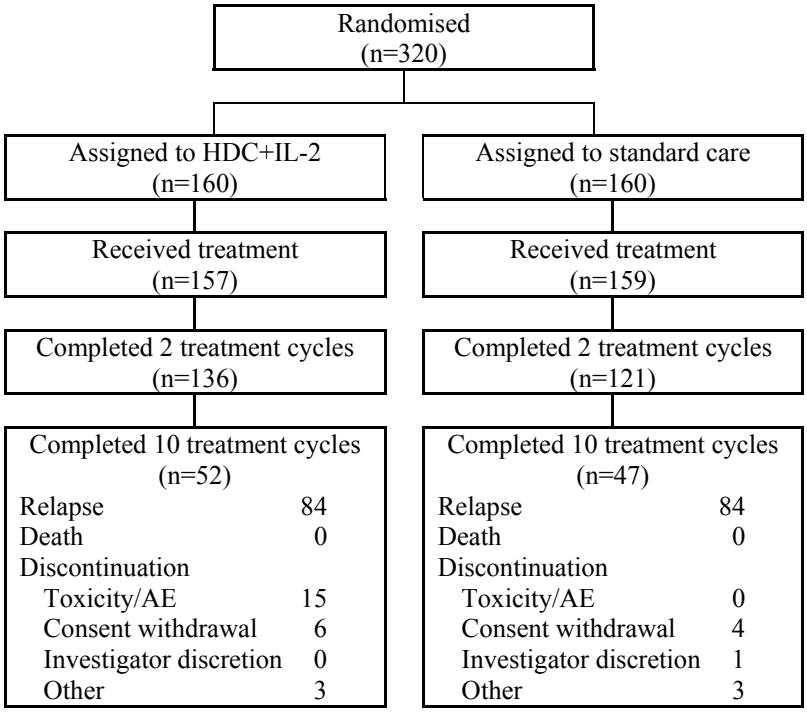
Randomisation
Central randomisation and stratification based on country and CR 1 *vs.* CR 1+ was performed.

Blinding (masking)
This study was not blinded due to the fact that there would be clearly observable physiological effects during HDC+IL-2 administration, such as skin flushing. However, sponsor staff and CRO data management were blinded to patient treatment.

Statistical methods
The primary analysis was performed using the log-rank test, stratified by country and CR stratum (CR 1 or CR 1+). The primary analysis was performed on the ITT population defined as all patients who were randomised, regardless of protocol violations. Secondary analyses to explore the impact of prognostic factors were conducted using standard survival analysis methods.

Results

Participant flow



Four patients (1 in the experimental arm, 3 controls) of the 320 patients dropped from the study 1, 7, 11 and 34 days after randomisation. The reasons were: one consent withdrawal, one lost to follow-up, one recurrent haematologic illness and one not eligible due to thrombocytopenia.

Recruitment
In the accrual period from June 1998 to August 2000, a total of 320 patients were randomly assigned to 2 treatment arms, 160 in each treatment group. Date of last patient last visit was October 2004. A total of 91 centres and 1 consortium of 9 centres enrolled patients with the range of 0-17 patients per centre.

Conduct of the study
The protocol was amended on four occasions a brief description of the principal changes is given below:
- 31st March 1998 – to make administrative changes, clarifications and minor changes e.g., a delay of one treatment cycle was allowed.
- 27th August 1999 – to extend the allowed period from completion of induction therapy from 2 to 3 months.

- 6th September 2000 – change of time of analysis from 12 months from last CR 1+ patient and 18 months from last CR 1 patient to 24 months after last patient enrolled. Removal of the proposed secondary analyses of relapse rates at 36 and 48 months.
- 1st November 2001 – change of analysis to 36 months after the last patient enrolled. Pooling of CR 1+ and CR1 patients due to poor enrolment of CR 1+. The primary endpoint remains duration LFS.

Thirty-eight percent (38%) of patients in the HDC+IL-2 group had enrolment deviations, somewhat higher than the control group (31%). No patients failed to meet basic AML diagnostic or CR criteria.

Baseline data
Baseline demographic and disease characteristics are displayed in Tables 12 and 13, respectively.

Table 12 Study MP-MA-0201 – Baseline demographic characteristics, ITT population

	HDC+IL-2 N=160	**Control N=160**
Age (years)		
mean (SD)	55.0 (14.00)	54.1 (14.26)
min, max	18.0, 81.0	18.0, 84.0
Age group (n [%])		
≤ 60	94 (59)	101 (63)
Race (n [%])		
white	153 (95.6)	149 (93.1)
black	3 (1.9)	4 (2.5)
Asian	3 (1.9)	2 (1.3)
other	1 (0.6)	5 (3.1)
Sex n (%)		
men	86 (53.8)	86 (53.8)
women	74 (46.3)	74 (46.3)
Height (cm)		
mean (SD)	170.9 (10.07)	170.5 (9.84)
min, max	148.0, 200.7	150.0, 197.0
Weight (kg)		
mean (SD)	77.2	75.9
min, max	43.0, 122.0	43.0, 138.9
Performance status (n [%])		
normal	125 (78.1)	114 (71.3)
symptoms without significant decrease in daily activities	35 (21.9)	46 (28.8)

Table 13 Study MP-MA-0201 – Baseline disease characteristics, ITT population

	HDC+IL-2 N=160	Control N=160
Days from AML diagnosis to randomisation		
mean (SD)	306.2 (288.1)	350.7 (448.0)
min, max	83.0, 1945	61.0, 2996
WBC at diagnosis ($x10^9$/l)		
mean (SD)	32.1 (54.8)	27.3 (51.7)
min, max	0.5, 426.0	0.4, 340.0
WBC category n (%)		
WBC > 20 x 10^9/l	62 (38.8)	48 (30.0)
WBC > 100 x 10^9/l	12 (7.5)	12 (7.5)
Number of courses to obtain current CR		
mean (SD)	1.2 (0.5)	1.3 (0.6)
min, max	0.0, 4.0	0.0, 4.0
Total number of CR		
1	130 (81.3)	132 (82.5)
2	27 (16.9)	21 (13.1)
3	3 (1.9)	4 (2.5)
> 3	0 (0.0)	2 (1.3)
Marrow blast percentage (after first induction course for current remission)		
mean (SD)	5.2 (11.0)	3.5 (5.7)
min, max	0.0, 80.0	0.0, 47.0
Percentage blast cells		
≤ 15%	149 (93)	149 (93)
> 15%	8 (5)	3 (2)
FAB classification group		
M0/M1/M5/M6/M7	59 (37)	56 (35)
M2/M3/M4	88 (55)	93 (58)
not classifiable	12 (8)	10 (6)
Karyotype at diagnosis, SWOG		
favourable	15 (9.4)	11 (6.9)
intermediate	76 (47.5)	75 (46.9)
unfavourable	18 (11.3)	18 (11.3)
missing	39 (24.4)	45 (28.1)
unknown	12 (7.5)	11 (6.9)
Days from consolidation to randomisation		
mean (SD)	75.4 (54.1)	72.2 (46.9)
min, max	20.0, 545.0	14.0, 468.0
Days from current CR to randomisation		
mean (SD)	149.9 (86.9)	134.4 (74.0)
min, max	6.0, 727.0	4.0, 553.0
Secondary AML		
yes	16 (10.0)	19 (11.9)
Extramedullary leukaemia		
yes	15 (9.4)	10 (6.3)
Autologous stem cell transplant		
yes	22 (13.8)	17 (10.6)
High-dose cytarabine therapy		
yes	107 (66.9)	109 (69.0)

FAB French-American-British classification of acute leukaemia
SWOG SouthWestern Oncology Group

Overall, patients in the 2 treatment groups were similar with respect to demographics or to disease characteristics. The majority of patients (83% of control and 81% of the HDC+IL-2 group) were in first remission or CR 1 at study entry. The mean number of induction/consolidation treatments required to achieve the current remission was 1 (ranged from 0 to 4) for each treatment group.

Similarly, the baseline demographic and disease characteristics were similar when the study population is broken into patient with CR 1 or CR 1+.

Numbers analysed
The primary efficacy analysis of all efficacy endpoints was conducted using the ITT population defined as all patients randomised (n = 320).

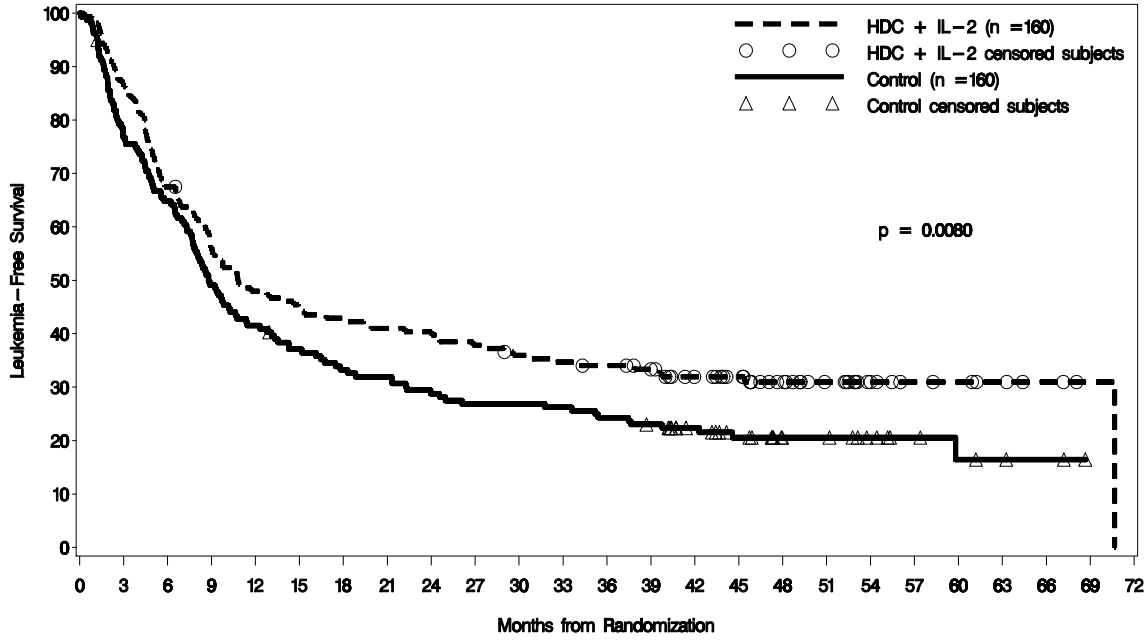
Outcomes and estimation
The primary efficacy and secondary analyses are summarised in Table 14 and in Figure 1.

Table 14 Study MP-MA-0201 – Leukaemia-free survival, ITT population

	HDC+IL-2 N=160	Control N=160	p-value
Days of LFS by quartiles of patients (95% CI)			
Median	324 (266, 550)	264 (231, 341)	0.0080[a]
LFS endpoint reached at time of analysis (n)	110	126	
Proportion of patients with LFS at 6-month intervals: Kaplan-Meier estimates			
6 months	67.5	64.8	0.61
12 months	47.9	41.5	0.25
18 months	42.9	33.2	0.08
24 months	40.3	28.8	0.03
36 months	34.0	24.7	0.06

a Log-rank test stratified by country and CR stratum

Figure 1 Study MP-MA-0201 – Leukaemia-free survival, ITT population



Results of LFS for patients with CR 1 at baseline are presented in Table 15. Figure 2 displays LFS for patients with CR 1 or CR 1+ at baseline, Figure 3 overall survival (OS) for the entire patient population and Figure 4 OS for patients with CR 1 at baseline.

Table 15 Study MP-MA-0201 – Leukaemia-free survival for patients with CR 1 at baseline, ITT population

	HDC+IL-2 N=129	Control N=132	p-value
Days of LFS by quartiles of patients (95% CI)			
Median	450 (293, 974)	291 (232, 406)	0.0113[a]
LFS endpoint reached at time of analysis (n)	103	82	
Proportion of patients with LFS at 6-month intervals: Kaplan-Meier estimates			
6 months	72.	66.7	0.34
12 months	52.5	43.9	0.17
18 months	47.8	36.2	0.06
24 months	44.7	31.6	0.03
36 months	39.9	26.2	0.02

a Log-rank test stratified by country and CR stratum

Figure 2 Study MP-MA-0201 – Leukaemia-free survival, ITT population

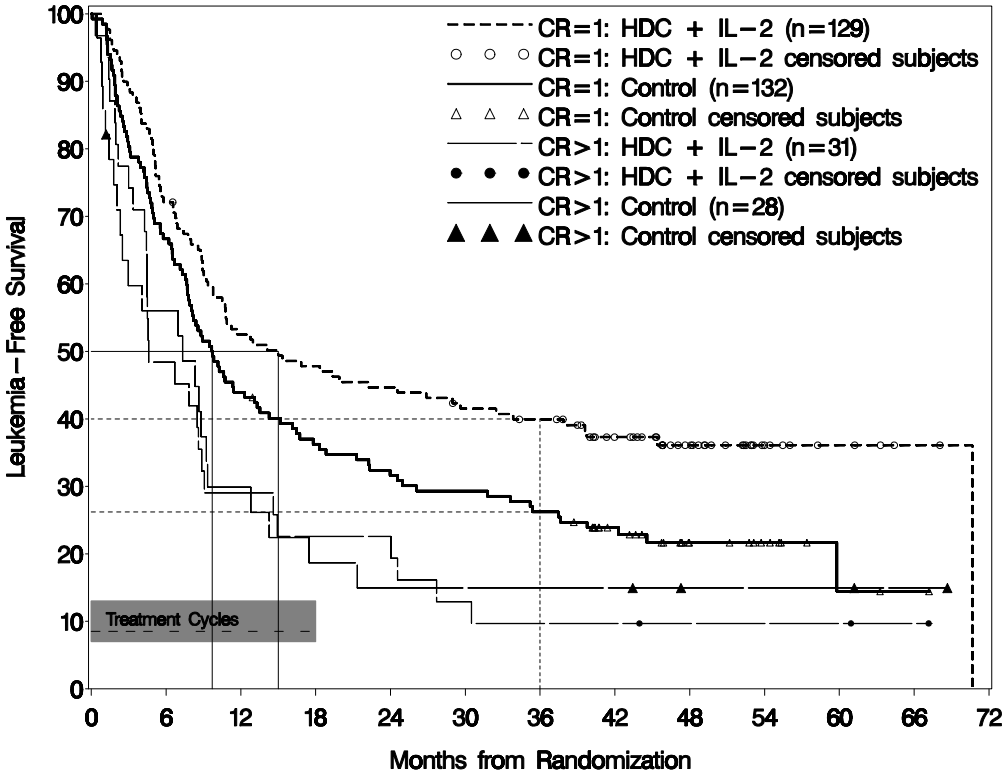


Figure 3 Study MP-MA-0201 – Overall survival, ITT population

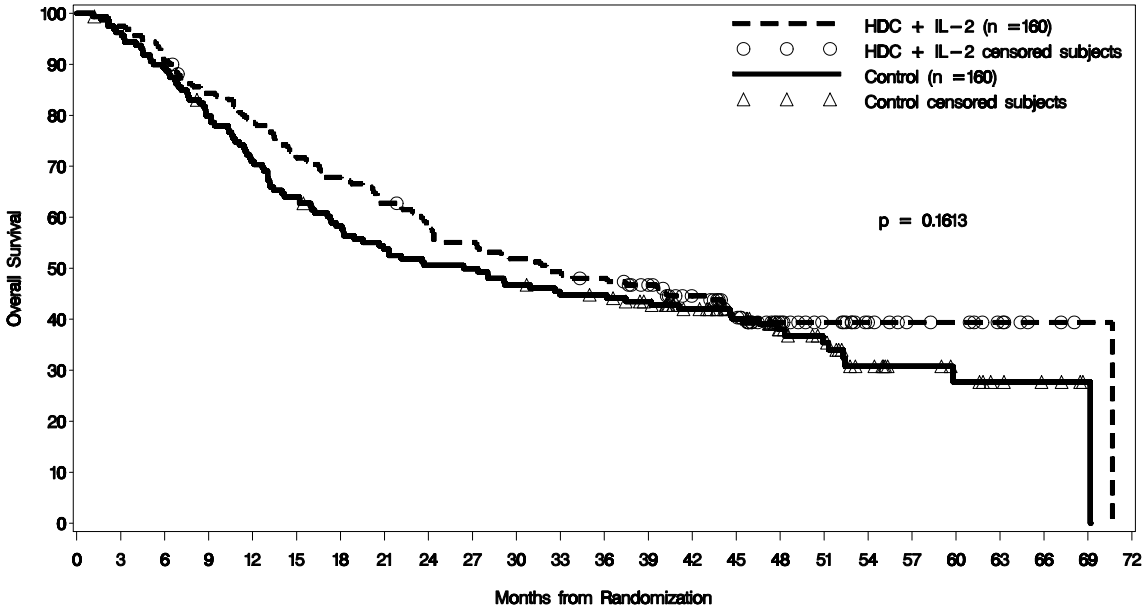
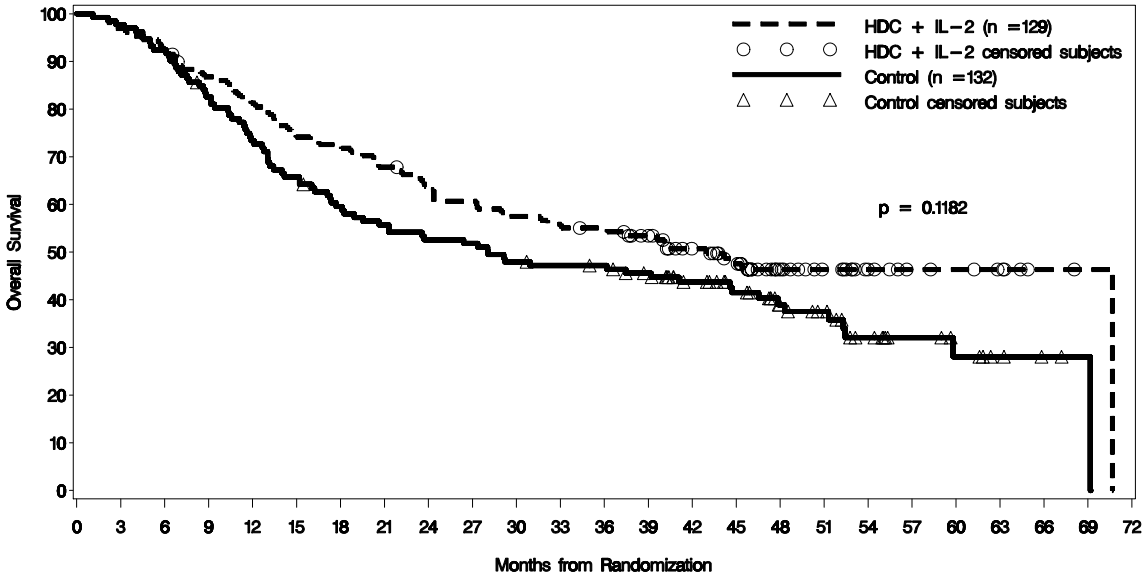


Figure 4 Study MP-MA-0201 – Overall survival for patients with CR 1 at baseline, ITT population



In response to the Day 120 list of questions, the applicant provided results of analyses of Quality of Life (QoL). The EORTC QLQ-C30 was used to evaluate the patients' QoL and was administered at baseline, before and at the end of the treatment periods of Cycles 3, 6 and 8 and at the study completion or at the early discontinuation visit. The QoL data analyses employed 7 scales for global QoL and personal functioning and 9 symptom scores which all showed modest differences that are consistent with the vasodilatory and immuno-inflammatory effects of HDC+IL-2. These analyses did not show clinically relevant loss of QoL due to this treatment regimen. Quality-of-life in HDC+IL-2-treated patients was maintained at levels similar to that of control patients receiving standard of care.

In response to the Day 180 list of outstanding issues, the applicant provided results of a sensitivity analysis censoring for deaths unrelated to AML relapse in the 261 patients with CR 1 using long-term follow-up data. Survival curve through 96 months showed a trend in favour of HDC+IL-2 over control ($p = 0.090$, stratified log rank test). At 5 years, the proportion of patients with CR 1 who died due to

AML were significantly lower in the group who received HDC+IL-2 than in those receiving no maintenance therapy (p = 0.037, Kaplan-Meier).

Ancillary analyses
In **Study MP-MA-0201**, there appeared to be an association between age 60 years or less, and treatment effect in the ITT and CR 1 populations. The log-rank test for LFS was statistically significant for patients up to 60 years of age (p = 0.02) but not for patients older than 60 years (p = 0.35).

For the overall population in **Study MP-MA-0201**, a significantly worse hazard for LFS was shown for men than for women (ratio 0.74, p < 0.036). Both men and women showed benefit from HDC+IL-2 treatment.

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

No analysis on pooled data from different studies or meta-analysis has been performed.

- Clinical studies in special populations

No study in special populations has been performed.

- Supportive study

Study AML-1 was a Phase II, non-randomised, uncontrolled, open-label, exploratory evaluation of feasibility, safety and efficacy of HDC+IL-2 to prevent relapse in patients with AML in remission. The trial was conducted between February 1993 (first patient first visit) and December 1999 (last patient last visit) in Sweden. This study is considered only supportive since the study design would not allow comparative analysis of efficacy.

Objectives: The primary objective was to determine the efficacy of IL-2 with the addition of HDC to extend the duration of leukaemia-free survival in patients with AML in remission. Secondary objectives were to evaluate the safety of the combination treatment and to evaluate feasibility of self-administration at home of this treatment.

Methods: Eligible patients were treated with IL-2 (1 μg/kg s.c., twice daily) and HDC (0.3-0.7 mg [starting dose] s.c., twice daily) in treatment cycles (3-week treatment plus rest periods of variable duration). Treatment cycles were given repeatedly for a duration of two years of complete remission or until relapse or death. Based on the medical status of the patient, individual adjustments of doses and duration of treatment and rest periods could be made.

Eight patients received IL-2 as a single agent for the first immunotherapy course. Another cohort of 30 patients received IL-2 as a single agent only during the first week of the first immunotherapy course. After the initial single drug IL-2 treatment, all patients were to receive the combination of IL-2 and HDC. Chemotherapy (low dose chemotherapy with cytarabine and thioguanine between the initial courses of immunotherapy) was given to some patients in addition study drugs.

Results: 39 patients were enrolled: 25 with CR 1 at baseline and 14 with CR 1+. A review of data by December 1999 showed relapse in 14 of the 25 patients with CR 1 at baseline and in 11 of 14 with CR 1+. Median leukaemia-free survival was 117 weeks for patients CR 1 at baseline and 49 weeks for patients CR 1+ at baseline. Leukaemia-free survival was in line with what has been reported for AML patients in the literature. Prognostic factors were overall not very favourable. The results of this initial study led to the development of the protocol for **Study MP-MA-0201**.

- Discussion on clinical efficacy

Study MP-MA-0201 showed a statistically significant difference in terms of LFS (p = 0.008, stratified log-rank test). For the CR 1 population, the difference in median survival was 159 days. Kaplan-Meier estimates of 3-year LFS in the CR 1 population were 26% in the control group and 40% in the HDC+IL-2 group (p = 0.02). No significant differences were observed in the CR 1+ population. No significant differences were observed in terms of overall survival.

The demonstration of efficacy of HDC in AML is entirely dependent on **Study MP-MA-0201**. In order that a single study may form the basis for granting of a marketing authorisation it should be adequately conducted and the results should be compelling.

A SAG-Oncology was consulted to advise on matters relating to the clinical relevance of maintenance treatment in adult patients with AML after the first remission, and in this patient population, whether IL-2 alone could be considered as an effective maintenance treatment and whether an improvement in LFS would represent a meaningful clinical benefit. In addition, the SAG-Oncology was asked to give its viewpoint on whether the non-clinical and clinical data demonstrated that histamine enhances/protects the activity of IL-2 and whether it would be feasible to perform further studies that seek to establish the effect size of histamine and IL-2 respectively in AML.

- The SAG-Oncology stated that there was no established maintenance treatment in adult patients with AML after first remission with the exception of ongoing investigational studies.
- There is no proof supporting that IL-2 alone is an effective maintenance treatment in adult patients with AML after the first remission. Several clinical studies, in general with a small sample size, have been conducted and none have demonstrated a benefit of IL-2 alone in this setting. However, there are larger, trials investigating this question and it should be noted that these studies have used varied dosing and schedules.
- LFS is an important clinical benefit endpoint, especially, for a patient population over 65 years of age. If LFS is the primary endpoint, overall survival should be a secondary endpoint and the clinical study must have sufficient evidence to allow concluding that overall survival is unlikely to be adversely affected by the treatment.
- The data provided do not demonstrate unequivocally that histamine enhances or protects the activity of IL-2. The proposed mechanism of action of histamine in this combination is plausible. The effect and the efficacy of histamine are supported with sparse data from very few non clinical experiments and the results of the clinical trials included in this application. The opinion on the robustness of the efficacy data presented diverged between participants at SAG-Oncology. Some were of the opinion that the single pivotal trial did not provide convincing evidence due to marked heterogeneity between participating centres/countries, insufficient support of pharmacological rationale, non-clinical data provided, absence of proof of concept in eradication minimal residual disease and finally the negative outcome of the clinical trials with HDC+IL2 in other indications questioned the validity of the rationale. Others considered that the biological rationale was sound and although the individual effects of the combination could not be delineated, the combination showed efficacy using a clinically relevant endpoint and given the unmet medical need and the manageable toxicity of the combination, the benefits were considered important, even in the absence of extensive supportive data. The negative results in solid tumours should not detract from the clinically relevant and statistically compelling results observed in AML based on good quality data confirmed by a GCP inspection.
- Many but not all SAG-Oncology members were of the opinion that a clinical study aiming at establishing the effect size of the combination histamine and IL-2 in treatment in adult patients with AML after the first remission could be performed and desirable. Others expressed the concern that the formal positive outcome for the primary endpoint is an issue because AML patients in this situation might be deprived of a potentially efficacious therapy for some years until further data might become available and further supportive clinical data could be acquired post-authorisation.

Clinical safety

- Patient exposure

Patients suffering from AML in first or subsequent remission and from metastatic melanoma (MM) constitute the main patient populations in the clinical development programme. In addition, smaller studies were conducted in renal cell carcinoma and hepatitis C. A total of patients of 1,188 were treated with HDC and 689 control patients (not exposed to HDC) were part in the clinical development programme (Table 16).

Table 16 Safety data set – Patient exposure

	Phase II and III n = 1188	MM+AML HDC+IL-2 n = 622	MM HDC 1.0 mg + IL-2 n = 426	AML HDC 0.5 mg + IL-2 n = 196
Total dose exposure of HDC (mg)				
Mean (SD)	172.4 (170.44)	117.7 (106.58)	115.1 (119.02)	123.2 (72.48)
Median	120	82.0	80.0	106.0
Min-Max	0-1322	2-994	2-994	8-294
Total exposure to HDC in days				
Mean (SD)	101 (91.46)	78.6 (70.94)	57.6 (59.51)	124.4 (72.33)
Median	64	50.5	40.0	108.5
Min-Max	0-661	1-497	1-497	8-308

Adverse events

The baseline demographic and disease characteristics of the patients with AML included in the safety database are similar to those of the patients of **Study MP-MA-0201** (Table 12 and 13). Table 17 gives an overview of adverse events in patient with AML.

Table 17 All Treated Patients in AML studies - Overview of adverse events

	HDC 0.5 mg+IL-2 n = 196	Standard of care n = 147
Number of patients with adverse event	195 (> 99)	140 (95)
Number of patients with a related adverse event	193 (98)	0
Number of patients with a severe or life-threatening adverse event	91 (46)	64 (44)
Number of patients with a serious adverse event	47 (24)	32 (21.8)
Number of patients with a AE associated with discontinuation of study drugs	59 (30)	1 (< 1)
Number of patients with a AE associated with death	3 (2)	3 (2)

The treatment-emergent adverse event (AE) which occurred in 5% or more of the patients in the Standard of Care versus the HDC 0.5 mg + IL-2 groups of patients are displayed in Table 18 below.

Table 18 All Treated Patients in AML studies - Summary of treatment-emergent adverse events in ≥ 5% patients

	HDC 0.5 mg+IL-2 n = 196	Standard of care n = 147
Blood and lymphatic system disorders		
Anaemia NOS	12 (6)	5 (3)
Eosinophilia	34 (17)	0
Neutropenia	11 (6)	4 (3)
Thrombocytopenia	38 (19)	13 (9)
Cardiac disorders		
Palpitations	18 (9)	3 (2)
Tachycardia NOS	23 (12)	2 (1)
Gastrointestinal disorders		
Abdominal pain NOS	12 (6)	6 (4)
Abdominal pain upper	14 (7)	6 (4)
Diarrhoea NOS	33 (17)	15 (10)
Dry mouth	11 (6)	0
Dyspepsia	27 (14)	5 (3)
Nausea	58 (30)	14 (10)
Vomiting NOS	29 (15)	6 (4)

	HDC 0.5 mg+IL-2 n = 196	Standard of care n = 147
General disorders and administration site conditions		
Chest pain	16 (8)	9 (6)
Fatigue	85 (43)	22 (15)
Feeling hot	22 (11)	0
Influenza like illness	31 (16)	6 (4)
Injection site bruising	18 (9)	0
Injection site erythema	69 (35)	0
Injection site granuloma	79 (40)	0
Injection site inflammation	25 (13)	0
Injection site pain	25 (13)	0
Injection site pruritus	39 (20)	0
Injection site rash	14 (7)	0
Injection site reaction NOS	42 (21)	0
Injection site swelling	13 (7)	0
Injection site urticaria	17 (9)	0
Oedema peripheral	11 (6)	7 (5)
Pain NOS	13 (7)	2 (1)
Pyrexia	82 (42)	22 (15)
Rigors	30 (15)	0
Weakness	14 (7)	9 (6)
Infections and infestations		
Herpes zoster	11 (6)	9 (6)
Influenza	10 (5)	10 (7)
Nasopharyngitis	4 (21)	23 (16)
Sinusitis NOS	13 (7)	9 (6)
Upper respiratory tract infection NOS	24 (12)	22 (15)
Investigations		
Platelet count decreased	9 (5)	8 (5)
Metabolism and nutrition disorders		
Anorexia	16 (8)	4 (3)
Musculoskeletal and connective tissue disorders		
Arthralgia	43 (22)	20 (14)
Back pain	2 (12)	15 (10)
Myalgia	36 (18)	3 (2)
Pain in limb	20 (10)	11 (8)
Neoplasms benign, malignant and unspecified (incl. cysts and polyps)		
Leukaemia relapse [1]	52 (27)	77 (52)
Nervous system disorders		
Dizziness	45 (23)	12 (8)
Dysgeusia	25 (13)	0
Headache NOS	112 (57)	20 (14)
Psychiatric disorders		
Anxiety	11 (6)	15 (10)
Depression	15 (8)	5 (3)
Insomnia	20 (10)	8 (5)
Respiratory, thoracic and mediastinal disorders		
Cough	49 (25)	18 (12)
Dyspnoea NOS	28 (14)	5 (3)
Nasal congestion	21 (11)	2 (1)
Pharyngitis	26 (13)	21 (14)
Rhinorrhoea	11 (6)	3 (2)
Skin and subcutaneous tissue disorders		
Erythema	17 (9)	3 (2)
Night sweats	11 (6)	6 (4)
Pruritus NOS	16 (8)	5 (3)
Rash NOS	26 (13)	7 (5)
Skin lesion NOS	5 (3)	9 (6)
Sweating increased	20 (10)	2 (1)

	HDC 0.5 mg+IL-2 n = 196	Standard of care n = 147
Vascular disorders		
Flushing	159 (81)	0
Hypotension NOS	24 (12)	4 (3)

1 Leukaemia relapse was considered to be efficacy endpoints; investigators did not consistently report these as AE and assessments of efficacy of HDC were not appropriately based on these datasets

The Grade 3/4 treatment-emergent AE that occurred in at least 1% of the patients in the AML studies are presented in Table 19.

Table 19 All Treated Patients in AML studies - Grade 3/4 treatment-emergent adverse events in ≥ 1% patients

	HDC 0.5 mg+IL-2 n = 196	Standard of care n = 147
Number of patients who had at least 1 Grade 3/4 AE	91 (46)	64 (44)
Leukaemia relapse [1]	26 (13)	40 (27)
Thrombocytopenia	19 (10)	3 (2)
Headache	13 (7)	0
Neutropenia	5 (3)	1 (< 1)
Pyrexia	4 (2)	1 (< 1)
Platelet count decreased	4 (2)	1 (< 1)
Diarrhoea	4 (2)	0
Influenza like illness	4 (2)	0
Eosinophilia	3 (2)	0
Injection site granuloma	3 (2)	0
Arthralgia	3 (2)	0
White blood cell count increased	2 (1)	3 (2)
Fatigue	2 (1)	2 (1)
Liver function tests NOS abnormal	2 (1)	1 (< 1)
Back pain	2 (1)	0
Injection site pain	2 (1)	0
Injection site pruritis	2 (1)	0
Weakness	2 (1)	0
Dizziness	2 (1)	0
Dysgeusia	2 (1)	0
Flushing	2 (1)	0
Nausea	2 (1)	0
Musculoskeletal pain	2 (1)	0
Febrile neutropenia	1 (< 1)	2 (1)
Cardiac congestive failure	0	2 (1)
White blood cell count decreased	0	2 (1)

[1] Leukaemia relapse was considered to be efficacy endpoints; investigators did not consistently report these as AE and assessments of efficacy of HDC were not appropriately based on these datasets

- Serious adverse event/deaths/other significant events

Deaths which occurred in the clinical development programme while the patients were in the study or within 28 days of the last dose of study drug are reported in Table 20.

Table 20 Phase II and III studies - Summary of deaths

	HDC [1] n = 1188		Non-HDC [1] n = 689	
	Number	Cause of death	Number	Cause of death
MP-MA-0201	4	AML	1	AML
AML1	None		None	
MP-8899-0104	16	Melanoma	18	Melanoma
MP-US-M01	12	Melanoma	11	Melanoma
	1	Cardiopulmonary arrest	1	Myocardial infarcts/cerebral infarcts
	1	Unknown	2	Liver failure
			1	Pulmonary embolus
			2	Study drug
MP-MA-0102	12	Melanoma	11	Melanoma
MP-MA-0103	14	Melanoma	None	
	1	Pneumonia		
MM2	8	Melanoma	None	
I-318-MA	1	Renal cell carcinoma	None	
	1	Pneumonia		
MP-MA-0405	1	Unknown	None	
MP-S01	4	Metastatic melanoma	None	

1 Studies MP-S02 and MP-S04 conducted in renal cell carcinoma are not included

Serious adverse events which were experienced by at least 3 patients in either treatment group in AML studies are presented in Table 21.

Table 21 All Treated Patients in AML studies - Serious adverse events experienced by at least 3 patients

	HDC 0.5 mg+IL-2 n = 196	Standard of care n = 147
Number of patients with at least 1 SAE	47 (24.0)	32 (21.8)
Blood and lymphatic system disorders	13 (6.6)	8 (5.4)
Febrile neutropenia	3 (1.5)	2 (1.4)
Thrombocytopenia	8 (4.1)	3 (2.0)
Cardiac disorders	4 (2.0)	4 (2.7)
General disorders and administration site conditions	10 (5.1)	6 (4.1)
Pyrexia	8 (4.1)	5 (3.4)
Infections and infestations	10 (5.1)	5 (3.4)
Pneumonia NOS	4 (2.0)	0 (0.0)
Musculoskeletal and connective tissue disorders	7 (3.6)	1 (0.7)
Back pain	4 (2.0)	0 (0.0)
Neoplasms benign, malignant and unspecified (incl. cysts and polyps)	17 (8.7)	13 (8.8)
Leukaemia relapse [1]	13 (6.6)	10 (6.8)

1 Leukaemia relapse was considered to be efficacy endpoints; investigators did not consistently report these as AE and assessments of efficacy of HDC were not appropriately based on these datasets

- Laboratory findings

The Grade 3 and 4 NCI post-baseline toxicities for selected haematology and chemistry laboratory tests for AML studies are presented in Table 22.

Table 22 All Treated Patients in AML studies - Grade 3/4 toxicities – selected haematology and chemistry laboratory tests

	HDC 0.5 mg+IL-2 n = 196	Standard of care n = 147
Haemoglobin	13 (7)	6 (4)
WBC count	30 (17)	22 (17)
Lymphocytes count	13 (9)	10 (11)
Platelet count	39 (23)	32 (27)
Alkaline phosphatase	0	0
Creatinine	1 (< 1)	1 (< 1)
SGOT (AST)	5 (2)	1 (< 1)
Total bilirubin	0	1 (< 1)

- Safety in special populations

Age

Overall, there was no major clinical differences between the incidences of AE in patients < 65 and ≥ 65 years of age in any of treatments (IL-2 alone, HDC+IL-2, HDC 0.5 mg+IL-2, HDC 1 mg+IL-2 or standard care) or when comparing across the treatments.

Gender

It did not appear to be any consistent relationship between the patient gender and the treatment administered (IL-2 alone, HDC+IL-2, HDC 0.5 mg+IL-2, HDC 1 mg+IL-2 or standard care) for almost all of the AE in which there was a difference of at least 5% between male and female patients. However, difference between males and females for headache NOS, nasal congestions, hypotension NOS and injection site erythema appeared to be consistent across the different treatment administered.

- Safety related to drug-drug interactions and other interactions

Study MP-MA-0503 was the only drug interaction study conducted (see section on pharmacokinetics).

- Discontinuation due to adverse events

The most frequently experienced AE that led to premature discontinuation of study drugs in patients in the AML studies were thrombocytopenia, platelet count decreased, neutropenia and pyrexia.

- Post-marketing experience

HDC has not been approved or marketed in any country.

- Discussion on clinical safety

A total 196 patients with AML have been exposed to HDC and IL-2 combination treatment, the size of this safety dataset is considered acceptable. Almost all patients received 0.5 mg of HDC.

The most common adverse drug reactions induced by the addition of HDC to IL-2 are flushing, hypotension, headache and injection site reactions, as identified in studies malignant melanoma where the comparison with IL-2 alone was possible.

Despite the higher rate of side effects in the experimental arm (33 *vs.* 17%) and the treatment constraints, the proposed treatment regimen does not appear to impact negatively on the quality of life of patients.

Very few deaths were reported during the treatment period or shortly after in the AML studies. All cases were attributed to the underlying disease and not related to study medication (1 death *vs.* 4 in the control and combination arms, respectively). The proportion of patients with SAE is slightly higher in the HDC+IL-2-treated patient group, 24.0 *vs.* 21.8%. Thrombocytopenia, infections and back pain were causes that were increased in this group.

The defence against infectious agents may be affected by HDC due to the phagocyte inactivation. Considering the overall treatment duration (18 months) and the therapy affecting the immune system it

could be postulated an increased risk of infection, as shown by the slightly increased number of SAE with infection as cause.

2.5 Pharmacovigilance

Detailed description of the Pharmacovigilance system

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

Risk Management Plan

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

Table Summary of the risk management plan

Safety concern	**Proposed pharmacovigilance activities**	**Proposed risk minimisation activities**
Hypotensive episodes	Routine pharmacovigilance	Patients should be monitored during treatment for possible clinical complications due to hypotension or hypovolemia (SPC section 4.2, 4.4).
Vasodilation	Routine pharmacovigilance	Patients should be monitored during treatment for possible clinical complications due to vasodilation (SPC section 4.2, 4.4).
Increased gastric acid secretion	Routine pharmacovigilance	Patients should be monitored during treatment for possible clinical complications due to increased gastric acid secretion (SPC section 4.4).
Local injection site reactions	Routine pharmacovigilance	Patients should be monitored during treatment for possible clinical complications due to local injection site reactions (SPC section 4.8).
Method of Administration	Routine pharmacovigilance	Detailed information on method of administration including administration rate, location and time between subsequent administrations as well as potential risk for hypotensive episode are provided (SPC section 4.2, 4.4, 4.8, 4.9).
Infections	Routine pharmacovigilance	Patients should be monitored during treatment for possible clinical complications such as neutropenia, febrile neutropenia, and sepsis (SPC section 4.2, 4.4).
Haematological Events (e.g. thrombocytopenia/eosinophilia)	Routine pharmacovigilance	The following information is provided in the SPC section 4.4,

		"Monitoring of laboratory test results is recommended including standard haematological and blood chemistry tests.". In the PL, patients are informed that blood tests will be performed during treatment. Patients should be monitored during treatment, monitoring of laboratory test results is recommended including standard haematological and blood chemistry tests
Interactions with other medicines (e.g. antihypertensive medicines, H1 and H2 blockers, tricyclic antidepressants, neuroleptics, histamine releasing medicines)	Routine pharmacovigilance	Patients should be monitored during treatment for possible interactions which are detailed in the SmPC and are appropriately addressed in the PL. *"Patients receiving the following medicinal products should be treated with caution (see section 4.5): Beta-blockers or other anti-hypertensive agents; H1 blocking agents and neuroleptics (anti-psychotics) with H1 receptor blocking properties; -Tricyclic antidepressants that may have H1 and H2 receptor blocking properties; -Monoamine oxidase inhibitors and anti-malarial and antitrypanosomal agents; -Neuromuscular blocking agents, narcotic analgesics, and various contrast media."* (SmPC section 4.4) There have been no specific studies on the effects of alcohol with IL-2+HDC treatments (SPC)
Patients with cardiac disorders	Routine pharmacovigilance	Patients with cardiac disease should be evaluated for VEFand wall function by echocardiography or nuclear medicine stress test and then treated with caution (see SPC section 4.4)
Patients with hepatic or renal Impairment	Routine pharmacovigilance	Patients with hepatic or renal impairment should be monitored during treatment for possible clinical complications as these patient populations have a disease which may itself affect drug metabolism or excretion (see SPC sections 4.4 and 5.2)

Paediatric population data	Routine pharmacovigilance	Ceplene has not been evaluated in the paediatric population (SPC section 4.1 and 4.2)
Pregnancy and Lactation data	Routine pharmacovigilance	No clinical data are available in pregnant women or on the effects on fertility and that it is not known if HDC is excreted in human breast milk. Due to the fact that Ceplene is to be administered in conjunction with IL-2, Ceplene must not be administered during pregnancy (SPC section 4.6, 5.2, 5.3).

The CHMP, having considered the data submitted in the application, is of the opinion that no additional risk minimisation activities are required beyond those included in the product information.

2.6 Overall conclusions, risk/benefit assessment and recommendation

Quality

The quality of the product is considered to be acceptable. Physicochemical and biological aspects relevant to the consistent clinical performance of the product have been investigated and are controlled in a satisfactory way. There are no unresolved quality issues, which have a negative impact on the Benefit:Risk balance of the product.

Non-clinical pharmacology and toxicology

Pharmacodynamic data are mainly based on bibliographic references. The effect on acute myeloid leukaemia (AML) is based on *in vitro* studies. Although there is a plausible scientific rationale for the efficacy of histamine dihydrochloride (HDC) in combination with interleukin (IL-2), a definite conclusion cannot be made on the basis of the non-clinical information.

The proprietary pharmacokinetic studies conducted were limited. Data showed similarities in the pharmacokinetic profile of histamine across the species studied (rats, rabbits, dogs, humans).

Non-clinical data reveal no special hazard for humans based on conventional studies of repeated-dose toxicity, local tolerance and genotoxicity. The toxicity associated with the use of HDC was related to exaggerated pharmacological responses in the species studied (rats, dogs, rabbits and monkeys) and were observed at exposures in excess of the maximum human exposure.

Histamine dihydrochloride is not considered to have any adverse effects on reproduction. The effects observed during the reproduction studies were similar to those noted during the toxicity repeat dose studies. Although doses were, in some cases, maternally toxic, this did not have any effects on the embryo and foetus.

Efficacy

The efficacy of the combination treatment of HDC and interleukin-2 (IL-2) was investigated in one pivotal study (**Study MP-MA-0201**) and showed in the ITT population that combination HDC+IL-2 resulted in a statistically significant increase of leukaemia-free survival (LFS). The effect was observed only in the population in first remission. In the subset of patients in CR 1, median LFS was increased by 22.7 weeks ($p = 0.0113$, stratified log-rank test) and LFS at 36 months was almost 40% in patients treated with the combination compared with 26% in the control group ($p = 0.02$). The applicant has suggested an indication accordingly.

The CHMP initially concluded that statistical significance of the outcome observed for the pivotal study on maintenance treatment with HDC+IL-2 cannot be considered statistically compelling. The clinical efficacy cannot be considered as established.

Safety

The safety profile of HDC combines common adverse drug reactions such as flushing, hypotension, headache and injection site reactions and a slightly increase rate of serious adverse events for thrombocytopenia, infections and back pain compared with the observational arm. However, the proposed treatment regimen does not impact negatively on Quality of Life of the treated patients, despite a treatment of 18-month duration with twice daily administrations during 3 weeks, followed by resting periods of 3 and then 6 weeks.

User consultation

The Patient Information Leaflet (PIL) for Ceplene (histamine dihydrochloride) has been tested in English in accordance with Articles 59(3) and 61(1) of Directive 2001/83/EC, as amended by Directive 2004/27/EC. The PIL for Ceplene (histamine dihydrochloride) was found to contain all the necessary information in a way that is accessible and understandable to those who participated in this test.

It is considered that the tested PIL meets the requirements set for User Testing.

Risk-benefit assessment

The demonstration of the efficacy of HDC in combination with IL-2 for preventing or delaying relapse in AML relies on one single pivotal Phase III study (**Study MP-MA-0201**). The combination demonstrated a statistically significant increase in LFS over a 36-month observation period in patients with acute myeloid leukaemia in remission. A subgroup analysis showed that the effect was only observed in the population of patients in first remission.

The contribution in the treatment effect of each individual drug has been evaluated neither in non-clinical nor in clinical study in the claimed indication. This has been seen as a major deficiency in the drug development.

The safety data of the combination treatment were not of major concern.

The applicant was invited to present an oral explanation to the CHMP to address the outstanding issues. In summary, the applicant argued that:

- There are several peer reviewed articles supporting the rationale of mechanism of action of HDC+IL-2
- Five randomised trials (n = 899) did not show a benefit of IL-2 alone (wide range of doses and schedules) for preventing the first relapse in AML patients
- LFS is a reliable marker to assess remission, commonly used in similar trials
- In **Study MP-MA-0201**, LFS was significantly prolonged in the combination treatment arm compared with the control arm and extensive sensitivity analyses confirmed that were not confounding prognostic factors
- The **Study MP-MA-0201** was not designed for concluding statistically on overall survival, however in the population of the claimed indication the overall survival is prolonged
- Negative study results in other indication are explainable by the higher of tumour burden of metastatic cancer compared with AML after the first complete remission.

Having considered the arguments and justifications presented by the applicant and the advice from the SAG-Oncology, the CHMP considered that the benefit risk of histamine dihydrochloride administered in conjunction with interleukin-2 for maintenance of remission in adult patients with acute myeloid leukaemia in first remission was not considered favourable due to the following grounds.

The application is dependent on one single pivotal study. This study, **Study MP-MA-0201**, cannot be considered to meet the requirements for approval with a single pivotal study according to the CHMP points to consider CPMP/EWP/2330/99 for the following reasons:

- The requirement of statistically compelling results is not fulfilled
- Supportive clinical data for efficacy are very limited and consist of a Phase II study evaluating in 39 patients with AML in remission, feasibility, safety and efficacy of HDC+IL-2 to prevent relapse
- The pharmacological rationale is weak in particular concerning the use of the combination and the supporting non-clinical data are not considered to be sufficient.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by majority decision that the risk-benefit balance of Ceplene administered in conjunction with interleukin-2, for maintenance of remission in adult patients with acute myeloid leukaemia in first remission to prolong the duration of leukaemia-free survival, was unfavourable and therefore did not recommend the granting of the marketing authorisation in the first instance.

2.7 Re-examination of the CHMP opinion of 19 March 2008

Following the CHMP Opinion concluding that the benefit risk of histamine dihydrochloride administered in conjunction with interleukin-2 for maintenance of remission in adult patients with acute myeloid leukaemia in first remission was not considered favourable, the applicant submitted detailed grounds for the re-examination of the grounds for refusal.

Detailed grounds for re-examination submitted by the applicant

The Applicant argued that data presented in the original application satisfactorily address each of the grounds for refusal raised by the CHMP and that the assessment should take into account the unmet medical need, the sound pharmacological rationale supporting the use of Ceplene/IL-2 immunotherapy, the claimed clinical benefits supported by robust and clinically meaningful data set, the acceptable safety profile and patient convenience

Concerning the requirement of statistically compelling results, the Applicant argued that the results of the pivotal Phase III 0201 of Ceplene/IL-2 treatment for remission maintenance in AML patients are scientifically valid, internally consistent, and robust. These characteristics, combined with the clear demonstration of clinical benefit on leukaemia-free survival in the target population, and viewed in the context of an orphan treatment for a poor prognosis disease, can be considered statistically as well as clinically compelling. The applicant also submitted the opinion of expert European biostatisticians to support its argumentation.

The applicant disagreed that supportive clinical data for efficacy are very limited and argued that the totality of data collected during the clinical development program of Ceplene and previously submitted in the Ceplene MAA is sufficient to demonstrate the safety, efficacy, and positive benefit-to-risk balance of this therapy for prevention of relapse in AML patients. According to the applicant, the published peer-reviewed preclinical and clinical pharmacodynamic data reviewed provide compelling evidence, and concluded that this evidence unequivocally supports the pharmacological rationale for the use of Ceplene/IL-2 in AML. The applicant has reviewed 5 clinical trials investigating different doses of IL-2 in patients with AML and concluded that these trials have not shown significant clinical efficacy of IL-2 treatment as monotherapy at doses ranging between 25 and 91 MIU/m^2/month.

Concerning the pharmacological rationale, the applicant reviewed and discussed the role of T cells and natural killer (NK) cells in eradicating leukaemic cells in AML patients, the rationale and results for IL-2 in AML, the mechanisms of T and NK cell inactivation and their implications for IL-2 immunotherapy in AML, the rationale and preclinical pharmacodynamic basis for improving IL-2 efficacy with HDC, as well as the clinical pharmacodynamic effect of HDC in improving IL-2-induced immunoactivation. The applicant also reviewed the likely mechanism of action of HDC by

overcoming cancer-related immunosuppression or immunosuppression in AML (in terms of dysfunctional T and NK cells recovered from AML patients) respectively based on in vitro or ex vivo data.

Report from the CHMP Scientific Advisory Group in Oncology

Following the request from the applicant, as agreed by the CHMP, a Scientific Advisory Group (SAG) meeting has been convened on 10 July 2008 to provide comments on the grounds for negative opinion and advice on the list of questions raised by the CHMP and adopted at the June 2008 CHMP meeting.

The SAG considered the CHMP grounds for refusal and agreed that the statistical significance of the log-rank test was convincing from a purely statistical perspective, but also concurred that there were only very little supportive clinical data, and that the pharmacological rationale, although plausible, was adding little to the clinical data. The SAG also agreed that the toxicity of the combination regimen was in itself manageable and not unacceptable in absolute terms, if clear evidence of efficacy could be established.

However, when judging the evidence of efficacy, two opposing views emerged. According to one view, the efficacy has been clearly established, and the clinical trial provided sufficient evidence to establish a positive benefit-risk in the target population. Since such an assessment is based on a single pivotal-trial, the evidence of efficacy is not overwhelming. However the large effect in terms of a relevant clinical endpoint, the lack of alternative established maintenance treatments and the acceptable toxicity profile were considered together as sufficient to establish an adequate level of efficacy of the combination of histamine+IL2. According to this view, although there are a number of issues that could be further clarified, the efficacy has been clearly demonstrated in the target population. The heterogeneity of the randomized study was considered to be no greater than in any other large AML study. Furthermore, it is a regular finding in every unselected AML study that one cannot classify in terms of risk a fair proportion of AML patients due to failed cytogenetics (i.e., no metaphases). It was also acknowledged that , subgroup analysis is of course of interest but cannot be required for registration if a predefined clinical relevant endpoint on the total population is met. However, the efficacy should be further characterised in subgroups of poor prognosis patients (e.g., patients >60 years old), e.g., through adequate studies, if necessary post-approval.

According to the opposing view, the efficacy cannot be considered established. The trial included a heterogeneous population in terms of known and unknown prognostic factors. Although the applicant showed that concerning known prognostic factors no important imbalances were apparent, reliable risk classification based on cytogenetics could only be determined in 69% of the patients due to missing data. Thus, important heterogeneity in the population recruited and imbalances between treatment arms cannot be excluded. The poor performance of the observation arm might indeed be due to such an imbalance. Given the lack of a second confirmatory trial, the problematic experience with similar immunotherapies, the lack of other clinical efficacy and pharmacological supportive data, there remain fundamental doubts about the efficacy of the combination and this could only be resolved with the conduct of a randomized clinical trial of similar design, designed and conducted according to state of the art standards in the treatment of AML.

The SAG agreed that historical comparisons are very difficult to interpret and can be in fact misleading. Historical comparisons cannot be used to conclude or even speculate on whether there exist or not any differences between histamine dihydrochloride+ IL-2, compared to IL-2 alone. The SAG considered that the more relevant question is rather concerning the effect of the combination of histamine dihydrochloride+IL-2 *versus* observation. Indeed, there is a catalogue of questions that could be of academic interest, trying to determine exactly the contributions of all the components of the combination, optimising the doses of the two agents, etc., but it is highly unlikely that all such answers could be obtained in a rare disease setting such as the maintenance treatment of AML. Given the large unmet medical need, a reasonable combination regimen with convincing evidence of adequate efficacy and safety would be of clear clinical usefulness in this disease setting, despite the unknown contribution of the individual agents. According to one view, the fact that other trials using higher doses of IL2 were negative and/or too toxic was viewed as indirect evidence of the contribution of histamine to the combination. According to other members the single pivotal trial using IL-2 plus histamine should be considered as one positive trial amongst five negative trials using IL-2 as single agent.

The SAG had divergent views on the need for a second randomized controlled trial to establish the benefit of the combination (see SAG comments on the CHMP grounds for refusal). There were two views concerning the feasibility of such a study. One view suggested that a second randomized controlled trial study should indeed be conducted according to state of the art standards, including stratification for known prognostic factors such as age, WBC count at diagnosis, cytogenetics and molecular markers (*flt-3* mutation, *MLL* partial tandem duplication), in order to allow proper assessment of the efficacy and adjustment for important prognostic factors. The opposite view maintained that the efficacy was not in question and that no second randomized controlled trial is necessary or even feasible. Any outstanding uncertainties about subgroups where the efficacy has been less convincingly shown (e.g., patients >60 years old) should be addressed as post-authorisation commitments (see SAG comments on the CHMP grounds for refusal).

Overall conclusion on grounds for re-examination

The CHMP assessed all the detailed grounds for re-examination and argumentations presented by the applicant and considered the views of the Scientific Advisory Group.

The CHMP considered all the new evidence submitted by the applicant and the argumentation put forward by the applicant and the SAG experts. The CHMP considered that the benefit-risk balance for the combination of Ceplene plus IL-2 was positive. The CHMP agreed that despite its limitations in terms of design, the results of study MP-MA-0201, can be considered to meet the requirements for approval with a single pivotal study according to the CHMP points to consider CPMP/EWP/2330/99, i.e., that the pivotal study can be considered as exceptionally compelling particularly due to the high quality of the conduct of the study, the clinically and statistically significant results, and the internal validity. The CHMP agreed that the concept of immune-surveillance of cancer is established and provides a plausible rationale for pharmacodynamic strategies to enhance antileukemic effects of T- and NK cells. However, the CHMP considered that further clinical data should be provided in order to provide a better understanding of the clinical efficacy of Ceplene in combination with IL-2, and to confirm the underlying mechanism of action. However, the CHMP, in agreement with the SAG, also recognised that certain relevant questions remain unanswered, in particular concerning the contributions to the effect of all the components of the combination, as well as optimising the doses of the two agents, etc., but it is highly unlikely that all such questions could be addressed comprehensively in a rare disease setting such as the maintenance treatment of AML. Thus, the CHMP proposed a marketing authorisation under exceptional circumstances, after having consulted the applicant.

In particular there is a need to gain more understanding with regard to the benefit-risk profile of Ceplene plus IL-2 combination and the role of Ceplene in such a combination. The applicant will provide clinical data to assess the immunologic and anti-leukaemic activity of Ceplene/IL-2 in adult AML patients in CR1, to study the effect in patients >60 years old compared to younger patients, and to assess the anti-leukaemic activity of Ceplene/IL-2 in adult AML patients with minimal residual disease. To this end, the applicant has committed to conduct the following studies:

- Multicenter Open-Label Clinical Pharmacology Study to Evaluate the Biomarkers and Pharmacologic Endpoints of Ceplene (Histamine Dihydrochloride) plus low dose Interleukin-2 in approximately 100 Adult Patients stratified by age great or less than 60 with Acute Myeloid Leukemia in First Complete Remission (CR), with well characterized Morphologic, Cytogenetic and Molecular profiles

- Open label clinical study to evaluate Minimal Residual Disease (MRD) for the assessment of the anti-leukaemic activity of Ceplene/IL-2 Ceplene (Histamine Dihydrochloride) plus low dose Interleukin-2 in approximately 150 Adult Patients stratified by age great or less than 60 with Acute Myeloid Leukemia in First Complete Remission.

The applicant has also committed to assessing the feasibility and if applicable to conducting a controlled clinical study in order to confirm the clinical benefit of the combination of Ceplene/IL-2 in adult AML patients in CR1:

- The applicant has also committed to determining the feasibility of conducting, in conjunction with collaborative groups in Europe and/or the United States, a Multicenter Randomized Open-Label Study to Evaluate the Safety and Efficacy of Immunotherapy with Subcutaneous Ceplene

(Histamine Dihydrochloride) plus low dose Interleukin-2 Versus a comparator arm to be determined in approximately 350 adult Patients (stratified by age great or less than 60) with Acute Myeloid Leukemia in First Complete Remission.

Divergent views were expressed by some CHMP members who considered that the benefit-risk balance could not be considered to be positive and that the grounds for refusal should be maintained, because the data presented are not sufficiently compelling to establish the efficacy of the product based on a single pivotal study, because of the absence of supportive data, and because relevant clinical data to confirm the pharmacological rationale for Ceplene + IL2 have not been submitted.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that:

– No additional risk minimisation activities were required beyond those included in the product information.

Similarity with authorised orphan medicinal products

In this application, the Applicant has provided arguments discussing the issue of similarity, in the context of Commission Regulation (EC) No 847/2000, regarding the orphan medicinal product Trisenox (arsenic trioxide) authorised in the EU for the treatment of acute promyelocytic leukaemia.

The CHMP concluded that:

- A marketing authorisation for the medicinal product Trisenox containing arsenic trioxide for induction of remission and consolidation in adult patients with relapsed/refractory acute promyelocytic leukaemia (APL) exists with orphan market exclusivity.

- Histamine dihydrochloride and arsenic trioxide are considered not to be similar with regards to the mechanism of action since they act on different pharmacodynamic targets.

- Histamine dihydrochloride is not structurally similar to arsenic trioxide as both molecules do not share the same principal molecular structure pursuant to Commission Regulation (EC) No 847/2000.

Therefore, the CHMP considered Ceplene not to be similar to any of the authorized orphan medicinal products (as defined in Art. 3 of Commission Regulation (EC) No 847/2000) for a condition relating to the proposed therapeutic indication.

Recommendation following re-examination

Based on the CHMP review of data on quality, safety and efficacy, the CHMP re-examined its initial opinion and in its final opinion concluded by a majority decision that the risk-benefit balance of Ceplene maintenance therapy for adult patients with acute myeloid leukaemia in first remission concomitantly treated with IL-2 was favourable and that the application satisfied the criteria for authorisation and recommended the granting of the marketing authorisation under exceptional circumstances.

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