

European Medicines Agency Evaluation of Medicines for Human Use

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ASSESSMENT REPORT

FOR

МЕРАСТ

International Nonproprietary Name: mifamurtide

Procedure No. EMEA/H/C/000802

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 IDM Pharma, S.A. submitted on 03 November 2006 an application for Marketing Authorisation to the European Medicines Agency (EMEA) through the centralised procedure for MEPACT, which was designated as an orphan medicinal product (EU/3/04/206) on 21 June 2004. MEPACT was designated as an orphan medicinal product in the following indication: treatment of osteosarcoma. The calculated prevalence of this condition was 0.5 per 10,000 EU population.

The applicant applied for the following indication: the treatment of high grade resectable nonmetastatic osteosarcoma in combination with chemotherapy.

The legal basis for this application refers to:

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

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

Protocol Assistance

The applicant received Protocol Assistance from the CHMP on 12 May 2005. Protocol Assistance pertained to the quality, non-clinical and clinical aspects of the dossier.

Licensing status:

A new application was filed in the following countries: USA

The Rapporteur and Co-Rapporteur appointed by the CHMP were:Rapporteur:Barbara van Zwieten-BootCo-Rapporteur:Eric Abadie/Pierre Demolis

1.2 Steps taken for the assessment of the product

- The application was received by the EMEA on 3 November 2006.
- The procedure started on 22 November 2006.
- The Rapporteur's first Assessment Report was circulated to all CHMP members on 8 February 2007. The Co-Rapporteur's first Assessment Report was circulated to all CHMP members on 9 February 2007.
- During the meeting on 19-22 March 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 23 March 2007.
- A clarification meeting with the Rapporteurs on the CHMP List of Questions was held on 4 April 2007.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 7 September 2007.
- The Rapporteurs circulated the Joint Assessment Report on the applicant's responses to the List of Questions to all CHMP members on 31 October 2007.
- During the CHMP meeting on 12-15 November 2007 the CHMP agreed on a list of outstanding issues to be addressed in writing and in an oral explanation by the applicant.
- A clarification meeting with the Rapporteurs on the CHMP list of outstanding issues was held on 28 November 2007.
- The applicant submitted the responses to the CHMP consolidated List of Questions on 21 December 2007.

- The Rapporteurs circulated the updated Joint Assessment Report on the applicant's responses to the list of outstanding issues to all CHMP members on 11 January 2008.
- During the CHMP meeting on 21-24 January 2008 the outstanding issues were addressed by the applicant during an oral explanation before the CHMP. During the same meeting the CHMP agreed on a second list of outstanding issues to be addressed in writing and in an oral explanation by the applicant. A GCP inspection, requested by the CHMP, was carried out at the following site(s): one investigator site in California, USA (inspected 11-13 June 2007), one investigator site in New York, USA (inspected 18-21 June 2007) and the sponsor site in California, USA (inspected 14-15 June 2007 / 14-18 April 2008). The final inspection reports were issued on 13 September 2007 and 9 June 2008.
- The applicant submitted the responses to the CHMP 2^{nd} list of outstanding issues 23 May 2008.
- The Rapporteurs circulated the updated Joint Assessment Report on the applicant's responses to the 2nd list of outstanding issues to all CHMP members on 20 June 2008.
- During the CHMP meeting on 23-26 June 2008 the CHMP agreed on a 3rd list of outstanding issues to be addressed in writing by the applicant.
- The applicant submitted the responses to the CHMP 3rd list of outstanding issues 20 August 2008.
- The Rapporteurs circulated the updated Joint Assessment Report on the applicant's responses to the 3rd list of outstanding issues to all CHMP members on 10 September 2008.
- During the CHMP meeting on 22-25 September 2008 the CHMP agreed on a 4th list of outstanding issues to be addressed in writing and at an oral explanation by the applicant. A clarification meeting with the Rapporteurs was held on 25 September 2008.
- The applicant submitted the responses to the CHMP 4th list of outstanding issues on 20 October 2008.
- The Rapporteurs circulated the updated Joint Assessment Report on the applicant's responses to the 4th list of outstanding issues to all CHMP members on 3 November 2008.
- During a meeting of a SAG Oncology on 6 November 2008, experts were convened to address questions raised by the CHMP.
- During the CHMP meeting on 17-20 November 2008 the outstanding issues were addressed by the applicant during an oral explanation before the CHMP.
- During the meeting on 15-18 December 2008 the CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a Marketing Authorisation to MEPACT on 18 December 2008. The applicant provided the letter of undertaking on the follow-up measures to be fulfilled post-authorisation on 15 December 2008.

2 SCIENTIFIC DISCUSSION

2.1 Introduction

Osteosarcoma is the most common primary malignancy of bone tissue, especially in children and adolescents, but the overall incidence in Europe is low (<2.4 per 100.000). Osteosarcomas comprise 56 percent of all bone cancers in individuals under the age of 20.

Staging incorporates the degree of differentiation as well as local and distant spread in order to estimate the prognosis of the patient. The histology grade of malignancy is divided into low grade and high grade. High-grade osteosarcoma accounts for approximately 20–22% of all primary malignant bone tumours. Occurrence is most commonly in the second decade of life with frequencies slightly higher in males than in females (1.5:1) and its peak frequency is observed in the adolescent.

The extent of the primary tumour is classified as either intra-compartmental, meaning it has basically remained in place, or extra-compartmental, meaning it has extended into other nearby structures.

Osteosarcoma can be localized or metastatic. Localized tumours are limited to the bone of origin, although local skip metastases may be apparent within the bone, indicating a worse prognosis. Radiologic evidence of metastatic tumour deposits in lung, other bones and other distant sites is found in 15% to 20% of patients at diagnosis; 85% to 90% of metastatic disease is in the lungs. The second most common site of metastasis is another bone.

Before the deployment of (neo)adjuvant chemotherapy, despite surgery and or radiotherapy, osteosarcoma had a 5 year survival rate of ca 15%. The explanation of this phenomenon is probably the presence of micrometastasis in the majority of osteosarcoma patients presenting without clinical metastasis. Due to the effects of (neo)adjuvant chemotherapy the 5 year's survival for patients with osteosarcoma is approximately 65 percent. Clinically detectable metastatic disease at initial diagnosis is the most consistent adverse prognostic factor: Overt metastasis at diagnosis has a major impact on patient survival: The estimated long-term survival proportion for patients with localized osteosarcoma is about two-thirds compared to 25 percent for patients with metastatic disease.

Since the eighties adjuvant chemotherapy is considered standard treatment component of resectable osteosarcoma, both in children and in adults. The choice of regimen and the optimal timing of application are still under investigation. Also, preoperative chemotherapy may be applied when a limb-sparing procedure is considered. Neoadjuvant treatment has been established in the early nineties with the aim to facilitate limb sparing surgery following initial systemic therapy. This neo-adjuvant treatment may result in down staging of the primary process as well as eradication of micrometastasis. It may also reveal efficacy of the chemotherapy by the extent of necrosis induced in the tumour (Huvos grade).

Current treatment regimen consist of ifosfamide, high dose methotrexate (HDMTX), cisplatin, and doxorubicin or (HD) methotrexate, doxorubicin, bleomycin, cyclophosphamide, and dactinomycin with/without cisplatin. Neo-adjuvant treatment has been established in the early nineties with the intent of facilitating limb sparing surgery following initial systemic therapy. The upfront addition of ifosfamide to HDMTX and doxorubicin (as well as cisplatin) has been found to improve initial tumour response rates, but the influence on overall and event-free survival remained unclear.

Response to neoadjuvant chemotherapy in general is variable and a major prognostic factor. Five-year survival rates for patients with an extremity sarcoma and a "good" response to chemotherapy (as stated, <10% viable tumor cells in the surgical specimen) are significantly higher than for those with a lesser response, although survival did not differ significantly in relation to histologic subtype.

Liposomal muramyl tripeptide phosphatidyl-ethanolamine (L-MTP-PE) consists of a liposomal embedded active ingredient muramyl tripeptide (MTP). The phosphatidyl ethanolamine (PE) was added to facilitate the integration of MTP into the liposomes. MTP is a synthetic derivative of muramyl dipeptide (MDP), the smallest naturally-occurring immunostimulatory component of the cell wall of primarily Gram positive bacteria like Bacillus Calmette-Guerin, and a major component in Freund's complete adjuvant. MTP-PE is lyophilised with a mixture of phospholipids (1-palmitoyl-2-oleoylphosphatidylcholine and 1,2-dioleoylphosphatidylserine) that, when constituted with saline,

provides the liposomal structure which is supposed to facilitate targeting the MTP-PE to tissue macrophages and the RES. The target cells of the liposomes seem the same cells that also express the receptor for MTP-PE and contain NOD2 as an intracellular effector protein. The presumed mode of action is the activation of macrophages by MTP-PE, rendering these cells tumoricidal, but the Applicant states that the mode of action is not fully understood.

The applicant applied for an indication for MEPACT in combination with post-operative multi-agent chemotherapy for children and adults for the treatment of high-grade resectable non-metastatic osteosarcoma (bone cancer) after surgery to remove the tumour. Following CHMP scientific review, the applicant changed the indication to: MEPACT is indicated in children, adolescents and young adults for the treatment of high-grade resectable non-metastatic osteosarcoma after macroscopically complete surgical resection. It is used in combination with post-operative multi-agent chemotherapy. Safety and efficacy have been assessed in studies of patients 2 to 30 years of age at initial diagnosis (see SPC section 5.1). The legal basis for this application refers to Article 8.3 of Directive 2001/83/EC, as amended ("complete and independent application").

MEPACT treatment should be initiated and supervised by specialist physicians experienced in the diagnosis and treatment of osteosarcoma. The recommended dose of mifamurtide for all patients is 2 mg/m^2 body surface area. It should be administered as adjuvant therapy following resection: twice weekly at least 3 days apart for 12 weeks, followed by once-weekly treatments for an additional 24 weeks for a total of 48 infusions in 36 weeks.(see SPC section 4.2).

MEPACT must be reconstituted, filtered using the filter provided and further diluted prior to administration. The reconstituted, filtered and diluted suspension for infusion is a homogenous, white to off-white, opaque liposomal suspension, free of visible particles and free of foam and lipid lumps. After reconstitution, filtering using the filter provided and further dilution, MEPACT is administered by intravenous infusion over a period of 1 hour. MEPACT must not be administered as a bolus injection (See SPC section 4.2). Instructions on reconstitution, filtering using the filter provided and dilution prior to administration are provided in the SPC (see SPC section 6.6.).

MEPACT has been granted Orphan Medicinal Product status in accordance to the conclusion of the COMP (EU/3/04/206, opinion dated 21/06/2004) in view of the prevalence of the condition (high grade resectable non-metastatic osteosarcoma after neoadjuvant therapy and definitive tumour resection in children and young adults) which was considered as <1 per 10,000 individuals in the EU.

2.2 Quality aspects

MEPACT is a liposomal formulation of a new chemical entity, mifamurtide, an immune modulator proposed for clinical use with adjuvant chemotherapy in the treatment of children and young adults with high grade resectable non-metastatic osteosarcoma after neoadjuvant therapy and definitive tumour resection. L-MTP-PE was first developed by Ciba-Geigy, Inc. in the early 1980s as a biological response modifier for the treatment of metastatic tumors. After licensing by another company, L-MTP-PE rights were purchased by IDM in 2003. The product is presented as lyophilisate for suspension for intravenous injection and is supplied in a carton that contains one dose of MEPACT supplied as lyophilisate in a 50 ml glass vial and one sterile Mifamurtide filter supplied in a blister. The recommended dose of mifamurtide for all patients is 2 mg/m² body surface area as per the SPC.

Active Substance

The INN name of the active substance is Mifamurtide corresponding to the molecular formula $C_{59}H_{108}N_6O_{19}P \cdot Na \cdot xH_2O$ where x = 0 to 5.

Mifamurtide, also called liposomal muramyl tripeptide phosphatidyl-ethanolamine (L-MTP-PE), is a liposomal formulation of active ingredient muramyl tripeptide phosphatidyl ethanolamine (MTP-PE), which is a fully synthetic lipophilic derivative of muramyl dipeptide (MDP).

MDP is the smallest naturally-occurring immunostimulatory component of the bacterial cell walls used in Freund's complete adjuvant. MTP-PE is lyophilised with a mixture of phospholipids [1-palmitoyl-2-oleoylphosphatidylcholine (POPC) and 1,2- dioleoylphosphatidylserine (OOPS)] that, when constituted with saline, provide the liposomal structure and facilitate targeting to tissue macrophages. Preliminary data have confirmed that MTP-PE is a specific ligand of NOD2, a receptor found primarily on monocytes, dendritic cells and macrophages.

MTP-PE is an amphipathic molecule, bearing a hydrophilic moiety (i.e. muramyl peptide) and a lipophilic moiety (i.e. dipalmitoyl phosphatidyl ethanolamine). MTP-PE behaves as a surface active, bilayer forming molecule that can associate at an oil-water interface and form micellar structures and intercalate into bilayers formed from other lipids, such as phospholipids.

It appears as an odourless white to off -white amorphous powder. The tripeptide possesses 4 chiral carbons, and also the muramyl moiety has 4 chiral carbons, all with a specific configuration. The substance is optically pure with a defined specific optical rotation when measured in chloroform/methanol 9:1.

It is soluble in chloroform, methylene chloride, t-butanol, and ethyl acetate, slightly soluble in methanol, and ethanol and insoluble in diethyl ether. In water it results in clear solutions with aggregated (micellar) structures. Its pKa is 2.5 to 3.5.

The current manufacturing process yields an amorphous solid with very low crystallinity. Since MTP-PE is processed from homogeneous solution and incorporated into liposome bilayers for administration, polymorph forms, if in existence, would disappear. Therefore, polymorphism of MTP-PE is not relevant and has not been studied.

• Manufacture

MTP-PE is synthesized from peptide, carbohydrate and lipid components. The synthesis of the drug substance is performed under GMP from five starting materials.

Three early and stable intermediates are manufactured separately and in bulk quantities, which can be stored long term. These early intermediates are prepared in sufficient quantity to make multiple batches of the drug substance from single batches of the early intermediates. The steps that may be considered as critical are described and validation results on three batches are submitted.

During synthesis, the drug substance is exposed only to organic solvents and is essentially free from bacterial contamination. Only at the last step of processing involving ultrafiltration for salt exchange and concentration is there a potential source of microbial contamination. However, the process is well controlled. A 0.22 μ m filtration unit and a 0.22 μ m membrane filter are in place before and after the ultrafiltration step to remove potential contaminants. The resulting MTP-PE drug substance powder is tested for bioburden and endotoxin prior to release for production of the drug product.

The commercial process for preparation of L-MTP-PE does not use any materials that are directly derived from animal sources. However, there are two animal derived phospholipases used during the synthesis of the excipients. The first phospholipase is produced by fermentation of a microorganism. The animal derived component, peptone, is introduced in the production medium used to produce the microorganism. The peptone used is derived from milk produced by healthy bovine and that such milk is comparable to the quality of milk used for human consumption as required by the guideline.

As far as the second phospholipase concerns, a certificate of origin for the bovine material was provided. Furthermore, a processing step that involves low pH treatment (2-3 pH solution) over 60 minutes is employed. This processing step was included to inactivate any putative viruses, if present.

• Specification

The specifications for the control of the drug substance includes tests for appearance (visual), identification (IR, TLC), specific rotation (polarimetry), pH and clarity of solution (PhEur), assay (HPLC), fatty acid analysis (GC), related substances (LC-MS/MS), residual solvents (GC), Ratio of Alanine to Isoglutamine (cation exchange-derivatisation-photometric determination), water content (Karl-Fischer), heavy metals (Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES)), microbial contamination (PhEur).

• Stability

Stability data on the active substance have been provided for three production scaled batches (postchange) and nine pilot scaled batches stored at -20 °C (18-24 months), 5 °C (18-24 months), and 25 °C/60% RH (6 months). The drug substance is normally packaged in 1000 ml glass containers with a polybutylene terephthalate (PBT) screw cap containing a polytetrafluoro-ethylene (PTFE) liner. However, the stability studies were performed in 2ml glass vials, blanketed with argon and sealed with Teflon coated butyl rubber stoppers. A re-test period of 24 months at -20 °C can be granted. The TLC method which has been used, is considered acceptable as the main degradation product is detected. The CHMP however, asked the applicant to provide stability data (at least of the proposed re-test period) in which the impurities have been measured by LC-MS/MS as part of the Follow-up Measures to which the Applicant committed.

Medicinal Product

• Pharmaceutical Development

The identification of the optimal liposomal formulation was based on preclinical and animal studies. The clinical trials of L-MTP-PE conducted under the sponsorship of Ciba-Geigy, and Jenner and US National Cancer Institute (for the pivotal study INT-0133) and the majority of the preclinical studies were all performed using the identified optimal formulation by mixing MTP-PE with the lipid excipients (consisting of a fixed POPC to OOPS ratio) in a defined ratio. This is the formulation proposed for the drug product discussed in this marketing application.

The product is a lyophilisate for suspension for injection, i.e., a dry mixture of 3 components to be constituted by hydration and mixing with 50 ml of 0.9% NaCl solution resulting in injectable multilamellar microspheres, to be injected after filtration and after adding to a 50 ml 0.9% NaCl infusion liquid.

L-MTP-PE is a formulation of muramyl tripeptide phosphatidyl ethanolamine (MTP-PE), which is a fully synthetic lipophilic derivative of muramyl dipeptide (MDP). The later is a naturally occurring component of bacterial cell walls. The product is supplied in vials, each one containing 5% overfill so one dosage form is expected to deliver 4 mg of MTP-PE and a mixture of two phospholipids: 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1,2-Dioleoyl-sn-glycero-3-phospho-L-serine monosodium salt (OOPS). The overage is intended to compensate for losses of volume of suspension in syringe, filter housing and infusion lines including drop counters etc.

Systemic administration of MTP-PE drug substance to animals results in activation of macrophages to bactericidal, virucidal, fungicidal, parasiticidal, and tumoricidal states. However, systemic administration of an aqueous solution of the amphiphilic MTP-PE, as micelles, was accompanied by deleterious side effects, stimulating development of a liposomal formulation.

However, the drug substance has been purposely designed to be a true phospholipid. It was demonstrated that the true phospholipid MTP-PE does not escape from the lipid bilayers and the MTP-PE molecule is not molecularly dissolved in aqueous solution. By using ultra-centrifugation experiments it was shown that no MTP-PE existed outside the liposomes. It was further demonstrated by immunoassay for MTP-PE, only functional for either free MTP-PE or MTP-PE at the surface of the liposomes, based on 4 batches, that only small parts (if any) of the MTP-PE exist in free form in the liposome suspension during the 7 days storage after constitution. In view of the presented data it is considered that it has been sufficiently demonstrated that measurement of free MTP-PE or release rate measurements of MTP-PE are not required as drug product specification.

In the very early development natural phospholipids used to manufacture a product in the form of a thin film but soon after synthetic excipients were employed and the product was manufactured as a sterile dry lyophilisate with a robust, porous structure. Synthetic phospholipids were preferred because they have a reproducible fatty acid composition with predictable properties for the formation of liposomes in contrast to phospholipids isolated from natural sources. Synthetic phospholipids also contain monounsaturated fatty acid chains that are more stable than the oxidation-sensitive polyunsaturated fatty acid chains contained in lipids from natural sources.

The two synthetic phospholipids used in the manufacture of the product are POPC (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine) and OOPS (1,2-dioleoyl-sn-glycerol-sn-phosphatidyl-L-serine monosodium salt. OOPS and POPC are non-compendial, inactive, excipients.

Both the polar head groups (choline and serine) and fatty acid chains (palmitic acid and oleic acid) of the lipids in L-MTP-PE are common, naturally occurring substances in man. POPC has a phosphatidyl choline structure similar to natural lecithins. It has the most frequently occurring fatty acid – palmitic acid – in 1-position and an unsaturated fatty acid - oleic acid - in 2-position. OOPS has a phosphatidyl serine structure similar to the anionic phospholipids found in many tissues. After lyophilisation, these lipids form a stable, dry lipid cake with a porous structure, providing a large surface area which is

optimal for contact with the constitution medium. The selected phospholipids have a phase transition temperature of about 5°C when in contact with water. This allows the in situ preparation of the liposome suspension at room temperature. The *in situ* constitution method was shown to yield multilamellar liposomes (MLVs) having the desired size range for optimal macrophage uptake and sustained release of MTP-PE to the cells. Size distribution studies showed that the MTP-PE liposomes have a monomodal size distribution and are chemically homogeneous, i.e., the same MTP-PE: POPC:OOPS ratio was found in both small (<1µm) and large (>5µm) liposomes. No unincorporated, non-liposomal MTP-PE was detected. The drug substance is an integral component of the liposomal bilayer.

Expected rates of hydrolysis are 75% for small unilamellar vesicles (SUVs), 50% for large unilamellar vesicles (LUVs), and 5% for multilamellar vesicles (MLVs). The potential impact of the varying ratios between SUVs - LUVs - MLVs towards a consistent drug dosage therapy has been sufficiently dealt, as well as relationship with degradation of POPC and MTP-PE.

The optimal ratio of of POPC to OOPS used in the product, was established by relevant studies of the pharmacologic effects in *in vitro* phagocytosis experiments with radiolabeled liposomes containing various ratios of the lipids. The ratio of MTP-PE to phospholipids was selected to optimize anti-tumor efficacy in a murine B16 melanoma model.

The exposed lipid on the outside of the liposome is susceptible to enzymatic hydrolysis.

Changes to the formulation during the drug product development were relatively minor and the provided information suggests that both pre-change and post-change products are very well comparable for all involved, very varying parameters. Before administration, the lyophilisate is reconstituted with Sodium Chloride Injection (0.9% NaCl) to form multilamellar liposomes suspension. The suspension is then filtered with the IDM spike filter prior to intravenous injection. The filter step intends to remove foam and reduce the content of particles $\geq 10 \ \mu m$ in the constituted liposomal suspension in order to prevent thromboembolic events after i.v. administration. The IDM spike filter is provided in the drug product package thus is considered part of this application.

The filters have little impact on the overall liposome size distribution, but remove or disperse large liposomes efficiently. Various filter materials have been tested for their compatibility with constituted L-MTP-PE. Results have shown straight pore filters of 3 μ m average pore size are particularly well suited; thus was chosen. The packaged filters are terminally sterilised by ethylene oxide. The release specification for the spike filter includes appropriate testing and specification for the residual ethylene oxide in accordance with ISO 10993-7. The IDM spike filter included with L-MTP-PE drug product is affixed with a CE mark showing it satisfies all essential requirements of applicable sections of Directive 93/42/CEE on the Medical Devices. Filters are released for use after meeting appropriate visual, dimensional, and functional tests.

POPC

POPC is an amphipathic molecule bearing a hydrophilic moiety (i.e., choline group) and a lipophilic moiety (i.e., the dipalmitoyl glycerol part). Such physico-chemical characteristics drive the solubility profile of POPC to behave as a bilayer forming agent. In aqueous media, it can be dispersed into liposomal structures.

It appears as a hygroscopic white to off-white odorless powder. There are no established polymorphs.

POPC is derived from phosphatidyl choline. Lecithin is usually used as a synonym for pure phosphatidyl choline, a phospholipid that is the major component of a phosphatide fraction isolated from either egg yolk or soy beans. Chemically, lecithin is phosphatidyl choline. Recommended doses range from 3 to 9 grams of phosphatidylcholine daily in divided doses.

Lecithin supplements containing 20 to 30% phosphatidyl-choline are used as a dietary supplement. Lecithin is also used for parenteral application and the product was shown to have no adverse effects.

Synthesis schemes were provided and sufficient information concerning critical steps and intermediates, residual solvents, specifications, analytical methods and stability has been provided. Impurities found in POPC appear to be of lipid structure. As such, they represent components found in natural lipids. No such components are known to cause any problems or safety concerns in the doses

finally administered. The amounts present are usually so low as individual components, that neither identification nor qualification is considered necessary.

Comparability test results between POPC batches from the two suppliers used throughout the product development were presented. In general the results indicate that POPC materials from both sources are highly comparable.

OOPS

OOPS appears as an odorless white to off-white powder. There are no established polymorphs. OOPS is derived from phosphatidyl serine. Phosphatidyl serine, a ubiquitous, endogenously occurring phospholipid, is a structural component of biological membranes of plants, animals and other life forms.

Phosphatidyl serine derived from both bovine brain and soy lecithin is used as a dietary supplement. Phosphatidyl serine derived from soy lecithin undergoes an enzymatic process that converts phosphatidyl choline to phosphatidyl serine. Because of the hypothetical possibility of bovine spongiform encephalopathy, the soy-derived phosphatidyl serine is preferred. Typical doses are 100 mg three times daily. Though with different route of administration, when phosphatidyl serine is used as a dietary supplement it is considered safe. It is generally accepted that "the use of phosphatidyl serine as a dietary supplement is safe provided that bovine-derived sources, if used, are not derived from bovine tissues from cattle born, raised, or slaughtered in any country where BSE exists."

Synthesis schemes were provided and sufficient information concerning critical steps and intermediates, residual solvents, specifications, analytical methods and stability has been provided.

Impurities seen in OOPS, are of lipid structure and as such, they represent components found in natural lipids. No such components are known to cause any problems or safety concerns in the doses finally administered. The amounts present are usually so low as individual components, that neither identification nor qualification is considered to be necessary.

Comparability test results between POPC batches from the two suppliers used throughout the product development were presented. In general the results indicate that POPC materials from both sources are highly comparable.

Adventitious Agents

The commercial process for preparation of L-MTP-PE does not use any materials that are directly derived from animal sources; except for the following two materials used during the manufacturing process for POPC and OOPS:

• Phospholipase A2 (PLA2) is used as reagent in the synthesis of POPC. Phospholipase A2 is isolated from porcine pancreatic gland.

• Peptone is used as a reagent in the production of Phospholipase D. Phospholipase D is then used in the manufacturing process of OOPS. Peptone is derived from bovine milk casein.

Regarding PLA2 a document from the manufacturer of PLA2 is available comprising statements that the porcine material is obtained from an approved and supervised slaughterhouse, from animals having received *ante mortem* and *post mortem* veterinarian inspections, and that storage and delivery is in frozen state, comprising enzyme activity unit specifications and microbiological specifications, and comprising a report stating that the risk of transmitting swine Hepatitis E Virus (swine HEV) is estimated to be insignificant. Considering the minute amount of these enzymes used in the MEPACT production and the rightly assumed inactivation by process steps of PLA2, POPC and MEPACT the issue of viral safety of the product is sufficiently covered.

Regarding Phospholipase D a statement is present from the manufacturer of Phospholipase D declaring that Phospholipase D is manufactured without materials of animal origin, i.e., the enzyme is TSE/BSE free, and that the peptone used is derived from milk produced by healthy bovine in New Zealand and that such milk is comparable to the quality of milk used for human consumption.

The provided statements are considered to be acceptable for the two materials from animal source, being used for the manufacturing of the excipients POPC and OOPS, respectively.

• Manufacture of the Product

The drug product is manufactured by aseptic processing under aseptic conditions in a grade A clean area. The active and inactive ingredients are formulated into bulk solution in an appropriate solvent. The bulk solution is filtered through a sterile 0.22 μ m nylon membrane filter and filled in sterilized, depyrogenated vials in a continuous manner. The filled vials are lyophilised, sealed, inspected and packaged. The bulk solution is typically compounded and filled within 48 hours. Before filtration of the bulk solution, samples for Quality Control and bioburden analysis are withdrawn.

• Product Specification

The release and shelf life specifications comprise requirements for both the dry lyophilisate as well as the constituted liposome suspension. The specifications include tests for appearance of vial content (visual), Mean of mass content per vial (weighing), Appearance of constituted liposomal suspension (visual), pH of constituted liposomal suspension (potentiometry), Particle (i.e., liposome) size and size distribution in constituted liposomal suspension (light diffraction), sub-visible particles (light obscuration particle count), identity of components (by TLC) within constituted liposomal suspension(for MTP-PE, POPC, OOPS by TLC), Water content (PhEur), Residual Solvent (PhEur), Sterility (PhEur), Bacterial Endotoxins (PhEur), assay Content of components within constituted liposomal suspension (for MTP-PE, POPC and OOPS by HPLC), Impurities (HPLC), Birefringence in constituted liposomal suspension (polarized light microscopy), Bioassay (biologic activity of MAC by their ability to generate TNF- α secretion).

The impurity limits included in the specifications for POPC and OOPS have been toxicologically qualified with the preclinical and clinical studies.

Batch analysis results were provided for 3 batches from IDM, 15 clinical batches from Ciba-Geigy and one additional batch from Jenner.

Sufficient data regarding the comparability of constituted liposome product of early clinical batches (from Ciba-Geigy) and post-change batches (from IDM) have been presented and appropriate testing and specifications were established.

• Stability of the Product

Long Term Stability

Stability data from five batches stored for up to 24 months at $(5\pm3 \text{ °C})$ and up to 12 months at accelerated $(25\pm2 \text{ °C} /60\pm5 \text{ % RH})$ storage conditions have been presented. These batches were manufactured by the commercial manufacturer using the proposed commercial process and scale.

Results showed no significant change from the release testing or from baseline. The data support the claimed self life, when the drug product is stored in accordance with the conditions specified in the SPC.

In addition, stability data from five older batches (Ciba-Geigy) stored for up to 24 months at 8°C and 25°C as well as three months at 40°C were provided as supportive information.

All stability studies have been performed with L-MTP-PE in the actual containers used for clinical studies and intended for commercial materials. Results of the chemical testing showed no change in MTP-PE, OOPS, and POPC content after up to 24 months storage at 5°C. MTP-PE and OOPS content after 12 months storage at 25°C showed an approximate 5% loss; MTP-PE content after 3 months storage at 40°C showed an approximate 10-12% loss; and, OOPS content after 3 months storage at 40°C showed an approximate 25% loss. The constituted liposomal suspension stored under the experimental conditions did not show any variation with regard to particle size distribution. After 3 months storage of the powder at 40°C, the appearance of the constituted liposomal suspension showed a yellowish color.

Short-Term Stability of the Liposome Suspension

The study showed that the reconstituted drug product from both Ciba-Geigy and IDM batches was stable for up to 14-24 hours. Storage of the constituted drug product for up to 14 hours showed no measurable degradation, and the stability profile is consistent across four lots of drug product when either stored in PVC or polyolefin infusion bags.

No adverse trend can be observed in the in-use stability study in the infusion bags. Values of MTP-PE assay fluctuation during the 24 hour storage period were within an acceptable window. The proposed 6 hours in-use time of the constituted product in the infusion bags is therefore acceptable. Stability of the constituted drug product has been demonstrated for time periods well in excess of those that can be expected in clinical practice and in excess of the proposed hold time as per the SPC.

A one-week short-term stability study of the liposome suspension prepared from three batches of MEPACT from the current manufacturer was presented to confirm the earlier findings from Ciba-Geigy for the stability of the constituted suspension of MEPACT in the vial. The reconstitution is performed according to the instructions proposed in the SPC. This study was also intended to show that the drug substance remains integrated in the liposomal layers (i.e., not released during storage.)

The data do not reveal change of the suspension in vials after 7 days of storage in the refrigerator or release of the drug substance from the liposomes.

Photostability of reconstituted Liposomes

A photostability study on reconstituted liposomes on one batch of drug product including test parameters on MTP-PE, POPC, Lyso-MTP-PE content was conducted. Results indicate that the constituted suspension is photostable up to 24 hours of indoor indirect daylight. The same batch was also tested in a temperature cycling study with results meeting the shelf life specifications.

Stability of Temperature Cycling of L-MTP-PE Lyophilisate

A temperature cycling study was also conducted to assess the effect of short-term temperature excursion outside of the label storage conditions, e.g., as may be experienced during shipping and handling, on product properties and stability of the product. The study was performed on one representative lot of L-MTP-PE vials. The product properties immediately after exposure to the thermal conditions all comply with stability acceptance criteria. This study will continue until the evaluation of product stored 24 months at label conditions after exposure in order to determine the long term effect of the thermal excursions.

In conclusion the submitted data support the self life of the product when stored under the conditions specified in the SPC and in addition sufficient in-use stability of the liposome suspension has been demonstrated as stated in the SPC.

Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the drug substance and drug product has been presented in a satisfactory manner. The quality of the active substance is considered sufficiently described and adequately supported by data. Sufficient information has been presented for the excipients POPC and OOPS which are considered novel excipients. Both comply with acceptable inhouse specifications in view of their synthesis. Full information on the synthesis and toxicologic profile of these excipients has been provided. As far as the drug product concerns the results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

2.3 Non-clinical aspects

Introduction

A total of 86 pharmacology studies (including primary and secondary pharmacodynamic and safety pharmacology evaluations), 12 pharmacokinetic (ADME) studies and 20 toxicology studies, including toxicokinetic assessments, have been submitted in support of the current marketing application for L-MTP-PE. The studies primarily used the i.v. route to model the intended clinical route of administration.

All non-clinical pharmacokinetic and toxicology studies were conducted with L-MTP-PE, the same test material used in all clinical studies and intended for marketing. Among all the safety pharmacology studies, only the QT intervals studies were GLP compliant. Other safety pharmacology studies were performed between 1984 and 1989 and were not GLP compliant. The majority of

toxicology studies, including the pivotal toxicology studies were conducted in accordance with international GLP guidelines.

The applicant sought EMEA protocol assistance on the pharmaceutical, preclinical and clinical development. Concerning preclinical aspects, the advice addressed safety pharmacology studies (QT prolongation following the requirements of ICH guideline S7B) and the lack of a complete genotoxicity program.

Pharmacology

• Primary pharmacodynamics

Pharmacotherapeutic group: Other cytokines and immunomodulators, ATC code: L03AX15

Mifamurtide (muramyl tripeptide phosphatidyl ethanolamine, MTP-PE) is a fully synthetic derivative of muramyl dipeptide (MDP), the smallest naturally-occurring immune stimulatory component of cell walls from *Mycobacterium sp*. It has similar immunostimulatory effects as natural MDP with the additional advantage of a longer half-life in plasma. MEPACT is a liposomal formulation specifically designed for *in vivo* targeting to macrophages by intravenous infusion.

MTP-PE is a specific ligand of NOD2, a receptor found primarily on monocytes, dendritic cells and macrophages. MTP-PE is a potent activator of monocytes and macrophages. Activation of human macrophages by MEPACT is associated with production of cytokines, including tumour necrosis factor (TNF- α), interleukin-1 (IL-1 β), IL-6, IL-8, and IL-12 and adhesion molecules, including lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1). *In vitro*-treated human monocytes killed allogeneic and autologous tumor cells (including melanoma, ovarian, colon, and renal carcinoma) (Key et al. 1982, Fidler et al. 1986, Talmadge et al. 1984, Talmadge et al. 1986) but had no toxicity towards normal cells.

In vivo administration of MEPACT resulted in the inhibition of tumour growth in mouse and rat models of lung metastasis, skin and liver cancer, and fibrosarcoma (Saiki et al., 1986, Karpoff et al. 2000, Thomas et al. 1995, Wange et al. 1995, MacEwen et al. 1988). Significant enhancement of disease-free survival was also demonstrated in the treatment of dog osteosarcoma and hemangiosarcoma with MEPACT as adjuvant therapy (MacEwen et al. 1989, Smith et al. 1993, Kurzman et al. 1995, Vail et al. 1995).

The applicant submitted the results of a study of L-MTP-PE on lung metastases in mice bearing Lewis Lung carcinoma primary tumour in the footpad. L-MTP-PE (0.75 or 7.5 mg/m² i.v., three times weekly/2 weeks) was started three days after either early (d 11) or late (d 17) removal of the primary tumour. Treatment with L-MTP-PE or with empty liposomes after early removal of the primary tumour resulted in decrease in the number of lung metastases compared to control. However, late removal of the primary tumour was associated with an increase in lung metastasis, and the effect was larger for mice treated with L-MTP-PE or with empty liposomes, compared to control.

An effect of L-MTP-PE was also observed using renal adenocarcinoma cells in mice receiving empty liposomes (Dinney et al., 1992).

In a fibrosarcoma tumor model, mice received a single local dose of thoracic irradiation, treatment with L-MTP- PE (3 mg/m² (1 mg/kg) i.v. twice weekly/4-weeks, or a combination of the two treatments five days after i.v. injection of fibrosarcoma cells. Treatment with L-MTP-PE alone did not decrease the median number of lung tumor colonies, nor significantly increase the animal survival. Although the treatment with irradiation alone significantly prolonged median survival (p = 0.01), the most significant results were obtained with the combined treatment (p = 0.001), where 60% of the mice survived up to day 140 and were found to be tumor-free (Saiki et al., 1986).

L-MTP-PE induced antitumour activity in a liver metastasis model of colon cancer in the rat (Thomas et al., 1995). However, using more tumour cells, L-MTP-PE enhanced tumour growth (Ref. Wang et al., 1995).

In a randomised study in dogs undergoing amputation for osteosarcoma, treatment with L-MTP-PE (2 mg/m², twice weekly/8 weeks) was associated with longer metastasis-free period and longer survival time compared to placebo liposomes (MacEwen et al., 1989). Similar results were observed when L-MTP-PE therapy was initiated after cisplatin therapy compared to placebo liposomes, but not

concurrently (Kurzman et al., 1995). Concurrent treatment with L-MTP-PE (1-2 mg/m² i.v. twice weekly/8 weeks) of metastatic splenic hemangiosarcoma after splenectomy with chemotherapy (doxorubicin/cyclophosphamide) prolongued disease-free survival and overall survival compared to chemotherapy plus liposome placebo (Vail et al., 1995). Canine monocytes could be activated to tumoricidal activity against canine osteosarcoma cells by L-MTP-PE in vitro and in vivo (Smith et al., 1993).

Treatment with L-MTP-PE in cats did not significantly alter either disease free interval or survival time (Fox et al., 1995)

In vitro and in vivo studies in mice (Fidler et al., 1989, Fogler et al., 1985, LeGrue et al., 1987) and dogs (Kurzman 1999) demonstrated that administration of L-MTP-PE leads to activation of macrophages resulting in enhanced cytotoxic activity against a variety of tumour cells (e.g. osteosarcoma) in vitro. Free and liposome encapsulated-MTP-PE activated murine (Gisler et al., 1982) and rat (Schroit et al., 1982) macrophages and spleen cells more efficiently than MDP *in vitro* and *in vivo*.

Human monocytes activated in vitro with L-MTP-PE became cytotoxic for several tumour cell types but continued to ignore normal cells (Barna et al., 1984)

NOD2 is an intracellular protein predominantly expressed in cells of the myeloid lineage, which is able to induce NF κ B activation and is implicated in innate immune defense against bacteria. NOD2 is a receptor for MDP (Girardin et al., 2003, Inohara et al., 2003) Preliminary *in vitro* data show that MTP-PE is a specific ligand of NOD2 suggesting that macrophage activation by L-MTP-PE is also mediated by NOD2.

MDP was observed to bind to NALP3 (Martinon F. et al., 2004), another receptor involved in inflammatory responses. Activation of monocyte/macrophage by L-MTP-PE was associated with secretion or transcription of a variety of cytokines, especially inflammatory cytokines (O'Reilly et al., 1991, Kurzman et al., 1993, Kleinerman et al., 1992, Maeda et al., 1993) such as TNF α (Dieter P., 1995), IL-1 α , IL-1 β , IL-6 (Fox, et al., 1994), and IL-8 (Asano T., 1994).

• Secondary pharmacodynamics

In vivo MTP-PE stimulated rabbit white blood cell proliferation (Wachsmuth et al., 1988). The proliferating monocytic cells were bone marrow-derived and migrated into tissue within 24 hours after MTP-PE administration. In addition, proliferation in the epithelium of the bile ducts and esophagus was also stimulated. Enhanced proliferation occurred more slowly and to a lesser extent in hepatocytes, hepatic interstitial cells, and renal epithelial cells, consistent with a regenerative process after an inflammatory or toxic event. Similar cell proliferations were observed in guinea pigs (Wachsmuth et al., 1989) and humans.

L-MTP-PE administrated before irradiation accelerated hematopoietic recovery from radiationinduced damage in mice (Fedorocko et al 1994, Mackova et al., 1993, 1997, 2002), with a synergistic effect with indomethacin. Oral administration of MTP-PE prevented doxorubicin-induced gastrointestinal toxicity in mice, preserving intestinal architecture (Killion et al., 1996)

• Safety pharmacology programme

In one rat study L-MTP-PE caused slight but significant increases in urine volume and electrolyte content (Buch, O. 1985). Liposomes at the high dose of 100 mg/kg induced a 50% increase of the very low density lipoprotein and the serum triglycerides, regardless of the presence of MTP-PE. Liver and total body weight were unaffected. A slight increase in plasma free fatty acid levels was seen with liposomes alone. Some changes in carbohydrate metabolism (transient increase in blood glucose, modifications in tissue glycogen) after L-MTP-PE administration attained statistical significance. There were no effects on liver enzyme by liposomes alone or L-MTP-PE (Muller, K. 1985; Albrecht, W. 1985). L-MTP-PE had no overt neurological or behavioural effects in mice or rats (Grewal et al., 1985).

On isolated atria preparation from guinea pigs, neither the frequency of spontaneous contraction nor the force of electrically-driven contraction was affected by L-MTP-PE. L-MTP-PE caused marginal and transient blood pressure reduction and bradycardia in cats (≥ 0.04 mg/kg (~ 0.5 mg/m²) i.v.), and only marginal changes in the QT intervals (highest dose 0.4mg/kg (~ 5 mg/m²)). With both drug-loaded

and unloaded liposomes, 50% of the cats presented ventricular extrasystoles, but the number of extrasystoles was increased in cats receiving L-MTP-PE. Some cats showed an increase in respiration rate and a moderate decrease in tidal volume.

In a single dose toxicity study in dogs (20 mg/m^2) prolongation of the PQ interval was observed in 2/4 dogs (no effect on QT interval was seen). There were no adverse cardiovascular changes observed by ECG following 3 or 6 month repeat dose administration in dogs (max dose respectively 0.1 mg/kg (2mg/m^2) and 0.5 mg/kg (10 mg/m^2)) and one month toxicity study in rabbits (max dose 0.1 mg/kg (1.2mg/m^2)).

There was no specific safety pharmacology study on the respiratory system in animals.

L-MTP-PE had no overt neurological or behavioural effects in mice or rats.

L-MTP-PE caused slight but significant increases in urine volume and electrolyte content in one rat study.

High doses of liposomes increased male rat serum triglycerides and very low density lipoprotein, no effects of L-MTP-PE were observed on serum lipids and lipoprotein levels, and only slight changes in carbohydrate metabolism (transient increase followed by a distinct decrease in blood glucose, modifications in tissue glycogen).

No antagonism of acetylcholine, barium chloride, histamine, noradrenaline, serotonin, apomorphine or physostigmine by L-MTP-PE was observed.

• Pharmacodynamic drug interactions

L-MTP-PE did not antagonize responses to acetylcholine, barium chloride, histamine, noradrenaline or serotonin when tested on isolated tissue preparations responsive to these agonists (Buch, O., 1985) Administration of L-MTP-PE did not antagonize apomorphine-induced hypothermia or physostigmine lethality in mice (Grewal et al., 1985)

Single or repeated doses of doxorubicin reduced the normal numbers of alveolar macrophages (AM) or peritoneal exudate macrophages (PEM) in mice. A reduction in the number of PEM was seen after i.p. injection of L-MTP-PE. 1-2 weeks after the last doxorubicin treatment PEM surviving the administration of up to 15 mg/kg doxorubicin were found to become tumoricidal if stimulated with L-MTP-PE.

Systemic administration of L-MTP-PE did not result in additive toxicity (measured as diminished blood leukocyte counts, altered leukocyte differentials, and decreased hematocrits) compared to chemotherapeutic treatment (ifosfamide (2.5 mg/kg), doxorubicin (10 mg/kg) or cisplatin (10 mg/kg)) and did not interfere with the anti-tumour effects of ifosfamide and doxorubicin. The myelosuppression normally observed following treatment with doxorubicin was prevented by combination treatment with L-MTP-PE.

In a lung-metastatic model, L-MTP-PE, cisplatin or cyclophosphamide, alone or in combination, significantly reduced the number of pulmonary metastases. However the therapeutic efficacy of cisplatin seemed reduced by L-MTP-PE administration.

In a murine ovarian tumour model, L-MTP-PE administration did not change the anti-tumour effect of cisplatin (up to 7.5 mg/kg). In addition no adverse effect on the effect of cisplatin (70 mg/m²) was observed in the dogs with osteosarcoma when L-MTP-PE was given concomitantly.

Coadministration of doxorubicin with L-MTP-PE resulted in an enhanced canine monocyte activation and cytotoxicity compared to doxorubicin or L-MTP-PE alone.

The in vitro response of human monocytes to 2 μ g/mL L-MTP-PE was not altered by prior or simultaneous incubation with 5- 500 ng/mL of doxorubicin (in vitro). Single-agent chemotherapy consisting of cisplatin, high dose methotrexate, cyclophosphamide, or doxorubicin given to patients before isolation of their monocytes did not interfere with the induction of tumoricidal activity by in vitro treatment with L-MTP-PE. To some extent, enhanced activation was observed in monocytes from patients following the administration of doxorubicin or cyclophosphamide. However, when patients had received doxorubicin and cyclophosphamide together on the same day, profound

suppression of in vitro activation was observed in 50% of the patients. This returned to normal by 3 weeks post-combination therapy.

Ibuprofen at dose levels of 40 μ g/mL suppressed the activation and the generation of the cytotoxic phenotype of monocytes in vitro. Low levels of ibuprofen (up to 10 μ g/mL), added to co-culture of monocytes and tumour cells once the monocytes were activated did not interfere with tumour killing (Fujimaki et al., 1993).

In a mouse tumour model repeated treatment of mice with diclofenac (i.p.) or with L-MTP-PE (i.v.) significantly suppressed tumour growth and increased the percentage of surviving mice. However, these effects were lost when diclofenac and L-MTP-PE treatment was combined.

Combined administration of indomethacin and L-MTP-PE prior to lethal irradiation protected 100% of the C57BL/6 mice from death, while no protection was seen with indomethacin alone and 80% protection was seen with L-MTP-PE (Fedorocko et al., 1996).

An in vitro study of the metabolism of L-MTP-PE and MTP-PE by cytochrome P450 in human liver microsomes indicated that L-MTP-PE and MTP-PE did not inhibit or induce cytochrome P450.

Pharmacokinetics

The pharmacokinetic data are derived from six studies, which were performed in the same four species (mice, rats, rabbits and dogs) that were used in most of the pharmacology and toxicology studies. The doses of L-MTP-PE investigated during the pharmacokinetic studies (in mice $1.2-4 \text{ mg/m}^2$ or 0.4-1.25 mg/kg, in rats $1.2-6 \text{ mg/m}^2$ or 0.2-1 mg/kg, in rabbits 1.2 mg/m^2 or 0.1 mg/kg, and in dogs $1-10 \text{ mg/m}^2$ or 0.05-0.5 mg/kg) were in the same order of magnitude as the doses of L-MTP-PE identified as the NOAEL (no observed adverse effect level) in rabbits and dogs, i.e., 0.1 mg/kg, and as the dose used in the Phase III clinical trial ($2-4 \text{ mg/m}^2$). The applicant performed a single dose study both with MTP-PE and L-MTP-PE in rats and dogs (study B56/1991). This study revealed that L-MTP-PE was extensively distributed to organs and tissues, whereas MTP-PE remained much more in the central plasma compartment.

- Methods of analysis.

Immunoassay

Plasma and serum concentrations of unchanged MTP-PE were measured using a chemoluminescence immunoassay (CLIA) (Gay et al., 1990, Gay et al., 1991). The CLIA assay was used to assess concentrations of MTP-PE in rat and dog plasma samples (Gay et al., 1993) and human serum samples (Landmann et al., 1994).

Radiometry

Preclinical animal pharmacokinetics investigations were mostly performed with both a tritium [³H] and a radio-carbon [¹⁴C]-labelled MTP-PE (Kocher, 1985; Wiegand, et al., 1986). Alternatively, the fate of L-MTP-PE was followed by labelling the liposomes with ⁶⁷Gallium-deferoxamine (⁶⁷Ga-DF) (Melissen et al., 1993)

– Absorption

As L-MTP-PE is administered by i.v. route, bioavailability is complete. Pharmacokinetic parameters were derived following single and repeated dosing of L-MTP-PE. Free MTP-PE was measured. Intravenous dosing was the only route of administration. The disposition of L-[³H]MTP-PE was studied in mice (Fogler et al., 1985), the disposition of L-[¹⁴C]MTP-PE was studied in rats (Gay et al., 1992; Wiegand et al., 1986), rabbit (Wiegand et al., 1986) and dog (Gay et al., 1992; Gygax et al., 1992). Shortly after i.v. administration, L-MTP-PE was cleared very rapidly from the circulation. The concentration of radiolabel in plasma followed a biphasic decrease, with a slower decrease during a later elimination phase.

In one study in dog, the mean plasma concentration-time profiles of total and free MTP-PE were superimposable at a dose of 2 mg/m² of L-MTP-PE, indicating that no liposomal drug was present in the circulation (Gygax D., 1992). The fact that free MTP-PE could sometimes be measured indicates leaking of MTP-PE from liposomes even during their very short residence time in the blood. In another study at doses of 1, 2 and 10 mg/m², plasma concentrations of total drug were higher than free drug, indicative the presence of liposomes in the circulation. Following administration of L-MTP-PE,

 $Cmax_{1hour}$ and AUC_{1-8h} for both free and total drug concentrations were consistent with a doseproportional relationship. L-MTP-PE and MTP-PE were administered separately to rats and dog. The AUC of MTP-PE following L-MTP-PE, is 10 less or 100 less of AUC of MTP-PE following MTP-PE in rat and dog respectively.

– Distribution

Distribution of L-MTP-PE was investigated in mice in two studies up to 24 h following i.v. administration of L-[3 H]MTP-PE and of MTP-PE in 67 Ga-DF radiolabelled liposomes (Fogler et al., 1985; Melissen et al., 1993). Organ-associated radioactivity indicated accumulation first in the lung, with 8% of the injected dose localized in the lung 5 minutes after administration. The liposomes distributed mainly in the liver (30% of the injected dose) and spleen (14-18% of the injected dose) over the 2 hours after administration. Investigation of the cell types involved in the uptake of liposomes showed that, in the liver, 83% of the liposomes were taken up by the macrophages (Kupffer cells). In the spleen, liposomes were taken up mainly by macrophages and dendritic cells.

The body distribution of L-MTP-PE was investigated in rats given intravenously L-[¹⁴C]MTP-PE or [³H]MTP-PE in ¹⁴C-labeled liposomes (Wiegand et al., 1984; Wiegand et al., 1986). Concentrations of the radiolabels were measured in organs after 5 and 30 minutes and 4, 6 and 24 hours. Radioactivity was distributed substantially to lung, spleen, liver and bone marrow. Radioactivity was rapidly cleared from the lung, while bone marrow, liver and spleen displayed a somewhat slower clearance of radioactivity. By 168 hours, low but significant residual ¹⁴C concentrations were found in all organs and tissues.

– Metabolism

No *in vitro* metabolism related experiments were submitted. Metabolism of L-MTP-PE was studied only in mice. When metabolism of L-MTP-PE was studied 60 minutes after i.v. injection of L-MTP-PE, practically all MTP-PE was intact in liver extracts. This persisted up to 4 hours and was observed in lung and spleen. The major peak of radioactivity extracted from urine, however, was MDP (Fogler et al., 1985)

– Excretion

The main route of excretion in rat, rabbit and dog for L-MTP-PE appeared to be urine.

The excretion of L-MTP-PE in urine was followed over 24 hours after the injection in mice (Fogler et al., 1985). Ten % of the ³H label was excreted by 24 hours.

The disposition of L-[¹⁴C]MTP-PE was investigated after intravenous administration to rats, rabbits and dogs (Wiegand et al., 1984). In all species, excretion of radioactivity was incomplete and ranged between 70 and 85% of the dose by day 7, and was still ongoing after 7 days. Excretion of ¹⁴C in the urine, however, was faster in rats than in rabbits and dogs.

No studies on excretion in milk were submitted.

Pharmacokinetic drug interactions

No pharmacokinetic drug interaction studies were submitted.

- Other pharmacokinetic studies

In Study B33/1983 the effect of liposome size on distribution to tissues and organs of rats was investigated. Liposomes larger than 5 micrometer showed high affinity to lung tissue. With decreasing size relatively more compound was distributed to liver and spleen.

Toxicology

A comprehensive panel of nonclinical toxicology studies was conducted in multiple species to assess the safety of L-MTP-PE or MTP-PE and to characterize any potential toxicity. Single dose and repeatdose studies were performed in mice, rats, rabbits and dogs. Teratogenic potential was investigated in rats and rabbits and mutagenicity was evaluated in an *in vitro* bacterial mutagenicity assay and in an *in vivo* micronucleus test in mice. Detection of chromosomal aberrations in mammalian cells was performed with the active agent, MTP-PE.

• Single dose toxicity

Single dose toxicity studies were performed in three species: mouse, hamster and dog. Rodents were comparatively insensitive as repeat i.v. doses up to 3,750 mg/m2 (1,250 mg/kg) of L-MTP-PE produced no mortality or overt signs of toxicity in mice. The rabbit and dog were selected as the primary species for toxicity studies based on preliminary experiments, which indicated that both species were more sensitive to the test compound than rodents. In the dog, administration of 10 mg/m2 of L-MTP-PE and above produced severe clinical signs and death within 24 hours of treatment, apparently due to infarction and extensive haemorrhage, especially from the gastrointestinal tract. This lethal syndrome occurred only in dogs given single injections of material. Tachyphylaxis to the pro-inflammatory response was described for various serum cytokines and body temperature.

• Repeat dose toxicity (with toxicokinetics)

Repeat dose studies were performed in five species: mice, rats, guinea pigs, rabbits, and dogs. Most of the studies were conducted in accordance with GLP guidelines.

In the mouse studies, L-MTP-PE at 10 mg/kg (30 mg/m^2) was administered intravenously for five consecutive days or twice weekly for one month. There was no treatment related mortality in the animals injected for 5 days, although weight gains during treatment were minimal in males. There were no signs of toxicity during treatment or gross pathology at the time of necropsy with either dosage regimen.

In the rats studies, daily administration of 0.1 to 1 mg/kg (0.6 to 6 mg/m²) of L-MTP-PE for two weeks did not show any sign of toxicity. Signs of pharmacological action were apparent in blood chemistry.

When guinea pigs were administered 0.1 to 25 mg/kg (2 to 500 mg/m²) of LMTP-PE twice weekly for four weeks all animals receiving 25 mg/kg were dead by 17 days after the start of treatment. Guinea pigs receiving 5 mg/kg or higher exhibited a febrile reaction and lost body weight.

Rabbits were administered intravenous doses of 0.001, 0.01, and 0.1 mg/kg (0.012, 0.12 or 1.2 mg/m^2), respectively) for one month. L-MTP-PE was tolerated at all dose levels without mortality. Gross and histopathologic changes occurred predominantly in the lungs, spleen, bone marrow, heart, and at injection sites. The changes in the lung, spleen and bone marrow were predominantly of an inflammatory nature, featuring infiltration/accumulation of leukocytes/macrophages around blood vessels, which is probably compatible with the macrophage-activating properties of LMTP-PE. However, intimal thickening and/or thrombus formation in pulmonary arteries was prominent in intermediate and high dose groups, although a tendency toward reversibility was observed at the end of the one month recovery period. It was concluded that the pulmonary arterial lesions were of uncertain etiology in rabbits that also have a background of spontaneous nodular pneumonitis and therefore the no-toxic-effect dose of the liposome-encapsulated material was considered to be 0.1 mg/kg. In the six month study, the treatment was tolerated without compound-related mortality and no salient changes in clinical or laboratory examinations up to and including a daily dose of 0.5 mg/kg. However, taking into account technical problems with administration and an infection with Encephalitozoon cuniculi, only 10 animals without any complications in total remained suitable for the purpose of the study. Due to technical and health problems, the objective of the study was not met.

Regarding the repeat dose studies performed in dogs, five subacute tolerance studies were initiated to test the various dosage regimens. Daily doses of 0.5 mg/kg (10 mg/m²) were injected for 5 consecutive days, or twice or 5 times a week for 4 weeks. A daily dose of 0.1 mg/kg (2 mg/m²) administered 5 times per week for a month was also studied. Results from these studies indicate that the toxicity related to multiple doses of 0.5 mg/kg was mild and centered around slight to moderate aberrations of haematology and clinical chemistry. Clinical signs were minimal and highly variable but included sporadic emesis, diarrhea, and pyrexia. The most prevalent clinical laboratory changes following one month dosing included slightly reduced erythrocytic parameters, leukocytosis often associated with neutrophilia or monocytosis, transient thrombocytopenia, slight hypokalemia, and increases in total serum globulin and cholesterol. Histopathological changes were minor and inconsistent, and arterial proliferative lesions were only present in one instance. Daily repeated doses of 0.1 mg/kg produced only minor fluctuations and this dose was essentially a no-observed-adverse-effect level.

The 3-month toxicity study conducted in dogs with intravenous doses of 0.001, 0.01, and 0.1 mg/kg, respectively (0.02, 0.2 or 2 mg/m²) confirmed the results observed in the tolerance studies. Adverse

effects were minimal. Slight decreases in albumins, increases in alpha- and beta and decreases in gamma-globulins, and slight leukocytosis were the only significant changes observed. There were no relevant gross or histological changes.

In 6-month pivotal study performed in dogs, overall, dogs in the 0.5 mg/kg (10 mg/m2) group showed signs of pronounced inflammatory response manifested as synovitis, pericarditis, inflammatory necrosis of the liver and vascular lesions accompanied by alteration and enlargement of lymph nodes. Considerable inter-individual differences were apparent in all these effects, which are probably a result of exaggerated pharmacological activity of the compound. Alterations related to this biological activity such as increased levels of C-reactive protein and fibrinogen and changes in the number of leukocytes were seen at all dose levels. The mild inflammatory response seen in the liver of some animals of the 0.1 mg/kg (2 mg/m2) group is also considered a reflection of the pharmacological activity since necrotic reactions were not apparent.

• Genotoxicity

The genotoxic potential of L-MTP-PE has been evaluated in in vitro and in vivo studies. L-MTP-PE was not a mutagen (data not shown).

• Carcinogenicity

Carcinogenicity studies were not submitted based on the applicant's justification that L-MTP-PE will be administered in conjunction with combination chemotherapy known to cause second malignancy (AML/MDS) in a proportion of patients. The applicant also claimed that L-MTP-PE does not present a known close chemical analogue with carcinogenic compounds and did not give rise to suspicious changes during the long-term toxicological and mutagenic-potential tests.

• Reproduction Toxicity

No studies have been submitted to assess the impact of L-MTP-PE on reproductive function. The applicant justification was that L-MTP-PE is being developed as adjunctive therapy in patients receiving chemotherapy including chemotherapy drugs reported to cause effects on fertility as well as foetal death and/or congenital anomalies. The applicant also pointed out that there were no results from repeat dose studies that suggested potential harmful effects which might lead to impairment of male or female reproductive function.

Reproductive effects were noted in developmental toxicity studies in rats and rabbits. No indication of treatment related embryo-foetal toxicity or teratogenicity was observed in rats at doses up to 0.8 mg/kg. In rabbits, there was an apparent association with growth retardation at doses beginning at 0.5 mg/kg. An increased incidence of abnormally ossified vertebrae was found. Skeletal examination also revealed slightly retarded ossification. A treatment-related reduction in food intake and maternal and foetal body weight gain was observed in another rabbit study. No treatment related external foetal abnormalities were found.

Globally, the low dose level of 0.1 mg/kg caused slight maternal toxicity, characterised by minor effects on body weight gain and food consumption during the first 6 days of treatment only. Only equivocal effects were seen at 0.2 mg/kg, but no definite conclusions regarding the no observable effect level could be made owing to the small group sizes used.

• Toxicokinetic data

Toxicokinetic data were submitted, mainly based on one study. Free and total concentrations of MTP-PE in plasma of beagle dogs have been measured on day 1, 86 and 178 during the repeated daily administration of 0.05, 0.1 and 0.5 mg/kg of L-MTP-PE for 6 months. Free and total AUC's of MTP-PE were consistent with a dose proportional relationship and have comparable values between day 1, 86, 178.

Exposure tended to be higher in rats than in dogs when given comparable doses.

• Local tolerance

Local tolerance was assessed in the single and repeat dose toxicity studies. L-MTP-PE was well tolerated following i.v. administration.

A non-GLP study was performed to investigate the skin sensitizing (contact allergenic) potency of

L-MTP-PE in albino guinea pig (Maurer, et al., 1987) No differences between the test groups and the vehicle treated controls (saline) were seen, after intradermal challenge application of L-MTP-PE. Additionally, there were no reactions suggestive of allergic or hypersensitivity reactions in the clinical trials.

• Other toxicity studies

Assessment of sensitization potential was performed in guinea pigs. There was no difference between L-MTP-PE groups and control. Additionally, there were no reactions suggestive of allergic or hypersensitivity reactions in the clinical trials.

Complementary non-GLP experiments (Study 20 and 21) were performed to evaluate the potential negative interactions that could occur in the simultaneous use of L-MTP-PE and chemotherapeutic drugs, especially with respect to enhancing hematologic toxicity of the chemotherapy agents. (Killion et al., 1992). According to the applicant, these studies showed that the intravenous administration of L-MTP-PE did not enhance the myelosuppressive and toxic effects of the chemotherapy drugs.

Ecotoxicity/environmental risk assessment

The applicant submitted an environmental risk assessment and justified that MEPACT does not pose a significant environmental risk.

Discussion on the non-clinical aspects

The applicant has submitted a number of published studies where MTP-PE was associated with monocytes and macrophages activation in vitro and in vivo. Systemic treatment with L-MTP-PE was tested in several tumour metastasis models in rodents. Trials of L-MTP-PE as adjuvant treatment have also been performed in dogs and cats. The treatment was variably effective in delaying tumour growth and enhancing animal survival. The exact mechanism by which MEPACT activation of monocytes and macrophages leads to antitumour activity in animals and humans is not yet known. Adequate information on the pharmacodynamic properties of MEPACT is provided in the SPC (see section 5.1)

Only the toxicology studies, which investigated QT intervals, included a GLP statement. This lack of GLP statement for the safety pharmacology studies is acceptable since several aspects of safety pharmacology have been assessed in GLP-compliant toxicology studies.

Only limited/marginal effects of L-MTP-PE on the cardiovascular system (prologation of PQ interval) and no effects on CNS have been observed. Some effects on the respiratory system have been noted. A moderate increase in respiration rate and reduced tidal volume were observed in anesthetized cats, transient irregular respiration was seen in rabbits and panting and an occasional respiratory distress was observed in dogs.

Respiratory distress has been mentioned in section 4.4 of the SPC. However the aetiology of this phenomenon is not known.

In general no adverse effects of L-MTP-PE treatment on efficacy of chemotherapeutic agents were observed. It may be possible to combine L-MTP-PE with doxorubicin, cisplatin, methotrexate, or cyclophosphamide as single agents. In contrast, a combination of chemotherapeutic agents (such as doxorubicin and cyclophosphamide) may suppress L-MTP-PE induced activation of monocytes. From these data is appears that doxorubicin is the preferred chemotherapeutic agent for a combination therapy with L-MTP-PE because doxorubicin even slightly enhanced the L-MTP-PE induced activation of human monocytes.

Ibuprofen does not seem to interfere with the efficacy of L-MTP-PE only in low doses. No studies on potential interactions with other relevant drugs prescribed during chemotherapeutic treatment such as corticosteroids or (other) anti-emetics have been presented.

L-MTP-PE was developed in order to increase the distribution of MTP-PE to organs and tissues. L-MTP-PE indeed significantly increased distribution to organs and tissues thereby making L-MTP-PE a more appropriate formulation than MTP-PE.

Pharmacokinetics of L-MTP-PE were studied both by means of radiolabelled material (14C and 3H) and cold material. L-MTP-PE was rapidly cleared from the central plasma compartment into tissues and organs. Distribution was mainly into lung, liver and spleen. There was no indication that

accumulation in plasma would take place. It is however, reasonable to assume that accumulation will take place in tissues and organs. Data on kinetics in pregnant and nursing animals were lacking. Plasma protein binding was not studied but it is reasonable to assume that it will be low.

Metabolism was only studied in mice. In liver tissue practically all MTP-PE was intact in liver extracts up to 4 hours and was also observed in lung and spleen. The major peak of radioactivity extracted from urine, however, was MDP.

Excretion mass balances were incomplete, most probably due to the use of inappropriate radiolabels. The main route of excretion in rat, rabbit and dog for L-MTP-PE related material appeared to be urine. Excretion in milk was not studied.

In sensitive species (rabbit and dog) the highest daily dose of liposomal mifamurtide that did not cause adverse effects was 0.1 mg/kg, corresponding to 1.2 and 2 mg/m2, respectively. The no-adverse-effect level for MEPACT in animals corresponds roughly to the 2 mg/m² recommend dose for humans.

Data from a six month dog study of daily intravenous injections of up to 0.5 mg/kg (10 mg/m2) MEPACT provide an 8- to 19-fold cumulative exposure safety margin for overt toxicity for the intended clinical dose in humans. Major toxic effects associated with these high daily and cumulative doses of MEPACT were mainly exaggerated pharmacological effects: pyrexia, signs of pronounced inflammatory response manifested as synovitis, bronchopneumonia, pericarditis and inflammatory necrosis of the liver and bone marrow. The following events were also observed: haemorrhage and prolongation of coagulation times, infarcts, morphological changes in the wall of small arteries, oedema and congestion of the central nervous system, minor cardiac effects, and slight hyponatraemia. MEPACT was not mutagenic and did not cause teratogenic effects in rats and rabbits. Embryotoxic effects were observed only at maternal toxic levels.

There were no results from general toxicity studies that suggested harmful effects on male or female reproductive organs. Specific studies addressing reproductive function, perinatal toxicity and carcinogenic potential have not been performed.

MEPACT does not appear to be a significant environmental concern because the estimated amount of MTP-PE to be release in the environment by each patient following use of the product is low, and because of the rarity of the condition. The main route of release will be waste water, and the PECsurface water for MEPACT was well below the threshold of the CHMP guideline for Phase I Assessment.

2.4 Clinical aspects

Introduction

The main study of clinical efficacy and safety was INT-0133, a randomised clinical trial. The applicant also submitted 11 phase I studies on PK, PD, safety and efficacy of L-MTP-PE in several concentrations in cancer patients. Also 7 phase II studies were submitted. These studies investigated the activity of different regimens of L-MTP-PE in patients with metastatic disease. Pharmacology results are taken from a total of eight studies in cancer patients (see Table 1).

Study ID	Phase	Patients	No. patients	Dose, regimen, duration
01	Ι	Metastatic cancer	38	$0.01-6 \text{ mg/m}^2$ 1h inf twice weekly
				4 weeks
02	Ι	Metastatic cancer	32 (4 PK)	0.01-12 mg/m ² 1h inf twice
				weekly 9 weeks
03	Ι	Metastatic cancer	48	0.05-9 mg/m ² 1h inf once weekly
				12 weeks
07	Ι	Adv malignant	30	1 or 4 mg 1h inf once weekly for
		melanoma		12 weeks
08	II	Relapsed	33	2 mg/m^2 1h infusion; twice
		osteosarcoma		weekly; 3-months

Table 1. Listing of clinical studies with pharmacokinetic and pharmacodynamic data.

				2 mg/m ² (titration up to 2 mg/m ² +2 mg) 1h inf; twice weekly 12 weeks followed once weekly 12 weeks.
09	II	Resectable melanoma high risk relapse	20	2 mg/m ² 1h inf twice weekly for 4 weeks before resection and 8 weeks after then 12 weeks once weekly
10	II	Relapsed osteosarcoma	12	2 mg/m ² (titration up to 2 mg/m ² +2 mg) 1h inf; twice weekly 12 weeks followed once weekly 12 weeks. + IFOS 1.8 mg/m ² or Cisplatin 120 mg/m ²
BR/MA1	Ι	Advanced metastatic	14 (14 PK)	4 mg 30 min inf, twice weekly 12 weeks
BR/MA2	II	NSCLC inoperable, recurrent, metastatic	12	1, 2, 4 mg 30 min inf, <u>once</u> weekly 12 weeks
BR/MB1	II	Resected Dukes'C colon adenocarcinoma	32	0.25, 1, 4 mg 30 min inf <u>once</u> weekly 24 weeks
BR/MC1	Ι	Metastatic recurrent colon carcinoma	24	0.25, 1, 4 mg 30 min inf, 13 weeks + 5-FU 370 mg/m ² and leucovorin 200 mg/m ² for 5 days every 4 weeks

GCP

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 trial INT-0133. No critical findings were observed and the overall compliance to current GCP standards was considered sufficient (see also discussion on clinical efficacy).

Pharmacokinetics

The information on the pharmacokinetics of L-MTP-PE submitted by the applicant was mainly based on biodistribution data from 4 patients (study 02) and serum concentrations from 14 cancer patients at a dose of 4 mg administered twice weekly (study BR/AM 1).

Study 02 was a single-center, open-label trial in adult patients with metastatic cancer refractory to standard therapy who had an estimated life expectancy of at least 12 weeks. The objectives were to determine the tolerability, immunomodulatory effects, antitumor activity of L-MTP-PE and, in select patients, the biodistribution of 99mTc-labeled L-MTP-PE, in patients with advanced metastatic malignancies. Thirty-two (32) patients were enrolled in the study, 21 males and 11 females with age ranging from 18 to 71 year (mean 51.4 years). The majority of patients had tumours of the gastrointestinal tract. Twenty patients completed the study and 12 were prematurely discontinued, primarily for reasons of unsatisfactory therapeutic response (progressive disease). Only four patients who had been infused with 1 mg of 99mTc-labeled L-MTP-PE either several days before infusion with 6 mg/m² of unlabeled L-MTP-PE or co-administered with unlabeled L-MTP-PE for a total dose of 6 mg/m^2 provided blood samples for the assessment of biodistribution. Blood samples were collected at the completion of infusion of L-MTP-PE (time 0) and at 1, 5, 10, 15, 30, 60, 120, and 240 minutes and 24 hours after injection. To assess biodistribution, total body images were obtained at 2, 6, and 24 hours following injection of 99mTc-labeled L-MTP-PE using a dual-headed gamma camera. Static spot images of the chest, abdomen, and pelvis in anterior and posterior views were obtained at the same imaging time slots as the total body images.

Study BR/MA 1 was of open and non-comparative design. The initial objective was to evaluate serum concentration-time profiles of free and total L-MTP-PE in patients with advanced metastatic cancer given repeated intravenous infusions of 4 mg of L-MTP-PE using serum remaining after the determination of serologic parameters. Patients with advanced metastatic cancers received 4 mg of L-

MTP-PE infused i.v. over 30 minutes twice weekly for 12 weeks. Blood samples were collected just before the start of infusion (hour 0) and 0.5, 2, 4, 6, 24, and 72 hours after the start of infusion. Additional samples were collected at various intervals between week 4 and week 12. Free drug concentrations were measured before disruption of the liposomes and total drug concentrations were measured after the release of MTP-PE from the disrupted liposomes. Serum concentrations of free and total MTP-PE were measured by an immunoassay that had a limit of quantitation of 0.1 nmol/l for free drug and 1.0 nmol/l for total drug. Within-assay and between-assay variations were in the range of 1.4% to 20.6% and recoveries were between 92.5% and 113.7%. Samples were analyzed for 14 patients, 7 males and 7 females with a mean age of 54.6 years (range 30 to 69 years).

Absorption

MEPACT is lyophilisate for suspension for injection. Therefore, bioavailability is per definition 100%.

• Distribution

In study 02, at 6 hours after injection of 99mTc-labeled liposomes containing 1 mg MTP-PE, radioactivity was found in liver, spleen, nasopharynx, thyroid, and, to a lesser extent, in lung. This radioactivity partially cleared by 24 hours. In 2 of the 4 patients, lung metastases were also observed. Plasma elimination of 99mTc-labeled L-MTP-PE was biphasic with an elimination half-life of 15 ± 9 min and $18 \pm 2h$ for the initial and terminal phases, respectively. Elimination of radioactivity was similar in plasma and red blood cells.

• Elimination

In study BR/MA 1, after infusion, total and free serum concentrations declined to values below the limit of quantitation within 24 hours. Serum concentration-time curves of free drug were lower than those of total drug, indicating the presence of liposomes in circulation. Mean serum concentrations measured at the end of the first infusion ($C_{0.5h}$) were 8.5 ± 6.9 nmol/l for total drug and 2.0 ± 0.8 nmol/l for free drug. Mean AUC values of the free drug after the first and last infusion were similar.

Figure 1 Mean serum concentration-time (hours) profiles of total and free MTP-PE after the first (week 1) and the last (week 11 or 12) intravenous infusion during 30 min of 4 mg L-MTP-PE (means of 7 to 9 patients, study BR/MA 1).



• Dose proportionality and time dependencies

Pharmacokinetics of L-MTP-PE have been measured only at a dose of 4 mg, which is somewhat higher than the proposed dose of 2 mg/m². Mean serum concentration-time curves of total and free MTP-PE after the first infusion on day 1 and after the last infusion during either week 11 or week 12 were comparable (Figure 1).

• Special populations

None of the intrinsic or extrinsic factors have been analysed for the pharmacokinetics of L-MTP-PE.

• Pharmacokinetic interaction studies

No formal interaction studies have been performed with MEPACT and other medicinal products.

• Pharmacokinetics using human biomaterials

No studies submitted.

Pharmacodynamics

• Mechanism of action

Immunomodulatory effects of L-MTP-PE were investigated in 11 phase I/II studies.

Liposomal MTP-PE is selectively taken up by macrophages and monocytes and slowly broken down within the cell. Cellular released L-MTP-PE may signal through the NOD2 receptor, an intracellular protein that induces NF- κ B activation and is implicated in innate immune defense against bacteria (Nardin et al. 2006).

Immunomodulatory effects of L-MTP-PE were investigated in 11 phase I/II studies. MTP-PE induced TNF α , IL-6, CRP and neopterin (usually early) during the treatment of most patients. According to the applicant, the observed induction was consistent with activation of macrophages by L-MTP-PE. The extent of induction varied greatly between patients and between studies e.g. mean maximal induction of IL-6 varied between 3- and 90-fold. According to the applicant, this may to some extent be explained that many of these assays were variable and not validated at the time these studies were performed.

• Primary and Secondary pharmacology

Effect of L-MTP-PE dose $(0.01 - 12 \text{ mg/m}^2)$ on immunodulatory effects were investigated in studies 01, 02, 03, 07, BR/MA2, BR/MB1 and BR/MC1 in patients with various forms of metastatic disease (Table 3). In general, MTP-PE administered at doses <0.25 mg/m² resulted in less induction of TNF α , IL-6, and IL-1 (02, BR/MB1, BR/MC1). There was considerable variability between studies but there was a trend that the rise in CRP levels increases with higher dose. In osteosarcoma patients treated with 2 mg/m² L-MTP-PE, CRP was elevated in all patients at 24 hours and elevations in CRP could be detected throughout the treatment course (study 08), (Kleinerman et al., 1992) whereas in study 10, only in 4/10 patients CRP was increased after the first administration (Kleinerman et al., 1995). At doses>1 mg/m² L-MTP-PE mean CRP levels were induced > 2-fold of the baseline.

Table 2. Mean % change from baseline CRP 24h after the first administration of L-MTP-PE, dose dependency

Study	01	02	BR/MA2 (1,2, 4 mg)	BR/MB1 (0.25,1,4 mg)	BR/MBC1 (0.25,1,4 mg)
Baseline CRP Dose	22#	5	7	0.6	2
0.01-0.25	-6 ± 56	141 ±323		501	40
mg/m2	N=8	N=2		N=6	N=4
0.4-1.8	29 ± 136	99 ± 186	-5 ± 20	573	319
mg/m2	N=6	N=5	N=2 (1 mg)	N=12	N=4
			147 ± 329		
			N=5 (2 mg)		
2-4 mg/m2		444 ± 589	221 ± 325	376	1081
_		N=8	N=4	N=13	N=10
2-6 mg/m2	50 ± 54	246 ± 283			
	N=8	N=2			

#. Baseline value for CRP was very high in study 01, in healthy subjects CRP levels are approximately 1 mg/dl.

Changes from baseline MTA 24h after the first administration of L-MTP-PE are shown in Table 3. In study 01, approximately half of the patients showed increases in MTA. The increase in activity was seen largely at doses less than 2 mg/m2 whereas in patients who received 2 to 6 mg/m2, and particularly in the patients treated at 6 mg/ m^2 , no effect on MTA was seen. MTA values in healthy volunteers were < 10%. At high baseline MTA values >35% (study 02 and BRMA1), MTA values decreased following L-MTP-PE infusion. Also in study BR/MA1, MTA declined during treatment with 4 mg L-MTP-PE in the 5 patients with initially high MTA(55±4%), whereas the 9 patients with initial low MTA (2±3%) showed a small, transient increase in MTA after the first administration of L-MTP-PE (Landmann et al., 1994). Highest MTA values were observed in week 4 in this study and in study 07 in weeks 5 to 7. In study 08, all except 1 patient had baseline value <10% cytotoxicity. 8/10 patients showed an increase in the absolute percent cytotoxicity value of more than 10% (12% to

48%)(Kleinerman et al. 1992). In study 09, in 9 out of 18 patients MTA increased more than 10%, which was correlated with clinical status (Fujimaki et al., 1993).

Study	01	02	03	07 (1, 4 mg)
Baseline MTA(%) \$ Dose	21	*	33	19
0.01-0.25 mg/m2	93 ± 219 N=4	182 ± 387 N=6	63 ±101 N=8	
0.4-1.8 mg/m2	31 ± 87 N=11	44 ± 139 N=8	545 ±1214 N=6	52# N=7
2-4 mg/m2		15 ± 150 N=4	-58 ±24 N=3	11# N=7
2-6 mg/m2	-24 ± 57 N=5			
6-12 mg/m2		45 ± 138	22 ± 118	

Table 3. Mean % change from baseline MTA 24 hours after the first administration of L-MTP-PE, dose dependency

\$Baseline MTA values for cancer patients were higher than reported for healthy controls(<10%)

* Baseline MTA value was different for the 4 dose groups: 11, 22, 30 and 41 from low to high L-MTP-PE.

#A higher increase in MTA was observed after 5 to 7 weeks. At both 1 and 4 mg MTA activity doubled from 18% baseline to 36% 24h post-dose.

Discussion on Clinical Pharmacology

After intravenous administration in 21 healthy adult subjects mifamurtide was cleared rapidly from plasma (minutes), resulting in a very low plasma concentration of total (liposomal and free) mifamurtide. The mean AUC was 17.0 +/- 4.71 h x nM and Cmax was 15.7 +/- 3.72 nM. In separate study in 14 patients, mean serum concentration-time curves of total and free mifamurtide that were assessed after the first infusion of MEPACT and after a last infusion 11 or 12 weeks later, were almost superimposable and the mean AUC values of the free mifamurtide after the first and last infusion were similar. These data indicate that neither total nor free mifamurtide accumulated during the treatment period (see SPC section 5.2).

At 6 hours after injection of radiolabelled liposomes containing 6 mg mifamurtide, radioactivity was found in liver, spleen, nasopharynx, thyroid, and, to a lesser extent, in lung. The liposomes were phagocytosed by cells of the reticuloendothelial system. In 2 of 4 patients with lung metastases, radioactivity was associated with lung metastases. Mean half-life of radiolabelled material was biphasic with an α phase of about 15 minutes and a terminal half-life of approximately 18 hours (see SPC section 5.2).

MEPACT is contraindicated in case of hypersensitivity to the active substance or to any of the excipients. MEPACT is also contraindicated in case of concurrent use with ciclosporin or other calcineurin inhibitors (see SPC section 4.5), or in case of concurrent use with high-dose non-steroidal anti-inflammatory drugs (NSAIDs, cyclooxygenase inhibitors) (see SPC section 4.5). The contraindications are adequately reflected in the SPC. (See SPC section 4.3).

No PK/PD studies with MEPACT have been performed in the target population. The applicant has committed to provide a final clinical study report of a clinical trial including PK/PD data obtained in the target population from the ongoing MTP-OS-403 study as a follow-up measure.

The pharmacokinetics of mifamurtide in patients with renal or hepatic impairment have not been formally studied. Caution should be used in these patients because dose adjustment information is not available. Continued monitoring of the kidney and liver function is recommended if MEPACT is used beyond completion of chemotherapy until all therapy is completed. (See SPC section 4.2). The applicant has committed to conduct a clinical study to assess the PK of MEPACT in patients with renal or hepatic impairment as a follow-up measure.

Interactions with other medicinal products and other forms of interaction are adequately described in the SPC (see SPC section 4.5).

Limited studies of the interaction of MEPACT with chemotherapy have been conducted. Although these studies are not conclusive, there is no evidence of interference of MEPACT with the anti-tumour effects of chemotherapy and vice versa

It is recommended to separate the administration times of MEPACT and doxorubicin or other lipophilic medicinal products if used in the same chemotherapy regimen.

The use of MEPACT concurrently with ciclosporin or other calcineurin inhibitors is contraindicated due to their hypothesised effect on splenic macrophages and mononuclear phagocytic function (see section 4.3).

Also, it has been demonstrated *in vitro* that high-dose NSAIDs (cyclooxygenase inhibitors) can block the macrophage activating effect of liposomal mifamurtide. Therefore the use of high-dose NSAIDs is contraindicated (see section 4.3).

Because mifamurtide acts through stimulation of the immune system, the chronic or routine use of corticosteroids should be avoided during treatment with MEPACT.

In vitro interaction studies showed that liposomal and non-liposomal mifamurtide do not inhibit the metabolic activity of cytochrome P450 in pooled human liver microsomes. Liposomal and non-liposomal mifamurtide do not induce the metabolic activity or the transcription of cytochrome P450 in primary cultures of freshly isolated human hepatocytes. Mifamurtide is therefore not expected to interact with the metabolism of substances that are hepatic cytochrome P450 substrates.

In a large controlled randomised study, MEPACT used at the recommended dose and schedule with other medicinal products that have known renal (cisplatin, ifosfamide) or hepatic (high-dose methotrexate, ifosfamide) toxicities did not exacerbate those toxicities and there was no need to adjust mifamurtide dose.

From the pharmacodynamic data presented, L-MTP-PE administration was fairly consistently associated with induction of TNF α , IL-6, CRP and neopterin and macrophage tumouricidal activity (MTA). These effects are consistent with activation of macrophages. No increase in MTA values was observed at L-MTP-PE doses above 2 mg/m², although the results should be interpreted with care because of high baseline levels. From the data presented, the optimal biological dose cannot be determined.

Clinical efficacy

• Dose response study(ies)

Two phase II studies were submitted. Study 08 and (1988-1992) included 33 osteosarcoma patients that developed or retained metastases despite chemotherapy, after primary tumour resection (Kleinerman et al., 1995). A historical comparison of efficacy endpoints was presented (data not shown).

Protocol 10 (1991-1992) included 12 osteosarcoma patients with persisting or developing metastases after or during chemotherapy (cisplatin or ifosfamide), after primary tumor resection. Protocol 10 studied the adverse events induced by L-MTP-PE when given in combination with cisplatin or ifosfamide only (data not shown). No efficacy parameters were analysed.

• Main study

The main efficacy study submitted was a phase III intergroup trial of doxorubicin, cisplatin and methotrexate with and without ifosfamide, with and without muramyl tripeptide phosphatidyl ethanolamine (MTP-PE) for treatment of osteogenic sarcoma (Meyers et al. 2005 and 2008; Romet-Lemonne et al. 2005; Anderson 2006).

METHODS

Study Participants

The main inclusion criteria were:

- Newly diagnosed (no greater than one month from diagnostic biopsy) fully malignant high-grade osteosarcoma of bone.

- Patients \leq 30 years old

- Patients with normal organ function (renal: serum creatinine ≤ 1.5 x normal or creatinine clearance or radioisotope GFR > 40 ml/min/m² or >70 ml/min/1.73 m² or an equivalent GFR as determined by the institutional normal range; hepatic: total bilirubin ≤ 1.5 x normal and SGOT (AST) or SGPT (ALT) < 2.5 x normal; cardiac: shortening fraction of $\geq 29\%$ by echocardiogram or ejection fraction $\geq 50\%$ by radionuclide angiogram; patients with a history of pericarditis or myocarditis were excluded).

Patients with low-grade osteosarcoma, parosteal or periosteal sarcoma were ineligible. Patients with metastatic disease were ineligible. No prior chemotherapy or radiation therapy were allowed. Patients should have had biopsy only to establish a diagnosis.

Treatments

This study was conducted in three phases (induction therapy; surgery; maintenance).

Phase 1 (Induction Therapy) Within 30 days of a new diagnosis of high-grade osteosarcoma, patients were randomly assigned to one of four treatment groups. Until week 10 patients received neoadjuvant induction therapy with one of two chemotherapy regimens: Regimen A consisted of doxorubicin, cisplatin, and methotrexate, and Regimen B consisted of doxorubicin, ifosfamide, and methotrexate.

Phase 2 Definitive surgery was performed between weeks 10 to 11, during which patients received no study medication.

Phase 3 Beginning at week 12, patients in Regimen A received maintenance therapy that consisted of the induction chemotherapeutics with or without L-MTP-PE. Maintenance therapy for patients in Regimen B consisted of the induction chemotherapeutics plus cisplatin, with or without L-MTP-PE. (See tables 4 and 5, Figure 2).

		Induction Phase (Weeks 0-9)	Maintenar (Weeks	nce Phase 12-36)
	Drug (Dose)	Number of	Number of	Duration of
		Doses/Courses	Doses/Courses	Treatment
Regime	en A			
	DOXO (25 mg/m2/day x 3)	2	4	20 Weeks
	CDDP (120 mg/m ²)	2	2	20 Weeks
	IFOS $(1.8 \text{ g/m}^2/\text{day x 5})$	None	None	
	MTX (12 g/m ²)	4	8	20 Weeks
	L-MTP-PE (2 mg/m^2)	None	None	
Regime	en A + L-MTP-PE			
	DOXO (25 mg/m2/day x 3)	2	4	20 Weeks
	CDDP (120 mg/m ²)	2	2	20 Weeks
	IFOS (1.8 g/m ² /day x 5)	None	None	
	MTX (12 g/m ²)	4	8	20 Weeks
	L-MTP-PE (2 mg/m ²)	None	48	36 Weeks ^b
Regime	en B		· · ·	
	DOXO (25 mg/m2/day x 3)	2	4	27 Weeks
	CDDP (120 mg/m ²)	None	4	27 Weeks
	IFOS (1.8 g/m ² /day x 5)	2	3	27 Weeks
	MTX (12 g/m ²)	4	8	27 Weeks
	L-MTP-PE (2 mg/m^2)	None	None	
Regime	en B + L-MTP-PE			
	DOXO (25 mg/m2/day x 3)	2	4	27 Weeks
	CDDP (120 mg/m ²)	None	4	27 Weeks
	IFOS (1.8 g/m ² /day x 5)	2	3	27 Weeks
	MTX (12 g/m ²)	4	8	27 Weeks
	L-MTP-PE (2 mg/m ²)	None	48	36 Weeks ^b

Table 4. Comparison of treatment arms in INT-0133

^a Patients underwent definitive surgery during Weeks 10-11, during which they received no study medication.

^b Twice weekly for 12 weeks, then once weekly for 24 weeks

 Table 5. Chemotherapy treatment schedule in INT-0133

											Week	of Treat	ment									
			Indu	tion										N	laintenar	ice						
	0	3	4	5	8	9		12	15	16	17	20	21	22	25	26	27	30	31	32	35	38
Regimen A	CDDP DOXO x 72 h	MTX	MTX	CDDP DOXO x 72 h	MTX	MTX		CDDP DOXO x 72 h	MTX	MTX	CDDP DOXO x 72 h	MTX	MTX	DOXO x 72 hrs	MTX	MTX	DOXO x 72 h	MTX	MTX			
Regimen A + L-MTP-PE	CDDP DOXO x 72 h	MTX	MTX	CDDP DOXO x 72 h	MTX	MTX		CDDP DOXO x 72 h L- MTP- PE	MTX L- MTP- PE	MTX L- MTP- PE	CDDP DOXO x 72 h L- MTP- PE	MTX L- MTP- PE	MTX L- MTP- PE	DOXO x 72 hrs L-MTP- PE	MTX L= MTP- PE	MTX L- MTP -PE	DOXO x 72 h L-MTP- PE	MTX L- MTP -PE	MTX L- MTP- PE	L- MTP- PE	L- MTP- PE	L-MTP- PE
Regimen B	IFOS x 5d DOXO x 72h	MTX	MTX	IFOS x 5d DOXO x 72h	MTX	MTX	gery	CDDP DOXO x 72 h	MTX	MTX	IFOS x 5d DOXO x 72h	MTX	MTX	CDDP DOXO x 72 h	MTX	MTX	IFOS x 5d DOXO x 72h	MTX	MTX	CDDP	IFOS x 5d	CDDP
Regimen B + L-MTP-PE	IFOS x 5d DOXO x 72h	MTX	MTX	IFOS x 5d DOXO x 72h	MTX	MTX		CDDP DOXO x 72 h L- MTP- PE	MTX L- MTP- PE	MTX L- MTP- PE	IFOS x 5d DOXO x 72h L- MTP- PE	MTX L- MTP- PE	MTX L- MTP- PE	CDDP DOXO x 72 h L-MTP- PE	MTX L- MTP- PE	MTX L- MTP -PE	IFOS x 5d DOXO x 72h L-MTP- PE	MTX L- MTP -PE	MTX L- MTP- PE	CDDP L- MTP- PE	IFOS x 5d L- MTP- PE	CDDP L-MTP- PPE

Patients assigned to receive L-MTP-PE in the maintenance phase received twice weekly i.v. injections for 12 weeks followed by once weekly injections for an additional 24 weeks. The starting dose of L-MTP-PE was 2 mg/m². According to the protocol, the dose could be escalated twice (to 2 mg/m² + 1 mg and then to 2 mg/m²+2 mg) until biologic markers of activity were seen: elevation of oral body temperature to at least 38.1°C within 24 hours of beginning drug administration, or the presence of grade 2 visible rigors lasting 30 minutes, or a significant elevation in CRP (>2x baseline) 24 hours post-dosing.

Figure 2: Study design



Objectives

The trial protocol stated multiple specific aims, including to improve the survival of patients with osteosarcoma, to compare the results of combined chemotherapeutic regimens with or without ifosamide, to test the effect of ifosfamide on histologic response; and to determine whether MTP-PE can improve disease-free survival for patients with osteogenic sarcoma.

Outcomes/endpoints

The primary endpoint stated in the protocol was disease-free survival for both treatment comparisons (MTP-PE maintenance and ifosfamide induction, respectively). Disease free survival (DFS) was defined as survival from randomization to relapse of osteosarcoma or death.

Sample size

The sample size calculation assumed 60% long-term survival, a Gompertz model with long-term DFS of 60% v. 72%, 2-sided significance level of .05, power of 80% and 2 years of flow-up, yielding 3.9 years of accrual or 585 patients.

Randomisation

Randomisation was stratified for LDH, involvement above knee or elbow, and prior amputation.

Blinding (masking)

None.

Statistical methods

Both primary analyses were pre-specified to use a logrank comparing the two levels of one factor while stratifying for the other (MTP-PE maintenance and ifosfamide induction, respectively).

In a separate cohort within the same study, patients with metastatic or unresectable disease could also be enrolled. These were to be considered separate from all efficacy analyses (no formal testing was to take place).

Results

Participant flow

A total of 793 patients were enrolled, 115 of whom had metastatic or unresectable disease. The 678 patients with non-metastatic resectable disease were referred to as the intent-to-treat (ITT) group and were the subject of the primary analysis of this study (INT-0133 pivotal). Eventually 664 patients were deemed eligible for study entry. 303 patients withdrew from the study during the treatment or follow up phases, mostly due to disease progression. 464 patients completed protocol therapy.

The intent-to-treat (ITT) dataset included 372 male and 306 female patients. The mean age of the patients was approximately 14 years and the majority of patients were white. Most subjects had a primary tumor site in either the femur or tibia. The applicant considers demographic characteristics to be similar across treatment groups.

Patients with metastatic or unresectable disease at baseline are analyzed separately (INT-0133"other"). Therefore, the primary analysis set was comprised of all randomized patients who did not have evidence of metastasis and who had (supposedly) resectable tumours at the time of randomization.

Clinical assessment and imaging study efficacy endpoints were designed to be completed at designated time points, i.e. each course of treatment.

Post-treatment follow-up was to continue after treatment every 3 months for 1 year, then every 6 months for 2 years, then once a year, and at relapse after completion of the study. Table 5 summarizes the length of actual follow-up for patients in the ITT dataset and table 6 shows the actual number of phase reports per patient per year after final treatment visit.

Recruitment

Intergroup Study 0133 (INT-0133, also referred to as CCG-7921 and POG-9351) was conducted by the Children's Cancer Group (CCG) and the Pediatric Oncology Group (POG) under the leadership of the U.S. NCI. The study was conducted at 178 centers, most in the United States. Subsequently the POG and CCG were combined to create the Children's Oncology Group (COG).

Conduct of the study

The original protocol was dated 4 March 1993. Seven amendments were made to the protocol. In general, these amendments extended the administration of L-MTP-PE for an additional 12 weeks, clarified procedures for the administration of L-MTP-PE and other study medications, further defined safety procedures, and made a variety of administrative clarifications and corrections. The amendments included also the addition of three originally unplanned interim analyses. During the time this trial was accruing patients, NCI implemented requirements that the Cancer Cooperative Groups, including POG and CCG, increase the rigor of the data monitoring committees and implement formal interim analysis plans. In addition, a proportional hazards regression model was used to assess interaction between the chemotherapy and L-MTP-PE therapy.

Baseline data

Patients' demographics are shown in Table 6.

	Regimen A	Regimen A + L-MTP-PE	Regimen B	Regimen B + L-MTP-PE
Gender				
Male	85	95	87	105
Female	89	72	79	66
Total	174	167	166	171
Age (years)				
Mean	13.8	14.0	13.5	13.8
Median	13.3	14.3	13.6	13.9
Range	4.0 - 30.1	4.9 - 29.2	4.2 - 30.6	1.4 - 30.4
Race				
White	116	109	120	105
Hispanic	20	27	16	22
Black	26	19	25	27
Oriental	4	4	0	б
Filipino	1	3	1	2
Other	7	5	4	9
Primary Tumor				
Arm – Humerus	18	21	16	21
Arm – Radius	2	0	4	5
Arm – Ulna	0	1	1	0
Arm	0	0	1	0
Leg – Femur	91	91	96	91
Leg – Tibia	40	45	38	45
Leg – Fibula	7	3	2	3
Leg	1	0	1	0
Other	8	5	7	4
Unknown	7	1	0	2

Table 6. Patients' demographics (pivotal study INT-0133)

Numbers analysed

The intent-to-treat dataset included 678 patients with non-metastatic and resectable disease. 664 patients were included in the eligible-patient data set.

Outcomes and estimation

Efficacy results were presented based on the primary analysis set as well as based on updated data sets. Results for disease-free survival and overall survival are shown in Tables 7-9 and Figures 3-4.

		2003	Data		2006 Data				2007 Data			
Treatment	# of Pts (deaths)	P- value ¹	Hazard Ratio	95% CI for HR ²	# of Pts (deaths)	P- value	Hazard Ratio	95% CI for HR	# of Pts (deaths)	P- value	Hazard Ratio	95% CI for HR
No MEPACT	340 (85)		1.00		340 (100)		1.00		340 (100)		1.00	
MEPACT	338 (63)	0.0183	0.67	(0.48, 0.94)	338 (73)	0.0352	0.72	(0.53, 0.98)	338 (73)	0.0313	0.72	(0.53, 0.97)
¹ P-value for ² Hazard ratio	¹ P-value for comparing "No MEPACT" with "MEPACT" from log-rank test stratified by ifosfamide use and randomization strata.											

Table 7: Overall Survival: 2003, 2006 and 2007 Datasets

	2003 Data				2006 Data				2007 Data			
Treatment	# of Pts (events)	P- value ¹	Hazard Ratio	95% CI for HR ²	# of Pts (events)	P- value	Hazard Ratio	95% CI for HR	# of Pts (events)	P- value	Hazard Ratio	95% CI for HR
No MEPACT	340 (126)		1.00		340 (133)		1.00		340 (133)		1.00	
MEPACT	338 (102)	0.0245	0.74	(0.57, 0.96)	338 (107)	0.0623	0.78	(0.61, 1.01)	338 (107)	0.0586	0.78	(0.61, 1.01)
¹ P-value for	¹ P-value for comparing "No MEPACT" with "MEPACT" from log-rank test stratified by ifosfamide use and randomization strata.											
² Hazard ratio	5				-							

 Table 8: Disease-Free Survival: 2003, 2006 and 2007 Datasets

Table 9. Four and six year survival probabilities (primary analysis set) – 2003 Dataset

Variable	# of Pts (events)	4-Year	6-Year							
Disease Free Survival Probability (95% CI)										
No L-MTP-PE (A/B)	340 (126)	0.609 (0.556, 0.667)	0.574 (0.517, 0.636)							
L-MTP-PE (A+/B+)	338 (102)	0.696 (0.646, 0.750)	0.661 (0.607, 0.720)							
Overall Survival Probability (95% CI)										
No L-MTP-PE (A/B)	340 (85)	0.773 (0.726, 0.823)	0.655 (0.591, 0.726)							
L-MTP-PE (A+/B+)	338 (63)	0.838 (0.796, 0.881)	0.768 (0.715, 0.826)							
Event Free Survival Probability (95% CI)										
No L-MTP-PE (A/B)	340 (126)	0.606 (0.553, 0.664)	0.578 (0.523, 0.640)							
L-MTP-PE (A+/B+)	338 (106)	0.686 (0.635, 0.740)	0.646 (0.591, 0.706)							

Figure 3. DFS in the primary analysis set (pivotal study INT-0133) - 2003 Dataset





Figure 4. OS in the primary analysis set(pivotal study INT-0133) - 2003 Dataset

Ancillary analyses

The results of subgroup analyses for overall survival and disease-free survival are shown in figures 5-8 (see discussion on clinical efficacy).



2007 Overall Survival: MEPACT vs. No MEPACT



Figure 6. Forest Plot: Disease-Free Survival, 2007 Dataset

2007 Disease-Free Survival: MEPACT vs. No MEPACT



Figure 7. Overall Survival by Treatment Assignment Primary Analysis Set 2006 Follow Up – 2006 Dataset



Figure 8. Disease-Free Survival by Treatment Assignment Primary Analysis Set – 2003 Dataset



Disease-Free Survival: Primary Analysis Set

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

None submitted.

• Clinical studies in special populations

None submitted.

• Supportive study(ies)

Patients with metastatic disease or unresectable primary disease were included in trial INT-0133 as a separate cohort (115 patients were included). Exploratory efficacy analyses were conducted and submitted (data not shown). Efficacy data from study 08 and Protocol 10 were also submitted (data not shown).

• Discussion on clinical efficacy

MEPACT significantly increased the overall survival of patients with newly-diagnosed resectable high-grade osteosarcoma when used in conjunction with combination chemotherapy when compared to chemotherapy alone. In a randomised phase III study of 678 patients with newly-diagnosed resectable high-grade osteosarcoma, the addition of adjuvant MEPACT to chemotherapy resulted in a relative reduction in the risk of death of 28% (p = 0.0313, hazard ratio (HR) = 0.72 [95% confidence interval (CI): 0.53, 0.97]).

The safety and efficacy of MEPACT have been established in children from the age of 2 years. It is not recommended for use in children below the age of 2 due to a lack of data on efficacy and safety in this age group. (See SPC section 4.2)

None of the patients treated in the osteosarcoma studies were 65 or older and in the phase III randomised study, only patients up to age 30 years were included. Therefore, there are not sufficient data to recommend the use of MEPACT in patients >30 years of age. (See SPC section 4.2)

During the assessment, the CHMP considered that because the present submission is based on the results of a single pivotal trial, INT-0133, the data would need to be of high quality and the results robust (CHMP/EWP/2330/99). A GCP inspection was requested to take place at 2 of the 178 INT-0133 investigational sites and the sponsor site with the scope to verify the data in the MAA for a sample of patients in investigator's sites as selected by CHMP. Special attention was to be given to the completeness of the dataset, particularly in terms of key efficacy data (primary endpoint), treatment

allocation and administration, and safety reporting. In addition, administrative aspects including randomisation, monitoring, and data entry were to be inspected at the sponsor's site Initially, full access to trial documentation was refused by the sponsor so that the validity of the clinical study could not be verified. The reason for this was that assurance of confidentiality was requested at the time of the inspection, which was not forthcoming from the inspectors as this would be considered within the scope of the scientific assessment of the marketing authorisation application. This constituted a critical finding and the GCP compliance of the pivotal study could not be confirmed. Upon further review, the sponsor provided written agreement to the applicant to fully cooperate with the EMEA. Following a justification submitted by the applicant, claiming that the access to trial documentation had been resolved, the CHMP requested a re-inspection of the sponsor of study INT-0133 and access to sponsor data centre and the requested documentation was provided. The GCP inspection took place as planned and there were several findings showing that the sponsor did not fully comply with its responsibilities for implementing and maintaining quality assurance and control systems with written SOPs to ensure that trials are conducted and data are generated, documented (recorded) and reported in compliance with the protocol, GCP and other applicable regulatory findings. Findings included the fact that no documents were available to substantiate on-site monitoring and auditing frequency (audits of each site were stated to be performed on a 3-yearly basis, with 10 patients or 12% of the sponsor's patients audited per site, whichever highest). Concerning the 2006 data set planned for the inspection, the inspectors concluded that this was no longer a true representation of the data available because additional follow-up was available since database lock. The original randomisation list was no longer available and had to be rebuilt. In some cases, events were reported without using the appropriate CRF forms. Inadequate procedures were used for the timely and complete reporting of study data. In some instances, the procedures in place to collect the most up to date data on overall survival were ineffective because of inadequate resolution of queries. Concerning the sites inspected, it had become clear that clinical trial data was not readily accessible. In isolated cases, the information could not be located even upon request. However, the inspectors found that overall all major deficiencies occurred in a limited number of cases and that there was no indication of a structural or pre-determined bias for one of the treatment arms. In conclusion, no critical findings were observed, the overall compliance to current GCP standards was considered sufficient, and the outcome of the inspection was that the database could be used in the scientific evaluation of the marketing authorisation application.

During the assessment, the CHMP also found that there were a number of unexplained findings and uncertainties about the treatment effect associated with MEPACT. In particular the mechanism of action of MEPACT has been claimed to be the aspecific activation of the mononuclear phagocyte system (MPS), but no direct evidence of MEPACT induced anti-tumoral cytotoxicity was available. Important differences in treatment effect had been observed for different age groups, gender and race. The treatment effect was different according to associated chemotherapy and appeared to be present only when MEPACT is used in conjunction with ifosfamide. A large number of patients have been censored in time-to-event analyses, but it was difficult to exclude that censoring is independent of outcome. The CHMP convened the scientific advisory group (SAG) for oncology to advise on a number of points. The SAG agreed that the claimed mechanism of a specific activation of the mononuclear phagocyte system (MPS) seems plausible and may be related to the clinical efficacy observed. The SAG agreed that it is difficult to speculate on the importance of any apparent effects in post hoc subgroup analyses. The apparent interactions with age, gender and race might be entirely due to chance. There is no strong pharmacological rationale for an interaction with any of those subgroups. However, it might be of interest to conduct further pharmacokinetic studies to explore intrinsic factors. No subgroup analyses have been presented by histological response to pre-operative chemotherapy. It would be interesting to investigate the effect of MTP by histological response to pre-operative chemotherapy, since this is an important prognostic factor. Also, important imbalances with respect to this factor should be ruled out. From the data presented on overall survival it is possible that there exists a quantitative interaction with ifosfamide. However, the SAG agreed that this quantitative interaction is likely to be small and of little clinical importance, although the precise magnitude is difficult to establish. What is important is that the clinical data presented are reassuring about the fact that there is no important qualitative interaction. The SAG agreed by consensus that the benefits of MTP are consistent regardless of the treatment arm used in the pivotal trial, although the treatment effect might be slightly different. Further pharmacokinetic studies could be conducted to address this point. Although missing data are always undesirable, in this context, the high level of censoring is

considered unbeneficial and could have been prevented despite the nature of the disease and the type of patient population. However, as long as the censoring mechanisms are the same between treatment arms, it should be possible to rule out major bias in the treatment estimate. From the data presented the SAG agreed that there are no signs of different follow-up and censoring mechanisms between treatment arms so this should not be a major concern. A more extended follow-up in terms of overall survival (OS) should be attempted with the aim of reducing the missing data. Overall, the SAG considered that the unexplained observations and uncertainties are well within the range of what is observed with other cancer products. The SAG agreed that based on the clinical efficacy data presented, treatment with MTP was associated with clinically significant benefits in the proposed indication. The SAG also agreed that the observed toxicity profile raised no particular concern, given the large unmet medical need and the sufficiently convincing efficacy data, and that it is considered important to conduct a number of additional studies post-approval to address the pharmacokinetics of MTP by age, to investigate the pharmacokinetics of ifosfamide in combination with MTP, to investigate the efficacy and safety of MTP for the treatment of adult osteosarcoma, to further explore the possibility of using MTP also before surgery (neoadjuvant setting), as well as to attempt to retrieve more OS data that is currently missing.

Clinical safety

The safety database also consisted of the 248 patients treated with MEPACT during the early phase single arm studies in patients with mostly advanced malignancies.

The applicant claimed that it is likely that undesirable effects also occurred in INT-0133, but they were not recorded because only serious and life-threatening adverse reactions were collected in that study. Although frequent and appropriate measures of safety and efficacy were required by protocol, not all results were collected in the CRFs. In addition, not all data recorded in the CRFs were entered into the the sponsor's database. The collection and reporting of data by course was tied to the chemotherapy courses during induction and maintenance. This meant that many events and toxicities that occurred during the course were not associated with specific dates.

• Patient exposure

The exposure to L-MTP-PE in pivotal study INT-0133 is shown in table 10.

		Regimen A with L-MTP-PE	Regimen B with L-MTP-PE
		(N=167)	(N=171)
Excluding patients with 0 do	ose		
Average L-MTP-PE dose	Ν	138	155
	Mean (SD)	3.1 (0.8)	3.1 (0.8)
	Median	3.0	3.0
	Min – Max	1.4 - 5.8	1.4 - 5.3
Cumulative L-MTP-PE	N	138	155
	Mean (SD)	120.4 (52.9)	117.6 (52.7)
	Median	132.2	117.6
	Min – Max	2.4 - 265.2	3.5 - 251.4
Including patients with 0 do	se		
Average L-MTP-PE	Ν	167	171
	Mean (SD)	2.6 (1.4)	2.8 (1.2)
	Median	2.9	3.0
	Min – Max	0.0 - 5.8	0.0 - 5.3
Cumulative L-MTP-PE	N	167	171
	Mean (SD)	99.5 (66.3)	106.6 (60.8)
	Median	115.0	110.4
	Min – Max	0.0 - 265.2	0.0 - 251.4

• Adverse events

Each of the 248 patients treated with MEPACT during the early phase single arm studies in patients with mostly advanced malignancies experienced at least one undesirable effect. Many of the most frequently reported undesirable effects as shown in the following summary table are thought to be related to the mechanism of action of mifamurtide. The majority of these events were reported as either mild or moderate. This profile is consistent whether summarising all early studies (n=248) or only those studies in osteosarcoma (n=51).

Adverse reactions are classified according to system organ class and frequency. Frequency groupings are defined according to the following convention: Very common ($\geq 1/10$), common ($\geq 1/100$ to < 1/10). Within each frequency grouping, undesirable effects are presented in order of decreasing seriousness (Table 11).

<u>Table 11</u>	<u>. Adverse</u>	reactions	associated	with	MEPACT	<u>in ≥</u>	<u>1/100 </u>	<u>patients</u>
							_	-

Infections and infestation	ns
Common:	Sepsis, cellulitis, nasopharyngitis, catheter site infection, upper respiratory tract infection, urinary tract infection, pharyngitis, <i>Herpes</i>
	simplex infection
Neoplasms benign, malig	gnant and unspecified (incl cysts and polyps)
Common:	Cancer pain
Blood and lymphatic sys	stem disorders
Very common:	Anaemia
Common:	Leukopenia, thrombocytopenia, granulocytopenia
Metabolism and nutritio	n disorders
Very common:	Anorexia
Common:	Dehydration, hypokalaemia, decreased appetite
Psychiatric disorders	
Common:	Confusional state, depression, insomnia, anxiety
Nervous system disorder	2°
Very common:	Headache, dizziness
Common:	Paraesthesia, hypoaesthesia, tremor, somnolence, lethargy
Eye disorders	
Common:	Blurred vision
Ear and labyrinth disord	lers
Common:	Vertigo, tinnitus, hearing loss
Cardiac disorders	
Very common:	Tachycardia
Common:	Cyanosis, palpitations
Vascular disorders	
Very common:	Hypertension, hypotension
Common:	Phlebitis, flushing, pallor
Respiratory, thoracic an	nd mediastinal disorders
Very common:	Dyspnoea, tachypnoea, cough
Common:	Pleural effusion, exacerbated dyspnoea, productive cough, haemoptysis,
	wheezing, epistaxis, exertional dyspnoea, sinus congestion, nasal
	congestion, pharyngolaryngeal pain
Gastrointestinal disorde	rs
Very common:	Vomiting, diarrhoea, constipation, abdominal pain, nausea
Common:	Upper abdominal pain, dyspepsia, abdominal distension, lower
	abdominal pain
Hepatobiliary disorders	
Common:	Hepatic pain
Skin and subcutaneous t	issue disorders
Very common:	Hyperhidrosis
Common:	Rash, pruritis, erythema, alopecia, dry skin
Musculoskeletal and con	inective tissue disorders
Very common:	Myalgia, arthralgia, back pain, pain in extremity
Common:	Muscle spasms, neck pain, groin pain, bone pain, shoulder pain, chest

	wall pain, musculoskeletal stiffness
Renal and urinary disor	rders
Common:	Haematuria, dysuria, pollakiuria
Reproductive system an	d breast disorders
Common:	Dysmenorrhoea
General disorders and a	administration site conditions
Very common:	Fever, chills, fatigue, hypothermia, pain, malaise, asthenia, chest pain
Common:	Peripheral oedema, oedema, mucosal inflammation, infusion site erythema, infusion site reaction, catheter site pain, chest discomfort, feeling cold
Investigations	
Common:	Weight decreased
Surgical and medical pr	rocedures
Common:	Post-procedural pain

Blood and lymphatic system disorders

Anaemia has most commonly been reported when MEPACT is used in conjunction with chemotherapeutic agents. In a randomised controlled trial, the incidence of myeloid malignancy (acute myeloid leukaemia/myelodysplastic syndrome) was the same in patients receiving MEPACT plus chemotherapy as in patients receiving only chemotherapy (approximately 2.5%).

Metabolism and nutritional disorders

Anorexia (21%) was very commonly reported in trials of MEPACT in late stage cancer patients.

Nervous system disorders

Consistent with other generalised symptoms, the most common nervous system disorders were headache (50%) and dizziness (17%).

Ear and labyrinth disorders

Although hearing loss may be attributable to ototoxic chemotherapy, like cisplatin, it is unclear whether MEPACT in conjunction with multi-agent chemotherapy may increase hearing loss.

A higher percentage of objective and subjective hearing loss was observed overall in patients who received MEPACT and chemotherapy (12 % and 7%, respectively) in the phase III study compared to those patients that received only chemotherapy (7% and 1%). All patients received a total dose of cisplatin of 480 mg/m² as part of their induction (neoadjuvant) and/or maintenance (adjuvant) chemotherapy regimen.

Cardiac and vascular disorders

Mild-moderate tachycardia (50%), hypertension (26%) and hypotension (29%) were commonly reported in uncontrolled trials of MEPACT. One serious incident of subacute thrombosis was reported in early studies, but no serious cardiac events were associated with MEPACT in a large randomised controlled trial.

Respiratory disorders

Respiratory disorders, including dyspnoea (21%), cough (18%) and tachypnoea (13%) were very commonly reported, and two patients with pre-existing asthma developed mild to moderate respiratory distress associated with MEPACT treatment in a phase II study.

Gastrointestinal disorders

Gastrointestinal disorders were frequently associated with MEPACT administration, including nausea (57%) and vomiting (44%) in about half of patients, constipation (17%), diarrhoea (13%) and abdominal pain.

Skin and subcutaneous disorders

Hyperhidrosis (11%) was very common in patients receiving MEPACT in uncontrolled studies.

Musculoskeletal and connective tissue disorders

Low grade pain was common in patients receiving MEPACT, including myalgia (31%), back pain (15%), extremity pain (12%) and arthralgia (10%).

General disorders and administration site conditions

The majority of patients experience chills (89%), fever (85%) and fatigue (53%). These are typically mild to moderate, transient in nature and generally respond to palliative treatment (e.g., paracetamol for fever). Other generalised symptoms that were typically mild to moderate and very common included hypothermia (23%), malaise (13%), pain (15%), asthenia (13%) and chest pain (11%). Oedema, chest discomfort, local infusion or catheter site reactions and 'feeling cold' were less frequently reported in these patients, mostly with late stage malignant disease.

Investigations

Increase in blood urea and blood creatinine was associated with MEPACT use in one patient with osteosarcoma.

Grade III-IV adverse events during study INT-0133

The most frequently reported grade 3 and 4 toxicities in the primary analysis set as well as in patients randomized to L-MTP-PE include low blood counts (47% ANC, 24% WBC, 29% platelets), stomatitis (46%), infections (22%), and abnormal liver enzymes (33% SGOT; 52% SGPT) (Table 12). The only other adverse events reported at a frequency greater than 10% were nausea and vomiting (18%) and objective hearing loss (12%). The same events occurred at comparable frequencies in the no-L-MTP-PE group with the exception of objective hearing loss, which occurred in 7% of the patients in the no-L-MTP-PE group (p=0.0478). All of these adverse events are typically associated with chemotherapy and are included in the toxicities list of the chemotherapy agents used in this study.

 Table 12: Grade III-IV Adverse Events by Individual Study Arm during study INT-0133

Adverse events	Arm A	Arm A + L- MTP-PE	Arm B	Arm B + L- MTP-PE	Total
Haematology					
second malignancy	4 (2%)	4 (2%)	3 (2%)	3 (2%)	14 (2%)
Kidney					
systolic BP	3 (2%)	1 (1%)	1 (1%)	0	5 (1%)
diastolic BP	3 (2%)	1 (1%)	3 (2%)	1 (1%)	8 (1%)
hematuria	0	0	1 (1%)	0	1
bladder	1 (1%)	0	0	0	1
Gastrointestinal					
stomatitis	94 (54%)	82 (49%)	61 (37%)	73 (43%)	310 (46%)
abdominal pain	4 (2%)	6 (4%)	3 (2%)	5 (3%)	18 (3%)
constipation	6 (3%)	7 (4%)	3 (2%)	3 (2%)	19 (3%)
diarrhea	6 (3%)	3 (2%)	5 (3%)	11 (6%)	25 (4%)
nausea and vomiting	36 (21%)	35 (21%)	23 (14%)	24 (14%)	118 (17%)
Pulmonary					
vital capacity	0	2 (1%)	1 (1%)	0	3
functional	0	1 (1%)	2 (1%)	1 (1%)	4 (1%)
clinical	0	0	2 (1%)	0	2
Cardiac					
rhythm	1 (1%)	3 (2%)	1 (1%)	1 (1%)	6 (1%)
echo	1 (1%)	2 (1%)	0	3 (2%)	6 (1%)
function	0	0	1 (1%)	0	1
hypertension	2 (1%)	1 (1%)	0	0	3
hypotension	3 (2%)	0	4 (2%)	1 (1%)	8 (1%)
Nervous					
Periph sensory	0	1 (1%)	5 (3%)	4 (2%)	10 (1%)
Cent cerebellar	6 (3%)	7 (4%)	8 (5%)	6 (4%)	27 (4%)
Skin	14 (8%)	7 (4%)	8 (5%)	6 (4%)	27 (4%)
Allergy	2 (1%)	3 (2%)	2 (1%)	3 (2%)	10 (1%)
Hearing					
objective deficit	8 (5%)	26 (16%)	16 (10%)	13 (8%)	63 (9%)

Adverse events	Arm A	Arm A + L- MTP-PE	Arm B	Arm B + L- MTP-PE	Total
subjective deficit	1 (1%)	10 (6%)	1 (1%)	2 (1%)	14 (2%)
Infection	48 (28%)	33 (20%)	33 (20%)	40 (23%)	154 (23%)
Fever	5 (3%)	2 (1%)	3 (2%)	4 (2%)	14 (2%)
Local reaction	2 (1%)	0	3 (2%)	1 (1%)	6 (1%)
Mood	6 (3%)	7 (4%)	3 (2%)	2 (1%)	18 (3%)
Weight change	4 (2%)	2 (1%)	0	4 (2%)	10 (1%)
Performance status	1 (1%)	1 (1%)	0	1 (1%)	3

Creatinine clearance

Creatinine clearance toxicity grade 3 and 4 was reported more frequently for patients assigned to the no-L-MTP-PE group. An examination of the four treatment groups for this categories is consistent for creatinine clearance, i.e. both groups receiving L-MTP-PE (Regimens A+ and B+) were lower than both groups not receiving L-MTP-PE (Regimens A and B). A decrease in creatinine clearance is not unexpected because of the known renal toxicity of the concomitant chemotherapy.

Hearing loss

Grade 3 and 4 objective hearing loss was observed primarily in treatments arms A and to a lesser extent also in arms with regimen B. A clinical significant higher loss was observed in with arm A treated with L-MTP-PE. Objective hearing loss was observed in 12% in the L-MTP-PE group (n=39) whereas in 7% of the patients in the no-L-MTP-PE group (n=24) (p=0.0478).

Immunological events

Five instances of potential allergic reaction to L-MTP-PE were reported or noted during review of INT-0133. Urticaria and macular, erythematous rash (grade 3) resulting in dose delay; erythema multiforme (grade \geq) resulting in dose discontinuation; hives (grade 1) resulting in dose mitigation to 2 mg/m2; allergic reaction to L-MTP-PE (NOS) grade 4 resulting in dose discontinuation; respiratory distress grade 2 resulting in dose discontinued.

• Serious adverse event/deaths/other significant events

In trial INT-0133, there were 148 deaths reported in the patients in the primary analysis group, mostly due to progressive disease. One other patient presumably died as the result of progressive disease. No deaths were attributed to L-MTP-PE. Most deaths were due to progressive disease.

Five instances of potential allergic reaction to L-MTP-PE were reported, including 2 cases of urticaria (66766, 63969), one erythema multiform (63890), one grade IV allergic reaction (65489) and one bronchospasm (60243). In four cases, reactions occurred late in the maintenance treatment. Except for urticaria, L-MTP-PE was discontinued. Rash and erythema occurred in 2% and 6% of treated patients during previous protocols including L-MTP-PE. One case of blister was recorded during phase I-II studies.

During INT-0133 clinical study, three patients experienced two pleural effusions (63737, 58228) and one pericardial effusion (68129). Although rare, pericardial and pleural effusions have been previously reported with L-MTP-PE. A total of 3 pleural effusions and one pericardial and pleural effusion were recorded during prior phase I-II studies. In toxicological studies, pericarditis was recorded. The aetiology of effusion could be related to the immune stimulation of L-MTP-PE.

Two episodes of seizure (61388) and painful spasms of extremities (65464) could be related to neurotoxicity of L-MTP-PE. One convulsion, one muscle spasm and one leukoencephalopathy were also recorded during phase I-II protocols including L-MTP-PE.

One case of cardiac grade II arrhythmia (68129) was reported during study int-0133. No other rhythm abnormality was recorded during phase I-II protocols. However tachycardia and bradycardia were commonly registered during all studies. They were frequently associated with initial febrile reaction. Patients experienced also hyper- or hypotension, nausea or vomiting, myalgia and pain, dyspnoea and chills. Severe flu like symptoms and arterial tension variation were dose limiting toxicity during phase I studies.

• Laboratory findings

From the phase II osteosarcoma studies, treatment-emergent adverse events associated with laboratory toxicities were reported by $\geq 5\%$ (3/51) of the 51 patients. The most common laboratory toxicity was leukopenia which was reported by 20% (10/51) of the patients. As noted above, this was also the adverse event that emerged as a potential SAE in several patients in protocol 10, where L-MTP-PE was used in conjunction with chemotherapy. Eight percent (4/51) of patients had at least one episode of a grade 3 or 4 leukopenia. Anaemia (12%, 6/51) was the second most common laboratory toxicity reported with grade 3 anaemia reported for 4% of patients (2/51). No grade 4 anaemia was reported. Neutropenia was reported by three patients who were enrolled in Protocol 10 where patients received either cisplatin or ifosfamide along with the L-MTP-PE. Treatment-emergent adverse events associated with laboratory toxicities that were reported by < 5% of the patients included 4% (2 of 51 patients) urine glucose, hyperkalemia, hypomagnesaemia; 2% (1 of 51 patients) pure red cell aplasia, blood creatinine increased, blood urea increased, urine specific gravity increased, hyponatremia, hypophosphatemia, proteinuria, ketonuria.

In trial INT-0133, the Grade 3 and grade 4 adverse toxicities regarding laboratory parameters were presented. A considerable increase in the number of blood transfusions is administered in the maintenance phase of arm B+L-MTP-PE when compared to the non-L-MTP-PE treated arm B (275 vs 341 respectively). In arm B, also the need for platelet transfusions and total parenteral nutrition (TPN) appeared higher in the L-MTP-PE-group.

Adverse	Arm A	$\frac{1}{1} \frac{1}{1} \frac{1}$	Arm B	Arm B + L-	Total
events		MTP-PE		MTP-PE	
Haematology					
white blood	40 (23%)	29 (17%)	44 (27%)	53 (31%)	166 (24%)
cell	~ /			~ /	
neutrophils	84 (48%)	75 (45%)	71 (43%)	85 (50%)	315 (46%)
platelets	56 (32%)	48 (29%)	43 (26%)	49 (29%)	196 (29%)
hemoglobin	14 (8%)	14 (8%)	14 (8%)	18 (11%)	60 (9%)
lymphs	2 (1%)	2 (1%)	3 (2%)	4 (2%)	11 (2%)
Coagulation				. ,	
PT	0	0	1 (1%)	0	1
PTT	0	0	0	0	0
Hepatic					
SGOT	48 (28%)	52 (31%)	66 (40%)	60 (35%)	226 (33%)
SGPT	84 (48%)	86 (51%)	102 (61%)	91 (53%)	363 (54%)
alkalin	4 (2%)	0	2 (1%)	5 (3%)	11 (2%)
phosphatase					
bilirubin total	19 (11%)	12 (7%)	17 (10%)	16 (9%)	64 (9%)
Pancreas					
amylase	1 (1%)	1 (1%)	0	1 (1%)	3
glucose	14 (8%)	4 (2%)	12 (7%)	10 (6%)	40 (6%)
Kidney					
BUN	2 (1%)	1 (1%)	0	2 (1%)	5 (1%)
creatinine	3 (2%)	4 (2%)	2 (1%)	2 (1%)	11 (2%)
Creatinine	5 (3%)	1 (1%)	9 (5%)	1 (1%)	16 (2%)
clearance					
Electrolytes					
sodium	5 (3%)	2 (1%)	2 (1%)	0	9 (1%)
potassium	10 (6%)	8 (5%)	11 (7%)	8 (5%)	37 (5%)
calcium	5 (3%)	2 (1%)	2 (1%)	3 (2%)	12 (2%)
magnesium	3 (2%)	6 (4%)	2 (1%)	3 (2%)	14 (2%)

 Table 13: laboratory data during study INT-0133
 Interview
 Interview

• Safety in special populations

No safety data from special populations were submitted.

• Safety related to drug-drug interactions and other interactions

No safety data related to drug-drug interactions and other interactions were submitted.

• Discontinuation due to adverse events

During phase I/II studies, six patients discontinued treatment after adverse event (rash and conjunctivitis for patient 1-5; myalgia, tachypnea and fever for patient 1-30; peripheral edema, vomiting, chills, fever for patient 3-31; dyspnea, hypotension, cough for patient 3-35; hypotension, pitting edema, dyspnea, cough, decreased urine output for patient 3-40; fever, pericardial and pleural effusion for patient 10-303). Four of these patients were receiving doses of L-MTP-PE form 4 to 6 mg/m², above the MTD. During protocols 08 and 10, thirty two patients discontinued L-MTP-PE due to an adverse event. In 14 cases, the causality relationship was related to L-MTP-PE. The most adverse events were expected, due to the immune stimulation related to L-MTP-PE.

During study INT-0133, eleven patients were withdrawn from the clinical study due to toxicity. As expected, regimen B (4 drug therapy) was associated with more withdrawal than regimen A (3 drug therapy). The addition of L-MTP-PE did not increase the number of withdrawal related to toxicity (4 patients with L-MTP-PE versus 7 patients without L-MTP-PE). However the number of withdrawals during the treatment phases by parent/patient was higher for the L-MTP-PE groups compared with those who did not receive L-MTP-PE. The administration of L-MTP-PE was scheduled to continue beyond the completion of chemotherapy. Review of the CRFs for these patients indicates that while many of the withdrawals were due to refusal of further L-MTP-PE therapy, they were not necessarily relayed to the completion of chemotherapy. In many of these instances the withdrawal was based on the patients' reactions to L-MTP-PE, such as fever and rigors.

	Regimen A	Regimen A +	Regimen B	Regimen B +	Total
		L-MTP-PE		L-MTP-PE	
Total	174	167	166	171	678
Induction phase	170	164	164	169	667
Withdrawn by	3	4	5	4	16
parent/patient					
Withdrawn by	3	1	0	0	4
physician					
Progressive	6	6	4	3	19
disease					
Withdrawn for	0	0	2	1	3
toxicity					
Maintenance	153	145	148	158	604
phase					
Withdrawn by	8	20	6	26	60
parent/ patient					
Withdrawn by	0	1	6	4	11
physician					
Progressive	9	8	7	9	33
disease					
Withdrawn for	1	1	4	2	8
toxicity					
Completed	130	108	120	106	464
protocol therapy					

Table 14: Patients exposure and withdrawal during study INT-0133

• Post marketing experience

No post-marketing data have been submitted.

• Discussion on clinical safety

Each of the 248 patients treated with MEPACT during the early phase single arm studies in patients with mostly advanced malignancies experienced at least one undesirable effect. Many of the most frequently reported undesirable effects were thought to be related to the mechanism of action of mifamurtide. The majority of these events were reported as either mild or moderate. This profile was

consistent whether summarising all early studies (n=248) or only those studies in osteosarcoma (n=51).

It is likely that undesirable effects also occurred in the large randomised study, but they were not recorded because only serious and life-threatening adverse reactions were collected in that study. In the pivotal study INT-0133 long term observations on safety are hampered by a low average number of approximately 1.4 and 1.5 assessment visits/year. Only grade 3 and grade 4 toxicities only in frequencies higher than 10% have been reported. The data on adverse events (AE) as provided do not differentiate for the time point at which these events were observed. The most frequently reported grade 3 and 4 toxicities encountered in this study (low blood counts, nausea and vomiting, stomatitis, infections, hearing loss and abnormal liver enzymes) were associated with the chemotherapy applied. A higher incidence of grade 3-4 objective hearing loss in patients receiving L-MTP-PE in combination with regimen A (doxorubicin, cisplatin, methotrexate) was observed.

In patients with a history of asthma or other chronic obstructive pulmonary disease, consideration should be given to administration of bronchodilators on a prophylactic basis. Two patients with pre-existing asthma developed mild to moderate respiratory distress associated with the treatment. If a severe respiratory reaction occurs, administration of MEPACT should be discontinued and appropriate treatment initiated (see SPC section 4.4).

Administration of MEPACT was commonly associated with transient neutropenia, usually when used in conjunction with chemotherapy. Episodes of neutropenic fever should be monitored and managed appropriately. MEPACT may be given during periods of neutropenia, but subsequent fever attributed to the treatment should be monitored closely. Fever or chills persisting for more than 8 hours after administration of MEPACT should be evaluated for possible sepsis (see SPC section 4.4).

Association of MEPACT with signs of pronounced inflammatory response, including pericarditis and pleuritis, was uncommon. MEPACT should be used with caution in patients with a history of autoimmune, inflammatory or other collagen diseases. During MEPACT administration, patients should be monitored for unusual signs or symptoms, such as arthritis or synovitis, suggestive of uncontrolled inflammatory reactions. (see SPC section 4.4).

Patients with a history of venous thrombosis, vasculitis or unstable cardiovascular disorders should be closely monitored during MEPACT administration. If symptoms are persistent and worsening, administration should be delayed or discontinued. Haemorrhage was observed in animals at very high doses. These are not expected at the recommended dose, however monitoring of clotting parameters after the first dose and once again after several doses is recommended. (see SPC section 4.4).

Occasional allergic reactions have been associated with MEPACT treatment, including rash, shortness of breath and Grade 4 hypertension. It may be difficult to distinguish allergic reactions from exaggerated inflammatory responses, but patients should be monitored for signs of allergic reactions (see SPC section 4.4).

Nausea, vomiting and loss of appetite are very common adverse reactions to MEPACT. Gastrointestinal toxicity may be exacerbated when MEPACT is used in combination with high dose, multi-agent chemotherapy and was associated with an increased use of parenteral nutrition (see SPC section 4.4).

There are no data from the use of mifamurtide in pregnant patients (see SPC section 4.7). Animal studies are insufficient with respect to reproductive toxicity (see SPC section 5.3). MEPACT should not be used during pregnancy and in women not using effective contraception.

It is unknown whether mifamurtide is excreted in human milk. The excretion of mifamurtide in milk has not been studied in animals. A decision on whether to continue/discontinue breast-feeding or to continue/discontinue therapy should be made taking into account the benefit of breast-feeding to the child and the benefit of MEPACT therapy to the woman.

No studies of the effects on the ability to drive and use machines have been performed. Some very common or common undesirable effects of MEPACT treatment (such as dizziness, vertigo, fatigue and blurred vision) may have an effect on the ability to drive and use machines (see SPC section 4.7).

No case of overdose has been reported. The maximum tolerated dose in phase I studies was 4-6 mg/m2 with a high variability of adverse reactions. Signs and symptoms that were associated with

higher doses and/or were dose limiting were not life-threatening, and included fever, chills, fatigue, nausea, vomiting, headache and hypo- or hypertension. In the event of an overdose, it is recommended that appropriate supportive treatment be initiated. Supportive measures should be based on institutional guidelines and the clinical symptoms observed. Examples include paracetamol for fever, chills and headache and anti-emetics (other than steroids) for nausea and vomiting (see SPC section 4.9).

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 (Table 15).

Safety issue	Proposed pharmacovigilance	Proposed risk minimisation activities
	activities	
1. Cardiotoxicity / vascular events	Monitoring through Surveillance Study, data from PK study in healthy volunteers, routine pharmacovigilance	<i>Warning in SPC</i> : Section 4.4 warns of risk of cardiovascular disorders, recommends close monitoring of susceptible patients.
2. Risk of bleeding / haemorrhage	Monitoring through Surveillance Study, routine pharmacovigilance	<i>Warning in SPC</i> : Section 4.4 warns of risk of haemorrhage, recommends close monitoring of clotting parameters.
3. Allergy and immunological events	Monitoring through Surveillance Study, routine pharmacovigilance	<i>Warning in SPC</i> : Section 4.4 warns of allergic reactions and recommends monitoring patients for signs.
4. Respiratory disorders	Monitoring through Surveillance Study, routine pharmacovigilance	<i>Warning in SPC</i> : Section 4.4 warns of a possibility of respiratory distress and mentions possible prophylactic and emergency measures.
5. Exacerbated gastro- intestinal toxicity leading to increased need for total parenteral nutrition	Monitoring through Surveillance Study, routine pharmacovigilance	<i>Warning in SPC</i> : Section 4.4 warns that patients treated with MEPACT may have an increased need for total parenteral nutrition.
6. Exacerbation of cisplatin- related ototoxicity	Monitoring through Surveillance Study (non-cisplatin patients), routine pharmacovigilance	<i>Comment in SPC:</i> Section 4.8 indicates that MEPACT may increase chemotherapy-associated hearing loss.
7. Neurotoxicity	Monitoring through Surveillance Study, routine pharmacovigilance	None
8. Flu-like symptoms (Masking of serious infection by MEPACT adverse reactions)	Routine pharmacovigilance	<i>Warning in SPC</i> : Section 4.4 warns that episodes of prolonged fever should be evaluated as sepsis.
9. Ocular events	Monitoring through Surveillance Study, routine pharmacovigilance	None
10. Chronic inflammation or autoimmune disease, such as synovitis or arthritis	Monitoring through Surveillance Study, routine pharmacovigilance	<i>Warning in SPC</i> : Section 4.4 warns of risk of pronounced inflammatory response, advises caution in patients with a history of autoimmune-related diseases and advises to monitor patients for related symptoms.
11. Hepatic and renal toxicities, including electrolyte imbalance	Monitoring through Surveillance Study, routine pharmacovigilance	Hepatic toxicity – <i>Recommendation in SPC</i> : Section 4.2 recommends monitoring of liver function until the end of MEPACT therapy. Renal toxicity – <i>Recommendation in SPC</i> : Section 4.2 recommends monitoring of kidney function until the end of MEPACT therapy
12. Myelosuppression	Monitoring through Surveillance	Warning in SPC: SPC Section 4.4 warns of

Table 15 Summary of the risk management plan

Safety issue	Proposed pharmacovigilance activities	Proposed risk minimisation activities
	Study, routine pharmacovigilance	episodes of neutropenia and neutropenic fever
13. Tolerability in conjunction with multi-agent chemotherapy (grade 1 and 2 events)	Collection of quality of life data as part of the Surveillance Study	None
14. Long-term safety information incomplete	Surveillance Study, routine pharmacovigilance	None

The applicant has committed to conduct a Surveillance Study (MTP-OS-501-R - Surveillance Study of patients with non-metastatic and resectable osteosarcoma).

The applicant has committed to closely monitor the off-label-use of MEPACT and report on this topic in detail in PSURs in accordance with the requirements set out in Article 24(3) of Regulation (EC) 726/2004, taking account of the guidance set out in Volume 9 (Pharmacovigilance) on the Rules governing medicinal products in the European Union.

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

Information on development, manufacture and control of the drug substance and drug product has been presented in a satisfactory manner. The quality of the active substance is considered sufficiently described and adequately supported by data. Sufficient information has been presented for the excipients POPC and OOPS which are considered novel excipients. Both comply with acceptable inhouse specifications in view of their synthesis. Full information on the synthesis and toxicologic profile of these excipients has been provided. As far as the drug product concerns the results of tests carried out indicate satisfactory consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in the clinic.

Non-clinical pharmacology and toxicology

Mifamurtide (muramyl tripeptide phosphatidyl ethanolamine, MTP-PE) is a fully synthetic derivative of muramyl dipeptide (MDP), the smallest naturally-occurring immune stimulatory component of cell walls from Mycobacterium sp. It has similar immunostimulatory effects as natural MDP with the additional advantage of a longer half-life in plasma. MEPACT is a liposomal formulation specifically designed for in vivo targeting to macrophages by intravenous infusion.

MTP-PE is a specific ligand of NOD2, a receptor found primarily on monocytes, dendritic cells and macrophages. MTP-PE is a potent activator of monocytes and macrophages. Activation of human macrophages by MEPACT is associated with production of cytokines, including tumour necrosis factor (TNF- α), interleukin-1 (IL-1 β), IL-6, IL-8, and IL-12 and adhesion molecules, including lymphocyte function-associated antigen-1 (LFA-1) and intercellular adhesion molecule-1 (ICAM-1). In vitro-treated human monocytes killed allogeneic and autologous tumor cells (including melanoma, ovarian, colon, and renal carcinoma), but had no toxicity towards normal cells.

In vivo administration of MEPACT resulted in the inhibition of tumour growth in mouse and rat models of lung metastasis, skin and liver cancer, and fibrosarcoma. Significant enhancement of disease-free survival was also demonstrated in the treatment of dog osteosarcoma and hemangiosarcoma with MEPACT as adjuvant therapy. The exact mechanism by which MEPACT activation of monocytes and macrophages leads to antitumour activity in animals and humans is not yet known.

In sensitive species (rabbit and dog) the highest daily dose of liposomal mifamurtide that did not cause adverse effects was 0.1 mg/kg, corresponding to 1.2 and 2 mg/m2, respectively. The no-adverse-effect level for MEPACT in animals corresponds roughly to the 2 mg/m2 recommend dose for humans.

Data from a six month dog study of daily intravenous injections of up to 0.5 mg/kg (10 mg/m2) MEPACT provide an 8- to 19-fold cumulative exposure safety margin for overt toxicity for the intended clinical dose in humans. Major toxic effects associated with these high daily and cumulative doses of MEPACT were mainly exaggerated pharmacological effects: pyrexia, signs of pronounced inflammatory response manifested as synovitis, bronchopneumonia, pericarditis and inflammatory necrosis of the liver and bone marrow. The following events were also observed: haemorrhage and prolongation of coagulation times, infarcts, morphological changes in the wall of small arteries, oedema and congestion of the central nervous system, minor cardiac effects, and slight hyponatraemia. MEPACT was not mutagenic and did not cause teratogenic effects in rats and rabbits. Embryotoxic effects were observed only at maternal toxic levels.

There were no results from general toxicity studies that suggested harmful effects on male or female reproductive organs. Specific studies addressing reproductive function, perinatal toxicity and carcinogenic potential have not been performed.

Efficacy

MEPACT significantly increased the overall survival of patients with newly-diagnosed resectable high-grade osteosarcoma when used in conjunction with combination chemotherapy when compared to chemotherapy alone. In a randomised phase III study of 678 patients (age range from 1.4 to 30.6 years) with newly-diagnosed resectable high-grade osetosarcoma, the addition of adjuvant MEPACT to chemotherapy either doxorubicin cisplatin and methotrexate with or without ifosfamide resulted in a relative reduction in the risk of death of 28% (p = 0.0313, hazard ratio (HR) = 0.72 [95% confidence interval (CI): 0.53, 0.97]).

Safety

The safety of liposomal mifamurtide has been assessed in more than 700 patients with various kinds and stages of cancer and in 21 healthy adult subjects. Each of the 248 patients treated with MEPACT during the early phase single arm studies in patients with mostly advanced malignancies experienced at least one undesirable effect. Many of the most frequently reported undesirable effects are thought to be related to the mechanism of action of mifamurtide. The majority of these events were reported as either mild or moderate. This profile is consistent whether summarising all early studies (n=248) or only those studies in osteosarcoma (n=51). It is likely that undesirable effects also occurred in the large randomised study, but they were not recorded because only serious and life-threatening adverse reactions were collected in that study.

Very common ($\geq 1/10$) adverse reactions associated with MEPACT were anaemia, anorexia, headache, dizziness, tachycardia, hypertension, hypotension, dyspnoea, tachypnoea, cough, vomiting, diarrhoea, constipation, abdominal pain, nausea, hyperhidrosis, myalgia, arthralgia, back pain, pain in extremity, fever, chills, fatigue, hypothermia, pain, malaise, asthenia, chest pain

From the safety database all the adverse reactions reported in clinical trials have been included in the Summary of Product Characteristics.

Having considered the safety concerns in the risk management plan, the CHMP considered that the proposed activities described in section 3.5 adequately addressed these.

• User consultation

A readability test was conducted and the results were submitted. The package leaflet had a minimum of 81% or greater pass rate in terms of locating and understanding the information. The user consultation was considered adequate.

Risk-benefit assessment

Based on the data presented, the CHMP concluded that MEPACT significantly increased the overall survival of patients with newly-diagnosed resectable high-grade osteosarcoma when used in conjunction with combination chemotherapy when compared to chemotherapy alone. Initially, the CHMP had concerns that there remained important uncertainties about the mechanism of action, that important interactions had not been ruled out, that the evidence of efficacy from the pivotal clinical trial was difficult to interpret due to missing data and incomplete long-term follow-up of all patients, and that due to these reasons the efficacy and safety could not be considered as established. However,

following the advice of the scientific advisory group and additional argumentations presented by the applicant during the oral explanation, the CHMP considered that although there were a number of uncertainties, e.g., about potential interactions with intrinsic or extrinsic factors, the effect of such potential interactions was considered to be small. Further pharmacokinetic studies will be conducted to address these issues. Similarly, although the pivotal trial data collection resulted in missing data and lack of extensive long-term follow-up, this was not considered to have introduced important bias favouring MEPACT. Further data from long-term safety studies will be provided. The CHMP considered that based on the clinical efficacy data presented, treatment with MEPACT was associated with clinically significant benefits in the proposed indication and that in view of the benefits the observed toxicity profile raised no particular concern, and concluded that the benefit-risk balance in the claimed indication was favourable.

A risk management plan was submitted. The CHMP, having considered the data submitted, was of the opinion that routine pharmacovigilance was adequate to monitor the safety of the product.

Recommendation

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considered by consensus that the risk-benefit balance of MEPACT was favourable in the indication "MEPACT is indicated in children, adolescents and young adults for the treatment of high-grade resectable non-metastatic osteosarcoma after macroscopically complete surgical resection. It is used in combination with post-operative multi-agent chemotherapy. Safety and efficacy have been assessed in studies of patients 2 to 30 years of age at initial diagnosis (see [SPC] section 5.1)" and therefore recommended the granting of the marketing authorisation