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

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WITHDRAWAL ASSESSMENT REPORT FOR VORAXAZE

International Nonproprietary Name:
glucarpidase

EMEA/H/C/681

This Withdrawal Public Assessment Report is based on the latest assessment report adopted by the CHMP with all information of a commercially confidential nature deleted.

This report should be read in conjunction with the "Question and Answer" document on the withdrawal of the application: the Assessment Report may not include all available information on the product if the CHMP assessment of the latest submitted information was still ongoing at the time of the withdrawal of the application.

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LIST OF ABBREVIATIONS

ALL	Acute lymphocytic laeukemia
CPG2	CPDG2, carboxypeptidase-G2, glucarpidase
DAMPA	2,4-diamino-N^{10}-methylpteroic acid
HDMTX	High dose methotexate
LV	leucovorin
MCB	Master Cell Bank
MTX	Methotrexate
sCR	Serum Creatinine
WBC	Working Cell Bank

I. RECOMMENDATION

Based on the review of the data on quality, safety and efficacy, the CHMP considers that the application for Voraxaze, an orphan medicinal product (EU/3/02/128), in the treatment of paediatric or adult patients experiencing methotrexate toxicity or at risk of methotrexate toxicity, is not approvable since "major objections" have not been solved, which preclude a recommendation for marketing authorisation at the present time.

The outstanding issues precluding a recommendation of marketing authorisation pertain to the following principal deficiencies:

- There have been repeated problems in the transfer of production from the manufacturer of the clinical trial batches, CAMR, to the manufacturer of the commercial batches, Eurogentec and deficiences in the validation of commercial scale production
- There are apparent differences between different analytical methods for determination of product
- The potential of glucarpidase to metabolise the MTX rescue agent folinic acid (FA) in the clinical setting, possibly leading to lower efficacy of FA, and its implications for interpretation of the efficacy data

II. EXECUTIVE SUMMARY

II.1 Problem statement

Methotrexate is an antimetabolite used in the treatment of certain neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis. Methotrexate inhibits dihydrofolic acid reductase. Delayed drug clearance has been identified as one of the major factors responsible for methotrexate toxicity. It has been postulated that the toxicity of methotrexate for normal tissues is more dependent upon the duration of exposure to the drug rather than the peak level achieved. When a patient has delayed drug elimination due to compromised renal function, a third space effusion, or other causes, methotrexate serum concentrations may remain elevated for prolonged periods. The potential for toxicity from high dose regimens or delayed excretion is reduced by the administration of FA (FA) during the final phase of methotrexate plasma elimination. Despite rigorous monitoring of plasma MTX concentrations and administration of antidote (FA) post high-dose treatment life-threatening toxicity occur in rare cases.

II.2 About the product

The rationale for use of glucarpidase in methotrexate toxicity is based on its ability to hydrolyze the carboxyl terminal glutamate residue from compounds such as methotrexate, producing glutamate and 2,4-diamino-N^{10}-methylpteroic acid (DAMPA). The enzymatic action of glucarpidase (carboxypeptidase-G2, CPG2 or CPDG2), a dimer with two subunits of Mr41 400 that require Zn^{2+} for activity, results in release of glutamate residues of a range of N-acylating moieties including peptidyl, aminoacyl, benzoyl, benzyloxycarbonyl, folyl and pteroyl groups. The enzyme was originally isolated from *Pseudomonas sp.* RS-16 but is now produced with recombinant techniques. Glucarpidase or carboxypeptidase G2 differs from the carboxypeptidase G1 in that it has similar Km values for folate, methotrexate and 5-methyltetrahydrofolate but a 10-fold lower affinity for 5-formyltetrahydrofolate.

II.3 The development programme/Compliance with CHMP Guidance/Scientific Advice

Carboxypeptidase has been known to prevent drug toxicity of methotrexate since 1970's. The development was, however, discontinued due to limited supply of the product.

Preclinical studies are limited as also would be expected due to the type of compound. The studies are primarily from the 1990ies, conducted by the National Cancer Institute in the USA.

No Scientific Advice has been sought during the development. No formal clinical development program has been presented. The efficacy and safety data are mainly derived from documentation of the results in two compassionate use programs.

II.4 General comments on compliance with GMP, GLP, GCP

It is not clearly stated in the dossier that the GMP inspection of Eurogentec was conducted at a time when production of glucarpidase was ongoing. As there were deviations seen to the defined range in operation for critical control parameters in the production of all three validation batches, and furthermore, an error made in the step of formulation of one batch of drug substance, it is considered important that functionality of batch record and the standard operation procedure are verified during production of drug substance. Thus, a product specific GMP inspection at Eurogentec was requested.

Non-clinical studies conducted were in compliance with GLP principles where justified.

The clinical studies were performed within the frame of a compassionate use program and on site monitoring was not performed on the entire study populations. Checking against source data has been performed in some individual cases and centers participating in the main clinical studies submitted in this application. In one ongoing study the cases included in the efficacy evaluation have been monitored.

Data management was performed in accordance with GCP.

III. SCIENTIFIC OVERVIEW AND DISCUSSION

III.1 Quality aspects

Drug substance

The drug substance glucarpidase is a recombinant protein of 41,400 D produced in *E coli*. The current manufacturer of the drug substance is Eurogentec, Belgium.

In the initial assessment two major issues were identified relating to the drug substance. An area of serious concern was the finding of repeated problems in the transfer of production from the initial manufacturer of the active substance, CAMR, UK, to Eurogentec. The transfer was effected trough the use of two pilot scale fermentation batches but the first batch failed to reproduce the results obtained at CAMR. After the introduction of modifications to the process procedure a second and successful fermentation run was conducted. Furthermore, going from production in the pilot to the commercial scale, additional problems were encountered, and due to unexpected behaviour of product, both the validation protocol and the production procedure had to be revised after manufacture of the first out of three validation batches.

Additional documentation was asked for to support consistency and control in production. Unless otherwise justified new validation data showing robustness of the process was requested. However, if the Applicant could use the experience obtained in the production of the first validation batch to explain why it was not possible to foresee e.g. the unexpected product loss in the diafiltration steps, to show how the introduced changes in the process affected product quality including the impurity profile and to clarify how the in process control system has been improved to prevent that unexpected events occur in future production, there was a possibility that a re-validation of the production process could be avoided.

In their response the company submitted results from two more batches. One of these was rejected due to failure in prefiltration bioburden indicating, taken together with the other deviations, that the production is not under full control. The concerns regarding the control of the consistency in production remains and refer to different areas such as the stringency in defining and adhering to the batch record, the functionality of the instructions given in the batch record for controlling consistent process and product performance in routine production, and the actions taken in case the acceptance criteria proposed for operation control are not met. A re-validation of the production process is requested including the validation of the proposed range in operation for control of different process steps. Consistency in the process performance should be supported by results from the analyses of product potency, purity and yield at different steps. Actions taken in case an intermediate product fails to meet the in process specifications/action limits should be outlined. Furthermore, the logistics in production should be reviewed. The review should cover both the situation when production can be operated according to the standard protocol, following the normal time frame, and when production may need to be interrupted for

handling batches under deviation. Storage of intermediates of the process should be indicated and data provided supporting that a prolonged storage of an intermediate does not affect product quality. Control of the performance of chromatography columns before use in purification should be described. The capacity of the process for removal of process-related impurities such as antifoam, complex media components, chromatographic media components, solvents and buffer components should be addressed as part of the validation studies.

Another concern raised in the initial assessment was that the applicant should demonstrate that the controls applied to the commercial-scale drug substance and drug product ensure that the manufacturing process is robust and commercial-scale batches are comparable to those used during pivotal clinical studies. The characterisation/comparability studies should be extended to include identification of the minor product-related substances/impurities being present in drug substance.

The comparability and characterisation studies showed various isoforms and low and high molecular weight forms of glucarpidase. The apparent difference in purity estimated in the SDS-PAGE with silver stain and Coomassie stain and GP-HPLC studies needs to be further elaborated. The minor components visible on the SDS-PAGE silver stained gels and the various isoforms visible in IEF studies should be characterised. Based on the identified structure, steps in production critical for their formation should be discussed and the applicant should review the need for analysis by IEF and SDS-PAGE with silver staining, during in-process control and specification testing or to support stability of product.

The comparability and characterisation studies have shown various isoforms and low and high molecular weight forms of glucarpidase being present in batches but these studies have not been pursued sufficiently far for identification the species resolved in different analyses or for confirmation of that batches share the same impurity profile. Neither is the relative amounts the product-related species represents in batches clear. Dependent on which assay/assay protocol is used for analysis, the estimated purity of a batch is seen differing by 25 % to 40 %.

To allow assessment of the comparability of batches and consistency in production, the characterisation studies should be extended to include the identification of the different product-related impurities/substances resolved in the analyses by SDS-PAGE, IEF and the weak ion exchange chromatography.
The applicant should set appropriate limits for each isoform in the drug substance specification and unless otherwise justified include it in the stability studies with appropriate limits.

A quantitative test should be applied controlling the levels of deamidated forms of the protein in drug substance and drug product, at release and end-of-shelf life.

The apparent difference in estimated purity of product seen in the analyses by SDS-PAGE, silver stain, SDS-PAGE, Coomassie stain (using 4 µg for analysis), and SDS-PAGE, Coomassie stain (using 10 µg for analysis), respectively, needs to be further investigated in order to identify the cause of the different results and to establish an appropriate protocol for use of the SDS-PAGE in analysis of the purity of product.

The applicant should address the concern that higher levels of these impurities are present in commercial-scale batches than in the batch used for clinical studies (CAMR 004) and should demonstrate that the controls applied to the commercial-scale drug substance and drug product ensure that the manufacturing process is robust and commercial-scale batches are comparable to those used during pivotal clinical studies.

Until this major objection has been satisfactorily resolved it is not possible to establish a storage period for the active ingredient.

A number of other concerns were also raised in the initial assessment. Most of these have been satisfactorily addressed but some still remain and some remaining aspects of others are now included in the major objections.

Drug Product

Voraxaze is supplied as a sterile freeze-dried preparation in 3 mL Ph. Eur. Type 1 glass vials. Each vial contains a nominal 1000 Units of glucarpidase, intended for injection after reconstitution with 1mL sterile normal saline. The solvent will not be included in the pack. Voraxaze is available in pack sizes of 1 or 4 vials.

The drug product is processed by a contract manufacturer, Cangene Corporation, Winnipeg, Canada. The product will be shipped to Biotec UK where it will be labelled and packaged for commercial use.

The composition of the bulk substance and drug product is identical and therefore production at Cangene includes solely the steps of thawing, sterile filtration, filling, lyophilisation, capping and the release testing for product sterility. Except for the step of lyophilisation, satisfactory validation studies were initially reported supporting acceptable control in the manufacture of drug product. An acceptable validation report for the lyophilisation has now been submitted.

It was identified in the initial round that already after three months, one batch failed to meet the specifications for drug product when stored under accelerated conditions. The Company held the possibility of a Maillard reaction between lactose and the enzyme as the likely explanation as to why the specification was not met and referred to error made during formulation of this batch. The mechanism by which the handling of this batch in production would enhance the Maillard reaction, however, was not discussed. Since lactosylation of the enzyme was detected also in the characterization studies being most pronounced in the CAMR batches, the Applicant was asked to review current knowledge on critical parameters controlling the Maillard reaction and make an assessment of the risk for the reaction to occur in drug product stored under refrigerated conditions, although by different kinetics. In response to this the applicant has submitted more stability data. In the extended characterisation studies, lactosylated forms of the enzyme were seen in all batches and the reference left in the response to the formation of these species under stress conditions seems therefore irrelevant. However, although the lactosylated forms have been found present, evidence on the Maillard reaction has only been seen in lot 2090301 where a mistake was made during formulation and the batch did not freeze dry correctly. Thus the proposed link between the chemical reaction and denaturation of the protein caused by malfunction of the lyophilisation step seems reasonable.

Until the major objection on control methods has been satisfactorily resolved it is not possible to establish a shelf life for the drug product.

As for the drug substance, a number of other concerns were also raised in the initial assessment. Most of these have been satisfactorily addressed but some remaining aspects of others are included in the major objections.

III.2 Non clinical aspects

Pharmacology

Carboxypeptidase G2 is a zinc-dependent enzyme closely related to the carboxypeptidase G1. The carboxypeptidase G class of enzymes hydrolyzes the C-terminal glutamate moiety from folic acid and analogs such as methotrexate, polyglutamate derivatives of folic acid, subfragments such as p-aminobenzoylglutamate and specific small peptides with C-terminal glutamate residues. Carboxypeptidase G enzymes have been isolated from a number of pseudomonas and they have all similar substrate specificities. Carboxypeptidase G2 (CPG2) differs from other carboxypeptidases in physical and kinetical properties, but as other carboxypeptidases has a role in cancer chemotherapy. The Km of the enzyme has been reported to 8 µM for methotrexate and 120 µM for 5-formyltetrahydrofolate. The potential usefulness of CPDG2 has been known since the 1970ies but the limited supply of the enzyme has prevented a more widespread use as a rescue agent.

The rationale for use of glucarpidase in methotrexate toxicity is based on the fact that the enzyme will hydrolyze the carboxyl terminal glutamate residue from compounds such as methotrexate, producing

glutamate and 2,4-diamino-N^{10}-methylpteroic acid (DAMPA), normally a minor metabolite of methotrexate and an inactive metabolite based on potential to inhibit dihydrofolate reductase activity. DAMPA and glutamate are metabolised by the liver, and thus use of Voraxaze provides an alternative route of elimination. Patients at risk for methotrexate toxicity are those with impaired renal function or with evidence of delayed elimination. In a population of patients methotrexate and metabolites may precipitate in renal tubules leading to life-threatening acute renal dysfunction. To counteract systemic MTX toxicity administration of FA is employed. However, FA may also rescue tumour cells along with patient's normal cells.

Methotrexate cannot be metabolised to inactive products in species such as mouse and human that lack certain hepatic enzymes. Glucarpidase will hydrolyze methotrexate to 2,4-diamino-N10-methylpteroic acid (DAMPA) that is metabolised by the liver and studies in mouse showed that intravenous glucarpidase could increase DAMPA levels from 0.212-0.607 µmol/l to 3.35-57.1 µmol/l postglucarpidase. In a 6 day mouse study the administration of glucarpidase appeared to provide some amelioration additional to FA of methotrexate toxicity as assessed by gross macroscopy of gastrointestinal changes (dilatation), but not by histology. Conclusive evidence of an "add-on" effect of glucarpidase to FA has thus not been possible to obtain partly due to difficulties in finding suitable animal model. In monkey intrathecally administered glucarpidase was found to counteract neurotoxicity induced by intrathecal methotrexate. In rabbit there was some evidence of rescue by intrathecal glucarpidase of methotrexate toxicity but there were signs of toxicity (including seizures and neurogenic pulmonary oedema) in animals which received both MTX and glucarpidase, and in animals treated with intrathecal DAMPA. There was concern that these effects may have been due to administration via cisternal puncture. In monkey, intravenous glucarpidase doses of 1 to 50 Units/kg were not related to any clear dose-dependent effects. A decrease in plasma methotrexate levels was seen already at a dose of 1 U/kg but more consistently from 5 U/kg. In parallel to the decrease in methotrexate, an increase in DAMPA levels was recorded. An adverse cardiovascular reaction was noted in the one animal given 50 U/kg, but with an uncertain relationship to glucarpidase administration. The effectiveness of intravenous glucarpidase on methotrexate toxicity as reflected in plasma kinetics was recorded in Rhesus monkey as a decrease in t1/2 of methotrexate from 5.8 minutes to 42 seconds and a >2 log decrease in plasma levels in 15 minutes. After repeated administration of glucarpidase in rhesus monkey, antibodies to the enzyme were produced and while no allergic reactions were reported a decrease in the effectiveness of glucarpidase was reported.

Glucarpidase (formerly carboxypeptidase G2), differs from the carboxypeptidase G1 in that it has similar Km values for folate, methotrexate and 5-methyltetrahydrofolate but a 10-fold lower affinity for 5-formyltetrahydrofolate (FA). This may be important when used in rescue regimens alongside FA when high dose methotrexate has led to toxicity. Further, there are reports in the literature that depending on differences in degradation of the active 6S and the inactive 6R stereoisomers of FA, the selectivity of glucarpidase may be overestimated and the protective effects of FA might be antagonised by the enzyme. Folinic acid is recommended not to be administered 2 to 4 hours before or after Voraxaze administration. These issues are further considered in the clinical sections.

Overall, preclinical data, although of varying quality is consistent with that the hydrolytic activity of glucarpidase either alone or in conjunction with FA, is effective in transforming methotrexate to inactive non-toxic metabolites *in vitro* and *in vivo*. Limited studies in rabbit and monkey were consistent with intrathecal administration of glucarpidase being able to provide rescue of intrathecal toxicity of methotrexate. The optimal dose of glucarpidase is unclear from preclinical data, but the enzyme *per se* seems devoid of any significant toxicity. No specific interaction studies are available, but data in the literature indicate that thymidine does not interfere with glucarpidase rescue.

Pharmacokinetics

The pharmacokinetics of glucarpidase are in principle limited to a study in rabbits that was also a comparability study. Glucarpidase is an enzyme and as such studies on metabolism, excretion or distribution are not considered meaningful. Pharmacokinetic data that included determination of maximum plasma levels, half-life and systemic exposure in mouse, rat, dog and monkey were consistent with that these species also are relevant models for human to use in toxicology studies. Because the

enzyme is to be used in methotrexate toxicity, possible species differences in metabolism of methotrexate are also relevant in this context. For example, methotrexate is extensively metabolised to the 7-hydroxy compound while this reaction is not initially considered to occur to any significant extent in humans or other species.

In order to study the effect of glucarpidase on FA in presence of MTX, the Applicant performed a new pharmacokinetic study in rabbits. Unfortunately, the results from this study are difficult to interpret for several reasons. In contrast to the results in humans, there was no marked effect of glucarpidase on FA in the absence of MTX in rabbits. The presence of MTX+glucarpidase indeed seemed to *increase* FA exposure, compared with when FA was given alone, and this effect increased at later FA doses. These observations might indicate another mechanism for the interaction between FA, MTX and glucarpidase in rabbits than in humans, and it is therefore difficult to extrapolate results from this study to the clinical situation.

Toxicology

The toxicity of glucarpidase to be used as rescue in methotrexate toxicity may not only be considered in terms of specific, "direct" toxicity but also as secondary toxicity, related to formation of DAMPA, normally a minor metabolite of methotrexate metabolism. The potential of DAMPA to cause toxicity is partially addressed in pharmacology studies where methotrexate was administered in combination with glucarpidase in mouse, rabbit and monkey studies. In monkey after intravenous administration no toxicity that could be attributed to DAMPA was reported. It was estimated that DAMPA levels in monkey studies (~51 µM) were in the same range as initial levels (2.33 to 18.73 µM) recorded in the clinic. However maximum human DAMPA levels of 890 µM have been measured in the clinic. Limited studies did not indicate any specific toxicity of glucarpidase when given by the intrathecal route, however, the DAMPA metabolite of methotrexate appeared to be associated with toxicity expressed as seizures and arrhythmia in rabbits. There was, though, concern that this may have been related to intrathecal administration *per se*. However, DAMPA produced by breakdown of MTX was not related to overt DAMPA-related toxicity. Additional studies using isolated rabbit heart and telemetric evaluation in dog did not indicate any significant, consistent adverse cardiovascular effects related to DAMPA administration. Glucarpidase by the intrathecal route was not toxic. In monkey, toxicity after intrathecal rescue by glucarpidase appeared limited. Adverse effects linked to glucarpidase in monkey were described as pleocytosis. The data in monkey suggest that intrathecal administration of glucarpidase could be valuable as rescue of overdose of methotrexate also providing a more rapid effect than lumbar CSF exchange or ventriculo-lumbar perfusion.

There are no studies available on potential for genotoxicity, carcinogenicity or reproduction toxicity. This is acceptable considering applicable guidelines and the pharmacological class. Further glucarpidase is to be used in cases of methotrexate toxicity or at risk of methotrexate toxicity with methotrexate being a substance with known genotoxic and reproduction toxicity potential. Overall, the proposed text in section 4.6 of the SPC is appropriate.

The repeated dose toxicity of glucarpidase by the intravenous route was investigated in rat and dog in a 3 and 14 day study. The dog appeared the most sensitive species with a NOAEL of 50 U/kg compared with a NOAEL of 5000 U/kg in rat. At the NOAEL in rat, Cmax was approximately 100 fold higher than corresponding value in humans at the MHD or approximately x14 higher based on allometric scaling. In dogs the NOAEL was equal to MHD or ½ based on surface area basis. Tolerability as reflected in NOAEL values appeared to differ in rat and dog with haematological reactions and congestion, haemorrhage of the gastrointestinal tract, lung and gall bladder in dog at high doses of 500 to 2500 U/kg. The differences may be partly explained by the longer duration (14 days with administration every 2nd day) of the dog study and a higher total dose. However, the total dose in dog that produced toxicity was lower than the highest non-toxic dose in rat. Test article-related changes in dog included increases in haemoglobin, and haematocrit and decreases in platelet and neutrophil counts. Increases in total bilirubin, blood urea nitrogen, creatinine, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltransferase, and triglycerides were also observed in mid and high dose males either dying or surviving to the end of the study. There were no treatment related effects on electrocardiogram recordings

observed in animals treated with 50 $U \cdot kg^{-1}$. In particular, there was no effect of treatment on QT or QTc intervals. There was no indication of any treatment-related deviation from pretest values in the surviving high dose females examined. The toxicity exhibited in the repeated dose study in dog was proposed to represent an exaggerated pharmacological effect of glucarpidase as a folic acid inhibitor. Also the immunogenicity in dog may have contributed to the additional toxicity in dog while immunogenicity was not evaluated in rat. As discussed, likely these factors significantly contribute to the toxic response in dogs. Other toxicity that was evident in preclinical studies was coupled to the liver evidenced as increases in liver enzymes. Glucarpidase has been shown to be immunogenic in humans. Data from old monkey studies indicate that the effectiveness of glucarpidase may decrease with high titers of anti-glucarpidase antibody.

No specific studies on immunotoxicity or local toxicity have been submitted. This is considered acceptable also taking into account that local toxicity is at least in part considered in the repeated dose toxicity studies. Local tolerance related to possible accidental administration via other routes was not assessed and studies are not considered necessary also in view of the expected close monitoring in the clinic.

From an environmental risk assessment perspective the conclusion that glucarpidase does not pose a potential risk to the environment seems justified.

III.3 Clinical aspects

Pharmacokinetics

Pharmacokinetic data in this application concerns both the primary efficacy endpoint – effect of glucarpidase on MTX/DAMPA plasma/serum concentrations, which is mainly discussed in the Clinical Efficacy section – and the pharmacokinetics of glucarpidase.

Assay methods for MTX and DAMPA

The simple assays used for routine analysis of MTX in many hospitals will over-estimate MTX concentrations after treatment with glucarpidase, due to cross-reactivity with the MTX degradation product, DAMPA. The proposed SPC mentions the risk of over-estimation of MTX concentrations. However, this might still pose a problem in clinical practice during monitoring of MTX concentrations for further FA treatment. This is further discussed below.

Determination of MTX and DAMPA concentrations in patient samples from the pivotal efficacy studies was made using validated HPLC assays at two central facilities. These methods could separate between MTX and DAMPA.

Glucarpidase was originally developed by the Centre of Applied Microbiology and Research (CAMR). The commercial batches will be manufactured by Eurogentec (bulk) and Cangene (product). The Applicant states that the NCI and Berlin studies were performed with the batch of glucarpidase (CAMR Lot 004) manufactured in February 1991 and used until June 2004. No pharmacokinetic data have been generated in man for glucarpidase manufactured by CAMR. However, pharmaceutical and non-clinical studies have been performed to demonstrate that CAMR Lot 004 and material produced by the commercial process are comparable. These data are discussed in the Quality and Non-clinical assessments.

Theoretically, glucarpidase might continue to metabolise MTX in plasma samples after they have been drawn from a patient. The NCI and Berlin protocols therefore required sites to inactivate glucarpidase in the samples by acid treatment prior to shipment to analysis centres for the HPLC assay of MTX concentrations. However, in the original application, it had not been unequivocally shown that, depending on sample handling, some part of the extensive degradation of MTX in the pivotal studies did indeed occur *ex vivo*, after blood samples had been drawn. However, additional *in vitro* data, provided with the response to day 120 LoQ, confirms that the effect of glucarpidase on MTX in blood and plasma is very rapid, with the major part of the degradation finalised already after 15 minutes. Thus, although the provided studies may not have been adequate to discriminate between different sample handling

procedures, the data altogether indicate that the main part of the degradation of MTX observed in blood samples obtained at 15 minutes after the glucarpidase dose from patients in the pivotal studies did likely occur *in vivo*, and not after the blood samples were drawn.

Pharmacokinetics of glucarpidase

Data on glucarpidase pharmacokinetics have been generated in one phase I, single-dose study in 8 healthy, adult subjects and 4 subjects with renal impairment, who did not receive MTX. Glucarpidase was administered at the therapeutic dose of 50 Units/kg as a short intravenous infusion. Glucarpidase concentrations in plasma were determined using validated enzyme and ELISA methods. The volume of distribution was about 60 ml/kg indicating distribution mainly to the blood volume. The elimination $t_{1/2}$ was about 9-10 hours. The elimination pathways have not been specifically evaluated, but this is acceptable for a protein drug. There were no apparent differences in pharmacokinetics between healthy subjects and subjects with creatinine clearance < 30 ml/min. The same dose is suggested for children and adults. As children were included in the efficacy/safety analysis, additional pharmacokinetic data is not considered necessary for this population. Apart from the interaction with FA discussed below, the risk for pharmacokinetic (metabolic) interactions with glucarpidase is expected to be low.

Interaction with FA

Folinic acid (FA) is a competitive substrate for glucarpidase, albeit with a lower affinity to the enzyme than MTX, and the proposed SPC therefore recommends that FA should not be administered within 2-4 hours prior to and after administration of Voraxaze. As the half-life of the active L-form of FA is short (35 minutes) and the initial effect of glucarpidase on MTX concentrations is rapid, the Applicant suggests that a 2- hour interval before and after glucarpidase administration, respectively, will avoid effects of *FA on glucarpidase*.

On the other hand, the potential effect of *glucarpidase on FA* was raised as a major concern at day 120. In response, the Applicant has performed a new study in healthy volunteers, where FA doses were administered at 2 to 26 hours after a glucarpidase dose. The exposure to and half-life of the active isomer, L-FA, was reduced by about 50%, when FA was administered 2 hours after glucarpidase. The activated form, L-5-methyl-tetrahydrofolate (L-5-MeTHF), decreased even more, with levels below the limit of quantification at all time-points. The effect of glucarpidase on FA was smaller the longer time there was between glucarpidase and FA administration. When the dose was given 26 hours after glucarpidase, the exposure to L-FA decreased by about 20% compared with FA given alone. However, the exposure to the active form, 5-Me-THF, was still 75% lower than when FA was administered alone for the 26 hour-post-glucarpidase FA dose, indicating that also low levels of glucarpidase are sufficient to have an effect on FA.

The Applicant suggests that the effect of glucarpidase on FA will be lower in presence of MTX, since MTX has a higher affinity to glucarpidase and will compete with FA for glucarpidase. There are, however, some obvious objections to this theoretical consideration. Given that MTX concentrations decreases extensively within 15 minutes of glucarpidase administration, it is doubtful whether there will be enough MTX left to compete with an FA dose given 2 or more hours after Voraxaze. Moreover, glucarpidase may be present in high excess, so that it can readily metabolise MTX as well as FA without competition.

In order to study the effect of glucarpidase on FA in presence of MTX, the Applicant performed a new pharmacokinetic study in rabbits. Unfortunately, the results from this study are difficult to interpret for several reasons and might indicate another mechanism for the interaction between FA, MTX and glucarpidase in rabbits than in humans (see Preclinical aspects above). It is therefore difficult to extrapolate results from the rabbit study to the clinical situation.

Thus, all the above considerations taken together, it is still not possible to exclude that glucarpidase might have negative effects on FA rescue treatment after a Voraxase dose. Although, in theory, it is reasonable to believe that an effective degradation of MTX in plasma is beneficial, it is not known how this affects MTX already taken up by cells. Without proven benefit of Voraxaze treatment compared with FA alone,

the potential risk of decreased efficacy of FA is a remaining major concern. This is further discussed under clinical efficacy below.

Pharmacokinetics of DAMPA

The pharmacokinetics of the MTX breakdown product, DAMPA, formed by glucarpidase, has been briefly discussed by the Applicant. The half-life of DAMPA reported in humans is 9-12 hours. Based on a study in monkeys, about half of the formed DAMPA is eliminated unchanged in the urine. Three metabolites have been identified in humans and monkeys, hydroxy-DAMPA, DAMPA-glucuronide and hydroxy-DAMPA-glucuronide. The pharmacokinetics of DAMPA is important in several aspects. As routine assays for MTX will over-predict MTX concentrations after administration of glucarpidase due to presence of DAMPA, it is important to include information in the SPC on the half-life of DAMPA. In response to day 120 LoQ, the Applicant has proposed a new paragraph in section 4.4., describing the problem.

The Applicant has performed *in vitro* studies to evaluate the effect of DAMPA on CYP450 enzymes. The results indicate that clinically relevant effects of DAMPA on CYP450 are unlikely at a single exposure.

Repeated doses of glucarpidase during the same MTX cycle are not recommended in the proposed SPC, but were occasionally given in the treatment programs, and might be given also in clinical practice. The additional doses appeared to be less effective than the first dose. The Applicant has discussed whether this might have been due to high levels of the DAMPA, possibly inhibiting the enzyme activity by negative feed-back, or to an impurity in MTX formulations, D-MTX, which is not a substrate for glucarpidase. However, no conclusions could be drawn.

Pharmacodynamics

The mechanism of action is enzymatic cleavage, by glucarpidase, of the MTX molecule, resulting in inactive e.g. non-toxic metabolites. The determination of MTX and the metabolite DAMPA in serum directly reflects the action of glucarpidase. The enzyme specificity of glucarpidase for MTX is not total and folates including the rescue medication FA (FA) are also substrates. The affinity of glucarpidase to FA is lower than to MTX, but the clinical consequences of the interaction with FA are potentially serious. The potential of glucarpidase to affect the pharmacokinetics of FA, being the cornerstone in clinical management of MTX toxicity, needs further investigation. As discussed under Pharmacokinetics above enough glucarpidase may still be present when an FA dose is given 2-4 hours after glucarpidase administration (as recommended by the applicant), to decrease the efficacy of FA. Considering the type of product and mode of action, neither the tolerability nor the metabolic interaction potential of glucarpidase, is expected to be a problem.

There is absence of dose-response data and in response to concerns raised in the primary assessment of the dossier.

The applicant is planning further studies in patients investigating plasma FA pharmacokinetics in patients treated with glucarpidase.

Anti-glucarpidase antibody formation

Sampling for anti-glucarpidase antibodies was made in the PK, NCI, Berlin and PD studies. The antibody formation post glucarpidase was 37-43% in the clinical studies and did not seem to be related to age or gender. *In vitro* data indicate that the antibodies may have some neutralising potency: serum from four of 22 patients with an antibody response inhibited glucarpidase enzymatic activity *in vitro*. The inhibition was to 28-58%. As the anticipated use of glucarpidase is one single dose, antibody formation may not be considered a critical issue. The issue regarding effect of anti-glucarpidase antibodies will be addressed in the planned Study (PR001-CLN-pro009). Awaiting the results from this study a warning is amended to section 4.4 of the SPC.

Clinical efficacy

No dose-response studies have been performed with glucarpidase. The key efficacy data included in this application comes from two clinical studies, the NCI Study (PR001-CLN-rpt002), in which the majority of patients were treated in the US, and the Berlin Study (PR001-CLN-rpt001), in which all the patients were treated in Europe or Israel. The majority of patients received only a single dose (7-73 units/kg) of glucarpidase by intravenous (IV) infusion over about 5 minutes. Patients of any age and irrespective of their underlying cancer diagnosis were eligible for treatment with glucarpidase if they were determined to be at risk of life-threatening MTX toxicity secondary to delayed MTX elimination and/or renal impairment following MTX administration.

The studies were conducted within the context of a named patient/compassionate use program. The emergency situation when a patient is at risk of life-threatening MTX-toxicity is obviously not ideal for randomised comparisons against placebo. The rarity of the condition and the occurrence of the ultimate clinical end point, lethal MTX-toxicity, add to the opinion that comparative studies are not feasible in this clinical situation.

Blood sampling for MTX was adequate and performed throughout at least 7 days after administration of glucarpidase. Sampling procedure and processing has been standardised and analysis performed at two central laboratories. As drug concentrations constitute the primary efficacy measure the quality of the data is crucial. The well established relationship between high MTX concentrations in plasma and potentially life-threatening toxicity makes MTX concentrations a suitable pharmacodynamic surrogate end point for effect, i.e. reduced toxicity. However, that assumes that no other factors play a role for the clinical effect. Potential toxicity of DAMPA, which is present in high levels post glucarpidase treatment, or potential interaction of glucarpidase with the rescue agent (FA), and possibly other unknown factors have to be ruled out. Preclinical data suggest that DAMPA is not a great problem. But entrapment of MTX within cells due to its conversion to a polyglutamated form could also be a reason why detoxification by elimination from plasma only is not enough

The cohort of patients with efficacy data to evaluate the primary endpoint (MTX determinations pre and post glucarpidase treatment) constitutes 103 patients. The total number of 230 patients recruited constitutes the population in which the MTX toxicity can be evaluated. The applicant has presented tables of demographic and disease- and treatment-related factors convincingly showing that the cohort of 103 patients with known MTX concentrations does not differ from the total population studied.

Glucarpidase produced a rapid, marked and sustained reduction in systemic MTX concentration in all three studies and >50% patients achieved a concentration that was below that known to be associated with serious toxicity in all post-glucarpidase samples (1μmol/L). The rapid decline in MTX concentration by approximately 2 logs shortly post glucarpidase administration is compelling evidence of the *in vivo* action of the enzyme in patients. The data is overwhelmingly consistent regarding this feature and no patient seem to behave differently. The PK of MTX during the follow-up period differs between patients and the proportion of patients with rebound concentration >0.1 μmol/L or >2-fold the first post-glucarpidase concentration was 39, 48 and 74 % in the NCI, PD and Berlin studies, respectively. The toxic implications of this are not possible to estimate, but it means that these patients will need further treatment with FA. Since FA is also a substrate for glucarpidase it should be considered that if it is still present there might be a risk that the antidote is inactivated. Furthermore, as mentioned above, the commonly used assays for determinations of MTX concentration are insufficient after glucarpidase administration, since they overestimate MTX concentrations (because of inability to differ between MTX and DAMPA). Thus, the clinical monitoring of MTX toxicity is interfered with and appropriate dosing of FA is difficult. The problem is however considered as minor since it will only lead to administration of higher doses of FA, which is not a toxic substance.

The MTX-related deaths in the NCI and Berlin studies affected 6 (12% of adult patients) and 24 % of the patients, respectively. In the PD study in total 3% died (6% in the adult population), and no children had a fatal outcome in this study. That is considered a large proportion of the treated patients. Without an untreated control group it is impossible to say if more would have died without glucarpidase, or if glucarpidase induced some of the cases by inactivating FA rescue medication. In analogy, the MTX toxicity data cannot be used to evaluate glucarpidase efficacy in terms of clinical benefit.

Dosing recommendations

The proposed SmPC and PI recommend that patients with evidence of delayed elimination of MTX, based on systemic (plasma or serum) MTX concentrations, be administered glucarpidase. In order to provide guidance on when MTX elimination should be considered delayed, the pharmacokinetics (PK) of high dose MTX have been modeled in order to provide a baseline of systemic concentrations at certain time points that are expected in patients with normal renal function. Based on the model a table of systemic concentrations of MTX predicted for subjects with 50% normal clearance rate was inserted in section 4.2 of the SPC, to guide the clinician. However, there is no clinical evidence behind recommending glucarpidase to all patients with the suggested degree of renal impairment. Thus, the dosing recommendations need some kind of validation by clinical toxicity data.

Clinical safety

Patients suffering from life-threatening MTX toxicity constitute a difficult population to detect additional toxicity in. Since glucarpidase is a protein and given only once, severe safety problems are not expected. The exposed population consists of 230 patients and 12 healthy volunteers, which should be sufficient to determine the safety profile of glucarpidase.

The frequency of AEs associated with glucarpidase is very small. Very few patients reported an AE that was considered to be related to glucarpidase: 9/180 (5%) in the NCI Study, 4/42 (10%) in the Berlin Study and 2/8 (25%) in the PD Study. No AEs related to glucarpidase were reported in the PK Study.

No listings of all AEs by study, or compiled data, were presented in the summary.

Three non-fatal SAEs were observed in the NCI Study, and in the Berlin Study, 10 SAEs were recorded affecting 4 patients. No serious AEs (SAEs) related to glucarpidase were reported in any study or in the literature.

The all cause mortality rates in the NCI and Berlin Studies were 10.6% and 42.9%, respectively. Of the 19 study deaths in the NCI Study, thirteen may be attributed to causes related to MTX treatment. Six deaths were considered to be due to other causes including disease progression. In the Berlin study 10 of the 18 deaths on study were considered to be related to MTX. Three patients died due to progression of the underlying malignant disease. Five patients died after subsequent chemotherapy or transplantation due to infections or cardiovascular causes. No deaths were considered by the investigators to be related to glucarpidase treatment in either the NCI or Berlin Studies. Furthermore, there were no deaths amongst the 8 patients reported in the PD Study (complete details of the entire PD study were included in the 120 responses). No deaths related to glucarpidase are reported in the literature. The absence of fatal toxicity related to glucarpidase was endorsed.

Antibody-formation is common after glucarpidase treatment. There is a concern regarding this issue as regards readministration of glucarpidase. Readministration of glucarpidase is thus not recommended.

Routine pharmacovigilance practice as suggested by the applicant in the submitted “Safety Specification and Pharmacovigilance Plan” is reasonable, based on the safety conclusions drawn regarding glucarpidase.

IV. ORPHAN MEDICINAL PRODUCTS

According to the conclusion of the COMP (Opinion dated 03/02/03) the prevalence of the “condition” delayed elimination of methotrexate (MTX) is 0.076 per 10 000 individuals in the EU. The proposed indication for Voraxaze is ‘the adjunctive treatment of patients experiencing or at risk of methotrexate toxicity’ and an Orphan drug designation has been granted in the EU (EU/3/02/128) for its use in this iatrogenic condition.

V. BENEFIT RISK ASSESSMENT

As detailed in the assessment and specified in the list of questions, there are outstanding major objections concerning the quality documentation.

Non-clinical studies are limited. The primary effect of glucarpidase has been shown and studies are generally sufficient in scope. In animal models, repeated doses of glucarpidase were related to immunogenicity and signs of adverse liver reactions. In a mouse study the administration of glucarpidase appeared to provide some amelioration additional to FA of methotrexate toxicity as assessed by gross macroscopy of gastrointestinal changes (dilatation), but not by histology and it is acknowledged that there is no readily available model to reproduce high methotrexate blood levels.

The pharmacokinetics of glucarpidase has been sparsely evaluated but the information is considered sufficient for an enzyme. The main, remaining pharmacokinetic concern instead relates to clinical efficacy, concerning the interaction with FA.

The lack of dose related outcome data remains an unsolved issue. Although there seem to be some information available since the dosing differed between 11 and 73 units/kg in the clinical studies the applicant did not provide more information regarding this issue. The proposed dose is not justified by any clinical data and it is not shown that repeated use of glucarpidase is beneficial.

High dose MTX is widely used and with adequate measures, including fluid treatment, alkalinisation of the urine and FA rescue, it can generally be handled with manageable toxic effects. It is also clear that some cases with severe MTX toxicity and prolonged excretion do occur despite these measures. Even though it is shown by the applicant that glucarpidase lowers MTX plasma concentrations, at present it has not been proven that glucarpidase is adding a beneficial effect in those cases. Neither comparative data with and without glucarpidase in patients, nor preclinical supportive data, is available.

It is not evident that lowering MTX concentrations in plasma is enough to counteract the toxic effects of MTX. MTX is glutamated intracellularly, thus trapped within the cells and might, at least to some extent, continue to exert its toxic effects despite the low plasma levels obtained with glucarpidase. However, the main issue is that the new PK data provided by the applicant show that the *in vivo* FA interaction cannot be neglected when the potential value of glucarpidase is estimated.

In summary, the benefit of glucarpidase is a rapid decrease of the MTX levels in plasma, it is not known whether intracellular concentrations really decrease (entrapment of polyglutamated drug) or if there is a reduction of MTX induced toxicity, due to absence of data. The toxicity profile of glucarpidase seems favourable. The main risk is the potential of glucarpidase to interfere with the rescue agent FA, which constitutes an essential part of the clinical management of MTX toxicity. A possible circumvention of such effect cannot be assumed based on the presented data. Thus, a positive benefit/risk cannot be concluded.